viruses 2016

At the Forefront of Virus–Host Interactions

26–28 January 2016

Pharmacenter, University of Basel
Basel, Switzerland

Program and Abstract Book

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Welcome by Dr. Eric O. Freed

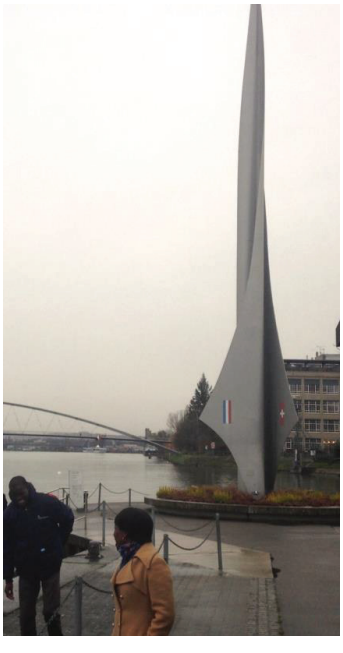
Dear authors and attendees,

The last few years have witnessed enormous progress in virology research, with major breakthroughs taking place across a wide range of virology-related disciplines. At the same time, the global impact of viral infections on human health has never been clearer. The conference *Viruses 2016 - At the Forefront of Virus–Host Interactions* will bring together leading virologists from around the world to share recent developments in their research. The sessions are organized in a theme-based manner, with related topics discussed from the perspective of diverse viruses, ranging from bacteriophage, plant, and insect viruses to animal viruses.

The *Viruses 2016 - At the Forefront of Virus–Host Interactions* will be held in Basel, Switzerland, from 26th to 28th of January 2016. It will comprise five plenary sessions to highlight the most exciting developments and the latest breakthroughs in virology.

Dr. Eric O. Freed
Conference Chair

Welcome by Prof. Dr. Thomas Klimkait



Dear colleagues planning to attend the Viruses 2016 conference "*At the Forefront of Virus–Host Interactions*"!

I am most honored to be able to invite you to the lovely city of Basel on the Rhine, the place where three countries meet at one point as seen in this photo. The picture of our "Three-Country-Corner" may serve as a symbol for the beauty of international collaboration in science, and I trust that this conference will be the place where a lively exchange of thoughts, vivid discussion of scientific work, and a multitude of interactions in an open spirit will yield valuable new contacts and ideas.

We have chosen the title "*At the Forefront of Virus–Host Interactions*" as this conference will especially focus on key viral principles. We truly hope that this basic science perspective will manage to complement the multitude of other events that focus on clinical and preclinical work, and that it will cross-fertilize to all disciplines from basic research to clinical application!

A number of well-known experts in their respective fields are attending with excellent speakers, and outstanding research abstracts! I am very much looking forward to a very productive conference with excellent presentations and discussions!

Dr. Thomas Klimkait
Co-Chair

Welcome by
Prof. Dr. Dr. h.c. Andrea Schenker-Wicki and
Prof. Dr. Edwin Constable

It is our pleasure and honor to welcome *Viruses 2016* to the Biozentrum of the University of Basel. Our University prides itself on its reputation and achievements in life sciences and translational sciences and we are particularly pleased that this conference, in an area of fundamental importance to human health, is taking place in Basel.

Since the Ebola outbreak in 2015, viruses have rarely been far from the news. These tragic epidemics emphasize the need for more information on the fundamentals of the molecular biology and genetics of viruses, as well as a better understanding of the viruses' interactions with host organisms and molecular treatments.

In the context of public health impact, the theme of "antiviral innate immunity" is of immense interest and importance, as are the sessions on interactions of viruses with membranes and replication organelles.

In the 124 years since the first description of the Tobacco Mosaic Virus in 1892, enormous strides in our understanding of viruses at all levels have been made. Nevertheless, conferences like this serve to emphasize that research in this area is at the cutting edge and of profound relevance to mankind.

We wish all participants a stimulating and enjoyable conference and hope that, in addition to fruitful scientific sessions and exchanges with colleagues, you will also find a little time to enjoy our campus and the city.

Prof. Dr. Dr. h.c. Andrea Schenker-Wicki
President
University of Basel

Prof. Dr. Edwin Constable
Vice President for Research
University of Basel

Acknowledgments

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An Open Acces Journal

Viruses (ISSN 1999-4915)

Special Issues Open for Submissions – 2016

1 GWAS and Beyond: Omics Research for Insights into Human-Virus Interactions and Pathogenesis
Guest Editor: Dr. Cheryl Winkler
Deadline: 30 September 2016

2 Lectins as Antiviral
Guest Editor: Dr. Barry O'Keefe
Deadline: 31 July 2016

3 Molecular Plant Virus—Insect Vector Interactions
Guest Editor: Assoc. Prof. Ralf Dietzgen
Deadline: 30 June 2016

4 Recent Progress in Bunyavirus Research
Guest Editors: Dr. Jane Tao and Dr. Pierre-Yves Lozach
Deadline: 27 February 2016

5 Recent Progress in Dengue Virus Research 2016
Guest Editors: Dr. Ali Amara and Prof. Felix Rey
Deadline: 31 July 2016

6 Recent Progress in Hepatitis E Virus Research
Guest Editor: Prof. Jacques Izopet
Deadline: 30 June 2016

7 Replicating-Competent Reporter-Expressing Viruses
Guest Editor: Dr. Luis Martinez-Sobrido
Deadline: 31 January 2016

8 RNA Packaging
Guest Editors: Dr. Roland Marquet and Prof. Polly Roy
Deadline: 31 May 2016

9 Use of Novel Cell Culture Models to Understand Viral-Host Interactions
Guest Editor: Prof. Charu Kaushic
Deadline: 30 June 2016

10 Viral Subversion of Stress Responses and Translational Control
Guest Editor: Dr. Craig McCormick
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11 Viruses 2016 - At the Forefront of Virus-Host Interactions
Guest Editors: Dr. Eric O.Freed and Prof. Thomas Klimkait
Deadline: 30 April 2016

12 Viruses and ERAD
Guest Editor: Prof. Jaquelin Dudley
Deadline: 31 July 2016

Young Investigator in Virology Prize 2016 competition to be opened soon! Visit Viruses web site for more information.

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The conference *Viruses 2016—At the Forefront of Virus–Host Interactions* (*Viruses 2016*) will be held from the 26th to the 28th of January 2016 in Basel, Switzerland. It will comprise five plenary sessions to cover the most exciting aspects and the latest developments in virology.

1.1 Conference Topics

- General Topics in Virology
- Antiviral Innate Immunity
- Non-Coding RNAs
- Interactions between Viruses and Membranes
- Replication Organelles

1.2 Conference Venue

Biocenter/PharmaCenter
Universität Basel
Klingelbergstrasse 50, CH-4056 Basel, Switzerland

1.3 Registration Desk

26 January–28 January 2016
07:30–17:30

Direct Telephone Line: +41 61 267 20 06

1.4 Wireless Internet Access

WLAN: unibas-event
Login: eventbzpz
Password: Virus-2016!

1.5 Directions and Map

1.6 Switzerland and the Tri-National-Region

Basel lies in the heart of Europe, on both banks of the Rhine. The city is the center of the idyllic border triangle of France, Germany and Switzerland—lying between the Swiss Jura, Germany's Black Forest and the Vosges in Alsace.

Basel is so easy to get to. Only a 10-minute drive from the city center, Basel's EuroAirport is served by a number of international airlines. Together with neighbouring Zürich Airport, it enjoys connections to all European airports and to more than 200 intercontinental destinations.

Located in the center of Europe, Basel is a major transportation hub. Its three railway stations not only offer excellent connections to far and wide but are also all situated in the very heart of the city. [Source: www.basel.com]

1.7 Basel

Where the Rhine, one of Europe's most important waterways, bends north and flows out of Switzerland towards the North Sea lies the charming city of Basel. This exceptional location at the heart of the three-country-triangle that joins Germany, France and Switzerland is what lends Basel its openness, economic strength and cultural diversity. [Source: www.bs.ch]

1.8 Best Connections

As far back as the Middle Ages, Basel became a major transportation hub thanks to its location on the Rhine and in the center of Europe. And still today, there is no way around Basel: The city lies at the intersection of the German and French rail and road networks. The trinational EuroAirport Basel-Mulhouse-Freiburg and the Rhine port connect Basel with the world. [Source: www.bs.ch]

1.9 Dynamic Economy

Again thanks to the Rhine, Basel developed into a prosperous center for commerce and trade fairs early on. Today, this city with a total area of only 37 square kilometres, inhabited by 200,000 people from 160 countries, is at the heart of the most dynamic economic region in Switzerland. [Source: www.bs.ch]

1.10 Fair Weather City

Next to the rich cultural offerings (museums with a global reputation, theater and concert halls, renowned architecture), the weather adds to the high quality of life: Nestled comfortably in the Rhine valley, Basel enjoys many more days of sunshine than the towns in central Switzerland. [Source: www.bs.ch]

1.11 The University of Basel

The University of Basel has an international reputation of outstanding achievements in research and teaching. Founded in 1460, the University of Basel is the oldest university in Switzerland and has a history of success going back over 550 years.

As a comprehensive university offering a wide range of high-quality educational opportunities, the University of Basel attracts students from Switzerland and the entire world, offering them outstanding studying conditions as they work towards their bachelor's, master's or PhD degrees. Today, the University of Basel has around 13,000 students from over a hundred nations, including 2,700 PhD students. The University of Basel has seven faculties covering a wide spectrum of academic disciplines. At the same time, the university has positioned itself amidst the international competition in the form of five strategic focal areas: Life Sciences, Visual Studies, Nanosciences, Sustainability and Energy Research and European and Global Studies. In international rankings, the University of Basel is regularly placed among the 100 top universities in the world thanks to its research achievements. [Source: www.unibas.ch]

1.12 Biocenter/PharmaCenter

The Biozentrum (Biocenter), is the largest department at the University of Basel's Faculty of Science. The primary focus of this interdisciplinary institute is basic molecular and biomedical research and teaching. The Biozentrum holds a leading position nationally and internationally and closely networks with partners from the academic world and industry.

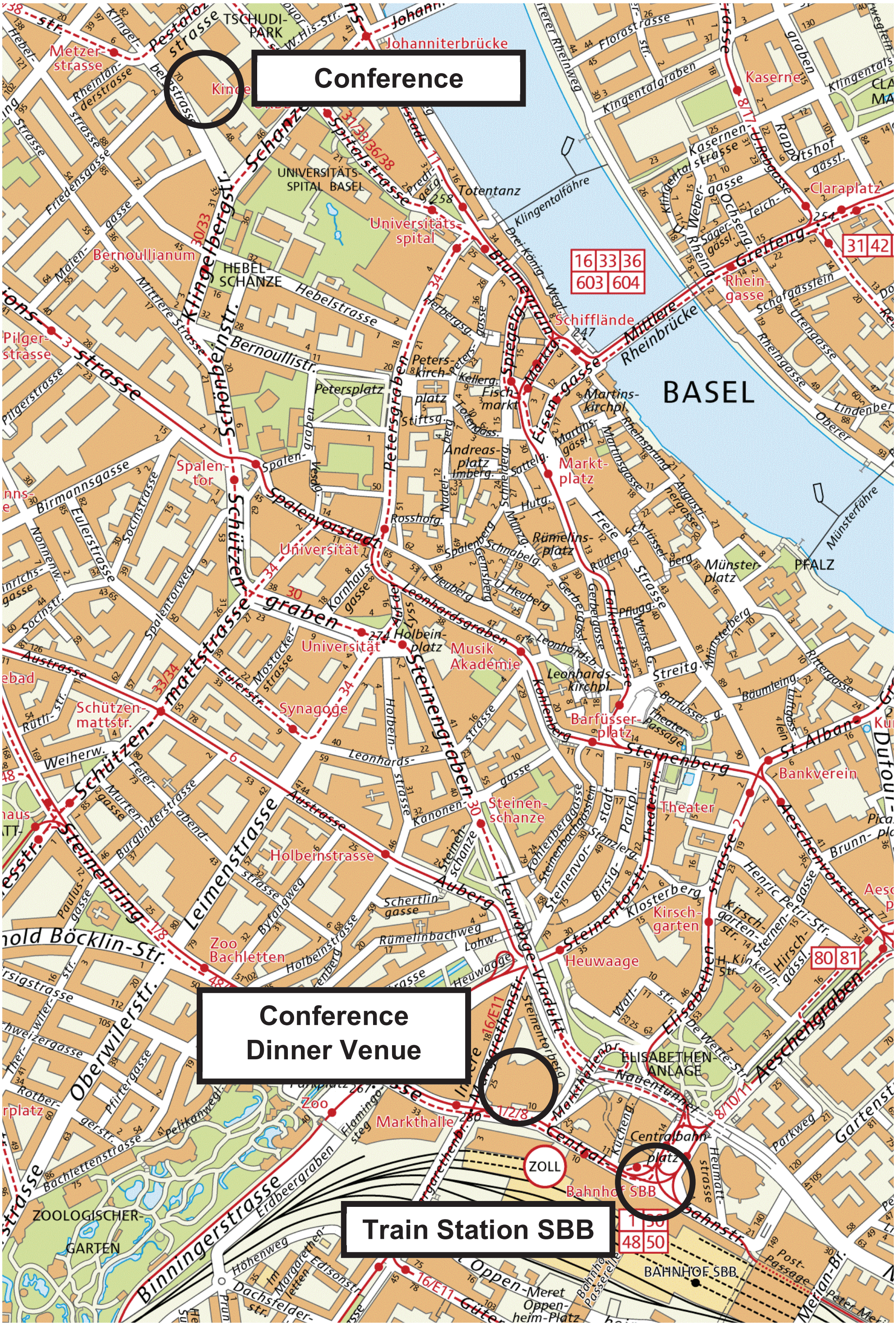
In 1971, at the time when the Biozentrum was founded, the visionary concept of developing an interdisciplinary research facility was unique. Today, some 40 years later, the success of this interdisciplinary approach to molecular and biomedical research remains evident. It continues to be the Biozentrum's greatest strength, along with its excellent facilities providing leading technologies and its highly motivated staff.

The Biozentrum is home to 30 research groups. These scientists, representing more than 40 nations, are engaged in investigating the molecular basis of biological processes. Their work covers a broad spectrum of activities, the scientific research is wide-ranging: How does a cell develop, how does it function and how are all its vital processes regulated? Can we make computer assisted models of these processes? How does a stem cell know what to become? How does a blood vessel form or the nervous system develop and how does the body defend itself against bacterial infections? Could the findings lead to new approaches in the treatment of serious diseases such as muscular diseases, Alzheimer's disease or cancer? Producing more than 200 scientific publications each year, the Biozentrum is regularly rated in the top 25% of the world rankings. Research at the Biozentrum is grouped into five major focal areas: Growth and Development, Infection Biology, Neurobiology, Structural Biology and Biophysics, as well as Computational and Systems Biology. These research areas, however, are not strictly separated from each other; new and relevant questions often arise at the overlap between the research fields, while the collaboration between teams and the expertise of each respective area leads to innovative solutions. This has contributed greatly to the scientific success of the Biozentrum. Both its funding and infrastructure make the Biozentrum internationally highly competitive and ensures research of the highest level.

The Biozentrum enjoys an excellent reputation for its scientific training, both nationally and internationally. Students are integrated into a research environment from the start of their academic career and gain first-hand experience of life as a scientist. Being able to link education with research makes the Bachelor's and Master's degree programs at the Biozentrum particularly attractive for many aspiring students. PhDs and postdocs, on the other hand, benefit from the Biozentrum's scientific success and the intensive, individual supervision.

The PharmaCenter Basel, The University of Basel Translational Science Platform, is the interdisciplinary center for excellence at the University of Basel. The PharmaCenter Basel aims to establish a leading research and teaching community in drug development, drug therapy and drug safety. Together with partners from the industry, the PharmaCenter Basel plans to translate increased knowledge about the molecular basis of disease into improved therapies. [Sources: www.biozentrum.unibas.ch and https://pharmacenter.unibas.ch/]

1.13 Location



1.14 How to Reach the Venue

Public Transport

From **EuroAirport Basel Mulhouse Freiburg** (15 minute journey)
Take the airport bus (No. 50) to the Kannenfeldplatz stop, where you have to change onto a No. 31, 36 or 38 bus going in the direction of Schifflände/Habermatten or Wyhlen Siedlung. Get off at the next stop, Metzerstrasse, and cross the road to the Biocenter/PharmaCenter.

From the **Basel SBB** (Swiss) and SNCF (French) train station (15 minute journey)
Take a No. 30 bus to the Kinderspital UKBB (children's hospital) stop and cross the road to the Biocenter/PharmaCenter–see Google Maps.

From the **Badischer Bahnhof** (German) train station: (10 minute journey)
Take a No. 30 bus to the Kinderspital UKBB (children's hospital) stop, and then walk to the Biocenter/PharmaCenter–see Google Maps.

By Car

Within Switzerland
Leave the motorway in the direction of the Unispital, drive through the tunnel and then across the viaduct. Keep on the main road, passing Spalentor, and carry straight on over the traffic lights. Turn left after about 500 m. The Biocenter/PharmaCenter is then on the right-hand side.

From France
After driving over the border in Saint-Louis, drive towards Basel-Kannenfeld as indicated. Stay on the main road (direction city), go straight on around the roundabout (direction city) and, after about 500 m, take the left-hand lane at Kannenfeldplatz. After only a few meters, take the right-hand lane and turn into Metzerstrasse. The Biocenter/PharmaCenter is then about 300 m ahead.

From Germany
Leave the expressway at exit Basel-St. Johann. After the tunnel, carry straight on for about 150 m. Turn left into Elsässerstrasse (direction city) and then, after 550 m, right onto St. Johanns-Ring (direction Augenspital). After 300 m, turn left into Klingelbergstrasse. The Biocenter/PharmaCenter is then on the left-hand side.
[Source: www.biozentrum.unibas.ch]

1.15 Inside the Biocenter/PharmaCenter

1.16 Conference Dinner

Wednesday, 27 January 2016, 19:00

Old Market Hall, Basel

The conference dinner will take place at the Old Market Hall next to Basel SBB. Dating from 1929, the landmark building with its magnificent cupola (the third largest of its type in the world) has been a symbol of the city for over 80 years. The inviting aromas of fresh bread, sweet fruits and freshly ground coffee can be experienced under the large cupola of the Old Market Hall. The attractive domed building has been in use as a market hall again since October 2013, featuring market stalls well-stocked with fresh products and appetising menus. Comfortable seating and free WLAN mean that eating, drinking and passing the time here is a unique opportunity. [Sources: www.bs.ch and www.fohhn.com]

Markthalle Basel

Viaduktstrasse 10

4051 Basel

http://www.markthalle-basel.ch

1.17 Visiting Basel and Dining Out

It is not easy to describe Basel in a few words. Descriptions for example such as the "cultural city of Switzerland" or "University town" are merely an attempt to give some sort of impression of the wealth of culture, history, relaxation and enjoyment to be found in the city. Whether it is a visit to one of the numerous museums, a dip in the Rhine or an evening at the theatre, allow yourself to be inspired by the joys that await you in Basel.

Art and Culture

Fondation Beyeler—www.fondationbeyeler.ch

In building Renzo Piano's museum in 1997, the Fondation Beyeler made its collection accessible to the public. The 250-odd works of classic modernism reflect the views of Hildy and Ernst Beyeler on 20th-century art and highlight features typical of the period: from Monet, Cézanne and van Gogh to Picasso, Warhol, Lichtenstein and Bacon. The paintings appear alongside tribal art from Africa, Oceania and Alaska.

Museum Tinguely—www.tinguely.ch

Situated directly on the Rhine, the Museum Tinguely, built according to plans by the Ticinese architect Mario Botta, houses the greatest collection of works by Jean Tinguely (1925–1991), one of the most innovative and important Swiss artists of the 20th century. The permanent exhibition presents a survey of his oeuvre spanning four decades. Special exhibitions show a wide range of artists and subjects including Marcel Duchamp and Kurt Schwitters who influenced Tinguely significantly, companions such as Arman, Niki de Saint Phalle, Yves Klein as well as current art trends along Tinguely's ideas.

Vitra Design Museum—www.design-museum.de

The Vitra Design Museum numbers among the world's most prominent museums of design. It is dedicated to the research and presentation of design, past and present, and examines its relationship to architecture, art and everyday culture. In the main museum building by Frank Gehry, the museum annually mounts two major temporary exhibitions. In conjunction with our alternating exhibitions, the Vitra Design Museum offers a variety of workshops and guided tours. [Source: www.basel.com/en]

Suggestions of Restaurants in Basel—www.basel.com

***Kohlmanns*—www.kohlmanns.ch**

It smells of fire, wood and freshly baked foods. The restaurant with its modern oak furniture is extremely cosy and is situated right at the Barfüsserplatz. *Kohlmanns* offers hearty Swiss and surprising regional specialities.

***Brasserie au Violon*—www.au-violon.com**

Lively brasserie with traditional and seasonal French cuisine served in a former prison.

***Zum Braunen Mutz*—www.braunermutz.ch**

The traditional tavern with bar and restaurant. Here you will meet original Basel locals of all generations.

***Der vierte König*—www.weinwirtschaft.ch**

In the restaurant *Der vierte König* you will find freshly cooked meals and a fine selection of bottled wines from all over the world.

***Kunsthalle*—www.restaurant-kunsthalle.ch**

The traditional restaurant *Kunsthalle*, where "Tout Bâle" feels at home serves seasonal delicacies.

***Käfer Stube* cuisine des alpes—www.kaefer-schweiz.ch**

Gourmet restaurant with regional products from all the alpine countries.

***Cheval Blanc*—www.lestroisrois.ch**

Refined seasonal cuisine and a selected wine list. Awarded with 19 points Gault-Millau and two Michelin stars. Summer terrace with a great view of the Rhine.

***Chez Donati*—www.lestroisrois.com**

For more than 50 years, the *Chez Donati* is an esteemed institution and the essence of fine Italian table culture in Basel.

***Brasserie Les Trois Rois*—www.lestroisrois.com**

The relaxed atmosphere and Swiss and French brasserie specialities make the city restaurant in the *Les Trois Rois*, a 5-star-superior deluxe hotel, a popular all-day rendezvous.

***Atelier* (*Der Teufelhof*)—www.teufelhof.com**

The restaurant charms by its modern and inspiring ambience. Enjoy a modern international cuisine with predominatly Swiss and regional products.

***Les Quatre Saisons*—www.lesquatresaisons.ch**

Treat yourself to some culinary delights in the newly renovated Restaurant *Les Quatre Saisons.* Head chef Peter Moser and his team apply a fresh sense of inspiration and a high level of commitment to their dishes, bringing together all of the elements necessary to create their unique cuisine–ingredients fresh from the market, original recipes and a great deal of passion.

Suggested Events

Parasols and Umbrellas—from Everyday Object to Work of Art—www.spielzeug-welten-museum-basel.ch/en/

The whole world is overwhelmed with cheap umbrellas from Asia with only a small artisan business being undertaken in Paris. With specific materials and a unique know-how the small shop offers parasols and umbrellas for all weathers and every occasion. They can be designed for protection against the rain or sun, for a wedding, a historic movie, a haute couture show or for a very particular special exhibition. This is the world of Michel Heurtault. He is Parisian by adoption and has gained an incredible knowledge on umbrellas—as well as acquiring the art of restoring historic umbrellas. Today his skills are deemed to be unique and have led to a worldwide reputation and to various awards, among them the Maître d'Art. The exhibition will display more than 400 museum pieces from bygone days as well as his latest creations. On display will be the various components of a parasol or an umbrella from the handle being in ornate shapes such as that of a dog or cat, old lace by the yard or historical embroidery and the framework made of various materials such as whalebone or metal. Parasols and umbrellas can enable us to look back to an evolving history of more than 4000 years. The oldest piece in the collection was manufactured by Jean Marius. At the beginning of the 18th century, Sun King Louis XIV granted Marius, the inventor of the three-way collapsible umbrella, a five-year royal monopoly on the manufacture of folding umbrellas.

The Bauhaus #itsalldesign—www.design-museum.de/en/information.html

With the major exhibition »The Bauhaus #itsalldesign« the Vitra Design Museum presents a comprehensive overview of design at the Bauhaus for the first time. The exhibition encompasses a multiplicity of rare, in some cases neverbefore-seen exhibits from the fields of design, architecture, art, film and photography. At the same time, it confronts the design of the Bauhaus with current debates and tendencies in design and with the works of contemporary designers, artists and architects.

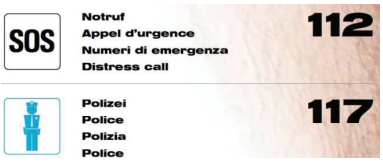
Cézanne to Richter. Masterpieces of the Kunstmuseum Basel—www.kunstmuseumbasel.ch/en

Basel's public art collection is of outstanding importance, especially in terms of the late 19th century and classical modernism.The large Oberlichtsaal and adjacent rooms of the Museum für Gegenwartskunst (Museum of Contemporary Art) is staging masterpieces from Paul Cézanne to Gerhard Richter. This wide-ranging overview vividly illustrates the main artistic developments in European painting up to the 1970s. The chronology serves as a guide to the 70 or so works which are less a didactic sequence of artistic movements and more a simultaneity of otherness that characterizes the modern period. The first works are by French artists who were seeking new visual languages beyond academic painting. The work by Paul Cézanne stands as an example of dogged artistic research.

Vogel Gryff, 27 January 2016—www.vogel-gryff.ch

Late in the morning of a wintry day in January, a raft with a very strange crew floats down the River Rhine. There are two drummers, two men carrying big flags, and two cannoneers, who repeatedly fire gun salutes. But the most important figure on the raft is a masked savage carrying an uprooted pine tree. He makes some dance steps constantly facing Kleinbasel, the city on the right bank of the River Rhine. Just below the Mittlere Brücke (the middle bridge) the savage is met by two other strange creatures: a lion dancing on his hind legs and a griffin with an awesome beak. At noon, the three figures dance on the bridge both jointly and alone to the sound of drums. They carefully avoid looking towards Grossbasel, to the left of the Rhine, or to cross the boundaries to this section of town. Wilder Mann (the savage man), Leu (the lion) and Vogel Gryff (the griffin) are traditional symbols or symbolic figures of three Kleinbasel societies. In earlier days, they served military and political purposes. Now, they are part of social life and help to strengthen community ties in the small district of Kleinbasel, eager to distinguish itself from the larger district on the left bank. The symbols as well as the processions—which used to happen spontaneously on different days—date back to the sixteenth century. Since 1841, when the societies moved their headquarters to the same building, the three celebrate together on the day allocated to the society presiding that year. Celebrations continue with a festive lunch for all members of the societies. In the afternoon and evening, the symbolic figures resume dancing in the streets of Kleinbasel. [Source: myswitzerland]

1.18 Emergency Information



Other useful numbers

Medical Emergency Center	+41 (0) 61 261 15 15
REGA air rescue service	1414

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Viruses (ISSN 1999-4915)

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2
Conference Program

2.1 Program at a Glance

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<tr><td>Coffee Break & Poster Session (even numbers)</td><td>Coffee Break & Poster Session (odd numbers)</td></tr>
<tr><td>Antiviral Innate Immunity</td><td>Interactions between Viruses and Membranes</td></tr>
<tr><td></td><td>Lunch</td><td>Lunch & Poster Session (odd numbers)</td><td>Lunch & Poster Session (even numbers)</td></tr>
<tr><td rowspan="4">Afternoon</td><td>Introduction
General Topics in Virology</td><td rowspan="2">Non-Coding RNAs</td><td rowspan="2">Replication Organelles</td></tr>
<tr><td>Coffee Break & Poster Session (odd numbers)</td><td>Coffee Break & Poster Session (even numbers)</td><td>Coffee Break</td></tr>
<tr><td rowspan="2">General Topics in Virology</td><td rowspan="2">Non-Coding RNAs</td><td>Replication Organelles</td></tr>
<tr><td>Closing Remarks</td></tr>
<tr><td></td><td></td><td>Conference Dinner</td><td></td></tr>
</table>

2.2. Detailed Program

Day 1: Tuesday 26 January 2016	
07:30–11:00	Check-in and Welcome Coffee
11:30–13:00	**Lunch**
13:00–18:00	**Session 1: General Topics in Virology**
Session Chair:	**Eric O. Freed**
13:00–13:20	Introduction—Eric O. Freed
13:20–14:20	Ari Helenius—Viral and Cellular Factors in Capsid Uncoating
14:20–14:50	Jesse D. Bloom—Next-Generation Approaches to Mapping the Constraints on Influenza Evolution
14:50–15:20	Julie K. Pfeiffer—How Gut Microbes Enhance Enteric Virus Infectivity
15:20–15:40	Stefan Finke—A Predicted Dynein Light Chain 1 Binding Motif in Rabies Virus L Protein is involved in Microtubule Reorganization and Primary Transcription
15:40–16:40	**Coffee Break and Poster Session (odd numbers)**
16:40–17:00	Joanna Parish—CTCF Regulates Differentiation-Dependent HPV Gene Expression
17:00–17:30	Jens Kuhn—Ebola Virus—Sex, Lies, and YouTube Videos
17:30–18:00	Thomas Klimkait—From Therapy to Eradication of Chronic Virus Infections? About HIV, HCV, and Other Role Models

Day 2: Wednesday 27 January 2016	
08:30–13:20	**Session 2: Antiviral Innate Immunity**
Session Chair:	**Andrea Cimarelli**
08:30– 09:00	Charles M. Rice—Unraveling the Multifaceted Roles of Innate Antiviral Effectors
09:00–09:30	Eric M. Poeschla—Broad Spectrum Antiviral Protection via RdRP-Mediated Stable Activation of MDA5-Dependent Innate Immunity
09:30–10:00	Ivan Marazzi—Regulation of Influenza Virus Replication by Host Factors
10:00–10:20	Andrew Mehle—The Unexpected Pro-Viral Role of "Anti-Viral" Genes during Influenza Virus Infection
10:20–10:40	Marlène Dreux—Antiviral Response by Plasmacytoid Dendritic Cells is Induced upon the Physical Contact with Cells Infected by Dengue Virus and via the Establishment of a Structural Platform at Contact
10:40–11:40	**Coffee Break and Poster Session (even numbers)**
11:40–12:10	Pierre Boudinot—Evolution of Antiviral Immunity: Insights from Comparison of Fishes and Mammals
12:10–12:40	Eva Harris—New Insights into Dengue Pathogenesis: How Dengue Virus NS1 Protein Triggers Endothelial Permeability and Vascular Leak
12:40–13:00	Marjolein Kikkert—Arteri- and Coronaviruses Employ Structurally Diverse Deubiquitinating Enzymes to Inhibit the Innate Immune Response in Infected Cells
13:00–13:20	Shan-Lu Liu—The Broad and Specific Antiviral Effects of IFITMs: Studies on Influenza A virus, Ebolavirus and HIV
13:20–14:20	**Lunch and Poster Session (odd numbers)**
14:20–18:30	**Session 3: Non-Coding RNAs**
Session Chair:	**Joanna Parish**
14:20–14:50	Ben TenOever—MicroRNA Biology in the Context of RNA Virus Infections
14:50–15:20	Maria Carla Saleh—A New Dimension in Insect Antiviral Immunity
15:20–15:50	Shou-Wei Ding—Mammalian siRNA Response to Virus Infection
15:50–16:10	Alexander A. Khromykh—Flavivirus Subgenomic RNA Captures XRN1 to Prevent Generation and Incorporation of Viral 3'UTR-Derived Immunostimulatory RNAs into Exosomes
16:10–17:10	**Coffee Break and Poster Session (even numbers)**
17:10–17:30	Jacob Gopas—MiR124 Contributes to Measles Virus Persistent Infection in Human Neuroblastoma Cells and Induces Apoptosis when Overexpressed
17:30–18:00	Bryan R. Cullen—Production of Functional Small Interfering RNAs by Human Dicer
18:00–18:30	Joan A. Steitz—Viral Noncoding RNAs: Insights into Evolution
19:00	**Conference Dinner**

Day 3: Thursday 28 January 2016	
08:30–13:20	**Session 4: Interactions between Viruses and Membranes**
Session Chair:	**Karyn Johnson**
08:30–09:00	Yohei Yamauchi—Mechanism of Influenza A Virus Uncoating During Host Cell Entry
09:00–09:30	Eric O. Freed—HIV Assembly and Maturation
09:30–10:00	Alexander Ploss—Determinants of Host Range Restrictions of Human Hepatotropic Viruses
10:00–10:20	Gabrielle Vieyres—ABHD5/CGI-58, the Causative Protein for the Chanarin-Dorfman Syndrome, Consumes Lipid Droplets to Support Assembly and Release of the Hepatitis C Lipo-Viro-Particle
10:20–10:40	Brett Lindenbach—Bacterial Effectors as Cell Biological Probes to Study the Replication of Positive-Strand RNA Viruses
10:40–11:40	**Coffee Break and Poster Session (odd numbers)**
11:40–12:10	Ry Young—The Role of Membrane Fusion in Phage Lysis
12:10–12:40	Margaret Kielian—Alphavirus Budding: How Viruses Remodel the Cell during Exit
12:40–13:00	Pawel Zmora—TMPRSS11A Activates Influenza and Emerging Coronaviruses and is Resistant to Serine Protease Inhibitor HAI-1
13:00–13:20	Thomas C. Mettenleiter—Molecular Basis of Herpesvirus Nuclear Egress: the Prototypic Vesicular Nucleo-Cytoplasmic Transport
13:20–14:20	**Lunch and Poster Session (even numbers)**
14:20–18:15	**Session 5: Replication Organelles**
Session Chair:	**Veronique Ziegler-Graff**
14:20–14:50	Paul Ahlquist—Protein, RNA and Membrane Interactions in Positive-Strand RNA Virus Genome Replication
14:50–15:20	Peter Nagy—Insights into the Assembly of the Tombusvirus Replicase: the Role of Co-Opted Host Proteins and Lipids
15:20–15:40	Inés Romero Brey—Architecture and Building Blocks of the Flaviviral Replication Organelles
15:40–16:40	**Coffee Break**
16:40–17:00	Eleni-Anna Loundras—Identification of Novel *Cis*- and *Trans*-Activities of FMDV $3D^{pol}$ Necessary for the Formation of Viral RNA Replication Complexes
17:00–17:30	Polly Roy—Disassembly and Assembly of Bluetongue Virus 1
17:30–18:00	Terence S. Dermody—Formation and Function of Reovirus Replication Organelles
18:00–18:15	Closing Remarks

3
Oral Presentation Abstracts

Session 1:
General Topics in Virology

Session Chair: Eric O. Freed

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. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. The full experimental details must be provided so that the results can be reproduced. We also encourage the publication of timely reviews and commentaries on topics of interest to the virology community and feature highlights from the virology literature in the 'News and Views' section.

Electronic files or software regarding the full details of the calculation and experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material.

Impact Factor: 3.353 (2014); 5-Year Impact Factor: 3.419 (2014)

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Viral and Cellular Factors in Virus Entry and Uncoating

Ari Helenius

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Over a period of many years, my research group has investigated the mechanisms of host cell entry of enveloped and non enveloped animal viruses using imaging, cell biological approaches, and automated siRNA screening. We find that viruses activate cellular signalling pathways that trigger a variety of endocytic mechanisms (clathrin-mediated, macropinocytosis, lipid raft-mediated, caveolar, macropinocytosis-like), which they exploit for cell entry. Penetration into the cytosol occurs from cytoplasmic organelles such as early and late endosomes, and the endoplasmic reticulum. Penetration is followed by intracellular transport of the viral capsids and uncoating of the genome. All steps require assistance from a number of cellular factors and processes. These provide possible targets for novel antiviral strategies. In the lecture, Simian virus 40, a polyoma virus, will be used as an example to illustrate the various steps.

Next-Generation Approaches to Mapping the Constraints on Influenza Evolution

Jesse Bloom

Division of Basic Sciences and Computational Biology Program, Fred Hutchinson Cancer Research Center, Seattle, WA Department of Genome Sciences, University of Washington, USA.

Influenza is characteristic of many viral pathogens in that it evolves rapidly. Successfully combating influenza therefore requires understanding the evolutionary pathways accessible to the virus. I will describe a combined experimental/computational approach to understand the constraints and selective pressures that shape influenza evolution. Specifically, my group is using high-throughput experiments to map the effects of all possible mutations to viral genes, and then developing computational algorithms that leverage these data to compute the likelihood of different evolutionary algorithms. I will show that these approaches can be used to improve the identification of mutations that have promoted past viral adaptation. I will also discuss how these approaches might be extended to better predict future viral evolution and quantitatively score the adaptation of viral variants to different hosts.

How Gut Microbes Enhance Enteric Virus Infectivity

Julie K. Pfeiffer

University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Enteric viruses encounter a vast microbial community in the mammalian digestive tract. We found that gut microbes are required for replication and pathogenesis of two unrelated enteric viruses, poliovirus and reovirus. Similarly, other groups have demonstrated that the mouse retrovirus MMTV relies on intestinal microbiota for transmission and murine norovirus relies on intestinal microbiota for replication. Therefore, enteric viruses from four different families (*Picornaviridae*, *Reoviridae*, *Retroviridae,* and *Caliciviridae*) benefit from intestinal bacteria. A common theme has emerged: Enteric viruses bind bacterial surface polysaccharides. We found that exposure to bacterial surface polysaccharides, including lipopolysaccharide (LPS) and peptidoglycan, enhanced poliovirus stability and cell attachment/viral receptor binding, providing one mechanism by which intestinal microbiota promote enteric picornavirus infection. Virion stabilization by bacteria may be important for transmission, since a mutant poliovirus with reduced binding to LPS had a fecal-oral transmission defect due to virion instability in feces. Our data suggest a model where picornavirus virions bind bacterial surface polysaccharides, enhancing cell attachment to promote infection and enhancing environmental stability to promote transmission to a new host. Recently, we visualized virion-bacteria interactions using electron microscopy and found that each bacterium binds several poliovirus or reovirus virions. These results raise the possibility that bacteria or bacterial polysaccharides may deliver virions to host cells to initiate the first viral replication cycle in the gut. Moreover, since bacteria are small compared to mammalian intestinal cells and each bacterium binds multiple virions, a bacterium may deliver more than one virion per host cell. Bacteria-mediated delivery of multiple virions into an intestinal cell is interesting considering that a limited number of virions are transmitted and therefore the first replication cycle of enteric viruses is likely initiated at an extremely low MOI. Bacteria may facilitate viral co-infection even when very few virions are present. Our recent data suggest that bacteria facilitate poliovirus co-infection *in vitro* and *in vivo*. We are now examining the genetic implications of bacteria-mediated viral co-infection.

A Predicted Dynein Light Chain 1 Binding Motif in Rabies Virus L Protein is Involved in Microtubule Reorganization and Primary Transcription

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To investigate the intracellular distribution of rabies virus (RABV; *Rhabdoviridae*) polymerase L, we expressed mCherry tagged L (mCherry-L) in the absence of other RABV proteins. Analysis of mCherry-L localization by confocal laser scan microscopy revealed that mCherry-L accumulated at microtubules (MT). Moreover, microtubule reorganization and acetylation correlated with mCherry-L accumulation at MTs, indicating that RABV polymerase L binds and leads to MT modification. *In silico* analysis revealed a putative dynein light chain 1 (DLC1) binding motif in L that could explain MT association through DLC1. Because DLC1 binding by polymerase cofactor P is known, those data further indicated that both components of the RABV polymerase complex are able to bind DLC1. Recombinant viruses with mutations in the respective P and L motifs showed that both motifs are required for regulation of primary transcription, indicating that DLC1 acts as an enhancer of transcription by binding to both components of the viral polymerase complex. Interestingly, DLC1 levels in infected cells were also regulated by both motifs. Finally, disruption of the L motif resulted in a cell type specific loss of MT localization, showing that DLC1 is involved in RABV L mediated reorganization of the cytoskeleton. Overall, we conclude that DLC1 acts as a transcription factor that stimulates primary RABV transcription by binding to both, RABV P and L protein. We further conclude that RABV L is able to influence MT organization and posttranslational modification. In infected neurons, MT manipulation by L may contribute to efficient intracellular transport of virus components.

CTCF Regulates Differentiation-Dependent HPV Gene Expression

Ieisha Pentland, Sally Roberts, Joanna Parish

University of Birmingham, Center for Human Virology, UK.

The CCCTC-binding factor (CTCF) is a multifunctional DNA binding protein that is involved in the genome wide organization of chromatin. CTCF has chromatin insulator properties by either blocking enhancer function or preventing the spread of heterochromatin. In addition, CTCF can block RNA polymerase II progression and thereby promote inclusion of weak upstream exons. Recent studies in Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) have demonstrated a role for CTCF in regulating viral gene expression. We have identified a strong and conserved CTCF binding site in the E2 open reading frame of high-risk human papillomavirus (HPV) types. Using three-dimensional organotypic raft cultures that recapitulate the complete HPV life cycle, we have shown that recruitment of CTCF to the E2 ORF in HPV18 is important for the regulation of HPV gene expression in differentiating epithelium. Subsequent mutation of this CTCF binding site alters the expression of early and late genes, thus affecting the growth and cell cycle of host cells. In summary, we have demonstrated a novel and important role of CTCF recruitment to HPV18 for the control of HPV gene expression during the virus life cycle.

Ebola Virus—Sex, Lies, and YouTube Videos

Jens H. Kuhn

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Until the end of 2013, ebolaviruses and other filoviruses were known to be exotic human and animal pathogens that cause geographic and temporally limited disease outbreaks each encompassing dozens to a few hundred cases. Although already somewhat part of pop culture due to semi-nonfictional books (e.g., *The Hot Zone*) or movies (e.g. *Outbreak*), filovirus research was limited to a scant few scientists typically working in maximum containment laboratories of government facilities. Consequently, less than ≈100 filovirus publications appeared per year. All of them could easily be peer-reviewed by experts, thereby guaranteeing at least a minimum of quality. This situation changed drastically with the still ongoing Western African Ebola virus disease outbreak that started in December of 2013 and thus far has caused 28,637 human infections and 11,315 deaths (CFR=39.5%). Publications surpassed 1,000 in 2014 and is approaching 2,000 in 2015. Here, I will discuss how the sheer number of manuscript submissions overwhelmed the scientific peer review process, resulting in countless publications that have been misleading public and policy makers with “facts” not founded on actual datasets. I will present several examples of what constitutes “common knowledge of Ebola” among non-filovirologists that actually do not stand the test of scientific scrutiny but have greatly influenced both ongoing research and outbreak control policies. Finally, I will appeal to the audience to consider novel ways of controlling scientific output during times of crises.

From Therapy to Eradication of Chronic Virus Infections? About HIV, HCV, and Other Role Models

Thomas Klimkait

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The inevitable chronicity of HIV infection in the affected patient has made this virus an excellent model for a better understanding of a multitude of general viral and antiviral aspects: The mechanisms and possible vulnerability of viral entry or the activation of key viral enzymatic functions in the HIV life cycle have been elucidated in depth. This has led to the development of today close to 30 potent and highly selective inhibitors. In addition, they helped turn the AIDS threat of an HIV infection into a treatable chronic condition with few side effects and near-to-normal life expectancy.

A similar drug development success is currently emerging for the devastating, organ-destructive HCV infection: The advent of directly acting agents (DAAs) is currently enabling for the first time extremely high rates of virus elimination and cure.

The current prospects of successful long-term treatment and even viral elimination will be reviewed, and new research avenues and their clinical status will the discussed. As one of the recent developments, potent novel concepts towards viral eradication are emerging. Yet, the new aim of eradicating viruses from chronic infection situations continues to pose enormous scientific challenges in various life threatening infections, particularly for HIV and HBV. These new approaches will be discussed as well as current shortcomings in the selectivity of inhibitors or deliberate activators of viral genes.

We will present recent laboratory evidence for a tropism-dependent clearing role of immune function on HIV persistence, and a concept for potential “selective elimination” will be discussed.

Session 2:
Antiviral Innate Immunity

Session Chair: Andrea Cimarelli

pathogens

Pathogens (ISSN 2076-0817) publishes reviews, regular research papers and short notes on all aspects of pathogens and pathogen-host interactions. There is no restriction on the length of the papers. Our aim is to encourage scientists to publish their experimental and theoretical research in as much detail as possible. Full experimental and/or methodical details must be provided for research articles. There are, in addition, unique features of this journal:

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Unraveling the Multifaceted Roles of Innate Antiviral Effectors

Charles M. Rice

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The painfully slow progress understanding hepatitis C finally yielded new oral therapies capable of eliminating the virus in more than 95% of those treated. In addition to helping drive this remarkable progress in the clinic, basic HCV studies have spawned several new directions in our group. One area concerns type I interferons and how they exert their amazing protective antiviral efficacy. Although we originally initiated this work to understand the now passé IFN-based cornerstone of HCV treatment, this effort expanded to include a number of viruses and approaches [1]. We have characterized the sensitivity of a large number of viruses to a library of ectopically expressed interferon-stimulated genes [2]. I'll highlight a few examples of new findings and some surprises that have changed my thinking about the evolution and function of intrinsic and innate immunity.

References

[1] Schoggins, J.W. *et al. Nature* **2014**, *505*, 691–695. [2] Dittmann, M. *et al. Cell* **2015**, 160, 631–643.

Broad Spectrum Antiviral Protection via RdRP-Mediated Stable Activation of MDA5-Dependent Innate Immunity

Eric M. Poeschla

Mayo Clinic College of Medicine, Rochester, Minnesota, USA; University of Colorado School of Medicine, Denver, Colorado, USA.

For many emerging and re-emerging infectious diseases, definitive solutions via sterilizing adaptive immunity may require years or decades to develop, if they are even possible. The innate immune system offers alternative mechanisms that do not require antigen-specific recognition or *a priori* knowledge of the causative agent. However, it is unclear whether effective stable innate immune system activation can be achieved without triggering harmful autoimmunity or other chronic inflammatory sequelae. Here, we show that transgenic expression of a picornavirus RNA-dependent RNA polymerase (RdRP), in the absence of other viral proteins, can profoundly reconfigure mammalian innate antiviral immunity by exposing the normally stringently membrane-sequestered RdRP activity to sustained innate immune detection. RdRP-transgenic mice have life-long, quantitatively dramatic upregulation of 80 interferon-stimulated genes (ISGs) and show profound resistance to normally lethal viral challenge. Multiple crosses with defined knockout mice (*Rag1, Mda5, Mavs, Ifnar1, Ifngr1,* and *Tlr3)* established that the mechanism operates via MDA5 and MAVS and is fully independent of the adaptive immune system. Human cell models recapitulated the key features with striking fidelity, with the RdRP inducing an analogous ISG network and a strict block to HIV-1 infection. This RdRP-mediated antiviral mechanism does not depend on secondary structure within the RdRP mRNA but operates at the protein level and requires RdRP catalysis. Importantly, despite lifelong massive ISG elevations, RdRP mice are entirely healthy, with normal longevity. Our data reveal that a powerfully augmented MDA5-mediated activation state can be a well-tolerated mammalian innate immune system configuration. These results provide a foundation for augmenting innate immunity to achieve broad-spectrum antiviral protection.

Regulation of Influenza Virus Replication by Host Factors

Adolfo García-Sastre, Ivan Marazzi

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Influenza A viruses are zoonotic pathogens that continuously circulate and change in several animal hosts, including birds, pigs, horses and humans. The emergence of novel virus strains that are capable of causing human epidemics or pandemics is a serious public health concern, and continues to cause problems all over the world. Viral replication depends on virus-host interactions resulting in co-opting of host factors and pathways for optimal viral replication as well as in inhibition of host pathways involved in viral restriction. These host factors often regulate not only influenza virus pathogenesis but also influenza virus host tropism. The identification and characterization of such virus-host interactions may result in novel antiviral strategies.

The Unexpected Pro-Viral Role of "Anti-Viral" Genes during Influenza Virus Infection

Vy Tran, Andrew Mehle

Medical Microbiology and Immunology, University of Wisconsin-Madison, USA.

During viral infections, a struggle exists between the host and the virus. Cells contain antiviral factors that selectively target and inhibit viral proteins and nucleic acids, whereas viruses neutralize these inhibitors and co-opt cellular factors for their own replication. The balance between these pro- and antiviral forces influences the outcome of viral infections and the course of diseases. We performed a genome-wide CRISPR knockout screen to identify cellular factors that regulate influenza virus infection, designing the screen to specifically query post-entry steps in the viral life cycle. To our surprise, the screen revealed that a large class of pro-apoptotic proteins and presumptive antiviral factors, including IFIT2 and IFIT3, are key enhancers of influenza virus replication. IFITs are a family of interferon-inducible proteins with isoform-specific antiviral activity against a broad array of viruses. We showed that both human and mouse IFIT2 knockout cells have a decreased capacity to support influenza virus replication compared to wild-type cells. Viral attachment was unaffected, consistent with our screen targeting post-entry steps. However, viral gene expression was reduced in the knockout cells beginning early in infection, resulting in ~100-fold drop in the production of infectious progeny. Remarkably, these knockout cells were almost completely resistant to virally induced cell death from a large collection of influenza A and influenza B viruses and spontaneously resolved the viral infection. Moreover, cells lacking the apoptotic activators Bax and Bak, which function downstream of IFIT2, also supported only low levels of replication and resist influenza-mediated cell death. We will describe the molecular mechanisms underlying the pro-viral function of IFIT2. Thus, while apoptosis is generally seen as a last-resort antiviral defense, our data suggest influenza virus has evolved to exploit the apoptotic cellular environment for maximal viral replication.

Antiviral Response by Plasmacytoid Dendritic Cells Is Induced upon the Physical Contact with Cells Infected by Dengue Virus and via the Establishment of a Structural Platform at Contact

Sonia Assil [1], Elodie Décembre [1], Andrew Davidson [2], Marlène Dreux [1]

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Plasmacytoid dendritic cells (pDCs) are known to be key players of the interferon (IFN) response against viruses. They function as sentinels of viral infection, predominantly by recognition of viral genomes. Recent work highlighted that cell-cell contact dependency is a hallmark of pDC-mediated antiviral state triggered by evolutionary distant viruses. Nonetheless the underlining mechanism is still enigmatic. In accordance, here we report that the sensing by pDCs of cells infected by dengue virus (DENV) also triggers a cell-cell contact-dependent antiviral response. We thus aim at defining the features of these contacts and thereby the transmission of the activating components to the pDCs. Confocal microscopy analyses revealed that surface viral proteins are transmitted, along with DENV genome, to pDCs. Consistently, functional analysis using recombinant DENV with point mutations demonstrates that the pDC response to infected cells required a surface viral protein-dependent secretion of viral genome, but not the production of infectious virions. Importantly, cell-cell contact between pDCs and DENV-infected cells display particular organization, including the localized clustering of both cellular and viral components. We demonstrate that DENV surface proteins form clusters at the cell-cell interface. Additionally, actin network, which also polarizes at the contact, is pivotal for both the establishment of cell-cell contacts and for pDC IFN response. Together our results suggest that actin network serve as a structural platform likely contributing to the polarization of immunostimulatory viral components at the contacts and their subsequent transmission and internalization by the pDCs by clathrin-dependent endocytosis. Therefore our results provide new insights on the cell-cell contact-dependent transmission of immunostimulatory elements to pDC and highlight novel features of innate immunity activation. This new concept may have broad importance for the many viruses that, like DENV, can disable the pathogen-sensing machinery within infected cells and can activate IFN response by cell-cell contact with pDCs.

Evolution of Antiviral Immunity: Insights from Comparison of Fishes and Mammals

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In vertebrates, antiviral innate immunity is orchestrated by type I IFNs. These master cytokines induce in turn a large number of interferon-stimulated genes (ISGs), which possess diverse effector and regulatory functions. The IFN system emerged in early vertebrates, as it is present in all tetrapods as well as in fishes but apparently not in other chordates. Comparative analyses of IFN pathways and ISG repertoire between fishes and mammals identify a conserved core system activated by various viruses. In addition to this core system, many ISGs belong to multigenic families, which diversified independently in each lineage. Altogether, these analyses draw an outline of the evolution of antiviral innate immunity in vertebrates. Among fish models, zebrafish has recently emerged as a powerful system for real time analysis of viral infection at the level of the whole organism. In this species, a model of Chikungunya infection leads to a massive type I IFN response, and allows to explore the concepts of infection tolerance and resistance. Tissue specific expression of ISGs and developmental patterns of response highlight how common selective constraints shape the IFN system in two groups of vertebrates with contrasting life styles and anatomical adaptations.

New Insights into Dengue Pathogenesis: How Dengue Virus NS1 Protein Triggers Endothelial Permeability and Vascular Leak

Henry Puerta-Guardo [1], Dustin Glasner [1], Kaycie Hopkins [1], Nori Ueno [2], Melissa Lodoen [2], P. Robert Beatty [1], Harris Eva [1]

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Dengue is the most prevalent arboviral disease in humans and a major public health problem worldwide. Systemic plasma leakage, leading to hypovolemic shock and potentially fatal complications, is a critical determinant of dengue severity. Recently, we described a novel pathogenic effect of dengue virus (DENV) non-structural protein 1 (NS1) in triggering permeability of human endothelial cells *in vitro* and systemic vascular leakage *in vivo*. We also showed that vaccination with recombinant DENV NS1 or passive transfer of NS1-immune serum protects against lethal DENV-induced vascular leak. In the endothelium, the endothelial glycocalyx like-layer (EGL) and tight junction (TJ) and adherens junction (AJ) proteins control barrier function critical for vascular homeostasis. Here, we examined the ability of DENV and the closely related West Nile Virus (WNV) NS1 to modulate endothelial permeability, the EGL, and TJ/AJ assembly/disassembly dynamics. DENV2 NS1 increased permeability of human pulmonary microvascular endothelial cells (HPMECs), human umbilical vein endothelial cells (HUVEC), human dermal microvascular endothelial cells (HMEC-1), and human brain microvascular endothelial cells (HBMEC), and human brain microvascular endothelial cells (HBMEC), as measured by Transendothelial Electrical Resistance (TEER). Interestingly, WNV NS1, which does not alter the permeability of HPMEC, HUVEC or HMEC monolayers, significantly reduced TEER of HBMECs. This is intriguing as WNV causes encephalitis and alters the blood-brain barrier, in contrast to DENV, which causes systemic vascular leak. We demonstrated that DENV2 NS1 but not WNV NS1 triggered increased expression/activation of cathepsin-L, a lysosomal cysteine proteinase, which then activated heparanase, an endo-β-d-glucuronidase that degrades heparan sulfate in the EGL, leading to altered distribution of sialic acid and the heparan sulfate proteoglycans, syndecan-1 and perlecan. Specific inhibitors of heparanase and cathepsin-L prevented NS1-mediated endothelial permeability and alteration of EGL components. Analysis of intercellular junction (TJ/AJ) integrity by immunofluorescence microscopy showed that DENV2 NS1 but not WNV NS1 induced the disassembly of TJ proteins, such as ZO-1 and claudin-5, and AJ proteins, such as beta-catenin and VE-cadherin. Our findings propose a new mechanism by which NS1 directly triggers endothelial vascular dysfunction through the early enzymatic activation and cleavage of the EGL and extracellular matrix as well as disassembly of intercellular junctions, resulting in increased plasma leakage that occurs in severe dengue disease.

Arteri- and Coronaviruses Employ Structurally Diverse Deubiquitinating Enzymes to Inhibit the Innate Immune Response in Infected Cells

Puck B. van Kasteren [1], Robert C.M. Knaap [1], Ben A. Bailey-Elkin [2], Tim J. Dalebout [1], Peter J. Bredenbeek [1], Louis Enjuanes [3], Eric J. Snijder [1], Brian L. Mark [2], Marjolein Kikkert [1]

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Objectives: The distantly related arteri- and coronavirus families (both belonging to the order Nidovirales) encode papain-like proteases (named PLP2 and PLpro, respectively) that play an essential role in the autoproteolytic maturation of their replicase polyproteins. In addition, *in vitro* experiments revealed that these same proteases can act as deubiquitinating enzymes (DUBs), suggesting a role in the inhibition of ubiquitin-regulated innate immunity. However, the pivotal role of these proteases in the viral replication process (which precludes their straightforward inactivation or deletion) has hampered the verification of the importance of their DUB activity during infection.

Methods: Recently, we obtained the crystal structures of both the equine arteritis virus (EAV) PLP2 and the Middle East respiratory syndrome coronavirus (MERS-CoV) PLpro, both in a covalent complex with ubiquitin. These structures revealed that the arterivirus DUB belongs to the Ovarian Tumor (OTU) superfamily, whereas the coronavirus DUB (as previously reported for other coronaviruses) belongs to the Ubiquitin-Specific Protease (USP) class of DUBs. Importantly, based on these crystal structures, we succeeded in designing mutations that specifically disrupt the DUB activity of these proteases, while leaving their function in polyprotein processing unaffected.

Results: Whereas the replication kinetics of viruses carrying these mutations were comparable to that of their respective parental viruses, we found a significantly increased expression of interferon beta mRNA and interferon-stimulated genes in cells infected with the DUB knockout-mutants compared to the parental controls.

Conclusions: Our work provides the first direct evidence that the DUB activity of structurally diverse arteri- and coronavirus proteases is important for the evasion of innate immunity during infection. Furthermore, the engineered separation of DUB and polyprotein processing activities might form the basis for the design of novel modified live virus vaccines with enhanced immunogenicity.

The Broad and Specific Antiviral Effects of IFITMs: Studies on Influenza A virus, Ebolavirus and HIV

Jingyou Yu [1], Minghua Li [1], Jordan Wilkins [1], Shilei Ding [2], Talia H Swartz [3], Anthony M. Esposito [3], Yi-Min Zheng [1], Eric O. Freed [4], Chen Liang [2], Benjamin K. Chen [3], Shan-Lu Liu [1]

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The interferon-induced transmembrane (IFITM) proteins broadly inhibit viral infection, yet the underlying mechanisms are still poorly understood. We have previously reported that this family of protein can change the membrane fluidity, likely explaining its broad antiviral effect through inhibiting viral fusion. Interestingly, we recently found that these IFITM proteins, particularly IFITM2 and 3, specifically interact with HIV-1 Env and inhibit its maturation. Consequently, the viral fusion activity, infectivity, as well as cell-to-cell transmission capability are blocked. Notably, knockdown of the endogenous IFITM expression in CD4+ T lymphocytes enhances HIV-1 transmission to Jurkat cells. Live cell imaging and flow cytometric analyses revealed that IFITM expression in donor cells impairs HIV-1 fusion in target cells and severely inhibits HIV-1 spread from cell to cell. By creating chimeras between IFITM1 and IFITM2, we discovered that the extended C-terminus of IFITM1 inherently suppresses its ability to inhibit HIV-1 cell-to-cell transmission. The inhibitory effects of IFITMs on HIV-1 cell-cell transmission can be extended to other HIV-1 strains, including some primary isolates, as well as to HIV-2 and SIVs. We also re-examined the effects of IFITMs on influenza A virus and Ebolavirus, and the results will be presented. Altogether, our new studies reveal some broad yet distinct effects of IFITMs on viral infection, and provide new insight into the mechanisms of action of IFITMs.

Session 3:
Non-Coding RNAs

Session Chair: Joanna Parish

Non-coding RNA (ISSN 2311-553X) is an open access journal which provides an advanced forum for research studies on non-coding RNAs and their regulatory roles. It publishes primarily original research papers, short reports, communications, snapshots and conference reports. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. The full experimental details must be provided so that the results can be reproduced. We also encourage the publication of timely reviews and of commentaries on hot topics of interest to the non-coding RNAs community.

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MicroRNA Biology in the Context of RNA Virus Infections

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The capacity to inhibit virus infection using a combination of pathogen-derived small RNAs and nucleases has proven an effective strategy in both prokaryotes and eukaryotes. However, for reasons that remain unclear, mammals have largely replaced this antiviral defense mechanism with the interferon-based system despite encoding the necessary machinery to elicit a small RNA-mediated antiviral response. This talk will focus on recent work examining the biology of small RNAs as it relates to the cellular response to mammalian virus infection.

A New Dimension in Insect Antiviral Immunity

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The field of insect immunity has been largely dominated by studies on antimicrobial immunity, while antiviral immunity received relatively little attention. The discovery of RNAi as an antiviral mechanism, first in plants and later in nematodes and insects, launched a new phase in studying host-pathogen relationships and immunity. Based on a combination of basic science and bioinformatic approaches, we analyze the mechanisms underlying viral tropism, systemic propagation of the antiviral signal and the basis of the persistence of the antiviral state. Furthermore, we examine whether the dsRNA-uptake pathway is conserved in mosquitoes and its relationship with viral immunity in that host.

Mammalian siRNA Response to Virus Infection

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The antiviral immunity controlled by RNAi pathway is induced in fungi, plants, invertebrates and mice following the detection of viral dsRNA as the non-self pathogen-associated molecular pattern. In antiviral RNAi, the viral dsRNA is further processed by Dicer into virus-derived small interfering RNAs (vsiRNAs) to guide virus clearance by Argonaute proteins. Consistent with a key antiviral function of the RNAi pathway, plant and animal viruses have evolved viral suppressors of RNAi (VSRs) as a counter-defense to promote infection at the level of either single cells or the whole organism. However, previous attempts in the past decade to identify human vsiRNAs were unsuccessful. We have recently developed a new strategy to identify mammalian vsiRNAs in mature somatic cells based on *in vivo* characterization of mouse vsiRNAs. Using this new approach, we show that *Influenza A virus* infection induces production of abundant vsiRNAs in distinct human somatic cells readily detectable by either deep sequencing or Northern blotting. The influenza vsiRNAs are predominantly 22 nucleotides long, are produced by human Dicer from virus dsRNA precursors, and are loaded in Argonaute proteins. We also show that non-structural protein 1 (NS1) encoded by *Influenza A virus* can suppress the biogenesis of the human vsiRNAs during infection. Our findings provide the first evidence that the antiviral RNAi response is conserved in humans.

Flavivirus Subgenomic RNA Captures XRN1 to Prevent Generation and Incorporation of Viral 3'UTR-Derived Immunostimulatory RNAs into Exosomes

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We previously showed that flaviviruses produce a unique subgenomic RNA (sfRNA) and established that it is generated as the result of incomplete degradation of viral genomic RNA by the cellular exoribonuclease XRN [1]. Strong secondary and tertiary structures located at the beginning of the 3'UTR stall XRN1 and prevent it from degrading the remaining 3'UTR RNA resulting in accumulation of sfRNA [2]. Generation of sfRNA facilitates virus replication and pathogenesis [1] and has been shown to inhibit host antiviral response [3], host mRNA degradation [4], and the RNAi pathway [5]. Here we show that XRN1 remains bound to sfRNA during West Nile virus infection and mutations that destabilize secondary structures essential for XRN1 stalling result in the release of XRN1 and restoration of its ribonuclease activity. This in turns leads to the generation of viral 3'UTR-derived RNA degradation products, which are incorporated into secreted exosomes and induce anti-viral activity in bystander cells. Transfection of A549 cells with a total RNA and a small RNA fraction isolated from exosomes produced from the mutant but not the wild type virus infection induced a strong antiviral effect. In addition, *in vitro* digestion of mutant but not wild type viral RNA with recombinant XRN1 followed by transfection of digested RNA products into A549 cells also induced antiviral activity against West Nile virus infection, recapitulating the effect observed in exosome-treated and exosomal RNA-transfected cells. Thus, we conclude that by capturing XRN1 on sfRNA flaviviruses prevent their 3'UTR region from degradation and incorporation of immunostimulatory RNA degradation products into exosomes in order to limit spread of antiviral activity to neighboring cells.

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MiR124 Contributes to Measles Virus Persistent Infection in Human Neuroblastoma Cells and Induces Apoptosis when Overexpressed

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The measles virus (MV) may invade the central nervous system and develop a non-cytopathic, persistent infection mainly within neurons. The factors that allow this otherwise highly cytopathic virus to persist remain largely unknown.

MicroRNAs (miRs) are a class of ~22 nt-long noncoding RNAs, transcribed from all multicellular organisms and some DNA viruses. Individual miRs may regulate several hundred genes.

Here we have studied the potential contribution of host cell-encoded miRs to maintenance of MV persistent infections in human neuroblastoma cells (UKF-NB and - UKF-NB-MV). We have shown that the MV, which does not encode miRs, modulates the expression- profile and levels of host cell-encoded miRs in persistently infected cells. MiR124 is strongly expressed in UKF-NB-MV but not in the non-infected UKF-NB cells. Cell division protein kinase 6 (CDK6) is an important regulator of cell cycle progression regulating G1/S transition. CDK6 is a target of mir124. We found diminished CDK6 protein expression in UKF-NB-MV as compared to control UKF-NB cells. In addition we show that UKF-NB-MV cells grow much slower than UKF-NB cells, possibly due to low CDK6 expression in these cells. When miR124-GFP was overexpressed in UKF-NB-MV cells, the cells died by apoptosis and CDK6 was further reduced. Conversely, when UKF-NB-MV cells were transfected with ANTAGOmiR124 to inhibit miR124 expression, they divided rapidly, CDK6 was up-regulated and apoptosis was not observed.

We hypothesize that persistent MV slows down cell division through induction of miR124 and down regulating CDK6. Over expression of miR124 in these cells induces apoptosis thus contributing to the conversion from persistent to lytic infection.

Production of Functional Small Interfering RNAs by Human Dicer

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While RNA interference (RNAi) functions as a potent antiviral innate immune response in plants and invertebrates, mammalian somatic cells appear incapable of mounting an RNAi response and very few small interfering RNAs (siRNAs) can be detected. To examine why siRNA production is inefficient, we have generated double knockout human cells lacking both Dicer and PKR. Using these cells, which tolerate double stranded RNA expression, we show that the overexpression of mutant forms of human Dicer lacking the amino-terminal helicase domain allows the processing of long dsRNAs to produce high levels of siRNAs that are readily detectable by Northern blot, are loaded into RNA induced silencing complexes and can effectively and specifically inhibit the expression of cognate mRNAs. However, even these more active Dicer mutants produce only modest levels of viral siRNAs in infected cells that are either unable to detectably inhibit virus replication (polio virus, PV) or exert only a modest inhibitory activity (influenza A virus, IAV). No inhibition of either PV or IAV replication was seen upon ectopic expression of WT Dicer. Interestingly, we observed that overexpression of the NS1 protein of IAV attenuated the production, but not the activity, of RISC-loaded siRNAs though the physiological significance of this remains unclear. We conclude that the production of siRNAs from long dsRNAs by WT Dicer is very inefficient but that this deficiency can be at least partially rescued by overexpression of helicase-deficient forms of human Dicer.

Viral Noncoding RNAs: Insights into Evolution

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Noncoding (nc)RNAs play pivotal roles in the regulation of gene expression, but exhibit a diversity of functions whether encoded by cellular or viral genomes. I shall present several recent stories related to non-coding RNAs produced by the oncogenic gamma herpesviruses. These illustrate the habit of viruses to exchange components with their host cells but then utilize them—sometimes in quite different ways—to enhance the viral life cycle. Thus, such studies provide insights into the evolution of both viruses and their host organisms.

One specific example is the highly abundant 170-nt ncRNA, EBER2, produced during both latency and lytic infection by EBV. EBER2 uses base pairing to interact with nascent transcripts arising from the terminal repeat (TR) region of EBV DNA in order to deliver the host B-cell transcription factor PAX5 to the TR chromatin. By doing so, EBER2 enhances lytic viral replication, which is known to be linked to the onset of EBV-associated tumors. We are currently looking for host ncRNAs that may fulfill the same functions as EBER2 in uninfected cells.

Another example comes from *Herpesvirus saimiri* (HVS), which co-transcribes microRNAs (miRNA) immediately downstream of viral small nuclear RNAs (snRNA). Therefore, the host-cell Integrator complex, which recognizes the snRNA 3'-end processing signal (3' box), generates the 5' end of the precursor (pre-)miRNA hairpins. Recently, we discovered that HVS pre-miRNAs carry yet another 3' box-like downstream sequence that is also essential for miRNA biogenesis. *In vivo* knockdown and rescue experiments confirmed that HVS pre-miRNA 3'-end processing depends on Integrator activity. To confirm the interaction between Integrator and its HVS pre-miRNA substrates *in vivo*, we employed RNA crosslinking and immunoprecipitation (CLIP) and developed an *in situ* proximity ligation assay (PLA), which can be generalized to localize specific transient RNA-protein interactions in cells. Surprisingly, in contrast to snRNA 3'-end processing, HVS pre-miRNAs 3'-end processing can be uncoupled from transcription, opening a unique opportunity to study host Integrator enzymology.

This work was supported by NIH grant CA16038. J. Steitz is an investigator of the Howard Hughes Medical Institute.

Session 4:
Interactions between Viruses and Membranes

Session Chair: Karyn Johnson

Editor-in-Chief: Prof. Dr. J. Peter W. Young - Department of Biology, University of York, Heslington, York YO10 5DD, UK.

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Mechanism of Influenza A Virus Uncoating During Host Cell Entry

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Influenza A virus (IAV) is an enveloped (-) ssRNA virus of great medical, social, and economic impact. After binding to the host cell surface, the virus is uptaken by clathrin-mediated endocytosis or macropinocytosis into vesicles.

(i) During endosome maturation IAV capsid is primed by the influx of H^+ and K^+ ions into the viral core via the M2 channel. This loosens the viral core and prepares the virus for cytosolic uncoating.

(ii) At late endosomes, low pH induces hemagglutinin (HA)-mediated viral fusion and exposes pre-packaged unanchored ubiquitin chains (a hallmark of misfolded protein aggregates) to the cytosol. A cytosolic histone deacetylase 6 (HDAC6) is recruited to the viral fusion sites via the HDAC6 ubiquitin-binding domain (ZnF-UBP). HDAC6 then binds viral matrix protein (M1), connecting the capsid to dynein, myosin motors and the cytoskeleton. The pulling force that is generated helps to break apart the capsid shell and to release the viral ribonucleoproteins (vRNPs) into the cytosol.

(iii) During acidification in endosomes, the homo-oligomeric structure of M1 undergoes an irreversible change in conformation. This results in M1 homo-dimers that have an open M1-M1 interphase. This interphase contains the antigenic site of the M1 uncoating monoclonal antibody, as well as, the interaction consensus of a nuclear import factor, the depletion of which blocks uncoating and infection. A mutant IAV strain with a point mutation in the consensus sequence became non-infectious.

Our findings indicate that IAV uses both aggresome processing and nuclear import machineries to efficiently uncoat the viral capsid following fusion at the surface of late endosomes. The understanding of host-mediated viral uncoating mechanisms can lead to the development of future novel antiviral strategies.

HIV Assembly and Maturation

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The assembly and budding of retroviruses involves a series of regulated steps, driven primarily by the viral Gag polyprotein precursor. In the case of HIV-1, assembly and budding occur predominantly at the plasma membrane. The HIV-1 Gag polyprotein precursor, Pr55Gag, is comprised of four major domains—matrix (MA), capsid (CA), nucleocapsid (NC) and p6—and two spacer peptides, SP1 and SP2. Assembly and budding from the host cell are driven by Pr55Gag; concomitant with particle release from the host cell plasma membrane, the viral protease cleaves Pr55Gag into its individual components, a process that triggers particle maturation.

The MA domain of Pr55Gag is the major viral determinant responsible for directing Gag to the plasma membrane. We showed a number of years ago that the host cell phospholipid PI(4,5)P2 plays an important role in directing Gag to the inner leaflet of the plasma membrane. The MA domain also plays a central role in the incorporation of the viral envelope (Env) glycoproteins into virions. Although Env incorporation is not required for Gag assembly *per se*, it is required for the formation of infectious particles. Our recent work has defined the structural requirements for Env incorporation. Specifically, the formation of MA trimers in the assembling virions appears to play a central role in Env incorporation. We are also investigating the role of host cell factors in HIV-1 Env glycoprotein incorporation. Results of these studies will be presented.

Determinants of Host Range Restrictions of Human Hepatotropic Viruses

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Infectious diseases account for at least 15 million human deaths each year. Many pathogens that cause disease in humans display a unique human tropism which is often poorly understood at the mechanistic level. The host range of a viral pathogen is determined by a number of parameters, including incompatibilities between orthologues of essential host factors from non-permissive species and viral proteins, the presence of dominant restriction factors, and differences in kinetics and magnitude of innate and adaptive immune responses. With particular focus on human hepatitis viruses we have performed gain-and-loss-of-function screens to define molecular determinants that restrict the viral host range. I will discuss how host adaptation approaches based on such discoveries can be harnessed to create (genetically) humanized animal models with inheritable susceptibility to human viral pathogens. Resulting small animal models can be utilized to study host responses at the organismal level as well as for testing novel therapeutic intervention approaches.

ABHD5/CGI-58, the Causative Protein for the Chanarin-Dorfman Syndrome, Consumes Lipid Droplets to Support Assembly and Release of the Hepatitis C Lipo-Viro-Particle

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Hepatitis C virus (HCV) assembly depends on cytosolic lipid droplets and on very low density lipoproteins (VLDL) synthesis, and virions are secreted as lipo-viro-particles. To elucidate how HCV usurps these pathways, we conducted a rational siRNA-based screen by selecting host genes involved in lipid droplet biology and VLDL secretion. Our screen identified several cellular pathways and functional protein clusters participating in the HCV replication cycle. The knockdown of nearly half of our candidates significantly inhibited HCV assembly or release. Among primary hits, ABHD5 knockdown repressed infectious HCV production similar to ApoE, a known HCV assembly factor. Importantly, this defect was rescued by expression of an RNAi-resistant ABHD5 variant. Moreover, ABHD5 expression had no effect on the virion specific infectivity but regulated both the rates of HCV assembly and release.

ABHD5 is a ubiquitously expressed protein that binds lipid droplets. Although ABHD5 folds as a lipid hydrolase, it lacks any intrinsic lipase activity. However, it can activate lipases for the mobilization and utilization of triglycerides from the lipid droplets. Moreover, it encodes a lysophosphatidic acid acyltransferase (LPAAT) motif. Finally, ABHD5 is responsible for the Chanarin-Dorfman syndrome, a rare inherited lipid storage disease associated with ichtyosis and liver steatosis.

In our Huh7-derived hepatoma cell line, ABHD5 was cytoplasmic and enriched at the lipid droplet surface and the Golgi. It also partially colocalized with the HCV replication and assembly machinery. Intriguingly, the Chanarin-Dorfman syndrome mutants did not support HCV production and showed an aberrant dispersed localization thorough the entire cell with enhanced nuclear staining and minimal overlap with HCV proteins. This striking difference was confirmed in live cells co-expressing WT and mutant proteins. Furthermore, ABHD5 overexpression reduced the size of the cytosolic lipid droplets, as observed by flow cytometry at the cellular level or confocal microscopy at the single droplet level. The

Chanarin-Dorfman syndrome mutants however did not consume lipid droplets and thus had lost his lipolytic activity. With a panel of targeted mutations we also excluded the role of the putative LPAAT motif, nuclear localization signal and phosphorylation site for the protein function in lipid droplet homeostasis or HCV production. Finally, with a combination of rationale approaches (RNA interference, lipase inhibitors) and proteomics (co-immunoprecipitation and mass spectrometry) we are currently working on the identification of the ABHD5 effector involved in hepatic lipid metabolism and HCV production.

In conclusion, we propose that ABHD5-mediated consumption of lipid droplets triggers the recruitment of triglycerides from the cytosolic lipid droplets to the nascent VLDL but also to the viral particle. This promotes the early step of HCV assembly but also the subsequent release of the lipo-viro-particle. Finally, our study provides the first association between the Chanarin-Dorfman syndrome protein, ABHD5, and an infectious disease and sheds light on the hepatic manifestations of this genetic disorder.

Bacterial Effectors as Cell Biological Probes to Study the Replication of Positive-Strand RNA Viruses

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Our understanding of virus-host interaction remains incomplete, in part due to limitations in existing experimental methods. While many cellular factors involved in replication of the *Flaviviridae* have been identified through genome-wide RNAi screening, identification of protein-protein interactions, and the use of pharmacological agents, limitations of these methods include variable knock-down efficiency, off-target effects, low reproducibility between genome-wide screens, false-positive interactions, and a relatively small and nonspecific pharmacopeia. Here we describe a new strategy to identify virus-host cell interactions by employing a large collection of bacterial effector proteins as a genetic toolkit to manipulate key cellular pathways with surgical precision. Many bacterial pathogens infect and survive within eukaryotic cells by injecting minute quantities of effector proteins, typically enzymes, into the cytosol of their hosts. These effector proteins have evolved to precisely and potently manipulate cellular pathways, prevent bacterial degradation, and favor bacterial replication. Remarkably, many bacterial effectors target the same cellular pathways utilized by positive-strand RNA viruses, including ER–Golgi and endolysosomal membrane trafficking, autophagy, and innate immunity. Importantly, effector proteins retain their function when ectopically expressed in mammalian cells and can be used to study cellular pathways independent of bacterial infection. Based on these known functional overlaps, we screened >300 bacterial effectors to identify cellular targets that enhance or inhibit the replication of hepatitis C virus (HCV) and yellow fever virus (YFV), two representative viruses in the family *Flaviviridae*. Our screen revealed multiple viral phenotypes, including inhibition or enhancement of virus replication in effector-expressing cells, as well as enhancement of virus replication in bystander cells. Of note, YFV is highly dependent on autophagy, while HCV replication is independent of autophagosome formation. Our data reveal several new aspects of HCV- and YFV-host interaction and provide proof-of-concept for an experimental niche wherein virologists can utilize bacterial effectors as tools to address a variety of cell biological questions.

The Role of Membrane Fusion in Phage Lysis

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In phage infections, lysis of the host is a programmed event independent of virion morphogenesis. It has long been known that phage lysis requires permeabilization of the cytoplasmic membrane by the holin, leading to degradation of the cell wall by the endolysin. Surprisingly, even after the degradation of the cell wall, lysis of Gram-negative hosts requires the function of a new class of proteins, the spanins, to disrupt the outer membrane (OM), else the progeny virions are left trapped in spherical cells bounded by the intact OM. The prototype two-component spanins are encoded by the *Rz* and *Rz1* genes of phage lambda. Uniquely, *Rz1* is completely embedded in the +1 reading frame of *Rz*. Rz is an inner membrane protein (i-spanin) with an N-terminal transmembrane domain (TMD), whereas Rz1 is an OM lipoprotein (o-spanin). The term spanin is derived from that fact that Rz and Rz1 form a complex via C-terminal interactions, thus spanning the entire periplasm. Both the i-spanin and o-spanin accumulate as homodimers covalently linked by intermolecular disulfide bonds. A model has been presented in which the Rz2-Rz12 hetero-tetrameric complexes are entrapped by the meshwork of the peptidoglycan until liberated by the muralytic action of the phage endolysin. This allows lateral oligomerization and conformational changes that result in fusion of the IM and OM. Experiments with spheroplasts labeled with different fluorescent proteins showed that the periplasmic domains of Rz and Rz1 can mediate efficient spheroplast fusion, thus strongly supporting the membrane fusion model. Mutational and suppressor analysis suggests that the Rz-Rz1 complex undergoes a collapsing conformational change during lytic function.

A second and completely distinct class of spanins is represented by gp11 of coliphage T1. Gp11 is the prototype unimolecular spanin (u-spanin), which has both an N-terminal OM lipoprotein signal, causing the mature lipoylated NTD to be sorted to the inner leaflet of the OM, and a C-terminal transmembrane domain that is embedded in the IM. Spheroplast experiments with gp11 have provided evidence that the u-spanin can also cause membrane fusion. We suggest that the two-component spanin complex and the u-spanin, although evolutionarily distinct, both achieve the final step in phage lysis the same way, by fusing the IM and OM and thus removing the final barrier to virion release by a topological strategy.

Alphavirus Budding: How Viruses Remodel the Cell during Exit

Margaret Kielian, Maria Guadalupe Martinez

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Alphaviruses are small, highly organized enveloped RNA viruses that contain an internal nucleocapsid and an external lattice of the viral E2 and E1 transmembrane proteins. Alphaviruses infect cells by endocytic uptake and low pH-triggered fusion. Replication occurs in the cytoplasm and the envelope proteins transit through the secretory pathway to the cell surface. The viral envelope is derived by budding through the plasma membrane in a process that excludes host membrane proteins. We engineered the alphavirus Sindbis virus with fluorescent protein labels on the E2 envelope protein to characterize virus assembly and budding in living cells. These studies revealed that virus infection induces dramatic reorganization of the cell cytoskeleton. Actin stress fibers were disassembled, focal adhesion complexes were disorganized, and short extensions that contain budding virus particles were induced. The microtubule (MT)-organizing center was disrupted, the organized MT network was lost, and long inter-cellular extensions containing both stable MT and actin were induced. These extensions released virus particles at the tip. Such extensions were also produced in non-infected cells expressing the capsid and envelope proteins, and were blocked by a mutation that inhibits the interaction of E2 with the capsid protein, a critical step in virus budding. The mechanisms by which virus infection signals and induces these striking rearrangements of the host cell cytoskeleton are being determined.

TMPRSS11A Activates Influenza and Emerging Coronaviruses and Is Resistant to Serine Protease Inhibitor HAI-1

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Influenza virus infection is a major health threat and the development of novel antivirals is an important task. The cleavage-activation of the influenza virus hemagglutinin (HA) by host cell proteases is required for viral infectivity and the responsible enzymes are potential targets for antiviral intervention. The type II transmembrane serine protease (TTSPs) TMPRSS2 was shown to be essential for pathogenesis of H1N1 and H7N9 influenza A viruses (FLUAV) in mice. In contrast, H3N2 viruses seem to be less dependent on TMPRSS2 for viral spread, suggesting that these viruses might exploit other TTSPs for their activation. Therefore, we addressed whether TTSPs, which so far have not been characterized in the context of viral infection, can activate FLUAV HA and the spike (S) protein of the emerging MERS-coronavirus (MERS-CoV).

We found that TMPRSS11A is expressed in the upper respiratory tract and cleaves and activates FLUAV HA and MERS-S upon directed expression in cell culture. TMPRSS11A activity was suppressed by the chemical and natural serine protease inhibitors camostat and plasminogen activator inhibitor 1 (PAI-1), respectively. In contrast, hepatocyte growth factor activator inhibitor type 1 (HAI-1) was not able to suppress TMPRSS11A-mediated HA cleavage. TMPRSS11A facilitated trypsin-independent FLUAV spread in HepG2 cells, indicating that endogenous levels of TMPRSS11A can be sufficient for HA activation. Finally, we obtained evidence that TMPRSS11a can counteract the host cell-encoded antiviral factor tetherin, suggesting that this protease and other TTSPs might promote viral spread via two mechanisms: activation of viral surface proteins and inactivation of tetherin. Collectively, our results identify TMPRSS11a as an activator of respiratory viruses and suggest that this protease could support viral spread in the host.

Molecular Basis of Herpesvirus Nuclear Egress: the Prototypic Vesicular Nucleo-Cytoplasmic Transport

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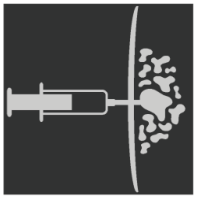
Herpesvirus nucleocapsids are assembled in the nucleus but mature to infectious virions in the cytosol. To gain access to the maturation compartment intranuclear capsids have to cross the nuclear envelope (NE). While nucleo-cytoplasmic transport usually occurs through nuclear pore complexes (NPC), the *ca.* 120 nm capsids are too large to pass through NPC. Thus, herpesviruses use a vesicle-mediated nucleo-cytoplasmic transport by budding of nucleocapsids at the inner nuclear membrane thereby obtaining a primary envelope, which then fuses with the outer nuclear membrane to release the capsid into the cytosol. Nuclear egress is mediated by the heterodimeric viral nuclear egress complex (NEC) which alone is sufficient for membrane bending and scission in artificial membrane systems as well as authentic nuclear envelopes. We have analyzed vesicle formation by the NEC *in situ* by multimodal imaging and determined the crystal structure of the NEC of the alphaherpesvirus pseudorabies virus. Our data unravel basic principles of vesiculation from the INM including the formation of two hexagonal lattices composed of multiple copies of the NEC which induce bending of the INM resulting in the formation of vesicles of distinct size capable to accommodate the viral nucleocapsid for transport. Although this herpesvirus-induced vesicular transfer through the NE has been thought unique, it may actually also be used for transport of other large cellular cargo, e.g. RNP complexes, through the NE.

Session 5:
Replication Organelles

Session Chair: Veronique Ziegler-Graff



vaccines

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Protein, RNA and Membrane Interactions in Positive-Strand RNA Virus Genome Replication

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Positive-strand RNA virus genome replication occurs on dramatically remodeled intracellular membranes, often inside invaginated, vesicular replication compartments (RCs). We are dissecting RC structure, assembly and function to better understand and control virus replication. The ~50 nm diameter, invaginated RC vesicles are formed on mitochondrial outer membranes by nodaviruses, which encode a single RNA replication protein A with an RNA polymerase domain, RNA capping domain, multiple essential self-interaction domains, and a mitochondrially-targeted transmembrane domain. In early steps separable from RNA synthesis, multiple protein A regions cooperate to recognize defined viral genomic RNA signals and recruit these RNAs to mitochondrial membranes. Subsequent RC vesicle formation requires protein A, RNA templates with additional initiation signals, and RNA synthesis.

Formation of similarly invaginated, ~70 nm RCs on ER membranes by bromoviruses (in the alphavirus superfamily) is directed by protein 1a, which has RNA capping ($1aN^{Cap}$) and helicase-like ($1aC^{Hel}$) domains. The 1a forms RCs by multimerizing, selectively binding ER membranes, and recruiting viral RNA templates and $2a^{Pol}$ polymerase. Genetic, fluorescence and electron microscopy results show that 1a also directly recruits host factors, including membrane-shaping reticulons and ESCRT proteins essential to form RC vesicles. The $1aN^{Cap}$ is a particular nexus of 1a-1a and 1a-membrane interactions. The 1a primarily binds membranes through an amphipathic helix in $1aN^{Cap}$, whose alternate interactions switch 1a between successive states controlling the order and balance of RC assembly steps. Ongoing mapping is revealing novel features of 1a-membrane interaction and showing that 1a multimerizing interactions are complex, involve homo- and hetero-typic binding, include mutually exclusive interactions implying alternate 1a conformations, and are essential for proper 1a localization, membrane remodeling, RNA template recruitment, and RNA synthesis.

Insights into the Assembly of the Tombusvirus Replicase: The Role of Co-Opted Host Proteins and Lipids

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Plus-stranded RNA viruses recruit cellular membranes and subvert cellular proteins involved in lipid biosynthesis to build viral replicase complexes (VRCs) and replication organelles. We use tombusviruses (TBSV), which are small (+)RNA viruses that serve as model plant viruses to study virus replication, recombination, and virus-host interactions using yeast (*Saccharomyces cerevisiae*) as a surrogate host. Several systematic genome-wide screens and global proteomic and lipidomic approaches have led to the identification of ~500 host proteins/genes that are implicated in TBSV replication. We find that sterols and phosphatidylethanolamine are important components of tombusvirus VRCs. We also present data that Tombusviruses co-opt cellular Oxysterol-binding protein related proteins (ORPs), and VAP proteins to facilitate the formation of membrane contact sites, where membranes are juxtaposed, to channel lipids to the replication sites. Using *in vitro* viral replication assay with artificial PE vesicles, we show stimulation of tombusvirus replication by sterols. Finally, we show evidence that TBSV usurps the ESCRT machinery to form spherules (vesicle-like structures) to build VRCs in a protected microenvironment involving peroxisomes and the cytosol.

Architecture and Building Blocks of the Flaviviral Replication Organelles

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Like all positive-strand RNA viruses, members of the family *Flaviviridae* extensively reorganize intracellular membranes to allow efficient RNA replication [1] 3D analysis of infected Dengue Virus (DENV) and Tick Borne Encephalitis Virus (TBEV)- cells by means of electron tomography (ET) have revealed that these flaviviruses remodel the endoplasmic reticulum (ER) membranes to build up their replication vesicles [2,3].

We have also previously shown that in contrast to DENV and TBEV and similarly to other positive-strand RNA viruses, Hepatitis C Virus (HCV) infection induces ER-derived double membrane vesicles (DMVs) that represent the major constituent of the cytoplasmic replication factory within the host cell [4]. Formation of this factory requires a concerted action of nonstructural proteins (NS) 3, 4A, 4B, 5A and 5B. Moreover, we found that the sole expression of NS5A was sufficient to induce DMV formation, albeit at low efficiency.

In a recent study we have further dissected the determinants within NS5A responsible for DMV formation [5]. We found that the RNA binding domain 1 (D1) as well as the amino-terminal membrane anchor are indispensable for this process. In contrast, deletion of NS5A D2 or D3 did not affect DMV formation, but completely disrupted RNA replication and virus assembly, respectively. To identify *cis*- and *trans*-acting factors of DMV formation we established a *trans*-cleavage assay. We found that induction of DMVs requires full length NS3 whereas a mutant lacking the helicase domain was unable to trigger DMV formation in spite of efficient polyprotein cleavage. Importantly, a mutation accelerating cleavage kinetics at the NS4B-5A site diminished DMV formation, while the insertion of an internal ribosome entry site (IRES) mimicking constitutive cleavage at this boundary completely abolished this process. These results suggest that regulated polyprotein cleavage is essential for efficient DMV biogenesis.

Our findings identify novel viral determinants governing the biogenesis of the HCV replication factory with possible implications for our understanding of how this central compartment of positive-strand RNA viruses is formed.

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Identification of Novel *Cis*- and *Trans*-Activities of FMDV 3D^{pol} Necessary for the Formation of Viral RNA Replication Complexes

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Foot-and-mouth disease virus (FMDV), a positive-sense single-stranded RNA virus in the family *Picornaviridae,* is the causative agent of foot-and-mouth disease, an economically damaging infection of cloven-hooved animals, with outbreaks resulting in large financial losses to the agricultural and livestock industries. Due to the highly contagious nature of FMDV, research with live-virus is restricted to a limited number of key facilities worldwide. As a result, the key events which precede the establishment of a viral replication complex are not well defined. This is despite the fact that the functions of many of the mature non-structural proteins necessary for viral genome replication are well understood. The development of FMDV sub-genomic replicons has allowed for the study of viral genome replication in a non-infectious system and helps elucidate the initial events in replication complex formation, together with the molecular interactions with the complex.

In this study, we have investigated the role of the viral RNA-dependent RNA polymerase 3D^{pol}, in the establishment of the viral replication complex in the context of a sub-genomic replicon. Using reciprocal complementation and immunofluorescent experiments we were able to identify distinct *cis* vs *trans* functional roles of FMDV 3D^{pol}. Our findings demonstrate that whereas 3D^{Pol} polymerization functions can be readily supplied in *trans,* a non-enzymatic *cis* preferential function of 3D^{pol} is involved in interacting with viral template RNA. Further experimentation showed that *cis* preferential functions can be rescued using complementary mutations in structured RNA elements. Using genetic complementation experiments we were able to investigate the precursor requirement for rescue of *cis* vs *trans* 3D^{pol} functions. Finally, we were able to use such 3D^{pol} mutations to probe the *cis* preferential protein-protein interactions in the replication complex. Using such data we propose an improved model in the initial events required for replication complex formation.

Disassembly and Assembly of Bluetongue Virus 1

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My lecture will be centered on the molecular dissection of Bluetongue virus, a model complex non-enveloped RNA virus with a view to understanding the virus disassembly/assembly pathways and replication. BTV is an insect-vectored emerging pathogen of wild ruminants and livestock (with mortality reaching 70% in sheep) in many parts of the world. The virion particle is an architecturally complex structure of four consecutive layers of protein surrounding a genome of ten double-stranded (ds) RNA segments. To understand BTV entry into the host we have used a multi-disciplinary approach including atomic and 3D structure reconstructions, protein engineering, synthetic biology and reverse genetics. Specifically, we have defined the individual steps involved in the sequential uncoating of the incoming core and, later, in the capsid assembly and packaging pathways which capture the multi-segmented viral genome. These findings illuminate BTV replication and lead directly to the design of novel assemblies and attenuated strains. Equally, they indicate the pathways that related viruses might use, including viruses that are pathogenic to man and animals, to provide an informed starting point for intervention or prevention.

Formation and Function of Reovirus Replication Organelles

Isabel Fernández de Castro [1], Raquel Tenorio [1], Mine Ikizler [2,3], Jonathan J. Knowlton [3,4], Bernardo A. Mainou [5], Paula F. Zamora [3,4], Liya Hu [6], B. V. V. Prasad [6], Cristina Risco [1], Terence S. Dermody [2,3,4]

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Most viruses that replicate in the cytoplasm of host cells form neoorganelles that serve as sites of viral genome replication and particle assembly. These highly specialized structures bring together viral replication proteins and nucleic acids, prevent activation of cell-intrinsic defenses, and coordinate release of progeny particles. Mammalian reoviruses are nonenveloped, double-stranded (ds) RNA viruses that serve as tractable models for studies of dsRNA virus replication and pathogenesis. Reovirus replication occurs in large cytoplasmic inclusions that are nucleated by viral nonstructural proteins sigma NS and mu NS, which in turn recruit viral structural proteins for dsRNA synthesis and assembly of viral progeny. We found that sigma NS forms octamers and preferentially binds viral single-stranded RNA. Alanine substitution of basic residues in the sigma NS RNA-binding region compromises fidelity of viral dsRNA synthesis, suggesting a new role for sigma NS in reovirus genome replication. Three-dimensional image reconstructions of transmission electron micrographs of reovirus-infected cells revealed that reovirus inclusions form within a membranous network. Viral inclusions contain filled and empty viral particles and microtubules and appose mitochondria and rough endoplasmic reticulum (RER). Live cell video-microscopy of reovirus-infected cells followed by correlative light and electron microscopy revealed that RER cisternae associate with the periphery and interior of early inclusions. Immunogold labeling of Tokuyasu cryosections showed numerous small vesicles and RER luminal proteins in the inclusion interior. Collectively, these data point to a deep remodeling of RER to provide a scaffold for viral and cellular proteins to coordinate viral genome replication and particle morphogenesis.

4
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1 Dicer Is Associated with Host Chromatin and Influences Gene Expression in Response to Influenza Infection

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Dicer plays an essential role in the RNA-interference (RNAi) pathway and is dynamically shuttling between nucleus and cytoplasm. While the significance of Dicer in regulation of antiviral RNAi in lower eukaryotes and genesis of microRNAs in mammals have been established, it remains elusive whether Dicer could also be involved in other innate antiviral pathways, particularly in the nucleus.

Influenza virus replicates viral genome in the nucleus and can serve as a perfect infection model for studying function of nuclear Dicer in response to infection. Using an influenza A virus infection model, we demonstrated that Dicer exerts anti-viral function through modulation of host IFN expressions in the nucleus. While depletion of Dicer leads to down-regulation of a subset of genes involved in IFN-β production in response to infection, ectopic expression of nuclear Dicer in influenza infected cells can enhance production of IFN-β. Interestingly, it appears that only nuclear-replicating influenza virus, but not the cytoplsmic-replicating Sendai virus, can stimulate translocation of Dicer to the nucleus. Furthermore, we found that Dicer is associated with host chromatin and SWI/SNF chromatin remodeling complex in non-infected cells, and such association could be interfered by the presence of viral RNAs in the nucleus. We hypothesized that Dicer maintains the chromatin status and it is important for the transcription switch of IFN genes in response to infection. Nucleus replicating virus, such as influenza A virus, adapts specific mechanism to counter antiviral activity exerted by nuclear Dicer. Molecular details for this mechanism will be discussed.

2 Induction of Nodamura Virus RNA Replication Complexes

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Our laboratory studies the mechanisms of viral RNA replication and replication complex (RC) assembly using Nodamura virus (NoV; family *Nodaviridae*). NoV replicates its bipartite positive strand RNA genome to tremendously high levels, in essence doubling the RNA content of a cell. Its replication is catalyzed by a viral RNA-dependent RNA polymerase (RdRp) that is encoded by the RNA1 genome segment. NoV RNA replication occurs in a wide range of eukaryotic host cells from both mammals and insects, suggesting that any host factors or processes involved must be highly conserved. In mice, NoV-infected muscle cells exhibit mitochondrial aggregation and rearrangement of mitochondrial structure, leading to disorganization of the muscle fibrils on the tissue level. However, the molecular basis for this pathogenesis and the role of mitochondria in NoV infection remained unclear until now. We showed using biochemical methods that the NoV RdRp interacts with membranes from a mitochondrial cellular fraction as an integral membrane protein. We used immunofluorescence confocal microscopy to demonstrate that the NoV RdRp and viral RCs localize to the outer mitochondrial membrane in transfected mammalian cells, consistent with previous reports of the related Flock House virus in insect and yeast cells. Unique to NoV is its ability to induce clustering of affected mitochondria in transfected mammalian cells, similar to that described for NoV-infected muscle tissue. The paralysis observed in NoV-infected animals may be a direct consequence of mitochondrial membrane rearrangement, mitochondrial clustering, and formation of the RCs. The mechanism by which the RCs arise in the presence of the NoV RdRp is currently under investigation.

3 Latency Reversing Agents and HIV Eradication: Can we Define the Optimal Therapy by Inversion of the Screening Process?

Valentino Cattori, Regina Hofmann-Lehmann

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More than thirty years after the identification of the virus, HIV infection is still incurable and patients are on lifelong antiretroviral therapy. HIV cure is hindered by the presence of latently infected cells, which can start shedding the virus after interruption of antiretroviral therapy. Potential HIV eradication therapies are mostly based on elimination of virus-infected cells after drug-induced reactivation of latent proviruses, but the test of latency reversing agents (LRAs) and of their combinations is difficult because of the lack of suitable cell culture models of HIV latency. To simplify the model, we treated cells derived from domestic cats with a combination of several LRAs, and judged the efficiency of the combinations by determining the sensitivity of the cells to feline immunodeficiency virus integration. In addition, we assessed the effect of LRA combination on the expression of several endogenous retroviruses of the cat.

Despite the limitations of the model, preliminary results showed that pretreatment of cells with LRA and following lentivirus infection can potentially predict the effect of LRAs on latently infected cells. We also could show that LRA-treatment does not cause a global activation of endogenous retroviruses.

This model may be used to evaluate the effect of LRA combinations in several different tissues ex vivo; to determine best combination for each tissue; and therefore the therapeutic combination (or sequence of LRA combination treatments) with the potential maximal effect *in vivo*.

4 Molecular Mechanisms Involved in Antibody-Dependent Enhancement of Dengue Virus Infection in Macrophages

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Dengue virus (DENV) is an emerging pathogen causing disease symptoms ranging from febrile illness to devastating hemorhagic manifestations. Increased disease severity is associated with pre-existing DENV antibodies and high circulating virus titers, which suggests that antibodies directly influence the infectious properties of the virus. The molecular mechanism by which antibodies enhance DENV infection however remains elusive.

We dissected how antibodies influence DENV infection in macrophages, the natural target cells for DENV replication. We applied live cell imaging and single virus tracking to unravel the route of cell entry and microarray analysis to identify the cellular responses upon DENV infection in the absence and presence of antibodies.

At conditions of antibody-dependent enhancement (ADE) of DENV infection, an increased number of infected cells and virus production was seen. However, no increased cell binding or cell entry was detected. Yet, DENV particles internalized via antibodies appear to have a higher chance to induce membrane fusion. Indeed, antibody-bound virions were observed to enter though a novel phagocytosis-like pathway that is distinct from entry in absence of antibodies. We observed that antibody-bound particles are captured and engulfed by the cell through active formation of actin-induced membrane protrusions. Macrophages actively sense and capture antibody-DENV particles located away from its cell body. The distinct route of entry and trafficking behaviour may increase the fusion potential of the virus. Additionally, microarray analysis showed that antibodies do not suppress antiviral signalling within infected cells. During the meeting, the critical determinants in DENV-ADE will be discussed.

5 New Potential Cellular Processes Altered During DENV2-Infection of Microvascular Endothelial Cells Revealed by Expression Profiles of Cytokines, Growth Factors and MicroRNAs

Diego Alejandro Alvarez-Diaz [1], Elizabeth Orozco-García [1], Andres Puerta-González [2], Clara Isabel Bermudez-Santana [2], Juan Carlos Gallego-Gómez [1]

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The most dramatic consequence of Dengue Virus (DENV) infection is a severe dysfunction of the microvascular endothelial cell (MEC) barrier leading to plasma leakage, hypovolemic shock and hemorrhage. However, the underlying cellular mechanisms responsible for the dysfunction of MECs are not well understood. In order to identify new potential cellular processes altered during DENV infection of MECs, the expression profiles of cytokines/growth factors and microRNAs were measured by Luminex Bead-based Multiplex Assays and Next Generation Sequencing respectively. Synchronized DENV2 infection (MOI-10) of HMEC-1 cells following 48 hours pos-infection (hpi), revealed the successfully replication of DENV2 in endothelial cells and the secretion of cytokines, chemokines and growth factors (IL-6, IL-8, FGF-2, GM-CSF, G-CSF, TGF-α, GRO, RANTES, MCP-1 and MCP-3). Culture supernatants of infected cells used as chemotactic stimulus increased the migration of non-infected HMEC-1 cells. A total of seven mature miRNAs were differentially expressed compared to time-matched mock-infected cells early at 3 hpi, hsa-miR-3607-3p and hsa-miR-3607-5p; and late at 24 hpi, hsa-let-7a-3p, hsa-miR-186-3p, hsa-miR-146a-5p, hsa-miR-21-3p and hsa-miR-484. Based on multifactorial analysis of cytokines, growth factors and migratory patterns increased in DENV-infected HMEC-1 cells in addition to network analysis of biological pathways regulated by the gene targets of the differentially expressed miRNAs, here it is proposed that DENV-infection alters the vascular barrier function by promoting increases in cytokines, growth factors and miRNAs that regulates biological process involved in vascular remodeling such as endothelial cell migration related to angiogenesis and endothelial-to-mesenchymal transition, adherent junctions interaction and VEGFR2 mediated vascular permeability.

6 Vesicular Trafficking of Incoming Human Papillomavirus Type 16 to the Golgi Apparatus Requires Gamma-Secretase Activity and Retromer Recruitment

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Human papillomavirus (HPV), a small non-enveloped virus containing a double-stranded DNA genome, is one of the most common sexually transmitted pathogens worldwide. Infection by high risk HPVs (such as HPV16) is strongly associated with all cervical cancer and many other types of cancers. Preventing tumor virus infection is an effective strategy to reduce the numbers of infected people and the resulting cancer cases. The route taken by papillomaviruses from the cell surface to the nucleus during infection is incompletely understood. To better track incoming virus, we developed an infectious HPV16 pseudovirus (PsV) with a 3xFLAG tag at C-terminus of minor capsid protein L2. By using this new reagent, we showed that HPV16 traffics sequentially from the cell surface to the endosome, the Golgi apparatus and the endoplasmic reticulum prior to nuclear entry. Our laboratory previously conducted a genome-wide siRNA screen and identified gamma-secretase and retromer as important cellular factors mediating HPV16 infection. Gamma-secretase cleaves many single-pass transmembrane proteins. Previous studies reported that inhibiting gamma-secretase activity prevented HPV16 infection, but the molecular mechanism and the stage of this inhibition still remain totally uncharacterized. We showed that inhibition of gamma-secretase does not interfere substantively with virus internalization, initiation of capsid disassembly, or virus entry into the early endosome, but gamma-secretase is required for localization of L2 and viral DNA to the Golgi apparatus and the endoplasmic reticulum. We showed that retromer is required for the retrograde trafficking of HPV16 to the Golgi apparatus during virus entry and that L2 binds directly to retromer via the conserved sequence motifs in its C-terminus. Either inhibiting retromer activity or preventing retromer/L2 interaction resulted in the accumulation of HPV16 in the early endosome, indicating that binding to retromer is required for the retrograde trafficking of HPV16 towards trans-Golgi network. Because the retromer binding sites are very conserved among all known HPV types, our study also suggested that virus has evolved to take advantage of this retrograde transport pathway for successful infection and that the virus is an unconventional retromer cargo.

7 *Drosophila* miRNA Star Strand Modulates Viral Replication

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MicroRNAs (miRNAs) are a class of small non-coding RNA that are critical regulators of diverse biological processes. During biogenesis, the miRNA precursor undergoes sequential cleavage to liberate 21-22 nt RNA duplexes. Generally one of the two miRNA strands in the duplex is found in higher abundance in the cell. The low abundance or the "star" strand (miR*) has traditionally been assumed to be degraded; however, improved deep sequencing methods have shown that miRs* can be present at physiologically relevant levels. In this study, we found that both strands of the miR-8 duplex are present in high copy numbers suggesting that they may both be functional regulators. To investigate whether the miR* strand (miR-8-5p) impacts host-virus interactions, we challenged *Drosophila melanogaster* flies and cells with the natural *Drosophila* pathogen Drosophila C virus (DCV). We found that miR-8-5p is down-regulated in both systems by the presence of DCV. Using inhibitors that repress miR-8-5p, viral replication increased, indicating that the miR-8-5p modulates viral replication. To understand how miR-8-5p affects the host-virus interaction, we predicted its putative targets using bioinformatics. The predicted targets include Jun-related antigen (Jra), a homolog of mammalian cJun of the cJun NH2-terminal kinase (JNK) pathway. Jra was verified to be a target of synthetic miR-8-5p. During DCV infection, Jra is up-regulated, concomitant to miR-8-5p down-regulation. This is consistent with Jra being a target of miR-8-5p and with the involvement of this interaction in virus infection. To further confirm that miR-8-5p modulates DCV infection through regulation of Jra, we are now analyzing virus infection in Jra mutant flies. Taken together these results suggest that the miR-8* strand is likely involved in modulation of viral replication via targeting of Drosophila host genes.

8 HCV Employs microRNAs to Modulate Lipid Metabolism in Liver Cells

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An estimated 150-200 million people worldwide are infected with hepatitis C virus (HCV), which can lead to serious complications including liver failure and hepatocellular carcinoma. We have recently shown that HCV affects the expression levels of several hepatic microRNAs (miRNAs), including miR-185 and miR-130b, causing changes to cellular metabolism in the liver. The modulation of liver metabolism results in differentially expressed host proteins, supporting replication of HCV.

One notable pathway modulated by HCV is fatty acid metabolism. This pathway is crucial for the later steps in HCV replication due to the enhanced requirement of fatty acids for reconstruction of the ER and formation of the membranous web. Furthermore, the viral membrane is derived from ER membranes and is dependent on a specific fatty acid composition.

Our studies suggest that fatty acid metabolism involves crucial host factor for the HCV life cycle. Transfection of Huh7.5 with miR-185 mimics affects the expression levels of several enzymes involved in lipid metabolism. Moreover, siRNA knockdowns of these host factors in Huh7.5 cells stably expressing an HCV full-length replicon shows reduced HCV protein levels. These data will be presented. Our understanding of the role of host factors associated with miRNAs and fatty acid metabolism in the antiviral host immunometabolic response will also be discussed.

9 Unambiguous Identification of Virus-miRNA Anteractions across a Broad Panel of Viruses

Troels K. H. Scheel [1,2], Joseph M. Luna [1,3], Matthias Liniger [4,5], Eiko Nishiuchi [1], Kathryn Rozen-Gagnon [1], Amir Shlomai [1], Gaël Auray [4,5], Markus Gerber [4,5], John Fak [3], Irene Keller [6], Rémy Bruggmann [6], Robert B. Darnell [3], Nicolas Ruggli [4,5], Charles M. Rice [1]

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Small non-coding RNAs have emerged as key players in modulation of viral infection. Numerous DNA viruses engage host microRNA (miRNA) machinery to manipulate host or viral gene expression, whereas RNA virus interactions remain poorly explored. An exception is the dependence of hepatitis C virus (HCV) on the liver-specific miRNA, miR-122, which has surfaced as therapeutic target. However, no broad investigation of virus-miRNA interactions has been done.

In this study, we used crosslinking immunoprecipitation (CLIP) of the Argonaute (AGO) protein to characterize strengths and specificities of miRNA interactions across 15 viral genomes. The alphaviruses bound a large proportion of the total AGO/miRNA pool through many interactions across the viral genome, however, due to equally high abundance of viral RNA, active sequestration appeared to be minimal. On the other hand, HCV and the pestivirus bovine viral diarrhea virus (BVDV) specifically sequestered relatively large proportions of the AGO/miRNA pool to the 5'UTR and 3'UTR, respectively. To circumvent the lack of paring of miRNAs to their cognate targets in traditional CLIP methods, we developed a method relying on miRNA-target chimeras to allow unambiguous identification of interactions. We validated this method on HCV, and confirmed binding of miR-122 to the 5'UTR, and miR-15 to a viable variant modified to bind this miRNA instead. Interestingly, we found that the BVDV 3'UTR interacted strongly with miR-17 and let-7. In contrast to alphaviruses, for which large proportions of target chimeras of many miRNAs appeared on viral RNA, HCV and BVDV specifically sequestered miR-122 and miR-17, respectively, with 40-50% of these miRNAs targeting viral RNA.

Thus, these findings may generalize the concept of RNA virus dependence on cellular miRNAs, highlight such interactions as therapeutic targets, and connect functional regulation of the transcriptome to miRNA sequestration.

10 Analysis of Transcriptional Profiles of Pig PBMCs in Course of Experimental Infection with three PRRSV Strains

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a causative agent of an important infectious disease causing serious economic losses to swine industry called PRRS (porcine reproductive and respiratory syndrome). The clinical signs of this syndrome include respiratory disorders, abortions and variable mortality in piglets. To compare the virulence of highly diverse East European strains belonging to subtype 2 (Russian strain ILI and Belarusian strain BOR) and Danish strain from classical subtype 1 (DAN) the experimental study enrolling infection of piglets was performed. The infection with strain BOR resulted in the in fever, higher clinical scoring and higher pathological scoring in lungs, while the infection with remaining two strains was clearly milder. Therefore, it was concluded that more in depth analysis of mechanisms determining the virulence of examined strains is necessary. Thus gene expression profiles of peripheral blood mononuclear cells (PBMC) of piglets infected with three PRRSV strains *vs.* control piglets were analysed by microarray analysis to gain insight into transcriptome changes after PRRSV infection. The genes were determined to be differentially expressed if the fold change (FC) was greater than 1.5 in up or down-regulation. Validation of microarray results was performed using RT-qPCR and showed high reliability of FC values determined by both methods. Microarray data were further analysed using the Ingenuity Systems Pathway Analysis program to determine most significantly affected immunological pathways and regulators. The patterns of activation score for particular immunological pathways analysed, as well as, upstream regulators activity were more similar in ILI and BOR infected groups, than in DAN group. Such results could be expected due to fact that BOR and ILI strains represent subtype 2, while DAN subtype 1. However, the clinical pictures of infection were different with the most severe in BOR infected pigs. Since the PRRSV interference with host defence mechanisms is at multiple levels, the divergence of PRRSV strains sequences can induce the variation of such activities and determine different infection course. Therefore, further analysis is required to deterimine genes variation in particular PRRSV strains, which may be crucial for the changes in the multifold interplay between the virus and host during PRRSV infection.

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11 Anchored Polyubiquitin Couples TRIM Intracellular Signaling to Virus Destruction

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In response to viral infection, several TRIpartite Motif (TRIM) proteins generate unanchored polyubiquitin (polyUb) moieties linked by Ub residue K63, which stimulate innate immune signaling cascades. The closely related TRIM5alpha and TRIM21 induce transcription of NF-κB-responsive genes after recognition of intracellular viruses, whereupon they also recruit proteasomes to mediate virus disassembly. A major question concerns the coordination of these events, as a system that spuriously generates free polyUb in the absence of infection would be detrimental for the host, while degradation of the virion before polyUb synthesis would dampen immune activation. To understand the mechanistic basis of TRIM restriction/signaling coupling, we employed an RNAi screen against the human E2 Ub-conjugating enzyme repertoire, and used viral infection assays as cellular readouts of TRIM5 and TRIM21 ubiquitination activities. Surprisingly, we found that these TRIMs recruit multiple non-redundant E2 enzymes and undergo a series of stepwise ubiquitination events. First, the E2 Ube2W monoubiquitinates TRIMs, allowing subsequent recognition by the Ube2N/Ube2V2 E2 pair, which elongates the monoUb into an anchored K63-polyUb modification. Genetic disruption of any component of this pathway abolishes anchored K63-polyUb as well as the ability to restrict viral infection. For TRIM5, this results in a specific loss of reverse transcription inhibition during infection by N-MLV; for TRIM21, loss of antibody-dependent intracellular neutralization of Adenovirus. We find that Ube2W is also required for TRIM-mediated stimulation of NF-κB, demonstrating that anchored chains precede the free K63-polyUb required for signaling. Such a system would require K63-polyUb liberation from TRIMs, and we demonstrate that the proteasome 19S-resident and degradation-coupled deubiquitinase Poh1/Rpn11 is required for TRIM-dependent immune responses following viral infections in human and mouse cell lines. We propose a model whereby attaching the polyUb stimulus of NF-κB signaling to TRIMs ensures innate signaling, can only occur concomitantly with virus disassembly at the proteasome.

12 Anti-Inflammatory Gene Expression Profile of Liver Sinusoidal Endothelial Cells in the Host Response to HCV Infection

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Introduction: Liver sinusoidal endothelial cells (LSEC) due to their extraordinary scavenger activity play a pivotal role in blood-borne virus clearance. LSECs account for the ~20% of liver *parenchyma* cells and are a unique organ-resident cell population with diverse functions, including degradation of bacterial by-products, antigen presentation and induction of immune tolerance. The purpose of this study was to apply systems biology approaches in evaluating the role of LSEC in the host response to HCV infection.

Materials and Methods: Poly(A) RNAs from HCV, MOCK or LPS treated primary human LSEC cultures were analyzed by RNA-sequencing (Illumina) to identify differentially expressed genes (DEG) and biological pathways. Cell transcriptomes were compared to similar analysis of mild (no fibrosis) and severe (cirrhosis) chronic hepatitis C infected liver specimens, hepatoma (Huh7.5) and THP1 cells.

Results: Following exposure to infectious HCV particles LSECs internalized virus, but failed to support HCV replication. LSECs displayed 754, 245 and 2543 DEGs at 8, 24 and 48h after exposure to HCV, respectively. Where LPS triggered an exceptionally potent activation, HCV in general induced a downregulation of pro-inflammatory genes. Critical innate immune response pathways were downregulated (RIG-I and TLR-signaling pathways), manifested by a diminished Pathogen recognition receptors (PRR) transcript expression and downregulation of genes encoding the adaptor proteins, JAK-STAT, NF-κB and IRF signaling cascades with a downstream reduced expression of cytokine gene expression. These changes are consistent with a decrease in the innate immune response. A molecular mechanism for this response is suggested by the following changes: upregulation of transcription factors essential for the induction of an anti-inflammatory state (such as *MAFB*, *NUPR1*, *IL33*, *KLF15*, *CEBPD*) and downregulation of those promoting inflammatory responses (*ETV1*, *MITH*, *HDAC9*, *EGR3*, *IRF6*) and upregulation of many immunomodulatory genes (*ACP5*, *A2M*, *C1QTNF1*, *NT5E*, *SERPING1*, *BMPER*) that may attenuate liver inflammation.

LSEC datasets were compared with Kupffer cell and Huh7.5 cell datasets to identify distinct gene expression profiles in HCV infected LSECs, KCs and hepatocytes. Macrophages demonstrated a broad increase in IL1β and NFκB-responsive proinflammatory networks,

which correlated with increasing severity of liver disease. In contrast, a subset of genes expressed in LSECs after HCV infection that were also found in HCV infected liver included pathways regulating immune responses, angiogenesis, adhesion, and ECM-organization.

Conclusion: Analysis of the host response of LSECs to HCV infection by RNA-seq gene expression analysis identified gene expression changes in liver non-parenchymal cells that might profoundly affect the host immune response to HCV. A portrait of changes in the expression of critical components of host immune and inflammatory pathways provided new evidence that LSECs may attenuate inflammation during HCV infection *in vivo*.

13 Antiviral piRNA Pathway Interactome in the Arbovirus Vector *Aedes Aegypti*

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ARthropod-BOrne viruses (arboviruses) pose a significant risk to human and animal health. Important emerging arboviruses such as dengue virus, chikungunya virus or Rift Valley fever virus, are transmitted by mosquitoes to vertebrate hosts. The infection in vertebrate cells is lytic and may have severe consequences to the host. In contrast, the infection in mosquitoes is non-cytopathic and RNA interference (RNAi) is considered to be the main mechanism that controls virus infection.

The small interfering (si)RNA pathway is thought to be the main RNAi pathway for controlling arbovirus infection in the vector; however, recent findings indicate the involvement of another, less characterized, RNA interference (RNAi) mechanism, at least in mosquitoes: the Piwi-interacting (pi)RNA pathway.

Using the mosquito-borne Semliki Forest virus (SFV) and mosquito *Aedes aegypti*-derived Aag2 cells, we found that knockdown of Piwi4 enhanced virus infection, but had no effect on piRNA production, indicating an antiviral role for the piRNA pathway. In contrast, knockdown of other core piRNA pathway genes resulted in decrease of SFV-specific piRNAs but little or no effect on virus infection. This illustrates the complexity of the piRNA pathway-virus interaction. Little is known about the involvement of other proteins in the pathway, their identity and functional importance. Thus, stable Aag2 cell lines expressing tagged piRNA pathway proteins were generated and used in proteomics studies followed by functional characterization of identified protein partners.

14 Assessment of the Antiviral Activity of MxA against Influenza A Virus

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Mx proteins belong to the family of dynamin-like, large GTPases and are primarily active against negative-stranded RNA viruses, including influenza A (IAV). Human MxA is able to form higher order oligomeric structures, however, the mode of action of MxA in terms of its oligomeric state and the interaction with viral proteins remains to be fully elucidated. Increasing evidence suggests that MxA requires auxiliary cellular factors for its antiviral activity. Recently, we have shown that MxA interacts with the DEAD-box helicase UAP56, which is an essential factor for efficient replication of IAV.

There is increasing evidence that NP represents the viral target of MxA. For instance, the resistance phenotype of human IAV segregates with a discrete cluster of surface-exposed amino acids in the NP. We showed that the dimeric form of MxA is able to form stable complexes with viral NP as well as UAP56 suggesting that the dimeric form of MxA plays a central role in antiviral function. In line with these findings, co-immunoprecipitation (Co-IP) experiments revealed that NPs of several MxA-sensitive or resistant strains exhibit different binding affinities to dimeric MxA. Furthermore, we observed that binding of MxA to UAP56 is greatly enhanced in the presence of NP.

Currently we characterize the interactions of MxA, NP and UAP56 by Co-IP and a tripartite split-GFP system. In particular, we investigate the effect of (i) GTPase activity mutants of MxA, (ii) ATPase and RNA binding activity mutants of UAP56 as well as (iii) NP mutants with altered MxA sensitivity. Furthermore, we assess the influence of these mutations on MxA antiviral activity.

15 Baculovirus Insect Midgut Entry: the Role of *per os* Infectivity Factors (PIFs)

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Baculoviruses infect insect midgut epithelial cells in the form of occlusion-derived virus (ODVs) particles. ODVs are assembled in the nucleus of infected cells and are occluded in viral occlusion bodies (OBs). For *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) at least seven conserved ODV membrane proteins are essential for oral infectivity. These proteins are called *per os* infectivity factors (PIFs). We have identified a multi-molecular complex composed of PIF1, PIF2, PIF3 and PIF4. Deletion of PIF1, PIF2 and PIF3 abolished complex formation, while deletion of PIF4 resulted in the detection of a stable sub-complex. P74 (PIF-0) is loosely associated with this PIF-complex by its identification as interaction partner of PIF1 by CoIP. With the same technique PIF-6 and three other proteins (P95, AC5 and AC108) were found as potential components of the PIF-complex. On the other hand, PIF-5 is not associated with this complex and may have a separate function in oral infectivity. To visualize the entry of ODVs into primary midgut epithelium cells and to study the role of individual PIF proteins therein, we constructed a labeled baculovirus with EGFP fused to the VP39 nucleocapsid protein. So far, we applied this technique to wild type AcMNPV and viruses with deletions of P74, PIF1 or PIF2 and studied *ex vivo* binding and fusion to midgut epithelial cells using live-imaging microscopy. The data showed that P74 was absolutely required for ODV binding. Viruses with PIF1 or PIF2 deletions, which are impaired in PIF-complex formation, were still able to bind midgut epithelial cells. However, these mutants did not enter these cells, suggesting that fusion was impaired. The results obtained with live-imaging complement the genetic and bio-chemical evidence for the role of PIFs in the oral infection process. The PIF-complex might mediate an ancient and well-conserved invertebrate virus entry mechanism, since PIF proteins are encoded by invertebrate viruses belonging to several other families as well (nudiviruses, hytrosaviruses and whispoviruses).

16 Characterization of Early Events in Bat Influenza Virus Infection

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Recently, sequences of two novel influenza A-like viruses were isolated from South American bats, challenging our understanding of influenza A viruses (IAV). Surprisingly, these bat influenza viruses, referred to as H17N10 and H18H11, are quite distinct from all other known IAV strains. In contrast to conventional IAV strains, the bat virus hemagglutinins (HAs) do not bind sialic acid, the canonical IAV receptor, and their neuraminidases (NAs) do not show neuraminidase activity. Moreover, major limitations to study these new influenza-like viruses come with the inability to rescue them by reverse genetics. However, the generation of chimeric bat viruses harboring glycoproteins (HA and NA) from known IAV strains was shown to be successful. In order to establish the best possible system for successful viral rescue and gaining knowledge on the biology of theseviruses, we have developed a flow cytometry-based system to study the binding of purified H17 and H18 to a panel of different bat and other mammalian cell lines, including those derived from *Sturnira lilium*, the bat species from which the H17N10 sequences originate. Furthermore, we have established a virus-like particle (VLP) based assay to examine the entry of pseudotyped H17N10 or H18N11 VLPs into different cell lines. In parallel, we are characterizing the susceptibility and productivity of the different cell lines upon infection with the chimeric bat influenza viruses. Altogether, the identification of cell lines susceptible to H17- or H18-binding, VLP entry and replication of chimeric bat viruses might contribute to the successful generation of infectious bat influenza viruses and provide a better understanding of bats as a potential reservoir of influenza viruses.

17 Characterization of Protein Intrinsic Disorder Involvement in RNA Virus Adaptation Using a Plant Virus Model

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Intrinsic disorder in proteins is defined as a lack of stable structure in physiological conditions. Born in the last decade, this concept is now widely accepted, and strongly states that disorder is ubiquitous in life and essential to a wide range of biological functions. For instance, disorder is mainly associated with multiple-interactions and high evolutive regions.

Large-scale computational studies show that intrinsic disorder is abundant in virus proteomes. Many experimental data support the biological reality of this structural feature. For instance, disorder has been experimentally associated to flexible molecular interfaces in some viral proteins, accounting for their multi-functionality. Another biological relevance of intrinsic disorder propensity in some viral proteomes resides in the enormous adaptability of viruses, and particularly RNA viruses. It is commonly accepted that high genetic diversity is crucial for RNA virus populations, enabling them to adapt to environmental changes. In this context, low topology constraints exerted on intrinsic disordered regions is expected to buffer numerous deleterious mutations. This tolerance to mutations could then be directly related to the adaptability of these viruses to overcome host resistances.

To test experimentally these assumptions, we designed a set of mutants of plant potyvirus Potato Virus Y (PVY) that contain various disorder degrees in the Viral genome-linked (VPg) protein, a key determinant of potyvirus adaptation.

Our experimental approach aims to estimate the contribution of intrinsic disorder to VPg-based PVY adaptation process. It consists in monitoring resistance breakdown apparition (*i.e* adaptation) in resistant host pepper plant genotype, inoculated either by wild-type PVY or by our VPg disorder-affected mutants.

Our results significantly demonstrate a positive correlation between VPg-disorder levels and PVY adaptive abilities to restore infection in pvr23-carrying resistant peppers. To our knowledge, this study provides the first experimental evidence of intrinsic disorder involvement in a plant RNA virus adaptive process.

18 Crucial Role of Glucose Regulator Protein 78 in the Japanese Encephalitis Virus Life Cycle

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Japanese Encephalitis Virus is a mosquito borne *Flavivirus* and major cause of encephalitis in several parts of South-east Asia, India and China. It claims around 10,000 deaths annually and among survivors around 50% develop lifelong neurological symptoms. JEV is an enveloped, positive stranded RNA virus. Virus infection begins with envelope protein binding to its specific receptor and internalization. In our present study we used a recombinant envelope domain 3 (ED3) of JEV Envelope protein as an exploratory molecule to identify JEV interacting membrane proteins and identified Glucose Regulator Protein 78 (GRP78) as a potential virus receptor by 2D gel electrophoresis and mass spectrometry. GRP78 is an important ER chaperon and present on cell membrane of several cell lines. Antibody inhibition and siRNA knockdown of GRP78 inhibits JEV entry in neuronal and epithelial cells. GRP78 also shows extensive colocalization with the JEV envelope protein in the ER of infected cells. By use of specific GRP78 inhibitors we observe a marked decrease in JEV RNA replication, translation of viral proteins and formation of mature virions suggesting an important role of GRP78 at a post-entry step. Our data suggests that GRP78 functions both as receptor and crucial modulator of JEV replication and highlights the potential of using GRP78 as a therapeutic target for developing antivirals.

19 Determining the Impact of Innate Immune Pathway on Viral Diversity and Evolution

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Host-pathogen interactions trigger selective pressures on both partners. Hosts face a vast array of parasites to which they must adapt, and it is widely believed that pathogens are a major selection pressure in all natural populations. The fact that immunity genes evolve faster, and often much faster, than other genes indicates that adaptive evolution is occurring. On the other hand, RNA viruses accumulate mutations at very high rates. Consequently, viral populations are very diverse and contain several genomes that diverge from the parental consensus sequence. Such diversity is critical for efficient transmission and full pathogenicity of the virus. It is thus likely that innate immunity, through an array of antiviral responses, shapes the diversity of viral populations. Our goal is to conduct a comprehensive study, under laboratory-controlled conditions, of the link between insect innate immunity and viral evolution using D. melanogaster as model insect and its natural pathogen Drosophila C virus (DCV). To do so, we generated mutant flies for the different components of the innate immune response on the same genetic background and serial passaged DCV in those flies. Viral population diversity was assessed by deep sequencing after serial passages. Results on the impact of the host immune system on viral evolution will be presented.

20 Effect of Folate Deficiency on Human Papillomavirus-Induced Carcinogenesis

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Several epidemiological studies highlighted the importance of micronutrient intake in cancer development. Folate, a water soluble B vitamin, has been shown to exert a protective effect in carcinogenesis. Deficiency in folate is associated with an increased incidence of several cancers, including cervical cancer, which are etiologically linked to Human Papilloma Virus (HPV) infection. In our study we aim to identify the molecular events upon folate deficiency that can promote HPV-induced carcinogenesis. Using HPV16E6E7-immortalized human keratinocytes, we observed that *in vitro* depletion of folate had an impact on cellular growth. Interestingly, cells cultured in low folate medium had an increased ratio of cells in the S-phase of cell cycle, in correlation with the activation of DNA damage response markers such as gH2AX. Additionally, genome wide methylation analysis of these cells showed that *in vitro* folate deficiency induced deregulation of the methylation profile, affecting numerous CpG islands within gene promoters. This result indicates that folate deficiency can also affect gene expression in HPV-immortalized keratinocytes in epigenetic terms. Furthermore, folate repletion in the culture medium led to higher growth rate and plating efficiency than the control cells, highlighting acquired pro-proliferative features during the folate deficiency period. Taken together, these observations suggest that folate deficiency may play a role as a cofactor in HPV-induced carcinogenesis. Further investigation is being conducted to characterize the molecular mechanisms underlying the observed events.

21 From Structure to Function: A Comparison of NEC Structures from Three Different Herpesviruses

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All herpesvirus capsids are assembled in the nucleus and migrate to the cytoplasm through a process called nuclear egress. This involves budding into the inner nuclear membrane (INM), translocation into the perinuclear space and subsequent membrane fusion with the outer nuclear membrane. The budding step is mediated via the nuclear egress complex (NEC), which consists of the conserved viral proteins UL31 and UL34. They represent a minimal membrane deformation and budding machinery and act by forming a hexagonal coat on the INM. I have determined the crystal structures of the NEC from herpes simplex virus 1 (HSV-1) and pseudorabies virus (PRV), and at the same time the structure of the NEC from cytomegalovirus (CMV) became available. A comparison of these structures allowed me to identify a flexible and structurally conserved α-helix in UL34 that most likely plays a role in regulating the oligomerization and budding step, as its presence interferes with the hexagonal coat formation on the membrane. Another interesting observation affects the orientation of UL31 and UL34 towards each other. In all three complexes, they are positioned in a slightly different way, which suggests a flexible hinge area. This mobility may be crucial for membrane deformation after the NEC forms a confluent coat. The membrane distal region of the NEC from each virus harbors a conserved, solvent-accessible α-helix in UL31 that presumably represents the capsid binding site. Mutational analysis is ongoing to understand the role of this helix in capsid binding.

22 Genetic Changes Associated with Paralysis in Enterovirus D68 Isolates from the 2014 Outbreak

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Enterovirus D68 (EV-D68) caused a severe respiratory illness outbreak in the United States in 2014. Reports of acute flaccid myelitis/paralysis in some children with detectable EV-D68 have raised concerns that genetic changes in EV-D68 could be contributing to increased disease severity and neurological symptoms. Using data and analytical tools in the Virus Pathogen Resource (ViPR; www.viprbrc.org) we performed a series of comparative genomic analyses of recent EV-D68 outbreak isolate sequences. Our results suggest that: 1) three distinct clades of EV-D68 were co-circulating during the 2014 outbreak with isolates associated with acute flaccid myelitis belonging to a single phylogenetic cluster - B.1.2; 2) all or the majority of isolates from the EV-D68 B.1.2 cluster have 28 unique substitutions that distinguish them from other isolates; 3) several of these substitutions are also observed at equivalent positions in paralysis-causing enteroviruses, including poliovirus, EV-D70 and EV-A71. These results suggest that the genetic substitutions found in isolates from the B.1.2 cluster may be responsible for the changes in EV-D68 symptomatology associated with the 2014 outbreak. Substitutions in the IRES element are being tested to determine if they preferentially affect translation efficiency in neuronal cell lines.

23 HIV-1 Gag Blocks Selenite-Induced Stress Granule Assembly by Altering the mRNA Cap-Binding Complex

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The host translational machinery is regulated by environmental stresses, which trigger multiple signaling pathways to activate either cell survival or apoptosis mechanisms. Cellular stress initiates the assembly of cytoplasmic aggregates called stress granules (SGs), dynamic accumulations of stalled translation pre-initiation complexes. Sodium selenite (Se) induces the assembly of non-canonical type-II SGs that differ in morphology, composition and assembly pathway than canonical type-I SGs. Inhibition of translation initiation by Se is dependent on the cap-binding activity of eIF4E-binding protein 1 (4EBP1). The subversion of host machineries is an essential part of the virus replicative process and, similarly to many other viruses, HIV-1 has evolved to subvert components of SGs to promote viral replication and eliminate host antiviral defenses, as we previously demonstrated. In the present study, we investigated the relationship between Se, SG assembly and HIV-1. We tested the effect of Se on HIV-1 and demonstrate that HIV-1 is able to block Se-induced SG assembly and specifically identify the structural protein Gag as the mediator of this blockade. Interestingly, in the presence of Gag, stressed cells demonstrated significantly reduced 4EBP1 binding to the 5′ mRNA m7G cap. The observed reduction in 4EBP1 m7G cap-binding was not caused by increased phosphorylation of 4EBP1 through activation of the mTOR pathway, but rather may be influenced by an interaction between HIV-1 Gag and eIF4E. Finally, upon treatment of HIV-1-expressing cells with increasing concentrations of Se, we observed a detrimental effect on HIV-1 replication, primarily due to effects of Se on post-budding virus maturation. This work identifies a novel mechanism by which HIV-1 Gag suppresses SG assembly. In additon, our *in vitro* data correlate with an intervention trial in which Se supplementation led to decreased viral loads.

24 Host Cell Potassium Channels are Essential for the Human Papillomavirus (HPV) Lifecycle

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HPV are the major causative agents of cervical cancer. Despite the availability of a vaccine against some cancer-associated HPV types no effective anti-viral treatments exist to treat the millions infected with this pathogen. A need exists to identify novel targets for anti-viral intervention. Host encoded ion channels play essential roles in epithelial cell biology and are the target for clinically available drug treatments ranging from heart disease to diabetes. Despite their abundance, a paucity of data exists examining the requirement for ion channels in the HPV life cycle and pathogenesis.

We provide evidence that potassium channels are essential for the HPV life cycle and are a potential target for anti-viral intervention. A screen of primary human foreskin keratinocytes (HFK) stably harbouring the high-risk HPV18 genome with a panel of broad range channel blockers identified a requirement for potassium channels in HPV early gene expression. Using a combination of more specific inhibitors and channel agonists we identified the specific host channel as a novel host factor required for the HPV life cycle. Moreover, using a sensitive flow cytometry assay we could demonstrate that the plasma membranes of HPV containing HFK are highly polarised and that the polarisation can be prevented using inhibitors of potassium channels.

As the HPV life cycle is inherently linked to the cell cycle status of the infected cell, we assessed the effects of modulating potassium channels on the keratinocyte cell cycle. These data demonstrate that the channels are absolutely required for G1/S transition in keratinocytes, which likely explains their impact on HPV biology. Supporting these findings we noted that the expression of specific channel sub-units was significantly increased in HFK harbouring HPV18 compared to uninfected donor cells. Currently we are screening clinical samples to determine whether these channels are increased in HPV-associated malignancies. We also investigated the potential of these channels for therapeutic intervention using a recovery assay. In these experiments, long-term treatment of HPV containing cells resulted in a significant decrease in HPV replication with no apparent impact on host cell physiology. In conclusion, we demonstrate the first link between host potassium channels and the HPV life cycle and identify a potential target for a therapeutic against HPV infection.

25 Human Microglia Cells Participate in Inflammatory Responses, Viral Reproduction and Transmission in Japanese Encephalitis Virus Infection

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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 (MG) are the brain-resident macrophages and are proposed to serve as reservoir for JEV. Using human primary MG and a new model of human monocyte-derived MG, the present study explores the influence of JEV in chemokine ligands/receptors expression by human MG and the role of MG in viral replication and transmission to another cell type. To achieve this work, the attenuated inactivated JEV as used in vaccines and two live JEV strains were employed. Both inactivated and live JEV induced important morphological changes on MG exerting large granules and round shape at 24 hours. Depending on the virus isolate, live JEV but not JEV vaccine induced production of CCL2, CXCL9 and CXCL10 in MG cell culture. In a dose dependant manner, JEV vaccine enhanced the expression of the chemokine receptors CCR2, CCR3, CCR4 and CX3CR1 on MG cell surface. Live JEV exclusively up-regulated CX3CR1 expression on MG with a maximal level at 24h post-infection (pi). Interestingly, JEV vaccine but not live JEV remarkably enhanced levels of HLA-DR expression on MG. Importantly, human MG were productively infected by JEV as confirmed with the presence of viral RNA in supernatants and cells up to 6 days pi. Interestingly, infectious JEV particles in supernatant diminished over time and supernatants were absent of infectious JEV after 6 days of infection. In contrast, at the same time, JEV-infected human MG were able to transmit infectious JEV to BHK-21 cells, a highly susceptible cell line to JEV. Altogether, the data show that human MG cells participate in inflammatory responses and viral reproduction upon JEV infection, which may be a source of neuronal infection. Importantly, JEV-treated cells express the fraktalkine receptor CX3CR1, a relevant chemokine receptor involved in cell migration and inflammation in the central nervous system. In conclusion, human MG may sustain JEV brain pathogenesis in long term infection.

26 Influenza A Virus PA-X Host Shutoff Protein is Selective for Host RNA Polymerase II Transcripts

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Influenza A viruses (IAVs) inhibit host gene expression by a process known as host shutoff. Host shutoff limits innate immune responses and may also redirect the translation apparatus to the production of viral proteins. Several IAV proteins participate in host shutoff, including recently discovered ribonuclease PA-X. We report that PA-X selectively targets host RNA polymerase II (Pol II) transcribed mRNAs, while sparing Pol I and Pol III transcripts. Interestingly, we show that unlike some other viral host shutoff ribonucleases, PA-X can also target Pol II-transcribed non-coding RNAs. Complete degradation of host mRNAs following PA-X-mediated endonucleolytic cleavage is dependent on the host 5'->3'-exonuclease Xrn1. IAV mRNAs are structurally similar to host mRNAs, but are synthesized and modified at the 3' end by the action of the viral RNA-dependent RNA polymerase complex. Infection of cells with the wild-type IAV or a recombinant PA-X-deficient virus revealed that IAV mRNAs resist PA-X-mediated degradation during infection. At the same time, loss of PA-X resulted in changes in the synthesis of select viral mRNAs and a decrease in viral protein accumulation. Collectively, these results significantly advance our understanding of IAV host shutoff, and suggest that the virus achieves selective degradation of host mRNAs by targeting some aspect of Pol II-dependent RNA biogenesis.

27 Inhibiting HIV Maturation: Role of Bevirimat and its Analogs

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One of the major problems faced during anti-retroviral treatment of HIV patients is evolution of drug-resistant viruses. Hence, identification of new antiviral targets continues to be a high priority for development of HIV therapeutics. Maturation inhibitors represent a new underdeveloped class of antiretroviral agents that block virus maturation not by targeting the PR enzyme itself but by binding the target of PR (Gag) and blocking a specific step in Gag processing. The first-in-class maturation inhibitor Bevirimat (BVM) was found to be ineffective against a subset of HIV-1 isolates in vitro as well as in clinical trials. The failure of the drug against these strains was attributed to polymorphisms mainly present in the CA-SP1 region of HIV-1.

To overcome this problem, a number of BVM derivatives (referred to as “second generation” maturation inhibitors) were generated by modifications in the C28 heteroatom. The BVM analogs were found to be more effective than the parental compound against HIV subtype C. Moreover, the BVM analogs displayed more potent antiviral activity than the parental compound. The BVM analogs delayed replication of HIV-1 clade C in HUT R5 cells. We have identified a panel of mutations in the Gag region. Efforts are underway to characterize the mutant viruses, which will guide in identifying the putative binding pocket of these compounds on HIV Gag and help in clinical development of these compounds.

28 Inhibition of HCV Protease and Viral Replication by Cell Penetrable, Humanized Nanobodies

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There is a need of new interferon-free, anti-HCV agents that are safe and broadly effective anti-HCV agents that can cope with genetic multiplicity and mutations of the virus. In this study, humanized-camel VHHs to genotype 3a HCV serine protease were produced and were linked molecularly to a cell penetrating peptide, penetratin (PEN). Human hepatic (Huh7) cells transfected with the JFH-1 RNA of HCV genotype 2a and treated with the cell penetrable nanobodies (transbodies) had a marked reduction of the HCV RNA intracellularly and in their culture fluids, fewer HCV foci inside the cells and fewer amounts of HCV core antigen in culture supernatants compared with the infected cells cultured in the medium alone. The transbodies-treated-transfected cells also had up-regulation of the genes coding for the host innate immune response (*TRIF*, *TRAF3*, *IRF3*, *IL-28B* and *IFN-β*), indicating that the transbodies rescued the host innate immune response from the HCV mediated-suppression. Computerized intermolecular docking revealed that the VHHs bound to residues of the protease catalytic triad, oxyanion loop and/or the NS3 N-terminal portion important for non-covalent binding of the NS4A protease cofactor protein. The so-produced transbodies have high potential for testing further as a candidate for safe, broadly effective and virus mutation tolerable anti-HCV agents.

29 Inhibition of Interferon Production and Signaling by Severe Fever with Thrombocytopenia Syndrome Virus NSs Protein

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Innate interferon (IFN) response that inhibits viral replication is the first-line host defense against viral infection. To circumvent this response, viruses have developed various counter-measures to antagonize IFN production and/or signaling. Severe fever-with-thrombocytopenia syndrome virus (SFTSV) is an emerging zoonotic pathogen initially identified in China and subsequently found in other parts of the world. SFTSV NSs protein is an IFN antagonist that has been shown to counteract type I IFN induction by targeting TBK1 and IKKε kinases and to impede IFN signaling by interacting with and sequestrating STAT2 in the cytoplasm. In this study, we demonstrated that SFTSV NSs protein suppresses both production and signaling of type I and type III IFNs by preventing STAT1 phosphorylation and activation. Infection with SFTSV or expression of its NSs protein potently inhibited not only the production of IFN-β induced by double-stranded RNA but also the activation of representative IFN-stimulated genes (ISGs) by IFN-α1, IFN-β, IFN-λ1 and IFN-λ2. In contrast, expression of NSs or infection with SFTSV had no influence on the activation of NF-κB signaling. Co-immunoprecipitation experiments indicated that NSs protein interacts with STAT1 and STAT2. NSs functioned not only to sequester STAT1 and STAT2 in the cytoplasm, but it also inhibited IFN-β-induced phosphorylation at serine 727 of STAT1. However, serine 701 phosphorylation was unaffected. Furthermore, STAT1 protein was inhibited at the transcriptional level. Chromatin immunoprecipitation assay showed that the recruitment of STAT1 and STAT2 to ISRE promoters of ISGs was attenuated when NSs was expressed. Taken together, our findings suggested that SFTSV NSs protein is viral antagonist of IFNs that potently suppresses IFN signaling by inhibiting phosphorylation and activation of STAT1. Our study has implications in the design and development of anti-SFTSV agents and vaccines. This work was supported by Hong Kong Research Grants Council-Natural Science Foundation of China Joint Research Scheme (N-HKU 714/12 and 81261160504), Hong Kong Research Grants Council Collaborative Research Fund (HKU1/CRF/11G), Hong Kong Medical Research Fund (HKM-15-M01) and 973 National Basic Research Program (2011CB504704).

30 Inhibition of Late but not Early Steps of Influenza A Virus Replication Allows Activation of Innate Immune Responses in Infected Cells and Priming of Secondary Antiviral Responses in Non-Infected Cells

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Influenza A viruses impact the public health and global economy by causing yearly epidemics and occasional pandemics. Several anti-influenza drugs are available and many are in development. However, the question remains which of these agents not only inhibit viral infection but also allow activation of host immune responses which can protect patients against reinfections. To answer this question, we studied the effect of saliphenylhalamide (SaliPhe), SNS-032, obatoclax, and gemcitabine on the development of antiviral responses in primary human macrophages. We showed that treatment with gemcitabine, which targets transcription and replication of viral RNA, but not with SaliPhe, SNS-032 or obatoclax, which inhibit virus entry, allowed for activation of antiviral responses in influenza A virus infected human macrophages. Moreover, immune modulators produced by gemcitabine-treated virus-infected cells primed secondary immune responses in non-infected human macrophages and human lung epithelial A549 cells. Thus, compounds that target late steps of virus infection could be beneficial for treatment of influenza patients. This strategy can be utilized in antiviral drug developmental programmes taking in consideration that immuno-modulating properties of compounds are stimulus-specific.

31 Insulin-Like Growth Factor-1 in Adipose Tissue Macrophages Contributes to Maintaining Adipose Tissue through Angiogenesis in Virus-Induced Obesity

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Some viruses are implicated in the induction of obesity. Among them, previous studies have confirmed that human adenovirus 36 (Ad36) is associated with increased adiposity, improved glycemic control, and induction of inflammation. The Ad36-induced inflammation is reflected in the infiltration of macrophages into adipose tissue. However, the characteristics and role of adipose tissue macrophages (ATMs) and macrophage-secreted factors in virus-induced obesity (VIO) are unclear. Although insulin-like growth factor-1 (IGF-1) is involved in obesity metabolism, the contribution of IGF secreted by macrophages in VIO has not been studied. Four-week old male mice were studied 1 week and 12 weeks after Ad36 infection for determining the characteristics of ATMs in VIO and diet-induced obesity (DIO). In addition, macrophage-specific IGF-1-deficient (MIKO) mice were used to study the involvement of IGF-1 in VIO. In the early stage of VIO (1 week after Ad36 infection), the M1 ATM subpopulation increased, which increased the M1/M2 ratio, whereas DIO did not cause this change. In the late stage of VIO (12 weeks after Ad36 infection), the M1/M2 ratio did not change because the M1 and M2 ATM subpopulations increased to a similar extent, despite an increase in adiposity. By contrast, DIO increased the M1/M2 ratio. In addition, VIO in wild-type mice upregulated angiogenesis in adipose tissue and improved glycemic control. However, MIKO mice showed no increase in adiposity, angiogenesis, infiltration of macrophages into adipose tissue, or improvement in glycemic control after Ad36 infection. These data suggest that IGF-1 secreted by macrophages may contribute to hyperplasia and hypertrophy in adipose tissue by increasing angiogenesis, which helps to maintain the ‘adipose tissue robustness’.

32 Interference with the Production of Fully Infectious Viral Particles Is a Conserved Property through Which Members of the IFITM Family of Antiviral Factors Inhibit RNA Viruses

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The InterFeron-induced Transmembrane Proteins 1, 2 and 3 (IFITMs) are a family of cellular proteins with a broad antiviral spectrum. The exact molecular mechanism through which IFITMs act remains unclear. However, IFITMs are known to act in target cells by trapping incoming viral particles in endosomal vesicles, impairing viral-to-cellular membrane fusion. Recently, our laboratory, along with others, identified an additional mechanism of inhibition by IFITMs against the HIV-1 retrovirus, whereby the presence of IFITMs in virus-producing cells resulted in their incorporation on the surface of HIV-1 virion particles and led to the production of virions of diminished fusogenicity, in the absence of defects in structural viral proteins incorporation and/or processing.

We now provide clear evidence that IFITMs similarly interfere with the production of fully infectious virions of representative members of *Retroviridae*, *Rhabdoviridae*, *Paramyxoviridae*, *Filoviridae*, *Flaviviridae*, *Bunyaviridae* and *Arenaviridae*, revealing the broad conservation of this antiviral mechanism against RNA viruses. Interestingly, the magnitude of the defects observed for the different viruses relied on the combination between IFITMs members, *ifitm* haplotypes and specific mode of virion assembly, indicating the existence of specificities that may fine tune the overall antiviral impact of IFITMs.
This study was carried out with the support of the ANRS and Sidaction.

33 Investigating the Interplay of bnAb Evolution and Autologous Virus Neutralization during HIV-1 Free Virus and Cell-to-Cell Transmission

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HIV-1 cell-to-cell transmission promotes high efficacy of infection and is less sensitive to neutralization by broadly neutralizing antibodies (bnAbs) compared to free virus spread. Testing a large panel of bnAbs and HIV-1 strains from subtypes A, B and C, we have recently shown that the activity of bnAbs during heterologous neutralization of cell-to-cell transmission not only depends on the bnAb epitope and virus strain targeted but is also influenced by the time window of bnAb action during the viral entry process. Whether these observations also apply for autologous neutralization of bnAbs and viral isolates from HIV-1 infected patients, however, still remains to be elucidated.

Using assay systems that allow for unambiguous discrimination between free virus and cell-to-cell transmission, we tested the neutralization of 14 longitudinally isolated virus strains by 12 autologous V1V2-directed bnAb variants derived recently from an HIV-1 superinfected individual, CAP256 [1].

In accordance with our previous results, the activity of most CAP256 bnAbs to inhibit cell-to-cell transmission of autologous virus strains was decreased compared to free virus spread. The extent of this activity loss, however, was markedly lower than we previously observed for heterologous neutralization by V1V2-directed bnAbs. Interestingly, the patient harbored several virus variants that were equally well neutralized during free virus and cell-to-cell transmission by all the autologous and heterologous V1V2-directed bnAbs tested. As equal activity in both transmission pathways is uncommon, this suggests a specific pressure of the CAP256 bnAbs on cell-to-cell transmission and virus evolution.

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34 Investigation of IFITM Incorporation into Influenza A Virus Particles and Its Consequences on Virus Infectivity

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Interferon-inducible transmembrane proteins (IFITMs) have been identified as potent antiviral factors inhibiting entry of a whole variety of viruses into their target cells, including influenza A virus (IAV). Recently it has been described that besides residing in target cells, IFITMs are also incorporated into newly produced HIV virions, decreasing their fusion capacity. In addition, IFITMs have been reported to interact with the HIV envelope protein, thereby interfering with its processing and incorporation into virions. Here, we describe studies on IFITM incorporation into IAV particles and its consequences on virus infectivity. We assessed the effect of IFITM incorporation on the envelope-mediated fusion process by comparing the entry efficiency of HIV virus like particles (VLPs) pseudotyped with envelope proteins of IAV, HIV or VSV in the presence or absence of IFITMs. To identify potential IFITM-mediated effects specific to influenza virions we also tested incorporation of IFITMs into IAV particles and the potential impact on virus infectivity. The results on incorporation and infectivity will be presented.

35 Investigation of the Effect and Roles of PPIG on the HIV-1 Replication

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It has been known that SR (serine/arginine) proteins are proteins involved in mRNA splicing and highly conserved. A number of SR proteins have been identified as splicing factors regulating the HIV-1 viral mRNA processing. However, peptidylprolyl isomerase G (PPIG) having a SR domain has not been known for its effect on HIV-1 mRNA processing and viral replication. To investigate the role of PPIG on HIV-1 splicing, we tested the effect of overexpression and knock-down of PPIG in 293T cells. We found that there was no change observed in HIV-1 transcription level and mRNA processing when PPIG was depleted. However, overexpression of PPIG decreased the level of HIV-1 mRNA transcription but not in the splicing. An overexpression of only the SR domain of PPIG resulted in similar to wild type. Furthermore, when VSV-G pseudotyped virus infected to cells overexpressed with a PPIG wild type or PPIG deletion mutants constructs wild type and only SR domain showed a decrease in Gag protein level but not with a PPIase domain. Taken together, our data suggest that PPIG might act as an inhibitor in HIV-1 production and replication by decreasing the viral transcription.

36 Investigations into Rotavirus Replication, Assembly and Exit from Mammalian Cells

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Rotavirus is one of the most common causative agents of gastroenteritis in young children. Rotavirus is a non-enveloped double strand RNA virus that replicates in the cytoplasm of host cells. Little is known regarding the nature of the replication and translation sites of Rotavirus RNA molecules, as well as the mechanisms by which these viruses are assembled and exit the cell. Here, using a combination of high-resolution imaging, proteomic and lipidomic approaches, we demonstrate that rotaviral RNA molecules translate, replicate and assemble within cytoplasmic domains surrounded by endoplasmic reticulum. Once the particles bud into the endoplasmic reticulum lumen we find that they are released from cells, non-lytically, in vesicles, via an unconventional secretory pathway. Our findings with Rotavirus parallel our previous findings with Poliovirus, Coxsackievirus and Rhinovirus [1], and widen the number of so-called non-enveloped viruses being released non-lytically from cells.

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37 Large-Scale Genomics Unveil Different Patterns of Evolution in Norovirus Genotypes

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Noroviruses are major pathogens associated with acute gastroenteritis worldwide. They are genetically diverse with two major genogroups (GI and GII) containing over 30 different genotypes. To gain insight into the evolutionary mechanisms involved in diversification of human noroviruses, we: (i) assembled a large database of sequences of the complete ORF2, which encodes the major capsid protein VP1, representing all known GI and GII norovirus genotypes; and (ii) developed a new platform to analyze complete norovirus genomes by Next-Generation Sequencing (NGS) to study intra- and inter-host evolution. Genotypes in which ≥10 representative sequences were available in public databases were evaluated in this study. Phylogenetic analyses of the amino acid sequences revealed that some (10/16) genotypes consisted of a single intragenotypic cluster, while others presented multiple intragenotypic clusters. GII.4 (the predominant norovirus genotype) presented the largest number of clusters, and those followed a temporal pattern of emergence where strains from one cluster circulated in the human population until they were replaced by strains of the next emerging cluster. The non-GII.4 genotypes presented single or multiple clusters, with some genotypes containing strains within a single cluster that differed by only a few residues over several decades and others containing distinct clusters that could co-circulate within the same epidemiological season. To expand our understanding of these apparently different mechanisms of diversification, we analyzed healthy individuals infected with GII.4 and GII.6 viruses in different settings using NGS. In the first set of samples, we analyzed the intra-host evolution in an individual following acute gastroenteritis who shed virus for over 4 weeks. The GII.4 viruses accumulated mutations as the infection progressed, while the GII.6 viruses remained stable during the prolonged shedding phase. In the second set of samples, we compared inter-host evolution in individuals from outbreaks where the causative agents were identified as GII.4 or GII.6 noroviruses, and showed that GII.4 viruses could rapidly evolve during the same season, while the GII.6 viruses did not. Together, our data support the existence of varying patterns of evolution among norovirus genotypes; the GII.4 viruses are more prone to change, while others (non-GII.4) tend to remain static over time. We propose that these evolutionary patterns influence the diversity and prevalence of individual genotypes in the human population.

38 LTR Point Mutations and Proviral DNA Level among Bovine Leukemia Virus Field Isolates from Poland

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Introduction and Aim: Bovine leukemia virus (BLV), a *Deltaretrovirus* in the *Retroviridae* family, is implicated as the causative agent of Enzootic Bovine Leucosis (EBL). Most of BLV infected cattle remain asymptomatic but one-third of them suffer from persistent lymphocytosis and around 1-5% develop lymphoid tumours. An official BLV control programme has been applied intensively in Poland since 2006 and consequently more than 90% of districts in the country already obtained EBL-free status. However a new phenomenon has been noted: the appearance of isolated BLV infected cattle in herds officially free from BLV. One of explanation is a presence of such kind of virus infection in population which results in rare cases of seronversion and low level of viral replication. It has been noted among other retroviruses that genetic variability in LTR might influence viral replication and also alter proviral load. Therefore, we carried out a genetic analysis of several BLV LTR sequences, representing virus isolates from emerging cases of BLV infection in herds already recognized as EBL-free, in relation to different level of proviral DNA.

Materials and Methods: PBLs were collected during two years period from blood samples of 62 cattle serologically positive for BLV in ELISA. LTR region of the provirus DNA was amplified using nested PCR and directly sequenced. The level of proviral DNA was measured by qPCR based on *pol* gene (TaqMan).

Results: The proviral load was calculated as the copy number of BLV *pol* gene per 1000 cell. The presence of provirus was confirmed in 54 samples and BLV copy number in these samples varied from 0,013 to 2311 copies per 1000 cell. Until now LTR fragment was amplified and sequenced successfully in 18 DNA samples. We analyzed sequences of whole LTR composed of U3, R, and U5 regions. A isolate was considered as a mutant when it possessed changes in comparison to the reference wild-type sequence represented by BLV-FLK. We found mutations located in the U3—TxRE2, TxRE3 (*tax-responsive element*) and TATAbox; in the R—within DAS region (*downstream activator sequence*) and the U5—where an interferon regulatory factor (IFR) binding site is located. Some of mutations were detected in one sample only but some of them were common for a few isolates.

Conclusion: Our *preliminary* studies indicated several variations within regulatory motifs in the LTR of BLV. Results of qPCR showed that most of these samples were characterized by rather high copy number of proviral DNA BLV, over 100 copies/1000 cell. Now we have been focused on amplification, cloning and sequencing of LTRs from low copy number

samples. Future studies will be carried out to examine if identified mutations are associated with different replication rate of virus.

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39 Mass Spectrometry and Bioinformatics to Study Viral Protein S-Acylation and Co-Evolution

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Spike proteins of many enveloped viruses are post-translationally modified with fatty acid residues bound via thio-ester bond to highly conserved cysteines. This lipid modification (S-acylation) is essential for virus replication affecting membrane fusion and/or progeny virus assembly reactions. Using mass spectrometry of anchoring fragments of Influenza virus glycoprotein hemagglutinin we demonstrated that a cysteine at the cytoplasmic border of the transmembrane domain (TMD) is acylated with stearate, while two cysteines in the cytoplasmic tail carry palmitates. Site-specific attachment of stearate to the TMD of other viral glycoproteins from different enveloped virus families was also found. Probably, it is a general feature of transmembrane proteins.

We also aimed to predict amino acid residues participating in viral proteins interactions during virus pathogenesis using bioinformatics tools. It was hypothesized that due to spatial limitations the amino acid residues located at the protein-protein interface should evolve in accordance. To find correlated mutations, we applied multiple sequence alignments of massive samplings from the Influenza Research Database (http://www.fludb.org/), amino acid frequency calculation and the EvFold program (http://evfold.org) searching co-evolving amino acid positions for the protein pairs of interest. To verify our predictions concerning proteins of Influenza virus envelope (in particular, matrix proteins M1 and M2 are taken into consideration), weve started experiments aimed to disturb the co-evolved amino acid position pairs and to follow the structural/ functional consequences. PCR mutagenesis and reverse genetics approaches as well as EM to study morphology of budding virions are the main methods being used so far.

40 Membrane Penetration by Non-Enveloped Viruses

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Virus entry and uncoating are stepwise processes which progressively dismantle the virus shell and eventually the capsid. They set free the viral genome and make it available for transcription, translation, or replication. Virus entry and uncoating involve many different host factors, including proteins, nucleic acids, sugars, ions and lipids. In addition, physical properties of the virus, such as stiffness and internal pressure, are important, and related to the way the virus assembles in an infected cell. This raises questions, such as 1) How do structure and stability of the virus change during entry, and what are the cellular cues that help to reduce virus stability? Our group has been interested in elucidating the mechanisms, by which viruses enter, uncoat and replicate in human cells. We have focused on non-enveloped DNA and RNA viruses, adenoviruses and picornaviruses [1–3]. Our work has shown that adenoviruses depend on distinct cellular cues to accomplish a step-by-step cell entry process from the plasma membrane to endosomes, through the cytosol and to the nucleus [4–11]. Adenoviruses rupture endosomal membranes, unlike enveloped viruses, which use low pH or receptors to induce conformational changes in their envelope glycoproteins, for example, and thereby deliver viral contents to the cytosol without apparent membrane damage. Adenovirus uncoating starts at the plasma membrane, where the virus receives mechanical cues upon binding to receptors which laterally move in the plasma membrane. This leads to exposure of the membrane lytic protein-VI from the virus [8,12,13]. Membrane rupture is also linked to dynamin-dependent virus endocytosis, but virus entry into the cytosol is independent of low endosomal pH, and does not involve Rab7-containing endosomes [14]. Yet, membrane rupture has been shown to require host factors regulating early to late endosome maturation [15]. Our recent studies now suggest a resolution of this puzzle. We show that virus-induced secretion of acidic lysosomes provides critical lipid cues for a two-step activation process of the viral membrane lytic protein-VI [16]. This involves a positive feedback loop between exposure of protein VI and lipid signaling, breaks open endosomal membranes, and releases incoming viruses to the cytosol, and eventually the nucleus for replication.

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41 MicroRNA-17 Is a Critical Host Factor for Pestivirus Replication

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Recently, replication of the pestivirus bovine viral diarrhoea virus (BVDV) was found to depend on the interaction of microRNA-17 (miR-17) with the 3' non-coding region of the viral genome (Scheel *et al.*, 21st International Symposium on Hepatitis C and Related Viruses, Banff, Canada). The miR-17 seed site identified in BVDV is conserved in the related pestivirus classical swine fever virus (CSFV). Therefore, the objective of this study was to explore the potential miR-17 requirements of CSFV for replication. To this end, CSFV with a defective miR-17 seed site was generated (CSFV-p3p4). Replication of the parent and mutant CSFV were analysed in the presence of locked antisense oligonucleotides (tinyLNA17) for miR-17 inhibition and of artificial miR-17p3p4 for seed site complementation, respectively. TinyLNA17 repressed the propagation of CSFV, suggesting that CSFV depends on miR-17 for efficient replication. Accordingly, replication of the CSFV-p3p4 mutant was strongly impaired. Complementation with artificial miR-17p3p4 restored the growth characteristics of CSFV-p3p4 completely. Luciferase-based miR-17 reporter assays and differential transcriptome analyses suggest a virus-mediated sponge effect on miR-17. Taken together, these data demonstrate that miR-17 is a critical host factor for replication of both, CSFV and BVDV.

42 Modulation of Host Pathways by MicroRNAs Differentially Expressed during Infection with Hepatitis C Virus

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Hepatitis C virus (HCV) infection-related morbidity and mortality is a major global health burden. MicroRNAs (miRNAs) have been shown to play an important role in HCV infection through modulation of host pathways essential for viral infection or through direct interactions with viral RNA.

The viral lifecycle of HCV relies heavily on lipid pathways for entry, replication and egress. Recently, our group has shown that 25-hydroxycholesterol, a sterol-lipid effector, exerts its antiviral effects in part though modulation of miRNAs involved in regulating lipid pathways. To further explore the role of miRNAs HCV infection, we utilized miRNA microarray analyses to identify miRNAs differentially expressed during treatment with 25-hydroxycholesterol and during infection with HCV. Transfection of synthetic mimics of differentially expressed miRNAs into Huh7.5 cells stably expressing a full length HCV replicon resulted in a 2-fold increase in HCV replication as measured by qPCR and an increase in HCV protein expression as determined by Western blot. Gene expression profiling analysis by microarray, following miRNA transfection of Huh7.5 cells expressing the HCV replicon, identified host pathways modulated by these miRNAs, individually. Overall, these results further elucidate the role of miRNAs during viral infection and the immunometabolic response to infection in the liver.

43 More Is Less: Enhancing Virus-Induced Membrane Fusion to Inhibit Viral Spread

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Interactions between the HIV-1 envelope glycoprotein (Env) on viral particles and the viral receptor/coreceptor on target cells trigger fusion of viral and host cell membranes, thus mediating virus entry. Transmission of viral particles occurs most efficiently if infected (virus producing) and uninfected (target) T lymphocytes transiently align, thus forming the so-called virological synapse (VS). When producer and target cell contact each other during VS formation they could, in principle, fuse and form a syncytium. Several safeguards put in place in virus producing cells, however, ensure that the majority of encounters between infected and uninfected cells resolve without fusion. Consequently, even if syncytia do form early in the infection process (as recently shown by intravital imaging of HIV-infected BLT mice by the Mempel group; [1], they do not grow beyond the four or five nuclei stage. Based on this finding and also based on our more recent *in vitro* analyses of how fusion-regulating factors affect HIV-1 transmission, we hypothesize that enhancing Env-induced membrane fusion and thus promoting the formation of large T cell syncytia will impair viral spread.

Using fusion enhancers that were identified when the Sodroski lab performed a screen to isolate fusion inhibitors [2], we have started to test this idea *in vitro*. Preliminary results of these studies, together with recent work by investigators studying varicella zoster virus [3], or measles virus [4], lend support to the concept that boosting (rather than repressing) membrane fusion, one of the key viral functions, can compromise virus production and thus merits to be considered as a potential anti-viral strategy.

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44 PB2-E627K Adaptation of Influenza A Virus Polymerase Complex Facilitates Viral Genome Trafficking in Mammalian Cells

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Adaptive mutations in the PB2 protein, such as the E627K substitution, are well known for cross species adaptation of avian influenza A virus. The PB2-E627K substitution significantly enhances viral polymerase activity and replication efficiency in mammalian cells. However, the detail mechanism of this adaptation process is not fully understood. Here, we propose that low replication efficiency in PB2-627E containing virus may attribute to the inhibition of viral genome trafficking in mammalian cells.

To explore molecular basis of PB2-627K adaptive substitution in this process in influenza virus replication, intracellular trafficking of viral RNA (vRNA) and viral proteins (PB2, PB1, PA, NP, NEP and M2) in cells infected with WSN-PB2-627K or WSN-PB2-627E viruses were examined using Immunofluorescence and Fluorescent In Situ Hybridization (IFA-FISH) Assay. It was found that, in the late hours post infection, vRNA of WSN-PB2-627K- virus infected cells were evenly distributed in cells; whereas in WSN-PB2-627E virus infected cells, predominant perinuclear vRNA accumulation was found locating near the microtubule-organization center (MTOC). Interestingly, various cellular marker, e.g., Rab11 (recycling endosomes), TGN46 (Trans-Goli apparatus) and LC3 (autophagosomes) were found densely packed proximate to the vRNA aggregates, suggesting the restriction of vRNA trafficking in WSN-PB2-627E virus infected cells may affect virus replication. Furthermore, electron micrographs shows distinct intracellular organization between WSN-PB2-627K and WSN-PB2-627E virus infected cells, in particular disorganization of mitochondria and microtubules were observed in WSN-PB2-627E but not WSN-PB2-627K virus infected A549 cells.

Our findings suggested that the PB2-627K adaptation of influenza virus may involve in mechanism to overcome hitherto unknown barriers which restrict avian influenza virus vRNA intracellular trafficking and virion release during virus replication.

45 Putative Packaging ATPase of *Acanthamoeba Polyphaga Mimivirus* L437 Interacts with Viral Recombinase and Type II Topisomerase

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Genome segregation and packaging are the critical steps in the life cycle of cellular organisms as well as viruses. Many of the recently discovered extremely large DNA viruses such as *Mimivirus* and *Pandoravirus*, of the Nucleo-cytoplasmic large DNA virus (NCLDV) superfamily, seem to have adapted a bacteria-like genome segregating and packaging mechanism. NCLDVs code for a DNA packaging motor that is related to FtsK/HerA motors of prokaryotes. We found that many NCLDVs, including *Mimivirus*, also encode a recombinase and a type II topoisomerase. These two components are the essential components of the prokaryotic genome segregation and packaging machinery. Here, by using the yeast two-hybrid system, we show that the putative packaging ATPase (gene product of L437) of *Mimivirus* physically interacts with one of the recombinases (L103) and subunit A of the type II topoisomerase (R480). In addition, the second copy of the putative packaging ATPase (L649) interacts with L437 packaging ATPase as well as with L103 and R480 subunit A. Interestingly, type II topoisomerase (R480) also interacts with one more recombinase (R771). We are currently validating these interactions by independent methods. These interactions could have important implications for the assembly and activation of complete genome segro-packasome machinery required for the resolution and encapsidation of the viral genome.

46 Role of Cyclin-Dependent Kinase 5 (CDK5) during DENV-2 *in Vitro* Infection

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Dengue is the most important mosquito-borne disease worldwide, since it is distributed over one hundred in tropical countries, both the Old and New World [1]. Dengue virus (DENV) is an enveloped RNA virus which requires the host-cell cytoplasmic machinery, including cytoskeleton elements (actin and microtubules) [2,3] to carry out a successful replication cycle. The CDK5 protein regulates the neuronal dynamics of microtubules; therefore, the knock-down by RNAi is used for gene therapy [4]. Connecting the neuronal role of CDK5 with the recent report in epithelial and endothelial cells [5], in the present study was evaluated the participation of CDK5 during DENV-2 *in vitro* infection using two approaches, the chemical inhibition of CDK5 (Roscovitine) and the gene knock-down using an artificial miRNA. The chemical inhibition of CDK5 using Roscovitine decreased viral titers of DENV-2 on the pre-treatment and post-infection stages in concentrations below 25 µM of a dose-dependent manner. Flow cytometry and fluorescence microscopy shown that Roscovitine decreased the viral envelope protein and induced changes in its subcellular distribution after the treatment 24 hours post-infection. In the same conditions, the artificial miRNA targeted to CDK5 protein, not showed apparent reduction on normal protein expression and not have any effect on DENV-2 infection, maybe due an off-target effect. Considering that Roscovitine is a potent and selective inhibitor of the cyclin-dependent kinases, among them CDK5, our data suggests that this protein is important for the DENV-2 replication cycle and can be disregarded as a potential target for developing host-targeted antivirals.

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47 Selection of High Titer Bovine Foamy Virus Variants and Identification of Key Adaptive Mutations

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Bovine foamy virus (BFV) is a non-primate member of the Spumaretroviruses. The four known BFV isolates are highly related to each other and their molecular biology is similar to that of the other foamy viruses (FVs). However, while the other FVs show high levels of cell-free infectivity, BFV is tightly cell-associated with an extremely inefficient transmission via cell-free virus particles.

To unravel the mechanism(s) responsible for the low level of cell-free BFV infectivity and in order to gain functional insights into the process of foamy virus particle formation and egress, BFV variants with enhanced cell-free infectivity were selected by serially passaging the original BFV Riems isolate in baby hamster kidney cells (BHK21 BFV indicator cell line) and MDBK (Madin-Darby bovine kidney) epithelial cells. BFV infectivity increased over time and plateaued at about 10^6 FFU/mL for BHK21 cells and 10^5 FFU/mL in MDBK cell. Concomitant to increased BFV titers, the BFV-specific cytopathic effect (CPE) was lost. From early, medium and late adaptive passages, either the *gag* and *env* genes encoding the FV budding machinery or almost the whole coding sequence of BFV variants with increased cell-free infectivity were cloned and sequenced. Whereas a pattern of consistent amino acid changes was detected in both genes, additional changes were apparently cell-type specific or occurred in a stochastic manner.

As expected, co-evolved Gag-Env pairs from the same proviral DNA showed a high likelihood of enhanced particle release (and high cell-free infectivity) while the combination of different Gag and Env protein only in rare cases resulted in productive particle release. Some clones showed a duplication of a potential budding-relevant late domain. All clones from late passages from MDBK- and BHK21-derived BFV variants carry one of two highly specific, in-frame deletions in the integrase which removes a BFV-specific C-terminal extension of IN that is absent in all other known retroviral IN proteins. Consistent genetic changes in *gag*, *pol* and *env* have been functionally characterized and their contribution to the high cell-free transmission phenotype determined.

48 Site-Specific Probes for Enteroviruses for Detailed Imaging in Light and Electron Microscopy

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We are focused on understanding the mechanisms and cellular factors behind enterovirus infection. Our previous results have suggested that enteroviruses follow a macropinocytic entry to host cells. In order to follow virus uncoating and other details in the infectious pathway, covalently conjugated probes for virus capsid or genome are needed. On the other hand, visualization of virus opening in cellular structures profits from dynamics probes that are released upon virus uncoating. We have developed a protocol for site-specific covalent conjugation of atomically monodisperse gold clusters with 1.5 nm metal core to viral surfaces (PNAS 2014). Water-soluble Au102(para- mercaptobenzoic acid)44 clusters, functionalized by maleimide linkers to target cysteines of viral capsid were conjugated to echovirus 1 and coxsackievirus B3 without compromising the infectivity. Quantitative analysis showed ordering of the bound gold clusters on the viral surface and a clear correlation between the clusters and the cysteine sites close to the viral surface. Another site-specific probe was developed for the hydrophobic pocket of enteroviruses (Nanoscale 2015). A derivative of Pleconaril was conjugated to fluorescent labels and Au102. The probe mildly stabilized the virus particle and caused a delay in the virus uncoating, but could not however inhibit the receptor binding, cellular entry or infectivity of the virus. The hydrophobic pocket binding was proven by STD and tr-NOESY NMR methods and TEM. The virus-fluorescent probe accumulated in endosomes but was seen to leak from the virus-positive endosomes from the capsid proteins suggesting that, like the physiological hydrophobic content, the probe may be released upon virus uncoating. Our results collectively thus show that the gold and fluorescently labeled probes may be used to track and visualize the studied enteroviruses during early phases of infection opening new avenues to follow virus uncoating in cells.

49 The HSV-1 Protein pUL31 Escorts Mature Capsids to the Nuclear Envelope for Egress by Membrane Budding

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Progeny capsids of herpesviruses leave the nucleus by budding through the nuclear envelope. Two viral proteins, the membrane protein pUL34 and the nucleo-phosphoprotein pUL31 form the nuclear egress complex (NEC) at the inner nuclear membrane (INM). This NEC is required for capsid egress out of the nucleus through primary envelopment at the INM followed by de-envelopment at the outer nuclear membrane (ONM). All pUL31 orthologs are composed of a diverse N-terminal domain with 1 to 3 basic patches and a conserved C-terminal domain. To decipher the functions of the N-terminal domain, we have generated several Herpes simplex virus mutants and show here that the N-terminal domain of pUL31 is essential with basic patches being critical for viral propagation. pUL31 and pUL34 entered the nucleus independently of each other via separate routes and the N-terminal domain of pUL31 was required to prevent their premature interaction in the cytoplasm. Unexpectedly, a classical bipartite nuclear localization signal embedded in this domain was not required for nuclear import of pUL31. In the nucleus, pUL31 associated with the nuclear envelope and newly formed capsids. Viral mutants lacking the N-terminal domain or with its basic patches neutralized still associated with nucleocapsids but were unable to translocate them to the nuclear envelope. Replacing the authentic basic patches with a novel artificial one resulted in HSV1(17+)Lox-UL31-hbpmp1mp2, that was viable but delayed in nuclear egress and compromised in viral production. Thus, while the C-terminal domain of pUL31 is sufficient for the interaction with nucleocapsids, the N-terminal domain was essential for capsid translocation to sites of nuclear egress and a coordinated interaction with pUL34. Our data indicate an orchestrated sequence of events with pUL31 binding to nucleocapsids and escorting them to the inner nuclear membrane. We propose a common mechanism for herpesviral nuclear egress: pUL31 is required for intranuclear translocation of nucleocapsids and subsequent interaction with pUL34 thereby coupling capsid maturation with primary envelopment. Our new herpesviral mutant HSV1(17+)Lox-UL31-hbpmp1mp2 represents an excellent tool to decipher the dynamic intranuclear transport of nucleocapsids as well as their viral and host interactions on the way to nuclear egress.

50 The Influenza NA Transmembrane Domain from Co-Evolution with the Head Domain to Post-Transcriptional Regulation

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Viral replication generally utilizes both the host cell machinery and gene regulation mechanisms. Enveloped viruses have the additional challenge of regulating the viral secretory proteins in addition to the cytosolic proteins. By co-expressing genes from influenza A viruses, we found that the RNA-binding protein NS1 substantially increases the production of the secretory glycoproteins hemagglutinin (HA) and neuraminidase (NA). The protein expression enhancement is mediated by the RNA-binding domain of NS1 and is dependent on the nucleotide composition of the N-terminal endoplasmic reticulum (ER)-targeting sequences of HA and NA. Synonymous mutations that increase the GC-content within the HA and NA ER-targeting sequences abolish NS1 regulation, indicating these 5' mRNA regions likely form structural elements, which limit synthesis when NS1 is absent. Comparing the GC-content profile of human ER-targeting sequences to their respective genes, and targeting sequences from bacteria, revealed several differences suggesting this is a general regulatory mechanism for human, as well as viral, secretory proteins. Together, this and prior work form our lab demonstrates that the ER-targeting sequence of NA (the transmembrane domain) has evolved on the amino acid level to facilitate folding and co-translational ER targeting and on the nucleotide level for post-transcriptional regulation by an RNA-binding protein.

51 The Lymphocytic Choriomeningitis Virus Matrix Protein PPXY Late Domain Drives the Production of Defective Interfering Particles

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Arenaviruses cause severe diseases in humans but establish asymptomatic, lifelong infections in rodent reservoirs. Persistently-infected rodents harbor high levels of defective interfering (DI) particles, which are thought to be important for establishing persistence and mitigating virus-induced cytopathic effect. Little is known about what drives the production of DI particles. We show that neither the PPXY late domain encoded within the lymphocytic choriomeningitis virus (LCMV) matrix protein nor a functional endosomal sorting complex transport (ESCRT) pathway is absolutely required for the generation of standard infectious virus particles. In contrast, DI particle release critically requires the PPXY late domain and is ESCRT-dependent. Additionally, the terminal tyrosine in the PPXY motif is reversibly phosphorylated and our findings indicate that this post-translational modification may regulate DI particle formation. Thus, we have uncovered a new role for the PPXY late domain and a possible mechanism for its regulation.

52 The Role of Retrograde Transport in *Vaccinia Virus* Replication

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Vaccinia virus (VACV), the prototypic poxvirus, undergoes a complex maturation process within the cytoplasm involving a "double-wrapping" step near the trans-Golgi network (TGN) to eventually form infectious enveloped virions (EEVs). The Golgi-associated retrograde protein complex (GARP) plays a key role in the retrograde transport of vesicles from endosomes to the TGN. This work investigated the role of retrograde transport and GARP in particular, in the replication cycle of VACV. GARP is a complex composed of four large heteromeric proteins: VPS51, VPS52, VPS53 and VPS54. The effects of GARP loss were investigated using mouse embryonic fibroblasts (MEFs) containing the hypomorphic VPS54 *wobbler* mutation, as well as VPS52-targetting siRNA in HeLa cells. GARP loss in both *wobbler* MEFs and HeLa cells lacking VPS52 resulted in a marked reduction in VACV replication and spread. This was due to a reduction specifically in the production of EEVs. Immunofluorescent studies were used to identify the mechanism by which GARP promotes EEV production, and revealed mislocalisation and aggregation of the EEV membrane protein B5 near the cell periphery. Western blotting revealed reduced levels of B5 in *wobbler* MEFs compared to wild type MEFs. These results identify the GARP complex as a pro-viral host factor required for VACV EEV production, and suggest that cellular retrograde transport pathways facilitate the recycling of viral membrane proteins, such as B5 from the surface of the cell to the viral wrapping centre near the TGN.

53 Tuning of PI3K/Akt Pathway by HIV-1 Nef and its Role in T Cell Biology

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Background and objectives: Human Immunodeficiency virus type 1 (HIV-1) primarily infects $CD4^+$ T cells and cells of monocyte/macrophage lineage. HIV-1 Nef is expressed early during viral life cycle. Nef protein is dispensable for viral replication *in vitro;* however, it plays an important role in viral pathogenesis in infected individuals. The presence of extracellular Nef has been detected in the serum of infected individuals. Nef modulates several signaling pathways involved in cell survival and cell death resulting in the survival of HIV-1 infected cells and killing of bystander cells. PI3K/Akt pathway is one of such pathways. We asked the question whether Nef can modulate PI3K/Akt pathway and its role in T cell biology.

Methods: We isolated peripheral blood lymphocytes (PBLs) from healthy donors and treated them with varying concentration of recombinant Nef (rNef) at various time points. In addition, we infected PBLs with HIV-1 wild clone and Nef deleted HIV-1 clone. We determine the interaction between Nef, PI3K, and Akt using pull down assay and confocal microscopy and levels of Akt activation were determined using western blotting. Furthermore, we quantified the IL-2 production and T cell proliferation in PBLs treated with rNef alone or in combination with T cell receptor (TCR) stimulation in the presence and absence of PI3K and Akt inhibitors.

Results: We found that Nef interacts with PI3K/Akt and activates Akt *in vitro*. Importantly, Nef exhibits synergism with TCR stimulation, as shown by increased IL-2 production and T cell proliferation in TCR-stimulated PBLs treated with rNef as compared to Nef or TCR stimulation alone. Hyperactivation of TCR-stimulated T cells by Nef was mediated by PI3K/Akt signaling.

Conclusions: Our data indicate that Nef can modulate Akt signaling in T cells via physical interaction and can enhance TCR response. Nef mediated enhancement of T-cell activation fuels the progression of the disease and could lead to new therapeutic approaches.

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54 Type I Interferon Treatment Inhibits the Formation of +RNA Virus Replication-Associated Membrane Structures

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Objectives: The antiviral innate immune response is built around the prompt recognition of pathogen-associated molecular patterns and the subsequent inhibition of virus replication until the adaptive immune response is activated for ultimate clearance of the virus. All plus-stranded RNA viruses hijack intracellular host membranes to form elaborate virus-induced membrane modifications, such as the network of interconnected ER-derived double membrane vesicles formed by corona- and arteriviruses. These membrane structures accommodate the viral replication machinery and devastate the interior of the cell during infection. We hypothesized that the innate immune system likely has ways to directly counteract the formation of these virus-induced membrane modifications.

Methods: Ectopic expression of selected arterivirus non-structural proteins (nsps) results in the formation of membrane structures, which are topologically identical to those observed during infection. Since they are formed in the absence of replicating viral RNA, a known target of interferon-induced responses, this set-up can be used to identify responses that specifically counteract the formation of virus-induced membrane structures. Using large-scale mosaic maps of 2D electron microscopy of cells expressing arterivirus nsp2-3, we assessed the effects of IFNβ treatment on both the quantity and the morphology of the membrane structures at the ultrastructural level.

Results: We found that type I interferon treatment significantly reduced the formation of virus-associated membrane modifications, while the expression level and subcellular localization of the nsps was not affected. Moreover, the morphology of nsp2-3 membrane structures was altered drastically upon interferon treatment. Data on several candidate interferon-stimulated genes that could be involved will be presented.

Conclusions - These data indeed show a direct inhibitory effect of type I interferon and/or interferon-stimulated genes on the formation of virus-induced membrane modifications. This identifies a novel host innate immune response mechanism that inhibits +RNA virus replication, which may provide a new target for antiviral therapy.

55 UBR Box N-Recognin-4 (UBR4) is a Human Host Factor Required by Mammalian Influenza A Viruses During Late Stages of the Viral Life Cycle

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As intracellular pathogen, Influenza A virus (IAV) is dependent on a large variety of cellular factors to complete its life cycle. Recently, several studies have been performed to identify host proteins required by IAV. Surprisingly, the overlap of host factors between the different screens was lower than anticipated. Likely, this is the result of variations in experimental procedure and selection methods of the individual studies. We revisited the raw data of four different published siRNA screens and identified by computational analysis an additional set of cellular proteins that are required by IAV. Amongst others UBR box N-recognin-4 (UBR4), a member of the N-recognin family, was a promising candidate which we chose for follow-up studies. UBR4 was identified as an interaction partner of the viral ion channel protein M2. Furthermore, siRNA-mediated knockdown of UBR4 protein levels reduced growth of the IAV strain A/WSN/33 in a lung epithelium cell line. Besides A/WSN/33, an H1N1 virus, the H3N2 virus strains A/Udorn/72 and A/HongKong/68 were shown to be sensitive to UBR4 depletion. Interestingly, avian influenza strains appeared to be less dependent on UBR4 expression. Notably, depletion of UBR4 in mouse lungs using peptide-phosphorodiamidate morpholino oligonucleotides (PPMOs) impaired IAV growth *in vivo* resulting in significantly reduced virus titers in lung homogenates compared to control and increased survival. In addition, the avian strain A/duck/England/1/1965 was shown to be, albeit not resistant, less sensitive to UBR4 knockdown compared to the human strain A/Puerto Rico/8/1934. Further experiments suggested that UBR4 is required during a late stage of the viral life cycle as nucleoprotein (NP) expression or the growth of a single cycle reporter virus remained unaffected by UBR4 knockdown. Transmission electron microscopy revealed that the production of virus buds and progeny virions was reduced by UBR4 knockdown while the morphology of virions was not affected.

56 UBXN1 Negatively Regulates Canonical NFkB Signaling by Inhibiting the Degradation of IkBa

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UBXN proteins likely participate in the global regulation of protein turnover, and we have recently shown that UBXN1 interferes with RIG-I-like receptor (RLR) signaling by interacting with MAVS and impeding its downstream effector functions. Here we demonstrate that over-expression of UBXN1 decreased lentivirus and retrovirus production by several orders-of-magnitude in single cycle assays, at the level of long terminal repeat-driven transcription. Independent of its effects on RLR signaling, UBXN1 blocked the canonical NFkB pathway by binding to Cullin1 (Cul1), inhibiting IkBa degradation. Multiple regions of UBXN1, including its UBA domain, were critical for this activity. Elimination of UBXN1 resulted in early murine embryonic lethality. shRNA-mediated knockdown of UBXN1 enhanced human immunodeficiency virus type 1 (HIV) production up to 10-fold in single cycle assays, but was inhibitory in multiple cycle assays in T cells using replication-competent virus, likely due to induction of antiviral genes activated by NFkB. In primary human fibroblasts, knockdown of UBXN1 caused prolonged degradation of IkBa and enhanced NFkB signaling, which was also observed after CRISPR-mediated knockout of UBXN1 in mouse embryo fibroblasts. Knockout of UBXN1 significantly up- and down-regulated hundreds of genes, notably those of several cell adhesion and immune signaling pathways. Reduction in *UBXN1* gene expression in Jurkat T cells latently infected with HIV resulted in enhanced HIV gene expression, consistent with the role of UBXN1 in modulating the NFkB pathway. The ability of UBXN1 to negatively regulate the NFkB pathway may have important implications for dampening the host immune response in disease processes and also re-activating quiescent HIV from latent viral reservoirs in chronically infected individuals.

57 Unraveling Molecular Pathogenesis of PPRV (Peste des Petits Ruminants Virus) Infection from Tissue Transcriptome of Infected Goats

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Peste-des-petits ruminants (PPR) is an acute, highly contagious, devastating transboundary list-A disease of OIE that mostly affects goats and sheep. It is characterized by erosive stomatitis, mucopurulent nasal discharge, pneumonia, gastro-enteritis and fetid diarrhea. PPR is caused by *Peste des petits ruminants virus* (PPRV) belongs to genus *Morbillivirus* of family *Paramyxoviridae.* After successful eradication of rinderpest, PPR gained wide spread concern for its eradication due to similarity of causative agents. In order to unravel the important molecules involved in pathogenesis global gene expression profiling was carried out. In this study goats were inoculated with a virulent PPRV (Izatnagar/94) isolate and tissue samples were collected immediately after the death for transcriptomics by RNA-Seq. Global gene expression of PPRV infected lung and spleen revealed enrichment of several pathways viz. apoptotic signaling, B cell and T cell activation, IFN-, IL signaling pathway. Network analysis revealed 51 and 37 differentially expressed highly connected (DEHC) genes in lung and spleen, respectively. DEHC network in lung showed connectivity among key immune genes - ISG15, LCK, IL7R, SOCS3, CBL whereas in spleen immune genes - ISG15, SRC, CDKN1A, HSPA5, HERC5 were found to be well connected in the DEHC network. A total of 74 unique DEHC genes were identified in both infected tissues. These unique genes on KEGG and REACTOME pathway analysis were enriched for cytokine signaling in immune system, CD28 co-stimulation, TCR signaling and regulation of KIT signaling indicated triggering of immune signaling cascades in the host on viral infection.

58 UPR Is Required Early during TBEV Infection to Trigger the IFN Response

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Flaviviruses are a major cause of disease in humans and animals worldwide. Tick-borne encephalitis virus (TBEV) is the most relevant arthropod-borne flavivirus endemic in Europe and is the etiological agent of tick-borne encephalitis; a potentially fatal infection of the central nervous system. In our recent work we demonstrated that TBEV is able to trigger the stress response of infected cells leading to the formation of stress granules (SG) [1]. We also found that the formation of SG in TBEV infected cells is delayed, following the same delayed kinetics of the IFN response [2]. Indeed, while TBEV replication is evident at early time points post infection, SG and IFN-β mRNA become detectable only after 16 hours. Transcriptome analysis of TBEV infected cells showed that, in addition to interferon and interferon stimulated genes, also genes of the unfolded protein response (UPR) were activated. Interestingly, the spliced form of Xbp1 and phosphorylation of PERK occurred early during infection (< 12h) indicating that the UPR occurs before induction of interferon. We then investigated the role of the UPR as an early cellular response to the infection and as a possible trigger of the interferon response. It was of interest to note that when cells were infected following treatment with Tunicamycin, a known inducer of the UPR, the IFN response was already active at 8 hours post-infection and the virus titres were significantly decreased. In this condition formation of stress granules was also anticipated with the same kinetics. These data suggest that TBEV is able to evade both the stress and interferon responses and that the UPR may play a critical and unexpected role in the delayed activation of both.

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59 Vimentin Dynamics in Enterovirus Infection

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Vimentin is the most commonly found intermediate filament in several cell types. Its expression is altered during development and in certain diseases. Vimentin has a high degree of homology among species suggesting that it has a vital role in cells. Several independent research groups have reported the association of vimentin with several viruses, especially affecting their replication and progeny virus production. Despite the abundance of these reports, a consensus on the role or the mechanism of between viruses and these observed vimentin dynamics is still missing. However, these reports indicate that vimentin can have more universal role in virus-cell interactions.

Human enteroviruses are large group of viruses from families of *Rhinoviruses*, *Echovirus*, Groups A and B of *Coxsackieviruses* and *Polioviruses*. They are one of the most common viruses infecting humans worldwide. Most commonly enterovirus infection leads to acute infection due to rapid lysing of the infected cells. However, in some cells, infection can also be persistent and lead to chronic infection in the tissue. The mechanism regulating lytic or persistent infection of enteroviruses is not known. There have been indications that viral capsid, viral genome and receptor usage would constitute to whether virus causes lytic or persistent infection. However, the cellular regulators behind this are still unknown.

Our results show that vimentin can have a dual regulating role during human enterovirus B-group infection. First, cell permissiveness to enterovirus infection can be modulated via PKC-mediated vimentin regulation that can also be induced by changing cell culture conditions. Second, enterovirus infection itself can modulate cellular vimentin network leading to accelerated cell death by virus.

60 A Host SUMOylation Response to Influenza Virus Infection

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Dynamic nuclear SUMO modifications play essential roles in orchestrating cellular responses to proteotoxic stress, DNA damage and DNA viruses. Here, we describe a non-canonical host SUMOylation response to the nuclear-replicating RNA pathogen, influenza virus, and identify viral RNA polymerase activity as a contributor to SUMO proteome remodeling. Using quantitative proteomics to compare SUMO responses to various stresses, we reveal that influenza virus infection causes unique re-targeting of SUMO to a diverse range of 63 host proteins involved in transcription, mRNA processing, RNA quality control and DNA damage repair. Depletion screening identified 10 of these virus-induced SUMO targets as potential antiviral factors, including C18orf25 and the SMC5/6 and PAF1 complexes. Mechanistic studies also uncovered a new role for SUMOylation of the PAF1 complex component, parafibromin (CDC73), in potentiating antiviral gene expression. Our global characterization of influenza virus-triggered SUMO redistribution provides an insight into understanding host nuclear SUMOylation responses to infection.

61 Analysis of Proteins from Isolated Mimivirus Factories at Different Stages of Infection by Mass Spectrometry

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The discovery of the giant amoeba-infecting virus *Acanthamoeba polyphaga* Mimivirus, along with the recent realization that microbe-size viruses are likely to be abundant, revived questions concerning the nature of viruses, their roles in the emergence of life, as well as their putative contribution to the generation of eukaryotic nuclei. The last point is of particular interest as many large DNA-viruses generate elaborate factories in the cytoplasm of their hosts, within which all viral transactions, including genome replication, RNA transcription, capsid assembly and genome encapsidation take place. Structural studies of such factories, generated by large DNA viruses such as Vaccinia and Mimivirus highlighted the extreme complexity of these viral assemblies, as well as their exquisite spatiotemporal organization. While we and other groups conducted extensive studies of the structural features of intracellular viral factories generated by the Mimivirus, the protein composition of these assemblies remained largely unknown. Here, we conducted a detailed Mass Spectrometry analysis of the protein composition of highly purified Mimivirus factories isolated at progressive post-infection time-points. Our studies provide important insights into the composition, generation, structure, and function of factories generated by diverse viruses, as well as characteristics of intracellular assemblies of macromolecules in general.

62 Bacteriophage Biosystem: Towards a Comprehensive Understanding of the Bacterial Virus-Host Interaction

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The Laboratory of Gene Technology (LoGT) has more than 15 years of experience identifying and analyzing bacterial viruses (or bacteriophages) infecting *P. aeruginosa*. These very diverse lytic bacteriophages infect their host and subsequently hijack the metabolism to produce progeny virus particles, before lysing the cell to release them.

Being deprived of an own metabolism, bacteriophages depend on their host cell's resources to fuel their replication. In the years of co-evolution, encode enzymes and other proteins influence replication, transcription and (post)translation by highly specific (in)direct interactions and processes. To overcome biochemical bottlenecks in specific metabolic processes for viral replication, and therefore were termed 'auxiliary metabolic genes'. As such the metabolic response to phage infection would vary depending on the number of AMGs in the genome of the phage. To gain additional insight in this matter, we studied the variations in an ubiquitous, gram-negative soil bacterium, *Pseudomonas aeruginosa*, under attack of six different lytic dsDNA phages by combining RNA sequencing, protein interaction approaches and "state of the art" metabolomics techniques. In this lecture, emphasis will be placed on examples of the molecular hijacking at the RNA, protein and metabolite level and how these integrate into the highly phage-specific response induced in *Pseudomonas*.

63 Cellular Receptors Targeted by Human Viruses to Infect Our Body

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A virus initiates infection by binding to one or several specific receptors at the host cell surface. These interactions play a major role in virus cellular tropism and transmission. Host viral receptors constitute, therefore, a major aspect of viral pathogenesis that could be exploited in future antiviral treatments. We have referenced all human virus receptors published in the literature to provide a complete picture of virus entry molecules in Human. We identified 134 interactions involving 56 human proteins and 12 kinds of carbohydrates that are used by 58 different viruses to infect our body. The interactions have been classified either as attachment/reversible or entry/irreversible. Most virus binding proteins are adhesion molecules, cellular receptors or lectins. The comparison of these interactions with viral entry mechanism: endocytosis clathrin-dependent or not, macropinocytosis, *etc*., suggested that the entry route is in majority dictated by the host protein bound rather than the nature of the virus itself. Notable differences are found between enveloped and non-enveloped viruses though. We further collected tissue expression data of human proteins to draw a map of human virus receptors per specific tissue. This is the first time that a comprehensive analysis of human receptor to viruses is being made by crossing human tissue expression data, virus tropism and entry molecular mechanisms.

64 Characterization of the Receptor-Binding Domain of Parvovirus B19 That Mediates Viral Uptake into Permissive Cells

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Parvovirus B19 (B19V) is known as the human pathogen causing the mild childhood disease erythema infectiosum. B19V shows an extraordinary narrow tissue tropism for erythroid progenitor cells in the bone marrow, which is determined by a highly restricted uptake into this erythroid differentiation stage. We have previously shown that the specific internalization is mediated by the interaction of the viral protein 1 unique region (VP1u) with a yet unknown cellular receptor. To locate the receptor-binding domain (RBD) within the VP1u, we analyzed the effect of truncations and mutations on the internalization capacity of the recombinant protein into UT7/Epo cells. Here we report that the N-terminal amino acids 5-80 of the VP1u are necessary and sufficient for cellular binding and internalization; thus, this N-terminal region represents the critical RBD required for B19V uptake. Using site-directed mutagenesis of conserved amino acids, we further identified a cluster of possible receptor-binding residues. *In silico* predictions and experimental results suggest that the RBD is structured as a rigid fold of three α-helices. We also found that dimerization of the VP1u leads to a considerably enhanced cellular binding and internalization compared to its monomeric form. Taken together, we identified the RBD that mediates B19V uptake and mapped functional and structural motifs within this sequence. The findings reveal insights into the uptake process of B19V, which contribute to understanding the pathogenesis of the infection and the neutralization of the virus by the immune system.

65 Comparative Studies on Retroviral and Retroviral-Like Cellular Proteases

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Exogenous retroviruses comprise a large family of enveloped RNA viruses. In the last stage of their replication, the Gag and Gag-Pro-Pol polyproteins of the virions are processed by the retroviral protease (PR) leading to viral maturation. Furthermore, several cellular proteins have been shown to be substrate of the PR and their processing has been proposed to have implications for the viral replication. The PR is a homodimeric aspartic protease that recognizes at least seven substrate residues. PRs, especially that of HIV-1 are targets for chemotherapy but effectiveness of protease inhibitors is severely hampered by the development of resistance. Even though the PRs are fairly specific, they apparently do not have consensus substrate sequences. Our comparative studies of eleven PRs representing each *genera* of retroviruses suggested a relatively conserved specificity in spite of the great sequence diversity, while HIV-1 PR clinical inhibitors are typically only weak inhibitors of the other retroviral proteases. Our previous studies have been complemented by studies on additional retroviral, retrotransposon and cellular dimeric retroviral-like aspartic (PEG10, Asprv1) proteases. These enzymes are also very weakly inhibited by PIs. Interestingly, many of the mutations occurring in HIV drug resistance introduce amino acids that can be found at the equivalent position in other retroviral or retroviral-like cellular proteases. Specificity studies of the enzymes revealed key determinants of the specificity and identified sequences that may lead to general substrates for these enzymes. These studies are expected to help the design of broad-spectrum inhibitors against retroviral and retroviral-like cellular proteases.

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66 Conserved Transcriptional Response in Ixodes ricinus Cells to Virus and Bacterial Infection

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In recent decades there has been an emergence and expansion of tick-borne diseases around the world, including tick-borne encephalitis (Europe) and human granulocytic anaplasmosis (HGA; North America and Europe). The primary tick vector in Europe is *Ixodes ricinus,* which is detected throughout the continent. Although much is known about the mammalian host response to infection with these bacterial and viral pathogens, there is limited knowledge of the cellular responses to infection within the tick vector. Tick-borne encephalitis in humans is caused by the flavivirus tick-borne encephalitis virus (TBEV) and a similar encephalitic disease in sheep is caused by the related virus, louping ill (LIV). HGA is caused by infection with the bacterium *Anaplasma phagocytophilum*, which also causes anaplasmosis in domestic animals. Recent studies have shown that *A. phagocytophilum* is able to inhibit apoptotic processes in tick and human cells, enabling infection to proceed. However, the tick cellular responses to infection with tick-borne flaviviruses have not been investigated.

In order to compare transcriptional responses to TBEV, louping ill virus and *A. phagocytophilum*, *I. ricinus* cells IRECTVM20 were separately infected with three different pathogens. Infected cell lysates were investigated using RNA sequencing. In each case, differentially regulated transcripts were identified and were similar for each pathogen at 0.95% (*A. phagocytophilum*), 2.96% (LIV) and 1.98% (TBEV) of total transcripts identified. Infection with all three pathogens stimulated significant transcriptional up-regulation of genes involved in the inhibition of the intrinsic apoptosis pathway including *heat shock protein 70* ($p<0.001$, $q<0.01$). Furthermore, infection also stimulated up-regulation of genes associated with innate immune response including *MAPK-activated protein kinase* ($p<0.001$, $q<0.01$) and *MAPK phosphatase 5* ($p<0.01$, $q<0.05$). Viral infection induced down-regulation of *trafficking protein particle complex 9* ($p<0.01$, $1<0.05$), representing an additional cellular mechanism to restrict apopotosis. These data provide an insight into key genes involved in orchestration of the tick cellular response to infection with different pathogens and possible pathogen-mediated effects that limit activation of tick cell apoptosis to promote pathogen survival.

67 Cross-Species Comparative Analysis of Dicer Proteins during Sindbis Virus Infection

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In plants and invertebrates, RNA silencing is a major defense mechanism against virus infections. The first event in RNA silencing is dicing of long double stranded RNAs into small interfering RNAs (siRNAs). The Dicer proteins involved in this process are phylogenetically conserved and have the same domain organization. Accordingly, the production of viral derived siRNAs has also been observed in the mouse, but only in restricted cell types. To gain insight on this restriction, we compare the dicing activity of human Dicer and fly Dicer-2 in the context of Sindbis virus (SINV) infection. Expression of human Dicer in flies inefficiently rescues the production of viral siRNAs but confers some protection against SINV. Conversely, expression of Dicer-2 in human cells allows the production of viral 21 nt small RNAs. However, this does not confer resistance to viral infection, but on the contrary results in stronger accumulation of viral RNA. We further show that Dicer-2 expression in human cells perturbs IFN signaling pathways and antagonizes PKR-mediated antiviral immunity. Overall, our data suggest that a functional incompatibility between the Dicer and IFN pathways explains the predominance of the IFN response in mammalian somatic cells.

68 Cutthroat Trout Virus: A Model for HEV

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In 1988, a small RNA virus was isolated from salmonid fishes and designated cutthroat trout virus (CTV). CTV is a non-enveloped positive-strand RNA virus, belonging to the *Hepeviridae* family. Its structure highly resembles that of hepatitis E virus (HEV) and has been shown to successfully replicate in cell culture. It has therefore been proposed as a model for HEV which may help to identify important molecular and cellular aspects of the HEV infection. To optimize the replication of CTV in cell culture, we infected a variety of cells to determine the most suitable cell-line for viral propagation. The rainbow trout gill (RTGill-W1) cell-line was able to produce up to 10^{10} genomic copies/mL within seven days in comparison to published data which reports viral titers of 10^{8} copies/mL within 20 days in Chinook salmon embryo (CHSE-214) cells. Furthermore, we show the detection of replication by using immunofluorescence. An antibody directed against the viral capsid protein (ORF2) was produced from the recombinant ORF2 protein. One week post-infection, clear ORF2 signal was detected in the cytosol of RTGill-W1 cells. Viral replication was also successfully detected using an antibody targeting dsRNA. Double staining experiments revealed that ORF2 and dsRNA signals are mainly located in the same cells. Additionally, a $TCID_{50}$ assay was established which allows for CTV inactivation studies. The $TCID_{50}$ analysis determined a titer of 5.92 log($TCID_{50}$/mL), indicating that low doses of virus still are infectious. These early results show the potential of the CTV model and how it can be used to study HEV.

69 Different Ways of Representing of Host Virus Interactions in ViralZone

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In the time course of infection, efficient viral replication and subsequent pathogenesis mostly depend upon a complex interplay between viral and host cellular proteins. In-depth knowledge of these molecular interactions is essential to understanding the biology of infection and for the design of new antiviral strategies. ViralZone is a database that brings together viral molecular biology knowledge with viral genomic and protein sequences. This resource integrates an extensive dataset of virus–host interactions which is represented in different ways. First, for each viral genus, a virus description page has been created containing a section dedicated to host-virus interactions. This section describes in a few sentences the molecular mechanisms by which specific viral proteins interfere with key cellular pathways. Additionally, we have generated a comprehensive dictionary for eukaryotic host-virus interactions. This controlled vocabulary has been detailed in 57 ViralZone pages, which contain an illustrated description of all host molecular pathways modulated by viruses. Finally, we have also created tables of host-virus interactions for several viruses including HIV or human herpesviruses in which each identified and relevant host-virus interaction is detailed.

70 Distinct Sets of PIWI Proteins Produce Arbovirus and Transposon-Derived piRNAs in *Aedes aegypti* Mosquito Cells

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The PIWI-interacting RNA (piRNA) pathway is of key importance in genome defense against "non-self" sequences, such as transposable elements (TEs), in most animal species. PiRNA biogenesis relies on a feed-forward amplification loop called the "ping-pong" mechanism, which amplifies the pool of piRNAs and mediates both transcriptional and post-transcriptional silencing of target sequences. To accomplish their function, piRNAs invariantly associate with the PIWI subclass of the Argonaute family.

Beside transposon-derived piRNAs, viral piRNAs with a ping-pong signature accumulate in *A. aegypti* somatic cells upon arthropod-borne (arbo) virus infection. The PIWI protein family in *Aedes* mosquitoes is expanded when compared to other model organisms, raising the possibility that individual PIWI proteins have functionally diversified in these insects and may have gained additional functions. We have recently identified Piwi5 and Ago3, but none of the other PIWI family members, as the essential effector proteins for piRNA biogenesis from Sindbis virus RNA in infected *Aedes aegypti* cells. In contrast, we showed that the production of piRNAs from transposons relies on a more versatile set of PIWI proteins, some of which do not contribute to viral piRNA biogenesis. We propose that specialized arms of the mosquito piRNA pathway may recognize and produce piRNAs from endogenous or exogenous parasitic RNAs

71 E6 Mediated Serine Phosphorylation of STAT3 is Critical for the Human Papillomavirus Lifecycle

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Human Papillomavirus (HPV) is the leading etiological cause of cervical cancer. Previous studies have demonstrated the activation of the transcription factor STAT3 by HPV in cervical cancer, but the mechanism of activation and the role of STAT3 during the productive virus lifecycle are currently unknown. Here, we show that, in primary human keratinocytes stably transfected with the HPV 18 genome, phosphorylation of serine 727 (S727) of STAT3 is elevated compared to normal human keratinocytes. Mechanistically, we show that the increase in S727 phosphorylation is due to the HPV E6 oncoprotein and overexpression of E6 in C33A cervical cancer cells led to an increase in the STAT3 responsive genes Cyclin D1, Survivin and Bcl XL. Using a panel of specific small molecule inhibitors we demonstrate that the increase in S727 phosphorylation is mediated by a MAPK pathway in HPV containing primary keratinocytes. Importantly, inhibition of STAT3 phosphorylation either by small molecule inhibitors or expression of a dominant negative STAT3 led to a significant reduction in HPV transcription and protein expression. Additionally, inhibition of STAT3 activation led to a reduction in p63 levels, a key transcription factor involved in keratinocyte proliferation and viral genome amplification. We are currently studying the impact of STAT3 activation on keratinocyte biology and the HPV lifecycle using organotypic raft culture models, which faithfully recapitulate a stratified epithelium. In summary, these data provide evidence for a critical role of STAT3 activation in the HPV lifecycle, which could lead to the identification of novel antivirals targeting HPV.

72 Epithelial Barriers of Murine Skin during Herpes Simplex Virus Type 1 Infection: The Role of Tight Junction Formation

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Keratinocytes of skin and mucosa represent the primary entry portals for herpes simplex virus type 1 (HSV-1) *in vivo*. We aim to understand how HSV-1 overcomes the barrier function of the host epithelia and to characterize the mechanisms underlying HSV-1 invasion into the stratified epithelia allowing the virus to reach its receptors and initiate infection. Our focus is on the impact of mechanical barriers that restrict viral invasion in the absence of immune responses. To analyze the initial infection steps in the epidermis, we established an *ex vivo* infection model using murine epidermal sheets as a tool to facilitate the analysis of cell determinants which confer protection against HSV infection [1]. Based on our observation that HSV-1 fails to establish successful *ex vivo* infection in complete skin samples, we mechanically wounded the tissue prior to infection to remove only the cornified layer from the underlying keratinocyte layers. Upon infection we observed only some infected cells in these lesions. To address the potential role of cellular junctions as further barrier for the HSV-1 infection, we initially performed infection studies in stratified cultures of Par3-deficient keratinocytes, which showed delayed tight junction maturation. Upon infection, the delay of junction maturation correlated with an increased number of infected cells as compared to wt. When E-cadherin, the major transmembrane protein of adherens junctions, is deleted in the epidermis, formation of tight junctions is even disturbed. Accordingly, we observed increased infection efficiency after infection of E-cadherin-deficient stratified cultures. Thus, we conclude that tight junction formation interferes with the successful viral access to cellular receptors in epidermis. Taken together, our results suggest tight junctions in addition to the cornified layer as mechanical barriers for HSV-1 to invade the epidermis.

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73 ER-Targeting Sequence Coding Regions Direct Secretory Protein Regulation by an RNA-Binding Protein

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Viruses generally utilize both the cellular machinery and regulation mechanisms to replicate. Enveloped viruses have the additional challenge of coordinating the expression of their viral secretory proteins. By co-expressing genes from influenza A viruses, we found the RNA-binding protein NS1 increases the production of the secretory glycoproteins HA and NA. The enhancement is mediated by the RNA-binding domain of NS1 and is dependent on the nucleotide composition of the HA and NA endoplasmic reticulum (ER)-targeting sequences. Synonymous ER-targeting sequence mutations that increase the GC-content abolish NS1 regulation, indicating these 5' mRNA regions likely form structural elements, which limit synthesis when NS1 is absent. Together, our results demonstrate that RNA-binding proteins can distinguish between ER-targeting sequence coding regions to regulate specific secretory proteins. Comparing the GC-content profile of human and bacterial targeting sequences to their respective genes revealed several differences suggesting this is a general regulatory mechanism for human secretory proteins.

74 Genetic Diversity of Bovine Herpes Virus Type 1 (BHV-1) Analyzed by Next Generation Sequencing of Unique Long genome Region

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Bovine herpesvirus 1 (BHV-1) is a cattle pathogen which causes significant economic losses in cattle industry. It is also an interesting model in alphaherpes viruses molecular biology research concerning latency, immune suppression and immune evasion. The development of various types of BHV-1 vaccines contributed to the understanding of immune response and protection against herpesvirus infection. Moreover, at present, the virus is being investigated as a potential human oncolytic vector. Therefore, it is important for the further studies of BHV-1 to know and understand precisely the sequence of virus genome. Many different strains of BHV-1, usually classified as belonging to one of the two basic genetic subtypes, 1.1 and 1.2, have been described. Diversity of BHV-1 strains and genomic sequences has been associated with diverse tropism and pathogenesis of the virus which can cause both respiratory and genital infections. However, no clear relation between sequence/function of individual genes and clinical effects of BHV-1 infection has been identified. Very little information about possible molecular evolution and geographic distribution of BHV-1 strains is available.

In present report, we analyzed the sequence of UL region of the genome of Lam, Jura and Schonboeken strains and compared it to the six BHV-1 belonging to either 1.1 or 1.2 subtypes. The genome assembly was made *de novo* using paired reads from a Miseq Illumina sequencer. We performed phylogenetic study that showed the evolutionary relation between all analyzed strains. Based on the molecular clock method, we computed the relative time of divergence from ancestral strain. These results indicate the possible dispersal of analyzed BHV-1 strains in Europe and their linking to American isolates.

75 HIV-1 Envelope Protein Can Overcome IFITM3 Inhibition

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The interferon-induced transmembrane (IFITM) proteins have been shown to inhibit a wide range of viruses including human immunodeficiency virus type 1 (HIV-1). As a membrane-associated protein, IFITM3 exerts its antiviral activity when present either in the virus target cells or when associated with virus particles. The ultimate effect is the impaired virus entry. This antiviral activity of IFITM3 represents an important mechanism in innate immunity against viral infections, since the ifitm3 knockout mice show much higher mortality in the event of influenza virus infection. However, a viral counter measure to overcome IFITM3 restriction has not been well characterized. In this study, we tested a panel of HIV-1 strains for their susceptibility to the inhibition of viral infectivity by IFITM3 and found that the AD8 strain was resistant as opposed to the IFITM3-sensitive strain NL4-3. Further studies showed that replacing the envelope gene of NL4-3 with that of AD8 rendered the chimeric virus resistant to IFITM3, which suggests that the Envelope protein modulates HIV-1 sensitivity to IFITM3. Results of mutagenesis experiments demonstrated that the V3 loop alone can determine the susceptibility of HIV-1 to IFITM3. Therefore, viral envelope proteins may assist viruses to evade the restriction of IFITM3 and likely other host restriction factors.

76 HIV-1-Mediated Endolysosome Translocation during Oxidative Stress via Rag-GTPase: Impact on Host Metabolism

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Mammalian target of rapamycin (mTOR) kinase couples metabolic and stress signals to pathways that mediate cellular growth and proliferation. mTOR maintains the activation of downstream effectors important for host cell metabolism including mRNA translation, cell proliferation and catabolic processes such as autophagy. Amino acid starvation leads to the inactivation of mTOR and induces its perinuclear localization, whereas during oxidative stress, mTOR controls the expression of stress-related factors. mTOR activation requires lysosome recruitment via Rag GTPases, and in earlier work we found that RagA co-precipitated with the HIV-1 RNP. Here, we demonstrate that HIV-1 modulates mTOR localization and activation. Using laser scanning confocal microscopy, we assessed the localization of mTOR in HIV-1-expressing cells. In oxidative stress induced by arsenite (Ars), a subpopulation of mTOR accumulated in the perinuclear region and colocalized with lysosomal-associated membrane protein-1 (LAMP-1) but not with stress granules. In striking contrast, a largely diffuse cytoplasmic localization of mTOR was found in HIV-1-expressing cells. Nevertheless, high levels of phosphorylated S6K-1 and 4E-BP1 were induced to the same extent by Ars-induced oxidative stress in both mock and HIV-1-expressing cells. Strikingly, RagA and RagB GTPase silencing impaired HIV-1's ability to prevent Ars-induced perinuclear clustering of lysosomes and had deleterious effects on viral production and infectivity. Moreover, HIV-1 markedly prevented mTOR/LAMP-1 perinuclear clustering during amino acid starvation but had little impact on mTOR activation levels in the absence of upstream signaling induced by nutrients, as judged by phosphorylation of mTOR and its downstream effectors. Finally, HIV-1 expression did not reverse mTOR inhibition in cells treated with different mTOR inhibitors. These findings demonstrate that HIV-1 modulates mTOR activity and localization by two distinct mechanisms: one that either targets a regulatory event upstream of mTOR and/or another that subverts endosomal membrane trafficking which in turn is dependent on the demonstrated interaction between HIV-1 and Rag-GTPase.

77 Human Genetic Predisposition to Tick-Borne Encephalitis Virus-Induced Disease: Possible Involvement of Polymorphism in Chemokine and Interleukin Genes

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Tick-borne encephalitis (TBE) is caused by single-stranded RNA virus from the *Flavivirus* genus. It is known that the result of virus-host interaction largely depends on the status of the host organism immune response (which is genetically predetermined). However, genetic predisposition to TBE is poorly studied in the human population. In this study, we examined whether four human genes encoding crucial components of antiviral immune response (chemokine ligand 2 (*CCL2*), interferon-γ-inducible protein 10 (*CXCL10*), interleukin 28B (*IL28B*), and interleukin 10 (*IL10*)) are involved in protective mechanisms against TBE virus. We determined genotypic and allelic frequencies for several single nucleotide polymorphisms (SNPs) in these genes and compared them in 132 non-immunized TBE patients (34 with fever, 60 with meningitis, 38 with severe forms) and in the control Russian population (221 Novosibirsk citizens). We found that the frequency of A/A homozygotes for the *IL28B* gene rs12980275 (A/G) SNP (located in 3'-flanking region) is significantly increased in TBE patients (60.6%) (especially in those with severe disease (71.4%)) as compared with the control group (47.5%) (P = 0.018 and 0.009). An increase in the A allele frequency for this SNP was also detected in TBE patients (77.2%) (and in patients with severe disease (84.3%)) as compared with the controls (65.8%) (P = 0.002 and 0.002). For the *IL10* gene rs1800872 (C/A) SNP (located in the promoter region), an increase in A/A homozygote frequency for patients with severe disease (10.5%) as compared with the control group (2.0%) was also found (P = 0.007). Our data suggest that the *IL28B* gene rs12980275 and *IL10* gene rs1800872 SNPs are associated with predisposition to TBE in Russian population.

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78 Identification and Characterization of a New Type of Inhibitor against the Human Immunodeficiency Virus Type-1 Nucleocapsid Protein

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The human immunodeficiency virus type-1 (HIV-1) nucleocapsid protein (NC) is an essential and multifunctional protein involved in multiple stages of the viral life cycle such as reverse transcription, integration of proviral DNA, and especially genome RNA packaging. For this reason, it has been considered as an attractive target for the development of new anti-HIV drugs. Although a number of inhibitors of NC have been reported thus far, the search for NC-specific and functional inhibitors with a good antiviral activity continues.

In this study, we report the identification of A1752, a small molecule with inhibitory action against HIV-1 NC, which shows a strong antiviral efficacy and an IC_{50} around 1 μM. A1752 binds directly to HIV-1 NC, thereby inhibiting specific chaperone functions of NC including Psi RNA dimerization and complementary trans-activation response element (cTAR) DNA destabilization, and it also disrupts the proper Gag processing. Further analysis of the mechanisms of action of A1752 also showed that it generates noninfectious viral particles with defects in uncoating and reverse transcription in the infected cells. These results demonstrate that A1752 is a specific and functional inhibitor of NC with a novel mode of action and good antiviral efficacy. Thus, this agent provides a new type of anti-HIV NC inhibitor candidate for further drug development.

79 Influence of Temperature Factor on *Peanut Stunt Virus* Pathogenesis

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Peanut stunt virus (PSV) belongs to the *Cucumovirus* genus in the family *Bromoviridae* and is transmitted by aphids. It is a serious pathogen of legumes, distributed worldwide. Temperature is one of the major factors shaping the plant-virus interactions, pathogenesis progress, and virus spread.

In this study, we analyzed influence of the temperature on PSV pathogenesis rate in *Nicotiana benthamian* in two temperature conditions supporting normal growth of plants.

The measurements of virus accumulation using RT-qPCR showed that the higher temperature causes rapid increase of viral RNAs levels in infected plants while at lower temperature this increase is slower. Proteomic analyzes done using 2D-DIGE/mass spectrometry approach revealed decrease in the amount of proteins involved in photosynthesis and carbohydrate metabolism in infected plants grown at higher temperature contrary to the proteins involved in plant reaction to stress which were largely more abundant.

80 Influenza a Virus Inhibits Type I Interferon-Mediated Signaling Independently of Its Block of General Host Gene Expression

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Type I interferons (IFNs) act as the first line of defense against viral infections. Upon secretion and receptor binding, they initiate a signaling cascade that eventually leads to the establishment of an antiviral state owing to the production of IFN-induced effector proteins. The type I IFN signaling cascade involves phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2. Phosphorylated STATs heterodimerize and translocate to the nucleus, where they activate transcription of IFN-responsive genes.

Previous studies have shown that influenza A virus (IAV) infection causes disruption of IFN-mediated signaling. Using a luciferase-based reporter assay, we found that overexpression of IAV non-structural protein 1 (NS1) causes a dramatic reduction of IFN-induced gene expression. However, STAT1 phosphorylation was not affected. Instead, immunofluorescence data demonstrated that NS1 interferes with IFN-induced nuclear translocation of STAT proteins.

We then assessed whether NS1 proteins from multiple IAV strains differ in their ability to suppress the signaling events in response to IFN. Intriguingly, most human and avian NS1 proteins that failed to inhibit general host gene expression remained effective IFN signaling antagonists. In addition, mutation of NS1 residues essential for interaction with CPSF30 and subsequent block in host mRNA maturation restored general gene expression, while still interfering with IFN-mediated signaling. Conversely, disruption of a conserved putative protein-protein interaction motif partially restored IFN signaling reporter activity.

Taken together, we suggest that IAVs have evolved multiple strategies to inhibit IFN-mediated signaling, relying on both general and specific suppression of host gene expression.

81 Influenza Virus Multivalency: From Single Ligand-Receptor Interactions to Full Virus Adhesion

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The dynamics of the polyvalent attachment of the influenza virus with its molecular receptor have been investigated by means of force spectroscopy using a force robot. A single molecule approach was used where a synthetized PEG ligand presenting the cellular receptor group (sialic acid) was probed against the surface of the virus to induce bond formation and rupture. This method revealed the tensile strength required to break a single bond between sialic acid and Hemagglutinin, the major virus protein. An increase in the contact time that the tip expended on the surface of the virus yielded a marked rise in the number of unbinding events observed in the force curves. The characteristic features of multiple bond rupture were recorded and the number of bonds formed between both surfaces could be estimated. This sequential unbinding of multiple receptors presented typical characteristics of a molecular Velcro, where bonds in parallel reinforce the adhesion force between both surfaces and tightens during rapid separation. Our results strongly suggest that binding dynamics of the virus is not only influenced by the multivalent nature of its surface but also by the length and flexibility of the spacer presenting the receptor. In an inverted experimental setup, with the virus bound to the tip and the receptors coating a flat surface, the adsorption and posterior detachment of whole virus particles was characterized. It was observed that although the shape of the force curves changed, the single molecule behavior could still be observed. These results will contribute to understand the mechanistic aspects of virus binding to spherical inhibitors, previously investigated in our group and are expected to provide some insights for the future design of more effective multivalent architectures.

82 Inhibition of Type I Interferon Induction by Sandfly Fever Sicilian Virus Virulence Factor NSs

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Phleboviruses are a group of emerging viruses with a wide spectrum of virulence: For example, Rift Valley Fever virus (RVFV) is highly pathogenic, whereas Sandfly fever Sicilian virus (SFSV) causes intermediate pathogenicity, and Uukuniemi virus (UUKV) appears to be apathogenic.

The major virulence factor of phleboviruses is the non-structural protein NSs, an inhibitor of the type I interferon (IFN) system. The extensively studied NSs protein of highly pathogenic RVFV has been reported to inhibit type I IFN induction at the level of host cell transcription, both specifically by recruitment of a SAP30-containing repressor complex and globally by sequestration and degradation of the TFIIH subunits p44 and p62, respectively. In contrast, the mode of action of the NSs protein of intermediately pathogenic SFSV has remained elusive.

We therefore aimed to characterize the IFN-inhibitory capacity of and the mechanism employed by the NSs of SFSV.

Previously, our group has identified multiple candidate host interactors for RVFV and SFSV by tandem affinity purification and mass spectrometry together with collaboration partners [1]. Intriguingly, these candidates also included interferon regulatory factor 3 (IRF3). We confirmed the protein-protein interaction between SFSV NSs and IRF3, while we found that other IRF family members were not targeted by SFSV NSs. Furthermore, SFSV NSs alone was sufficient to abrogate IRF3 activation and IFN-β promoter activity.

In summary, we were able to show that Sandfly fever Sicilian virus (SFSV) also encodes a NSs protein that efficiently inhibits the induction of type I interferon. Different to the highly pathogenic RVFV, which induces a general host transcription shutoff, however, SFSV specifically targets IFN promoter activation.

Thus, although these phleboviruses are highly related, their NSs proteins display remarkably diverse strategies of counteracting the type I interferon system. Hence, we hypothesize that the quality of NSs-host factor interactions correlates with the virulence levels of phleboviruses.

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83 Investigating Group A Rotaviruses in Farm Animals in Ghana

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Group A rotaviruses are the most common cause of acute gastroenteritis among infants and children younger than five years of age, and accounts for about half a million death per annum. The viruses are also a common cause of infectious diarrhoea in young animals. The disease burden of rotaviruses is worse in developing countries than developed ones, which has primarily been attributed to poor sanitation practices. Even though rotaviruses have been shown to be specie-specific, various studies suggest that stains that used to be in animals are now being detected in humans. Two rotavirus vaccines that have been licensed and recommended for use worldwide by the WHO are less effective in developing countries compared to developed countries. In Ghana, and many other developing countries, there is close interaction between humans and domestic and/or farm animals, or with the feces of these animals. There is therefore the possibility of the same rotavirus strains inhabiting humans and animals alike. This study therefore seeks to determine reservoirs of group A rotavirus strains in farm animals (pig and cattle) and to investigate the molecular diversity of the virus strains. Animal farms located in Accra, Ghana, were identified and stool samples were collected from young animals from pig and cattle farms. The samples were tested for Group A rotaviruses using the Proflow™ Rotavirus-Adenovirus enzyme immunoassays (EIA). Double-stranded RNA (dsRNA) was extracted from the EIA positive samples, and the extracted RNA analyzed by PAGE. Subsequently, the positive samples were subjected to quantitative and conventional semi-nested multiplex polymerase chain reaction (PCR) and sequencing. Ten out of the total number of samples tested (~10%) were EIA positive for rotavirus; all of which were from pigs. One of the samples tested positive for adenovirus in addition to rotavirus (co-infection). Only 20% of the EIA positive samples could be amplified by PCR. The amplified genes were sequenced and identified as *G5P* [13]. This study demonstrates for the first time the detection and characterization of rotavirus in farm animals in Ghana.

84 Itraconazole Inhibits Enterovirus Replication by Targeting the Oxysterol Binding Protein (OSBP)

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Enteroviruses (e.g. poliovirus, coxsackievirus, enterovirus-71, and rhinovirus) are a large genus in the picornavirus family of (+)RNA viruses and pose a serious health threat for which currently no antiviral therapy is available. Enteroviruses hijack host proteins and lipids to build so-called replication organelles (ROs), on which viral genome replication takes place. RO formation is critical to the replication of enteroviruses, but the underlying molecular mechanisms are poorly understood. Recently, phosphatidylinositol-4-phosphate (PI4P) and cholesterol were the first lipids shown to be critical for enterovirus replication. To identify novel host factors involved in enterovirus replication, we screened a drug library for novel inhibitors of enteroviruses. We identified itraconazole (ITZ), an antifungal drug that also has anticancer activity, as a broad-spectrum inhibitor of enterovirus genome replication. We demonstrate that ITZ inhibits viral RNA replication through the novel target oxysterol-binding protein (OSBP), a protein that is recruited to Golgi membranes through PI4P and that shuttles cholesterol and PI4P between ER and Golgi membranes. The importance of OSBP for enterovirus replication is underscored by the findings that knockdown of OSBP inhibited virus replication and that OSW-1, a specific OSBP antagonist, also inhibited enterovirus replication. Overexpression of OSBP counteracted the antiviral effects of ITZ and OSW-1, confirming that these compounds inhibit replication by targeting OSBP. We show that ITZ binds OSBP and inhibits its lipid shuttling function *in vitro*. In infected cells, OSBP was recruited to ROs depending on PI4P lipids, similar to its Golgi recruitment in uninfected cells. Importantly, ITZ affected PI4P and cholesterol levels at ROs, indicating that ITZ also inhibited OSBP-mediated lipid shuttling in infected cells. Together, we identified OSBP as a novel molecular target of ITZ and point to an essential role of OSBP-mediated lipid exchange in virus replication that can be targeted by antiviral drugs.

85 Junin Virus Blocks Pkr-Mediated Phosphorylation of Eif2alpha

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Arenaviruses are important human pathogens for which FDA-approved vaccines and effective antiviraltherapeutics do not exist. We comprehensively mapped the arenavirus nucleoprotein-human protein interactome as ameans to uncover critical host-pathogen interactions that can be therapeutically targeted. Bioinformatic analysis ofinteracting host protein partners revealed that host translation appears to be a key biological process engaged by thearenaviruses during infection. In particular, dsRNA activated protein kinase (PKR), a well characterized inhibitor oftranslation initiation via phosphorylation of eIF2α in response to virus infection, was identified in the screen. Infection withthe New World Junin virus Candid 1 (JUNV C#1) leads to increased expression of PKR as well as its redistribution to viralreplication factories. Functional characterization of the role of PKR in Junin virus infection revealed that PKR becomesphosphorylated following infection, yet it is unable to phosphorylate eIF2α, even in response to high doses of thesynthetic dsRNA poly(I:C). This blockade of PKR function is highly specific as the Old World lymphocytic choriomeningitisvirus (LCMV) was unable to inhibit eIF2α phosphorylation. Interestingly, siRNA silencing of PKR, a protein with welldocumented antiviral activity, resulted in a modest inhibition in the production of infectious virus particles compared tocells expressing normal levels of PKR. Not only does this indicate that JUNV C#1 is able to potently inhibit the antiviralactivity of PKR, it suggests that the hijacking of this kinase may be beneficial to the viral life cycle.

86 KSHV Modulates the IRE1-XBP1 Axis of the Unfolded Protein Response during Lytic Replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the infectious cause of the endothelial cancer Kaposi's sarcoma, and two rare B-cell lymphoproliferative disorders. KSHV hijacks multiple cellular stress responses during infection, which is thought to aid tumor initiation, but the precise mechanistic details are unclear. One cellular stress response linked to KSHV infection is the unfolded protein response (UPR), which is activated in response to endoplasmic reticulum (ER) stress and acts to restore homeostasis. One of the sentinels of ER stress is the endoribonuclease inositol-requiring enzyme (IRE) 1, which upon activation splices out a 26-nucleotide region of xbp1 (X-box binding protein 1) mRNA, shifting the reading frame to translate the active transcription factor XBP1s. In addition to transactivating UPR genes, XBP1s also drives the expression of the KSHV latent-lytic switch gene, K-RTA, which results in reactivation from latency and initiation of lytic replication. Thus, it appears that KSHV has evolved a mechanism to respond to ER stress. Here, we demonstrate that K-RTA-mediated lytic replication in two different KSHV model cell lines, BCBL-1 and iSLK.219, can induce xbp1 splicing. However, despite splicing, XBP1s protein failed to accumulate and cellular XBP1s-target genes were not upregulated. Moreover, ectopic expression of XBP1s inhibited the release of infectious viral progeny. Therefore, while XBP1s plays an important role in reactivation from latency, it inhibits later steps in lytic viral replication. Our findings suggest XBP1 may be downregulated by products of the lytic viral gene expression program to mitigate its deleterious effects and permit efficient viral replication.

87 Mappig Translational and Transcriptional Changes during Influenza Virus Infection

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Influenza virus is a major human pathogen, annually affecting millions of people worldwide. During influenza infection there is a dramatic shutoff of cellular protein synthesis which is mediated by different mechanisms; cleavage of capped RNA from cellular pre-mRNAs (cap-snatching), degradation of the cellular mRNA and inhibition of cellular mRNA splicing and export. Interestingly, it was proposed that there is a preferential selective translation of viral proteins on the expense of host mRNA, but the exact mechanism/s and the extent for which different host mRNAs are affected from influenza infection was never globally studied.Our goal was to systematically elucidate translational and transcriptional changes that occur during influenza infection both in the virus and the host. To this end, we infected A549 cells with influenza PR8 for several time points and generated libraries of ribosome-protected mRNA fragments (footprints). In parallel we preform RNA-Seq measurements that allow the quantification of total RNA levels. By measuring both mRNA levels and footprints distribution for every gene along infection we were able to characterize the translation and transcriptional changes and their relative contribution along infection.

Overall our data show that during infection viral genes are not translated more efficiently than their host counterparts and that the main mechanism of host shut-off is mediated by host mRNA degradation. Although most of the regulation we observed was due to changes in mRNA expression, by calculating the translational efficiency (TE) for the expressed host genes we identified a dynamic translational regulation for a subset of host genes. By clustering this subset of genes based on their TE values, we revealed few distinct temporal profiles. One upregulated cluster was enriched in genes resistant to eIF2a phosphorylation (a central cellular response to stress that reduces translation). Looking at the effect of eIF2a phosphorylation we found a distinct kinetic pattern along infection. Surprisingly, we show significantly reduced viral titers in cells that express a constitutively active, nonphosphorylatable eIF2alpha. Taken together, our results imply to a model in which the translational state of the host cell at the time of infection influences viral propagation.

88 Modeling Varicella Zoster Virus Latency and Reactivation Using Human Embryonic stem Cell-Derived Neurons

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Most adults worldwide harbor latent varicella zoster virus (VZV) in their ganglia and reactivation can cause herpes zoster. VZV latency in sensory and autonomic neurons has remained enigmatic and difficult to study, and experimental reactivation has not yet been achieved. We have previously shown that human embryonic stem cell (hESC)-derived neurons are permissive to productive and spreading VZV infections. We now demonstrate that hESC-derived neurons can also host a persistent non-productive infection lasting for weeks which can subsequently be reactivated by multiple experimental stimuli. Quiescent infections were established by exposing neurons to low titer cell-free VZV either by using acyclovir or by infection of axons in compartmented microfluidic chambers without acyclovir. VZV DNA and low levels of viral transcription were detectable by qPCR for up to 7 weeks. Approximately 5% of neuronal nuclei harbored VZV genomes by fluorscent *in situ* hybridization (FISH).

Quiescently-infected human neuronal cultures were experimentally induced to undergo renewed viral gene and protein expression by growth factor removal or by inhibition of PI3Kinase activity. Strikingly, incubation of cultures induced to reactivate at a lower temperature (34^{o}C) resulted in enhanced VZV reactivation resulting in spreading, productive infections. Reactivated neurons could be transfered to new cultures and infect surrounding cells. Comparison of VZV genome transcription in quiescently infected to productively infected neurons using RNASeq revealed preferential transcription from specific genome regions, especially the duplicated regions. These experiments establish a powerful new system for modeling the VZV latent state, and reveal a potential role for temperature in VZV reactivation and disease.

89 Molecular Basis for Specific viral RNA Recognition and 2'-O Ribose Methylation by the Dengue Virus NS5 Protein

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Dengue virus (DENV) causes several hundred million human infections and more than 20,000 deaths annually. Neither an efficacious vaccine conferring immunity against all four circulating serotypes nor specific drugs are currently available to treat this emerging global disease. Capping of the DENV RNA genome is an essential structural modification that protects the RNA from degradation by 5' exo-ribonucleases, ensures efficient expression of viral proteins and allows escape from the host innate immune response. The large flavivirus NS5 protein (105 kDa) has RNA methyl-transferase activities at its N terminal region, which is responsible for capping the virus RNA genome. The methyl transfer reactions are thought to occur sequentially using the strictly conserved flavivirus 5' RNA sequence as substrate (GpppAG-RNA), leading to the formation of the 5' RNA cap: G0pppAGRNA→ m7G0pppAG-RNA (named "cap-0") →m7G0pppAm2'-OG-RNA (named "cap-1"). To elucidate how viral RNA is specifically recognized and methylated, we determined the crystal structure of a ternary complex between the full-length NS5 protein from dengue virus, an octameric cap-0 viral RNA substrate bearing the authentic DENV genomic sequence (5'-m7G0pppA1G2U3U4G5U6U7-3') and S-adenosyl-L homocysteine (SAH), the by-product of the methylation reaction. The structure provides for the first time a molecular basis for specific adenosine 2'-O methylation, rationalizes mutagenesis studies targeting the K61-D146-K180-E216 enzymatic tetrad as well as residues lining the RNA binding groove and offers novel mechanistic and evolutionary insights into cap-1 formation by NS5, which underlies innate immunity evasion by flaviviruses.

90 Mutational Analysis of Conserved Motifs of the Rift Valley Fever Virus Nucleoprotein Indicates Novel Functional Residues

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Rift Valley fever virus (RVFV, *Phlebovirus, Bunyaviridae*) is an important pathogen of both humans and livestock. RVFV transmission by mosquitoes across sub-Saharan Africa and the Arabian Peninsula has a significant impact on the socio-economics of these areas. RVFV nucleocapsid (N) protein has a number of identified functions, the most important of which are the ability to encapsidate viral RNA and to form higher-order multimeric structures. These functions are crucial for successful virus replication. Alignment of phlebovirus N sequences revealed conserved residues with as yet undetermined function, some of which are surface-exposed based on the crystal structure. These residues were selected for mutational analysis and the ability of the mutants to multimerise and promote RVFV replication in a minigenome assay was assessed. We found that all the N mutants generated were non-functional in minigenome assays, but still multimerised. The nature of the mutations being surface exposed suggests that they will not be involved in RNA binding. This indicates that the nucleocapsid may have currently unknown protein-protein interactions with other viral or host proteins, which are essential for successful virus replication in mammalian host cells. Immunoprecipitation assays and proteomics approaches are being developed to identify these novel N protein interactions, which will aid in the future development of new intervention strategies.

91 Mutations Reducing Cytotoxicty of Chikununya Virus Affect Viral RNA Replication and Antiviral Interferon Response

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Chikungunya virus (CHIKV, family *Togaviridae*) is a mosquito-transmitted Old World alphavirus. It has positive-stranded RNA genome about 11.8 kb that encodes for four non-structural proteins (nsP1–4) and five structural proteins. The ns-proteins are translated from genomic RNA and are the essential components of the viral replicase complex. CHIKV nsP2 is a multifunctional protein: proteolytically processes the viral ns-polyprotein; possesses NTPase, RNA triphosphatase, and RNA helicase activities and counteracts interferon (IFN) signaling. In vertebrate cells it induces cytopathic effects such as shutdown of transcription and translation. By these reasons mutations in the nsP2 region are involved in establishing a persistent infection and are shown to prolong survival of the host cell.

We introduced a P718G mutation (PG) in CHIKV nsP2 and selected for additional mutations that together with PG resulted in a CHIKV replicon with a non-cytotoxic phenotype. We found that all known enzymatic activities of CHIKV nsP2, as well as its RNA-binding capability, were compromised by these mutations leading to a reduced capacity for RNA replication. Combination of different cytotoxicity reducing mutations resulted in more pronounced effects. In infected cells CHIKV, harboring such mutations, caused early and prominent production of IFNβ indicating that mutant viruses were unable to suppress this antiviral response.

Selected mutations were also analyzed using uncoupled replicase expression/RNA replicaton assay. This method allows to analyze effect of the mutations on the ability of viral replicase to perform RNA synthesis. In this system the mutations caused only moderate reduction of viral replicase activity and failed to induce excessive IFN response. Most likely it indicates that in the context of virus infection IFN response leads to suppression of RNA replication. Our results give us new insights into interaction of alphavirus and innate immune response pathways.

92 Phosphoproteomic Exploration of IAV Infection Reveals NUP98 as a Crucial Host-Factor for Viral Replication

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Coordinated early activation of particular signalingsignaling pathways and proteins has been shown to be crucial for influenza A virus (IAV) infection. Thus, the characterization of virus-induced modifications in the phosphorylation of cellular proteins could lead to the identification of promising targets for antivirals development. In order to obtain a comprehensive view of the signalingsignaling events and the changes in host-cell phosphorylation upon IAV entry, we have conducted a proteome-wide SILAC-based quantitative phosphoproteomic screen of A549 cells at 5 and 15 minutes after infection with IAV. We have quantified around 3000 phosphorylation sites from 1300 different proteins and identified infection-induced changes in the phosphorylation of an important subset. Bioinformatic analysis has revealed that both ErbB and MAPK signalingsignaling pathways are activated as early as 5 min after the initial contact of the virus with the cells and that this early activation is accompanied by significant changes in the phosphorylation of key proteins of the cytoskeleton and the vesicle-mediated transport. Surprisingly, the phosphorylation of different components of the nuclear import machinery is significantly altered as well at such a short time post-infection. Among them, we have focused on the characterization of the role of the 98 KDa nucleoporin (NUP98) in viral replication. We are currently investigating its contribution to the trafficking of vRNPs from the cytoplasm to their replication sites in the nucleus and how the activity of this protein is regulated by phosphorylation during IAV infection.

93 Prediction of Conserved Long-Range RNA-RNA Interactions in Full Viral Genomes

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Long-range RNA-RNA interactions (LRIs) play an important role in viral replication. Only a few of these interactions are known in a limited number of viral species. Up to now, it has been impossible to screen a full viral genome for LRIs experimentally or *in silico*. Most bioinformatical tools are unable to predict pseudoknots, but known LRIs are often part of cross-reacting structures. We present LRIscan, an easy to use tool for the prediction of long-range interactions in full viral genomes based on a multiple genome alignment. LRIscan is able to find interactions spanning thousands of nucleotides, but can also determine interactions in local sequences. We applied our tool to a full genome alignment of 106 Hepatitis C viruses (HCV) and an alignment of 12 Tombusviruses. We were able to find all previously known LRIs of HCV and 6 out of 8 known LRIs in Tombusviruses, whereas the two missing LRIs are not conserved across Tombusvirus species. Strikingly, we identified a conserved interaction between the apical loops of SLII and DLS of Hepatitis C viruses. This possible initial interaction can be extended to include 62 interacting base pairs to build a potential genome circularization between the 5'UTR and 3'UTR. With LRIscan, we provide the user for the first time the opportunity to decrease the huge amount of theoretically possible interactions and to find LRIs in specific regions of interest. LRIscan is therefore a useful tool to help virologists to decrease the amount of costly wet lab experiments. Wet lab experiments, which subsequently verify the functionality of these newly identified interactions, could improve our understanding of the viral replication mechanism.

94 Remodeling of Intracellular Membranes in Bunyamwera Virus-Infected Mosquito Cells

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Viral infections frequently induce pronounced structural remodeling in target cells. Viruses that replicate in the cytoplasm modify and use cell organelles for replication of the viral genome and particle assembly, through viral protein interaction with cell membranes. Arboviruses are prominent pathogens in humans that are transmitted by arthropod vectors. Bunyamwera virus (BUNV), a well-characterized member of the arbovirus group, is an enveloped negative single-stranded (ss)RNA virus that serves as a model to study cell membrane remodeling events. Here we used rBUNV-eGFP(Gc), a recombinant virus with enhanced green fluorescent protein (eGFP) fused to viral glycoprotein C (Gc), to study how the *Aedes albopictus* mosquito-derived C6/36 cell line responds throughout *in vivo* Bunyamwera virus infection. Live cell videomicroscopy of rBUNV-eGFP(Gc)-infected cells, followed by correlative light and electron microscopy, showed marked remodeling of intracellular membranes (convoluted membranes) and indicated a very close connection between large vacuoles and viral inclusions. Videomicroscopy analyses during the acute stage of infection showed that infected cells transmitted Gc signal pulses through the filopodial network to neighboring cells. Further studies will be performed by immunogold labeling in Tokuyasu cryosections of rBUNV-eGFP(Gc)-infected mosquito cells to determine how Bunyamwera virus proteins interact with intracellular membranes. These data will help to clarify the strategies that viruses use to take advantage of cell membranes and organelles for viral morphogenesis and to spread their progeny in the host.

95 Role of SAMHD1 SUMOylation in its Antiviral and Enzymatic Activities

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Background: SAMHD1 is a dNTPase and RNAse involved in a genetic auto-immune disorder called Aicardi-Goutières Syndrome which is characterized by improper immune activation resulting from the accumulation of intracellular DNA.Recently, SAMHD1 has been reported to block HIV-1 infection in non-dividing cells such as quiescent T lymphocytes and myeloid cells (monocytes/macrophages). In contrast, the closely related HIV-2 or SIV viruses readily replicate in these cells. In fact, HIV-2 and SIV, but not HIV-1, express Vpx, an accessory protein that interacts with SAMHD1 and simultaneously recruits the CUL4-DCAF1-DBD1 Ubiquitin E3 ligase complex. This E3 ligase complex poly-ubiquitinates SAMHD1 that is ultimately degraded by the proteasome.The ability of SAMHD1 to restrict HIV-1 infection is inhibited by phosphorylation of Thr592. *In vivo*, this modification also impairs the RNase, but not the dNTPase activity. Interestingly, *in vitro*, mutation of Thr592 is not sufficient to block the RNase activity, suggesting other factors may be involved.

Observations: We found that SAMHD1 is modified by SUMO proteins by immunoprecipitation assays. Accumulation of SUMO-modified forms is enhanced upon proteasome inhibition. By site-directed mutagenesis we validated the SUMOylation sites identified by mass spectrometry studies. Proximity ligation assay revealed that the interaction between SAMHD1 and SUMO proteins occurs in the nucleus. Next, we generated cells stably expressing WT or SUMO-site mutant SAMHD1 and tested their susceptibility to HIV-1 or HIV-2/SIV.

Conclusions: We found that SAMHD1 is SUMOylated and modification likely occurs in the nucleus. We are currently addressing the phenotype of SUMO-site SAMHD1 mutants to establish if this modification is implicated

96 Severe Acute Respiratory Syndrome Coronavirus 3a Protein Activates NLRP3 Inflammasomes by Promoting ASC Ubiquitination

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Severe acute respiratory syndrome (SARS) coronavirus and Middle East respiratory syndrome (MERS) coronavirus cause highly lethal respiratory diseases associated with the induction of a storm of pro-inflammatory cytokines. Exactly how SARS-CoV induces these cytokines is not understood. In light of the activation of NLRP3 inflammasomes by influenza A virus M2 protein, we asked whether SARS coronaviruses might also encode inflammasome-activating proteins. In this study, we show that SARS coronavirus 3a protein activates NLRP3 inflammasomes through a new mechanism mediated by ASC ubiquitination. Activation of NLRP3 inflammasomes requires two signals that trigger NF-κB activation and interleukin 1β cleavage. 3a protein is capable of activating both signals. On one hand, it promotes proteolytic activation of p105 to generate p50. The inhibition of this activity of 3a protein by deubiquitinases DUBA and A20 suggested the involvement of TRAF3 and TRAF6. On the other hand, interleukin-1β (IL1β) secretion was elevated when 3a protein was expressed in the HEK293 cells reconstituted for the NLRP3 inflammasome system. Comparison of 3a mutants indicated that TRAF-binding but neither caveolin-binding nor ion channel activity is required for its inflammasome-activating property. The interaction of 3a protein with ASC, TRAF3 and TRAF6 was confirmed. 3a, TRAF3 and ASC were found to colocalize to discrete punctate structures in the cytoplasm. TRAF3-dependent ASC ubiquitination was more pronounced in the presence of 3a. Taken together, SARS-CoV 3a protein is a potent activator of NF-κB and NLRP3 inflammasomes. Our study not only derives novel mechanistic insight on the induction of pro-inflammatory cytokines by SARS-CoV, but also provides new strategies for developing specific antivirals and anti-inflammatory agents to combat SARS and MERS. Supported by HMRF (13121032, 14130822 and HKM-15-M01) and RGC (HKU1/CRF/11G, N-HKU712/12 and T11-707/15-R).

97 Shining a Light on Sequential Events of Influenza a Virus Entry

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Influenza viruses hijack the host cellular machinery to mediate successful viral propagation. Dissecting the not well-understood complex virus-host interactome offers promising possibilities to develop anti-viral drugs. Based on the results of several high-throughput screens, certain host-factors have been selected for detailed follow-up studies. Three small-molecule inhibitors targeting identified host cell kinases have been shown to interfere with viral replication of different influenza subtypes, thus being potential anti-viral drugs. Taking advantage of confocal laser scanning microscopy and quantitative single cell analysis, their anti-viral effect was traced to early infection steps. Binding was quantified immediately after infection by measuring the fluorescence intensity of directly labeled viruses on the cell surface. In addition, internalization of labeled viruses was assessed by subsequent indirect immunofluorescence (IIF) staining of Influenza Hemagglutinin in non-permeabilized cells. Thus, internalized viruses, which show the single label fluorescence, are distinguished from bound viruses showing two co-localizing signals. Moreover, viral fusion was detected by labeling viruses simultaneously with two fluorescence lipophilic dyes generating a FRET pair. Before fusion only the photon emission of the FRET acceptor is observed, whereas after fusion FRET donor and acceptor are spatially distributed, leading to detectable donor fluorescence. The next step of infection, the viral uncoating, was analyzed by IIF staining of Influenza Matrix protein 1 (M1). Using automated machine learning software, scoring of the characteristic M1 dispersal phenotype was performed. Finally, import of the viral genome into the nucleus was detected by IIF labeling of the Influenza nuclear protein (NP). Through automated cell segmentation, fluorescence signals of NP in the cytoplasm and nucleus were separately analyzed in order to determine effects on the genome import.

98 Spatio-Temporal Dynamics of Yellow Fever Virus Infection *in Vivo*

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Arthropod-borne flaviviruses cause major health and economic concerns worldwide. Although vaccines or treatments currently do not exist for many flavivirus infections, the yellow fever virus (YFV) live-attenuated vaccine strain, termed YFV-17D, is one of the most efficient vaccines ever developed. However, the cellular and molecular factors governing YFV-17D attenuation in humans are poorly described. Here, we take advantage for the first time of an immunocompromised mouse model engrafted with components of a human immune system (NRG-HIS mice) to characterize the dynamics and host determinants of YFV-17D infection *in vivo*. YFV-17D RNA was detectable in the serum of infected NRG-HIS mice, reaching a peak at 11 days post infection, while no increase in viremia could be observed in non-engrafted NRG mice or in immunocompetent mice. Of the multiple organs examined in NRG-HIS mice, viral replication was observed only in the spleen—an organ highly enriched in human immune cells in NRG-HIS mice—thus providing evidence for specific interactions between YFV-17D and components of the human immune system (HIS). Through RT-qPCR and RNA-sequencing, we were also able to observe the up-regulation of several human interferon stimulated genes in human peripheral blood mononuclear cells (PBMCs) of NRG-HIS mice infected with YFV-17D. Comparison with previously published transcriptomic data from the PBMCs of human vaccines highlighted similarities between the two models. To gain new insights on the interaction between YFV-17D and the HIS, we have taken advantage of a novel technology, Prime RNA flow, to characterize the spatio-temporal dynamics of YFV-17D RNA within the HIS. We observed that over the course of infection, YFV-17D positive-sense RNA was predominantly associated with human rather than murine PBMCs. Consistent with our previous findings, this observation was more prominent in the spleen of these animals. Moreover, YFV-17D RNA preferentially localized with specific cellular subsets of the HIS, which thus may have a role in the mechanisms of viral attenuation. In simultaneously detecting the positive- and negative-sense viral RNA strands of YFV-17D, we also characterized the dynamics of YFV-17D replication within the HIS over the course of infection. We are currently performing an exhaustive single-cell transcriptomic profiling of particular human immune cells carrying YFV-17D RNA in order to identify previously unknown immune signatures regulating flavivirus attenuation. Altogether, humanized mice represent a unique platform to uncover the molecular mechanisms governing viral pathogenesis and immunogenicity in humans.

99 SQSTM1/p62 Regulates HTLV-1 Tax-Mediated NF-κB Activation

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Human T-Leukemia Virus-1(HTLV-1)-mediated cellular T-cell transformation relies on the activation of the NF-κB pathway by Tax, the HTLV-1 transactivator protein. NF-κB activation by Tax requires Tax poly-ubiquitination and interaction with cellular factors, such as Optineurin (OPTN) and Tax1-binding protein 1 (TAX1BP1). The recent identification of both OPTN and TAX1BP1 as selective autophagy receptors sharing high sequence similarities with sequestosome-1 (SQSTM-1/p62), a well-described selective autophagy receptor and an NF-κB signaling adaptor, led us to hypothesize that Tax could hijack selective autophagy receptors for an efficient NF-κB activation. Using immunoprecipitation and confocal imaging of endogenous SQSTM/p62 in Tax-expressing cells or in HTLV-1 chronically infected T-cell lines, we show that Tax interacts with SQSTM-1/p62. Interestingly, this interaction is independent of Tax ubiquitination, which is unexpected regarding the classical model of selective autophagy. Tax-mediated activation of NF-κB in p62-deficient cells was significantly reduced compared to wild type cells, indicating that SQSTM/p62 potentiates Tax activity. Using atg7 silencing, we show that autophagy-related pathways are indeed required for an effective NF-κB activation by Tax. Surprisingly however, over-expression of SQSTM-1/p62 leads to a dramatic reduction of NF-κB induction by Tax, along with a decrease in the amount of soluble Tax caused by both degradation and vesicular sequestration of Tax. Altogether, our results reveal the double-edged consequences of Tax / SQSTM/p62 interaction, and highlight the complex relationships that this viral protein establishes with cellular pathways.

100 Structural Basis of Membrane Budding by the Nuclear Egress Complex of Herpesviruses

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During nuclear egress, herpesvirus capsids bud into the inner nuclear membrane forming perinuclear viral particles that subsequently fuse with the outer nuclear membrane, releasing capsids into the cytoplasm. This unusual budding process is mediated by the nuclear egress complex (NEC) composed of two conserved viral proteins, UL31 and UL34. Earlier, we discovered that the herpesvirus nuclear egress complex (NEC) could bud synthetic membranes *in vitro* without the help of other proteins by forming a coat-like hexagonal scaffold inside the budding membrane. To understand the structural basis of NEC-mediated membrane budding, we determined the crystal structures of the NEC from two herpesviruses. The hexagonal lattice observed in the NEC crystals recapitulates the honeycomb coats within the budded vesicles. Perturbation of the oligomeric interfaces through mutagenesis blocks budding *in vitro* confirming that NEC oligomerization into a honeycomb lattice drives budding. The structure represents the first atomic-level view of an oligomeric array formed by a membrane-deforming protein making possible the dissection of its unique budding mechanism and the design of inhibitors to block it.

101 TaqMan Low Density Arrays Showed that One in Five Human Norovirus Clinical Cases were Multiple Infections with Other Pathogenic Gastroenteric Micro-Organisms

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Human norovirus (hNoV) has been estimated to contribute to 18% of all the infectious intestinal diseases (IIDs) worldwide, causing about three million cases of gastroenteritis per year in the UK. Routine detection of hNoV is performed by PCR, and specimens are often also analyzed by fecal culture for the detection of *Escherichia*, *Campylobacter*, *Salmonella* and *Shigella* species. A panel of 26 IID pathogens was designed for the TaqMan low-density array (TLDA) platform by the Public Health England (PHE) Diagnostic Laboratory in Cambridge (UK). The panel was tested on 773 residual stool samples extracted using SymphonyDP automatic extractor (QIAgen) coupled with MagNA Lyser (Roche) bead-beating pre-treatment; the purified nucleic acids were amplified by the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) on TLDA cards (Thermo Fisher). Clinical data was retrieved for hNoV PCR-positive patients through the eHospital electronic medical records system (EPIC). Cohen's kappa, Mann-Whitney U and Chi-square tests were performed to identify agreement and differences between groups. The TLDA/MaGna Lyzer approach identified 30 hNoV and 148 other IIDs more cases than the routine screening. hNoV was the most prevalent single IID agent and was identified as a single infection in 112 cases, and 32 cases were infected with hNoV and another micro-organism. *Dientamoeba fragilis* was the most common co-infecting organism with hNoV (n=9). Triple infections with hNoV were less frequent (n=4) and 75% of them included *Yersinia enterocolitica*. There were no differences by age or sex, between patients with single hNoV infections and those co-infected with hNoV and other organisms. The present study provided evidence for infections and co-infections missed by routine screening amongst hospitalized patients, which has implications for infections control. Further work is required to understand whether co-infections might be associated with a more severe outcome or with susceptibility markers.

102 The Contribution of Cellular Receptors to HSV-1 Entry into Skin Cells

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Cellular entry of Herpes simplex virus type 1 (HSV-1) includes the interaction of several viral glycoproteins with various cell surface receptors. First, virions attach to heparan sulfate proteoglycans (HSPGs) which is thought to facilitate the interaction with cellular receptors leading to fusion of the viral envelope with the cell membrane. As HSV-1 can enter its human host via epithelia of skin or mucosa, we focus our infection studies on epidermal keratinocytes and dermal fibroblasts, the two most abundant cell types in skin or mucosa. *Ex vivo* infection of nectin-1-deficient epidermis revealed nectin-1 as the major but not exclusive receptor for HSV-1. Similar observations were made in dermal fibroblasts. Herpes virus entry mediator (HVEM) was identified as alternative receptor that had a more limited role in epidermis and also acted less efficiently than nectin-1 in dermal fibroblasts [1]. To investigate the role of attachment receptors for HSV-1 entry into target cells we treated epidermal sheets with the HSPG analog heparin prior to infection to interfere with viral attachment. We observed a concentration-dependent inhibition of infection suggesting a role of viral binding to HSPGs in epidermis. In addition to HSPGs, the macrophage receptor with collagenous structure (MARCO) was shown to promote adsorption of HSV-1 to epithelial cells [2]. We also observed that polyinosinic acid (poly(I)), a ligand for class A scavenger receptors, has protective effects against HSV-1 entry. However, our experiments in MARCO-deficient epidermis and dermal fibroblasts revealed efficient infection of both WT and MARCO-deficient cells suggesting that viral binding to MARCO has no impact on efficient HSV-1 entry into skin cells. Thus, it remains to be shown how poly(I) treatment of skin cells interferes with infection efficiency.

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103 The Coronavirus Endoribonuclease Is a Potent IFN-Antagonist and Prevents Activation of the OAS-RNaseL Pathway

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The innate immune system critically relies on the detection of foreign signatures on invading pathogens to subsequently initiate an IFN-mediated antiviral response. Viruses have accordingly evolved versatile mechanisms to avoid the presentation of their immunogenic structures to innate immune sensors or they encode viral antagonists to counteract the antiviral effects of IFNs. Coronaviruses are large RNA viruses that replicate in the host's cytoplasm and efficiently evade innate immune sensing in other cell types than plasmacytoid dendritic cells. We show here that the CoV endoribonuclease (EndoU) is essential to antagonize cytoplasmic RNA sensing to prevent type-I interferon expression and to counteract dsRNA-mediated activation of the OAS-RNaseL and PKR pathways. In particular, mutant viruses harboring active site substitutions in the EndoU of human coronavirus (HCoV) 229E and mouse hepatitis virus (MHV) are severely attenuated and elicit an early and unleashed IFN response. In addition we observe a pronounced activation of the OAS-RNaseL pathway upon infection with EndoU-deficient mutant viruses, which results in remarkable degradation of rRNA. Accordingly, EndoU-deficient viruses can only retain replication in cells that are deficient in IFN expression or sensing, and in cells lacking RNaseL and PKR. Collectively, these data establish a fundamental role for the highly conserved coronaviral EndoU in the evasion of innate immune responses, namely to prevent accumulation of dsRNA and subsequent activation of cytoplasmic sensors, such as Mda5, OAS, and PKR.

104 The Expression of Human Coronavirus NL63 N, M and ORF 3 Proteins in Insect Cells

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NL63-CoV was first isolated from a seven month-old baby with bronchiolitis in early 2004. The virus has since been detected in up to 10% of all respiratory tract samples collected in different countries indicating a global distribution. Proteins N, M and ORF 3 are known coronavirus structural proteins with essential roles in virus replication and assembly. Here we aim to express NL63-CoV N, M and ORF3 proteins using a baculovirus expression system. Bioinformatic tools were used to identify short, potentially immunogenic peptide sequences within the NL63-CoV N, M and ORF3 amino acid sequences. N-terminus and C-terminus specific MAP- and KLH-conjugated peptides were synthesized and used to immunize female Balb/C mice. Mouse antisera, containing polyclonal antibodies (PAbs) against these antigens, were screened by a direct ELISA and Immunofluorescences Assay (IFA). Subsequently, spleens of immunized mice were harvested and splenocytes were fused with Sp2/0Ag14 myeloma cells. Monoclonal antibodies (MAbs) produced were screened for specificity against the peptide antigens, as well as full length NL63-CoV proteins using ELISA and IFA. Next, the full-length NL63-CoV genes were amplified by PCR from a NL63-CoV recombinant vector. pFastBac-NL63 constructs were generated by cloning each of the three full-length amplicons into a pFastBac l vector, respectively. Recombinant bacmids were obtained by transposition and recombinant baculoviruses were produced in Spodoptera frugiperda (Sf9) cells. Expression of recombinant N, M and ORFs proteins in the insects cells were confirmed with the pAbs and mAbs produced. The recombinant baculoviruses will be used to study the processing of the three NL63-CoV proteins, as well as studying the role of each in virus-like particle formation.

105 The Molecular Pathogen-Host Relationship of Human Papillomavirus Type 16 E6 Variants and Organotypic Epithelia

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Human papillomaviruses (HPVs) are a group of small double-stranded DNA viruses that infect host epithelia and have variable risks for causing cancer. While low-risk HPVs typically cause benign warts, high-risk HPVs cause nearly all cervical cancers and are found in ano-genital as well as head and neck cancers. HPV16 is the most common high-risk genotype, but not all infections persist and progress to cancer. To understand this diversity our lab investigates naturally-occurring HPV16 E6 oncogene variants with either low or high risk for cancer progression, denoted as the lower-risk European Prototype (EP) or higher-risk Asian-American (AA) variant. These E6 variants are a result of only three non-synonymous single-nucleotide polymorphisms (SNPs) and are named based on their geographical origins of discovery. We used our previously characterized 3D organotypic skin model to facilitate an HPV infectious cycle combined with "-omics" technology to investigate the molecular basis of these variant-specific pathogen-host relationships. RNA-Seq informatics analyses were used to characterize the viral and host transcriptomes. Intriguingly, viral transcription profiles revealed early viral integration of the AA but not the EP variant into the human genome, confirmed by high E6 oncogene transcription levels and detectable viral-human fusion transcripts. Likewise, differential gene expression analysis uncovered a signature for host chromosomal instability. Since viral integration is a key phenomenon for host cell transformation, variant-specific integration mediated by host genetic instability could represent a new paradigm in HPV variant biology. Current and future experiments will focus on further characterization of the integration mechanism and molecular landscape using genomics and epigenomics technology. Overall, our basic science work has strong impact for understanding how a small number of genetic variation in pathogens can lead to significantly different disease outcomes in host tissue.

106 The Picornavirus Encephalomyocarditis Virus Modulates the Host Lipid Landscape and Promotes Replication Organelle Formation by Hijacking PI4KA and OSBP

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Positive-strand RNA viruses are masters of reprogramming the host lipid metabolism to generate “replication organelles” (ROs) that support virus genome amplification. By which mechanisms picornaviruses of the genus Cardiovirus develop their ROs is currently unknown. We obtained evidence that the cardiovirus encephalomyocarditis virus (EMCV) converges on the same host pathway used by the distantly-related flavivirus HCV, by hijacking the ER-localized phosphatidylinositol 4-kinase III alpha (PI4KA) and oxysterol-binding protein (OSBP). EMCV protein 3A interacts with PI4KA and recruits it to ROs to locally generate elevated levels of phosphatidylinositol 4-phosphate (PI4P). PI4P proved important for the recruitment of OSBP, which subsequently delivers cholesterol to ROs in a PI4P-dependent manner. Furthermore, we report the isolation of EMCV escape mutants that can replicate independent of PI4KA and PI4P lipids. Sequence analysis revealed two independent single point mutations in the viral protein 3A, A32V and A34V, which recapitulated the resistant phenotype to knockdown or pharmacological inhibition of PI4KA when introduced in back wt EMCV. In cells with blocked PI4KA activity, the mutants no longer induced PI4P accumulation at ROs, which were also devoid of OSBP and cholesterol. Remarkably, the single point mutations in 3A only provided minimal cross-resistance to OSBP inhibitors, suggesting that a function of OSBP possibly unrelated to PI4P/cholesterol shuttling might be important for EMCV replication. Our findings have important evolutionary implications, by revealing a conserved phenomenon of resistance shared by cardioviruses and enteroviruses, which we previously showed to also become independent of PI4KB-mediated PI4P accumulation by acquiring single point mutations in their 3A protein.

107 Virus-Cell Interactions under Conditions of Adeno-Herpetic Co-Infection and Performance Features of Cidofovir in These Conditions

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The wide distribution of adenoviruses and herpesviruses and their similar tropism create the possibility of multi-viral infections with the development of mixed infections. Herpes simplex virus can persist in the body constantly after initial infection. It causes different clinical forms of disease including acute and chronic recurrent infections. An important aspect of adenoviral infection is its ability of multiple clinical manifestations and latency. The aim of the work was to study the effect of mixed infection on a replication of viral DNA, viral proteins synthesis and the level of infectious virus in epithelial MDBK cells. Comparing co-infected epithelial MDBK cells with mono-infected cells, the decrease of HAdV-5 and HSV-1/US infectious titers (2.6 lg and 1.6 lg respectively) was observed. The inhibition of the virus cytopathic effect on cells was also detected. It is known that one of the key points of the virus reproduction is the virion protein synthesis. An insufficient number of viral proteins inhibits viral morphogenesis process and the formation of new infectious viral particles. Expression characteristics of certain structural adenovirus and herpes simplex virus proteins in MDBK cells were received. A significant inhibition of herpesvirus glycoproteins synthesis (64%) and inhibition of adenoviral protein hexon expression (17%) were observed. The behavior of viruses in response to the treatment with antiviral drugs in the case of mixed infection has not been studied. A 29% decrease of cidofovir (concentration 32 μg/mL) effectiveness in MDBK cells with mixed HAdV-5 - HSV-1/US infection comparing to MDBK cells with herpes simplex virus infection was observed. At the same time, the activity of the drug was unchanged in the case of adenovirus infection. The abnormal action of the drug in the co-infected cells indicates the need for further study of antiviral compounds. These compounds can be inefficient in medical practice and since they may lead to the development of viral resistance.

108 Translation of Kaposi's Sarcoma-Associated Herpesvirus (KSHV) Lytic Gene Products are Resistant to mTORC1 Inhibition

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma (KS), the leading AIDS-related malignancy. Several KSHV lytic gene products stimulate activation of mTORC1 upstream of Rheb-GTPase. mTORC1 inhibitors, such as rapamycin, cause tumor regression in KS patients and tumor xenograft models. This regression correlates with dephosphorylation of mTORC1 kinase targets and decreased expression of VEGF. KSHV has also been shown to promote assembly of eIF4F during lytic reactivation. Here we sought to determine whether mTORC1 activation and eIF4F formation are required for translation of host and viral mRNAs. We found that mTORC1 activity was required for efficient reactivation from latency, but dispensable for genome replication and release of progeny virions. During KSHV lytic reactivation, the mTORC1-4EBP1-eIF4F axis was intact, yet KSHV gene products were efficiently translated when eIF4F was disassembled through mTORC1 inhibition. Polysome analysis and puromycin labelling of nascent polypeptides demonstrate that there is a reduction in global translation during lytic replication, but global translation is resistant to further inhibition by mTOR inactivation. We extracted RNA from the translating polysome fractions for RNA-seq analysis to determine whether there is mTORC1-dependent regulation of host and viral messages in the context of lytic replication. We employed a m7'GTP affinity purification LC-MS/MS approach with stable isotope dimethyl labelling to investigate alterations in the composition of the translation initiation complexes during KSHV lytic replication. Accumulating evidence suggests that KSHV can assemble alternative translation initiation complexes that resist the effects of mTORC1 inhibition, thereby facilitating translation of viral mRNPs and the production of infectious progeny.

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109 Translational Control of APOBEC3G/F Restriction Factors by the HIV-1 Vif Protein

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The human immunodeficiency virus type 1 (HIV-1) requires the concerted contribution of many cellular factors to achieve efficient replication. Similarly, mammalian cells express a set of proteins called restriction factors to suppress viral replication. Among these factors, the family of APOBEC3 (Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 or A3) proteins and in particular A3G and A3F are the most efficient against HIV-1. They belong to a large family of cytidine deaminases that catalyze the deamination of cytidines to uridines in single stranded DNA substrate during HIV-1 retrotranscription. The antiviral activity of A3G/F is counteracted by the HIV-1 Vif (Viral infectivity factor) protein. Vif significantly reduces their expression in cell and their incorporation into viral particles by 1) recruiting an E3 ubiquitin ligase complex to induce their degradation by the proteasome, and 2) regulating their translation. Up to now, mechanisms by which Vif regulates the translation of A3G/F are unknown.

To address the role of Vif in the regulation of A3G/F translation, we tested the importance of the untranslated regions (UTRs) of A3G/F mRNA in the translational inhibition. HEK 293T cells were transfected with wild-type and mutated constructions of A3G/F mRNAs (ΔUTRs, Δ5'UTR, Δ3'UTR and ΔSL) in presence or absence of Vif. These experiments were also performed with a proteasome inhibitor (ALLN) in order to distinguish the proteasomal degradation pathway from the translational inhibition.

Although the translation of wild-type A3G/F mRNA is significantly reduced by Vif, we showed that the suppression of their 5'UTRs does not allow these mRNAs to be regulated by Vif anymore, suggesting the importance of the 5'UTR for translational repression. Within this 5'UTR, the two distal stem-loops are required for the inhibition. Moreover, the repression is strictly dependent on A3G/F 5'UTR as its replacement by heterologous 5'UTRs prevents translation inhibition. Interestingly, we found that A3G is also translationally regulated by Vif in HIV-1 chronically infected lymphoid cells (H9). This effect of Vif on A3G/F translation is conserved amongst HIV-1 isolates, whereas CBF-b, an essential factor in Vif-induced A3G/F proteasomal degradation, is not required to down-regulate A3G/F translation. Finally, we observed a strong correlation between the level of A3G/F protein translation in cell, their incorporation into viral particles, and the infectivity of released virions.

Experiments are in progress to identify with precision the mechanisms of A3G/F translational regulation and determine Vif domains involved in this process. Regulating the translation of A3G/F could thus be considered as a new target to restore a functional expression of A3G/F and viral restriction.

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110 Trend of Proliferative and Cancer Inducing Genes in Fibrosis to Cirrhosis in HCV 3a Infected Patients Liver Samples

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HCV chronic infection is a leading health problem worldwide. Limited data available for disease progression from mild to moderate, and to severe fibrosis compared to HCC make it difficult to detect at early stages. The present study elucidates the mechanism of disease progression from early (F1) to moderate (F2, F3), and to severe fibrosis (F4) in HCV genotype 3a infected patients. Microarray gene expression profiling was carried out on HCV infected biopsy samples compared to normal liver samples, using operon human whole genome microarray chip to find out the host genes transcriptionally regulated by the virus. A total of 157 genes were found differentially regulated in the four fibrosis stages compared to control group and were classified in 36 categories according to their biological functions. HCV infection differentially regulated the expression of genes involved in apoptosis, cell structure, signal transduction, proliferation, metabolism, cytokine signaling, immune response, cell adhesion and maintenance, and post translational modifications by pathway analysis. Gene expression pattern, and associated pathway analysis helped to elucidate the potential genes involved in step-wise disease progression. Increasing trend of proliferative and cell growth related genes while shutting down of immune system showed disease progress to mild to moderate to advanced stage cirrhosis. There are more chances of developing liver cancer in patients infected with HCV genotype 3a in a systematic manner. The identified gene set can act as disease markers for prediction whether the fibrosis lead to cirrhosis and its association with end stage liver disease development.

111 Infection of Human Cells by Bovine Herpesvirus (BHV-1) with Restricted Host Range.

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Bovine herpesvirus 1 (BHV-1) is a widespread pathogen of domestic and wild cattle. BHV-1 is a member of the subfamily *Alphaherpesvirinae* of the *Herpesviridae* family and it is investigated not only as an animal pathogen but also as a good model for molecular studies on human viruses (such as HSV-1 or VZV), belonging to the same family. One of the interesting properties of BHV-1 is its strictly restricted host range compared to other herpesviruses, including HSV-1. The natural host of BHV-1 is cattle and it is propagated only in cells of bovine origin. However, in our previous studies of herpes viral immune evasion, we managed to obtain productive BHV-1 infection in a human cell line, MelJuSo (Koppers-Lalic, 2003). It has been also suggested that BHV-1 can be used as a human oncolytic vector due to its capability of replication in human transformed cell lines (Cuddington, 2015).

Here we report that BHV-1 successfully infects various cells of human origin: highly modified human melanoma cells—MelJuSo, more primary human keratinocytes—HaKat, neural precursors hNSC, as well as neuroblastoma SH-SY5Y cells. We compared the replication and spread of BHV-1 in human and in bovine cells (MDBK, KOP, bovine PBMC and primary bovine fibroblasts). Furthermore, we analyzed the role of viral proteins: envelope glycoprotein E and protein kinase US3 during virus infection. Both these proteins are engaged in virus transmission and spread in cell culture. For our studies we constructed a wide panel of fluorescent BHV-1 mutants with fluorescent tags fused to Us3 or to envelope and capsid proteins. We observed a number of functional differences in biological activity of BHV-1 in various cells and we confirmed that BHV-1, contrary to its established species-specificity, is capable to infect and kill many types of human cells.

112 Segment 7 and 8 mRNAs from Highly Pathogenic Influenza A/Brevig Misson/1918/1 (H1N1) Interact with a Different Set of Cellular Splicing Proteins than Those from Less Pathogenic Influenza A/Netherlands/178/95 (H3N2).

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Influenza A replicates in the cell nucleus where two of the eight genomic segments produce mRNAs that can be spliced. We have previously reported that pre-mRNAs of both segment 7 and 8, derived from the highly pathogenic influenza A/Brevig Misson/1918/1 (H1N1), are spliced to a lower extent than those from an isolate of lower pathogenicity (influenza A/Netherlands/178/95 (H3N2)). Thus, splicing efficiency correlates with pathogenic properties of influenza viruses. We believe that sequence variation among influenza A types affects the efficiency of splicing by interfering with the binding of splicing regulatory proteins to segment 7 and 8 mRNAs. Segment 7 and 8 encodes the M1/M2 and NS1/NS2 proteins respectively, and we speculate that the difference in the M1/M2 and/or NS1/NS2 protein levels plays a role in influenza virus pathogenesis. Identification of cellular factors that control splicing of influenza virus mRNAs is therefore of interest. In order to identify such factors, we performed pull-downs with oligos extending the entire length of segments 7 and 8 of highly pathogenic influenza A/Brevig Misson/1918/1, and of less pathogenic influenza A/Netherlands/178/95. Several proteins of the hnRNP- and SR-protein families were found to bind both segment 7 and 8 mRNAs, such as nRNPA1, hnRNPG, hnRNPI/PTB, hnRNPL and TRA2β. Interestingly, a number of these factors interact specifically with binding sites on mRNAs from either highly pathogenic influenza A/Brevig Misson/1918/1, or from the less pathogenic influenza A/Netherlands/178/95. Binding sites will be further mapped, and mutated in expression plasmids to determine their role in the influenza splicing process in human cells. We conclude that influenza viruses with different pathogenic properties interact with different cellular RNA binding proteins.

113 Uncoating Cues at Virus Entry in African Swine Fever Virus Infection

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We search for targets at early stages of infection aiming to discover how large double stranded DNA viruses overcome host cell defenses and reorganize cellular structures to initiate replication. African swine fever virus (ASFV) enters the cell by endocytosis aided by macropinocytosis and within a few minutes after infection, viral decapsidation occurs at the acid pH of late endosomes as a first uncoating step. Events following, including the virus exit from the endosome to the cytosol, require an intact cholesterol endosomal efflux. In fact, cholesterol is required at several steps of the virus life cycle starting from virus entry. ASF virus reorganizes cholesterol landscape of the cell to the perinuclear replication site where the viral factory is built. These results add to a growing body of evidence pointing out cholesterol efflux and the endosomal membrane as crucial players for the start of viral replication in several virus models.

114 Aberrations in Glycosylation Pattern of HCV Envelope Protein E2 Isolated from Patients.

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Despite recent development of new generation target-specific antivirals, hepatitis C virus (HCV) infections are still one of the most serious global health problems. HCV envelope glycoproteins E1 and E2 play an essential role in HCV infection. Both proteins are highly glycosylated with N-linked glycans and they contain up to 5 (E1) and 11 (E2) conserved glycosylation sites. Glycosylation is considered to be important for some key processes such as interaction with cellular receptors or sensitivity to neutralization by antibodies. Many published results suggest that glycan shield masks epitopes for neutralizing antibodies and glycan shifting within conserved linear E2 region (412-423) helps the virus to hide itself from neutralizing antibodies. However, identification of an exact mechanism of how HCV escapes the immune defense to establish persistent infection remains elusive. To address this question, we generated a pool of HCV quasi-species containing different E2 glycoprotein variants derived from children patients previously characterized in terms of interferon–ribavirin therapy outcome. Randomly selected clones were sequenced and checked for the glycosylation status. Comparison of obtained sequences revealed alterations in the glycosylation pattern, especially loss of a N1 glycosylation site in a non-responders group. In contrast to previously described *in vitro* results, we observed repeatable N-X-N/R pattern instead of amino acid substitution N417S/N417G/N417T within the N1 glycosylation site sequence. Such altered glycoprotein was only weakly recognized by broadly neutralizing antibody AP33. Due to the fact that AP33 and other neutralizing antibodies share the same linear epitope on E2 glycoprotein, we can assume that mutations in the E2 sequence leading to the loss of N1 glycosylation site can contribute to the mechanism of HCV immune escape.

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