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The 3'UTR of WNV genome is an excellent target for antiviral drugs

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Why West Nile virus?

Zika virus

Yellow fever virus

Flavivirus

West Nile virus

Dengue virus

St. Louis Encephalitis Virus

West Nile virus has spread worldwide during the last two decades…

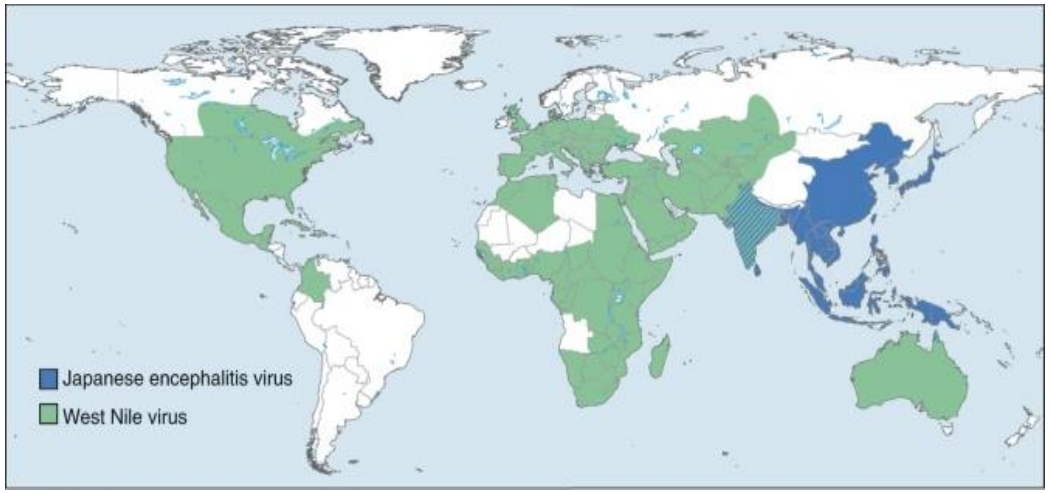
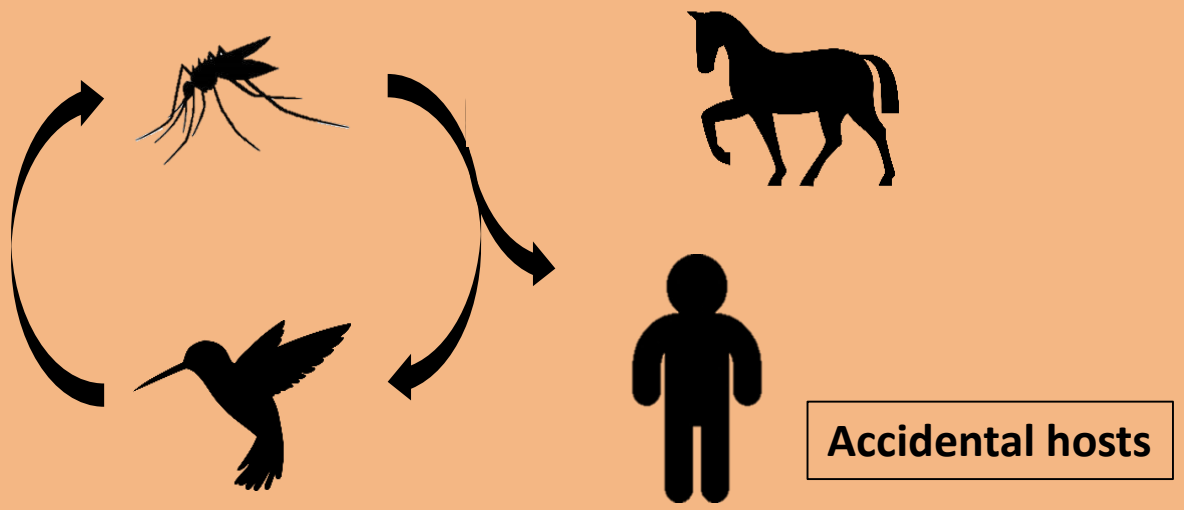


Figure from Pierson, T.C., Diamond, M.S. (2020). Nat Microbiol 5, 796–812

Why West Nile virus?

Transmission cycle



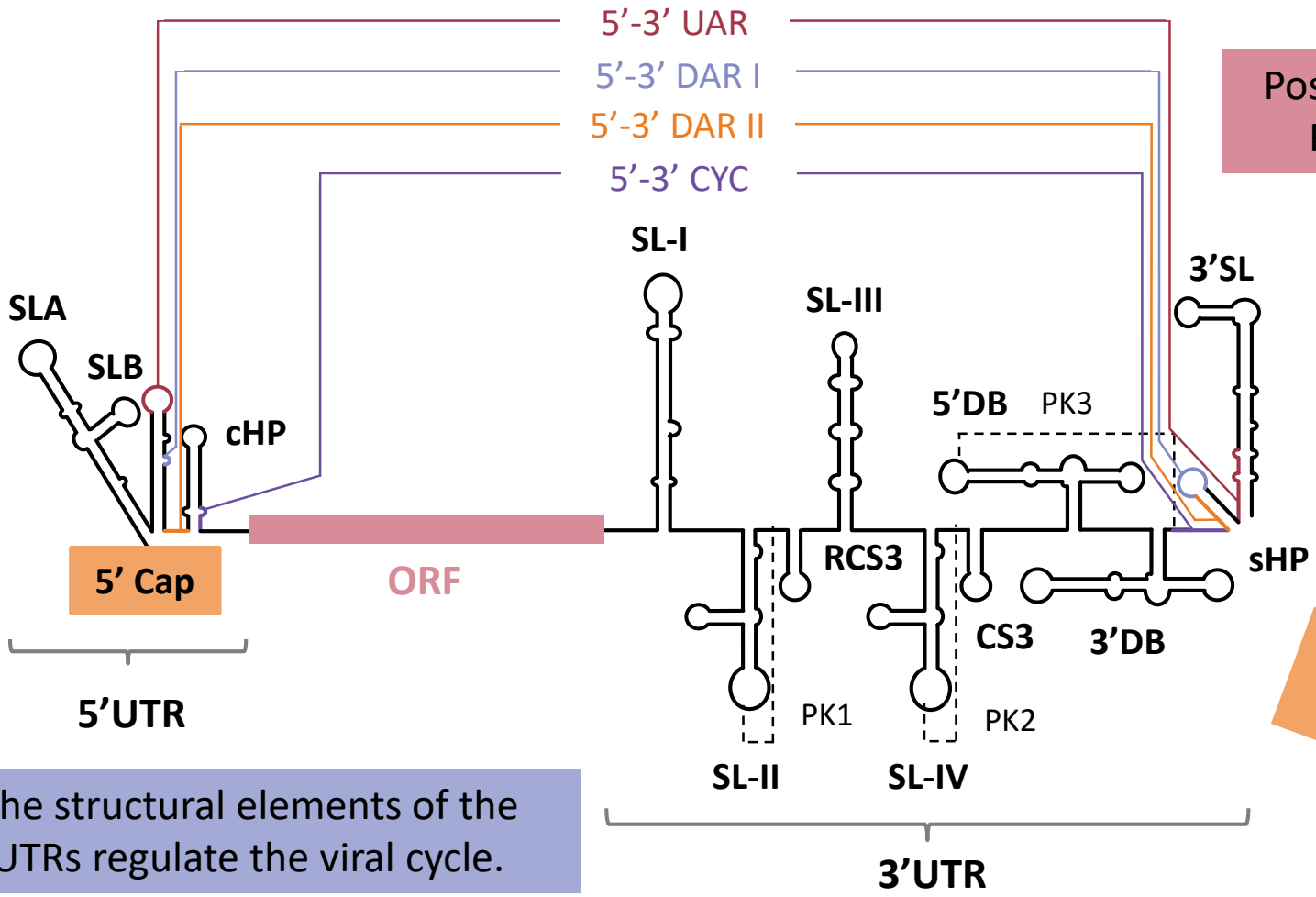
~ **80%** of WNV infections in humans are asymptomatic.

~ **20%** infected people develop Nile fever.

1/150 suffer serious neuroinvasive diseases.

There are no specific vaccines or treatments for WNV.

Knowing West Nile virus…

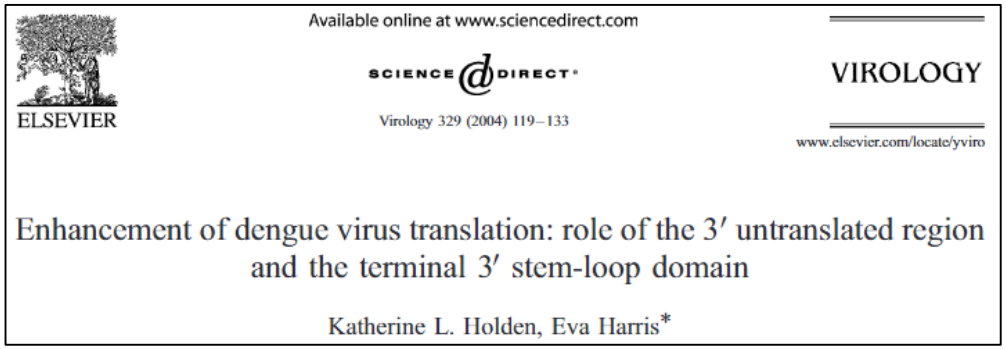


Positive-strand RNA virus

Absence of a polyA tail

The structural elements of the UTRs regulate the viral cycle.

Translation mechanism in *Flavivirus*



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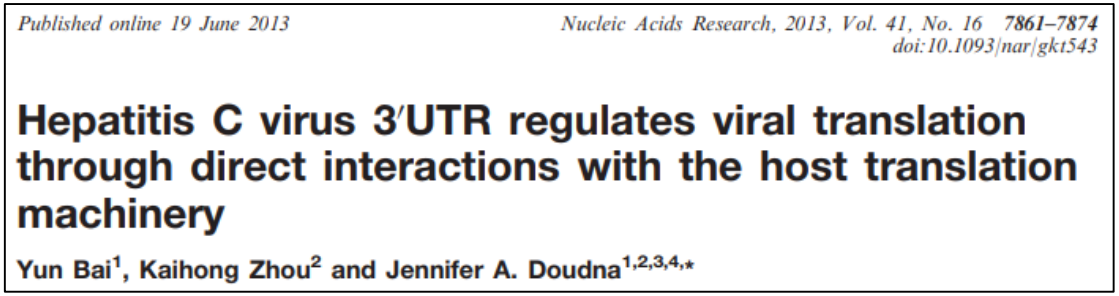
Enhancement of dengue virus translation: role of the 3′ untranslated region and the terminal 3′ stem-loop domain

Katherine L. Holden, Eva Harris*

The **3'UTR region** of different flaviviruses enhances viral translation, but…

…How it does?

In **Hepatitis C virus**, the 3'UTR region is capable of binding the 40S ribosomal subunit...



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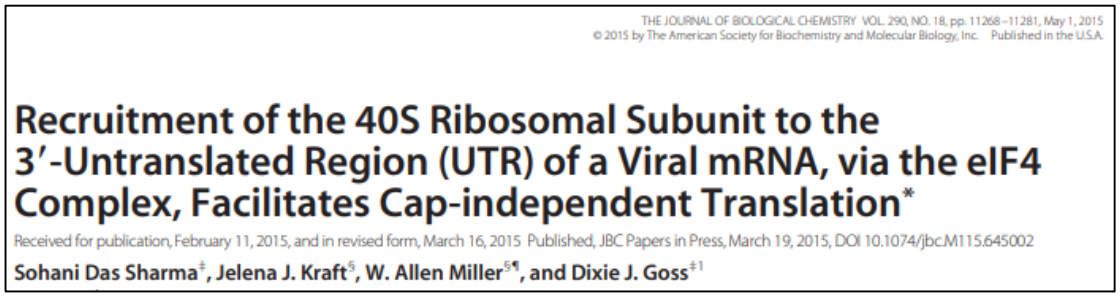
doi:10.1093/nar/gkt543

Hepatitis C virus 3′UTR regulates viral translation through direct interactions with the host translation machinery

Yun Bai[1], Kaihong Zhou[2] and Jennifer A. Doudna[1,2,3,4,*]



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 18, pp. 11268–11281, May 1, 2015

Recruitment of the 40S Ribosomal Subunit to the 3′-Untranslated Region (UTR) of a Viral mRNA, via the eIF4 Complex, Facilitates Cap-independent Translation*

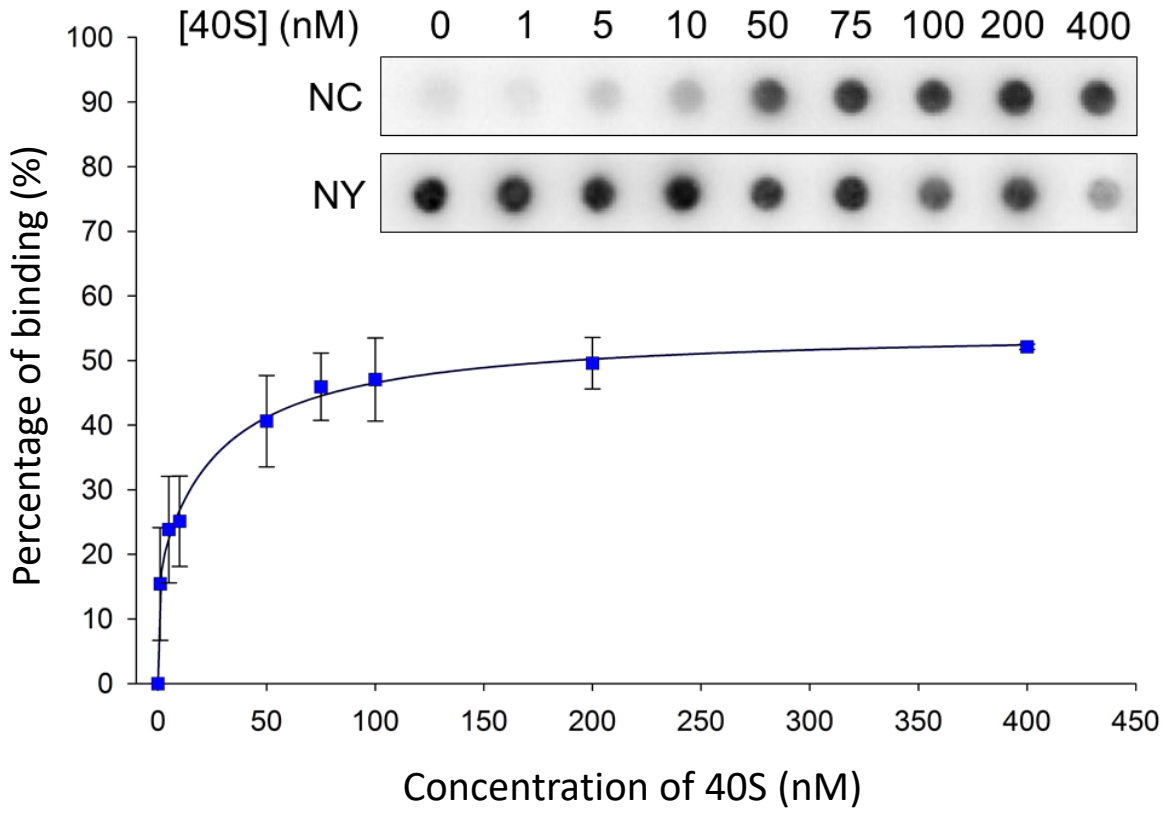
Received for publication, February 11, 2015, and in revised form, March 16, 2015 Published, JBC Papers in Press, March 19, 2015, DOI 10.1074/jbc.M115.645002

Sohani Das Sharma[‡], Jelena J. Kraft[§], W. Allen Miller[§¶], and Dixie J. Goss[‡1]

The 3'UTR region of the **Barley Yellow Dwarf virus** binds the 40S subunit and transfer it to the 5'UTR region by long-distance RNA-RNA contacts.

The 3'UTR of the WNV recruits the 40S ribosomal subunit

Two theoretical binding sites for the 40S subunit in the 3'UTR region...

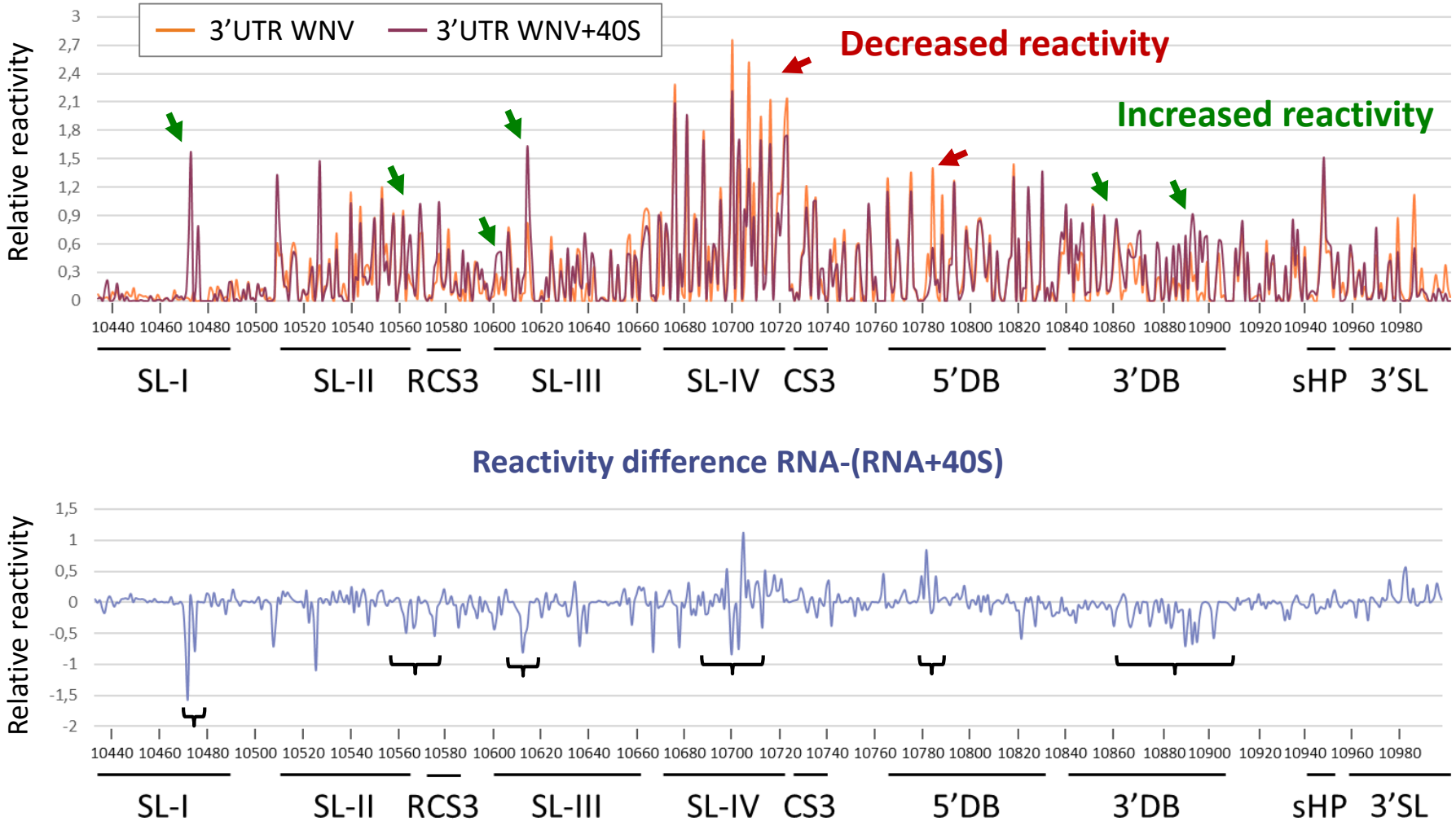


$$Y = \frac{B_{max}1 \cdot X}{K_d1 + X} + \frac{B_{max}2 \cdot X}{K_d2 + X}$$

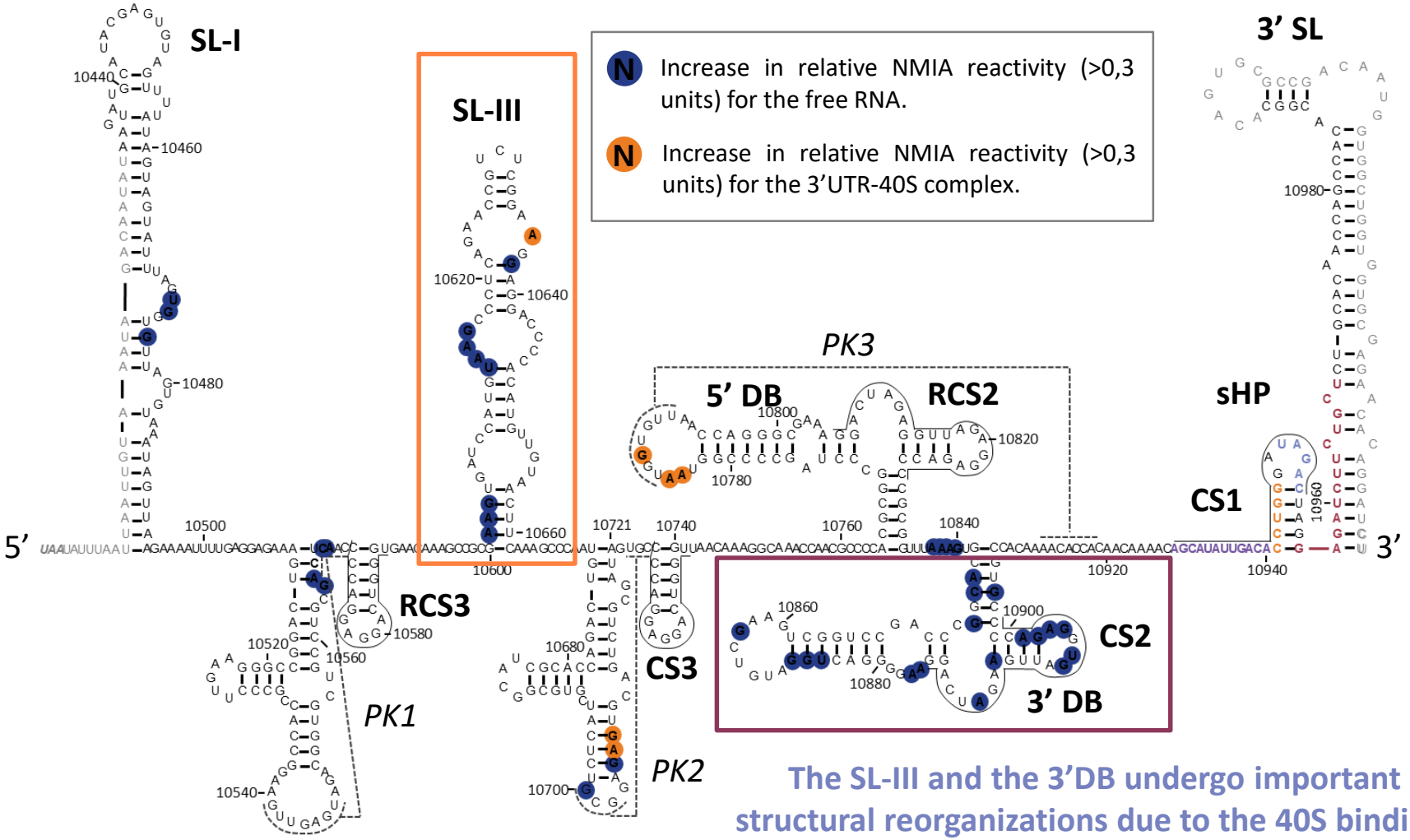
Sitio 1	Sitio 2
$K_d1 = 0{,}23\ nM$	$K_d2 = 28{,}83\ nM$
$B_{max}1 = 17{,}64\%$	$B_{max}2 = 37{,}29\%$

...with very different characteristics.

The 3’DB plays a key role in the recruitment of the 40S subunit



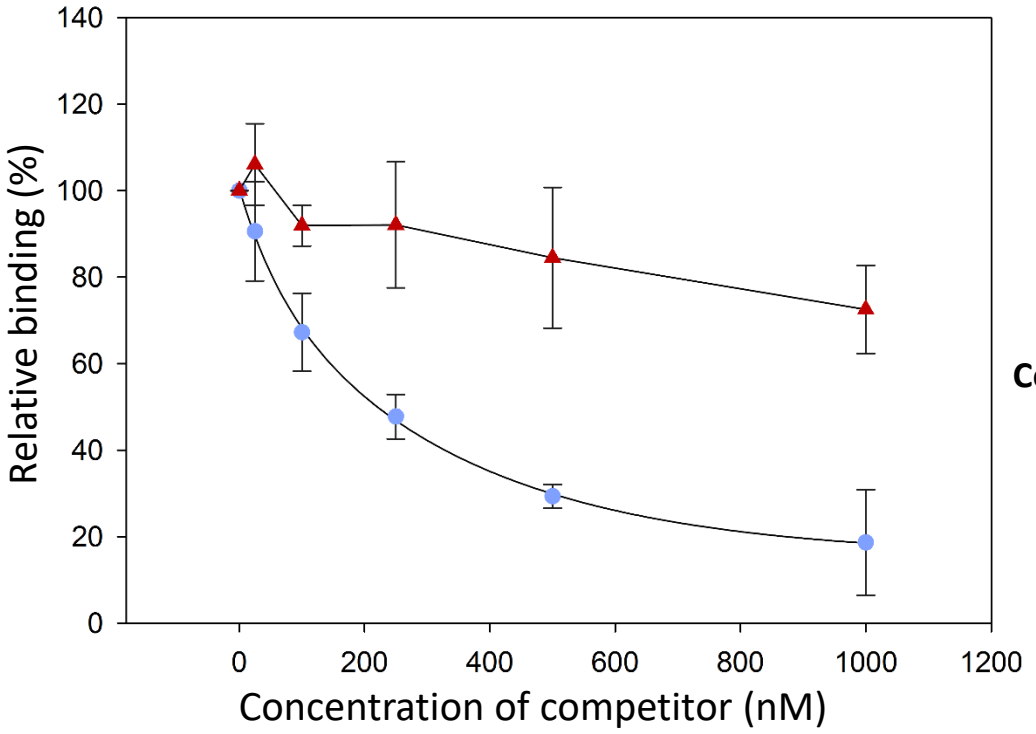
The 3’DB plays a key role in the recruitment of the 40S subunit

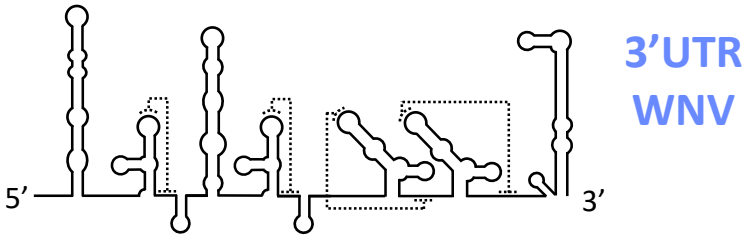


The SL-III and the 3’DB undergo important structural reorganizations due to the 40S binding.

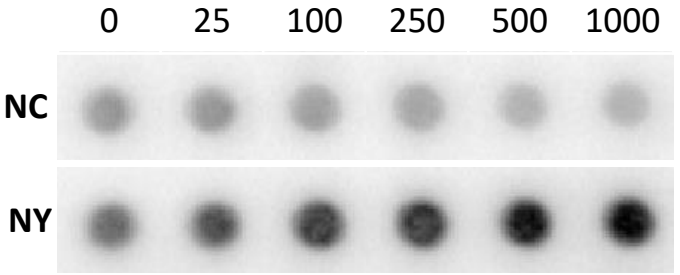
Binding of the 40S to the 3’UTR WNV is specific

Unlabeled 3’UTR WNV and Non-related RNA.





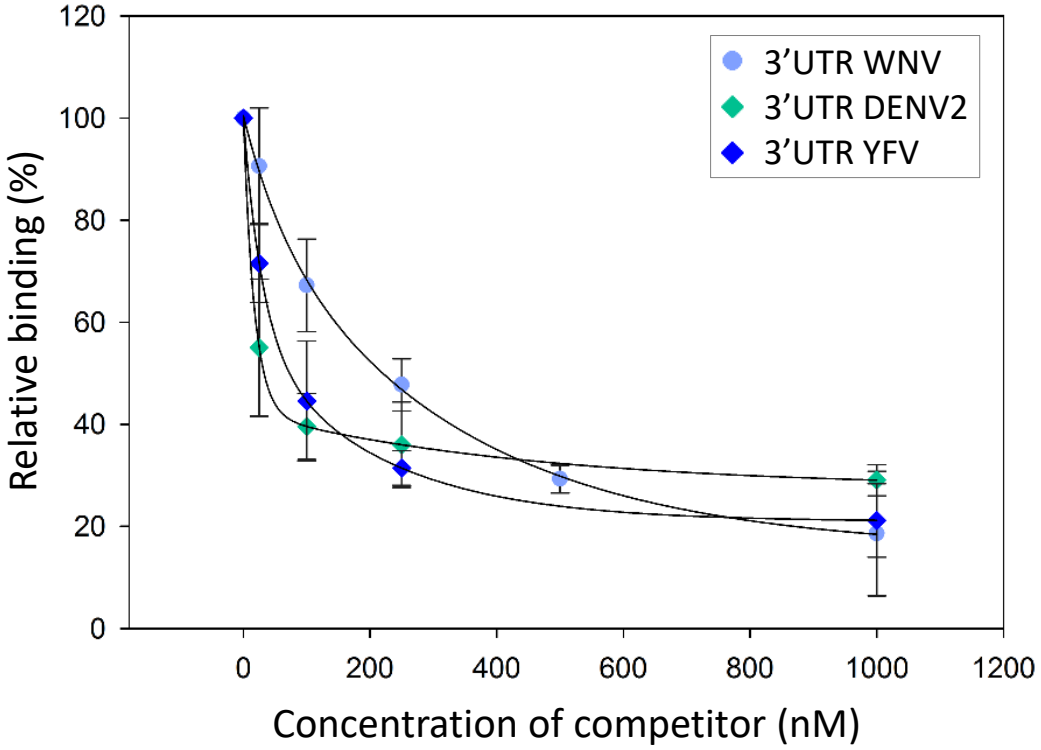
Concentration of unlabeled RNA 3’UTR WNV (nM)

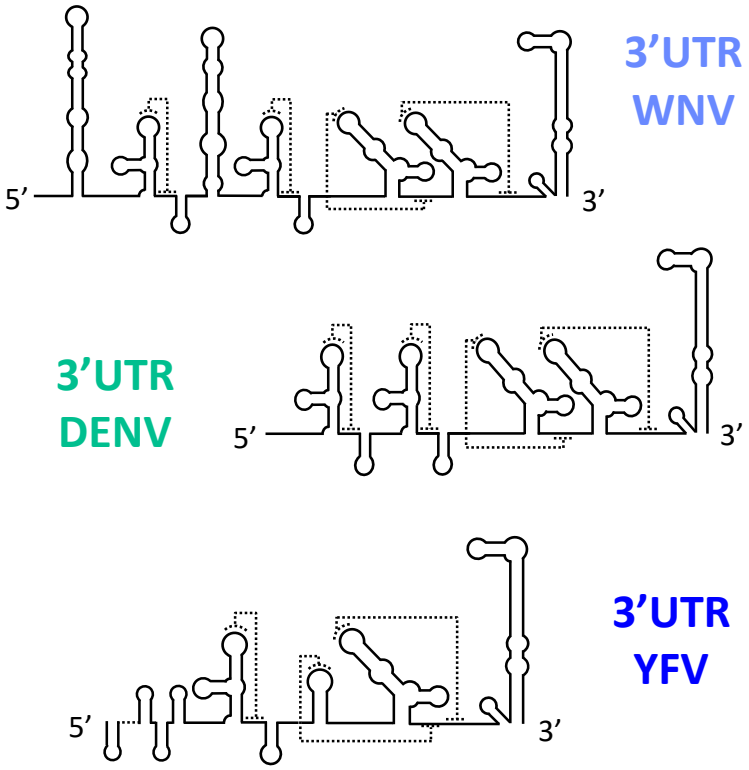


Increasing concentrations of unlabeled 3’UTR WNV displaced the binding of the labeled RNA to the 40S subunit, while the presence of a molar excess of a non-related RNA did not affect significantly to this binding. Indicating the binding of the 40S subunit to the 3’UTR region of WNV is specific.

Flaviviruses recruit the 40S through their 3’UTR

Unlabeled 3’UTR DENV and 3’UTR YFV.





The 3’UTR regions of both DENV and YFV displace the 3’UTR WNV-40S binding, suggesting that the recruitment of the 40S by the 3’UTR region is a common mechanism in *Flavivirus*.

Is the binding of the 40S subunit to the 3'UTR mediated by RNA or ribosomal proteins?

1. Incubation of the 3'UTR WNV with 40S

40S

Radioabeled 3'UTR WNV

5' 3'

3'UTR WNV – 40S complex

5' 3'

With (or without) the unlabeled 3'UTR as competitor

2. Cross-linking.

Irradiation with UV light

Covalent bond

5' 3'

3. Treatment with proteinase K.

Proteinase K

Degradation of ribosomal proteins.

5' 3'

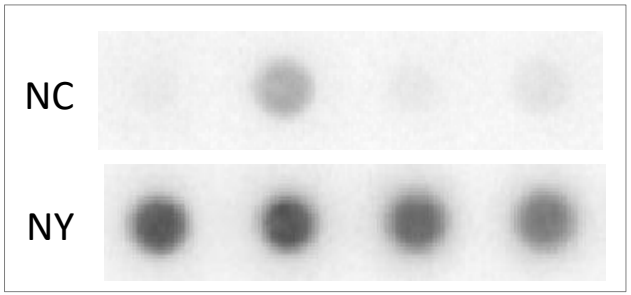
4. Double differential filter retention.

Ribonucleoprotein complexes

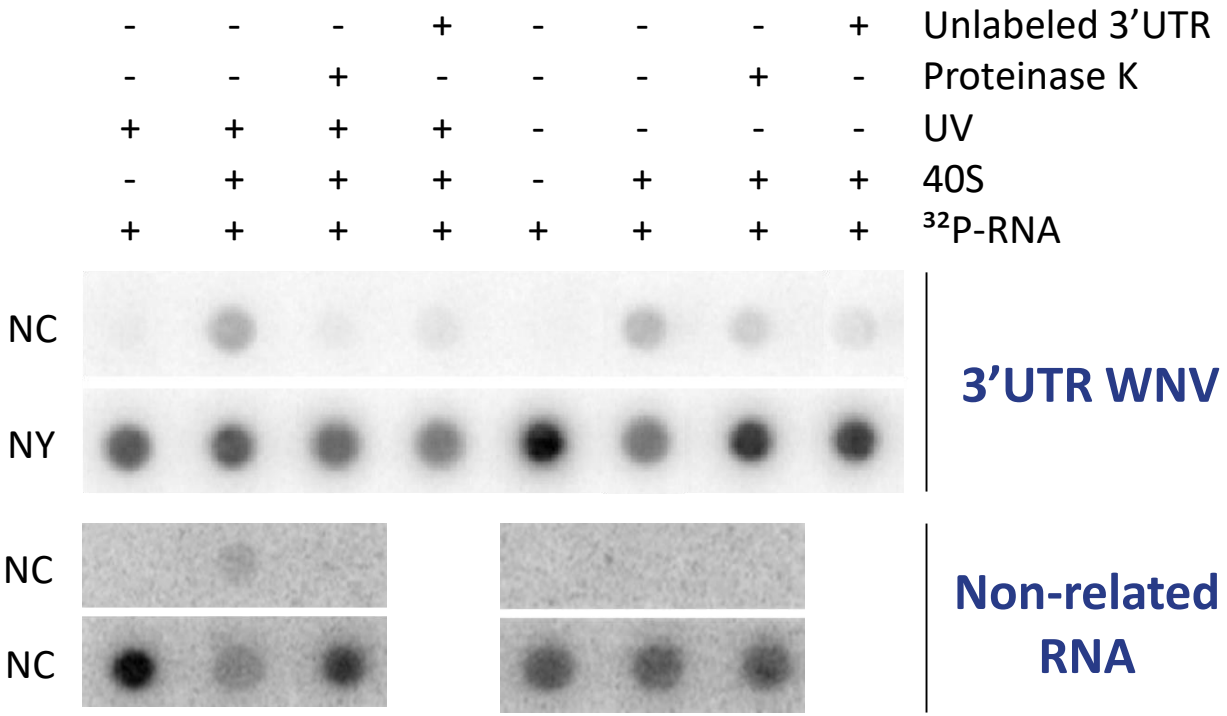
Free RNA

NC

NY



The recruitment of the 40S subunit by the 3’UTR region of WNV depends on ribosomal proteins



The binding of the 40S ribosomal subunit to the 3’UTR WNV depends on protein factors existing in the 40S complex.

Identification of ribosomal proteins involved in the interaction with the 3'UTR WNV

1. Incubation of the 3'UTR WNV with 40S

40S

Radioabeled 3'UTR WNV

5' 3'

3'UTR WNV – 40S complex

5' 3'

2. Cross-linking.

Irradiation with UV light

Covalent bond

5' 3'

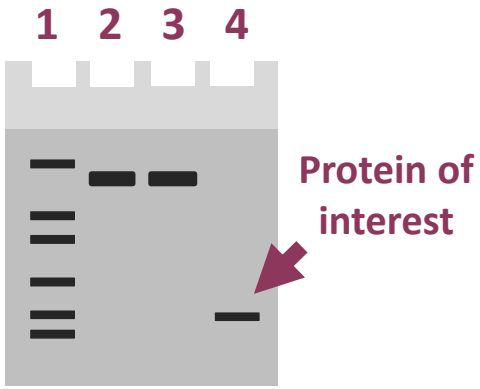
3. Treatment with RNase A.

RNase A

Label transfer from the 3'UTR WNV RNA to the protein of interest.

5' 3'

4. SDS-PAGE

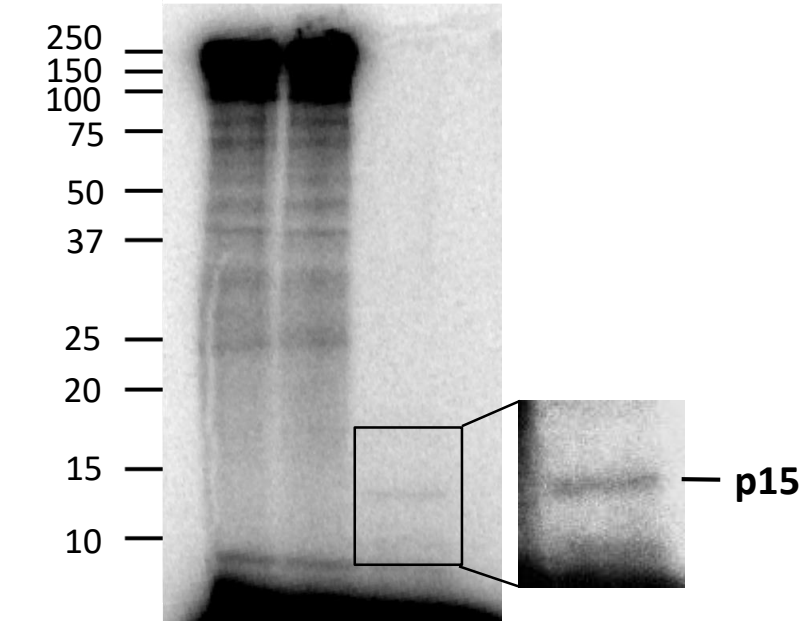


1. Molecular weight marker.
2. Cross-linked RNA.
3. Cross-linked RNA+40S.
4. Cross-linked RNA+40S treated with RNase A.

The 40S subunit binds to the 3’UTR region through a protein of about 15 kDa

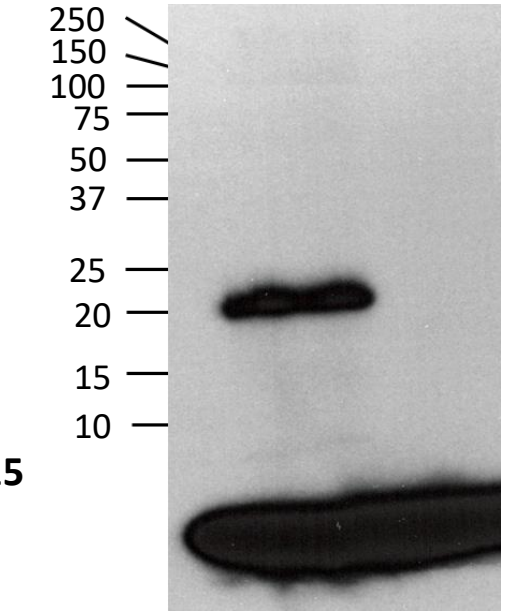
3’UTR WNV

-	-	+	RNase A
+	+	+	UV
-	+	+	40S
+	+	+	^{32}P-3’UTR WNV



Non-related RNA

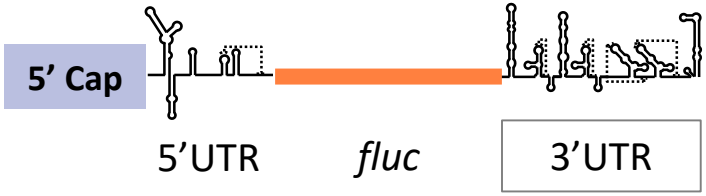
-	-	+	RNase A
+	+	+	UV
-	+	+	40S
+	+	+	^{32}P-Non-related RNA



The RNase A treatment does not completely degrade the 3’UTR of WNV incubated with the 40S subunit and exposed to UV light. A band corresponding to a 15 kDa ribosomal protein appears instead.

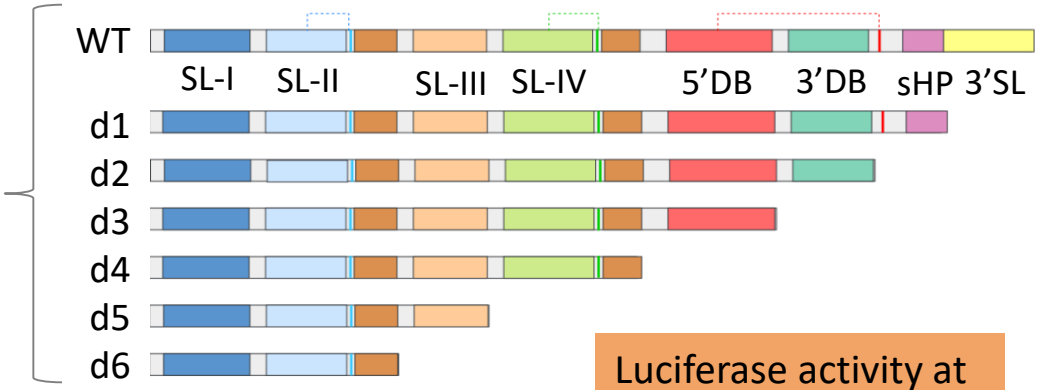
The 3’DB domain is essential in the regulation of translation

WNV RNA-FLUC

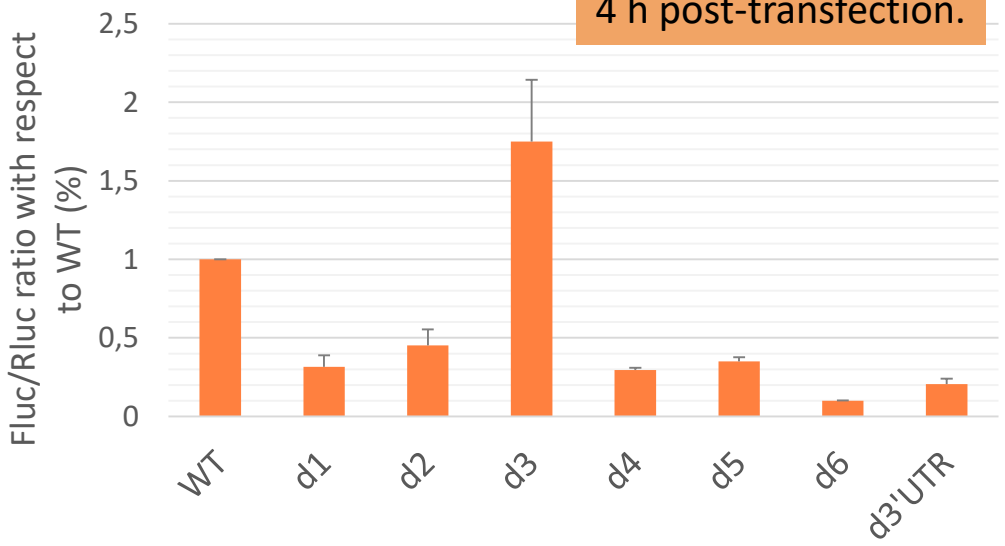


Transfection internal control

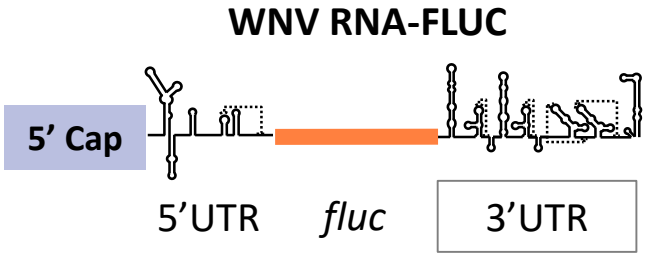
The results of the functional assays show a general decrease of translational efficiency when the 3’UTR is shortened from its 3’ end. However, deletion of the 3’DB domain enhances viral translation, even above the levels obtained with the WT construction.

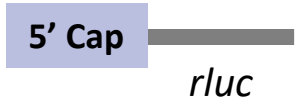


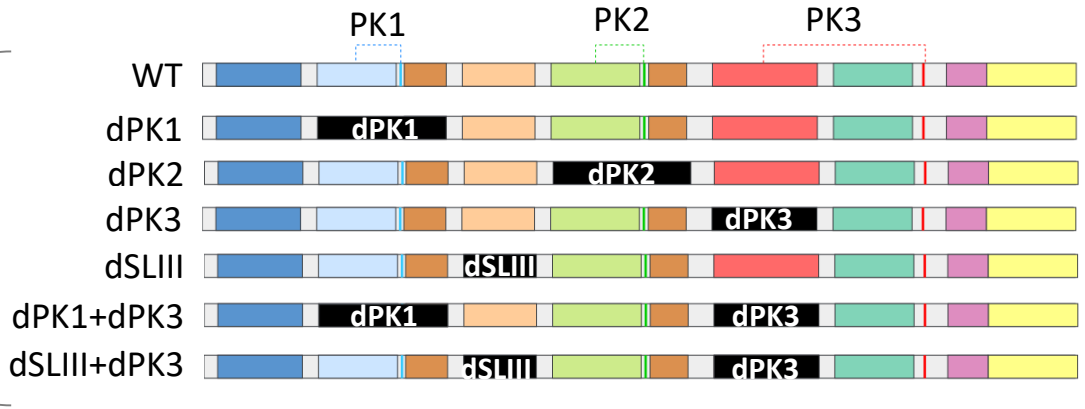
Luciferase activity at 4 h post-transfection.



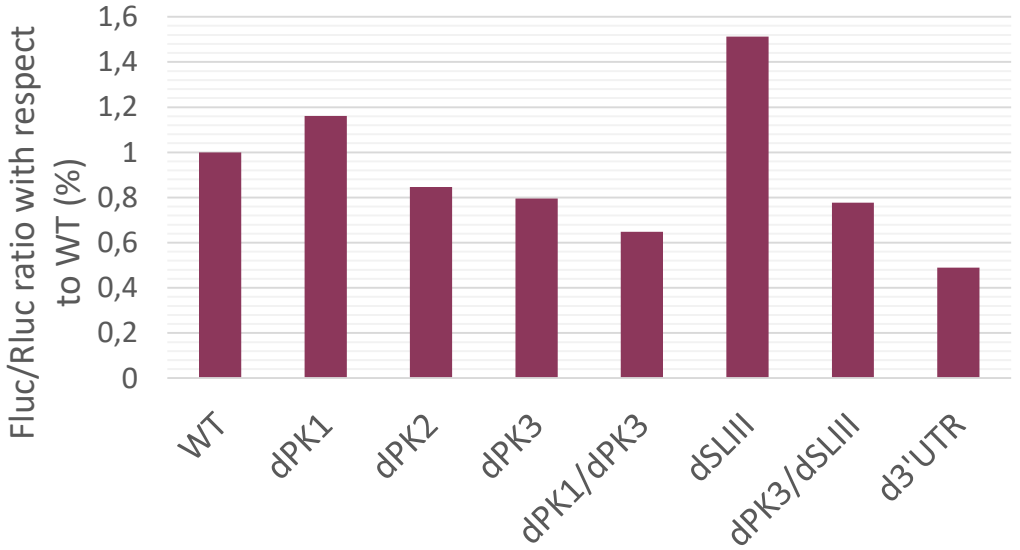
Deletion of the SL-III element enhances viral translation



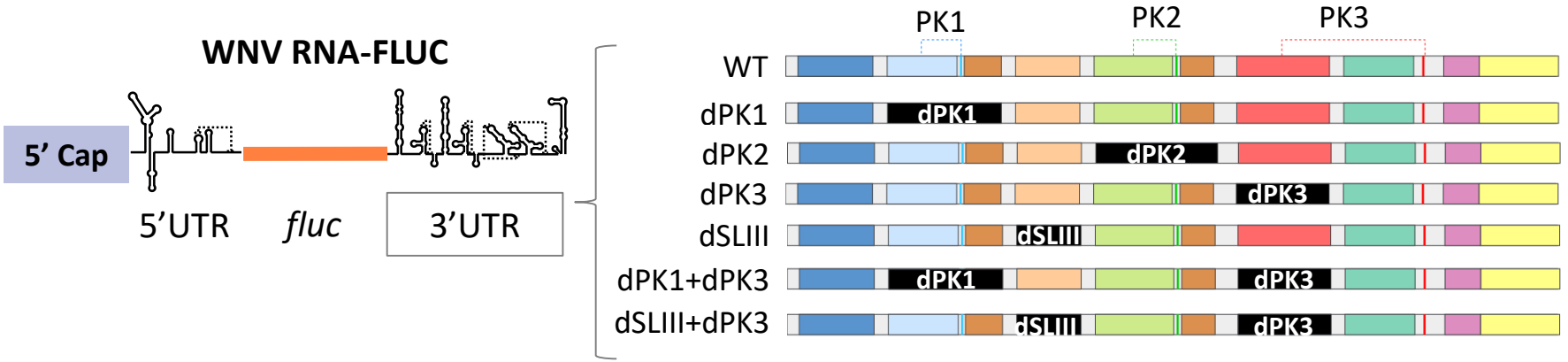


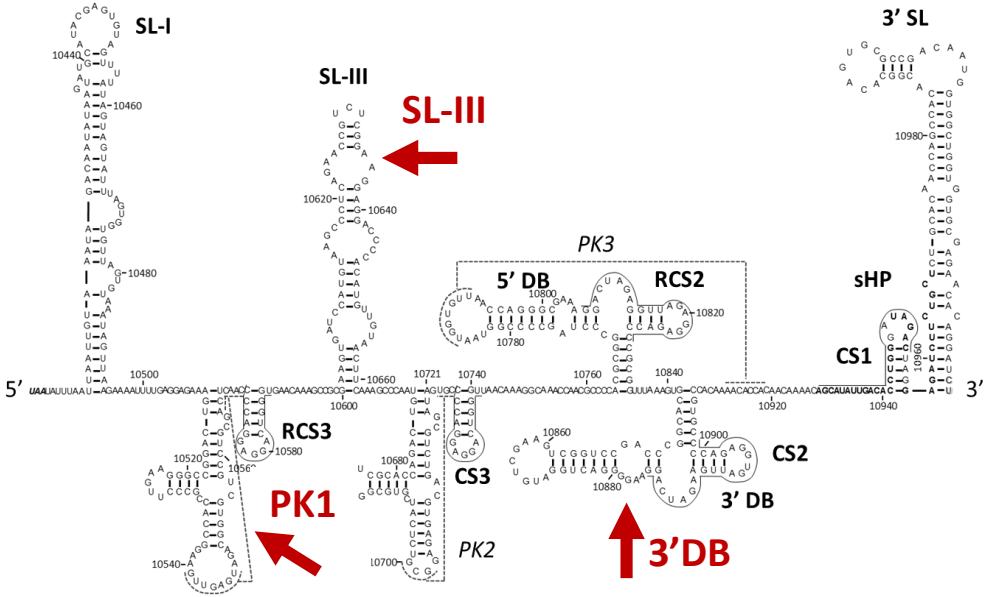


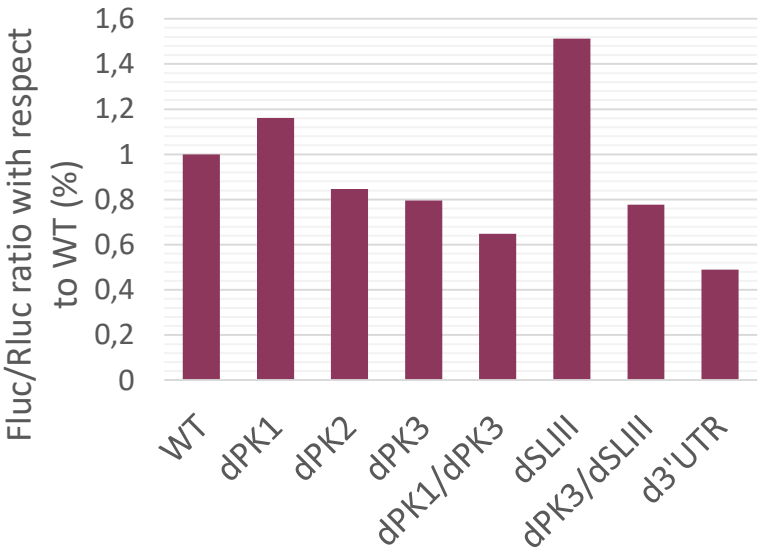
Deletion of the SL-III element causes a significant increase in the levels of translation with respect to the wild-type construction, suggesting that this could have a suppressive function of viral translation.



Deletion of the SL-III element enhances viral translation

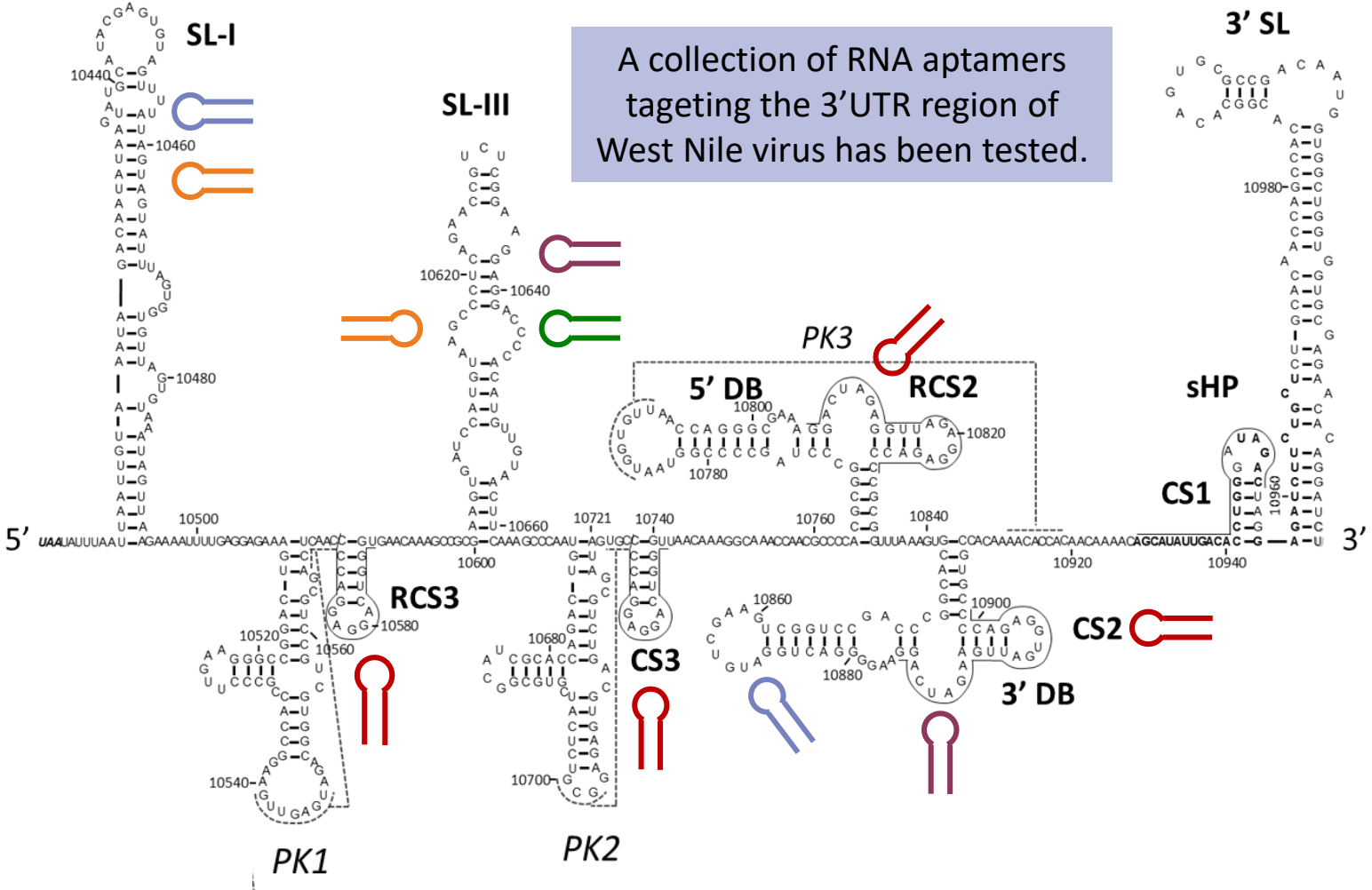




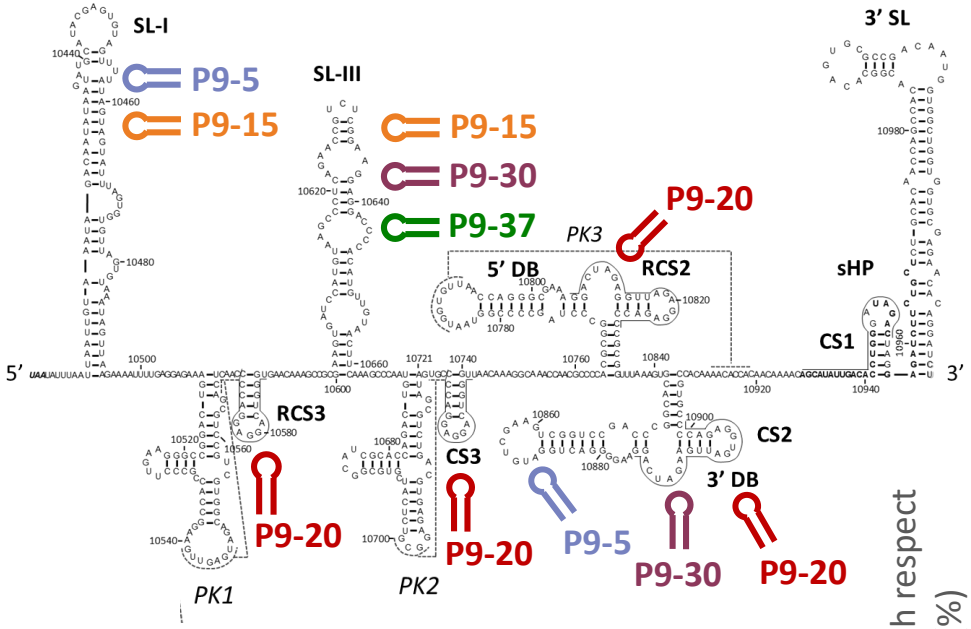


Aptamers that target the SL-III element enhance viral translation

A collection of RNA aptamers tageting the 3'UTR region of West Nile virus has been tested.



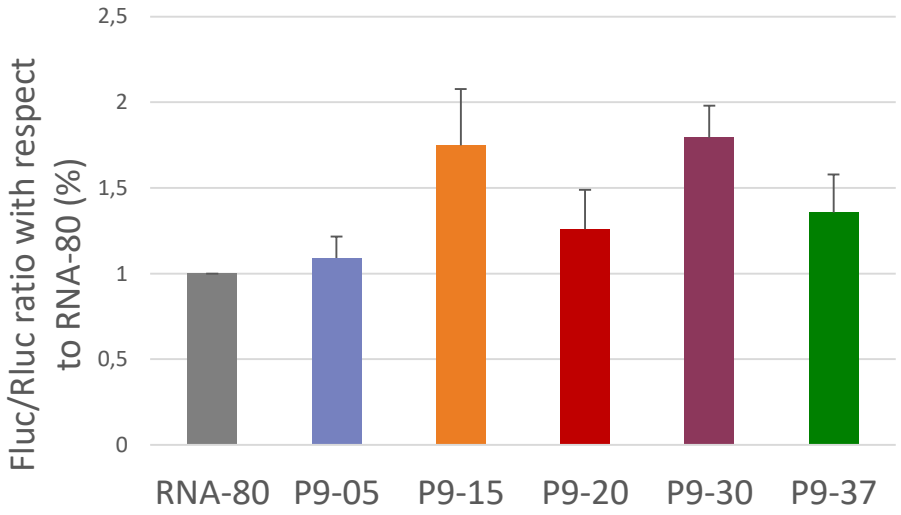
Aptamers that target the SL-III element enhance viral translation



Aptamers P9-15, P9-30 and P9-37 increased viral translation...

...All of them target the **SL-III element**.

The SL-III element could inhibit viral translation in order to promote the transition to the replication phase.



Conclusions

- The binding of the 40S ribosomal subunit to the 3'UTR region is **specific** and it seems to be a **general mechanism of translation regulation** of *Flavivirus*.
- In the 3'UTR of WNV the existence of **two binding sites to the 40S subunit** has been predicted. Binding of the 40S subunit leads a structural reorganization of the 3'UTR in which the **3'DB is a key element**.
- The binding between the 3'UTR of WNV and the 40S subunit is **mediated by ribosomal proteins**.
- The recruitment of the 40S subunit by the 3'UTR can be **a potential target for the development of specific treatments** against the virus.

Aptamers targeting specific elements of the 3'UTR efficiently interfere with the regulation of translation in WNV.

Acknowledgments

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PID2019-104018RB-100/ AEI /10.13039/501100011033