Viruses 2018 – Breakthroughs in Viral Replication

Faculty of Biology

University of Barcelona

Spain

7 – 9 February 2018

Conference Chair

Eric O. Freed

Conference Co-Chair

Albert Bosch

Organised by

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viruses
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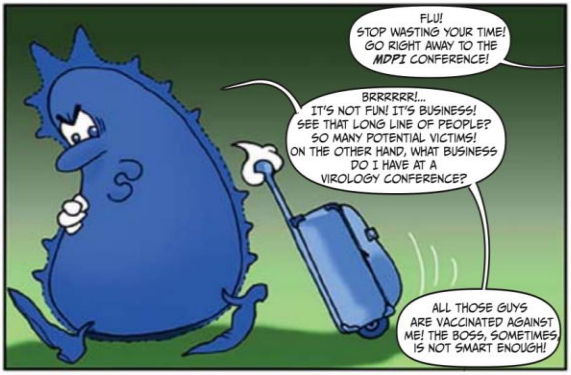

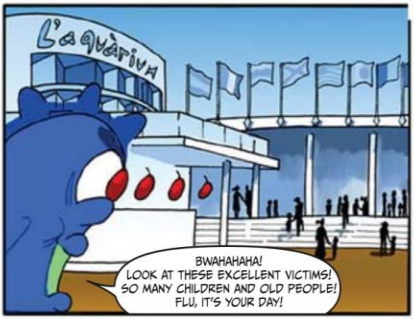

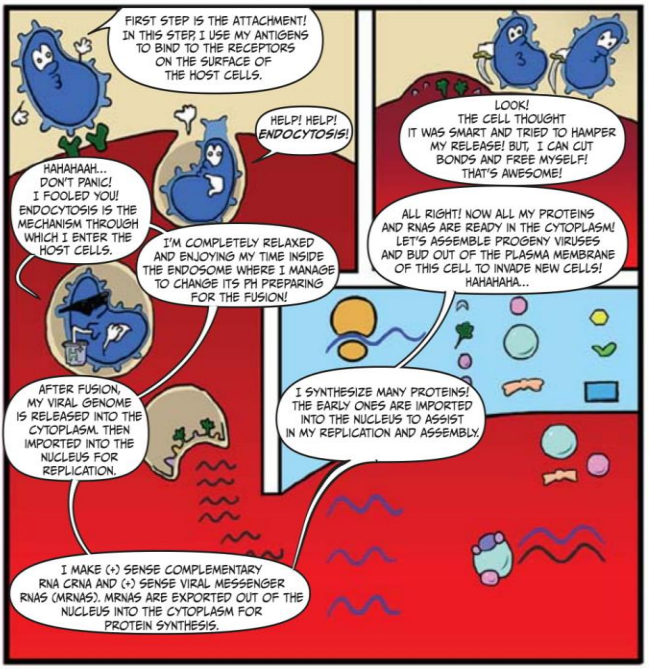

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7 – 9 February 2018, Barcelona, Spain

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Wednesday 7 February 2018: 08:00 - 12:30 / 14:00 - 18:00 / Conference Dinner: 20:30

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Conference Programme

Wednesday 7 February

08:00 – 08:45	Check-in
08:45 – 09:00	Opening Ceremony by Eric O. Freed and Albert Bosch

Session 1 – General Topics in Virology
Chairs: Thommas Klimkait and Joanna Parish

09:00 – 09:30	**Ralf Bartenschlager** "New insights into *Flaviviridae* – Host Cell Interactions"
09:30 – 09:45	**Rachel Whitaker** "CRISPR-Cas Co-evolutionary Dynamics in Natural Host-virus Populations"
09:45 – 10:00	**Michael Bauer** "Ubiquitination-dependent Adenovirus Capsid Disassembly at the Nuclear Pore Complex"
10:00 – 10:15	**Moriah Szpara** "Genomic and Phenotypic Diversity Associated with Neonatal HSV-2 Disease"
10:15 – 10:45	**Coffee Break**
10:45 – 11:15	**Rosa Pintó** "Hepatitis A Virus Codon Usage: Implications for Virus Biology and Phenotype"
11:15 – 11:45	**Michael S. Diamond** "CRISPR-Cas9 Screens Define Novel Host Factors Required for Infection by Arthritogenic Alphaviruses"
11:45 – 12:00	**Itay Rousso** "Reverse Transcription Mechanically Induces HIV-1 Capsid Disassembly"
12:00 – 12:15	**Heinrich Gottlinger** "SERINCs and HIV Infectivity"
12:15 – 12:30	**Inés Romero Brey** "Visualizing the 3D Architecture of the Hepatitis A Virus Replication Organelles"
12:30 – 14:00	**Lunch**

Session 2 – Structural Virology - **UPDATE**

Chair: Delphine Muriaux and Kay Choi

14:00 – 14:15	**Yohei Yamauchi** "An Influenza Uncoating Epitope Confers Capsid Stability"
14:15 – 14:45	**Felix A. Rey** "Relations between Cell–Cell Fusion, Eukaryotic Evolution and Class II Enveloped Viruses Identified by Structural Studies"
14:45 – 15:00	**Kelly Lee** "Dynamics Bridge Structure, Function, and Phenotype in Viral Entry Machines"
15:00 – 15:15	**Hadas Cohen Dvashi** "Structure-based Rational Design of Broad Range Immunotherapy Targeting TfR1-tropic Arenaviruses"
15:15 – 15:30	**Matteo Castelli** "Unraveling the Structure-function Relationship of Hepatitis C Virus Fusion Machinery by in Silico Structural Modeling"
15:30 – 16:00	**Coffee Break**
16:00 – 16:30	**Eric O. Freed** "HIV-1 Assembly, Release, and Maturation"
16:30 – 17:00	**Leo James** "Viral Genome Hide and Seek; Encapsidation versus Host Immunity"
17:00 – 17:15	**Ana Joaquina Perez-Berná** "Correlative Study of Cellular Modification Induced by Hepatitis C Infection by Cryo Soft X-ray Tomography and Infrared Microscopy"
17:15 – 17:30	**Diego Ferrero** "Supramolecular Arrangement of the Zika Virus NS5 Protein"
17:30 – 17:45	**Jamil Saad** "Towards Elucidating the Structural Basis for Envelope Incorporation into HIV-1 Particles"
20:30	**Conference Dinner**

Thursday 8 February

Session 3 – Virus Replication Compartments
Chair: Stefano Aquaro and Nathalie Arhel

08:30 – 09:00	**Sandra Weller** "Roles of UL12 and ICP8 in the Production of Herpes Simplex Virus DNA that can be Packaged into Infectious Virus"
09:00 – 09:30	**Sara Sawyer** "Spillover: How Viruses Adapt at the Animal–Human Interface"
09:30 – 09:45	**Thejaswi Nagaraju** "Mapping Epstein-Barr Viral Genome Replication Spatially and Temporally during the Viral Lytic Cycle"
09:45 – 10:00	**Jean-Francois Eleouet** "Respiratory Syncytial Virus RNA Synthesis in Cytoplasmic Inclusion Bodies: Organization and Functioning"
10:00 – 10:15	**Griffith Parks** "Cytoplasmic-Replicating Parainfluenza Virus Sensitizes the Nuclear Compartment to DNA-Damaging Agents; Implications for Oncolytic Virus Therapies"
10:15 – 10:45	**Coffee Break**
10:45 – 11:15	**Clodagh O'Shea** "Cracking Nuclear Codes: The 5D viral-cellular genome structures and interactions that drive pathological proliferation"
11:15 – 11:45	**Elena I. Frolova** "Assembly of Viral Replication Complexes: Roles for Viral and Host Proteins"
11:45 – 12:00	**Luis DaSilva** "ESCRT Machinery Activity is Required for Oropouche Virus Assembly at the Golgi Complex"
12:00 – 12:15	**Montserrat Bárcena** "Common Themes in the Ultrastructure and Function of the Coronavirus Replication Organelle"
12:15 – 12:30	**Catherine Eichwald** "Core or Viroplasm? Dissecting Rotavirus VP2 and NSP5 Interaction"
12:30 – 14:00	**Lunch**

Session 4 – Replication and Pathogenesis of RNA viruses

Chair: Josep Quer and Richard Sutton

14:00 – 14:30	**Amelia Nieto** "Epigenetic Control of Influenza Virus: Role of H3K79 Methylation in Interferon-induced Antiviral Response"
14:30 – 15:00	**Ian Goodfellow** "Identification of *Trans* Acting Factors Involved in the Norovirus Life Cycle"
15:00 – 15:15	**Rachel van Duyne** "HIV-1 Envelope Glycoproteins Confer Broad Resistance to Antiretrovirals in vitro"
15:15 – 15:30	**Natalya Teterina** "MicroRNA-based Suppression of Neurotropic Flavivirus Replication: Application for the Development of Live Attenuated Vaccine"
15:30 – 15:45	**Mariana Batista** "Adaptation of NrHV to the Mouse Host Using Selective Innate and Adaptive KO Strains"
15:45 – 16:00	**Fernando Ponz** "Differential Developmental Symptoms in Turnip Mosaic Virus-infected Hosts is Associated with the Dynamic Association of the Viral P3 Protein to the Endoplasmic Reticulum"
16:00 – 16:15	**Pietro Scaturro** "An Orthogonal Proteomic Screen of Zika virus Uncovers Novel Host-dependency Factors"
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16:30 – 18:30	**Apéro and Poster Session**

Friday 9 February

Session 5 – Genome Packaging and Replication/Assembly

Chair: Juan José López-Moya and F.L. Cosset

08:30 – 09:00	**K. Andrew White** "Intra-genomic RNA-based Regulation in a Plus-strand RNA Virus"
09:00 – 09:30	**Adam Zlotnick** "Assembly and Disassembly of HBV: Biology Recapitulates Physics"
09:30 – 09:45	**Kay Choi** "Crystal Structure of the Dengue Virus Promoter RNA"
09:45 – 10:00	**Vibhu Prasad** "Adenovirus Activation of the Unfolded Protein Response Sensor Ire1 Enhances Immediate Early Viral Transcription to Promote Viral Persistence and Lytic Egress"
10:00 – 10:15	**Myra Hosmillo** "Identification of cis-acting RNA Sequences Involved in Norovirus VPg-dependent RNA Synthesis"
10:15 – 10:45	**Coffee Break**
10:45 – 11:15	**Andrew Ward** "Structure, function, and immune recognition of the enigmatic HIV envelope glycoprotein"
11:15 – 11:30	**Alexander Borodavka** "Protein-Assisted RNA Folding Mediates Specific RNA–RNA Genome Segment Interactions in Segmented RNA Viruses"
11:30 – 11:45	**Brett Lindenbach** "The Hepacivirus and Pestivirus NS3 Helicases Act as Motor Proteins to Power RNA Encapsidation During Virus Particle Assembly"
11:45 – 12:00	**Solène Denolly** "The Amino-Terminus of the Hepatitis C Virus (HCV) p7 Viroporin and its Cleavage from Glycoprotein E2-p7 Precursor Determine Specific Infectivity and Secretion Levels of HCV Particle Type"
12:00 – 12:15	**José Almendral** "Differential Phosphorylation and N-terminal Configuration of Capsid Subunits in Parvovirus Assembly and Viral Trafficking"

12:15 – 14:00	**Lunch**

Session 6 – Antiviral Innate Immunity and Viral Pathogenesis
Chair: Shan-Lu Liu and Eric Poeschla

14:00 – 14:30	**Carolyn Coyne** "Stem Cell-derived Enteroids to Model Enterovirus-GI Interactions"
14:30 – 15:00	**Sara Cherry** "Harnessing Genomic Approaches to Explore the Interface between Viruses and Hosts"
15:00 – 15:30	**Urs Greber** "Towards Understanding 'Cell-to-Cell' Variability of Viral Gene Expression"
15:30 – 15:45	**Eric Snijder** "Middle East Respiratory Syndrome-Coronavirus Employs the Deubiquitinating Activity of its nsp3 Papain-like Protease to Suppress the Innate Immune Response"
15:45 – 16:15	**Coffee Break**
16:15 – 16:45	**Frank Kirchhoff** "HIV Accessory Factors: More than One Way to Skin a Cat"
16:45 – 17:15	**Alain Kohl** "Arboviruses and Antiviral RNAi Pathways in Mosquito Cells: Progress in Understanding Regulation and Effectors"
17:15 – 17:30	**Susana Guerra** "ISG15 Governs Mitochondrial Function in Macrophages following Vaccinia Virus Infection"
17:30 – 17:45	**Pilar Domingo-Calap** "Virus-virus Interactions Driven by Innate Immune Responses"
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18:00 – 18:15	**Closing Remarks**

Welcome by Eric O. Freed and Albert Bosch

Dear Colleagues,

It is with great pleasure that we welcome you in Barcelona to the conference "Viruses 2018: Breakthroughs in Viral Replication".

The importance of viruses to human health has never been more apparent, and significant progress is being made in understanding virus replication, structure, transmission, pathogenesis and antiviral immunity. This conference will bring together leading virologists from around the world to share their recent findings. During this exciting three-days event, meeting participants will have the opportunity to present posters and short talks on their work and discuss their research in a relaxed, collegial environment.

The conference is sponsored by MDPI, the publisher of the open-access journal *Viruses* and follows the very successful meeting "Viruses 2016: At the Forefront of Virus-Host Interactions" held in January 2016 in Basel, Switzerland.

We very much wish you enjoy this exciting meeting in Barcelona.

Best regards,

Eric O. Freed
Conference Chair

National Cancer Institute
NIH - Frederick, MD, USA

Albert Bosch
Conference co-Chair

Enteric Virus Laboratory
School of Biology
University of Barcelona, Spain

General Information

Viruses (ISSN 1999-4915) is an open access journal which provides an advanced forum for studies of viruses. It publishes reviews, regular research papers, communications, conference reports and short notes. *Viruses*'s aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. The journal's main subject areas include topics such as virology, bacteriophage, vaccines, viral immunology, virus structures and dynamics, etc.

Among other databases, *Viruses* is indexed by the Science Citation Index Expanded (Web of Science) and MEDLINE (PubMed).

Journal Webpage: http://www.mdpi.com/journal/viruses
Impact factor: 3.465 (2016); 5-Year Impact Factor: 3.640 (2016)

Viruses 2018 – Breakthroughs in Viral Replication will be held at the Faculty of Biology, University of Barcelona, on February 7 – 9, 2018.

This exciting three-day event seeks to gather leading virologists from around the world and to create a forum where they can share their most recent findings in the field of virology. This is an opportunity to discuss important breakthroughs in the field; broaden your knowledge, meet scientists from other areas and perhaps develop new mutually beneficial collaborations.

Conference Venue

Faculty of Biology, University of Barcelona
Avinguda Diagonal, 643, 08028 Barcelona, Spain

Registration Desk

The desk for registration, information and distribution of documents will be open from 08:00 on 7 February 2018.

Certificate of Attendance

Upon request, the participants of the event will receive an electronic Certificate of Attendance by email once the event is concluded.

Barcelona and Catalonia

Catalonia has become one of the favourite tourist destinations of Spain, mainly because of Barcelona, a city that never sleeps and knows how to please the big majority. With a history among the oldest in Europe, Barcelona offers a mixture of inland and seaside charms that panders the interests of everybody. The variety of artistic treasures, Romanesque churches and the works of famous artists such as Dalí, Gaudí, Miró or Picasso will make of your visit to the city a remarkable experience.

Barcelona is the capital and largest city of Catalonia and Spain's second largest city, with a population of over one and half million people (over five million in the whole province).
This city, bathed by the Mediterranean Sea, has become one of most cosmopolitan cities of Europe which has transformed it into the very modern, yet incredibly old city.
This beautiful city is full of what European cities are known for (outdoor markets, restaurants, shops, museums and churches) and which makes it the perfect scenario to get lost in its picturesque streets and avenues. Moreover, Barcelona's extensive and reliable Metro system will take you to more far-flung destinations. The core centre of the town, focused around the *Ciutat Vella* ("Old City"), provides days of enjoyment for those looking to experience the life of Barcelona while the beaches the city was built upon provide sun and relaxation during the long periods of agreeably warm weather. [Source: www.wikitravel.org].

Plaza Espanya (Source: www.viajero-turismo.com)

The Faculty of Biology, University of Barcelona

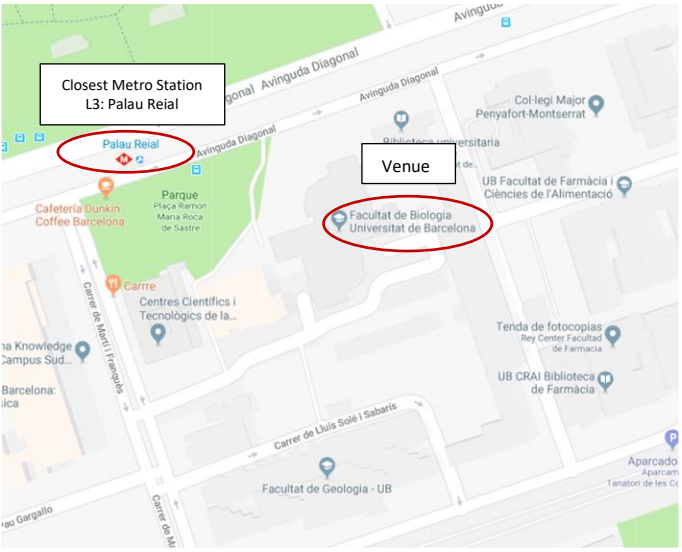
The conference will be held at the Faculty of Biology of the University of Barcelona. Established in 1974, it became a pioneering institution in Spain and first appeared as a result of increasing knowledge in the field of life sciences at a time of relentless diversification.

The University of Barcelona (UB) is the most formidable public institution of higher education in Catalonia, catering to the needs of the greatest number of students and delivering the broadest and most comprehensive offering in higher educational courses. The UB is also the principal centre of university research in Spain and has become a European benchmark for research activity, both in terms of the number of research programmes it conducts and the excellence these have achieved.

Having been founded in 1450, the University's own history is closely tied to the history of Barcelona and Catalonia, it combines the values of tradition with its position as an institution dedicated to innovation and teaching excellence: a university that is as outward-looking and cosmopolitan as the city from which it takes its name. For these reasons, it plays a direct and active part in the urban fabric of Barcelona, becoming a hub of cultural activity for the city itself. [Source: www.ub.edu].

How to Reach the Venue

Address: Avinguda Diagonal, 643, 08028 Barcelona, Spain



Venue Location (Source: www.google.es/maps/)

Conference Dinner

Wednesday 7 February, 20:30

The conference dinner will be held at Abrassame, a cutting-edge restaurant specialised in Mediterranean cuisine which, in addition to its location at the picturesque terrace of Arenas de Barcelona, will make of your evening at the restaurant an experience to remember.

Abrassame is located at *Gran Via de les Corts Catalanes 373 – 385*, only a few minutes away from one of Barcelona's most emblematic must-sees, Montjüic. You can easily reach the restaurant from the conference venue either by taxi or by Metro. If you were to choose the second option, the easiest way to get there is by taking the Metro Line L3 at Palau Reial Station and drop off at Espanya Metro Station after 6 stops.

Contact persons during the event

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Emergency Information

All emergencies in Spain: 112 (no area code needed)

Ambulance (Ambulancia) and health emergencies: 061 or 112
Fire brigade (Cuerpo de bomberos): 080 or 112
Spanish National Police (Policía nacional): 091

Pathogens (ISSN 2076-0817; CODEN: PATHCD) is an international, open access journal of pathogens and pathogen-host interactions published quarterly online by MDPI. It publishes reviews, regular research papers and short notes on all aspects of pathogens and pathogen-host interactions. *Pathogens's* aim is to encourage scientists to publish their experimental and theoretical research in as much detail as possible. Some of the journal's main subject areas include topics such as identification and characterization of pathogens, pathogen invasion and host defenses, transmission of pathogens, vectorology, immune defense mechanisms, etc.

Pathogens is indexed I the Emerging Sources Citation Index (ESCI – Web of Science), Scopus and PubMed Central.

Journal Webpage: http://www.mdpi.com/journal/pathogens

This Conference's **Best Oral Presentation Award** is sponsored by *Pathogens*.

Abstracts

Session 1: General Topics in Virology

Session Chairs – Thommas Klimkait and Joanna Parish

New Insights into *Flaviviridae* – Host Cell Interactions

Ralf Bartenschlager

Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany

Members of the *Flaviviridae* virus family comprise a large group of enveloped viruses with a single-strand RNA genome of positive polarity. Several genera belong to this family including the *Hepacivirus* genus, of which hepatitis C virus (HCV) is the prototype member and the *Flavivirus* genus, comprising both Dengue virus and Zika virus. Viruses of these genera differ in many respects such as the mode of transmission or the course of infection, being predominantly persistent in the case of HCV, or acute self-limiting in the case of flaviviruses. Although the fundamental replication strategy of *Flaviviridae* members is similar, during the last few years important differences have been discovered, including the way in which these viruses exploit cellular resources to facilitate viral propagation. These differences might be responsible, at least in part, for the various biological properties of these viruses, thus offering the possibility to learn from comparisons. In my talk, I will focus on some aspects related to the replication cycles of flavi- and hepaciviruses and how they exploit their host cells to achieve robust virus propagation.

NOTES

CRISPR-Cas Co-Evolutionary Dynamics in Natural Host–Virus Populations

Rachel Whitaker, Samantha DeWerff

University of Illinois at Urbana-Champaign, Urbama, IL, USA

We explore a unique virus–host interaction and uncover mutualistic outcomes of infection and how these effect population dynamics. To study this, we are utilizing the model system of the thermophile *Sulfolobus islandicus* and its virus *Sulfolobus*-spindle shaped virus 9 (SSV9) isolated from Kamchatka, Russia. We have demonstrated that in the natural population of *Sulfolobus* there is almost complete immunity to SSV9, and yet chronic infections persist. In chronic infection, the host can grow and replicate in the culture; however, it does so while continually producing new infectious viral particles. We show that chronically infected host from lab-controlled infections. In growth curves of pure culture, we have shown that there is a cost to infection, in which the infected strain is unable to reach the same carrying capacity of the un-infected ancestor. However, in competitions between an infected and an un-infected host, the infected host quickly fixes in the population, even when initially rare. These two results suggest that there is a trade-off in fitness for the infected host. In nature, this could be seen as beneficial, where the virus acts as natural weapon to prevent the colonization of the environment of a susceptible strain. We hypothesize that this is also beneficial to the chronic virus in a highly immune population, whereby it promotes its own vertical fitness.

NOTES

Ubiquitination-Dependent Adenovirus Capsid Disassembly at the Nuclear Pore Complex

Michael Bauer [1,2], Justin W. Flatt [1,3], Urs F. Greber [1]

[1] *Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland*
[2] *Life Science Zurich Graduate School, ETH and University of Zurich, Zurich, Switzerland*
[3] *Institute of Biotechnology and Department of Biosciences, University of Helsinki, Helsinki, Finland*

Adenoviruses (AdVs) deliver their genomes to the nucleus for replication. During cell entry, they engage a stepwise uncoating process which culminates in the dismantling of the capsid at the nuclear pore complex (NPC), releasing linear double-stranded DNA for nuclear translocation. The underlying mechanism of virion uncoating involves the binding to the nucleoporin Nup214 and kinesin-mediated pulling on the capsid, yet host factors that guide the overall process are largely unknown. Here, we have identified a cellular E3 ubiquitin ligase (E3L) that is required for successful import of genomes into the nucleus. Light and electron microscopy experiments indicate that E3L is involved in the separation of genomes from capsids docked at NPCs. Furthermore, we found that the E3L RING domain is essential for efficient uncoating and infection. CRISPR/Cas9 knockout of E3L in HeLa cells prevents docked virions from uncoating at NPCs, whereas later doxycycline-induced expression restores the final disassembly step. Intriguingly, inhibition of the proteasome impairs capsid disruption in the context of E3L functionality. The data indicate that E3L-mediated ubiquitination and the proteasome work in concert to release the AdV genome from the virus capsid. Through proteomics, we are exploring whether E3L and the proteasome unlock the docked virion by licensing an activating factor, removing an inhibitor, or directly modifying the capsid.

NOTES

Genomic and Phenotypic Diversity Associated with Neonatal HSV-2 Disease

Moriah Louise Szpara [1], Lisa Nowoslawski Akhtar [2], Christopher D. Bowen [1], Daniel W. Renner [1], Utsav Pandey [1], Ashley N. Della Fera [2], David W. W. Kimberlin [3], Mark N. Prichard [3], Richard J. Whitley [3], Matthew D. Weitzman [2]

[1] *Pennsylvania State University, PA, USA*
[2] *Children's Hospital of Philadelphia, Philadelphia, PA, USA*
[3] *University of Alabama at Birmingham, Birmingham, AL, USA*

Around 1 in 3200 births results in neonatal infection with herpes simplex virus type 1 or 2 (HSV-1 and HSV-2), often from mothers who are not aware of their infection or of active viral shedding. Half of these infants experience disseminated multi-organ or invasive disease in the central nervous system (CNS), while the remainder have more limited infections of the skin, eyes, or mouth (SEM). Invasive CNS and disseminated HSV infections are associated with much higher rates of permanent life-long morbidity and mortality. The viral and/or host factors that contribute to invasive HSV disease outcomes are not known. We evaluated ten HSV-2 isolates cultured from neonates with a range of clinical presentations, to reveal the level of viral diversity within this vulnerable population. These isolates were compared at both a phenotypic and a genomic level. To measure viral fitness independent of host immune factors, we measured viral growth characteristics of each isolate in cultured cells. We then sequenced the complete viral genomes of all ten neonatal HSV-2 isolates. We found extensive genomic diversity in these neonatal isolates, including amino acid variations in more than 90% of the proteins encoded by HSV-2. Phenotypic analyses revealed that a subset of invasive CNS isolates had a large plaque morphology in vitro, which correlated with a faster rate of spread from cell-to-cell. When the genomes of these isolates were compared to other neonatal isolates, we found that large plaque CNS isolates shared a non-synonymous variation in glycoprotein I (gI). HSV gI functions in cell-to-cell spread of virus, including spread between neurons. Ongoing work aims to understand the potential contribution of these genetic and phenotypic differences to the clinical outcomes observed in neonatal HSV disease in vivo. This study represents the first-ever application of comparative pathogen genomics to neonatal HSV disease.

NOTES

Hepatitis A Virus Codon Usage: Implications for Virus Biology and Phenotype

Rosa Pintó, Montserrat de Castellarnau, Anna Altisent, Susana Guix, Albert Bosch

University of Barcelona, Barcelona, Spain

Codon usage bias is universal to all genomes. Hepatitis A virus (HAV) codon usage is highly biased and deoptimized with respect to its host. Accordingly, HAV is unable to induce cellular translational shutoff and its internal ribosome entry site (IRES) is inefficient. Codon usage deoptimization may be seen as a hawk (host cell) versus dove (HAV) game strategy for accessing transfer RNA (tRNAs). HAV avoids the use of abundant host cell codons and thereby eludes competition for the corresponding tRNAs. Instead, codons that are abundant or rare in cellular messenger RNAs (mRNAs) are used relatively rarely in its genome, although intermediately abundant host cell codons are abundant in the viral genome. Rare codons in the capsid coding region slow down the translation elongation rate and, in doing so, intrinsically modulate capsid folding, which is critical to the stability of a virus transmitted through the fecal–oral route. HAV is a paradigmatic example of what has been proposed as a codon usage “code” for protein structure.

NOTES

CRISPR-Cas9 Screens Define Novel Host Factors Required for Infection by Arthritogenic Alphaviruses

Rong Zhang [1], Arthur S. Kim [1], Julie M. Fox [1], William B. Klimstra [2], Rebecca Rimkunas [3], Rachel H. Fong [3], James E. Crowe, Jr. [4], Benjamin J. Doranz [3], Daved H. Fremont [1], Michael S. Diamond [1]

[1] *Washington University School of Medicine, St. Louis, MO, USA*
[2] *University of Pittsburgh, Pittsburgh, PA, USA*
[3] *Integral Molecular Inc., Philadelphia, PA USA*
[4] *Vanderbilt University, Nashville, TN, USA*

Arthritogenic alphaviruses comprise a group of enveloped RNA viruses that are transmitted to humans by mosquitoes and cause debilitating acute and chronic musculoskeletal disease, which has substantial impact on quality of life. To define host factors contributing to the entry and pathogenesis of arthritogenic alphaviruses, we conducted a genome-wide CRISPR-Cas9 screen using a chikungunya (CHIKV) 181/25-mKate2 virus. Genomic DNA was extracted for deep sequencing after iterative rounds of viral infection, cell sorting and propagation. We analyzed the frequency of sgRNA sequences, and selected top-ranking genes for further study. Validation experiments with several gene 'hits' demonstrate corroborating phenotypes in clonal cell lines. Multiple alphaviruses and other unrelated enveloped/non-enveloped viruses are being used to define the specificity of gene candidates. In my talk, I will discuss the identity and functions of the top "hits" from the screens that are required for CHIKV attachment and internalization. The identification and characterization of host factors required for CHIKV infection may help to develop countermeasures for this globally expanding family of arthritogenic alphaviruses.

NOTES

Reverse Transcription Mechanically Induces HIV-1 Capsid Disassembly

Itay Rousso

Department of Physiology and Cell Biology, Ben-Gurion University of the Negev, Beer-Sheva, Israel

The RNA genome of human immunodeficiency virus type 1 (HIV-1) is enclosed inside a capsid shell that disassembles within a cell in a process known as. Although HIV-1 uncoating has been linked to reverse transcription of the viral genome in target cells, the mechanism by which uncoating is initiated is unknown. Using time-lapse atomic force microscopy, we analyzed the structure and physical properties of isolated HIV-1 cores during the course of reverse transcription in vitro. We found that during reverse transcription, the pressure inside the capsid increases, reaching a maximum after 7 h. High-resolution mechanical mapping reveals the formation of a coiled filamentous structure underneath the capsid surface. Subsequently, this coiled structure disappears, the stiffness of the capsid drops precipitously, and the cores partially or completely rupture. We propose that the transcription of the relatively flexible ssRNA into the more rigid RNA–DNA hybrid elevates the pressure within the core, which induces capsid disassembly.

NOTES

SERINCs and HIV Infectivity

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HIV-1 Nef is an accessory protein that is crucial for efficient virus spreading in vivo. Although not essential in vitro, Nef promotes HIV-1 replication in quiescent primary $CD4^+$ T cells. One well-documented function of Nef is to enhance the specific infectivity of progeny virions, but the mechanism has long remained obscure. Recently, we identified the multipass transmembrane proteins SERINC3 and SERINC5 as novel antiviral host factors that are incorporated into HIV-1 progeny virions when Nef is absent. Virion-associated SERINC5, in particular, potently inhibits the specific infectivity of progeny virions. Nef counteracts the anti-HIV activity of SERINC5 by inhibiting its cell-surface expression, and consequently its incorporation into virions.

More recently, we observed that the ability to inhibit HIV-1 infectivity is conserved among vertebrate SERINC5 proteins, whereas the sensitivity to downregulation by Nef is not. Furthermore, our results indicate that sensitivity to Nef is, at least in part, governed by intracellular loop 4 (ICL4) of SERINC5. For instance, a Nef-resistant frog SERINC5 acquired sensitivity to Nef when its ICL4 was replaced by that of human SERINC5. Conversely, the ICL4 of frog SERINC5 conferred resistance to Nef when transferred to human SERINC5. Additionally, human SERINC5 became largely resistant to Nef when certain residues in its ICL4 were mutated. Taken together, our results identify the ICL4 of SERINC5 as a major determinant of Nef responsiveness.

NOTES

Visualizing the 3D Architecture of the Hepatitis A Virus (HAV) Replication Organelles

Inés Romero Brey [1], Steffen Klein [1], Nicole Schieber [2], Katharina Esser-Nobis [1], Yannick Schwab [2], Rainer Gosert [3], Volker Lohmann [1], Ralf Bartenschlager [1]

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Electron tomography (ET) has enormously contributed to increase our knowledge about how viruses interact with their cellular host and, as a result of such an interaction, how the cell landscape and their membranes are being extensively remodeled.
Here we have applied ET to unravel the cytoarchitecture of the replication organelles induced by Hepatitis A virus (HAV), a member of the family *Picornaviridae*. Our tomographic analysis of cells expressing the HAV replicase proteins revealed that the HAV-induced structures have a rather tubular morphology. These structures are found tightly apposed to each other, forming well-defined cytosolic clusters surrounded by ER, mitochondria, and lipid droplets (LDs). Occasionally, double-membrane vesicles (DMVs) were also found in the periphery of these clusters, which seem to be formed from single-membrane tubules (SMTs). The presence of two types of viral-induced structures reflects striking similarities with other members of the family *Picornaviridae*, such as enteroviruses and cardioviruses.
In addition, by means of focused ion beam-scanning electron microscopy (FIB-SEM), we have also imaged HAV-infected cells that were previously selected by means of fluorescence microscopy. This correlative analysis provided us with detailed ultrastructural information, not only about the architecture of the HAV-induced replication factories, but also about other cell organelles.

NOTES

Abstracts

Session 2: Structural Virology

Session Chairs – Delphine Muriaux and Kay Choi

An Influenza Uncoating Epitope Confers Capsid Stability

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Influenza A virus (IAV) entry is a step-wise process that is regulated by a variety of viral and cellular cues. To quantify each step of IAV entry, we employ immunofluorescence-based assays. IAV capsid uncoating begins in the mildly acidic environment of endosomes where the M2 ion channel mediates viral core acidification and M1 conformational change. After viral fusion at late endosomes, the cellular aggresome processing machinery completes the capsid uncoating process. A M1 monoclonal antibody (HB64) allowed the preferential detection of capsid uncoating but the underlying mechanism was not clear.

Here, we mapped the epitope of HB64 to the N-terminus of M1. Based on previous structural studies, this epitope is located at the interphase of the M1 dimer. It is masked at neutral pH (extracellular environment) and exposed at low pH (endosomes), a phenomenon that explains the enhanced HB64 binding to uncoated virus particles. A 1.9 Å resolution crystal structure of M1 with a single point mutation in the epitope led to the loss of a surface cavity and increased flexibility of loop L1. M1 VLP studies showed that capsid assembly and stability were compromised in this point mutant, suggesting that acid-exposed capsid epitopes may help us understand the assembly/disassembly paradox.

NOTES

Relations between Cell-Cell Fusion, Eukaryotic Evolution and Class II Enveloped Viruses Identified by Structural Studies

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Structural studies have revealed that the membrane fusion proteins used by enveloped viruses to enter cells belong to one of three structural classes (I, II and III) of homologous proteins, irrespective of the virus genome type and replication strategy. This finding illustrates the mosaic nature of viral genomes, which are a collection of fragments derived from different origins. One special case are the class II fusion proteins found in the *Togaviridae*, *Flaviviridae* and in several families of the *Bunyavirales* order. Class II fusion proteins were also detected in *C. elegans* retroviruses, which are otherwise clearly related to mammalian retroviruses through the Gag-Pol gene, although mammalian retrovirus have a class I fusion protein envelope gene. The cell-cell fusion protein EFF-1, responsible for syncytia formation to form the skin during *C. elegans* embryogenesis, was also shown by X-ray crystallography to be homologous to the class II viral proteins, suggesting a possible virus-to-cell transfer via reverse transcription and integration into the cellular germ-line, as documented in mammals for *syncyti*ns, which are class I fusion proteind derived from mammalian retroviruses and required for placenta formation via trophoblast fusion. The recent structural characterization of the ancestral gamete fusogen HAP2, present in male gametes across the main branches of eukaryotes except for fungi, showed that it is also homologous to class II fusion proteins—a further illustration of the impact of virus-cell genetic exchanges, which appear to have been at the origin of sexual life on earth. In this presentation, I will review the evolutionary aspects relating cell-cell fusion processes and enveloped viruses.

NOTES

Dynamics Bridge Structure, Function, and Phenotype in Viral Entry Machines

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The fusion glycoproteins that decorate the surface of enveloped viruses undergo dramatic conformational changes over the course of engaging cellular receptors and during cell entry. These dynamic events ultimately drive the fusion of viral and cellular membranes, leading to the delivery of the genetic cargo. While methods such as X-ray crystallography have provided static structures of their pre- and post-fusion states, acquiring a mechanistic understanding of how fusion glycoproteins drive membrane fusion requires analytical approaches that enable dynamic intermediate states to be probed. We are using hydrogen/deuterium-exchange mass spectrometry (HDX-MS) and cryo-EM to probe fusion protein function and pathways of conformational change in influenza and HIV. HDX-MS allows one to measure protein dynamics under native conditions with sequence-specific resolution. We employed this approach to investigate fusion glycoprotein activation in response to receptor binding (HIV) or exposure to acidic pH (influenza), as well as their inhibition by neutralizing antibodies. Our results reveal structural features of fusogenic intermediates and the conformational pathway that is traversed by the fusion machines. We also observe major strain-specific variations in the dynamic profile of pre-fusion and activated spikes for influenza HA and HIV Env glycoproteins. These differences in the dynamics of structural epitopes impact their recognition by receptors and antibodies.

NOTES

Structure-Based Rational Design of Broad Range Immunotherapy Targeting TfR1-Tropic Arenaviruses

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Arenaviruses that circulate in rodent populations can cause life-threatening hemorrhagic fevers when infecting humans. Due to their efficient transmission, arenaviruses pose a severe risk for outbreaks and might be exploited for bioterrorism. Therefore, effective countermeasures against these viruses are highly desired. Despite the fact that all pathogenic arenaviruses from South America utilize transferrin receptor 1 (TfR1) as a cellular receptor, their viral glycoproteins are highly diversified, impeding efforts for isolating cross-neutralizing antibodies. To tackle this problem, we rationally designed an immunoadhesin molecule which mimics a TfR1 ortholog from rodent host that is better recognized by these viruses compared with the human TfR1. This molecule serves as a decoy and effectively neutralizes various strains of pathogenic arenaviruses such as Sabia, Machupo, Guanarito, and Junin. Due to its generic design, this immunoadhesin offers a potential broadly reactive immunotherapy against new strains that could emerge in the future.

NOTES

Unravelling the Structure–Function Relationship of Hepatitis C Virus Fusion Machinery by in Silico Structural Modeling

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The direct structural characterization of hepatitis C virus (HCV) surface glycoproteins E1 and E2—the constituents of its entry machinery—is limited to the study of highly truncated constructs. Conversely, E1 and E2 full-length structures (either alone or in complex), and a clear picture of the domains involved in the fusion process are not defined, hampering the development of strategies to interfere with HCV entry.

The results reported here describe a structural model comprising the entire E1 and E2 ectodomains that was generated in silico using both computational predictions and biological data. In detail, an extensive alanine scanning library covering 553 out of the 555 E1 and E2 residues was generated and tested for the binding to a panel of conformational human monoclonal antibodies (mAb), allowing identification of the localization of their epitopes as well as the disulfide connectivity underlying E1E2 native conformation. Subsequently, E1 and E2 secondary, tertiary, and quaternary structure was predicted through a panel of freely-available algorithms. The computational and experimental results were merged and converted into distance restraints to generate ab initio a fully-glycosylated, covalently-bound, E1E2 structural model validated by mapping immunogenic and functional domains.

The predicted structure highlights novel heterodimerization interfaces, provides information on the structural features regulating E1 and E2 immunogenicity, and identifies the CD81-mediated disulfide isomerization steps fundamental for HCV entry, allowing for the first time to correlate the structure of each domain to its function. The model represents a step towards the rational design of immunogens and drugs inhibiting HCV entry.

NOTES

Structure, Function, and Immune Recognition of the Enigmatic HIV Envelope Glycoprotein

Andrew Ward [1,*], Christopher Cottrell [1], Hannah Turner [1], Bartek Nogal [1], Kimmo Rantalainen [1], Zachary Berndsen [1], Sasha Moola [1], Matteo Bianchi [1], Jeong Hyun Lee [2], Elise Landais [1], Pascal Poignard [3], Ian Wilson [1], Lars Hangartner [1]

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The HIV-1 envelope glycoprotein spike on the surface of the virion mediates host cell recognition and entry, and is the target for all broadly neutralizing antibodies (bnAbs). Many of the functional properties of Env are disadvantageous for HIV vaccine design; such as membrane association, meta-stability, heterogeneneity (soluble vs. TM glycosylation), and epitope accessibility. Hence, a molecular understanding of the function of Env can inform structure-based vaccine design. Toward that end, various soluble Env spike constructs that structurally and antigenically mimic native Env have now been generated, allowing atomic level understanding of the molecular bases of receptor-based conformational changes, as well as neutralization by bnAbs. Additionally, several of these soluble trimers have been deployed and tested in animal immunization studies as vaccine candidates. Here, I will describe how my lab uses cryoEM to solve structures of Env complexes to understand the structure, function, and immune recognition of the spike. CryoEM is ideally suited for such studies because of the throughput, sample requirement, and ability to analyze complex and heterogeneous samples. The structural information that we generate can subsequently be used for immunogen design and re-design, with the goal of programming immunity in a rational manner.

NOTES

Viral Genome Hide and Seek; Encapsidation Versus Host Immunity

Leo James

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For many viruses, the most complex and hazardous infectious step begins immediately after entry into a cell. Viruses must avoid being detected and disabled by host immunity but actively recruit host proteins to act as co-factors. Encapsidation is a near universal solution to this problem and extensively employed to protect fragile genomic cargo. In my talk, I will discuss some of the consequences of encaspidation from both a host and pathogen perspective. I will describe how the HIV capsid manages to shield its genome whilst undergoing reverse transcription. A remarkable system of selective dNTP import simultaneously excludes nucleic acid sensors and facilities rapid DNA synthesis. Capsids represent both a barrier to sensing internal viral molecules and a target in their own right. An example of a potent host mechanism that circumvents the capsid by combining adaptive and innate immunity will be presented. Against the simplicity of a protein shell, hosts utilize complex sensing networks and synergize their responses.

NOTES

Correlative Study of Cellular Modification Induced by Hepatitis C Infection by Cryo Soft X-ray Tomography and Infrared Microscopy

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Hepatitis C virus is an enveloped RNA virus. One of the hallmarks of HCV infection is the formation of a "membranous web". Recent results in our groups have shown the first complete cartography of the control and HCV-infected cells at 40 nm resolution. We have performed full-field cryo soft X-ray tomography (cryo-SXT) (MISTRAL-beamline) in the water window to investigate, in whole unstained cells, the morphology of the membranous rearrangements induced by the HCV replicon in conditions close to the living physiological state. All the alterations could be reverted by a combination of sofosbuvir/daclatasvir, which are clinically approved antivirals (DAAs) for HCV infection.

Using synchrotron-based infrared microscopy (MIRAS-beamline) analysis in combination with the high-resolution cryo-SXT, we have correlated the typical structure associated to the development of the infection, with the chemical composition such as protein secondary structure and oxidation in the presence and in the absence of infection. The correlation maps showed an important increase in the oxidation in all the infected cytoplasm as well as the chemical identification of the specialized structures during the infection. So the correlated studies between MISTRAL–MIRAS-beamlines could clarify the chemical nature of the viral structures during the infection and also during the healing process.

NOTES

Supramolecular Arrangement of the Zika Virus NS5 Protein

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Zika virus (ZIKV) has explosively emerged over the past year, causing a series of epidemics across the Western Hemisphere. Neonatal microcephaly associated with ZIKV infection has already caused a public health emergency of international concern. As with other members in the *Flaviviridae* family, ZIKV relies on its NS5 protein for RNA capping (by methyltransferase N-terminal domain) and genome replication (by RNA-dependent RNA polymerase, (RdRP) domain), being an attractive antiviral target.

The crystal structures of the ZIKV NS5 protein in two different space groups evidenced conserved protein self-interactions to form dimers and also higher-order fibrillar oligomers. Complementary analyses by small-angle X-ray scattering (SAXS), mass spectrometry, negative staining TEM, and atomic force microscopy (AFM) have been employed to further characterize the NS5 assemblies. Additionally, point mutations and deletions in the NS5 open reading frame (ORF) allowed us to confirm the interfaces between NS5 monomers within a dimeric structure in solution, as well as the dimer–dimer interactions to form higher-order assemblies.

The quaternary arrangement of ZIKV NS5 provides a model to explain the coordination between the different protein activities, and paves the way for exploring new structure-based inhibitors that would interfere with the intermolecular NS5–NS5 interactions.

NOTES

Towards Elucidating the Structural Basis for Envelope Incorporation into HIV-1 Particles

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One of the least-understood phenomena in retroviral assembly is the mechanism by which the envelope (Env) protein is recruited and incorporated into virus particles. The cytoplasmic tail of HIV-1 gp41 (gp41CT) plays important roles in virus replication, including meditation of Env intracellular trafficking and incorporation into virions—the mechanisms of which are poorly understood. Studies have suggested that Env incorporation into HIV-1 particles is mediated by interactions between the MA domain of Gag and gp41CT, and that MA trimerization is an obligatory step in the assembly of infectious HIV-1 virions. We have devised new approaches that allowed for the successful preparation of the gp41CT protein and determination of the solution structure in micellar solution. Structural data revealed that the N-terminal 45 residues are unstructured and not associated with the membrane. However, the C-terminal 105 residues form three membrane-bound amphipathic α-helices with distinctive structural features such as variable degree of membrane penetration, hydrophobic and basic surfaces, clusters of aromatic residues, and a network of cation-π interactions. This work filled a major gap by providing the structure of the last segment of HIV-1 Env, and provided insights into the mechanisms of Env mobility and conformation on the virion surface and incorporation into virus particles. To characterize the interactions with the MA domain of Gag, we established a system in which gp41CT and MA trimer are reconstituted in a membrane mimetic. Structural, biochemical, and biophysical data on gp41CT and its interaction with membrane and MA will be presented.

NOTES

Abstracts

Session 3: Virus Replication Compartments

Session Chairs – Stefano Aquaro and Nathalie Arhel

Roles of UL12 and ICP8 in the Production of Herpes Simplex Virus DNA That Can Be Packaged into Infectious Virus

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The formation of head-to-tail concatemers during DNA replication is an obligate step in the production of infectious HSV; yet little is known about the actual mechanism by which they are formed. Several lines of evidence from our laboratory suggest that HSV produces concatemers by a DNA recombination-dependent mechanism. We have identified a two-component HSV recombinase comprised of a 5′ to 3′ exonuclease (UL12) and a ssDNA annealing protein or SSAP (ICP8). This complex is reminiscent of the λ phage Red α/β recombinase known to be required for recombination-dependent replication in lambda and for its ability to promote in vivo recombination-mediated genetic engineering (recombineering). Another challenge that HSV faces during the establishment of a lytic infection is to avoid triggering host DDR pathways that are antiviral. Components of both C-NHEJ and HRR pathways have been shown to be antiviral, and HSV has evolved mechanisms to inactivate them. It has been recognized for some time that the viral immediate early protein ICP0 plays an important role in counteracting intrinsic antiviral mechanisms. Recent work suggests that HSV has evolved another way to counteract the antiviral effect of C-NHEJ. Genetic, biochemical and pharmacological studies on the roles of UL12 and ICP8 during the HSV life cycle will be discussed.

NOTES

Spillover: How Viruses Adapt at the Animal–Human Interface

Sara Sawyer

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The spillover of viruses to new host species can lead to the emergence of new viruses and new diseases. For instance, the HIV pandemic began with a series of spillover events of simian immunodeficiency virus (SIV) from African primates to humans. Spillover, at its very core, is an evolutionary process whereby a virus moves from one host ecosystem to another, and must adapt to its new environment. Genetic differences between old and new hosts constitute the selective engine that drives the evolutionary process of spillover. A first-principles understanding of virus spillover will require that we understand to what viruses are adapting as they move between species. We study this process over an array of virus families so that common themes emerge. In this talk, we will describe current work on HIV and dengue viruses, both of which emerged from primate reservoirs.

NOTES

Mapping Epstein-Barr Viral Genome Replication Spatially and Temporally During the Viral Lytic Cycle

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Epstein-Barr virus (EBV)'s lytic cycle rarely arises spontaneously. We have developed cells in which the viral lytic cycle can be induced efficiently and in which the viral DNA can be detected by its binding to a fluorescently-tagged Lac repressor (Chiu et al. 2013). The viral DNA is amplified in sites whose sizes we have now been found to grow linearly over time but whose centers remain fixed with respect to the nuclear envelope. The rate of viral DNA synthesis in these sites was measured with pulses of EdU followed by their detection with "click" chemistry, and showed a linear phase which lasted for approximately 30 h. The early viral DNA synthesis was difficult to measure because of the asynchrony in the induction of EBV's lytic cycle, but appeared to be exponential which accords with the accumulation of synthesized viral DNA detected by live-cell imaging. We have examined the modes of EBV DNA synthesis during the lytic cycle to test the hypothesis that its early, apparently exponential phase, is mediated by a Theta mode which then converts to a rolling circle mode. Density-shift experiments using pulses of BrdU shorter than the generation time for a single viral replicon yielded only light-light and heavy-light viral DNA during the early phase of the lytic cycle, while they also yielded heavy-heavy viral DNA species late in the lytic cycle. These findings support there being two distinct modes of EBV DNA synthesis during the viral lytic cycle.

NOTES

Respiratory Syncytial Virus RNA Synthesis in Cytoplasmic Inclusion Bodies: Organization and Functioning

Charles-Adrien Richard [1], Vincent Rincheval [2], Mickaël Lelek [3], Benjamin Bailly [4], Marie Galloux [1], Christophe Zimmer [3], Ralf Altmeyer [5], Marie-Anne Rameix-Welti [2], Christina Sizun [6], Jean-Francois Eleouet [1]

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Respiratory syncytial virus (RSV) RNA synthesis occurs in cytoplasmic inclusion bodies (IBs) in which all the components of the viral RNA polymerase (L, P, N, and M2-1) are concentrated. By using conventional and super-resolution imaging, we have investigated the ultrastructure of IBs. We found that IBs are the main site of viral RNA synthesis, and that newly-synthesized viral mRNA and the viral transcription anti-terminator M2-1 concentrate in IB sub-compartments, called "IB associated granules" (IBAGs). In contrast, viral genomic RNA, the nucleoprotein N, the Large polymerase L, and its cofactor the phosphoprotein P are all excluded from IBAGs. We also found that RSV P protein recruits M2-1 to IBs and that M2-1 dephosphorylation—required in a cyclic manner for efficient viral transcription—is achieved by a complex formed between P and a cellular phosphatase. When the P–Phosphatase interaction was disrupted, M2-1 remained phosphorylated but was excluded from IBAGs, indicating that only dephosphorylated M2-1 is competent for viral mRNA binding, and hence for a proposed mRNA sorting function. Finally, a chemical compound which was selected as an RSV transcriptase inhibitor was found to be capable of disrupting the IBAGs.

NOTES

Cytoplasmic-Replicating Parainfluenza Virus Sensitizes the Nuclear Compartment to DNA-Damaging Agents—Implications for Oncolytic Virus Therapies

Griffith Parks, Candace Fox

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Parainfluenza virus type 5 (PIV5) is an RNA virus which replicates exclusively in the cytoplasm, with no need for the cell nucleus. Infection of human laryngeal cancer cells with the cytopathic PIV5 P/V mutant results in >90% cell death, but some cells survive and continue to proliferate as persistently infected (PI) cells. We have tested the hypothesis that PI cells harboring the cytopathic PIV5 virus have altered cell death pathways. In challenge experiments with external inducers of apoptosis, PI cells were more sensitive to cisplatin-induced DNA damage and cell death, which correlated with defects in both DNA damage signaling pathways (Chk1) and in the nuclear translocation of damage-specific DNA binding protein 1 (DDB1). PIV5 mutant infection upregulated p53 and WIP1 (wild-type p53-induced phosphatase 1), which serves to regulate the balance between DNA repair/survival and apoptosis. This sensitivity was also seen with acute infection with human parainfluenza virus 2. We present a model whereby PIV5 blocking of innate immune responses in the cytoplasm results in enhanced sensitivity to DNA damage in the nucleus. Our studies suggest approaches to enhance the potency of RNA virus therapy vectors, and highlight unforeseen potential risks of oncolytic vectors.

NOTES

Cracking Nuclear Codes: The 5D viral-cellular genome structures and interactions that drive pathological proliferation

Clodagh O'Shea

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Adenoviruses are important pathogens as well as critical vectors, vaccines and oncolytic viral therapies in genomic medicine. Adenoviruses capsids contain 36 kb linear double stranded DNA genomes with early (E) and late (L) transcriptional modules that are all read, transcribed, replicated and packaged in the cell nucleus. However, it is now clear that linear DNA sequence information alone is not sufficient to predict the functions of either cellular or viral genomic DNA in the nucleus. In the nucleus, cellular DNA assembles with nucleosomes into chromatin structures that interact in spatially defined 3D topologically associated domains (TADs) to determine if genes are active or silent through still poorly understood mechanisms.

A fundamental question is what is the structure, 3D interactions and organization of viral and cellular DNA in the nucleus? To address this, we developed a new DNA labeling method, ChromEMT, which enables the chromatin ultrastructure and 3D organization of DNA to be visualized at high resolutions though unprecedented 3D volumes of the nucleus in situ. We have exploited ChromEMT to reveal the structure of viral and cellular DNA in the nucleus and new insights into gene silencing/activation in infected cells.

We will also discuss how Ad5 oncoproteins, E1B-55K and E4-ORF3, target critical 3D hubs and interactions in the nucleus to drive the assembly of E2A viral replication domains and break cellular genome compartmentalization. The assembly of nascent E2A domains also triggers the MRN dependent activation of a global ATM response and induces a novel transcriptional program that may limit viral infection and spread in vivo.

NOTES

Assembly of Viral Replication Complexes: Roles for Viral and Host Proteins

Elena Frolova

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Positive-strand RNA viruses encode a small set of proteins that are required for replication of their genomes and viral particle formation. However, these proteins alone are insufficient for the formation of functional replication complexes and need to be complemented by host factors, which are specific for different viral species. Host factors play indispensable roles in RNA replication and other steps of infection development. Alphavirus-specific nonstructural protein 3 (nsP3) contains a long, intrinsically disordered hypervariable domain (HVD) that recruits cellular proteins and viral genomic RNA into the replication complex assembly process. The New World and Old World alphaviruses encode HVDs that demonstrate essentially no homology at the amino acid level and interact with distinct sets of host factors. For geographically isolated, pathogenic alphaviruses, such as Venezuelan equine encephalitis virus (VEEV) and chikungunya virus (CHIKV), the most important interacting proteins were found to be the FXR and G3BP family members, respectively. These proteins share common characteristics, which determine their role in alphavirus replication, namely, an ability for RNA-binding and for self-assembly into large complexes. Importantly, the members of each family have redundant functions in viral RNA replication and only the knockout of expression of all of the members from each family have deleterious effects on VEEV or CHIKV replication. Another New World alphavirus, eastern equine encephalitis virus, demonstrates an additional level of redundancy, and its HVD interacts with both FXRs and G3BPs. Other HVD-interacting cellular proteins have additional strong proviral functions. Manipulation of protein binding sites changes the cell specificity of alphavirus replication.

NOTES

ESCRT Machinery Activity Is Required for Oropouche Virus Assembly at the Golgi Complex

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Peribunyaviridae is one of the largest families of RNA viruses, with several members that cause mild-to-severe diseases in humans and livestock. In this study, we analyzed the assembly pathway of Oropouche virus (OROV), a peribunyavirus of the orthobunyavirus genus that is the etiologic agent of a frequent arthropod-transmitted human viral disease in Latin American countries. Our electron microscopy analyses showed that the assembly of OROV involves budding of virus particles toward the lumen of Golgi cisternae. These Golgi subcompartments become enlarged and physically separated from Golgi stacks, forming Oropouche viral factory (Vf) units. As revealed by conventional confocal and superresolution microscopy, while these Vfs lack typical early and late endosome markers, they are enriched in Golgi proteins and endosomal complex required for transport (ESCRT) elements. Moreover, further microscopy and viral replication assays showed that functional ESCRT machinery is required for efficient Vf morphogenesis and production of infectious OROV particles. Although many enveloped viruses have evolved to usurp elements of the ESCRT machinery for assembly and budding, to our knowledge, this is the first report showing the recruitment of ESCRT components to the Golgi complex and their involvement in the assembly/egress of an Orthobunyavirus.

NOTES

Common Themes in the Ultrastructure and Function of the Coronavirus Replication Organelle

Eric J. Snijder [1], Ronald W. A. L. Limpens [1], Adriaan H. de Wilde [1], Frank F. G. A. Faas [1], Helena J. Maier [2], Abraham J. Koster [1], Montserrat Bárcena [1]

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During coronavirus infection, the viral RNA-synthesizing machinery associates with modified endoplasmic reticulum membranes, which are often collectively referred to as the replication organelle (RO). While double-membrane vesicles (DMVs) seem to be a pan-coronavirus RO motif, studies to-date describe an assortment of additional membrane elements that seem to differ per coronavirus genus. These include convoluted membranes (CMs), induced by alphacoronaviruses and betacoronaviruses, and zippered ER and double-membrane spherules, reported only for gammacoronaviruses. Despite much speculation, it is still unclear with which element(s) of the coronavirus RO viral RNA synthesis is associated. We show here that double-membrane spherules are also formed during alphacoronavirus and betacoronavirus infections and that CMs consist of labyrinthine zippered ER, suggesting that all CoV may induce the same basic membrane modifications with variations in their detailed configuration. Metabolic labeling of newly-synthesized viral RNA followed by autoradiography revealed that viral RNA synthesis is associated with DMVs in cells infected with MERS-CoV (betacoronavirus) and IBV (gammacoronavirus). In contrast, no newly-synthesized RNA signal was associated with spherules, CM, or zippered ER. Our results demonstrate that DMVs are bona fide replication compartments and raise new questions about the possible roles of the other RO elements in the coronavirus replication cycle.

NOTES

Core or Viroplasm? Dissecting Rotavirus VP2 and NSP5 Interaction

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In the course of rotavirus replication, the core–shell protein VP2 is also an essential component of the cytosolic viral factories—namely, viroplasms. VP2 spontaneously forms core–shell structures, but when co-expressed with the viroplasm matrix protein NSP5, VLSs are formed. The VLSs are cytosolic inclusions which are morphologically identical to replicative viroplasms but composed only of NSP5 with VP2 or NSP2. We hypothesized that the NSP5 interaction with VP2 disrupts spontaneous cores to allow the formation of viroplasms. By dissecting VP2, we found that amino acid regions 103–135 and 840–881 are required for VLS formation with NSP5. We further examined VP2 region 103–135 to identify highly-conserved amino acids of all known rotavirus families, and found that point mutation L122A (SA11 strain) hampers NSP5 hyperphosphorylation. Moreover, while this mutant showed impaired VLS formation and NSP5 interaction, it was still able to form core–shell structures. The VP2L122A over-expression in MA104 cells reduces rotavirus infectivity. In a similar approach, the analysis of NSP5 showed that regions 34–80 and 130–198 are necessary for association with VP2. The conserved interaction domains identified for both VP2 and NSP5—essentials for the replication initiation—make them excellent targets for the development of novel and broad-range antivirals.

NOTES

Abstracts

Session 4: Replication and Pathogenesis of RNA viruses

Session Chairs – Josep Quer and Richard Sutton

Epigenetic Control of Influenza Virus: Role of H3K79 Methylation in Interferon-Induced Antiviral Response

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Influenza virus establishes a network of virus–host functional interactions, which depends on chromatin dynamic and therefore on epigenetic modifications. Using an unbiased search, we analyzed the epigenetic changes at DNA methylation and post-translational histone modification levels induced by the infection. DNA methylation was unaltered, while we found a general decrease in histone acetylation, which correlates with transcriptional inactivation and may cooperate with the impairment of cellular transcription that causes influenza virus infection. A particular increase in H3K79 methylation was observed and the use of an inhibitor of the specific H3K79 methylase, Dot1L enzyme or its down-regulation, increased influenza virus replication. The antiviral response was reduced in conditions of Dot1L inhibition, since decreased nuclear translocation of the NF–kB complex, and IFN-b, Mx1 and ISG56 expression was detected. The data suggested a control of antiviral signaling by methylation of H3K79 and consequently, influenza virus replication was unaffected in IFN pathway-compromised, Dot1L-inhibited cells. H3K79 methylation also controlled the replication of another potent interferon-inducing virus such as vesicular stomatitis virus, but did not modify the amplification of respiratory syncytial virus that poorly induces interferon signaling. Epigenetic methylation of H3K79 might have an important role in controlling interferon-induced signaling against viral pathogens.

NOTES

Identification of *trans* Acting Factors Involved in the Norovirus Life Cycle

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Noroviruses are a significant cause of gastroenteritis and are thought to cause >200,000 deaths per year in children under 5 years of age. Due to the lack of long lasting immunity, all age groups are susceptible to norovirus infection, leading to a high socioeconomic impact. Despite this burden, therapeutics and vaccines for noroviruses are currently lacking.

Our understanding of the biology of noroviruses has significantly improved in recent years, at least in part due to the discovery of a murine norovirus model that combines a robust cell culture and reverse genetics systems with a homologous host that can be genetically modified. Human norovirus replicons and recently developed culture systems in both B cells and stem cell-derived intestinal organoids, have greatly improved the tools with which to dissect the norovirus life cycle. We have used a combination of these experimental systems to gain better insights into the norovirus life cycle. Powerful comparative analysis between MNV, other model caliciviruses and human norovirus has allowed us to uncover host cell factors essential for the norovirus life cycle. Using epitope tagged viruses, we have been able to investigate the composition of the norovirus replication complex and begun to identify cellular components that contribute to the norovirus life cycle. The combination of reverse genetics, model systems and stem cell derived organoids has provided unprecedented insight into the biology of these economically important pathogens.

NOTES

HIV-1 Envelope Glycoprotein Mutations Confer Broad Resistance to Antiretrovirals in Vitro

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We have identified compensatory mutations within the human immunodeficiency virus (HIV)-1 envelope (Env) glycoprotein that rescue defects in replication conferred by mutations in Gag. Despite exhibiting severe deficiencies in cell-free particle infectivity, these Env mutants replicate with wild-type (WT) kinetics and are capable of efficient cell-to-cell transmission. The goal of this study was to characterize the ability of the Env mutant viruses to confer a broad replication advantage and determine whether they can escape inhibition by antiretrovirals (ARVs). We propagated the Env mutants in T-cell lines and measured replication kinetics in the presence or absence of ARVs. We observed that the Env mutants displayed markedly reduced sensitivity to multiple classes of of ARVs—ritonavir (RTV), nelfinavir (NFV), dolutegravir (DTG), tenofovir (TFV), and rilpivirine (TMC)—at drug concentrations that block or delay WT virus replication. Long-term passage of WT virus in the presence of several of these inhibitors resulted in the selection of ARV-escape mutants containing substitutions in Env. This escape occurred in the absence of resistance mutations in the enzyme targets of these drugs. These results demonstrate that mutations in Env can broadly contribute to drug resistance in vitro.

NOTES

MicroRNA-Based Suppression of Neurotropic Flavivirus Replication: Application for the Development of Live Attenuated Vaccine

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The mosquito- and tick-borne viruses within the *Flaviviridae* family comprise important emerging and re-emerging pathogens (Japanese encephalitis (JEV), tick-borne encephalitis (TBEV), West Nile (WNV), St. Louis encephalitis (SLEV), and Zika (ZIKV) viruses) that have caused outbreaks of severe neurologic disease in humans. In recent years, microRNA-targeting has become an effective strategy for selective control of tissue-tropism and pathogenesis of both DNA and RNA viruses. The purpose of this study is to apply this strategy to control the neuropathogenesis of flavivirus infection. To restrict flavivirus replication, we inserted target sequences for host tissue-specific microRNAs into viral genomes. Here, we demonstrated that genome targeting of neurotropic flaviviruses for cellular microRNAs expressed in the brain results in selective restriction of viral neurovirulence and neuroinvasiveness and complete abolishment of virus neurotropism in highly permissive newborn mice. Simultaneous microRNA co-targeting of two or three distantly located regions of viral genome (ORF and 3'NCR) had an additive effect on the reduction of virus neuropathogenesis in the CNS and resulted in a more potent attenuation of the virus. Such co-targeting improved the genetic stability of viruses during prolonged persistence in immunodeficient animals. We believe that microRNA-mediated silencing of virus replication can reinforce the safety of newly developed and existing vaccines.

NOTES

Adaptation of NrHV to the Mouse Host Using Selective Innate and Adaptive KO Strains

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In 2014, Norway rat hepacivirus (NrHV) was discovered in NYC rats, and is the first hepacivirus able to infect and replicate in the liver of laboratory mice. NrHV infection in mice shares virological and immunological features with hepatitis C virus (HCV) infection in humans. However, NrHV does not generate a chronic infection in completely immune-competent mice. Trying to overcome this limitation, we aim to better understand the dynamics of NrHV replication in the new host and to select for adapted variants able to overcome the wild-type (WT) mouse immune system. Specifically, we tested innate and adaptive immune system knock-out (KO) mice and monitored viremia and liver damage over time. Interferon type I receptor, IRF1, and B cell KOs developed a stable chronic infection. $CD8^{-/-}$ mice showed drastically reduced viremia throughout the infection, but were still infected 3 months post-infection. $IFNGR^{-/-}$ mice presented an acute resolving infection, similar to the WT mice, and an increase in ALT activity was related to the presence of $CD8^{+}$ T cells. In conclusion, NrHV infection control is CD8-dependent, but clearance requires factors other than IFNg production. $CD8^{-/-}$ mice, which show a significant immune pressure without clearance, might be a good candidate for viral adaptation, but further studies are necessary. Financial Support: FAPESP.

NOTES

Differential Developmental Symptoms in *Turnip Mosaic Virus*-Infected Hosts Is Associated with the Dynamic Association of the Viral P3 Protein to the Endoplasmic Reticulum

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Intracellular trafficking of viral replication complexes (VRCs) towards the periphery of the infected cell to enable virus exit is a critical and highly-regulated step in the life cycle of many viruses infecting eukaryotic cells. In the case of plant viruses, the presence of the cell wall forces this process to be directed towards characteristic plant organelles named plasmodesmata—intercellular extensions of the endoplasmic reticulum physically connecting adjacent cells. Recently, the P3 protein of *Turnip mosaic virus* (TuMV)—a Picorna-like virus of the Genus *Potyvirus* in the family *Potyviridae*—has been implicated in viral replication, in particular in VRCs movement towards plasmodesmata. Our work with two isolates belonging to two major strains of TuMV has shown that they induce differential development-related symptoms when infecting Arabidopsis plants. One of the strains strongly arrests growth and development of the host, while the other induces growth delay and alterations in the architecture of the flower stalk. The viral determinant of the differential plant development is the P3 protein. The results of subcellular localization of P3 showed that the dynamic association of the protein to the endoplasmic reticulum is different between both TuMV isolates. Both the differential host development and the type of subcellular association are strain-interchangeable, with just a single amino acid change in P3. The implications of the subcellular role found for this viral protein in the understanding of the host developmental plan will be discussed.

NOTES

An Orthogonal Proteomic Screen of Zika Virus Uncovers Novel Host-Dependency Factors

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Zika virus (ZIKV) has recently emerged as a global health concern due to its widespread diffusion and its association with severe neurological symptoms and microcephaly in newborns. However, the molecular mechanisms responsible for ZIKV pathogenicity remain largely elusive. Here, we used primary human neural progenitor cells (hNPC) and the neuronal cell line SK-N-BE2 in an integrated proteomics approach to characterize cellular responses to viral infection on proteome and phosphoproteome levels, as well as affinity proteomics to identify cellular targets of all ZIKV proteins. This approach identified 388 ZIKV-interacting proteins, unraveling ZIKV-specific and pan-flaviviral activities as well as host factors with known functions in neuronal development, retinal defects, and infertility. Moreover, our time-resolved phosphoproteome analysis identified 1295 phosphosites specifically up- or down-regulated upon ZIKV infection, indicating a profound dynamic modulation of fundamental signaling pathways such as AKT, MAPK/ERK, and ATM/ATR, and providing plausible mechanistic insights into the proliferation arrest elicited by ZIKV infection. Our integrative approach highlights non-structural protein 4B (NS4B) as one of the disease determinants likely targeting fundamental neuronal differentiation processes, and identifies a number of previously undisclosed ZIKV host-dependency factors, providing a comprehensive framework for a system-level understanding of ZIKV-induced perturbations at the protein and cellular pathway level.

NOTES

Abstracts

Session 5: Genome Packaging and Replication/Assembly

Session Chairs – Juan José López-Moya and François-Loïc Cosset

Intra-Genomic RNA-Based Regulation in a Plus-Strand RNA Virus

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Intragenomic, long-range RNA–RNA interactions within the plus-strand RNA genomes of Tombusviruses regulate and coordinate different essential viral functions. Remarkably, these viruses employ a complex network of no fewer than six distinct long-range RNA–RNA interactions, which span distances from ~1 kb to several thousand nucleotides in their ~4.8 kb ssRNA genomes. The viral events modulated by such RNA-based interactions include translation of viral proteins, translational readthrough, RNA genome replication, and transcription of two viral subgenomic mRNAs. The multi-faceted RNA-based regulatory scheme used for control of subgenomic mRNA1 transcription will be presented.

NOTES

Assembly and Disassembly of HBV: Biology Recapitulates Physics

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Hepatitis B Virus (HBV) capsid assembly and disassembly are spontaneous reactions. Intuitively, they should mirror one another. However, there is remarkable hysteresis separating them. Assembled capsids persist under conditions where they could never be assembled. The gap between these two reactions has important implications for virus biology and understanding regulation of assembly. Observation of assembly is consistent with a nucleated reaction with metastable intermediates. Assembly reactions for empty particles frequently overshoot the 120-dimer T = 4 capsid and then relax back to an icosahedral shell. Assembly-directed antiviral compounds (Core protein Allosteric Modulators, CpAMs) can allosterically activate assembly leading to empty particles. These CpAMs also take advantage of the relative stability of defective particles to generate aberrant structures. Observation of disassembly identifies some of the same intermediates observed during assembly, yet requires extreme conditions. We show how allostery can be the basis of hysteresis while the geometry inherent to an icosahedron can be the basis of common assembly and disassembly intermediates.

NOTES

Crystal Structure of the Dengue Virus Promoter RNA

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Flaviviruses such as dengue and zika viruses pose significant threats as emerging diseases, yet no antiviral therapies are available and existing vaccines are of limited utility. Flavivirus genome replication occurs in a replication complex that contains viral nonstructural (NS) proteins, host proteins, and the viral RNA genome. At the heart of the replication complex is NS5, a virally encoded protein consisting of methyltransferase and RNA-dependent RNA polymerase domains. During viral genome replication, NS5 specifically recognizes structural features of the 5'-end of the viral genome, known as stem loop A (SLA), which acts as a promoter to initiate RNA synthesis. Following synthesis of (+) strand RNA, NS5 again recognizes the SLA on the nascent strand and methylates the 5' guanine cap. The predicted 'Y'-shaped secondary structure of SLA is conserved in many flaviviruses, suggesting that flaviviruses use a general mechanism of SLA-mediated RNA replication. However, little is known about how NS5 engages SLA for polymerase and methyltransferase reactions during genome replication. We recently determined the crystal structure of dengue virus SLA—the first structure of this flavivirus promoter. The SLA is 65 Å long and 50 Å wide, and forms a "T"-shaped three-way junction. In light of this new structure, we have identified essential SLA regions that are important for maintaining the fold of SLA as well as regions important for NS5 binding. Based on these observations, we propose a structure-based model of the SLA and viral polymerase NS5.

NOTES

Adenovirus Activation of the Unfolded Protein Response Sensor Ire1 Enhances Immediate Early Viral Transcription to Promote Viral Persistence and Lytic Egress

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Adenoviruses (AdVs) deliver a double-stranded viral DNA genome to the nucleus of host cells, and replicate at low levels in persistent infections, or at high levels in lytic infections. The first viral gene to be expressed in the nucleus is the immediate early gene E1A, which transcriptionally activates all other viral promoters, and many host genes. E1A expression can be suppressed by interferon, which precludes lytic replication and gives rise to viral persistence. We previously found that chemically enhancing the unfolded protein response (UPR) increases AdV gene expression and lytic virus release, which required Ire1 and XBP1. Here, we show that human AdV activates Ire1 in cancer and normal cells. Ire1 activation required the initial expression of E1A, and E1A activated early viral gene E3/19K. Expression of the single transmembrane glycoprotein E3/19K was sufficient to activate Ire1, and the soluble domain of E3/19K and Ire1 interacted in the endoplasmic reticulum, as shown by split-GFP complementation assays. Activated Ire1 spliced the XBP1 mRNA into an mRNA encoding transcriptionally active factor XBP1. Using chromatin-immunoprecipitation (ChIP) assays, we show that XBP1 binds to the E1A promoter. Importantly, the reduction of either Ire1 or XBP1 levels by RNA-interference in persistent AdV-infected human diploid fibroblasts reduced the infection. In addition, analyses of viral plaques in Ire1 knock-out Crispr/Cas9 HeLa cells demonstrated that Ire1 enhanced the lytic AdV spreading. Collectively, the data show that the induction of the UPR promotes lytic and persistent AdV infections via a novel positive feedback loop between cellular and viral proteins, involving E3//19K triggered Ire1 and XBP1. This or similar feedback loops for transcriptional enhancement may be widespread in viruses, owing to the notion that XBP1 binding sites are found in different promoters of diverse members of the *Adenoviridae*.

NOTES

Identification of cis-acting RNA Sequences Involved in Norovirus VPg-Dependent RNA Synthesis

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Despite their socioeconomic impact, our understanding of the biology of noroviruses has been somewhat limited, due primarily to their inability to be efficiently cultured in the laboratory until relatively recently. The identification of murine norovirus (MNV)—which can replicate efficiently in immortalized cells and for which several robust reverse genetics systems have been developed—has transformed our understanding of the molecular detail of viral genome translation and replication.

Using MNV as a model system, we have previously shown that while norovirus negative sense RNA is likely primed via a de novo mechanism, positive sense RNA is VPg primed and that the RNA is linked to a highly conserved tyrosine residue at position 26. However, the mechanism by which this linkage occurs and the template for norovirus VPg nucleotidylylation has yet to be fully characterized.

We have now interrogated the role of evolutionarily conserved RNA structures at the 3' end of the viral genome in the viral life cycle. Using mutagenesis and reverse genetics, we identified a series of mutations within the ORF3 coding region that were deleterious for viral replication. Further studies revealed that these mutations function by reducing the ability of the viral negative sense RNA to function as a template for VPg nucleotidylylation. Biochemical analysis indicates that this complex required NS5 (VPg), NS6 (3C-like protease), and NS7 (the viral RNA-dependent RNA polymerase), but that precursor forms may also function in the nucleotidylylation reaction.

NOTES

HIV-1 Assembly, Release, and Maturation

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Our research is focused primarily on the late stages of the HIV-1 replication cycle. This work involves identifying and characterizing functional determinants in the major structural polyprotein, Gag, required for virus assembly, budding, and maturation; elucidating mechanisms of envelope (Env) glycoprotein trafficking and incorporation into virions; and defining the pathway by which Gag targets the plasma membrane and associates with the lipid bilayer. We also have a long-term interest in host cell factors that influence retroviral assembly and release; these include factors that promote virus replication (e.g., the cellular ESCRT machinery) or are late-acting antiviral factors (e.g., tetherin, ISG15, TIM-1, MARCH8, and GBP5). Finally, we have also been developing a new class of anti-HIV inhibitor known as maturation inhibitors. These molecules act by blocking a late step in the protease-mediated processing of the Gag precursor protein during virus maturation. The study of maturation inhibitors, and resistance mutations that arise during HIV-1 propagation in the presence of these compounds, has provided fundamental insights into the molecular mechanisms of HIV-1 assembly and maturation.

NOTES

Protein-Assisted RNA Folding Mediates Specific RNA–RNA Genome Segment Interactions in Segmented RNA Viruses

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Segmented RNA viruses, including influenza viruses and rotaviruses, are ubiquitous human, animal and plant pathogens. A major challenge in understanding their assembly is the combinatorial problem of a non-random selection of a full genomic set of distinct single-stranded (ss)RNAs. This process involves multiple, complex RNA–RNA and protein–RNA interactions, which to date have been obscured by non-specific binding and aggregation at concentrations approaching in vivo assembly conditions. To interrogate specific inter-segment interactions in rotaviruses, we employ two-color fluorescence cross-correlation spectroscopy (FCCS) for detecting stable RNA–RNA interactions taking place in complex RNA and protein mixtures. We show that binding of the rotavirus non-structural protein NSP2 to ssRNAs results in RNA conformational rearrangements conducive to forming stable contacts between RNA segments. To identify the sites of inter-segment interactions, we developed an RNA–RNA SELEX approach for mapping the RNA sequences mediating stable inter-molecular base-pairing between the interacting ssRNAs. Our findings elucidate the molecular basis underlying inter-segment interactions in rotaviruses, paving the way for studying genome packaging of other segmented RNA viruses. The integrated approach expands the arsenal of techniques much needed for delineating dynamic RNA–RNA interactions involved in the assembly of large ribonucleoprotein complexes.

NOTES

The Hepacivirus and Pestivirus NS3 Helicases Act as Motor Proteins to Power RNA Encapsidation during Virus Particle Assembly

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Most positive-strand RNA viruses encode RNA helicases; however, the precise functions of these enzymes in vivo have not been elucidated. We hypothesized that the HCV NS3 helicase activity directly contributes to virus assembly; however, addressing this question has been difficult because helicase activity is essential for RNA replication, which is a prerequisite for virus assembly. To overcome this obstacle, we developed a genetic complementation strategy to separate the functions of the NS3 helicase in RNA replication from its role in virus assembly. First, we identified HCV NS3 mutants that replicated efficiently but were incapable of producing virus particles. Second, we demonstrated that these mutants could produce virus particles when supplied with a second assembly-competent form of NS3 in *trans*. Third, extensive mutational analysis on the *trans*-complementing NS3 revealed that the helicase RNA binding and NTPase activities are required for HCV nucleocapsid formation and virus assembly. Finally, parallel analysis on related pestiviruses confirmed that the role of the RNA helicase activity in virus assembly is conserved for other members of the *Flaviviridae*. These findings indicate that the helicase domain acts as a molecular motor to package the viral genome during nucleocapsid assembly.

NOTES

The Amino-Terminus of the Hepatitis C Virus (HCV) p7 Viroporin and its Cleavage from Glycoprotein E2-p7 Precursor Determine Specific Infectivity and Secretion Levels of HCV Particle Type

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Viroporins are small transmembrane viral proteins with ion channel activities modulating properties of intracellular membranes. Hepatitis C virus (HCV) encodes a viroporin, p7, acting during assembly, envelopment and secretion of viral particles. p7 is produced by cleavage from the HCV polyprotein but also exists as an E2p7 precursor, of poorly defined properties. In this study, we have explored how the retarded cleavage between E2 glycoprotein and p7 could regulate their functions associated to virion assembly and/or perturbation of cellular membrane processes. Specifically, we demonstrate that p7 is able to regulate the cell secretory pathway, which induces the intracellular retention of HCV glycoproteins and favors assembly of HCV particles. Our study also identifies a novel assembly function located at p7 amino-terminus that is unmasked through E2p7-regulated processing. Indeed, by coordinating the encounter between HCV non-structural protein NS2 and NS5A, respectively responsible for bringing E1E2 glycoproteins and viral nucleocapsids components to assembly sites, we found that p7 amino-terminus controls both the levels of different types of released viral particles and the envelopment of infectious particles. Altogether, our results underscore a critical post-translational control of assembly and secretion of HCV particles that governs their specific infectivity.

NOTES

Differential Phosphorylation and N-Terminal Configuration of Capsid Subunits in Parvovirus Assembly and Viral Trafficking

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Parvoviruses are nonenveloped eukaryotic nuclear viruses containing a 5 kb single-stranded (ss) DNA genome in a 25 nm-diameter icosahedral (T = 1) capsid made from two-to-three polypeptides. The parvovirus minute virus of mice (MVM) was used to study the roles that phosphorylation and N-terminal domains (Nt) configuration of capsid subunits may play in icosahedral nuclear viruses assembly. In synchronous MVM infection, capsid subunits newly assembled as two types of cytoplasmic trimeric intermediates (3VP2 and 1VP1:2VP2) harbored a VP1 phosphorylation level five-fold higher than that of VP2, and hidden Nt. Upon nuclear translocation at S phase, VP1-Nt became exposed in the heterotrimer and subsequent subviral assembly intermediates. Empty capsid subunits showed a phosphorylation level restored to VP1:VP2 stoichiometry, and the Nt concealed in their interior. However ssDNA-filled virus maturing at S/G2 lacked VP1 phosphorylation and one major VP2 phosphopeptide, and exposed VP2-Nt. Endosomal VP2-Nt cleavage resulted in VP3 subunits devoid of any phospholabel, implying that incoming viral particles specifically harbor a low phosphorylation status. Phosphorylation provides a mechanistic coupling of parvovirus nuclear assembly to the cell cycle.

NOTES

Abstracts

Session 6: Antiviral Innate Immunity and Viral Pathogenesis

Session Chairs – Shan-Lu Liu and Eric Poeschla

Stem Cell-Derived Enteroids to Model Enterovirus-GI Interactions

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The human gastrointestinal (GI) tract is a complex organ, with an epithelial surface that must provide a protective and immunological barrier in a complex and diverse microbial environment. Enteroviruses are leading causes of human infections worldwide, particularly in infants and children, and infect primarily via the fecal-oral route. These viruses, which include poliovirus, coxsackievirus, echovirus, enterovirus D68 (EV-D68), and enterovirus 71 (EV71), are small, single-stranded RNA viruses belonging to the *Picornaviridae* family. The events that surround enterovirus infections of the human GI epithelium remain poorly understood, largely owing to the lack of human-based models that recapitulate the complexity of the GI tract. We have utilized human and mouse stem cell-derived enteroids to define the cell biological and immunological events associated with enterovirus infections of the GI tract using coxsackievirus B (CVB), echovirus 11 (E11), and EV71. Our studies have revealed both species-specific and virus-specific events associated with enterovirus infections of the GI tract, including differential roles for type I and III interferons (IFNs) in the control of enterovirus infections and virus-specific tropism for cell types present in the GI epithelium. Together, our studies provide insights into enterovirus infections of the human intestine, which could lead to the identification of novel therapeutic targets and/or strategies to prevent or treat infections by these highly clinically relevant viruses.

NOTES

Harnessing Genomic Approaches to Explore the Interface between Viruses and Hosts

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Our driving interest is to discover the spectrum of cellular factors at the virus–host interface and to elucidate the mechanisms by which pathogens subvert this cellular machinery while evading recognition. We use a combination of functional genomics coupled with cutting-edge molecular approaches to define both the players and mechanisms involved. We have performed a large number of genome-wide RNAi screens and these data have revealed fundamental insights into the plethora of pathways engaged to block infection. Furthermore, we have compared and contrasted host factor dependencies across diverse human arthropod-borne viruses exploring genes and pathways active in both insects and humans. We use the model organism Drosophila for its powerful genetic tools and focus on conserved genes that play parallel roles in both vectors and humans. Using this system, we have identified complex mechanisms involved in viral RNA recognition and restriction. In addition, we have developed a system to explore enteric immunity to arboviruses using the Drosophila system where we have uncovered new roles for the microbiota in shaping antiviral defense. We will discuss our new findings on the pathways and players involved in arbovirus–host interactions and antiviral immunity.

NOTES

Towards Understanding 'Cell-to-Cell' Variability of Viral Gene Expression

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Viruses are intimately linked with their hosts [1]. Although virions are largely inert as long as they are extracellular, they increase complexity when they contact a host cell, and trigger an infection. Virions enter cells with the help of attachment factors, receptors and facilitators, and they release their genome from a protective protein coat [2,3]. Many DNA viruses, such as adenoviruses, replicate in the nucleus. This unconditionally requires the transcription of viral genes in the nucleus [4].

Human adenoviruses cause self-limiting respiratory, ocular and gastro-intestinal tract infections in immune-competent individuals, where infections can persist for many years and produce low levels of virions [5]. Immune control of adenovirus infections occurs by cross-reacting cellular and humoral responses, including interferon [6]. In immune-deficient individuals, adenovirus infections are increasingly linked to higher morbidity and mortality. In patients, adenovirus infections occur as sequential single infections or concomitant co-infections, and can give rise to recombinant virus types. Such recombinants can rapidly expand, and are clinically identified as so-called 'new' isolates, often poorly controlled by the immune system. Effective treatments of adenovirus disease are currently not available.

Here, I review the mechanisms of adenovirus entry and gene delivery into epithelial and immune cells [3,7,8]. I will then discuss the heterogeneity in viral transcription. Our earlier findings indicated that chemical activation of the unfolded protein response (UPR) boosts the transcription of the immediate early viral transactivator E1A by enhancing the levels of the transcription factor XBP1, as part of the canonical UPR following ER stress [9]. We further reported that the positive transcription elongation factor beta (pTEF-beta) comprising Cdk9 presents a post-exposure anti-viral drug target for clinically tested chemical compounds [10]. Using highly sensitive single cell, single transcript assays we show that the cell-to-cell variability of immediate early E1A transcription correlates at least in part with particular phases of the cell cycle. Evidence for a positive feedback loop boosting E1A expression will be discussed. This feedback loop involves early viral gene products besides E1A, the UPR sensor Ire1 in the endoplasmic reticulum, and XBP1. These results open the possibility that the UPR contributes to the lytic outbreak of adenoviruses from persistently infected cells.

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NOTES

Middle East Respiratory Syndrome-Coronavirus Employs the Deubiquitinating Activity of Its nsp3 Papain-Like Protease to Suppress the Innate Immune Response

Robert Knaap [1], Nadia Oreshkova [1], Tim Dalebout [1], Javier Gutiérrez [2], Raúl Fernández [2], Isabel Sola [2], Luis Enjuanes [2], Eric Snijder [1], Marjolein Kikkert [1]

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Replication of Middle East respiratory syndrome coronavirus (MERS-CoV) requires the nsp3-based papain-like protease (PLpro) to cleave multiple sites in the viral replicase polyproteins. Additionally, MERS-CoV PLpro exhibits deubiquitinating and interferon-stimulated gene 15 (ISG15)-deconjugating activity, which presumably counteract host antiviral responses. Guided by a crystal structure of MERS-CoV PLpro in complex with ubiquitin, PLpro mutations were designed that should disrupt binding of ubiquitinated substrates, thus blocking PLpro's deubiquitinase (DUB) activity without affecting viral polyprotein cleavage. Using recombinant DUB-knockout MERS-CoV, we examined the specific impact of PLpro's DUB activity on the innate immune response. Replication of DUB-knockout mutant and wild-type control was comparable in cell lines either able or unable to mount an innate immune response. However, compared to wild-type virus infection, strikingly increased mRNA levels for IFN-β and ISGs were measured in cells infected with the DUB-knockout mutant. Moreover, preliminary data showed a clearly increased survival rate of mice infected with the DUB-knockout mutant relative to wild-type MERS-CoV-infected animals, supporting the conclusion that the DUB-knockout mutant is attenuated. Mouse samples are currently analysed to assess virus replication and immune responses. This study provides the first direct evidence that the DUB activity of MERS-CoV PLpro contributes to viral innate immune evasion.

NOTES

HIV Accessory Factors: More than One Way to Skin a Cat

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Retroviruses have evolved effective strategies to evade the host immune response, such as high variability and latent infection. In addition, primate lentiviruses such as HIV-1 and its simian precursors have acquired several "accessory" genes that antagonize antiviral host restriction factors to allow continuous and efficient viral replication despite apparently strong innate and acquired immune responses. Here, I summarize some of our current knowledge on the acquisition and function of the viral *vpr, vpu*, and *nef* genes. First, I will briefly discuss the multi-functionality of these viral factors and the evolution of specific properties of pandemic HIV-1 strains that most likely contribute to its effective spread and high virulence. Second, I will present data supporting that HIV and other primate lentiviruses use alternative Vpu-, Vpr-, or Nef-dependent strategies to modulate NF-κB-dependent expression of viral and antiviral genes. Finally, evidence will be presented that Vpu suppresses superinfection and the stimulation of effective innate immune responses by several cooperative mechanisms, including the modulation of DNA damage responses to suppress the accumulation of nuclear viral cDNA species. Altogether, accumulating evidence illustrates not only the striking plasticity and multi-functionality of HIV and SIV accessory proteins, but also shows that they functionally synergize and use alternative mechanisms to counteract cellular defence mechanisms.

NOTES

Arboviruses and Antiviral RNAi Pathways in Mosquito Cells: Progress in Understanding Regulation and Effectors

Alain Kohl

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Arboviruses such as Zika, dengue (*Flaviviridae* family) and chikungunya (*Togaviridae* family, genus Alphavirus) are transmitted by mosquitoes and more specifically *Aedes aegypti*, as the key vector. There has been considerable progress over the last decade to understand how vector mosquitoes control virus replication, and the central role of RNA interference (RNAi) pathways is now well established with the exogenous siRNA and piRNA pathways of importance. Here, I will present recent insights into antiviral RNAi processes in response to arbovirus infection in Aedes aegypti-derived cells and/or mosquitoes in response to both alphaviruses and flaviviruses. This has allowed us to draw comparisons highlighting similarities but also some differences with regards to RNAi responses between these two groups of positive strand RNA arboviruses. Recent work has also given us novel insights into the regulation of RNAi pathways by cellular proteins, which I will discuss.

NOTES

ISG15 Governs Mitochondrial Function in Macrophages Following Vaccinia Virus Infection

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The interferon (IFN)-stimulated gene 15 (*ISG15*) encodes one of the most abundant proteins induced by interferon, and its expression is associated with antiviral immunity. To identify protein components implicated in IFN and ISG15 signaling, we compared the proteomes of *ISG15*$^{-/-}$ and *ISG15*$^{+/+}$ bone marrow-derived macrophages (BMDM) after vaccinia virus (VACV) infection. The results of this analysis revealed that oxidative phosphorylation (OXPHOS) and mitophagy were pathways altered in *ISG15*$^{-/-}$ BMDM treated with IFN. Mitochondrial respiration, ATP, and reactive oxygen species (ROS) production was higher in *ISG15*$^{+/+}$ BMDM than in *ISG15*$^{-/-}$ BMDM following IFN treatment, indicating the involvement of ISG15-dependent mechanisms. An additional consequence of *ISG15* depletion was a significant change in macrophage polarization. Although infected *ISG15*$^{-/-}$ macrophages showed a robust proinflammatory cytokine expression pattern typical of an M1 phenotype, a clear blockade of NO production and arginase-1 activation was detected. Accordingly, following IFN treatment, NO release was higher in *ISG15*$^{+/+}$ macrophages than in *ISG15*$^{-/-}$ macrophages, concomitant with a decrease in viral titer. Thus, *ISG15*$^{-/-}$ macrophages were permissive for VACV replication following IFN treatment. In conclusion, our results demonstrate that ISG15 governs the dynamic functionality of mitochondria, specifically, OXPHOS and mitophagy, broadening its physiological role as an antiviral agent.

NOTES

Virus–virus Interactions Driven by Innate Immune Responses

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Innate immune responses are the first line of defense against viruses. Among them, interferon (IFN)-regulated responses drive the outcome of infection in a coordinated process of signaling to neighbor cells to locally suppress viral spread. In the host–pathogen race, viruses have evolved different strategies to evade the immune system (e.g., IFN blockade). Owing to their high mutation rates, RNA viral populations are known to be a cloud of variants that will interact both within and between cells. These virus–virus interactions set the stage for the evolution of viruses. Using vesicular stomatitis virus (VSV), we have analyzed how population structure determines virus–virus interactions using a wild-type virus (able to block the IFN response) and a variant which is unable to block the IFN cascade. Inefficient IFN blockade by a given virus variant is expected to reduce not only its own spread, but also the spread of viruses infecting neighboring cells by triggering cell entry into an antiviral state. This produces intra-host, social-like interactions in the virus. Importantly, the ability of an IFN suppressing trait to evolve may be hampered by the presence of non-suppressors in the same population. Such an inability of a cooperative trait to evolve despite its obvious long-term benefits is akin to the well-known tragedy of the commons.

NOTES

Interferon Inhibits Flavivirus Replication via IFI6, a Chaperone-Dependent ER Membrane Effector

Blake Richardson[1], Maikke Ohlson [1], Jennifer Eitson [1], Ian Boys [1], Katrina Mar[1], Pamela De La Cruz Rivera [1], Ashwani Kumar [2], Chao Xing [2], John Schoggins [1]

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The endoplasmic reticulum (ER) is an architecturally diverse organelle that serves as a membrane source for the replication of multiple viruses. Flaviviruses induce unique single-membrane ER invaginations that house the viral replication machinery. Whether this virus-induced ER remodeling is vulnerable to antiviral pathways is unknown. Here, we show that flavivirus replication at the ER is targeted by interferon (IFN)-mediated immunity. Through genome-scale CRISPR screening, we uncovered an antiviral mechanism mediated by a functional gene pairing between *IFI6*, an interferon-stimulated gene (ISG) cloned over 30 years ago, and *HSPA5*, the ER-resident HSP70 chaperone. We reveal that IFI6 is an ER-localized integral membrane effector that is stabilized through interactions with HSPA5. During infection, IFI6 colocalizes with the viral replication scaffold and prevents the formation of virus-induced ER membrane invaginations, thereby suppressing multiple flaviviruses, including yellow fever virus, West Nile virus, dengue virus, and Zika virus. Strikingly, IFI6 has little effect on other mammalian RNA viruses, including the related *Flaviviridae* member hepatitis C virus, which replicates within ER-derived double membrane exvaginations. These findings support a model in which the IFN response is armed with a membrane-targeted effector that discriminately blocks the establishment of virus-specific ER microenvironments required for replication.

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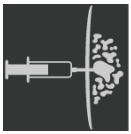

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Abstracts

Poster Exhibition – Session 1: General Topics in Virology

1. Autophagy Participates in Dengue Virus Production by Promoting Viral Uncoating Process

Li-Wei Chu, Yueh-Hsin Ping

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Dengue virus (DENV) is a mosquito-transmitted RNA virus belonging to the flaviviridae family and is one of the most significant human viral pathogens, especially in tropical areas. Unfortunately, because of insufficient understanding of the interactions between DENV and host cells, antiviral therapies treating DENV infection are currently not yet in sight. Following the fate of individual virus particles using single-virus tracking (SVT) technology allows us to probe dynamic interactions between virus and cellular factors, and to dissect the steps of the infectious process. Recently, we have established a real-time multi-fluorescence imaging system to track a single fluorescence-labeled DENV particle in a living cell and elucidated a novel autophagy-mediated intracellular transportation of DENV. We further dissected the uncoating process of DENV by visualizing the acidification of a virus-containing vesicle and the membrane fusion between the viral envelope and membrane of the transport vesicle. SVT assay showed that the intensity of pHrodo Red-labeled DENV elevated when the DENV particle was co-localized with an autophagosome, suggesting that DENV-containing autophagosomes undergo acidification. Moreover, FRET imaging of dual-fluorescence labeling DENV particles also revealed that member fusion of DENV particles was detected within autophagosomes. Taken together, we not only uncovered new roles of autophagy in the DENV infection process, but also provided invaluable feedback to the development of novel biophotonics approaches.

2. Abnormal Hepatocellular Organelles Remain to Be Observed in Sustained Virological Response Patients

Haruyo Aoyagi [1], Hiroko Iijima [2], Francesc Puig-Basagoiti [1], Xin Zheng [1], Yu Ting Kao [1], Hossam Gewaid E. [1], Takuma Zaitsu [1], Mami Matsuda [1], Koichi Watashi [1], Ryosuke Suzuki [1], Takahiro Masaki [3], Nobuhiro Aizawa [2], Noritomo Shimada [4], Keizo Kato [5], Akihito Tsubota [6], Ayako Mimata [7], Yuriko Sakamaki [7], Shizuko Ichinose [7], Kenjiro Wake [8], Masamichi Muramatsu [1], Takaji Wakita [1], Hideki Aizaki [1]

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Background: Over 90% of patients with chronic hepatitis C achieved a sustained virological response (SVR) after direct-acting antivirals (DAA). However, approximately 10% of SVR-patients developed hepatocellular carcinoma (HCC) within 10 years. Therefore, carcinogenesis after SVR remains problematic. This study aimed to elucidate the liver pathology after SVR and to reconsider the management of the increasing number of SVR-patients.
Methods: Viral genomes were analyzed by RT-PCR and cellular ultrastructures were assessed using transmission electron microscopy (TEM) in liver biopsies of SVR-patients treated with IFNα.
Results: HCV RNA was detected in three out of 13 samples of liver tissue after SVR. Moreover, we detected latent HCV RNA in two out of 11 SVR-HCC patients. Abnormal organelles such as nuclear membrane disruption, cristae destruction in swollen mitochondria, as well as membrane vesicle formation of the ER were identified in the hepatocytes of SVR-patients. The dilated and degranulated ERs were increased in SVR-HCC patients as compared with SVR-noHCC patients. The hepatic stage of fibrosis in SVR-HCC patients did not improve compared to SVR-noHCC patients.
Conclusions: Abnormal hepatocellular organelles in SVR-patients indicate a persistent disease state (post-SVR syndrome). Long-term follow-up of patients is recommended after achieving SVR.

3. A Novel and Promising Chemical Compound with Anti-Rotavirus Activity

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The search for effective antivirals against rotavirus (RV) gastroenteritis is important in reducing the still high morbidity and mortality of RV infections in developing countries. In this study, we describe the anti-RV activity of ML-60218, a small molecule known as RNA polymerase III inhibitor. In cell-based assays, ML-60218 was found to disrupt viroplasms, the cytoplasmic viral factories where viral genome replication and assembly of progeny double-layered particles (DLPs) take place. This phenotype correlated with a reduction in accumulated viral proteins and viral genome segments and with inhibition of infectious progeny virus production. In vitro, ML-60218 caused dose-dependent damage of the structural integrity of purified DLPs, whose transcriptional activity was also compromised in a dose-related manner. The higher-order structures formed by the DLP outer layer protein VP6 were found severely damaged by ML-60218, whereas VP6 trimerization was not affected. ML-60218 is the first chemical compound found capable of (i) disrupting viroplasms and (ii) compromising the stability of DLPs by targeting the viral protein VP6. This molecule thus represents a starting point towards the design of a new class of potent and selective anti-RV compounds. (Copyright © American Society for Microbiology, JVI Accepted Manuscript Posted Online 15 November 2017 J. Virol. doi:10.1128/JVI.01943-17).

4. Analysis of the Mechanical Properties of HIV-1 Capsid and Their Impact on the Uncoating Process

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The human immunodeficiency virus (HIV-1) core consists of a protein shell called a capsid that encapsulates the virus genome. After the virus enters into the host cell and prior to the import of the viral dsDNA into the nucleus, the capsid has to disassemble in a process called uncoating. Several studies showed that uncoating is promoted by reverse transcription (RT) and regulated by cellular factors. Mutations that alter the stability of the capsid affect the uncoating rate and impair HIV-1 infectivity. Our atomic force microscopy (AFM) mechanical properties measurements of wild-type (WT) and hyperstable mutants cores showed that mutation that increases stability and delays uncoating also increases the physical stiffness of HIV-1 capsid. In addition, we analyzed the morphological and stiffness changes of WT and hyperstable cores during reverse transcription using time-lapse AFM. We found that hyperstable cores stayed intact whereas WT cores disassembled. Based on our findings, we propose that mechanical properties of HIV-1 capsid must be well balanced in order to enable capsid uncoating followed by infection.

5. Building a Mathematical Model of Virus Entry

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To accomplish infection, a virus particle must overcome the various intrinsic physical barriers presented by a cell. This is achieved through the molecular choreography of virus entry, whereby a series of coordinated virus–host interactions allow the virus particle to attach, engage, and penetrate the cell. The precise mechanism of entry by any given virus is defined by its interactions with specific cell-surface receptors. Understanding these interactions provides insights into virus tropism, and may guide the rational design of vaccines and therapies.

The coordinated step-wise nature of virus entry is highly amenable to mathematical modelling, with each step being expressed as a discrete probabilistic event. Using hepatitis C virus (HCV) as a model system, we have built a mechanistic mathematical model of virus entry.

In comparison to many viruses, the entry pathway of HCV is unusually complex, involving at least four essential cellular components. We focused on CD81 and SR-B1—two receptors necessary for the early stages of virus entry. We used antibody-mediated blockade and over-expression to modulate the availability of either receptor, and then measured the effect on virus titre. In addition, we determined the number of virus particles that attached during these experiments, allowing us to calculate the probability of a virus particle achieving virus entry. We also evaluated virus–receptor interactions using soluble versions of the major HCV glycoprotein. Informed by these observations, we then constructed a mathematical model of the early stages of HCV entry and fitted this to our experimental data.

This approach has allowed us to investigate the likely mechanisms of HCV entry, predict the efficiency of this process, and infer the stoichiometries of HCV–receptor interactions. Aside from gaining specific insights into the nature of HCV entry, this work also provides a blueprint for similar investigations of other virus systems.

6. Coreceptors HIV-1 Mediated Apoptosis Occurs in Macrophages Infected by X4- but Not by R5-Viruses

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Apoptosis induction is associated with HIV-1 binding with CXCR4 (X4) or CCR5 (R5) (essential step during HIV-1 replicative life cycle) in different target cells. For this purpose, apoptotic induction was evaluated in MDM infected by X4 and R5 viruses. Apoptosis induced by X4-viruses starts from day 6, peaks 40–50% at day 10, whereas is 1.65% in R5-infected MDM. In line with this, Western blotting analysis was conducted and the p38 activation at 30' after infection in MDM infected X4-virus, but not with R5 strains was shown. Moreover, the CXCR4 antagonist AMD3100 inhibited apoptosis and proapoptotic MAPK/p38 activation. Microarray analysis showed modulation of proapoptotic and cancer-related genes induced by X4 strains starting at 24 h after infection, whereas R5 viruses modulated the expression of genes not correlated with apoptotic-pathways. Overall, these results shed light on the biological mechanism leading to MDM survival during HIV-infection. Abortive infection with X4 strains, and their consequent depletion from reservoirs, may explain a major pathogenic role of R5 viruses in all (but terminal) phases of the HIV-1 disease and provide important implications for the therapy of HIV infection.

7. Detection of Human Bocavirus mRNA in PBMCs from Patient with Acute Lower Respiratory Tract Infection

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Human bocavirus type one (HBoV1) is a parvovirus discovered in 2005, and is considered as a respiratory pathogen. Although HBoV1 genomic sequences are most commonly detected in samples from the respiratory tract, the replication cycle and HBoV1 target cells are still not completely studied.
In this study, we report on a 17-month-old boy with life-threatening acute lower respiratory tract infection (LRTI) admitted to the Children's Clinical University Hospital of Riga, Latvia. DNA from nasopharyngeal aspirate (NPA), stool, whole blood, and corresponding cell-free blood plasma was extracted and tested for HBoV1-specific NS1 gene sequence using polymerase chain reaction (PCR). Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) and mRNA reverse-transcribed into cDNA, where the presence of HBoV1 was also analysed by PCR targeting HBoV1 NS1 gene. All DNA and cDNA samples tested were HBoV1-positive.
The presence of HBoV1 DNA in whole blood and cell-free blood plasma shows that virus is not only present in the respiratory tract, but is circulating in the blood. Detection of HBoV1 mRNA indicates HBoV1 transcription in the PBMCs of patients with acute LRTI, and could be used as a marker of active HBoV1 infection.

8. Functional Study of Hepatitis C Virus to Identify Mechanisms of Antibody Evasion by Hypervariable Region 1

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It is estimated that 2–3% of the world population is infected with hepatitis C virus (HCV), and despite recent advances in HCV therapy, over 500,000 people die each year from virus-induced liver disease. The extensive sequence diversity of envelope proteins E1 and E2 of HCV along with shielding of important epitopes by hypervariable region 1 (HVR1) of E2 are believed to be major obstacles to developing a vaccine against HCV.
--Using cultured viruses expressing the E1/E2-complex of H77 (genotype 1a) with HVR1 from isolates of genotype 1-4, we show that different HVR1 sequences confer divergent levels of HVR1-mediated neutralization occlusion. We show that these differences can be determined by specific combinations of residues in the C-terminal part of the HVR1 sequence.
Furthermore, we show that differences in the remaining part of the envelope glycoproteins have an impact on the level of occlusion mediated by HVR1, and we found two positions in E2 that seem to have a role in this.
These findings suggest that the total effect of HVR1-mediated neutralization occlusion is not just an intrinsic property of the HVR1 sequence itself, but is determined by a complex interplay between HVR1 and the remaining part of the envelope glycoproteins.

9. Genetic and Antigenic Characterization of *Piscine orthoreovirus* (PRV) from Rainbow Trout (*Oncorhynchus mykiss*)

Dhamotharan Kannimuthu [1], Vendramin Niccolo [2], Turhan Markussen [1], Øystein Wessel [1], Ingvild Nyman [1], Maria K. Dahle [3], Torstein Tengs [1], Espen Rimstad [1]

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Piscine orthoreovirus (PRV-1) infects erythrocytes and causes the disease heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon. PRV is ubiquitous in farmed salmon, but it has resisted cultivation in cell cultures. A novel piscine orthoreovirus (PRV-3) was detected in rainbow trout with a disease resembling HSMI. The complete coding sequences of the ten genome segments of PRV-3 were obtained by NGS. We analyzed the coding regions and protein sequences of the PRV infecting rainbow trout and compared to the PRV of Atlantic salmon. Phylogenetic analysis shows that PRV-3 belongs to a separate cluster to PRV-1 and PRV-2 (from Coho salmon), indicating that PRV-3 is adapted to a different host. Detailed analyses of all known proteins encoded by PRV-3 revealed that most predicted secondary structures and functional domains are conserved in between PRV-3 and PRV-1. Virus particles were purified from in vivo infected erythrocytes by CsCl gradient ultracentrifugation. Rabbit antiserum against purified PRV-3 cross-reacted with PRV1 and vice versa. Polyclonal antisera raised against PRV-1 sigma 1, sigma 3, sigma NS, mu 1C, mu NS, and lambda 1 cross-reacted to PRV-3. This is the first report describing the genetic and antigenic relationship between PRV strains.

10. HCV Resistance-Associated Substitutions (RAS) Testing: Cut off at 15%?

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Background: Despite the effectiveness of new generation direct-acting antivirals (DAAs) for treating hepatitis C virus (HCV) infection, a non-negligible percentage of patients (5–10%) experience treatment failure due to the selection of resistance-associated substitutions (RASs). Clinical guidelines recommend the physicians to assesses HCV resistance using population sequencing by the presupposition that only an RAS present in more than 15% of the viral population has an impact on treatment outcome.
Methods: By the comprehensive analysis of HCV samples using next-generation sequencing as a routine management in our laboratory, we report on two HCV G1a patients harboring mutants at a frequency lower than 15% at baseline that failed treatment with Harvoni® (ledipasvir/sofosbuvir) at 8 and 12 weeks, respectively. All DAA target genes (NS3, NS5A, and NS5B) were analyzed in both baseline and failure samples.
Results: NS5A RAS Y93H and E62D detected at baseline at frequency of 3.5% and 3.7%, respectively, were selected at 100% in corresponding treatment failure patients. Furthermore, NS3 RAS Q80L, S122G, and R155K detected at frequency below 10% of viral population were also selected at 100% in viral relapse.
Conclusions: Minority variants at frequencies below the established cut-off value of 15%, which remain unnoticed when testing with population sequencing, may be relevant in treatment decisions, and detailed information should be provided by NGS to guide re-treatment strategies. Moreover, NS3 RAS were also co-selected at failure, even though antiviral treatment was directed against NS5A and NS5B targets, suggesting that RAS studies should include all HCV DAA target regions, regardless of the treatment.

11. Hsp90 Dictates Viral Sequence Space by Balancing the Evolutionary Tradeoffs between Protein Stability, Aggregation, and Translation Rate

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The acquisition of mutations is central to evolution, but the detrimental effects of most mutations on protein folding and stability limit protein evolvability. Molecular chaperones, which suppress aggregation and facilitate polypeptide folding, are proposed to promote sequence diversification by buffering destabilizing mutations. However, whether and how chaperones directly control protein evolution remains poorly understood. Here, we examine the effect of reducing the activity of the key eukaryotic chaperone Hsp90 on poliovirus evolution. Contrary to the predictions of a buffering model, inhibiting Hsp90 increases population sequence diversity and promotes the accumulation of mutations reducing protein stability. Explaining this counterintuitive observation, we find that Hsp90 offsets the evolutionary tradeoff between protein stability and aggregation. Lower chaperone levels favor sequence variants of reduced hydrophobicity, thus decreasing protein aggregation propensity, but at a cost to protein stability. Notably, reducing Hsp90 activity also promotes clusters of codon-deoptimized synonymous mutations at inter-domain boundaries, likely to promote local ribosomal slowdown to facilitate cotranslational domain folding. Our results reveal how a chaperone can shape the sequence landscape at both the protein and RNA levels to harmonize the competing constraints posed by protein stability, aggregation propensity, and translation rate on successful protein biogenesis.

12. Human Papillomavirus Seroprevalence and Association with Anal HPV Infection and Squamous Intra-Epithelial Lesions in Australian Gay and Bisexual Men

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Background: Gay and bisexual men (GBM) are at disproportionately high risk of anal cancer. The precursor lesions—high-grade squamous intra-epithelial lesions (HSILs)—are very common, and it is evident that not all HSIL progresses to cancer. The serological response to anal human papillomavirus (HPV) in GBM has not been well characterised.
Methods: The Study of the Prevention of Anal Cancer is an ongoing cohort study of GBM aged 35 years and older. At six visits over three years, anal samples are collected for cytology, HPV DNA testing, and histology. Baseline serum was tested for HPV L1, E6, and E7 antibodies for 10 HPV types. Seroprevalence and associated predictors were analysed.
Results: 588 of 617 participants were included in this analysis. Of these, 436 (74.2%) were seropositive for at least one of the 10 HPV types. Almost half had L1 antibodies to HPV6 (48.5%), over a third to HPV11 (36.4%) and HPV16 (34.5%). HIV-positive men were more likely to be HPV L1 seropositive. For most high-risk HPV types, HSIL detection was highest among participants who were HPV serology and DNA positive. There was a borderline significant association between the presence of HPV16 E6 antibodies and prevalent HSIL (OR 2.97, 95% CI 0.92–9.60, $p = 0.068$).
Conclusions: HPV L1 seropositivity was common in this cohort of older GBM. These results suggest that HPV L1 seropositivity—in conjunction with anal HPV DNA—is a useful predictor of concurrent HSIL. The apparent association between HPV16 E6 antibodies and prevalent HSIL is a finding with potential clinical significance that needs further exploration.

13. Influenza Neutralization Fingerprinting Utilizing an Engineered Reporter Virus-Based High-Throughput Neutralization Assay

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The isolation and characterization of broadly neutralizing anti-influenza antibodies aids the development of new vaccines. However, the standard assay to measure influenza antibody response requires high-containment laboratories due to the use of live viruses and substantial hands-on time, posing a challenge for automation. Here we develop a high-throughput, safe influenza virus neutralization assay using the engineered influenza viruses carrying a fluorescent reporter instead of viral polymerase PB1, thus limiting their replication on cells expressing PB1. To characterize the breadth of neutralizing antibody responses in humans, we generated a panel of engineered reporter H1N1 (*N* = 18) and H3N2 (*N* = 24) influenza viruses expressing representative HA and NA circulating since 1930, and defined the serum neutralization profiles of 36 healthy individuals. For H1N1, we found that most individuals have neutralization titers not only against the viruses they were exposed to, but also against ones circulated before their birth or after sample collection. In contrast, neutralization breadth against H3N2 viruses was strictly limited to viruses circulating during each individual lifetime. Using the influenza virus panel, we generated the neutralization fingerprints of monoclonal antibodies that would facilitate the delineation of the specificity and composition of neutralizing antibodies in sera, and help in designing vaccines capable of providing broader protection against influenza viruses.

14. Localisation of the Human Coronavirus NL63 Nucleocapsid Protein

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Human coronavirus NL63 (HcoV-NL63) was identified in 2004 and has been linked with both upper and more severe lower respiratory tract illness. The multi- domain coronavirus nucleocapsid protein (N) plays an integral role in viral replication and assembly. Given its importance and abundant expression within infected cells, N is one of the most studied CoV structural proteins. Previous studies show that N localises to the nucleus in most CoV species, with the exception of SARS-CoV and HcoV-NL63. In this study, NL63-CoV N was expressed in a mammalian system. The N gene was cloned into pXJ40-FLAG and pXJ40-HA vectors. Following transfection, the expression of N in Cos-7 cells was confirmed by Western Blot (WB) and Immunofluorescence (IF). N-specific monoclonal antibodies were generated in a murine system for protein detection. Immunoblotting detected a ~50 kDa protein, and IF showed N in the nucleus of transfected cells; no expression was seen in the cytoplasm. Cytotoxicity was observed in Cos7 cells expressing HcoV-NL63 N. The subcellular localisation pattern of N appears to be nuclear. DNA counter-staining indicates the absence of nucleic acid at sites of N localisation, which suggests nucleolar accumulation of the protein, or a subcellular nucleolar localisation pattern for HcoV-NL63 N.

15. Protein–Protein Interaction Network in Herpes Simplex Virus Type 1

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Human herpesviruses infect over 90% of the worldwide population. The currently nine known human species differ in infective strategies, but they all cause lifelong infections with sporadic reactivations. Infection symptoms range from mild to very severe and life-threatening conditions. The high prevalence and possible severity of their infections, coupled with the lack of antiviral therapy capable of eradicating the virus from the host, make these viruses an important socioeconomic burden. Using network biology, we have been working to extend our understanding on the physical and functional relationships among herpesvirus proteins upon infection, crucial to develop better alternatives to tackle these infections. We designed a computational pipeline for reconstruction and analysis of protein–protein interaction (PPI) networks that combine both computational predictions and experimentally supported interactions. A web server has been designed to make all our interactomics data publicly available.

16. Revisiting the Implication of Beta-Karyopherins in HIV-1 Nuclear Import

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The import of cellular proteins into the nucleus via the nuclear pore is carried out by karyopherins/importins: Kapb1/Impb, Kapb2/Transportin/TNPO1, Kapb3/Imp5/RanBP5, Imp4/RanBP4, Imp7/RanBP7, Imp8/RanBP8, Imp9, Imp11, Imp12/TRN-SR2/TNPO3, Imp13.
The HIV pre-integration complex (PIC) translocates into the nucleus as proviral DNA associated to select viral and cellular proteins. Multiple putative NLSs were identified in HIV-1 proteins, and several karyopherins are proposed to recognize the viral PIC, but their direct contribution to PIC nuclear translocation is uncertain, and knowledge of the minimal machinery required for HIV nuclear import eludes us still.
Since no viral protein/Kapb complex was identified to mediate HIV nuclear import, we reasoned that it may instead be mediated by a nucleic acid molecule or motif. The central DNA Flap—a single strand overlap that is generated by strand displacement at the end of reverse transcription—has been reported to promote HIV-1 nuclear import. In addition, HIV-1 was shown to associate with 3'-defective tRNAs for nuclear import, suggesting that it may highjack a cellular nuclear import pathway involved in the quality control of RNA molecules. In this study, we tested the implication of Kapbs known to participate in the nuclear import of nucleic acids or proteins involved in nucleic acid processing.

17. The Effects of Autophagy on H5N1 Infection of Immature Dendritic Cells

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H5N1-induced cytokine dysregulation may be a main reason for high mortality. Our past study demonstrated that dendritic cells capture H5N1 to infect other cells via DC-SIGN. H5N1-infected cells produced considerable amounts of cytokines and even caused severe cell death. Recent studies showed that H5N1 could cause autophagic cell death in lung epithelial cells, but autophagy is not involved in virus-induced cytokine and viral replication. We therefore compared H5N1 infection in immature dendritic cells and epithelial cells to determine whether autophagy plays essential roles in H5N1-induced cell death, cytokine secretion, and viral replication in immature dendritic cells, and whether DC-SIGN is involved.

We found a considerable decrease in H5N1-induced cell death and cytokine secretion in both H5N1-infected immature dendritic cells and lung epithelial cells. However, we also demonstrated that autophagy blockade reduced viral replication only in immature dendritic cells, but not in lung epithelial cells. Furthermore, we used a DC-SIGN expressing cell line and anti-DC-SIGN mAb or downstream molecule inhibitor to investigate whether DC-SIGN is involved in H5N1-mediated autophagy. Our results proved that DC-SIGN had no direct influence on H5N1-induced autophagy. The effects caused by autophagy between H5N1-infected dendritic cells and epithelial cells will be further studied.

18. The Production of Cytokines in Whole Blood Cultures Following Exposure to Human Coronavirus NL63 and SARS-CoV Nucleocapsid Proteins

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Human coronavirus (HCoV) nucleocapsid protein (N) is a multi-domain, multi-functional protein important for the formation of the viral ribonucleocapsid core, replication and assembly, and other viral processes. Previous studies reported that SARS-CoV N elicits a strong immune response in humans, and DNA vaccination of N can induce both humoral and cellular immune responses in mice. In vitro stimulation of whole blood culture (WBC) with specific antigens is used for several purposes, including assessing human cellular immune responses to pathogens. A WBC assay provides a more physiological environment, which may provide a broader assessment of serum biomarkers. HCoV-NL63 and SARS-CoV nucleocapsid genes were cloned, and N was expressed in a baculovirus expression system; the purified antigens were then used to screen WBCs for cytokine production in an immunoassay. Sera from 40 healthy, non-smoking, male individuals were screened, and IL-6 was used as a biomarker for inflammation, IL-10, and IFN-gamma for humoral and cell mediated immunity, respectively. Total cells from WBCs were incubated with HCoV-NL63 and SARS-CoV N (10 µg/mL) overnight at 37 °C. Preliminary studies on the N protein have shown that it is able to elicit an inflammatory response, observed by an increased production of IL-6. These results were also observed in a pilot study preceding this one, where exposure to N was found to stimulate production of IL-6 in lipopolysaccharide (LPS)-unstimulated samples ($p < 0.05$), but had no effects on IL-10 and IFN levels. The results thus indicate that N has the potential to evoke a broad-based cellular immune response.

19. The Role of the Deubiquitinase USP7 in Influenza Virus Entry

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Endocytosis is a common entry strategy for many clinically relevant viruses, including Influenza A virus (IAV). However, the mechanism by which these viruses escape the endosomes and gain access to the cytosol is not well characterized. We previously showed that histone deacetylase 6 (HDAC6)—a cytoplasmic deacetylase with ubiquitin-binding activity—supports IAV capsid disassembly during cell entry. In this study, we hypothesized that deubiquitinating enzymes (DUBs, proteases that remove ubiquitin and edit ubiquitination events) may also play a role in IAV entry. Using small-molecule inhibitors and RNA interference in human airway cells, we identified ubiquitin-specific protease 7 (USP7) as an IAV pro-viral factor. We further employed quantitative IAV entry assays based on confocal microscopy, high-throughput immunofluorescence microscopy, and FACS, and concluded that USP7 was required at a post-fusion, pre-nuclear step of entry. Moreover, USP7 was not required for IAV entry when endocytosis was bypassed. IAV uncoating is supported by actin filaments and myosin II, microtubules, and dynein. Since USP7 has been described as a regulator of actin assembly at endosomes, it may regulate IAV uncoating. This project is relevant for novel therapeutic interventions aimed at targeting cellular rather than viral proteins. Moreover, it will help us attain a better picture of how ubiquitination events regulate virus uncoating and cell entry.

20. The Virologist in His Labyrinth: How to Introduce the Complex of Virology to the Lay Community

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Background: Low virology literacy is associated with higher health and economic challenges. The current global antimicrobial resistance (AMR) phenomena and vaccine-hesitancy or refusal "epidemiology" illustrate this point nicely. These crises could have been averted had greater, more wide reaching efforts been made to communicate the scientific facts effectively to the general public. Extensive efforts have been made recently to engage communities with science through animations and games, and, while these models have proved effective and engaging, they fail to significantly improve literacy. Looking for a model that can gently introduce the readers to complex vocabulary and afford a prime opportunity for stimulated self-learning, we found that comics do so and more. Aim: We aim to create a series of scientifically accurate, attractive, engaging, fact-filled comics re the science behind viral diseases, and explore their impact. Methods: 1000 participants completed a survey concerning their past, current and future acceptance for comics and other digital learning models. Results: Our data showed comics to be an acceptable learning/teaching model (>82%). It also showed that the most appealing learning model was animations 38%, followed by comics (36%), then info-graphics (16%) then games (10%). Recommendation: The conservative virology educational systems should seek ways to effectively integrate comics into the public life. They also have to evaluate robustly whether virology comics, if supported and sustained, through repeated engagement can generate a more impactful and lasting individual interest in, and engagement with virology.

21. Towards a Protein-Based Zika Virus Vaccine

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The recent outbreak of Zika virus (ZIKV) has highlighted the urgency of a vaccine. Our aim is to develop protein-based ZIKV vaccine candidates, e.g., virus-like particles (VLPs) and domain III of viral envelope protein (E-DIII).
VLPs are the product of prM and E proteins self-assembling in structures that resemble native virions, but lack the viral genome. We expressed VLPs in mammalian or insect cell systems using wild-type E sequence or a mutant that abrogates E trimerization with the aim of focusing the immune response against Envelope Dimer Epitopes.E-DIII domain is the main target for highly specific neutralizing antibodies. We expressed and purified a codon optimized E-DIII fused to SV5-tag, and to the human IgG heavy chain constant domain 3 (γCH3) to allow its dimerization. Mice were vaccinated with the purified protein and a single boost was found to be sufficient to induce neutralizing antibodies recognizing ZIKV E. The vaccinated mice are currently assessed for protection against ZIKV infection. The results of these studies will be discussed.
In parallel, we generated ZIKV E-specific antibodies. We identified a ZIKV E specific region around the N154 glycosylation site, in an exposed loop (the 150 loop) on the protein surface that has a sequence that is remarkably distinct from the related flaviviruses. A peptide corresponding to the 150 loop was used to generate a rabbit polyclonal antiserum, and two mouse monoclonal antibodies. The latter recognised overlapping epitopes in the 150 loop. These antibodies are currently under characterisation and they have been assessed for purification of ZIKV VLPs and virions as affinity chromatography platforms.

22. Variability in Disinfection Resista ation Pathway Suppre urrently Circulating *Enterovirus B* Serotypes

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The environmental persistence and resistance to disinfection influences the dissemination of human enteric viruses. Susceptibility to disinfection is known to vary between viruses and even between closely related strains, yet the extent of this variation and the underlying reasons remain unknown.
Nine viruses belonging to coxsackievirus B5, B4, and B1 serotypes were isolated from wastewater. Five disinfection treatments were applied to each virus: two inducing genome damage (UV_{254} and sunlight), two targeting both viral proteins and genomes (free chlorine and chlorine dioxide), and one inducing non-oxidative protein denaturation (heat). Then, the disinfection resistance of the different viruses was compared.
The resistance of the tested viruses differed according to the treatment, with genome-damaging disinfectants inducing the least and oxidative disinfectants inducing the greatest variability in resistance. For most treatments, the extent of resistance was roughly associated with serotype. However, even among strains of a single serotype, differences in resistance could be observed, and the response of the lab strain to disinfection frequently did not correspond to that of the environmental isolates. Finally, we partly rationalized the observed patterns by linking resistance to differential viral features, including capsid structure and stability, presence of oxidizable residues in genome and proteins, and replicative fitness.
Overall, our results demonstrate that even small differences in the genome and protein composition of a virus can lead to phenotypic features that affect their response to disinfection. This implies that disinfection requirements for any given virus family need to be evaluated based on multiple and currently circulating strains.

23. Virus Entry Complex Changes during Tissue Culture Adaptation of Human Herpesvirus-6A Identified by Whole-Genome Deep Sequencing and Structural Modelling.

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Tissue-culture adaptation of viruses can modulate infection. Laboratory passage and BACmid cloning of human betaherpesvirus, cytomegalovirus, HCMV have resulted in genomic alterations affecting virus infectious entry and cellular tropism with implications for pathology and vaccines. In this study we have examined tissue-culture adaptation in the related human betaherpesvirus, the Roselovirus reference genome from human herpesvirus 6A, HHV-6A, strain U1102. This virus also has an endogenous human form via "cloning" within the germline subtelomeric chromosomal-integration in approximately 1% of human populations. Therefore, understanding the effects of laboratory passage is essential to understanding relationships between exogenous and endogenous virus during infection and pathology. Using whole-genome next-generation deep-sequencing Illumina-based methods, we compared the original clinical isolates to tissue-culture passaged, BACmid-cloned virus and endogenous "cloned" virus genomes. This re-defined the reference virus genome and showed BACmid virus rearrangement. As shown for HCMV culture effects, SNPs accumulated during tissue culture passage, which affected the virus entry complex, mediating cellular fusion and favouring cell-associated spread. We constructed molecular models based on homologous tertiary structures and investigated effects on the multimeric fusion complex. Results show a mutational effect on the trimeric interfaces, which may influence the post-fusion structure for cell-associated spread, affecting infection intervention and vaccine design.

24. An Influenza Uncoating Epitope Confers Capsid Stability

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Influenza A virus (IAV) entry is a step-wise process that is regulated by a variety of viral and cellular cues. To quantify each step of IAV entry, we employ immunofluorescence-based assays. IAV capsid uncoating begins in the mildly acidic environment of endosomes where the M2 ion channel mediates viral core acidification and M1 conformational change. After viral fusion at late endosomes, the cellular aggresome processing machinery completes the capsid uncoating process. A M1 monoclonal antibody (HB64) allowed the preferential detection of capsid uncoating but the underlying mechanism was not clear.

Here, we mapped the epitope of HB64 to the N-terminus of M1. Based on previous structural studies, this epitope is located at the interphase of the M1 dimer. It is masked at neutral pH (extracellular environment) and exposed at low pH (endosomes), a phenomenon that explains the enhanced HB64 binding to uncoated virus particles. A 1.9 Å resolution crystal structure of M1 with a single point mutation in the epitope led to the loss of a surface cavity and increased flexibility of loop L1. M1 VLP studies showed that capsid assembly and stability were compromised in this point mutant, suggesting that acid-exposed capsid epitopes may help us understand the assembly/disassembly paradox.

Abstracts

Poster Exhibition – Session 2: Structural Virology

25. Structural and Biochemical Characterization of Non-Structural Protein 3 from Flavivirus

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Flaviviruses are positive sense single-strand RNA viruses transmitted by arthropods. Many flaviviruses are significant human pathogens such as Dengue virus (DENV) and emerging zika virus (ZIKV). Currently, there is no specific nor broad-spectrum antiviral available against flavivirus infections. The non-structural protein 3 (NS3) is a mulitfunctional protein necessary in viral replication and polyprotein processing. The N-terminus of NS3 is a serine protease and C-terminus is a NTPase/Helicase. Due to its essential role in the virus life cycle, NS3 is an attractive drug-target. Here, we report the crystal structures of NS3 protease from ZIKV and full length NS3 from DENV along with the biochemical characterization of these proteins. These results advance our current understanding of flavivirus polyprotein processing and accelerate structure-based antiviral drug discovery against ZIKV.

26. Analysis of HIV-1 gp120 *N*-glycosylation in the Context of Structure and Function

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HIV-1 envelope (Env) is a trimeric herterodimer composed of gp41, the transmembrane domain, and gp120, the outer envelope domain. Both gp120 and a portion of gp41 are covered in oligosaccharides which encompass 50% of the mass of gp120. These *N*-glycosylation sites (NGS) play a key role in virus entry into host cells and as a shield against broadly neutralizing antibodies (bnAbs). The "glycan shield" that is formed by the inter-linking network of *N*-glycans serves as the primary interface between the virus and host, yet the glycosylation patterns that form the shield are not well understood. We have previously demonstrated that the expression of Env gp120 in a variety of cell lines results in differential *N*-glycosylation of the many potential sites within the protein and that the differential glycosylation affects Env recognition by antibodies from the sera of HIV-1-infected subjects (*JBC* 285, 20860, 2010). To characterize the extent of glycosylation at each NGS, we applied our high-resolution mass spectrometry workflow to perform a quantitative site-specific analysis for four Env variants. Based on this analysis, we are able to see glycosylation patterns based on structural location and observe changes in the glycosylation pattern when the system is perturbed by differences in local *N*-glycan sequon patterns. By use of this method, we were able to characterize and parameterize combinations of sequons that are interdependent and play a crucial role in the assembly of a functional trimer. These variants were analyzed using infectivity and neutralization assays. Structural modeling was used to determine the glycan interface with the most abundant glycoforms observed by MS at these sites. This work will report on the process of interpreting a dense amount of *N*-glycan heterogeneity data and how the heterogeneity changes under different conditions in the context of the protein's structure and activity.

27. Characterization of Influenza A Polymerase Bound to Positive and Negative Promotors

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The influenza A virus genome consists of eight segments of single-stranded, negative-sense RNA. Each viral RNA (vRNA) segment is associated with the polymerase, and multiples copies of the nucleoprotein to form the ribonucleoprotein (vRNP). These vRNPs are the essential units for transcription and replication.
After cell infection, the vRNPs migrate to the nucleus where transcription and replication take place. Replication occurs through an intermediate complex containing the positive sense, complimentary copy, of the genomic vRNA called cRNP. It is expected that the major difference between v- and cRNP would be the conformation of the polymerase. To isolate the two complexes that contain c and v promoters independently, we use a method based on the high affinity binding of the *Pseudomonas aeruginosa* bacteriophage PP7 coat protein (cpPP7) to the translational operator RNA hairpin of PP7. We have reconstituted in vivo two different recombinant polymerase-RNA complexes by transfection of the polymerase and two different short virus-like RNA fragments containing the sequence of the PP7 translational operator hairpin whether in positive or negative sense.
To purify the different polymerase-RNA complexes, we use a purification step using a STREP-tagged-cpPP7 protein that binds only the right sense of the translational operator RNA hairpin of PP7 in each of the two different constructions. Finally, and after purification, the structure of those complexes will be solved by cryo-electron microscopy.

28. Phage Display Antibodies against Ectromelia Virus That Neutralize Variola Virus: Selection and Implementation for p35 Neutralizing Epitope Mapping

Ivan Ilichev [1,3], Yana Khlusevich [2], Andrey Matveev [1,2,3], Dmitrii Pyshnyi [2], Georgiy Shevelev [2], Ivan Baykov [2], Leonid Bulychev [4], Nikolai Bormotov [4], Vera Morozova [1,2,3], Nina Tikunova [2]

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In this work, five phage display antibodies (pdAbs) against ectromelia virus (ECTV) were selected from the vaccinia virus (VACV)-immune phage-display library of human scFv antibodies. ELISA demonstrated that selected pdAbs couldrecognize ECTV, VACV, and cowpox virus (CPXV). Atomic force microscopy visualized binding of the pdAbs to VACV.Three of the selected pdAbs neutralized variola virus (VARV) in the plaque reduction neutralization test. Western blotanalysis of ECTV, VARV, VACV, and CPXV proteins indicated that neutralizing pdAbs bound orthopoxvirus 35 kDaproteins, which are encoded by the open reading frames orthologous to the ORF H3L in VACV. The fully human antibody fh1A was constructed on the base of the VH and VL domains of pdAb, which demonstrated a dose-dependent inhibition of plaque formation after infection with VARV, VACV, and CPXV. To determine the p35 region responsible for binding to neutralizing pdAbs, a panel of truncated p35 proteins was designed and expressed inEscherichia coli cells, and a minimal p35 fragment recognized by selected neutralizing pdAbs was identified. Inaddition, peptide phage-display combinatorial libraries were applied to localize the epitope. The obtained dataindicated that the epitope responsible for recognition by the neutralizing pdAbs is discontinuous and amino acid residues located within two p35 regions, 15–19 aa and 232–237 aa, are involved in binding with neutralizing anti-p35 antibodies. This study was supported by the Russian Scientific Foundation (project #16-14-00083).

29. Reovirus Non-Lytic Egress Is Mediated by a Complex Structure Formed from Membranes and Actin

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Mnbvc cvMammalian orthoreoviruses (reoviruses) are nonenveloped viruses and a prototype member of the Reoviridae family. Generally, nonenveloped viruses are assembled in the cytoplasm and exit infected cells by lysis. However, recent evidence suggests that some nonenveloped viruses, including reovirus, are released without lysis, indicating that these viruses use alternative routes for egress. To further understand the host factors and cellular pathways involved in reovirus cell exit, we imaged infected cells at late times postinfection using a variety of microscopic techniques. Confocal microscopy showed that reovirus egress areas are located in discrete regions at the basal surface of infected cells. We used high-pressure freezing (HPF) for better ultrastructure preservation and observed that mature viral particles assembled in cytoplasmic factories are recruited to a membranous intermediate not previously described. Transport from these intermediates to the cell surface is mediated by smaller membranous carriers in which mature virions are attached to filaments and membranes. Electron tomography showed that reovirus exits cells when these carriers fuse with the plasma membrane. Super-resolution microscopy using stimulated emission depletion showed that actin filaments surround viral inclusions and membranous carriers. Actin was detected in purified virus preparations by immunoblotting and immunogold labeling. Incubation with the actin-inhibiting drug jasplakinolide altered the morphology of viral inclusions and the integrity of viral particles. Collectively, our results suggest that reovirus uses a previously undescribed membrane-engaged, actin-dependent, non-lytic egress mechanism. We are currently investigating how mature virions are selectively recruited by membranous carriers at the periphery of viral inclusions for transport to egress areas.

30. Sequential Conformational Changes in Influenza Hemagglutinin during Fusion Activation

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Membrane fusion is critical for influenza virus infection and is driven by the surface glycoprotein hemagglutinin (HA). When exposed to acidic pH inside endosomes, HA activates and refolds, bringing the virus and host membranes into apposition and inducing them to merge. Structures of the pre- and post-fusion conformations of HA illustrate the initial and final states of one isolated component of the fusion machinery, however these static structures do not elucidate how the intact machinery in virions functions to drive the fusion process. We employed hydrogen/deuterium-exchange mass spectrometry to monitor HA fusion activation using intact X-31 H3N2 influenza virions. Activation is initiated by fusion peptide release, which leads to the formation of a stable but dynamic intermediate. This precedes dissociation and local unfolding of the globular head, which is the final step that enables fusion subunit refolding to the low energy post-fusion state. By contrast, intermediate states were not observed with the soluble HA ectodomain (BHA) where fusion activation was abrupt and two-state. These results suggest that intact HA in virions is stabilized through its transmembrane domain, enabling formation of the dynamic intermediate. Our new structural dynamic findings highlight the importance of studying membrane fusion using intact virions.

31. Solution Structure of the HIV-1 gp41 Cytoplasmic Tail, and Development of Techniques for the Characterization of Interactions with the Matrix

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The surface envelope glycoprotein (Env) of human immunodeficiency virus type 1 (HIV-1) is composed of a trimer of gp120 glycoproteins non-covalently bound to a trimer of gp41 transmembrane proteins. The C-terminal cytoplasmic tail of gp41 (gp41CT) is involved in multiple facets of Env function including immune evasion, mediation of Env intracellular trafficking, and incorporation into assembling virions, mechanisms of which are poorly understood. An abundance of genetic and biochemical studies suggest that Env incorporation is mediated by interactions between the matrix domain (MA) of Gag and gp41CT. The lack of structural data on gp41CT has been a barrier to investigating the molecular mechanism of Env incorporation at the molecular level. We have recently devised new approaches that led to successful preparation of a recombinant gp41CT protein and allowed for determination of the solution NMR structure in a membrane mimetic (micelles). We found that gp41CT consists of two independent domains, an unstructured 45 amino acid N-terminus that is not associated with the membrane, and a 105-amino acid C-terminus that forms three amphipathic α-helices, which are tightly associated with the membrane. This work fills a major gap by providing the structure of the last unknown segment of HIV-1 Env, which will provide insights into the mechanisms of Gag-mediated Env incorporation. In an attempt to establish a viable system to characterize MA-gp41CT interactions by structural and biophysical tools, we engineered and produced MA-containing constructs and prepared lipid nanodiscs to use as a membrane platform to form a complex. At the meeting, we will present preliminary data on the characterization of the MA/gp41CT/membrane complex by NMR, biochemical and biophysical methods.

32. Structure and Interactions of the Essential 3'X Terminal RNA Domain of Hepatitis C Virus

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The untranslated regions (UTRs) flanking the ORF of the hepatitis C virus (HCV) genomic RNA play essential functions in the viral cycle. The 5'UTR includes an internal ribosome entry site, while the 3'UTR contains a 98-nucleotide terminal domain called 3'X. This latter domain has been suggested to act as a dynamic switch, signaling the transition between replication, translation and possibly packaging processes of the virus.
Two highly conserved sequences of domain 3'X, DLS and *k*, are involved in viral RNA dimerization and in a distal base-pairing contact with an upstream 5BSL3.2 stem-loop, respectively. On the basis of nuclear magnetic resonance spectroscopy and small-angle X-ray scattering results, we show that domain 3'X forms a structure composed of two coaxially-stacked SL1' and SL2' stem-loops [1,2]. This fold facilitates primer-independent viral replication by exposing terminal unpaired nucleotides after the SL1' stem, as well as viral RNA dimerization by exposing a palindromic DLS segment in the apical loop of subdomain SL2'. Indeed, at higher ionic strength, the domain forms symmetric dimers comprising an extended 110-nucleotide SL2' duplex [1,2]. Using this evidence as well as information about the structure of the distal contact formed between 3'X and 5BSL3.2 [3], we suggest how domain 3'X contributes to regulate the life cycle of HCV through its monomer, dimer and 5BSL3.2-bound conformations.

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2. Cantero-Camacho, A.; Fan, L. Wang, Y.-X.; Gallego, J. *RNA* **23**, 1465-1476 (2017).
3. Cantero-Camacho, A.; Gallego, J. *Submitted*.

33. Structure of Elongated Flexuous Viral Nanoparticles Exploited for Nanobiotechnological Functionalization

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A deep knowledge of virion structure is useful not just because of the information itself and its implications in disease development, but also because it enables and facilitates the development of viruses as nanotools in nanobiotechnology. Functionalization of viruses as nanoparticles is rapidly and strongly emerging as a powerful technology with multiple applications (Yury Khudyakov, Paul Pumpens (2015) *Viral nanotechnology*, CRC Press). In this context, plant viruses show clear advantages such as biosafety, fast and massive production using plants as bioreactors, and low cost. Many applications have been developed using icosahedral viruses, but rod-shaped viruses have also attracted attention as viral nanoparticles (VNPs). In our work, we are developing the functionalization of *Turnip mosaic virus* (TuMV) nanoparticles, not a rigid rod-shaped plant virus, but a flexuous one. TuMV allows the multiple-display of different molecules for several applications related with biomedicine and crop-related disease control, among others. Knowledge of the detailed structure of the TuMV coat protein (CP), has allowed different approaches to be developed for chemical conjugation of compounds different in size and chemical nature. Progress towards this goal and current achievements for different applications will be described and discussed.

Poster Exhibition – Session 3: Virus Replication Compartments

34. Chikungunya Virus nsP3 Hypervariable Domain Recruits the Cellular DHX9 DExD/H Box Helicase to Replication Complexes to Promote Viral Translation

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RNA helicases—particularly DExH-box helicase 9 (DHX9)—are enzymes with DNA and RNA duplexes unwinding activities which are essential for several cellular processes such as replication, translation, RNA processing, and transport. Because of its central role in RNA metabolism, DHX9 is hijacked by RNA viruses in order to assist transcription, trafficking, and translation of viral RNAs. Based on yeast-two hybrid studies suggesting that DHX9 could be a potential binding partner of chikungunya virus (CHIKV) nsP3 protein, the objective of the present study was to determine the outcome of DHX9 in CHIKV-infected cells and to question the functional role played by this helicase during CHIKV replication. Here, we show that CHIKV nsP2 and the hypervariable C-terminal domain of nsP3 indeed interacted with DHX9. While expression of nsP2 decreased DHX9 detection in the nucleus, nsP3 relocalized DHX9 into cytoplasmic foci. In CHIKV-infected cells, DHX9 co-localized and interacted with viral complexes formed by viral RNA and nsPs. These complexes also contained the Ras-GAP SH3 domain binding protein (G3BP), which was previously identified as a CHIKV replication cofactor. Silencing experiments showed that while DHX9 negatively controls viral RNA synthesis, it is also required for optimal nsPs translation. Altogether, the current study identifies DHX9 as a novel cofactor for CHIKV replication in human cells that differentially regulates the various steps of the CHIKV life cycle.

35. Coarse-Grained Molecular Models of Positive Strand Viral RNA Replication Compartments

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Positive strand virus genome replication occurs within membranous replication compartments derived from cellular membranes. Striking 3D electron microscopy tomography data from flavivirus- and nidovirus-infected cells has provided valuable information about the gross architecture of these viral RNA replication compartments, but how these replication compartments are organized and how RNA replication may occur within the compartment is unknown due to the resolution gap between electron microscopy used to visualize the replication compartment ultrastructure and X-ray crystallography approaches used to solve molecular structure. Using molecular modeling based on existing 3D EM tomography data, we developed atomic-scale three-dimensional models of viral replication compartments and viral dsRNA intermediates that will allow us to begin exploring the constraints inherent to genomic RNA replication within a small membranous compartment. The new coarse-grained dsRNA model we developed allows for microsecond analysis of dsRNA compaction within vesicles, assessment of electrostatic potentials within vesicles, and determination of the amount of outward pressure exerted on the vesicle by the dsRNA. This model provided critical new insight into the forces at play within the replication compartment that cannot be determined using current experimental approaches. The results of this study provide new tools for understanding how positive strand RNA viruses may replicate their genomes within the confines of small membranous replication compartments.

36. Elucidating the Function of Host Trafficking Protein GBF1 in Chikungunya Virus Replication

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Chikungunya virus (CHIKV) is a reemerging arbovirus responsible for massive epidemics of arthritic disease. In an siRNA screen, we identified components of COPI vesicular transport as host factors that promote CHIKV replication. siRNAs targeting a subunit of the COPI coatomer as well as GBF1—a guanine nucleotide exchange factor required for COPI trafficking—diminished CHIKV infectivity and replication. Treatment of infected cells after viral adsorption with a specific inhibitor of GBF1 (golgicide A, GCA) diminished viral replication in multiple cell types, indicating that GBF1 functions at a post-entry replication step. GCA also inhibits the replication of several other alphaviruses, suggesting a common mechanism of action for GBF1. RNA viruses remodel intracellular host membranes to form distinct viral replication compartments that serve as sites of viral RNA synthesis. During CHIKV infection, GBF1 partially co-localizes with dsRNA, a marker of replication compartments, both at the plasma membrane and within intracellular structures. Additionally, treatment of infected cells with GCA resulted in delayed formation of CHIKV replication compartments as well as a substantial decrease in viral yield. These findings point to a requirement for GBF1 in CHIKV replication compartment formation and subsequent RNA synthesis, and highlight a potential new target for therapeutic intervention. the Role of GBF1 in the Replicat

37. Encephalomyocarditis Virus Replication Organelles: Variations on a Picornavirus Theme

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Like other positive-stranded RNA viruses, picornaviruses generate distinct cytosolic membrane structures that are dedicated to viral RNA synthesis. While these replication organelles (ROs) have been extensively studied for enteroviruses, the ROs induced by other picornavirus genera remain largely uncharacterized. Here we assessed the three-dimensional architecture of the ROs induced by encephalomyocarditis virus (EMCV)—a member of the *Cardiovirus* genus that is distantly related to enteroviruses. Connections between early single-membrane EMCV ROs and the endoplasmic reticulum (ER) were revealed, establishing the ER as a likely membrane donor organelle. These early single-membrane ROs seem to transform into double-membrane vesicles (DMVs) along infection. Both single- and double-membrane structures were found to support viral RNA synthesis, and progeny viruses accumulated in close proximity, suggesting a spatial association between RNA synthesis and virus assembly. Further, we explored the role of phosphatidylinositol-4-phosphate (PI4P, a critical host factor for both enterovirus and cardiovirus replication) in RO biogenesis. In contrast with recent findings in enteroviruses, low PI4P conditions did not delay the emergence of cardiovirus ROs in a resistant EMCV mutant. Our results show that despite striking similarities in the ultrastructure and transformation of their ROs, cardioviruses and enteroviruses diverge in their requirements for RO biogenesis.

38. Expression and Cleavage of MERS-Coronavirus nsp3-4 Polyprotein Induces the Formation of Double-Membrane Vesicles That Mimic Those Associated with Coronaviral RNA Replication

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Coronavirus RNA synthesis is associated with membranes derived from the endoplasmic reticulum which are predominantly transformed into double-membrane vesicles (DMVs). The mechanism underlying the biogenesis of these remarkable structures remains largely unknown. Previous work on Severe Acute Respiratory Syndrome-coronavirus (SARS-CoV) suggested that the viral nonstructural proteins nsp3, nsp4, and nsp6 would all be required for DMV formation. Here we investigated the minimum viral protein requirements for the formation of Middle East Respiratory Syndrome-coronavirus (MERS-CoV) DMVs. Using electron tomography, we demonstrate that co-expression of nsp3 and nsp4 is required and sufficient for MERS-CoV DMV formation, and that this conclusion can in fact be extended to SARS-CoV. Co-expressing MERS-CoV nsp3 and nsp4 either as individual subunits or as a self-cleaving nsp3-4 precursor resulted in comparable DMVs, and also induced zippered ER that appeared to wrap into nascent DMVs. While the addition of the third transmembrane protein (nsp6) did not noticeably change the membrane structures observed, inactivation of the nsp3-4 polyprotein cleavage precluded DMV formation, thereby establishing a suggestive link between polyprotein processing and membrane remodeling. These findings provide important insight into the biogenesis of coronavirus DMVs, evidence strong similarities with another nidovirus group (arteriviruses), and highlight possible general principles in DMV formation.

39. Immunohistochemical Evidence of Human Parvovirus B19 Infection in the Post-Mortem Brain Tissue of the Elderly

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Neurologic manifestations of human parvovirus B19 (B19) infection have been reported. The parvoviruses are dependent on help from host cells to replicate. The presence of B19V DNA in human brain tissue has been determined in recent studies, and therefore the cellular source of parvovirus replication in the human brain should be clarified.

Brain tissue autopsy samples were selected from 24 elderly individuals with morphological signs of encephalopathy. The grey and white matter obtained from temporal and frontal lobes were sectioned and immunohistochemical reaction using anti-B19 monoclonal antibody, and quantitative estimation of immunopositive cells was performed. Calculations were performed using SPSS 23.0.

There were significantly ($p = 0.009$) more B19-positive astrocytes in the white matter of the frontal lobe compared to the temporal lobe. In the frontal lobe white matter, a statistically greater number ($p < 0.001$) of B19-positive astrocytes compared to oligodendrocytes was found: 3.00 (1.00; 5.00) vs. 2.00 (1.00; 3.00), respectively.

We found an increased number of B19-positive astrocytes in the white matter as compared to gray matter of the frontal lobe as well as compared to selected regions of the temporal lobe. Our data demonstrate that the B19 invade the central nervous system with astrocytes being the target cell, and this occurs with advanced age.

40. Infectious Pancreatic Necrosis Virus Entry into CHSE-214 and SHK-1 Cells

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Infectious pancreatic necrosis virus (IPNV) is the etiologic agent of infectious pancreatic necrosis, which affects several salmonid species.This disease has great economic impact in salmon-farming countries. IPNV is a small icosahedral non-enveloped particle that contains a bipartite double-stranded RNA genome and belongs to the *Birnaviridae* family.

To determine the internalization mechanism used by IPNV in CHSE-214 and SHK-1 cells, we blocked the molecular components associated to different endocytic pathways using specific inhibitors and determined their effect on virus infection. IPNV infection was measured by immunofluorescence using an anti-VP2/VP3 antibody and epifluorescence microscopy. The functionality of each endocytic pathway was checked using specific fluorescent tracers and visualized by laser scanning confocal microscopy.

We found that IPNV stimulated the fluid phase uptake and virus particles co-localized with the soluble dextran-Texas red tracer. In addition, changes in the distribution of actin F were observed after inoculation of the virus and the infection was blocked by the disruption of actin dynamics with cytochalasin D. In addition, viral internalization was significantly reduced when the Na+/H+ anti-porter bomb NHE-1 was inhibited with 5-(*N*-ethyl-*N*-isopropyl) amyloride. Taken together, these data indicate that IPNV enters the cells by macropinocytosis. To examine the role of macropinocytosis regulators, additional inhibitors were tested. The results suggest that IPNV-induced macropinocytosis is regulated differently in CHSE-214 and SHK-1 cells. Thus, in CHSE-214 cells, Rho Rac1 GTPase would be involved, while in SHK-1 cells the GTPAse Rho Cdc42 would have a regulatory role.

41. Phosphatidate Phosphatase LPIN1 Is Rate Limiting for Functional Hepatitis C Virus Replicase Complex Formation

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The hepatitis C virus (HCV) replication cycle is closely tied to lipid metabolism, and its infection causes profound changes in lipid homeostasis. LPIN1 and LPIN2 cooperate to maintain hepatic lipid homeostasis. They are members of a phosphatidate phosphatase enzyme family (lipins), which play a pivotal role in glycerophospholipid biosynthesis and may also act as transcriptional regulators of lipogenic genes.

We studied the role of these proteins in the HCV replication cycle by RNA interference studies. Using surrogate models for HCV infection as well as confocal and electron microscopy, we concluded that lipin1 is rate-limiting for the generation of the membranous compartment that hosts the viral replicase in a step downstream of primary translation that leads to early RNA replication. cDNA overexpression experiments indicated that lipin1β cDNA is sufficient to partially rescue HCV infection in lipin1-deficient cells and that mutant cDNA expressing a catalytically inactive lipin1 could not restore HCV infection susceptibility, indicating that lipin1 phosphatase activity is required for efficient HCV infection. In contrast to lipin1, lipin2 silencing caused non-specific inhibition of virus entry and virus propagation in different infection models, including HCV. Our results indicate that HCV subverts lipin1 to promote membrane biosynthesis in order to to favor HCV RNA replicase membrane association.

42. Spatial Translation Control of Cellular and Viral RNA Translation during Chikungunya Virus Infection

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The life cycle of positive strand RNA viruses is tightly connected to the endoplasmic reticulum (ER). The ER and the cytosol represent distinct biological environments for translation, with different regulatory factors affecting protein expression. How and whether (+)RNA viral infection affects this tightly regulated spatial control of translation is mostly unknown. The emerging Chikungunya virus (CHIKV) replicates in spherules derived from the cytosolic membrane and expresses the non-structural and structural proteins from the genomic and the subgenomic RNA, respectively. By combining compartment fractionation with two genome-wide techniques (RNA-Seq and ribosome profiling analyses), we show that CHIKV infection differentially affects the two translation compartments on several layers, including mRNA steady-state, location, and translation. The consequences of this differential spatial regulation on CHIKV infection will be discussed.

43. The Dynamics of both Filamentous and Globular Mammalian Reovirus Viral Factories Rely on the Microtubule Network

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Mammalian reovirus viral factories (VFs) form filamentous or globular structures, depending on the viral strains. Here, we demonstrate that both kinds of VFs condense into a perinuclear position. However, filamentous VFs reach this position faster than globular ones. These dynamic differences correlated with the capacity of a filamentous virus strain to transit its VFs from globular to filamentous morphologies. Interestingly, we show that globular VFs coalesce with each other, thereby gaining in size and decreasing in number. Moreover, globular VFs rely on an intact microtubule network and dynein for their structural assembly, maintenance, and perinuclear condensation. By using different transfection ratios of μNS and a filamentous version of μ2 to mimic filamentous VFs at various infection stages, we determined the dependency on MT network for the perinuclear condensation of VF-like structures (VFLSs). Additionally, increased amounts of μNS promote the perinuclear positioning of VFLSs, which directly correlates with an increase in acetylated tubulin levels.

44. The Interplay between Zika Virus and Host Valosin-Containing Protein (VCP/p97)

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Infections with flaviviruses constitute a major public health concern worldwide. Notably, infection with Zika virus (ZIKV)—closely related to dengue virus (DENV)—can cause congenital microcephaly in addition to other neurological complications. Most importantly, no antivirals or vaccine are available.

In order to generate an intracellular environment favorable to viral replication, flaviviruses induce replication factories (RFs) through the remodeling of endoplasmic reticulum (ER) membranes driven by enigmatic mechanisms. The non-enzymatic NS4B flaviviral protein which is absolutely required for RNA replication and is localized in DENV and ZIKV RFs might be involved in such morphogenesis, possibly through interactions with host factors.

The recent mass spectrometry-based elucidation of DENV NS4B interactome in infectious conditions (Chatel-Chaix et al., Cell Host & Microbe, 2016) identified valosin-containing protein (VCP/p97) as a cellular partner of NS4B. VCP/p97 is an AAA+-type ATPase which is notably involved in protein homeostasis by retro-translocating ER proteins to the cytosol, unfolding proteins for degradation, or disassembling protein aggregates. Since DENV and ZIKV are genetically close, we hypothesized that VCP associates with ZIKV NS4B and that it regulates RF biogenesis and more generally viral replication. Confocal microscopy-based analysis revealed that VCP strikingly redistributes to NS4B-containing large structures (presumably RFs) in Huh7.5 cells infected with Asian or African ZIKV strains. Interestingly, pharmacological inhibition of VCP with NMS-873 drastically impaired ZIKV replication strongly supporting that VCP is required for ZIKV life cycle. Ongoing experiments aiming at characterizing the role of VCP in ZIKV replication will be presented.

Poster Exhibition – Session 4: Replication and Pathogenesis of RNA Viruses

45. Human Placenta Susceptibility to Respiratory Syncytial Virus Infection

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The human respiratory syncytial virus (RSV) is a common respiratory virus that usually causes mild cold-like symptoms, but can be especially threatening for infants and older adults. Strong epidemiologic evidence suggests that early-life infections with this virus predispose individuals to chronic respiratory dysfunction and asthma, possibly related to the persistence of the virus itself or to its effects on lung development. Recent studies have shown vertical transmission of RSV in rats from mother to offspring and persistence of virus in lungs after birth, which indicates a possible transplacental transmission throughout the main elements of this tissue: trophoblast cells and villous core stroma cells. In the present study, we demonstrate that human placental explants are susceptible to RSV infection by the immunodetection of viral F protein in the villous core stroma cells, although there was no positive sign in trophoblast cells. Because last trimester placental trophoblast cells are not permissive to RSV infection, we used two cellular models of the trophoblast of human first-trimester placenta (HTR8/SVneo and BeWo cells) to assess its susceptibility to infection, and we determined that the viral replication cycle is taking place in these cell lines. These results indicate that trophoblast cells of terminal placenta are not infected with RSV, but other cellular types such as villous core stroma cells may be; and finally, results indicate that the trophoblasts of early pregnancy are more permissive to RSV infection.

46. Molecular Determinants for the Pathogenicity of H7N9 Influenza Viruses in Mammals

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Interspecies transmission of avian influenza viruses to mammals poses a continuous threat to agriculture and public health. The emergence of H7N9 in China has resulted in human cases with various clinical outcomes, ranging from asymptomatic to fatal infections. Mechanisms that led to the increased mammalian infectivity and pathogenicity remain to be explored. Here we generated a series of reassortants from two closely related viruses: the virulent A/Shanghai/01/2013 (SH1) and the avirulent A/Shanghai/5/2013 (SH5). Infection in the C57BL/6 mouse model indicated that PB2, PB1, NA, and M genes were determinants for the viral virulence. SH1-derived PB2 and PB1 genes could lead to increased polymerase activities, while PB1-L598M, PB2-E191K, and PB2-E627K mutations could independently enhance the polymerase activity and the pathogenicity of SH5 in mice, causing more severe pneumonia and higher virus replication. Notably, PB2-191K was highly conserved in early H7N9 influenza virus isolates but not in the other subtypes, suggesting a possible role in the genesis and emergence of this novel H7N9 virus. The virulence markers reported here may serve as indicators for virus surveillance and the potential targets for treatment and prevention of the H7N9 virus.

47. Rift Valley Fever Phlebovirus Nucleoprotein Proteomic Studies Identify Important WNT Pathway Interactions

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Rift Valley fever phlebovirus (RVFV, *Phenuviridae, Bunyavirales*) is an important pathogen of both humans and livestock. The transmission of RVFV by mosquitoes across sub-Saharan Africa and the Arabian Peninsula has a significant impact on the socio-economics of these areas. Research interests have primarily focused on identifying interacting partners of the non-structural protein (NSs), encoded within the viral S RNA segment. However, the interaction partners of the nucleocapsid protein (N) remain largely unknown. Using a proteomics-based approach, we identified 24 potential mammalian host-derived N protein interaction partners. Following an siRNA screen utilising a viral minigenome system, the cellular proteins β-Catenin, Polyadenylate binding protein 4, Scaffold attachment factor B, and Annexin A2 appeared to be important for the formation of functional ribonucleoprotein (RNP) complexes. As β-Catenin is a known effector molecule of the WNT pathway, analysis following RVFV infection and minigenome transfection on the WNT pathway indicated a cell-specific inhibition of the pathway. This was evidenced by a direct interaction of β-Catenin with RVFV N protein and evidence of relocalisation of β-Catenin from the plasma membrane. Understanding the fundamental biology, followed by further characterisation of these interactions, will aid the future development of new intervention strategies.

48. Uneven 5′- and 3′-End Stoichiometry in Potyviral Protein Production

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Potato virus A (PVA) has a positive sense genome linked to a viral protein called VPg at its 5′ end. Most of the multifunctional PVA proteins are produced from an open reading frame encoding a large polyprotein. Ectopic expression of VPg with PVA RNA enhance viral translation. The translational boost gets even stronger when VPg and PVA RNA are co-expressed either with translational initiation factor eIF(iso)4E or a ribosomal stalk protein P0. A long-standing paradigm is that potyviral proteins are produced in equimolar quantities as part of the polyprotein, yet the turnover of individual proteins can be regulated separately. Here, we show that VPg-mediated translational enhancement is not equimolar in all parts of the PVA genome. It is specifically involved in enhancement of 3′-end expression. Using reporter gene constructs, we show that *Renilla* luciferase (Rluc) expression gets significantly enhanced when located immediately upstream of coat protein (CP) gene, but not when placed upstream of the HCPro gene. VPg-mediated enhancement of PVA RNA accumulation occurs similarly regardless of the Rluc gene position in PVA icDNA, suggesting that the location of the reporter gene is not responsible for the phenomenon. On the same line we can observe increased CP production from the 3′-end of PVA RNA when VPg is co-expressed while simultaneously CI production from the middle of PVA RNA stays steady. Based on our observations, we cautiously propose that a translational mechanism may exist to produce CP with an uneven stoichiometry for particle assembly during potyvirus infection.

49. Zika Virus Replicates in Proliferating Cells in Anchoring Villus Explants from First-Trimester Human Placentas, Potential Sites for Dissemination of Infection at the Uterine–Placental Interface

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Maternal Zika virus (ZIKV) infection with prolonged viremia leads to fetal infection and congenital Zika syndrome. We reported that ZIKV infects primary cells from human placentas and fetal membranes. Here, we describe studies of viral replication in numerous explants of anchoring villi and basal decidua from first-trimester human placentas and mid-gestation amniotic epithelial cells (AmEpCs) (Tabata et al., J Infect Dis, 2017). Explants and AmEpCs were infected with American and African ZIKV strains at low multiplicities, and ZIKV proteins were visualized by immunofluorescence. Titers of infectious progeny, cell proliferation, and invasiveness were quantified. In anchoring villi, ZIKV replicated in proliferating cytotrophoblasts in proximal cell columns, dividing Hofbauer cells in villus cores and invasive cytotrophoblasts, but frequencies differed. Cytotrophoblasts in explants infected by Nicaraguan strains were invasive, whereas those infected by the African prototype MR766 largely remained in cell columns, and titers varied by donor and strain. In basal decidua, ZIKV replicated in glandular epithelium, decidual cells, and immune cells in lymphatic vessels, suggesting a route for trafficking infected cells during early pregnancy. ZIKV-infected Hofbauer cells frequently occurred in pairs and expressed Ki67, indicating replication in dividing cells, and Ro3306, an inhibitor of G2/M reduced virus output. Recent studies revealed that NS1 from American strains permeabilizes villus cores and could enable virion infiltration. We conclude that ZIKV infection in early pregnancy targets the proliferating cell column and invasive cytotrophoblasts and Hofbauer cells, amplifying infection and enabling transplacental transmission.

50. 18-(Phthalimide-2-yl) Ferruginol Affects Dengue Virus Infection Acting as a Potential Host-Targeted Antiviral

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Introduction: In search of new antiviral alternatives to control dengue virus infection, so-called host-targeted antivirals (HTAs) have become highly relevant, and the research that includes them is flourishing. In the same line of study, we have previously reported that the semi-synthetic compound 18-(phthalimide-2-yl) ferruginol has relevant anti-dengue activity in post-infective stages, showing a dramatic reduction in viral plaque size. The aim of this study is to determine the potential mechanism of 18-(phthalimide-2-yl) ferruginol during dengue virus infection and their relationship with HTAs.
Methodology: The antiviral effect of this molecule during cell pre-treatment and post-infection steps was evaluated by PFU assay. Alterations in actin filaments and microtubules were predicted by molecular docking and confirmed using advanced fluorescence microscopy and image analysis. Changes in the amount of viral envelope protein were also determined.
Results: After cell pre-treatment for 24 h, the compound showed antiviral activity. Under these treatment conditions, the molecule induced dramatic changes in the reorganization of actin filaments and slightly altered the microtubules pattern, without nuclear fragmentation. Additionally, this compound significantly reduced the amount of viral envelope protein after 12 h.p.i. Molecular docking analysis showed that the molecule binds with moderate affinity (kcal/mol) to the β-tubulin subunit (−9.7) and actin monomers (−9.3), as well as proteins upstream in the actin polymerization pathway, PI3K (−8.8) and Rho A (−9.6).
Conclusion: Molecular docking and biological assays indicate that 18-(phthalimide-2-yl) ferruginol has a potential mechanism of action related to HTAs affecting the actin polymerization.

51. A Comparison of Early Entry Requirements in Calicivirus Isolates of Differing Pathogenicities

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Feline calicicvirus (FCV) is a member of the Caliviridae family, and occurs in cats worldwide. Virulent systemic (VS) FCV strains have been associated with severe disease, with mortality rates up to 79%, but the differences in the pathogenesis of VS and non-VS strains is unknown. As recent work has suggested that differences in viral attachment and entry could be involved, we investigated FCV genomic release via the endosomal pathway. This was achieved via the use of a novel RNA release assay. Whereas the F9 vaccine strain required a low pH for genomic release, a representative VS-FCV strain was more unstable, releasing its genome at neutral pH. Virus growth in the presence or absence of chloroquine was examined to investigate the role of endosomal acidification in infection; while F9 failed to replicate in the presence of chloroquine, growth of the VS-FCV strain was not inhibited. Similarly, no inhibition was observed when further field isolates of VS-FCV were tested. Preliminary results suggest that the VS-FCV capsid might be more unstable following receptor binding, and this capsid instability may play a role in pathogenesis.

52. Activation of Adenosine Monophosphate-Activated Protein Kinase (AMPK) Inhibits West Nile Virus and Zika Virus Infection

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Mosquito-borne flaviviruses are a group of RNA viruses that constitute global threats to human and animal health. Replication of these pathogens is intimately associated to intracellular membrane rearrangements, and hence with lipid synthesis. Adenosine monophosphate-activated protein kinase (AMPK) is a master regulator of energy homeostasis and lipid metabolism, and thus constitutes a potential therapeutic target for multiple human diseases. The activation of this protein kinase positively regulates fatty acid oxidation and autophagy, whereas it negatively regulates lipid synthesis. Considering the strict dependence on cellular lipid metabolism for flavivirus replication, we have evaluated the effect of pharmacological activation of AMPK on the infection of two medically relevant flaviviruses: West Nile virus (WNV) and Zika virus (ZIKV). Our results showed that activation of AMPK resulted in the reduction of both WNV and ZIKV infection. These results point to drugs promoting AMPK activation as novel antiviral candidates that merit further evaluation.

53. Cell Type-Dependent Utilization of FIP1C for HIV-1 Envelope Glycoprotein Incorporation into Virions

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The lentiviral envelope glycoprotein complex (Env) is a critical determinant of viral infectivity. In most physiologically relevant cell types, the Env cytoplasmic tail (CT) is required for Env incorporation into virions, but in others the CT is not required. The role of the CT was recently linked to the endosomal trafficking factor, Rab11 family interacting protein 1C (FIP1C). We found that FIP1C is expressed in the Jurkat T-cell line but not in MT-4 cells. Furthermore, Env incorporation in progeny virus produced by Jurkat is higher than in virus produced by MT-4. In MT-4, the requirement for the Env-CT and FIP1C-dependent Env incorporation is overcome by inherently higher HIV protein expression. In Jurkat, knockdown of FIP1C resulted in a reduction in virion infectivity. These data are consistent with a role of FIP1C in promoting Env incorporation in non-permissive cell lines and hPBMCs. These findings suggest a role of Env recycling in Env incorporation into virions in physiologically relevant cell types. The data obtained from this study shed light on the mechanism of HIV-1 Env incorporation, thereby providing key insights into the trafficking pathways utilized by Env during particle assembly.

54. Cloning and Formation of Tools for Studying Zika Virus

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Zika virus is an emerging mosquito-borne arbovirus with recent devastating epidemics. Therefore, the current study was designed to address the issues concerning ZIKV replication, interaction with host, and spread in infected organism. First, we expanded a recently published set of reverse genetics tools for this virus (ref1) by constructing additional recombinant genomes of ZIKV (Asian clade, isolate BeH819015 from Brazil) stably expressing different marker proteins including ZsGreen, ox-GFP, and ox-BFP. Using these tools, a set of ZIKV recombinants harboring mutations that may be associated with neurotoxicity and/or rapid spread of infection, was developed. The construction of a novel set of ZIKV clones harboring marker-protein tagged NS4A is in progress. New ZIKV replicon vectors harboring mCherry, Nanoluc, or ZsGreen markers were also constructed and tested. Fragments of ZIKV genome corresponding to individual proteins were cloned into a eukaryotic expression vector for the expression of tagged versions of these proteins. The expression clones for the production of the full ns-region of ZIKV in mammalian cells were constructed with the aim of using them as a source of active replicase in ZIKV trans-complementation system; the latter is based on the use of ZIKV replicons harboring deletions similar to those previously used for Kunjin replicon (ref2); in addition, shorter template RNAs have been designed and tested. Using the shortest template, consisting of ZIKV UTR sequences and Nanoluc reporter, it was observed that ZIKV is virtually unable to use such a template. This contrasts with findings made using replicases of alphaviruses assayed in a similar setup, indicating a cis-preference of ZIKV replicase and/or a need for the expression of at least of some proteins, such as NS5, from the template RNA itself. These studies have provided us with important tools for studying ZIKV virus infection as well as a first insight into the template RNA requirements of ZIKV replicase.

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55. Evaluation of the Role of AMPk during Respiratory Syncytial Virus (RSV) Infection in the BeWo Cell Line

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AMP-activated kinase (AMPk) is a key factor in lipid metabolism, and several studies indicate that some viruses can modify their activity to promote lipogenesis. However, it has not yet been established that respiratory syncytial virus (RSV) can induce metabolic reprogramming in cells of the placenta. The aim of this study was to demonstrate the effect that RSV infection has on the lipid metabolism in placental cell culture. We evaluated the active form of AMPk and some metabolic targets in BeWo cells during infection. We confirm that the phosphorylation of AMPk is drastically reduced in infected cells, and therefore the inactive form p-ACCA1 was decreased. In addition, the targets of AMPk such as FAS and HMGCR were overexpressed on infected cells. On the other hand, the lipid droplets co-localize with VSR nucleoprotein in a perinuclear pattern. Finally, using an AMP analog (AICAR) significantly reduced the viral expression of the M2-1 protein in a dose-dependent manner. Suggesting that RSV can induce AMPk-dependent lipid metabolic changes during infection and pharmacological targets of AMPk could be used as a possible antiviral strategy to study in the future.

56. Fibroblast Growth Factor Receptor Signaling Modulates Dengue Virus Replication and Virion Production

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Dengue virus (DENV) is the etiological agent of dengue fever, dengue hemorrhagic fever, and dengue shock syndrome, and is currently the most important arboviral pathogen affecting humans. Estimates from the world health organization (WHO) indicate that DENV causes more than twenty thousand deaths annually around the globe. In spite of its medical relevance, no vaccine or antiviral drugs against DENV are available. Since DENV extensively exploits host cell pathways in all steps of its replication cycle, in the present study we analyzed the human kinome by an RNAi-based screen to identify host kinases that are either promoting or suppressing DENV replication in human cells. Among the identified kinases, silencing of fibroblast growth factor receptor 4 (FGFR4) was found to be detrimental to viral replication. Interestingly, pharmacological inhibition of FGFR4 enhanced the production of infectious DENV particles, indicating a role in suppressing virion assembly and release. Further analysis showed that blocking FGFR4 signaling enhanced both intra- and extra-cellular infectivity, arguing for a role of this pathway in viral assembly and/or maturation.

57. Fidelity of RNA-Dependent DNA Synthesis of Retroviral Reverse Transcriptases: Assessment and Limitations

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In M13mp2 *lacZ*α forward mutation assays measuring intrinsic fidelity of DNA-dependent DNA synthesis, wild-type human immunodeficiency virus type 1 (HIV-1) reverse transcriptases (RTs) of group M/subtype B showed >10-fold higher error rates than murine leukaemia virus (MLV) and avian myeloblastosis virus (AMV) RTs. An adapted version of the assay was used to obtain error rates of RNA-dependent DNA synthesis for several RTs, including wild-type HIV-1_{BH10}, HIV-1_{ESP49}, AMV, and MLV RTs, and the high-fidelity mutants of HIV-1_{ESP49} RT K65R and K65R/V75I. Our results showed that there were less than two-fold differences in fidelity between the studied RTs, with error rates ranging from 2.5 x 10^{-5} to 3.5 x 10^{-5}. These results were consistent with the existence of a transcriptional inaccuracy threshold, generated by the RNA polymerase while synthesizing the RNA template used in the assay. A modest but consistent reduction of the inaccuracy threshold was achieved by lowering the pH and Mg^{2+} concentration of the transcription reaction. Despite assay limitations, we conclude that HIV-1_{BH10} and HIV-1_{ESP49} RTs are less accurate when copying DNA templates than RNA templates. Analysis of the RNA-dependent mutational spectra revealed a higher tendency to introduce large deletions at the initiation of reverse transcription by all HIV-1 RTs except the double-mutant K65R/V75I.

58. Functional Mapping of the Replicase of Providence Virus, an Insect RNA Virus That Also Replicates in Plants and Animals

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Providence virus (PrV) is a non-enveloped (+ve) ssRNA virus (Family: *Carmotetraviridae*) originally discovered as a persistent infection in a lepidopteran midgut cell line. PrV infects human tissue culture cells, and can also establish a productive infection in plants. PrV has a monopartite genome, encoding three ORFs: at the 5′ end is p130 (unknown function), which overlaps with p104 (the viral replicase), followed by p81, encoding the capsid protein precursor. PrV capsids are typical of an animal (tetra)virus, but the replicase—with a carmo-like RNA-dependent RNA polymerase (RdRp) domain—is most related to those of plant viruses belonging to the *Tombusviridae* family. The PrV replicase is expressed via a readthrough stop in the p104 ORF, resulting in the translation of the N-terminal p40 and the full-length p104, with the RdRp domain at its C terminus. In this study, we mapped the functional domains of the PrV replicase using wild-type and deletion derivatives of p104 and p40 fused to EGFP in *Spodoptera frugiperda* (Sf9) cells. We used co-immunoprecipitation assays and immunofluorescence confocal microscopy to demonstrate that p40 self-interacts and interacts with p104 via an N-terminal protein interaction domain, anchoring in target membranes via two membrane spanning domains. These domains are required for the recruitment of p40 and p104 to the site of replication on membranes of the Golgi apparatus and secretory vesicles. Our findings highlight similarities between the replicases of PrV and tombusviruses, supporting the hypothesis that the PrV non-structural proteins originate from a tombus-like plant virus.

59. Glucose Regulated Protein 78 (GRP78) Interacts with Zika Virus Envelope and Is Required for a Productive Infection

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Zika virus (ZIKV) is a member of the Flaviviridae family which until recently was a relatively obscure tropical disease. However, as ZIKV has been shown to be the causative agent of fetal abnormalities and Guillain-Barré syndrome in outbreaks across the Americas, efforts towards delineating important factors in the viral lifecycle have increased. Combining protein pull-down from human A549 cells with mass spectrometry, it was found that ZIKV envelope (Env) interacts with glucose-regulated protein 78 (GRP78). This interaction was confirmed via Western blot. Additionally, immunofluorescence of ZIKV-infected cells revealed that GRP78 re-localises following infection, and co-localises with Env.
In general, flaviviruses such as Japanese encephalitis virus (JEV) and dengue virus (DENV) are known to co-opt members of the unfolded protein response—including GRP78—to enhance viral infectivity and propagation.
To determine the importance of this interaction during ZIKV infection, A549 or Huh7 cells were treated with epigallocatechin gallate (EGCG, a small-molecule inhibitor of GRP78), or specific siRNA targeted against GRP78 prior to infection with a NanoLuc-expressing reporter or wild-type virus. Inhibition or depletion of GRP78 significantly reduced both virus luciferase readings and viral titres, indicating that functional GRP78 is required for efficient ZIKV infection of mammalian cell culture.

60. Human Hepatic Stellate Cells Are Permissive for Hepatitis C Virus Infection/Replication and Play Important Roles in Fibrosis

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Hepatitis C virus infection is a major cause of chronic liver disease, including hepatitis, fibrosis, and cirrhosis. During fibrosis, hepatic stellate cells (HSCs) play important roles in the control of extracellular matrix synthesis and degradation in fibrotic livers. However, whether HCV directly influences the fibrogenic process of HSCs is unknown. The goal of this study was to clarify whether human HSCs are susceptible to HCV infection.

To assess whether HSCs can be infected by HCV directly, Twnt4 cells were incubated with cell-culture-derived infectious HCV (HCVcc). We could not detect HCV-positive signals in HSCs by either the mCherry fluorescent system or RT-qPCR after infection with HCVcc. To investigate whether HCV is capable of cell-to-cell transmission, HCV-infected Huh7 cells were co-cultured with HSCs, which resulted in several HSCs showing HCV-positive signals. Furthermore, after co-culture of SGR cells with HSCs for 4 days, we detected HCV-positive signals in HSCs cells. We also detected HCV proteins and RNA in purified exosomes from the supernatant of SGR cells. Then, exosomes were purified from SGR cells' supernatant, and incubated with HSCs and Huh7 cells. Nuclear translocation of mCherry signal was observed in HSCs cultured with exosomes. In addition, we found that the expression of several liver fibrosis markers was up-regulated in SGR-Twnt4 cells.

We demonstrated that intact HCV RNA might be transferred from Huh7 cells to HSCs by exosomes, which could represent a novel mechanism of viral spread and liver fibrosis.

61. Immunogold Transmission Electron Microscopy Reveals That Human Respiratory Syncytial Virus (Human Orthopneumovirus) Co-Localizes with Cell Surface Nucleolin and Not CX3CR1

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In spite of growing evidence supporting the essential role of cell surface nucleolin in respiratory syncytial virus (RSV) cell fusion, there remains some debate as to whether other molecules may be functioning as an "RSV receptor", with cell fusion being more of a stochastic process after viral attachment to the cell membrane. One such candidate is CX3CR1, a putative receptor for the RSV G protein. In order to shed light on this, we used immunogold transmission electron microscopy to document the co-localization of RSV with nucleolin or with CX3CR1 after viral challenge. Results in polarized MDCK cells and human airway epithelial air–liquid interface cell cultures showed that RSV co-localizes with nucleolin at the cell surface and within the cytoplasm, while CX3CR1 only co-localizes with RSV within the cytoplasm. As a control we also looked for RSV immunogold co-localization with Caveolin-1 and found none. In conclusion, these findings further validate nucleolin as a cellular receptor and novel therapeutic drug target for RSV.

62. Influenza A Virus NS1 Protein-Induced Upregulation of microRNAs to Modulate Host Inflammatory Response and Promote Viral Infection

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Viruses antagonize and co-opt cellular process to ensure efficient replication. The non-structural (NS1) protein of influenza A viruses (IAV) is a virulence factor that has multiple functions during viral infection. It is well established that NS1 plays a major role in limiting both interferon (IFN) production and the IFN-induced proteins. Here, we demonstrate a new role of NS1 that is involved in the modulation of the NF-κB signaling pathway and in promoting viral replication. Genome-wide microarray indicated that IAV infection upregulated the expression of ~20 miRNAs by at least twofold. Among these up-regulated miRNAs, miR-146a and miR-139-5p, which are known to regulate NF-κB expression, are significantly downregulated in cells infected with virus mutant lacking the NS1 protein (delNS1). Pre-treatment of infected cells with exogenous IFNβ has no significant effect on miR-146a and miR-139-5p expression, arguing that these miRNAs are downregulated by the presence of IFNβ. We further demonstrated that NS1 protein can activate the promoter activity of miR-146a in a strain-specific manner. Importantly, propagation of IAV was strongly impaired in the presence of miR-146a and miR-139-5p inhibitors, while miR-146a and miR-139-5p mimics showed a positive impact on virus growth. NF-κB activity is necessary for viral replication at the early stage of infection. However, too much NF-κB triggers the immune response, which is not favorable for viral survival. Our findings seem to suggest a novel mechanism, that IAV has evolved a protective strategy by exploiting specific miRNAs to modulate the inflammatory response within a tolerable limit and thereby promote replication.

63. Initiation of Translation of the mRNAs of Infectious Pancreatic Necrosis Virus

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Infectious Pancreatic Necrosis (IPN) is a salmonid disease causing losses in salmon farming worldwide. Infectious pancreatic necrosis virus (IPNV) is a non-enveloped icosahedral virus with a genome (gRNA) composed of two segments of uncapped and non-polyadenylated double-stranded RNA (ds)RNA: RNA-A and RNA-B. Remarkably, the 5-ends of each strand of the viral RNA is linked covalently to the viral genome-linked protein (VPg). The model of synthesis of RNA indicates that the viral mRNA is equal to the +ss gRNA. The characteristics of these mRNAs being linked to the Vpg in their 5'end and being non-polyadenylated make them different from cellular mRNA, and must be relevant for their translation.

IPNV infection inhibits cellular mRNA translation without affecting viral mRNA translation. Moreover, only the Cap-dependent and not the EMCV internal ribosome entry site (IRES)-dependent translation is inhibited, suggesting that IPNV mRNAs exert an alternative mechanism to the Cap-dependent to translation initiation. We had shown that the translation of mRNA-A is commanded by an internal ribosome entry site in the 5'UTR. Besides, there is evidence suggesting that Vpg could be involved in the translation of viral mRNAs. These results show that the translation initiation of IPNV mRNAs may use different mechanisms to recruit the translation machinery.

64. Initiation, Extension, and Termination of RNA Synthesis by the Polymerase of Nipah Virus

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Paramyxoviruses represent a family of nonsegmented negative-strand RNA viruses encompassing a broad range of human pathogens, among which Nipah virus (NiV) has emerged as a global public health concern due to its high mortality rate. Currently, no approved small molecule therapeutics or vaccines are available for NiV infection. Here, we present the first report of an active purified NiV polymerase complex assembled from the multifunctional L protein bound to its phosphoprotein cofactor. Using negative-stain electron microscopy, we obtained the first molecular snapshot of a paramyxovirus polymerase complex, which shows morphological and size similarities to related RNA polymerases. We used this active enzyme to elucidate the specific sequence elements driving recognition of the 3'-terminal genomic promoter, initiation of RNA synthesis, primer extension, and transition to elongation mode. Evidence of transcription termination was supported by NiV polymerase stuttering, leading to polyadenylation in response to gene-end and polyuridine signals on the template strand. The lack of available antiviral therapy for NiV prompted us to identify the triphosphate forms of R1479 and GS-5734—two clinically relevant nucleotide analogs—as inhibitors of NiV polymerase activity by delayed chain termination. These findings help reveal the mechanism and structure of an RNA polymerase from a previously uncharacterized virus family.

65. Interchangeability of Functional Domains within the Alphaviral Nonstructural Polyprotein

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Alphaviruses are arthropod-borne positive-strand RNA viruses of the family *Togaviridae*. Several alphaviruses are human pathogens, causing debilitating symptoms of various severity and duration. The four alphaviral nonstructural proteins (nsPs) act together as a replicase complex to ensure viral RNA replication and transcription. Each nsP with its structural and functional modules—domains—is quite well characterized, but the interactions between them and the exact infection mechanism remain largely unknown. We used homologous domain swapping to further examine the importance and interactions of chosen replicase protein domains in alphaviral replication. In principle, we swapped genome regions of Semliki Forest virus that code for various ns polyprotein domains for corresponding chikungunya virus or Sindbis virus sequences. To our surprise, most swap-mutants were viable and able to produce infectious particles. After adapting less-successful viruses, we identified combinations of ten compensatory mutations, five of which considerably improved the rescue efficiency of infectious virus as individual mutations. We observed that beneficial mutations often arose in ns polyprotein cleavage sites. Thus, we contemplate the most likely explanation for these changes to be untolerably fast cleavage in the nsP1/2 site in swap-viruses where a part of the cleavage site or domains involved in processing have been swapped.

66. Internal Disequilibria and Phenotypic Diversification during Replication of Hepatitis C Virus in a Non-Coevolving Cellular Environment

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The genetic variability of RNA viruses constitutes a major challenge for the control of RNA viral diseases. Long-term virus evolution in an unperturbed cellular environment can reveal features of virus evolution that cannot be revealed by comparing natural viral isolates. We have explored adaptation, mutant spectrum dynamics, and phenotypic diversification of hepatitis C virus (HCV) by subjecting the virus to 200 serial passages in human hepatoma Huh-7.5 cells. Adaptation of HCV to the cells was evidenced by an increase in progeny production. The rate of accumulation of mutations in the genomic consensus sequence deviated from linearity, and mutant spectrum analyses revealed a complex dynamics of mutational waves, which was sustained beyond passage 100. The virus underwent several phenotypic changes such as enhanced cell killing, a shift towards higher virion density, increased shut-off of host cell protein synthesis, and resistance to multiple drugs. Despite replication in a non-coevolving cellular environment, the virus exhibited internal population disequilibria that did not decline with increased adaptation to the host cells. The diversification of phenotypic traits suggests that disequilibria inherent to viral populations may provide a selective advantage to viruses that can be fully exploited in HCV towards an unpredictable viral evolution in the complex liver environment.

67. Investigation of the Role of GBF1 in the Replication of ssRNA(+) Viruses

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GBF1 has emerged as a host factor required for the replication of ssRNA(+) viruses of different families. Its role in viral replication is still unknown. ssRNA(+) viruses replicate their genome in rearranged membranes. GBF1 is essential at the onset of replication, but is not involved in the formation of membrane rearrangements. GBF1 is a guanine-nucleotide exchange factor for Arf family members. Recently, we identified Arf4 and Arf5 (class II Arfs) as host factors required for the replication of hepatitis C virus (HCV), a GBF1-dependent virus. To assess if a GBF1/class II Arfs pathway is conserved among ssRNA(+) viruses, we investigated yellow fever virus (YFV), Sindbis Virus (SV), coxsackievirus B4 (CVB4), and coronavirus 229E (CoV229E). We found that GBF1 is essential for YFV, CVB4, and CoV229E, but not SV. However, the depletion of Arf1, Arf3, Arf4, or Arf5 had no impact. The depletion of Arfs pairs suggested that class II Arfs could be involved in YFV and SV infection, as for HCV, but not for CVB4 and CoV229E. In addition, another Arf pair appears to be essential for YFV and SV, but not for other viruses. We conclude that the mechanism of action of GBF1 in viral replication does not appear to be conserved.

68. Novel Approach for Development of Viral Mutant Swarm Production Assay and Phenotypic Characterization of Hepatitis E Genotype1 Mutant Swarms

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Replication in most RNA viruses is known to be error-prone (ep), thereby generating a repertoire of viral genomes which are genetically linked through mutation, interact at the functional level and collectively contribute to the characteristics of the virus' biological behavior. These are known as quasispecies, also termed as 'mutant swarms'. The complexity and composition of mutant swarms in vivo are thought to contribute to virus adaptability, persistence and drug resistance.

Despite constant efforts, no robust cell-culture system has been established for gt1, due to which molecular mechanisms underlying its life cycle are poorly understood. The aim of this work is to establish a novel, simple and robust method for the generation of full-genome RNA mutant swarms and their phenotypic characterization. Our approach combines the advantages of ep-PCR with reverse genetics to obtain sufficient amounts of RNA mutant swarms. The mutation rate has increased significantly in full-genomes amplified in imbalanced dNTP ep-PCR reactions. Phylogenetic analysis on full-length genomes and gt1-4 reference sequences allowed us to ascertain the close relationship of full-genomes to gt1 strains. Ep-PCR-amplified and genetic-diversified full-length HEV genomes were templates for infectious RNA mutant swarm synthesis (gt1).

Further, we characterized the phenotypic behavior of mutant swarms. When expressed in hepatoma cells, mutant swarms have shown remarkable difference compared to Wild-type (Wt) in terms of viral RNA accumulation (intracellular and extracellular), viral protein expression and host responses. Hepatoma cells infected with mutant swarms showed high amounts of ORF1 protein in an immunofluorescence assay. Host genes, Viperin, OAS1, RIGI and IFITM3 showed a >2 to 10-fold increase in their expression levels.

69. Out-of-Frame or Truncated Additional Gene Products Generated by Polymerase Slippage during Virus Replication in Plants

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Polymerase slippage during replication of human RNA viruses (*Ebolavirus*, *Hepatitis C virus)* can result in nucleotide insertions or deletions, leading to the expression of overlapping open reading frames (ORFs) and/or truncated proteins with premature stops.
We are studying similar mechanisms in plant viruses, starting with members of the family *Potyviridae*, characterized by single-stranded positive-sense RNA genomes expressed through a large ORF encoding a polyprotein autoproteolytically cleaved into mature products. All members of the family present an additional out-of-frame ORF called PIPO, yielding a P3N-PIPO product associated with virus movement, and expressed via polymerase slippage at a conserved $G_{1\text{-}2}A_{6\text{-}7}$ sequence. Recently, we characterized in *Sweet potato feathery mottle virus* an equivalent mechanism that results in the expression of another out-of-frame P1N-PISPO, which displays RNA silencing suppression activity, suggesting that the strategy might be commonly used by potyvirids to enhance the coding capacity of their genomes. To further explore the extent of this, we are analyzing polymerase slippage frequencies for insertions/deletions in $G_{1\text{-}2}A_{6\text{-}7}$ motifs found in other viruses such as *Coccinia mottle virus*, a cucurbit-infecting ipomovirus, and studying the effects of multiple infections between potyvirid and unrelated viruses on slippage frequency.

70. Papain-Like Protease 2 (vOTU) Chimeras between Highly-Pathogenic JXwn06 and Ingelvac PRRS MLV Strains of Porcine Reproductive and Respiratory Syndrome Virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) papain-like protease 2 (PLP2; in nonstructural protein 2) is a member of the viral ovarian tumor (vOTU) protease superfamily. The enzyme has deubiquitinating (DUB) and deISGylating activity, and is proposed to utilize these activities to circumvent the innate immune response. Previous work indicated that the 215 amino acid vOTUs of JXwn06 strain (JX) of HP-PRRSV and Ingelvac PRRS modified live vaccine (MLV) showed strain-specific deubiquitinating activity but little deISGylating activity. Importantly, HP-PRRSV alone demonstrated a clear preference for lysine 63-linked poly-Ubiquitin, strongly associated with innate immune response regulation.

We now report the development of viruses to examine the contribution of the vOTU amino acid domain to virulence. Two chimeric viruses (rMLV-JXOTU and rJX-MLVOTU) as well as their parental viruses (rJX and rMLV) were successfully produced and rescued by visible cytopathic effect, and then confirmed by immunofluorescence analysis, targeted RT-PCR, Northern blot, and next-generation sequencing (NGS) at passage 3 and 10. In vitro studies demonstrated that the rescued chimeric viruses (rMLV-JXOTU and rJX-MLVOTU) possessed peak viral titers of 1.4×10^7 and 5.5×10^5 pfu/mL at passage 3 on MARC-145 cells, respectively, showing similar replication rates to the rescued parental viruses rMLV (8.5×10^6 pfu/mL) and rJX (1.5×10^6 pfu/mL) in MARC-145 cells. Other viral characteristics were also similar to the respective parental viruses. These two chimeric viruses along with the parental viruses were used to infect porcine alveolar macrophage cells. The chimeric and parental viruses induced variable RNA expression of IFN-α and IFN-β, and a higher expression of RIG and ISG15 genes. The chimeric viruses could also not be distinguished by the amount of ubiquitin protein detected in infected cells nor in the DUB activity seen. These viruses will be evaluated in swine to examine the contribution of the PLP2/OTU domain to virulence.

71. Persistent Replication of Astrovirus MLB1 on the Huh-7.5 Hepatoma Cell Line

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Since its discovery in 2008, little has been found out about Astrovirus MLB clade pathogenesis. Yet, while these viruses belong to a family of enteric viruses, they have been identified as causative agents of infection of the central nervous system in two reports. Here, we present data on their propagation and replication on two different human cell lines.
Cell culture propagation was attempted using two astrovirus MLB strains issued from clinical stool specimens and replication was monitored by quantification of viral genomes by specific real-time reverse-transcription PCR assay (qRT-PCR). A MLB2 strain showed an increase in viral genomes of more than 2 logs after two passages on A549 cells and 3 logs after three passages on Huh-7.5 cells, suggesting adaptation to both cell types. However, propagation of a MLB1 strain on Huh-7.5 resulted in a low but steady detection of viral genomes in the supernatant over six serial passages despite a high increase of intracellular viral RNA, suggesting persistent replication of viruses which may be strongly associated to cells. Efforts to propagate both MLB strains on the CaCo-2 cell line—usually the most permissive cell line for astroviruses—failed, with the absence of genome detection after two passages.
Conclusion: Our preliminary results suggest that Huh-7.5 and A549 cell lines are more permissive than the CaCo-2 cell line for astrovirus MLB replication. In addition, there are clues indicating that astrovirus MLB1 accumulates in the cell with a smaller fraction being detectable in the supernatant.

72. Re-directing Bovine Viral Diarrhea Virus miRNA Tropism Using Random Seed Site Mutagenesis

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Hepatitis C virus (HCV) binds the liver-specific microRNA (miR)-122 to its 5' UTR, enhancing RNA translation, stability, and replication. Bovine viral diarrhea virus (BVDV)—a related pestivirus—binds cellular miRNAs let-7 and miR-17 to two seed sites (S1 and S2) in its 3' UTR.

Here, we show that BVDV critically depends on miR-17 but not let-7. Bidirectional luciferase reporters confirmed a pro-translational role for miR-17. Randomization of S2 led to the selection of a virus with two let-7 sites (2xlet-7) in a miR-17 deprived environment; deep sequencing revealed other sub-dominant seed sites. The 2xlet-7 virus was sensitive to let-7 inhibition and S2 mutagenesis. Unlike the wild-type, this virus was slightly attenuated by S1 mutation. S1/S2 randomization in all combinations with miR-17 or let-7 seed sites led to partially miRNA-dependent viruses. miRNA interactions of mutants were confirmed by Argonaute (AGO) cross-linking and immunoprecipitation (CLIP). This will further demonstrate whether BVDV "sponging" of the cellular miR-17 pool can be re-directed to let-7.

In conclusion, BVDV critically depends on miR-17, but miRNA tropism can be re-directed. Given the opposite roles of miR-17 and let-7 in cell proliferation, the 2xlet-7 virus provides a promising tool to study the impact of miRNA "sponging" on the host.

73. Reverse Genetic System Approach to Understand the Replication and Pathogenesis of Infectious Salmon Anemia Virus (ISAV)

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The infectious salmon anemia virus (ISAV) is a piscine *Orthomyxovirus*, and is the major cause of Atlantic salmon outbreaks, producing billions of dollars in economical losses in the Chilean industry. Segment 6 encodes the HE protein, which also contains the highly polymorphic region (HPR) sequence in the stalk domain, in contrast with Influenzavirus which posseses a highly-conserved stalk region in the HA protein. Although there have been commercial vaccines since 2008, in the last 3 years different viruses belonging to genotypes HPR0, HPR3, HPR7b, and HPR14 have been reported in Chilean outbreaks. Some evidence shows that avirulent ISAV HPR0 is a genetic precursor of the virulent strains. Due to the viral genome segment reassortment and the quasispecies population, it is difficult to understand the role of segment 6 and the HPR region in the viral pathogenesis. To-date, no virus studies have been conducted where only segment 6 of each viral strain is exchanged while maintaining the same genetic backbone. For the first time we generate the above-cited synthetic ISAV strains using our reverse genetic system and report an interesting replication rate difference and co-evolution of segment 6 in ASK cells. This study was supported by Grant FONDECYT 1161006 (CONICYT, Chile); MECESUP-USACH USA1555, DICYT, DGT and VRIDEI (USACH).

74. RNA Circularization during Viroid Replication

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Circular RNAs are attracting scientific interest given their involvement in gene expression regulation and human diseases. Viroids are plant pathogens constituted by a circular non-coding RNA. Like viruses, viroids hijack host factors and structures to complete the infectious cycles and—particularly for RNA circularization—they recruit the nuclear DNA ligase 1 or the chloroplastic tRNA ligase, depending on viroid type. Eggplant latent viroid (ELVd) replicates in the plant chloroplasts by a rolling-circle mechanism. A host DNA-dependent RNA polymerase transcribes viroid strands that are cleaved by hammerhead ribozymes. Finally, viroid monomers are circularized by the chloroplastic tRNA ligase. To gain insight into the sequence and structural requirements in the ELVd circularization reaction, we performed a mutational analysis using an *Escherichia coli*-based expression system. Analyzed mutations affected the upper strand of the ligation domain in the minimum free energy conformation of the ELVd linear intermediate. All mutant ELVd forms were circularized with the same efficiency as the wild type, indicating that either the only requirement for ELVd circularization is the terminal 5′-hydroxyl and 2′,3′-cyclic phosphodiester groups produced by the hammerhead ribozymes or, if a particular folding is necessary, this must be transitorily adopted by the lower strand of the linear replication intermediate.

75. Rubella Virus Strain-Specific Differences in Metabolic and Interferon-Response Pathways Addressed in iPSCs as a Model for Viral Impairment of Early Embryogenesis

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The underlying mechanisms of rubella virus teratogenicity are still poorly understood. The lack of a suitable animal model points towards human-specific factors. Recently human induced pluripotent stem cells (iPSCs) were introduced by our group as an in vitro cell culture model for the viral impact on the very early phase of human embryogenesis. Here, we further evaluated the suitability of iPSCs as such a model through the characterization of different RV clinical isolates. On Vero as RV cytopathic effect-susceptible cell lines, these strains were arranged in two groups, one with and one lacking cytopathic alterations. Thereafter, metabolic extracellular flux analysis of RV-infected endothelial and epithelial cell lines was performed. Although a comparable set of genes was altered at their transcription level by all RV strains, only the strains positive for cytopathic alterations induced a significant increase in basal oxygen consumption rate and spare respiratory capacity. RV strains associated with metabolic alterations also evoked a stronger interferon response on primary cell lines than those lacking metabolic alterations. Notably, the RV strain positive for metabolic alterations established a higher initial infection rate in iPSCs than the others. Transcriptomic analysis of RV-infected iPSCs and derived embryonic lineages revealed a differential gene expression profile. These data support the notion that iPSCs iare a suitable in vitro cell culture model representing the blastocyst stage until early gastrulation. The use of different RV strains on iPSCs will extend our current knowledge on viral (teratogenic) alterations during early embryogenesis.

76. The Ebola Virus Delta Peptide Acts as an Enterotoxin in Vivo

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The Ebola virus (EBOV) delta peptide—a 40-residue partially-conserved nonstructural polypeptide—is produced in abundance during Ebola virus disease (EVD). Conserved residues in the C-terminus of delta peptides from related species of filoviruses are rich in aromatic and cationic residues, with the potential to form an amphipathic α-helix characteristic of membrane-permeabilizing peptides, while the conserved pair of cysteines determines the active disulfide-linked hairpin structure that is essential for EBOV delta peptide's viroporin activity. EBOV delta peptide is cytotoxic, and permeabilizes different cell types and synthetic lipid bilayers in vitro. The high structural relatedness of EBOV delta peptide to the cytolytic peptide of the nonstructural protein 4 (NSP4) of rotavirus—a known viral enterotoxin—led us to hypothesize that EBOV delta peptide can act as an enterotoxin. We tested this hypothesis in an established adult mouse model of *Vibrio cholerae*-induced diarrhea. EBOV delta peptide induces potent diarrhea that peaks at around 9-to-12 h post-injection into the ileum. Histological analysis shows severe damage to the cellular architecture of the small intestine. We show that EBOV delta peptide is an enterotoxin that plays an important role in EVD pathogenesis, and is a novel target for the development of therapeutic countermeasures.

77. The Effect of Alphavirus Infection on the Cellular Deubiquitylases Transcriptomes

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Emerging and re-emerging viral infections are causing great concern across the globe. In recent years, Chikungunya virus (CHIKV)—a member of the *Alphavirus* family which causes the disease Chikungunya fever—has caused particular concern due to the spread of the virus across South-East Asia, the Indian sub-continent, and more recently to the Caribbean and Central/South America. Currently, no vaccines or antivirals are available. Characterisation of how viruses interact with their host cells may to help elucidate potential targets for antiviral therapy. The process of reversible ubiquitination, carried out by a large group of enzymes called deubiquitylases (DUBs), plays a key role in the majority of the cellular process. Viruses are known to target this system to aid their replication. Using Semliki Forest virus (SFV, a model alphavirus), we have investigated the effect of alphavirus infection on cellular DUB transcript levels in HeLa cells. Out of 36 DUBs tested to-date, 7 showed an increase in mRNA levels post SFV infection. All seven DUBs were also confirmed to increase after CHIKV infection. Experiments are currently underway to investigate if this increase is reflected at the protein level. The work is being extended to investigate DUB expression in fibroblast cells and HEK293 cells after SFV and CHIKV infection. Selected DUBs are being followed up to determine their functional role during infection. This may lead to the identification of potential targets for anti-alphavirus therapy.

78. The Recruitment of Mouse Hepatitis Virus (MHV) Nucleocapsid Protein to Replication-Transcription Complexes Plays a Key Role in Infection

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Coronaviruses (CoV) are enveloped viruses with a single-stranded positive RNA genome, and cause numerous pathologies in mammals. The nucleocapsid protein (N) of CoV is a multifunctional protein that is known to interact with replication and transcription complexes (RTCs). To shed light on the importance of N protein–nsp3 interaction during the CoV life cycle, we have identified the domains of mouse hepatitis virus (MHV) N protein involved in its association with RTCs to create specific binding point mutants. In particular, we have found that the N protein mainly binds to nsp3 and that its N1b and SR regions mediate this interaction in a gRNA-independent manner. N protein variants carrying point mutations in these domains fail to be recruited to RTCs and have a dominant-negative effect of MHV infection by impairing virus replication and progeny. Our data thus show that N protein recruitment to the RTCs plays an important role in MHV life cycle.

79. Understanding the Mechanism of Persistence: Activating the Replication of an RNA Virus in Persistently Infected Mammalian Cells

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Providence virus (PrV) is a small, positive-sense, single-stranded insect RNA virus that belongs to the family Carmotetraviridae. This virus was originally isolated from a lepidopteran cell line, but is capable of establishing persistent infections in mammalian cells. PrV has a monopartite genome encoding three ORFs: at the 5' end is p130, a protein of unknown function, which overlaps with the viral replicase ORF, p104, and p81, encoding the capsid protein precursor, is located at the 3' end of the viral genome. The replicase ORF is translated into two proteins through the activity of a readthrough stop—a truncated N-terminal accessory protein, p40, and the full-length replicase encoding the RdRp domain at its C-terminus. In this study, we investigated the mechanism of persistent PrV infections in human cervical (HeLa) cells. PrV replication was assessed by confocal immunofluorescence microscopy using the presence of p40 and double-stranded RNA, indicative of a replicating viral RNA to quantify viral replication. Treatment of persistently infected HeLa cells with Triton X-100 and NP-40—both non-ionic detergents—resulted in increased levels of detectable p40 and dsRNA, and therefore viral replication. Treatment with saponin and digitonin showed a modest increase in p40 and dsRNA levels. In contrast, tween 20, sodium dodecyl sulphate, or hexadecyltrimethylammonium bromide did not induce increased levels of viral replication. We propose that treatment of mammalian cells with non-ionic detergents results in the formation of membrane-bound vesicles, which activates PrV replication in persistently infected cells.

80. Structural Analysis of the Human RED–SMU1 Splicing Complex and Potential for Host-Directed Anti-Influenza Therapy

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Transcription and replication of the influenza A virus (IAV) RNA genome occurs in the nucleus of infected cells. We showed previously that the RED–SMU1 splicing complex regulates the splicing of the viral NS1 mRNA into the NS2 mRNA encoding the essential NS2/NEP protein. In cells depleted for RED or SMU1, the production of infectious IAVs was reduced by 2-logs, therefore designating the RED–SMU1 complex as a promising drug target (PMID 24945353).

Here, using in vitro and cell-based protein–protein interaction assays, we further characterized the human RED–SMU1 interface, and we solved the crystal structure of a minimal REDmid–SMU1Nter complex. Ectopic expression of the short REDmid domain in cultured cells led to disruption of the RED–SMU1 complex and decreased IAV replication. These data provided the rationale to screen in silico a set of small compounds for binding at the SMU1Nter–REDmid interface. We thereby identified several compounds that disrupt the RED–SMU1 complex and specifically inhibit IAV replication whilst preserving cell viability. Overall, our data demonstrate the potential of destabilizing the RED–SMU1 complex as a novel antiviral therapy, which could be active against a wide range of IAVs and be less likely to select for resistance mutants.

81. New Inter-Cellular Mechanism for the Transmission of Japanese Encephalitis Virus by Human Microglia Cells

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Japanese encephalitis virus (JEV) is a neurotropic flavivirus and a major cause of mortality and morbidity in humans. JEV-infected patients exhibit strong inflammation of the brain and an accumulation of viral particles in the hypothalamus and hippocampus. Microglial cells, the brain-resident macrophages, represent the first line of defence during brain insults (including viral infection), but may serve as reservoir for JEV. Using a model of human monocyte-derived microglia, the present study explores the role of human microglia in JEV (Nakayama strain) propagation and transmission to another cell type. Although infectious JEV particles in supernatant diminished over 6 days post-infection, levels of viral RNA increased in both cells and supernatants. However, virus particles recovery occurred upon co-culture of the latter JEV-infected human microglia with BHK-21 cells—a highly susceptible cell line to JEV. Importantly, viral transmission required cell–cell contact conditions, where dsRNA was possibly the source of viral infectious material. Mechanistically, the axis CX_3CR1-CX_3CL1 was critical in the interactions between microglia–BHK-21 cells allowing viral transmission. Altogether, the data show a novel role of JEV-infected human microglial cells in viral propagation upon interaction with susceptible cell type, which may be a source of neuronal infection. Finally, the fraktalkine receptor CX_3CR1 is a relevant chemokine receptor involved in cell migration and inflammation in the central nervous system. In conclusion, human microglia may sustain JEV brain pathogenesis in late stages of infection.

82. The Vpu-Interacting Protein ATP6V0C Regulates Expression of Tetherin and HIV-1 Release

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The HIV-1 accessory protein Vpu enhances virus release by counteracting the host restriction factor tetherin, and this antagonism involves Vpu-mediated down-regulation of cell-surface tetherin. To further understand the role of host proteins in Vpu function, we carried out yeast two-hybrid (Y2H) screening and identified five Vpu-interacting proteins, including the small glutamine-rich tetratricopeptide repeat-containing protein (SGTA) and the V0 subunit C of vacuolar ATPase (ATP6V0C). Previously, we reported that overexpression of SGTA inhibited HIV-1 release and stabilized a 23-kDa non-glycosylated tetherin species (Waheed et al., Sci. Rep. 2016). In the current study, we examined the role of ATP6V0C in Vpu-mediated tetherin degradation and HIV-1 release.

We observed that knockdown of ATP6V0C in HeLa cells impairs Vpu-mediated tetherin degradation and HIV-1 release. This inhibition of HIV-1 release imposed by ATP6V0C knockdown can be rescued by knockdown of tetherin. Interestingly, knockdown of other V-ATPase subunits has differential effects; knock-down of ATP6V0C" has no effect on HIV-1 release or tetherin degradation, whereas knockdown of ATP6V0A1 inhibited tetherin degradation and HIV-1 release. Overexpression of ATP6V0C—primarily in the absence of Vpu—resulted in stabilization of 26-kDa, high-mannose-enriched tetherin. The stabilization of 26-kDa tetherin is specific to ATP6V0C, as overexpression of ATP6V0C" has no effect on the expression of 26-kDa tetherin. Further, the ATP6V0C-mediated stabilization is specific to tetherin, as overexpression of ATP6V0C did not stabilize CD4 or interfere with its Vpu-mediated down-regulation. Immunofluorescence localization studies showed that the ATP6V0C-stabilized 26-kDa tetherin was sequestered in LAMP1-enriched intracellular lysosomal compartments. These studies indicate that the Vpu-interacting protein ATP6V0C plays a role in the regulation of tetherin expression and HIV-1 assembly and release.

83. A New Biotinylation System to Investigate Enveloped Virus Assembly

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We have developed a BirA biotin ligase-based system that enables tracking of sub-populations of a protein of interest over time. We spatially restrict BirA localization using fused targeting domains to enable location-based biotinylation of the AVI-tagged (biotin acceptor motif) protein of interest. Controlled pulses of exogenous biotin followed by chase periods permit pulse-labeling of the AVI-tagged protein with biotin. Combining these two features, we are able to biotinylate specific subcellular populations of the AVI-tagged protein and track their fate in a time-resolved manner. Enhanced and stringent retrieval of the spatially and temporally-defined biotinylated protein populations is achieved using Streptavidin-based probes. We have validated our new targeted BirA biotin pulse-chase system using the alphavirus Cp protein, and will apply this technology to investigate virus assembly and budding.

84. A Reverse Genetics System for Rotavirus

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Despite recent new developments, there is still a need for an effective reverse genetics system for rotavirus (RV). Most systems based on the use of a helper-virus require a selective marker for the genome segment (gs) of interest. Our approach is based on the incorporation of an exogenous gs encoding EGFP fused to NSP5 separated by the Tobacco Etch Virus protease (TEVp) cleavage site (TS) and expressed in MA104 cells from a Recombinant T7-Vaccinia Virus (rVV). In vivo processing by TEVp yields the fully functional cleaved NSP5 and EGFP. After the generation of reassortant viruses upon co-infection of the rVV-EGFP/NSP5 with RV, the cleared lysate was used to infect MA104 cells expressing TEVp. Cells infected with RV harbouring the recombinant segment and sustaining viral replication are expected to be EGFP positive. The cells were FACS-sorted for EGFP expression and further cultured with fresh non-infected MA014 cells. These infection-sorting cycles are repeated several times to isolate the reassortant RV. Our system can be used to study the role in infection of single proteins. It could also be applied to any genome segment and any virus as long as the size of the recombinant gene is compatible with packaging into infective particles.

85. Adenovirus Coupled Assembly and Packaging at the Periphery of the Replication Center

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Adenovirus (AdV) is one of the most complex icosahedral, nonenveloped viruses. The elaborated capsid architecture is the product of a correspondingly complex assembly process, of which many aspects remain unclear. In particular, it is still not settled if assembly and packaging occur in a sequential or a concerted manner. Here, cells infected with AdV type 5 (Ad5) wild type (wt) were studied at different post-infection times and compared with an Ad5 mutant (Ad5/FC31), which has a delay in the packaging process. Immunofluorescence and immunoelectron microscopy assays were carried out to determine the localization of viral DNA, packaging, core and capsid proteins in infected cells. The results indicate that all assembly factors can be found in an area previously recognized as the peripheral replicative zone, which could therefore be the AdV assembly factory. Assembly intermediates observed in this region support the concerted assembly and packaging model. This assembly process could be divided in two pathways, one for only capsid proteins and another one for viral DNA and core proteins. Only when both pathways are coupled by correct interaction between packaging proteins and the genome, is the viral particle produced. The mutation in Ad5/FC31 decouples these pathways, generating empty capsids and speckled bodies, which are accumulations of unpackaged cores.
This work is supported by grants BFU2016-74868-P from the Spanish Agencia Estatal de Investigación, the European Regional Development Fund, BFU2013-41249-P and the Spanish Adenovirus Network (AdenoNet, BIO2015-68990-REDT) from Ministerio de Economía y Competitividad of Spain.

86. Cd302 and Cr1l Restrict Hepatitis C Virus Infection of Murine Hepatocytes and Contribute to the Human:Mouse Species Barrier

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Hepatitis C virus (HCV) has a narrow host range and mice are refractory to HCV infection. Human entry factor transgenic (hEFT) mice with blunted innate immunity support only low level HCV replication, indicating that additional uncharacterized factors restrict HCV infection of mice. A murine liver cDNA library was transduced into human hepatoma cells which were modified to undergo apoptosis upon HCV replication. Iterative rounds of viral selection identified murine Cd302 and Cr1l as potent restriction factors of HCV infection with cross-genotype potency. Combined over-expression of both factors resulted in a dramatic drop in the ability to infect permissive cells, comparable to the restriction observed for the most potent human restriction factor, IRF1. However, RNAseq revealed divergent mechanisms of action for the observed IRF1 and Cd302/Cr1l restriction phenotypes. Domain deletion identified Cd302/Cr1l regions associated with the anti-HCV phenotype. While no effect was observed on HCV RNA replication, entry of lentiviral pseudoparticles bearing HCV glycoproteins was impeded, indicating a block in the early stages of infection. Constitutive high-level expression of Cd302/Cr1l mRNA and protein in mouse livers and hepatocytes was observed. Both proteins localized to the cell-surface and were non-interferon inducible. The magnitude of the antiviral response to HCV increased with Cd302/Cr1l silencing in hEFT mouse hepatocytes when compared to control infections, due to enhanced exposure to viral PAMPs and priming of innate immune responses upon silencing. The human homologs showed differential levels of restriction: Cd302 was also a potent HCV restriction factor whereas Cr1l had no effect. Levels of human homolog expression in human hepatocytes also differed from the pattern observed in murine livers. These newly identified murine restriction factors contribute to the HCV

human:mouse species barrier and represent targets for gene knock-out to facilitate the generation of a fully permissive immunocompetent small animal model for HCV. In addition, Cd302 and Cr1l represent a previously unappreciated layer of constitutive antiviral protection in the murine liver.

87. Commonalities in Mechanisms of Action between Three Distinct Classes of Human Cytomegalovirus DNA Cleavage/Packaging Inhibitors

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Herpesvirus terminase is a three-subunit enzymatic complex that packages concatemeric (replicative) viral DNA into capsids, then cleaves the DNA to produce intranuclear capsids containing mature viral genomes that are protected from exogenous nuclease. Three inhibitor classes target the human cytomegalovirus terminase: the halogenated benzimidazoles, tomeglovir, and letermovir. Recent licensure of letermovir for use in stem cell transplant patients has validated terminase as an important antiviral target and rekindled interest in terminase function and mechanisms of its inhibition. Despite profound structural differences, there are apparent commonalities in the mechanisms of action of all three inhibitor classes. First, there is significant overlap between subdomains of terminase subunits in which resistance mutations cluster, and some mutations confer cross-resistance to all three inhibitors (Chou, AAC 2017 61(11)). Second, all three inhibitors can induce formation of supergenomic viral DNA that appears to result from cleavage site skipping. Third, while DNA-containing nucleocapsids are formed in the presence of halogenated benzimidazoles and letermovir, evidence suggests that the encapsidated DNA is abnormal and is not nuclease-protected. These findings suggest that the three current inhibitor classes target terminase by a similar or common mechanism. Discovery of additional terminase inhibitors may reveal distinct mechanisms of inhibition and perhaps novel terminase functions.

88. Curaxin CBL0100 Blocks HIV-1 Replication and Reactivation through Inhibition of Viral Transcriptional Elongation

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Despite combination antiretroviral therapy (cART), acquired immunodeficiency syndrome (AIDS), predominantly caused by the human immunodeficiency virus type 1 (HIV-1), remains incurable. The barrier to a cure lies in the virus' ability to establish a latent infection in HIV/AIDS patients. Unsurprisingly, efforts for a sterilizing cure have focused on the "shock and kill" strategy using latency-reversing agents (LRAs) to complement cART in order to eliminate these latent reservoirs. However, this method faces numerous challenges. Recently, the "block and lock" strategy has been proposed. It aims to reinforce a deep state of latency and prevent sporadic reactivation ("blip") of HIV-1 using latency-promoting agents (LPAs) for a functional cure. Our studies of curaxin 100 (CBL0100), a small-molecule targeting the facilitates chromatin transcription (FACT) complex, show that it blocks both HIV-1 replication and reactivation in in vitro and ex vivo models of HIV-1. Mechanistic investigation elucidated that CBL0100 preferentially targets HIV-1 transcriptional elongation and decreases the occupancy of RNA Polymerase II (Pol II) and FACT at the HIV-1 promoter region. In conclusion, CBL0100 is a newly identified inhibitor of HIV-1 transcription that can be used as an LPA in the "block and lock" cure strategy.

89. Deep Mutational Scan of the Highly Conserved Influenza A M1 Matrix Protein Reveals Substantial Intrinsic Mutational Tolerance

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Influenza A matrix protein, M1, plays key roles in many stages of the virus's infectious cycle: directing assembly of budding virions, determining morphology, and directing intracellular trafficking of vRNPs. In nature, M1 is highly conserved, exhibiting >95% amino acid identity amongst all influenza A subtypes. We seek to understand whether there are significant functional constraints on M1 leading to this high sequence conservation or whether the protein inherently tolerates mutations that are not sampled in nature. We performed a deep mutational scan (DMS) of influenza M1 using an M1 codon mutant virus library, which undergoes a M1 functional selection through viral replication during cell infection. We observe that M1 has low mutational tolerance at several sites that correspond to residues that have been reported to be functionally critical. However, surprisingly, substantial tolerance of mutations is observed over large spans of M1, including the C-terminal two-thirds. We are also combining our analysis of M1 sequence mutation tolerance with cryo-electron tomography (cryo-ET) to understand M1-determinants of virion assembly and morphology. A DMS of subpopulations from the mutant library that are biased towards different particle morphologies is being performed in parallel with cryo-ET imaging of virus ultrastructure.

90. Gag Induced PIP2 Nanoclustering during HIV-1 Assembly: from Model Membranes to Infectious Virus in CD4 T-Cells

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During infection, HIV-1 is mainly replicating in CD4 T-cells. The viral Gag protein is targeting the cell plasma membrane to self-assemble during viral particle biogenesis. In addition to a myristic acid, its N-terminal MA domain harbours a highly basic region that recognizes acidic phospholipids, more specifically $PI(4,5)P_2$. For decades, it has been considered that HIV-1 assembly occurred in pre-existing nano-domains of the plasma membrane enriched in cholesterol and sphingomyelin. We have recently shown that unmyristoylated Gag was generating $PI(4,5)P_2$ and cholesterol-, but not sphingomyelin-enriched nanodomains on model membranes. Here, using line scanning and super-resolution STED-microscopy-based Fluorescence Correlation Spectroscopy (FCS), we determine HIV-1 assembly locations and simultaneously measure the dynamics of $PI(4,5)P_2$ in and out of these HIV-assembly sites. We performed this approach on plain Gag-$PI(4,5)P_2$ model membranes and in Gag transfected and HIV-1 infected Jurkat T-cells. We observed that in Gag transfected as well as in HIV-1 infected cells, $PI(4,5)P_2$ is nanoclustered during virus assembly thus demonstrating that the viral Gag protein is the driving force for $PI(4,5)P_2$ clustering. These results strongly confirms that cellular lipids are spatially sorted by the virus in order to facilitate its assembly.

91. HCMV Envelope Protein gpUL132 Controls Viral Production through Efficient Viral AC Formation

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Abstract: The human cytomegalovirus UL132 open reading frame encodes a 270-amino acid type I envelope glycoprotein, gpUL132, that is required for efficient virus production. Deletion of UL132 from the HCMV genome resulted in a pronounced deficit in virus yield with an approximately 2–3 log decreases in infectious titer. To determine the role of gpUL132 in the virus life cycle, we first studied the characteristics of the ΔUL132 HCMV mutant. Using density gradient centrifugation in potassium-tartrate, we observed that ΔUL132 extracellular particles banded at different densities compared to wild-type (wt) HCMV particles. Additional studies indicated that the defects in the ΔUL132 mutant virus resulted from the altered morphogenesis of the membranous viral assembly compartment (AC), rather than deficits in virus entry, genome replication, or cell-to-cell spread. Expression of gpUL132 in trans rescued the defects in the AC in cells infected with the ΔUL132 mutant virus and infectious virus production. Importantly, we utilized a fusion protein combining the ecto- and transmembrane domains from an irrelevant protein, TrkB, with the cytosolic domain from gpUL132 for these experiments, which demonstrates that the cytosolic domain of gpUl132 is sufficient to rescue the defects in AC formation in cells infected with the ΔUL132 mutant. These findings argue that gpUL132 is essential for HCMV AC formation and highlight its importance for viral production.

92. Host-Specific Differences in Rift Valley Fever Virus Genome Packaging

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Arboviruses have evolved to replicate in both insects and mammals. An important part of the arbovirus life-cycle is the packaging of the viral genome into virions. For bunyaviruses, which comprise a tri-segmented RNA genome, incorporation of all three segments is required to produce infectious virus. Using single-molecule RNA fluorescence in situ hybridization (FISH), we recently showed that the zoonotic Rift Valley fever virus (RVFV) uses a non-selective genome packaging strategy to produce progeny in mammalian cells. Here, we evaluated the intracellular spatio-temporal distribution of RVFV genome segments in insect cells and analysed the genome segment composition of virions produced in these cells. The results reveal that RVFV genome segment replication and recruitment in insect cells is highly similar to what we previously observed in mammalian cells. However, comparing insect cell-derived virus with virus produced in mammalian cells revealed striking differences. The average total of genome segments per virion and the percentage of virions containing all three segments was significantly higher in virions produced in insect cells. Altogether, this study suggests that RVFV genome packaging is more efficient in insects compared to mammals, explaining the ability of RVFV to replicate to higher infectious titers in the former.

93. Identification of Small Molecules Inhibiting the Dimerization of HCMV DNA Polymerase Processivity Factor UL44

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Human cytomegalovirus (HCMV) is a leading cause of severe disease in immunocompromised individuals. Despite the availability of several drugs, pharmacological treatment is associated with toxicity and the emergence or resistant strains. Therefore, it is essential to identify new potential targets of therapeutic intervention. One such target is represented by the dimerization of HCMV DNA polymerase processivity factor UL44. Indeed, UL44 plays an essential role in viral replication by tethering the DNA polymerase holoenzyme to the DNA and its dimerization is absolutely required for DNA binding and OriLyt-dependent DNA replication.

The aim of this study is to identify small molecules (SMs) that hinder viral replication interfering with UL44 homodimerization. Therefore, we first confirmed that UL44 forms dimers in a cellular context by Fluorescent and Bioluminescent resonant energy transfer assays, and that single amino acid substitutions affect the dimerization process. Subsequently, using the recently published crystal structure of UL44 homodimers, we performed a virtual screening to identify SMs potentially interfering with UL44 homodimerization. The ability of 18 SMs, selected from the virtual screening results, to interfere with UL44 dimerization was subsequently assessed in vitro using GST-pulldown and thermal shift assays. The effect on viral replication was assessed by Plaque Reduction Assays using AD169 and by fluorimetric analysis using YFP-tagged recombinant viruses. Finally, the effect on cell viability and growth was assessed by MTT assay.

94. Lethal Mutagenesis of Flaviviruses: Zika Virus and Usutu Virus Differ in Their Relative Sensitivity to Three Mutagenic Nucleosides

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Flaviviruses constitute an increasing source of public health concern with new pathogens causing human disease, rapidly spreading to diverse geographic areas. Despite lethal mutagenesis having been extensively studied with different viruses, there is not yet any data on the antiviral effect associated with increased mutagenesis in Zika virus (ZIKV) and Usutu virus (USUV), two recent emerging threats. In this study, we demonstrate that both viruses are highly sensitive to three different mutagenic ribonucleosides—favipiravir, ribavirin and 5-fluorouracil—while they remain unaffected when treated with a mutagenic deoxyribonucleoside. Serial passage of ZIKV in the presence of these compounds resulted in the rapid extinction of infectious virus (in less than four passages), suggesting that this pathogen is highly sensitive to mutagenesis. USUV extinction is only achieved when a 10-fold dilution is applied between every passage but not in experiments involving the serial transfer of undiluted sample, indicating that this flavivirus may be more resistant to increased mutagenesis than ZIKV. Although both viruses are affected by the same three drugs, ZIKV is relatively more susceptive to serial passage in the presence of purine analogues (favipiravir and ribavirin) while USUV replication is suppressed more efficiently by 5-fluorouracil. These differences in the sensitivity to mutagenesis positively correlate with the relative increases in mutation frequencies observed in the viral populations treated. We will discuss the implications of our results on the fidelity of flavivirus replication, and the rational design of antiviral therapies based on lethal mutagenesis.

95. Probing Lipid and Protein Dynamics at Individual HIV-1 Assembly Sites

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Human Immunodeficiency Virus type 1 (HIV-1) assembly at the plasma membrane brings together individual virus components in a process organized by the viral structural protein Gag. The protein distribution at virus assembly sites has been studied in depth by both electron and super-resolution microscopy approaches. However, currently, little is known about the dynamics of participating lipids and proteins during assembly, with information only available from studies on model membrane systems. Here, we present a novel approach to study the molecular dynamics of bona fide virus assembly sites based on a combination of super-resolution STED microscopy and Fluorescence Correlation Spectroscopy (FCS). STED-FCS allows for the investigation of diffusion dynamics of lipids and proteins at subdiffraction scales and has already been applied to study protein dynamics on the surface of individual virus particles (Chojnacki et al. 2017). We have adopted STED-FCS to probe lipid and protein diffusion inside and outside virus assembly sites in Jurkat T-cells infected with fully infectious HIV-1. We found trapping of Env and MHC-I proteins as well cholesterol inside these sites whereas sphingomyelin or phosphatidylethanolamine did not interact with the Gag assembly sites. Our experiments introduce a powerful tool for future studies of lipid and protein diffusion and interaction dynamics at individual virus assembly sites in a fully infectious virus model.

96. Protein-Assisted RNA Folding Mediates Specific RNA–RNA Genome Segment Interactions in Segmented RNA Viruses

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Segmented RNA viruses, including influenza viruses and rotaviruses, are ubiquitous human, animal and plant pathogens. A major challenge in understanding their assembly is the combinatorial problem of a non-random selection of a full genomic set of distinct single-stranded (ss)RNAs. This process involves multiple, complex RNA–RNA and protein–RNA interactions, which to date have been obscured by non-specific binding and aggregation at concentrations approaching in vivo assembly conditions. To interrogate specific inter-segment interactions in rotaviruses, we employ two-color fluorescence cross-correlation spectroscopy (FCCS) for detecting stable RNA–RNA interactions taking place in complex RNA and protein mixtures. We show that binding of the rotavirus non-structural protein NSP2 to ssRNAs results in RNA conformational rearrangements conducive to forming stable contacts between RNA segments. To identify the sites of inter-segment interactions, we developed an RNA–RNA SELEX approach for mapping the RNA sequences mediating stable inter-molecular base-pairing between the interacting ssRNAs. Our findings elucidate the molecular basis underlying inter-segment interactions in rotaviruses, paving the way for studying genome packaging of other segmented RNA viruses. The integrated approach expands the arsenal of techniques much needed for delineating dynamic RNA–RNA interactions involved in the assembly of large ribonucleoprotein complexes.

97. Shuttling of Influenza a Virus Nuclear Export Protein: Dynamics of Oligomerization and Nuclear Export Studied by Fluorescence Correlation Spectroscopy

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The influenza virus genome is composed of eight single-stranded negative-sense RNAs forming viral ribonucleoprotein complexes (vRNP). Following transcription and replication of vRNPs in the nucleus of infected cells, newly assembled vRNPs are exported in a CRM1-dependent manner with the assistance of the viral export complex, consisting of the matrix protein 1 (M1) and the nuclear export protein (NEP). Whereas M1 does not possess any nuclear export signal (NES) but one nuclear localization signal (NLS), NEP exhibits two NESs at its N-terminus. To date, neither the stoichiometry of export-relevant viral proteins on single vRNPs, nor the regulation mechanism of the viral protein NEP are entirely understood. Therefore, to advance understanding of the mechanism that influenza viruses use for nuclear export and genome assembly, we aim to quantify NEP oligomerization- and export dynamics in an infection-relevant lung epithelial cell-line (A549) using fluorescence correlation spectroscopy (FCS). Here, we predict a regulation mechanism for NEP shuttling between the cytoplasm and nucleus of infected cells based on shielding and exposure of its two NESs, a mechanism commonly used by proteins transporting cargos between these two compartments. We observe concentration-independent, but phosphorylation-dependent dimerization of NEP in the cytoplasm, whereas in the nucleus a lower NEP dimer but higher monomer fraction is observed. Eventually, the influence of different viral components such as membrane-bound Hemagglutinin (HA), vRNAs or whole vRNPs on dimerization and shuttling of NEP will be further investigated.

98. The HCV NS5A Interacting Proteins TBC1D20 and Rab1 Have a Role in Lipid Droplet Formation

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Hepatitis C virus (HCV) is a positive strand RNA virus that persistently infects human hepatocytes. Lipid droplets (LD), dynamic intracellular lipid storage organelles, were identified as essential organelles for infectious HCV production. HCV non-structural protein 5A (NS5A) was previously shown to interact with the Rab1-GAP TBC1D20. This interaction was found to be indispensable for viral replication. We found that a subpopulation of Rab1 is associated with LDs. Furthermore, overexpression of its dominant-negative (DN) form abolished all steady state LDs. In infected cells, Rab1-DN induced the elimination of NS5A from viral replication sites. Loss of function mutations in TBC1D20, were found to be associated with Warburg Micro syndrome (WARBM), a severe genetic disorder. Interestingly, fibroblasts from WRBM patients and mouse fibroblasts with similar mutations, show enlarged LDs upon induction of LD formation, further linking Rab1 to LD biogenesis. This phenotype is rescued by transfection of TBC1D20 but not by a non-functional TBC1D20 (R105A) mutant. Also, Rab1-GFP and diacylglycerol O-Acyltransferase 2, an LD-associated enzyme that catalyzes the last step in triglyceride synthesis, were found in close proximity using FRET analysis. Our results support a model whereby Rab1 regulates the redistribution of biogenesis-essential factors to facilitate LD biogenesis.

99. The Role of the Pro-Rich Loop of CA in HIV-1 Assembly, Maturation and Infectivity

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HIV-1 assembly is driven by the viral structural polyprotein Gag that forms the immature lattice at the plasma membrane of infected cells. Assembly of the immature Gag lattice is driven, in part, by interactions between the capsid (CA) domains of Gag. Upon the cleavage of Gag molecules by the viral protease (PR), the CA protein forms a cone-shaped core in mature virions. We characterized the role in the viral replication cycle of a highly exposed Pro-Pro-Ile-Pro (PPIP) motif (CA residues 122–125) in the loop connecting helices 6 and 7 of the N-terminal domain of CA. Two mutations, P122A and I124A, significantly decreased infectivity, impaired replication activity and caused defects in Gag processing and virion morphology. The defects in assembly and release were also observed for PR-deficient P122A and I124A particles. We selected and characterized viruses containing compensatory mutations that are able to restore viral replication competency in the original mutants. Interestingly, the viral revertants exhibited variable replication activity in different cell types. Structural changes in host protein binding sites in in vitro-assembled mutant capsid cores were identified. Overall, these data demonstrate that the Pro-rich loop is important for the formation of the immature Gag lattice and for early post-entry events.

100. HCV NS5B phosphorylation

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Introduction: Previous studies from our laboratory (Valero-Hernández et al; AAC 2016, 23;60(6):3540-50) have shown that HCV NS5B could be phosphorylated *in vitro* by the cellular Ser/Thr kinase Akt. In addition, Akt and NS5B coimmunoprecipitate and colocalize. Furthermore, Akt regulates HCV infectivity. With these antecedents, we addressed the following objectives:

a) To identify residues in the primary amino acid sequence of HCV NS5B phosphorylated by Akt.
b) To check the effect of such phosphorylation on the *in vitro* polymerase activity of NS5B.

Results: NS5B peptides with Ser or Thr amino acids phosphorylated by Akt were identified by phosphoproteomics: S29 (or less probable S27), T53, T267 (or less probable S269), and S282.

To verify the effect of NS5B phosphorylation in the polymerase activity, we obtained several point mutants in which these residues (S27, S29, T53, S267, S269 and S282) were replaced by glutamic acid (phospho-mimetic) or by alanine. All these changes except T267E drastically decreased the polymerase activity of NS5B *in vitro*.

Conclusions: NS5B is phosphorylated *in vitro* by Akt. Ser/Thr residues phosphorylated by Akt were at positions 29 (or 27), 53, 267 (or 269), and 282. All these residues, except T267, are totally conserved through different genotypes. Proteins carrying mutations mimicking phosphorylated residues showed reduced RNA-polymerase activity than wild type NS5B. This result was in accordance with those previously obtained in our laboratory using Akt inhibitors in cell culture in the context of HCV infection.

In conclusion, Akt phosphorylates NS5B *in vitro,* and NS5B phophorylated by Akt showed reduced polymerase activity. Further experiments should be done to determine if NS5B phosphorylation acts as a switch from replication to assembly or NS5B phosphorylation is a mark to be added to those NS5B molecules that are going to do other regulatory actions instead of RNA replication into the cell.

101. Temperature-Sensitive Mutants as a Tool to Dissect the Influenza RNA-Polymerase Targeting to the Nucleus

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The influenza virus RNA-dependent RNA polymerase catalyzes genome replication and transcription within the nucleus. Efficient nuclear import and final assembly of the polymerase subunits PA, PB1 and PB2 are critical steps in the virus life cycle. We found that the PA linker plays a key role in the efficient transport of the PA+PB1 dimer into the nucleus. A large series of PA linker mutants engineered by substitution to proline codon or codon deletion displayed a temperature-sensitive (ts) phenotype, a phenotype we found to be associated to a block of the PA+PB1 dimer transport into the nucleus at restrictive temperature (39.5°C). PA virus mutants and additional PB1 mutants engineered at the PA linker interface exhibited reduced growth at 39.5°C versus 37°C/33°C, suggesting an alteration of folding kinetics parameters. The ts-phenotype was also associated with reduced efficiency of replication/transcription as measured in a minireplicon assay. While selection of revertant viruses was successful with substitution mutants, we failed to obtain an escape mutant with codon-deleted PA mutants. Complementation assays using PA and PB1 mutants and importin-beta IPO5 revealed residues associated to the formation of a stable complex involved in the efficient transport of PA+PB1 into the nucleus.

Poster Exhibition – Session 6: Antiviral Innate Immunity and Viral Pathogenesis

103. *Aedes aegypti* SUMOylation Pathway Suppresses Arbovirus Replication

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Mosquito-borne arboviruses pose an important threat to human and livestock health globally, as highlighted by recent outbreaks of Zika, chikungunya, and dengue viruses. Currently, the interactions between the mosquito antiviral responses and arboviruses are poorly understood; deciphering this will be crucial to the development of novel methods to limit arbovirus replication and transmission.

The small ubiquitin-related modifier (SUMO) pathway in mammals can either enhance or suppress the replication of a range of viruses (including dengue virus), yet the activity andbiological significance of this pathway in mosquitoes remain uncharacterised.

Biochemical comparison of the *Aedes aegypti* SUMOylation pathway with the *H. sapiens* SUMOylation pathway identified the conserved activity of the SP-RING domain in *Ae*PIAS, confirming its function as a SUMO ligase. Importantly, we found SUMO to be ubiquitously expressed throughout *Ae. aegypti* tissues that act as sites of arboviral replication. In vitro depletion experiments of the *Ae*SUMOylation pathway significantly enhanced replication of Zika and Bunyamwera viruses. This is the first study to highlight a vital role of the SUMOylation pathway in restricting the replication of arboviruses in *Ae. aegypti*.

104. *p400* Is Antiviral and Regulates the siRNA Pathway in *Aedes aegypti* Mosquitoes

Floriane Almire [1], Melanie McDonald [1], Joy Kean [1], Claire Donald [1], Alma Mcdonald [1], Mathilde Lauréti [1], Margus Varjak [1], Sandra Terry [1], Marie Vazeille [2], Anna-Bella Failloux [2], Alain Kohl [1], Emilie Pondeville [1]

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Aedes aegypti female mosquitoes are vectors of arboviruses such as Zika virus (ZIKV) and chikungunya virus (CHIKV). *Ae. aegypti* mosquitoes possess different immune pathways, of which the major small interfering exogenous (si)RNA pathway limits arbovirus replication. To identify new antiviral genes in *Aedes*, we screened mosquito cells from candidates previously shown to be involved in the siRNA pathway in the fly *Drosophila melanogaster*. Among candidate genes, we identified the gene *p400* (also called *domino*) as antiviral in mosquito cells and also in vivo in *Ae. aegypti* females. Moreover, p400 is regulating in vitro and in vivo the expression of *argonaute-2 (ago-2)*, a key player of the siRNA pathway, strongly suggesting that p400 is antiviral by mediating the exogenous siRNA pathway. Tissue-specific analysis of *p400* and *ago-2* gene expression and immunofluorescence assays show that p400 and ago-2 are both expressed in the same different female tissues. Interestingly, p400 regulates the siRNA pathway in a tissue-specific manner. These findings provide a novel insight into the regulation of the aedine antiviral exo-siRNA pathway.

105. Nitazoxanide Induces IRF-1 to Inhibit Human Norovirus Replication and Exhibits Potent Synergy with Ribavirin

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Norovirus is the main cause of nonbacterial gastroenteritis worldwide and results in more than 200,000 deaths annually. Despite being self-limiting, it could develop chronic infection and become debilitating and life-threatening in immunocompromised patients. The lack of a specific therapy promoted the development of antiviral drugs against norovirus. Recently, nitazoxanide (NTZ) has been used empirically in the clinic and demonstrates effectiveness against norovirus infection. However, the definitive anti-norovirus effect and the mechanism-of-action remain to be further investigated.

By using a human norovirus (HuNV) replicon model, we find NTZ and its active metabolite, tizoxanide (TIZ), exhibited inhibitory activity against HuNV RNA replication, with mean IC_{50} values of 1.080 and 0.942 μg/mL, respectively. Mechanically, NTZ activated cellular immune response and induced a subset of interferon-stimulated genes (ISGs), especially IRF-1. After NTZ treatment, there was a ~12-fold increase of IRF-1 mRNA and ~3.28-fold increase of IRF-1 protein, which is comparable to 1000 IU/mL of IFNα treatment. IRF-1 overexpression exerted potent inhibition of HuNV replication, whereas IRF-1 knockdown partially abrogated the antiviral activity of NTZ, demonstrating that NTZ specifically induced IRF-1 to combat HuNV. Furthermore, we explored the combination of NTZ with ribavirin; the latter was proven to be effective against HuNV infection in vitro and in vivo. NTZ synergistically suppressed HuNV with ribavirin and completely cleared HuNV replicons from host cells after long-term treatment.

Our results demonstrated that NTZ induced potent antiviral ISGs, in particular IRF-1, to combat HuNV replication. Nitazoxanide monotherapy and combination therapy with ribavirin are promising treatments against norovirus infection.

106. ZIKV Interactions within Murine "myelinating" CNS and PNS Co-Cultures

Stephanie Louise Cumberworth [1], Jennifer Barrie [2], Madeleine E. Cunningham [2], Verena Schultz [2], Daniely Paulino Gomes de Figueiredo [2], Adrian J. Wilder-Smith [2], Benjamin Brennan [1], Christopher Linington [2], Susan C. Barnett [2], Hugh J. Willison [2], Julia M. Edgar [2], Alain Kohl [1]

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The Zika virus (ZIKV) outbreak originating in Brazil (2015) was characterised by an unprecedented upsurge in severe neurological disorders. Zika congenital syndrome encompasses an expanding spectrum of neurodevelopmental manifestations of neonates, predominantly affecting the central nervous system (CNS). Peripheral nervous system (PNS) complications have also been described in adults. Herein, we present a "myelinating" multi-cell-type co-culture system to model ZIKV-mediated neurotropism and pathogenesis within the CNS and PNS. Co-cultures are derived from embryonic day-13 mouse (background strain A129) spinal cord (CNS) or dorsal root ganglion (PNS), either wild-type or knockout for *Ifnar*1, and infected with a Brazilian ZIKV isolate (ZIKV/H.sapiens/Brazil/PE243/2015). Infections were performed at pre- and post-myelinating stages of CNS culture development. Consequently, we were able to ascertain cell populations targeted during ZIKV infection and their relative susceptibility to infection in the presence or absence of type I interferon signalling. Our findings indicate that ZIKV impedes myelination within the CNS when co-cultures are challenged at a time point which reflects the developmental stage of a late foetal/early post-partum brain.

107. Adenovirus Types C5, D26, and B35 Neutralizing Antibodies (NAbs) Titre in Healthy Adults and Children in Burkina Faso: Impact of Adenovirus-Based Vaccines Derived From "Rare" Types

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Adenovirus (AdV)-based vectors are one of the most efficient antigen delivery tools. However, pre-existing adenovirus immunity can greatly modify AdV's efficacy and toxicity. Despite encouraging preclinical and Phase I/II trial results, Human AdV type C5 (HAdV-C5) vectors used in three Phase II trials targeting HIV were prematurely interrupted because of the lack of efficacy and a higher rate of HIV acquisition in HAdV-C5 seropositive recipients. Subsequently, a major impetus for vaccine development favored AdV types with low seroprevalence in Europe and North America (e.g., HAdV-D26 and HAdV-B35). Numerous AdV-based vaccine candidates using HAdV-D26 and HAdV-B35 are being evaluated in humans. Here, we show that HAdV-D26 neutralizing antibody (NAb) titre is indeed low in the French and North American population, but high (73%) in the Burkinabé population. In contrast, Ad35 serology seems low for all populations tested. Using an experimental ex vitro model based on dendritic cell activation by immune complexed AdVs, our data indicated that West Africa is not the ideal region for HAd26-D26-derived vaccines. Our work provides insight into the possibility of using specific AdV types for vaccines according to the geographical area populations that may be at risk for pathogen outbreaks, and also emphasizes the gaps in our knowledge of AdV seroprevalence.

108. Antimicrobial Peptides Can Bind Human Adenovirus Types and Modify the Innate Immune Response of Human Dendritic Cells

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Adenoviruses (Ads) infect mucosal surfaces, leading to mild symptoms in most immune-competent individuals. Despite robust immune responses, many Ads establish decades-long persistent infections. To understand the innate immune response to Ads, we previously characterized the impact of serum components on Ad interaction with dendritic cells (DCs). Here, we asked if antimicrobial peptides (AMPs) influence the innate immune response to Ads, because a handful of AMPs can function as immunomodulators during viral infection. Using SPR, we found differential binding of three AMPs (lactoferrin, HNP-1, and LL-37) to Ads from species C, D, and B. Lactoferrin and HNP-1 binding increased virus uptake/delivery to the nucleus of DCs, which was mirrored in most cases by increased TNF secretion and up-regulation of mRNAs that code for cytokines and inflammasome components (e.g., *IL1b, TNF, AIM2, NLRP3*). The DC-challenged secretome contained an array of pro-inflammatory cytokines (including IL-1β) and chemokines. Interestingly, caspase-1 activation and IL-1β secretion were associated with the generation of NLRP3 inflammasome without loss of membrane integrity. This mechanism is in contrast to that seen by antibody-complexed HAds, which activate the AIM2 inflammasome and lead to DC pyroptosis. Together, our data point towards an immunomodulatory role(s) of AMPs during HAdV infections.

109. Beyond Retroviruses: Potent Restriction of Flaviviruses by Primate TRIM5

Kristin McNally [1], Abhilash Chiramel [1], Sara Sawyer [2], Nicholas Meyerson [2], Shelly Robertson [1], Vanessa Montoya [1], OMayra Mendez [1], Gail Sturdevant [1], Sonja Best [1]

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TRIpartite Motif protein-5a (TRIM5α) is an important cellular inhibitor of retroviruses that functions in a highly host and virus species-specific fashion by binding to incoming virus capsids to disrupt uncoating and reverse transcription. However, TRIM5α is thought to be specific for retroviruses, with human TRIM5a only possessing limited function against HIV-1. We previously showed that a mouse paralog of primate TRIM5α, TRIM30D, restricts specific flaviviruses in the tick-borne encephalitis virus (TBEV) serogroup. Thus, we tested whether primate TRIM5α could also restrict flavivirus replication. Expression of either human or rhesus macaque TRIM5α potently inhibited replication of TBEV and closely related viruses by up to 1000-fold, but not dengue (DENV), yellow fever (YFV) or Zika (ZIKV) viruses. TRIM5α bound and degraded specific viral nonstructural proteins involved in RNA replication, revealing a remarkable plasticity of TRIM5α in recognition of diverse viral molecular-patterns. CRISPR/Cas-deletion of *TRIM5* expression in human HAP1 cells rescued $2\log_{10}$ replication of sensitive viruses in the presence of type-I IFN, suggesting that human TRIM5α is functional and participates in antiviral responses. Evolution by emerging flaviviruses including DENV, YFV and ZIKV to evade TRIM5 may facilitate the establishment of urban transmission cycles in humans without the need for an intermediate amplifying primate host.

110. Caspase-Dependent Suppression of Type I Interferon Signaling Promotes KSHV Lytic Replication

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Type I interferon (IFN) responses are a key antiviral defense triggered by the recognition of foreign nucleic acids by pathogen sensors. Thus, in order to successfully establish infection, viruses must block or reduce type I IFN responses. Consistent with this idea, there is little induction of type I IFN during lytic infection with Kaposi's sarcoma-associated herpesvirus (KSHV)—the causative agent of Kaposi's sarcoma (KS), an AIDS-defining malignancy and the leading cause of cancer death in sub-Saharan Africa. Here we report that KSHV takes advantage of a cellular caspase-dependent pathway to limit type I IFN induction during reactivation of lytic infection. We have found that during KSHV lytic reactivation, caspase activity prevented transcription and secretion of the type I IFN IFNβ by interfering with signaling through the TBK1/IKKε-IRF3 pathway. In addition, inhibition of caspases reduced overall viral gene expression, viral DNA replication, and virion production, which was rescued when IFNb was knocked down. These results indicate that caspase activity promotes KSHV replication by suppressing IFNb induction. We found a similar effect of caspase activity in both iSLK.219 epithelial cells and TREx-BCBL1 lymphoma cells—two cell culture models of KSHV infection. Interestingly, we did not observe an increase in cell death during lytic infection, despite activation of apoptotic caspases, suggesting that apoptosis is blocked during KSHV lytic infection. Overall, our results show that caspase-mediated regulation of pathogen sensing machinery is an important mechanism exploited by KSHV to evade innate immune responses.

111. Characterization of the IFN Antagonistic Abilities of European Tick-Borne Flaviviruses: A Focus on Louping Ill and Tick-Borne Encephalitis Virus

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Louping ill virus (LIV; *Flavivirus, Flaviviridae*) is an important—but poorly characterized—animal pathogen of economic concern within the UK. Transmitted by ticks, LIV predominantly causes disease in ruminants and grouse, while human cases are rarely reported. LIV is closely related to another *Flavivirus*—tick-borne encephalitis virus (TBEV), a significant human pathogen that is prevalent throughout Europe. The mechanisms that underpin host restriction in these viruses are poorly understood. In an effort to fully characterize LIV, we sequenced 20 LIV genomes and performed phylogenetic analysis to determine the recent evolutionary history of the virus. It has previously been shown that the TBEV non-structural (NS) genes do not act as type-I interferon (IFN) antagonists. Therefore, we investigated possible antagonistic actions of the LIV NS proteins. Using a luciferase-based reporter assay, we identified NS2A as a type-I IFN antagonist, exerting its effect at the level of TBK1, within the IFN induction cascade. We also identified and modeled a subgenomic flavivirus RNA (sfRNA) that is produced during LIV infection, and have shown that this also acts as a type-I IFN antagonist. Investigating the molecular mechanisms that underlie LIV infection aids our understanding of the evolution of Flaviviruses and the molecular determinants of host restriction.

112. De Novo Expression of Intron-Containing HIV-1 RNA Triggers Innate Immune Activation

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A hallmark of HIV-1 infection in vivo is systemic chronic immune activation, which has been postulated to lead to HIV-associated non-AIDS complications. Despite long-term viral suppression by anti-retroviral therapy (ART), immune activation and type I IFN (IFN-I)-dependent inflammatory state persists in the majority of HIV-infected individuals, and is associated with excess risk of mortality and morbidity. In this study, we report that the establishment of productive infection in macrophages results in the induction of IFN-I-dependent pro-inflammatory responses. Upregulation of IFN-I responses was suppressed by pre-treatment with AZT or raltegravir but not indinavir, suggesting that a post-integration step in the viral life cycle triggered innate immune activation in macrophages. Interestingly, infection of macrophages with Rev-deficient HIV-1 that fails to export intron-containing unspliced viral RNA (usRNA), but not HIV-1 mutants which lacked expression of Gag-pol, Vif, Vpr, Vpu, Env or Nef, abrogated induction of IFN-I responses. Furthermore, rescue of nuclear export of usRNA by addition of the MPMV constitutive transport element (CTE) in Rev-deficient HIV-1 failed to induce IFN-I responses, suggesting that Rev–CRM1-dependent nuclear export of HIV-1 usRNA activates host sensing mechanisms. Ability of cells to distinguish intron-containing HIV-1 usRNA from self mRNA was dependent on localization of non-self HIV usRNA at peripheral membrane sites and trigger non-receptor tyrosine kinase-initiated signaling cascades. These findings suggest the presence of a novel innate sensing mechanism that detects newly transcribed HIV-1 usRNA, and that persistent expression of viral usRNAs in long-lived tissue-resident macrophages might contribute to the chronic inflammatory signature in ART-suppressed individuals.

113. Decay Accelerating Factor as a Virulence Determinant in Influenza a Virus Infection

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The complement is no longer considered a mere killer of infected cells and pathogens, but is viewed as a key player in immunity. It bridges innate and adaptive responses, and orchestrates the intensity of immunological and inflammatory processes by communicating with immune cells. Interactions are beginning to be fully appreciated, and their identification is crucial, as excess complement activation is associated with severe outcomes in many infections. The complement must be selective enough to avoid mounting a potent attack against the host. The self-targeting deleterious effects of the complement are avoided via a series of so-called regulators of complement activation (RCA) whose function is perfect for viral targeting. Amongst the RCAs, complement decay-accelerating factor (DAF or CD55) and CD59 block the complement cascade at central and terminal points, respectively, and localise ubiquitously at the apical surface of polarised cells. The lack of DAF and CD59 is associated with over-stimulation of the complement, resulting in increased inflammatory cytokines and worse outcomes in several models of infection and autoimmunity. We found that, conversely to what was observed in these models, in IAV infection the lack of DAF, but not of CD59 (used as control), mitigates the disease outcome. Our results suggest a completely novel mechanism that bypasses the well-established immune evasion strategy of protecting virions from complement-mediated attack through incorporation of RCAs in their envelopes. In fact, our data shows that DAF-deficient mice display less inflammatory signs in the lungs, and resolve the inflammation faster without affecting viral clearance. Mechanistically, we have evidence that DAF contributes to the recruitment of neutrophils and monocytes. Our results contribute to better defining virulence factors in IAV infection and understanding how components of the complement communicate with other arms of host immunity.

114. Dengue Virus Infection Alters the Activation Phenotype of Dendritic Cells Exposed to Conditioned Media of Dermal Fibroblasts

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Dengue virus (DENV) enters the host during the mosquito feeding process, and thus the skin is the first organ that comes in contact with the virus and its primary replication site. However, little is known regarding the innate immune response in this organ and how it modifies the microenvironment in response to infection. The aim of our study is to evaluate the activation phenotype of monocyte-derived dendritic cells (moDCs) infected with DENV, previously activated by human dermal fibroblasts (HDFs). In order to reach this objective, HDFs were activated with poly(I:C) and the conditioned media was used to culture moDCs for 24 h. After that, the moDCs were infected with DENV at 2 MOI and 48 hpi, and activation markers were evaluated by flow cytometry. Our results show that moDCs express maturation and activation markers such as CD40, CD83, and HLA-DR. In response to DENV, CD40 is down-regulated, while DC83 and HLA-DR are retained and no expression of CD80 and CD86 is observed. These results suggest that the microenvironment generated by HDFs affects the activation phenotype moDC in response to DENV, possibly becoming dysfunctional at initiating an adaptive immune response.

115. Development of *Aedes aegypti* Mosquito Cell Lines Stably Expressing Zika Virus Proteins

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Recent outbreaks of Zika Virus (ZIKV) infection in the Americas have led to a global public health alarm. Causing febrile illness similar to other arboviral infections, it was recently associated with cases of microcephaly in neonates of mothers infected during pregnancy. With the threat of superinfection or co-infection with other arboviruses transmitted by the same *Aedes* mosquito vector, effort has been made in studying the molecular interaction of ZIKV and its vector. We constructed plasmids that encode for different V5-tagged ZIKV proteins and zeocin resistance markers to construct cell lines based on *Aedes aegypti* Aag2 cells. The produced cell lines had stable expression of ZIKV proteins, as shown by protein half-life and immunoprecipitation assays. Confocal microscopy was able to show protein localization and distribution within the developed mosquito cell lines. Further experiments were designed to assess the functionality of the cell lines. To simulate superinfection, the cell lines were infected with Semliki Forest Virus (SFV) or ZIKV at low multiplicities of infection (MOIs) to know the effect of ZIKV proteins on viral replication. RNA interference activity of the cell lines was also evaluated to determine the role of ZIKV proteins in evading mosquito immunity.

116. Epstein–Barr Virus Infection of Epithelial Cells Induces APOBEC3C and Generates Mitochondrial DNA Mutation

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Background: Epstein–Barr virus infects 90% of adults and is associated with various types of cancer. The oncogenic role of EBV in an epithelial tumor has not been fully elucidated compared with B lymphoma. The anti-viral APOBECs (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) may induce C-to-T nucleotide mutation not only in the viral, but also in the host or mitochondrial genome. Expressions of APOBEC3A (A3A), 3C, 3G, and 3H which show cytoplasmic localization. were induced upon EBV infection. Their expression induced mitochondrial DNA (mtDNA) mutation, and reduction of mtDNA copy number in epithelial cells. Since A3C induced mtDNA mutation most frequently, A3C was disrupted to clarify its role in EBV infection.
Method: The A3A, A3C, A3G and A3H expression vector was transfected into epithelial AGS cells expressing CD21 receptor (AGS-CD21). The frequency of C-to-T mutation was evaluated by the 3D-PCR method. Cas9 protein and the A3C-specific guide RNA complex were transfected into AGS-CD21 cells for gene disruption (A3CKO). A3C protein expression was examined by Western blotting; DNA mutations were examined by sequencing. AGS-CD21 and A3CKO cells were infected with a recombinant Akata EBV expressing eGFP. The copy number of mtDNA was calculated by qPCR.
Result: A3A, A3C, and A3G induced mtDNA mutations in epithelial cells. Frequency of mutation was highest at A3C. A3C transfection induced C-to-T mutation in the mtDNA D-loop in a dose-dependent manner. A3CKO cell clones showed similar morphology to the parental cell. The frequency of mtDNA mutation by EBV infection was lower in A3CKO clones than wild-type cells. The copy number of mtDNA was not reduced by EBV infection of A3CKO clones.
Discussion: A3CKO clones showed infrequent mtDNA mutation. The mtDNA mutation during EBV infection should have significance in the viral lifecycle or fate of latently EBV-infected cells including transformation or apoptotic cell death.

117. HIV-1 Activation of Innate Immunity Depends Strongly on the Intracellular Level of TREX1 and Sensing of Incomplete Reverse Transcription Products

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TREX1 has been reported to degrade cytosolic immune-stimulatory DNA (including viral DNA generated during HIV-1 infection), but the dynamic range of its capacity to suppress innate immune stimulation is unknown, and its full role in the viral life cycle remains unclear. A main purpose of our study was to determine how the intracellular level of TREX1 affects HIV-1 activation and avoidance of innate immunity. Using stable over-expression and CRISPR-mediated gene disruption, we engineered a range of TREX1 levels in THP-1 monocytes. Increasing the level of TREX1 dramatically suppressed HIV-1 induction of interferon-stimulated genes (ISGs). Productive infection and integrated proviruses were unchanged or increased. Knocking out TREX1 impaired viral infectivity, increased early viral cDNA, and caused log-fold increases in HIV-1 ISG induction. cGAS knockout abrogated all ISG induction. Moreover, cGAS knockout produced no increase in single cycle infection, establishing that HIV-1 DNA-triggered signaling is not rapid enough to impair the initial ISG-triggering infection cycle. Disruption of the HIV-1 capsid by PF74 also induced ISGs, and this was TREX1 level-dependent, required reverse transcriptase catalysis, and was also eliminated by cGAS gene knockout. Thus, the intracellular level of TREX1 pivotally modulates innate immune induction by HIV-1. Partial HIV-1 genomes are the TREX1 target, and are sensed by cGAS. The nearly complete lack of innate immune induction despite equal-to-increased viral integration observed when the TREX1 protein level is experimentally elevated indicates that integration-competent genomes are shielded from cytosolic sensor-effectors during uncoating and transit to the nucleus.

118. HIV-1 Modulates Host Cell Metabolism by Controlling Late Endosome/Lysosome Positioning and Motility

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The major barrier to the eradication of HIV-1 is the elimination of viral reservoirs—the establishment and maintenance of which have been shown to be dependent on mammalian target of rapamycin (mTOR) complexes (C)1 and C2. mTORC1 serves as the cell's major metabolic hub that couples nutrient sensing to cellular homeostasis, and is the main regulator of autophagy—a catabolic process critical for cell maintenance. The effects of HIV-1 on autophagy during infection are complex and depend on the cell type and on the status of the cell, but collectively contribute to viral persistence and immune evasion. In previous studies, we showed that HIV-1 activates mTORC1 and promotes late endosome/lysosome (LE/Lys) repositioning, which aretwo key mechanisms that directly regulate autophagy. In this work, we further investigate the molecular mechanisms of the HIV-1-mediated modulation of autophagy. Using a combination of pH-sensitive lysosomal dyes and high-resolution confocal microscopy in live cells, we show that HIV-1 generates a cellular environment that resists autophagy induced by various stressors, including oxidative stress and starvation. We also provide evidence that HIV-1 hinders LE/Lys acidification, resulting in a defect in LE/Lys degradative activity and motility. We then demonstrate that HIV-1 loses its ability to prevent autophagy upon disruption of LE/Lys trafficking, suggesting that HIV-1 depends on its ability to commandeer LE/Lys to inhibit autophagy. Furthermore, by inducing autophagy with the IMPase inhibitor lithium chloride, HIV-1's ability to inhibit autophagy was supressed, and as a consequence viral replication was drastically reduced. To conclude, a greater understanding of HIV-1's ability to commandeer mTOR, LE/Lys positioning, and the repercussions on autophagy and host cell metabolism will provide new insights into the development of a functional cure for HIV-1.

119. Human Cytomegalovirus-Encoded GP39 Targets MAVS- and STING-Signaling Pathways to Evade Host Interferon Immune Response

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Human cytomegalovirus (HCMV) has developed highly sophisticated immune evasion mechanisms that target both the innate and adaptive immune responses. However, the HCMV-encoded proteins involved in this immune escape have not been fully elucidated. Here, we report that HCMV glycoprotein GP39 inhibits the IFN-β response by targeting the mitochondrial antiviral-signaling protein (MAVS)- and stimulator of interferon genes (STING)-mediated signaling pathways. GP39 accumulation in mitochondria attenuates the mitochondrial membrane potential, leading to the promotion of MAVS leakage from the mitochondria. Furthermore, GP39 disrupts STING oligomerization and STING-TBK1 association through competitive interaction. Intriguingly, GP39 blocks interferon regulatory factor 3 (IRF3) nuclear translocation and its cytoplasmic domain is essential for inhibiting IRF3 activation. A mutant HCMV lacking US7-16 showed impairment in the antagonism of MAVS/STING-mediated IFN-β expression, reversible by the introduction of GP39. Our findings reveal that the HCMV GP39 acts as an antagonist of IFN signaling to persistently evade host innate antiviral responses.

120. Humoral Immune Responses to Adenovirus Induce Tolerogenic Dendritic Cells That Promote Regulatory T Cells and Influence Viral Persistence

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Following repeated encounters with ubiquitous viruses such as human adenoviruses (HAdVs), robust humoral and cellular immune responses are thought to act together to combat subsequent infections. Yet, HAdVs can establish persistent, subclinical, infections that last for decades in healthy individuals. However, in T-cell-, but not B-cell-compromised individuals, reactivation of persistent HAdV constitutes a life-threatening risk. This dichotomy led us to ask if there is a path between B and T cell communication that HAdVs exploit to foster their persistence. To address HAdV persistence, we show that healthy adults harbor HAdV-specific regulatory T cells (T_{regs}). Because peripheral-induced T_{regs} are generated by tolerogenic dendritic cells (DCs), we investigated the involvement of immunoglobulin-complexed (IC)-HAdVs, that induce DC maturation or pyroptosis. Using transcriptome and secretome profiling, and pharmacological, biochemical, genetic, molecular, and cell biological approaches, we demonstrate that IC-HAdVs induce tolerogenic DCs that can drive naïve T cells to mature into T_{regs}. We propose that IC-HAdVs favor HAdV persistence by exploiting a novel path in the communication between B and T cell responses. Our results could impact therapy and pre-screening for high-risk individuals undergoing immunosuppression.

121. Identification of Tripartite Motif Proteins That Regulate Antiviral Innate Immune Response in Human Primary Plasmacytoid Dendritic Cells

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Interferons (IFN) are key components of innate immune response and the first line of antiviral defense. IFN response is triggered by the detection of viral RNA or DNA by host pattern recognition receptors (PRRs), which initiate signaling cascades resulting in the secretion of IFN by infected cells. By inducing the expression of hundreds of genes, IFNs establish an antiviral state in surrounding cells, thus preventing viral spread.

Tight control of IFN production is crucial in order to mount an efficient antiviral defense while preventing detrimental effects. Among other mechanisms of control, tripartite motif (TRIM) proteins have recently emerged as important regulators of intracellular immune signaling. It is still unclear however, whether TRIM proteins have a real impact on antiviral defense in relevant cells in vivo.

We therefore sought to determine the capacity of TRIM protein to regulate IFN response in plasmacytoid dendritic cells (pDCs) which constitute by far the main IFN-producing cell subset. To do this, we systematically quantified the expression of all TRIM genes in steady-state and HIV-1 or influenza virus-infected primary pDCs. By further silencing the endogenous expression of relevant candidates, we identified TRIM family members acting as positive or negative regulators of IFN response in human pDCs.

122. LGP2 Holds A "PACT" with the RNA Silencing Machinery

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The RIG-I-like receptors trigger inflammatory and antiviral responses by sensing non-self RNA molecules produced during viral replication. To-date, three RLR members have been identified: RIG-I (retinoic acid-inducible gene-I), MDA5 (melanoma-differentiation-associated gene 5), and LGP2 (laboratory of genetics and physiology 2). Upon ligand recognition, MDA5 and RIG-I trigger a signaling cascade through mitochondrial antiviral-signaling protein (MAVS) on the mitochondria and peroxisomes, whereas LGP2 is involved in regulating MDA5 and RIG-I activities. LGP2 regulation of RIG-I and MDA5-dependant type-I interferon signaling is a matter of controversy. We show that LGP2 interacts with different components of the RNA silencing machinery. Particularly, we identified a direct protein–protein interaction between LGP2 and interferon-inducible double-stranded RNA-dependent protein kinase activator A (PACT). The LGP2–PACT interaction is mediated by the regulatory C-terminal domain of LGP2, and is necessary for inhibiting the RIG^{-I-} and amplifying the MDA5-responses. We describe a point mutation within LGP2 that disrupts LGP2–PACT interaction and leads to the loss of LGP2 regulatory activity over RIG-I and MDA5. These results provide a model in which PACT–LGP2 interaction regulates RIG-I and MDA5 inflammatory response and allows cellular RNA silencing machinery to coordinate the innate immune response.

123. Modulation of the NF-kB Signaling Pathway by the HIV-2 Envelope Glycoprotein and Its Incomplete BST-2 Antagonism

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HIVs exploit the transcription factor NF-kB to ensure efficient LTR-driven gene transcription. However, NF-kB is primarily known to act as a key regulator of the proinflammatory and antiviral responses. Interestingly, retroviruses activate NF-kB during early stages of infection to initiate proviral genome expression while suppressing it at later stages to restrain the expression of antiviral genes. During HIV-1 infection, diverse viral proteins such as Env, Nef and Vpr have been proposed to activate NF-kB activity, whereas Vpu has been shown to inhibit NF-kB activation.

It is still unclear how HIV-2 regulates the NF-kB signaling pathway during its replication cycle. To date, no study has investigated either the effects of the HIV-2 Env protein on NF-kB activation, or the impacts of the Env-mediated anti-BST-2 antagonism on the modulation of the NF-kB signaling pathway. Here, we tested the potential impacts of HIV-2 Env on the NF-kB activity.

Importantly, we demonstrate for the first time that the HIV-2 Env induces NF-kB activation in HEK293T cells. Furthermore, the anti-BST-2 activity of the late HIV-2 Env is not sufficient to completely inhibit NF-kB activity. This study could provide new insights into the understanding of the enhanced immune antiviral responses in HIV-2 disease as compared to HIV-1.

124. Molecular Characterization of Hepatitis C Virus Resistance-Associated Substitutions after Interferon-Free Treatment Failure by Massive Parallel Sequencing

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Introduction: At present, more than 60,000 chronically Hepatitis C-infected patients have started direct-acting antiviral (DAA) treatments in Spain. It has been estimated that around 2% will fail because of the selection of viral resistant-associated substitutions (RASs), thus conditioning future therapeutic options due to the cross-resistance with inhibitors of the same family.
Methods: More than 160 treatment failure patients were recruited from 29 Spanish hospitals with collaboration of CIBER of Liver and Digestive Diseases

(Ciberehd). All serum samples were genotyped, and RASs were analyzed by next-generation sequencing (NGS).
Results: 90% of the patients showed RASs in at least one analyzed region (NS3, NS5A, and/or NS5B). The subtype distribution among all treatment failure samples was 53% G1b, 16% G1a, 17% G3a, 9% G4d, 1% G2j, 1% G2c, 1% G4a, and 2% mixed infections. The most prevalent RASs were detected in positions 80, 122, 155, and 168 in the NS3 region, positions 28, 30, 58, and 93 in the NS5A region, and positions 159 and 316 in the NS5B region. Moreover, resistance substitution S282T—which has been directly associated with resistance to sofosbuvir (SOF), despite causing a dramatic viral fitness decay in cell culture experiments—has been detected in 4% of SOF-treated patients.
Conclusion: The extensive characterization of the spectrum of substitutions by massive parallel sequencing is an essential tool to identify resistance-associated substitutions, combination of variants in the same sequence, and their frequency during direct-acting antiviral-based treatment failures. This information will help in designing the most accurate retreatment option for these patients.

125. Nef Antagonizes TIM-Mediated Inhibition of HIV Release: Role of SERINC

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The T cell immunoglobulin and mucin domain (TIM) proteins inhibit the release of HIV-1 and other enveloped viruses by interacting with cell- and virion-associated phosphatidylserine (PS). Here, we show that the Nef proteins of HIV-1 and other lentiviruses antagonize TIM-mediated restriction. TIM-1 more potently inhibits the release of Nef-deficient relative to Nef-expressing HIV-1, and the ectopic expression of Nef, or knockdown of TIM proteins, relieves restriction. HIV-1 Nef does not significantly downregulate TIM-1 expression, but promotes its internalization from the plasma membrane. Intriguingly, depletion of SERINC proteins attenuates TIM-mediated restriction of HIV-1 release, especially that of Nef-deficient viruses, indicating that Nef counteracts TIM-1, at least in part, through SERINCs. Consistent with this model, MLV glycoGag and EIAV S2 proteins also counteract TIM-mediated inhibition of HIV-1 release. Collectively, our work reveals a new role for Nef in antagonizing TIM-1, and highlights a complex interplay between Nef and HIV-1 restriction by TIMs and SERINCs.

126. Pathological Modelling of Tick-Borne Encephalitis Virus Infection Using Brain Cells Derived from Human Fetal Neural Progenitors

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Tick-Borne Encephalitis Virus (TBEV), a member of the Flaviviridae family, genus *Flavivirus*, is the major arbovirus of health interest in Central/Northern Europe and North-Eastern Asia. The virus is responsible for febrile illness, and in some cases, for neurological manifestations that may cause permanent disability or death. Although neurons have been described as the primary target of infection, TBEV-induced neuropathogenesis remains poorly understood. Here, we used an in vitro model of neural cells differentiated from fetal human Neural Progenitor Cells (hNPCs) to question the mechanisms by which the virus damages the human brain. Our results show that TBEV replicates in this heterogeneous culture. Both neurons and glial cells (astrocytes and oligodendrocytes) are susceptible and permissive to the virus. In neurons, a cytopathic effect was observed as early as 72 h post-infection, followed by a continuous apoptotic cell loss for the length of the study (14 days). Whereas glial cell survival was not impaired, cells were affected, as a hypertrophy—suggesting a reactive stage—was observed. By showing that neurons were impaired in their survival and glial cells enter a reactive stage upon TBEV infection, we demonstrated that our in vitro model recapitulates two main events occurring in vivo. We further set up monocultures of both neurons and astrocytes and are currently investigating the role of astrocytes in neuronal death. Thereafter, to decipher the cellular response to infection, we analyzed the expression levels of antiviral response genes using a PCR array. We reported an overexpression of viral sensors, cytokines and Interferon-Stimulated Genes, indicating an activation of the antiviral response. Using RNAi approaches, we are currently determining the involvement of each viral sensor in TBEV recognition. Thus, we developed a new in vitro model of TBEV infection that we are currently using to deepen our understanding of TBEV-induced neuropathogenesis.

127. PML Is a Restriction Factor of Enterovirus 71 Replication through Inhibition of Autophagy

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The PML (promyelocytic leukemia) protein—also known as TRIM19—functions as the major organizer of PML nuclear bodies (NBs) in most mammalian cells, and plays important roles in antiviral activities against both DNA and RNA viruses. In the current study, we found that the down-regulation of PML rendered HeLa cells more susceptible to infection by enterovirus 71, and the overexpression of PMLIII or PMLIV isoform inhibited viral protein expression, leading to viral titers 2 to 3 log units lower in comparison to the control. Using short interfering RNAs, we demonstrated that the downregulation of either PMLIII or PMLIV isoform increased both viral protein VP1 expression and virus production. We showed that PML repressing EV71 replication was in part mediated through inhibition of autophagy, and PML deficiency triggered autophagy. We further observed that EV71 infection resulted in the reduction of PML, which is independent of the proteasome pathway. Instead, PML degradation was mediated by virus protease $3C^{pro}$. We conclude that PML contributes to a cellular antiviral effect through inhibition of autophagy, which is countered by the disruption of PML nuclear bodies mediated by viral protease $3C^{pro}$.

128. R5-Tropic HIV Resistance in a Subset of Elite and Viremic Controllers

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Elite and Viremic Controllers (EC/VCs) are able to control HIV. Our aim is to identify EC/VCs with intrinsic resistance to HIV and perform cell and genetic studies to determine if there is an associated hereditary basis.

$CD4^+$ T cells from EC/VCs were purified, activated, and infected with replication-defective HIV of different tropisms, analyzed after 3 days by flow cytometry as %YFP+ transduced cells and results were compared with healthy donors (CTRL) and progressors on therapy (prog). CCR5 and CCR2 surface levels and RNA expression levels were analyzed; $CD4^+$ T cells from two EC family members were similarly analyzed.

For most samples, there was no resistance to any of the pseudotyped HIV particles in activated $CD4^+$ T cells. However, 16% of EC/VCs showed 4-fold resistance, on average, specific to R5-tropic virus. Decreased CCR2 and CCR5 RNA levels in EC/VCs with the R5 resistance phenotype were observed compared to CTRL and prog. CCR2 and CCR5 mRNA levels were positively correlated ($r = 0.93$) and not correlated with lnc LOC102724297. R5 resistance was not observed in macrophages and depended how the T cells were activated.

Resistance specific to R5-tropic HIV was observed in some family members of the index EC, associated with CCR2 and CCR5 down-regulation, suggesting a common regulatory mechanism. Half-lives of CCR5/CCR2 RNA were not altered, excluding a post-transcriptional mechanism. RNA-Seq suggested global down-regulation encompassing ~500 kbp of the chromosome 3 locus. Current efforts are focused on determining whether this is due to a transcriptional vs. epigenetic mechanism.

129. RNA-seq Analyses of Picornaviral RdRP-Transgenic Mice Reveals Critical Regulatory Pathways

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Cellular recognition of pathogen-associated molecular patterns such as viral dsRNA leads to the activation of interferon-stimulated genes (ISGs), which have diverse sensor-effector functions that protect the host. While transient ISG upregulation is critical for antiviral immunity, constitutive ISG activation can trigger harmful autoimmunity. We previously used RNA microarrays to show that transgenic mice expressing a picornavirus RNA-dependent RNA polymerase (RdRP-mice) exhibit quantitatively dramatic, life-long, and MDA5-MAVS-dependent upregulation of many ISGs (*PLoS Path* 2016, PMID 26633895). A most intriguing feature is that unlike other constitutive MDA5 activation states in mice and humans, the mice develop no autoimmune or autoinflammatory problems. They also do not differ from wild-type (WT) mice in longevity, habitus, behavior, or various other health assessments. Here, we used RNA-seq to compare patterns of gene expression between four biological replicates each of WT and RdRP-mice. We identified 156 and 273 genes that were upregulated and downregulated in RdRP-mice relative to WT mice, respectively. Of the upregulated genes, ~79% (123) are known ISGs, 12 of which were up by more than 50-fold. Functional enrichment analyses revealed that RdRP-mice are upregulating genes that lead to the activation of the innate immune system, type I interferon response, and defenses to viruses, and the inhibition of viral infection, inflammation, morbidity, and mortality, consistent with the relative health of RdRP-mice. Finally, we used Ingenuity Pathway Analyses (IPA) to predict the activation or inhibition of molecular pathways associated with genes that were differentially expressed. We identified potential upstream regulators of these pathways, and biological functions that they likely influence. We will also present comparisons of results of these RNA-seq analyses to other publicly available RNA-seq datasets in mice with and without harmful autoimmune phenotypes to determine commonalities and differences. The results of this study advance our understanding of how mice expressing RdRP can exhibit lifelong upregulation of ISGs without experiencing the negative consequences of autoimmunity.

130. SIV Infection of Tissue Macrophages Is Critical for TB Reactivation in Rhesus Macaques

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Tuberculosis (TB) and HIV/AIDS profoundly affect the immune system and synergistically accelerate disease progression. It is believed that $CD4^+$ T cell depletion by HIV is the major cause of immunodeficiency and reactivation of latent TB. Previous studies demonstrated that blood monocyte turnover concurrent with tissue macrophage death from virus infection, better predicted AIDS onset than $CD4^+$ T cell depletion in SIV-infected macaques. Here, we describe the contribution of macrophages to the pathogenesis of *Mycobacterium tuberculosis* (*Mtb*)/HIV co-infection. We found that increased monocyte/macrophage turnover and levels of SIV-infected lung macrophages correlated with TB reactivation. All *Mtb*/SIV co-infected monkeys exhibited declines in $CD4^+$ T cells regardless of reactivation or latency outcomes, negating lower $CD4^+$ T cell levels as a primary cause of *Mtb* reactivation. Results suggest that SIV-related damage to macrophages contributes to *Mtb* reactivation during co-infection. This also supports strategies to target lung macrophages for the treatment of TB.

131. Study of the Interactions of Zika Virus with the Antiviral Responses by Experimental Evolution

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Zika virus (ZIKV) infection causes neurological diseases and birth defects, representing an important threat for human health. Recent studies demonstrated that interferon (IFN) response is pivotal for the control of ZIKV spread, and that its in utero transmission and protects against ZIKV-induced neurological diseases. To delineate the genetic interactions with the IFN response in ZIKV genome and the viral escape mechanisms, we conducted an unbiased genomic approach based on the study of the evolution of the entire viral quasi-species in response to experimental modulations of the host antiviral signalling. First, we set up an experimental cell culture system to exert a selective pressure on ZIKV replication by the Toll-like receptor (TLR)-3-induced antiviral signaling. We uncovered that cell culture passaged ZIKV populations adapt to the antiviral response, as determined by viral resistance to TLR3-induced restriction by five iterative passages. We observed that passaged viruses no longer induce IFN response, as assessed by the absence of interferon-stimulated gene upregulation, concomitantly with an increased ability to replicate (approximately ten-fold as compared to input virus). Importantly, we developed methods for high-resolution analysis of the viral evolution and reconstruction of viral genome haplotypes to define the molecular events leading to the viral escape from these responses.

132. The Human Cytomegalovirus Gp39 Inhibits TLR4-Mediated Innate Response

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The innate immune system involves innate immunological sensors, such as Toll-like receptors (TLRs), which recognize exogenous pathogens or self-molecules as ligands in our body via infection or tissue damage to activate pro-inflammatory cytokines, such as interleukin-6 (IL-6) or IL-8, and type of interferons (IFNs) to clear harmful components. Despite having mature defense mechanisms, human cytomegalovirus (HCMV) evades healthy immune response during infection, and this evasion allows it to establish latency in the host. Here, we report a role of gp39, a novel HCMV glycoprotein, in the TLR4 signaling pathway. We demonstrate that gp39 physically suggests that the ability of HCMV to target TLR4 signaling reveals a critical aspect of HCMV function, with possible importance for immune escape and viral persistence.

133. The Transcriptome Response to Salmon Gill Poxvirus Infection

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Salmon gill poxvirus disease (SGPVD) is a disease in Atlantic salmon (*Salmo salar*) caused by salmon gill poxvirus (SGPV), a large DNA virus containing >200 genes. SGPVD is often acute, leading to high mortality. The virus infects the gill epithelial cells, and a common observation is apoptosis and proliferation of the epithelium resulting in a reduced respiratory surface.
We collected gill samples from a natural SGPVD outbreak in Atlantic salmon presmolts at the early phase of infection, during the acute mortality phase, and at the resolving phase of the disease. Selected samples were subject to histological examination, SGPV qPCR, and gene expression profiling using an Atlantic salmon oligonucleotide microarray.
The gene expression pattern within the acute phase of SGPVD was in line with pathological findings, and included upregulation of pro-apoptotic and proliferative genes, along with changes in ion channel and mucus-protein expression. The interferon-regulated antiviral response was strongly upregulated in diseased gills, and chemokine expression patterns changed dramatically, but gene expression patterns did not indicate lymphocyte recruitment. These data provide grounds for novel insight into the infection mechanisms and host interaction of SGPV.

134. The Ubiquitin-Proteasome System at Early Stages of African Swine Fever Infection

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Ubiquitination and proteasome activity have been recognized as essential elements for the initial establishment of virus infections.
African swine fever virus (ASFV) is a double-stranded DNA virus similar to poxvirus that causes a deadly disease of swine with very high socioeconomic impact. The disease is endemic to Africa, but it spread out through Caucasus to several eastern European countries 10 years ago. It continues to spread westwards and has reached several EU countries.
We have analyzed the potential consequences of proteasome inhibition early after virus entry in Vero cells and in porcine macrophages. In fact, proteasome function appeared to be essential during the first 6 hours of the infection cycle. The presence of proteasome inhibitors before this time point caused severe reductions in viral infectivity. Additionally, under proteasome inhibition genome replication, late gene expression and viral production were severely reduced.
We analyzed ASFV uncoating, which is achieved by a sequential program engaging molecular cues at the endosomal pathway. However, when viral cores are released out of the endosome, the function of the proteasome could be necessary for final core breakdown to start replication. In fact, we found that inhibition of the final stage of the proteasome pathway blocked a post-internalization step with accumulation of intact viral cores. It might be possible that similar to vaccinia virus, ASFV core-associated and/or viral proteins involved in DNA replication may be targets for the ubiquitin-proteasome pathway.

135. Translational Inhibition of the Restriction Factor APOBEC3G by the HIV-1 Vif Protein

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In order to defend themselves against viral infections, human cells express proteins called restriction factors which counteract different steps in the life cycle of various viruses. Amongst these restriction factors, APOBEC3G (A3G) inhibits retroviruses such as HIV-1 (Human immunodeficiency virus type 1) by inducing hypermutations during the reverse transcription of the viral genome, either leading to its degradation or making the virus incapable of producing functional viral proteins. However, HIV-1 expresses the protein Vif which counteracts A3G using different strategies, such as ubiquitination followed by proteasomal degradation, reduction of transcription, or inhibition of its translation. The mechanism of this translational inhibition is still unknown, and the aim of our study is to identify cellular proteins that might be involved in this process. In a first step, we pull down A3G mRNA from HEK293T cells using a set of complementary, biotinylated oligonucleotides followed by the identification of associated proteins by mass spectrometry. Then, we will compare the identified proteins, or protein complexes in the presence or absence of Vif to check whether Vif affects their composition. The identification of possible targets or partners of Vif is an important step in deciphering the mechanism used by Vif to mediate translational inhibition of A3G.

136. Upregulation of DNA Cytosine Deaminase APOBEC3B by Adenoviruses

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APOBEC3 proteins (Apolipoprotein B Editing Catalytic subunits 3, or A3s) are interferon-induced DNA cytosine deaminases playing a role in innate immune defense against viruses. Numerous recent studies have converged upon the APOBEC3B member as an important source of mutations in several human cancers and notably in lung tumors. APOBEC3B is upregulated in these tumors compared to normal tissue but the mechanisms are unknown. We tested the hypothesis that viral respiratory viruses can cause APOBEC3B upregulation.
We demonstrate that infection of lung cancer cell lines and immortalized normal bronchial epithelial cells with two common adenoviral strains (HAdV-C2 and HAdV-B3) promote the upregulation of APOBEC3B at the transcriptional and protein levels. Messenger RNA abundance of the other APOBEC3 members did not increase upon infection. Interestingly, the APOBEC3B protein produced upon infection with HAdV-C2 showed a slight difference of molecular weight compared to the APOBEC3B protein induced upon infection with the HAdV-B3 strain. Analysis of the APOBEC3B transcripts revealed the presence of a unique isoform.
Experiments are ongoing to understand the difference of molecular weight and to test the functional activity of the induced APOBEC3B proteins.

137. Zika Virus Induced Small RNA Response in *Aedes aegypti* Cells

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The recent outbreak of Zika virus (ZIKV) in South America caused severe medical damage to societies. ZIKV is spread by *Aedes aegypti* mosquitoes, and thus it is important to understand virus–vector interactions. Analysis of ZIKV infection in mosquito cells indicated that two RNA interference pathways are involved: the Piwi-interacting RNA (piRNA) and exogenous short interfering RNA (exo-siRNA) pathways, which are characterised by the production of virus-derived small RNAs of 25–29 and 21 nucleotides, respectively. ZIKV-specific piRNA-sized small RNAs lacked the characteristic piRNA ping-pong signature motif, and were bound to Ago3 but not to Piwi5 or Piwi6. Silencing of PIWI proteins indicated that the knockdown of Ago3, Piwi5, or Piwi6 did not enhance ZIKV replication, and only Piwi4 displayed antiviral activity. However, the latter do not bind piRNAs on its own. ZIKV-specific siRNAs were loaded into the exo-siRNA pathway effector Argonaute 2 (Ago2); yet, the knockdown of Ago2 did not enhance virus replication. If Dcr2—an enzyme responsible for cleaving dsRNA into siRNAs—was knocked out, ZIKV replication was increased compared to control cells. These results suggest that ZIKV can evade mosquito innate immunity mediated by small RNAs, and it is therefore crucial to understand these virus–vector interactions in greater detail.

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