

# Interplay between viral transcription and host translation



**Gabriel Demo, PhD.**

Demo lab



**Central European Institute of Technology (CEITEC), Masaryk University**

National Institute  
of Virology and Bacteriology

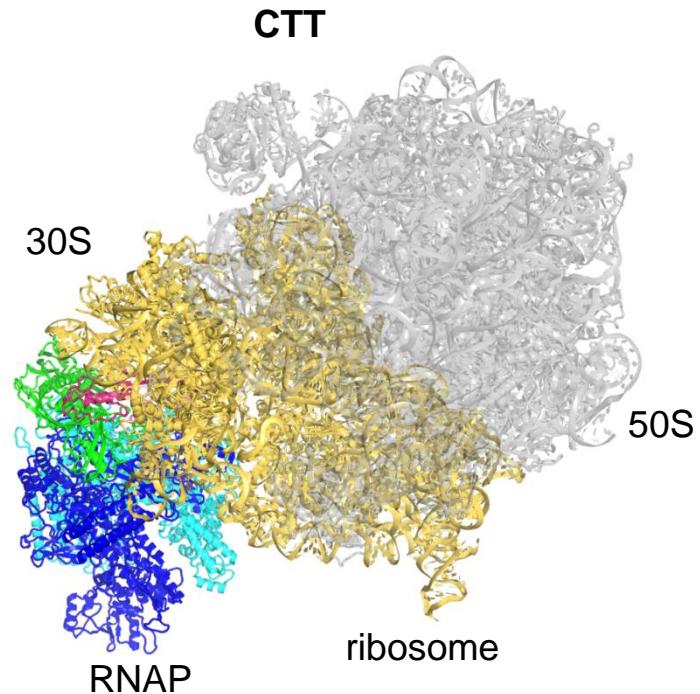
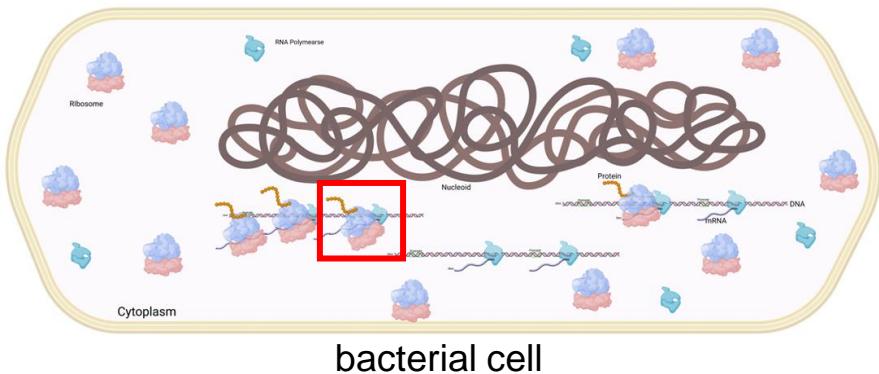


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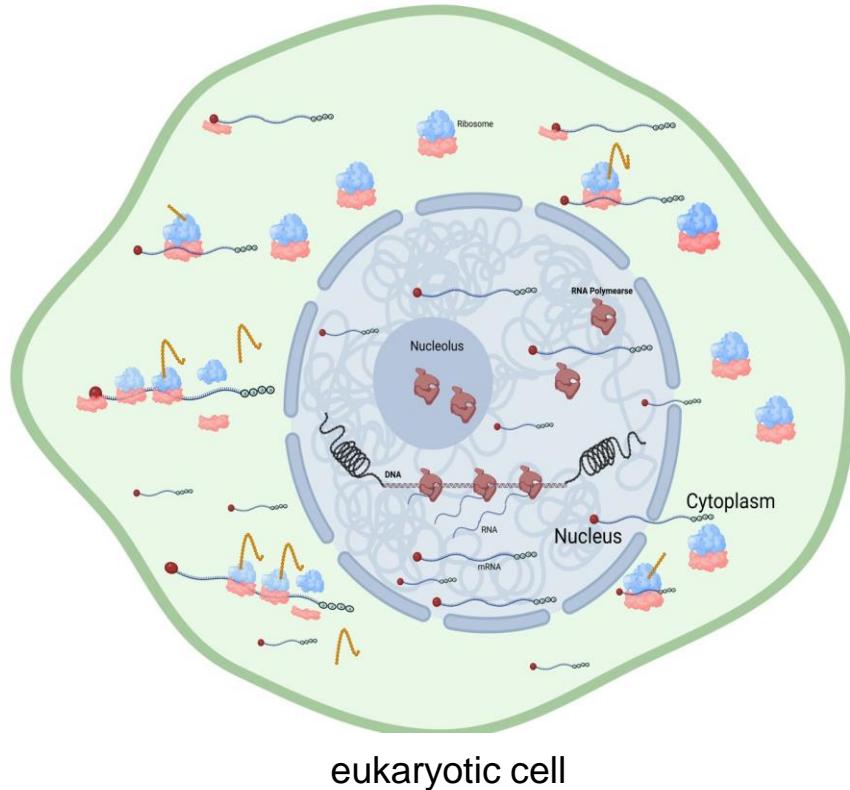
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# Bacterial Transcription-Translation Coupling (CTT)



Kohler R., et al. (2017). *Science*.  
Demo G., et al. (2017). *eLife*.  
Webster MW., et al. (2020). *Science*.  
Wang C., et al. (2020). *Science*.

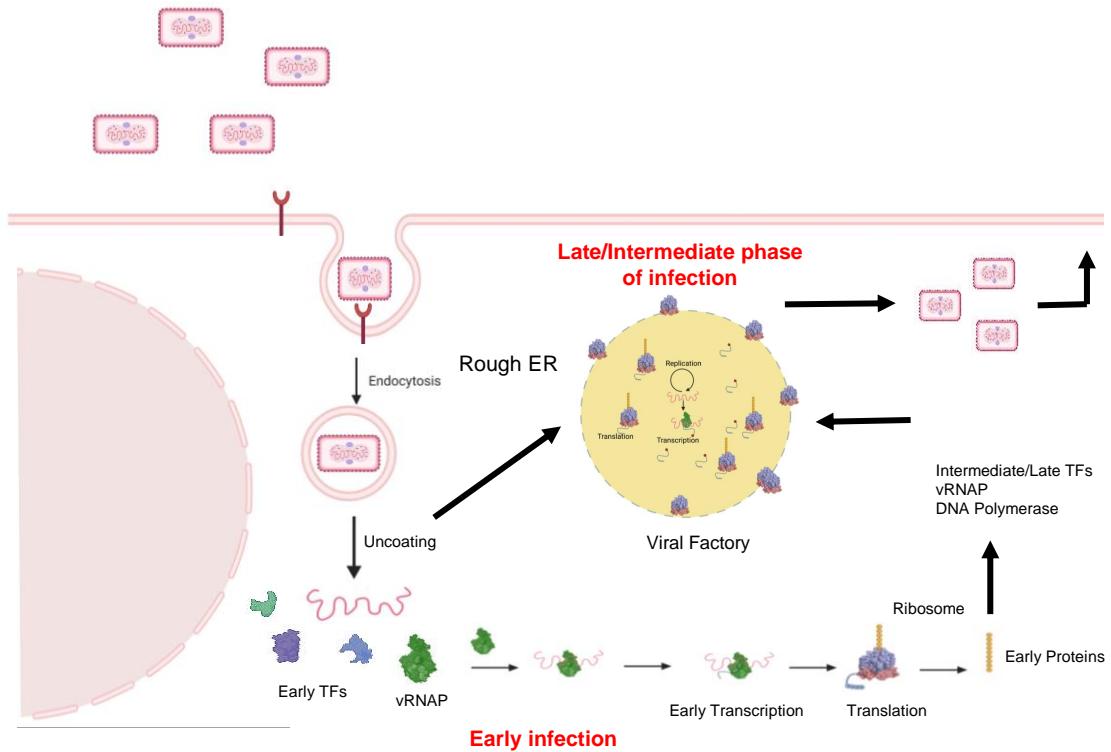
# Coupled transcription-translation in eukaryotes?



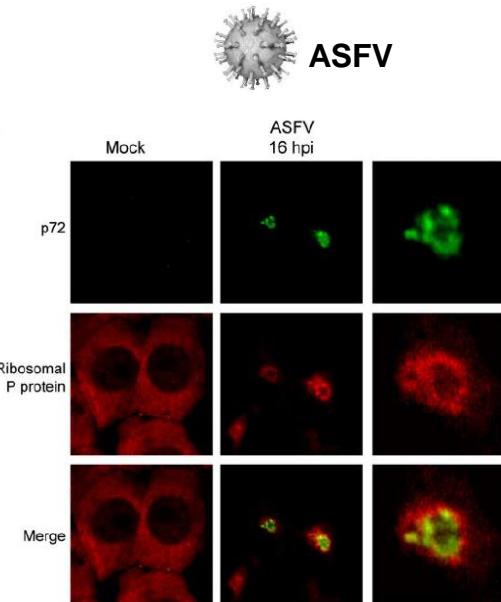
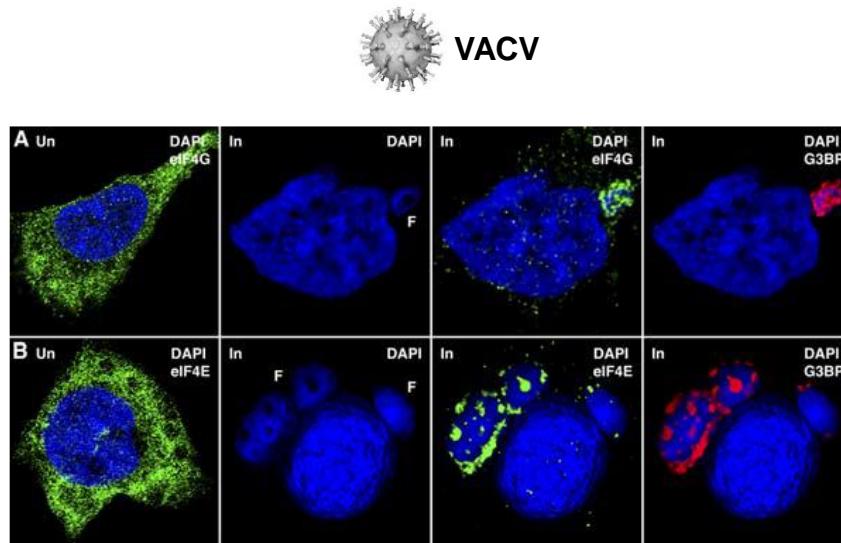
# Viral infection



poxviruses



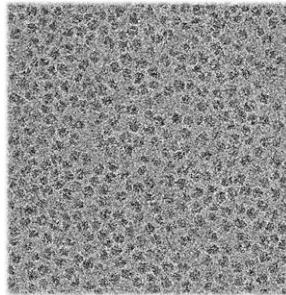
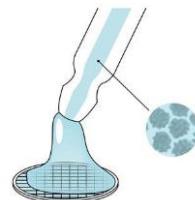
# Co-localization of host translation machinery in viral factories



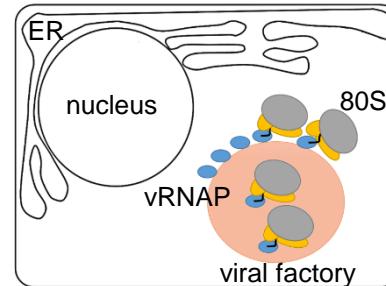
# Structural biology approach

## Single particle cryo-EM

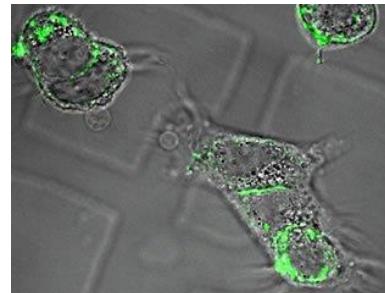
*In vitro* complex formation



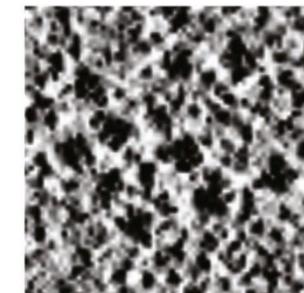
## cryo-ET



Virus-infected mammalian cell

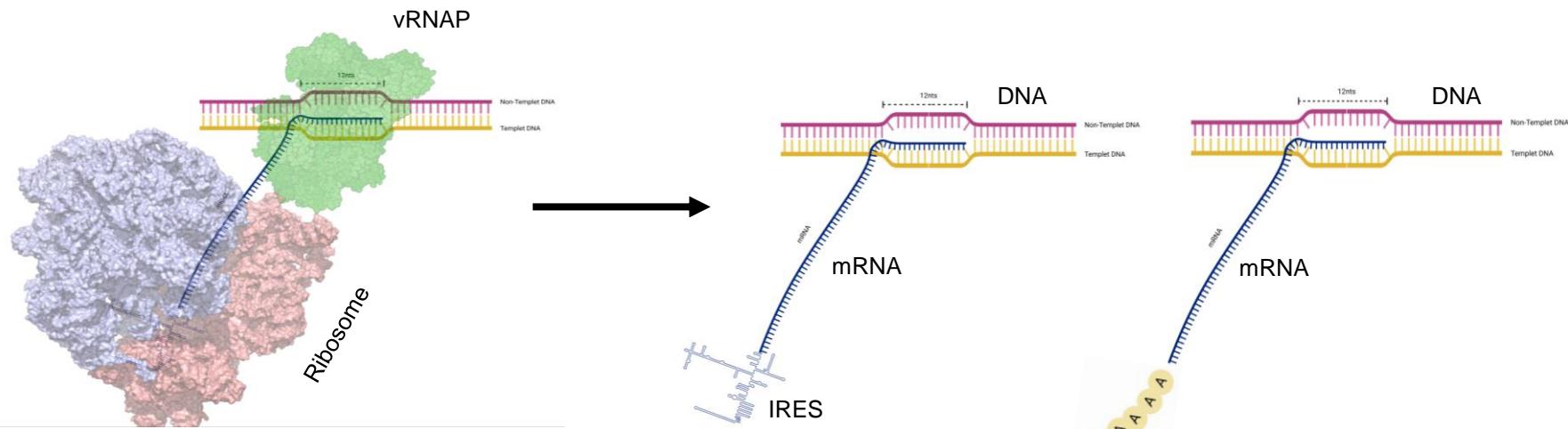


CLEM microscopy

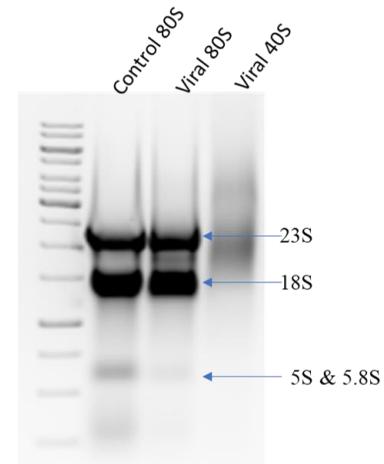
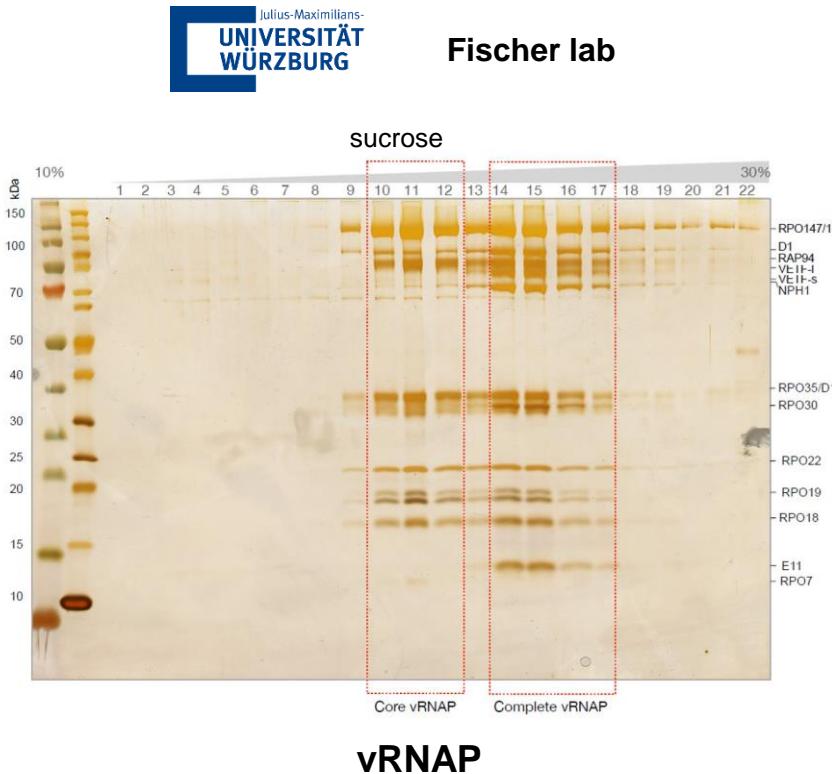
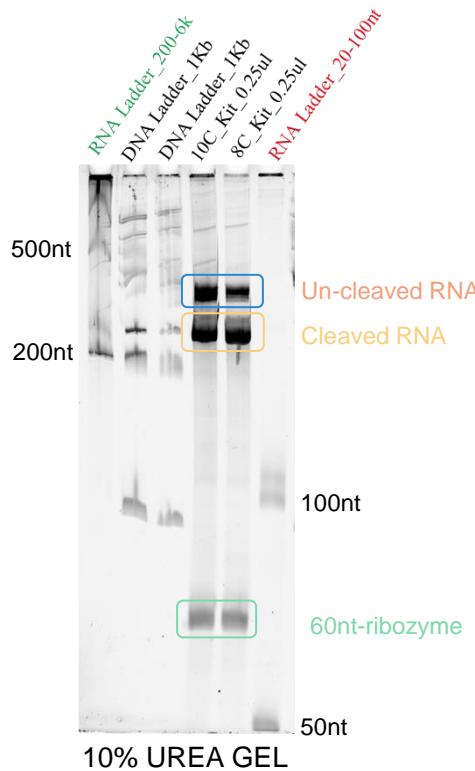


Tilt series tomogram

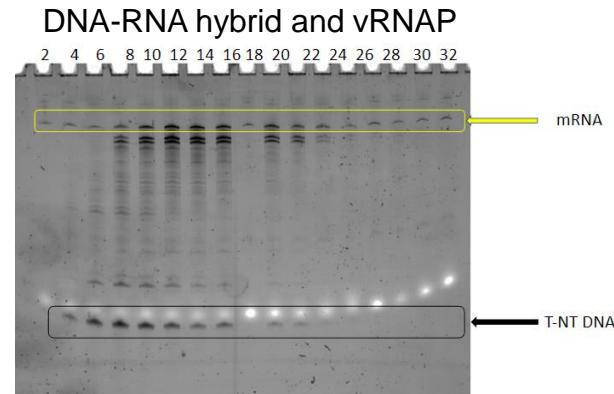
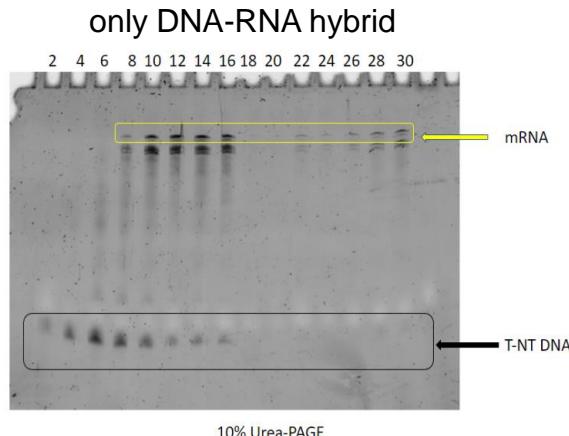
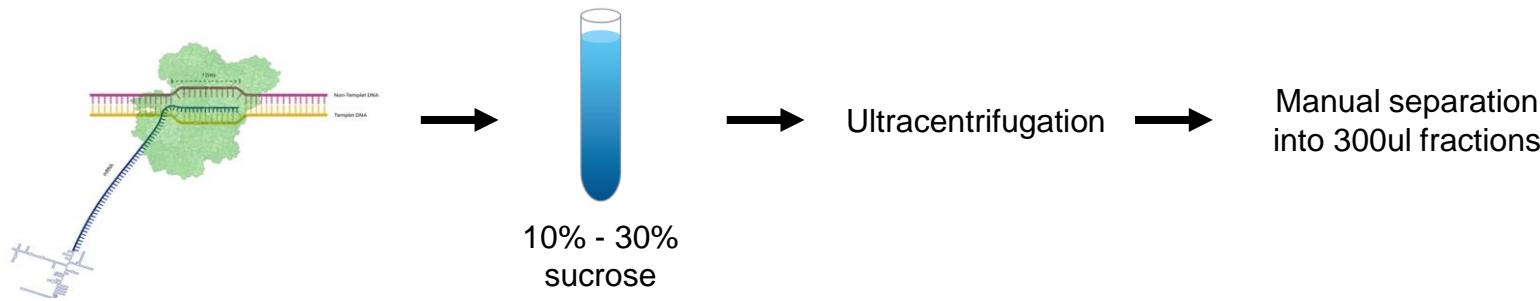
# *In vitro* reconstitution of CTT with vRNAP and ribosomes



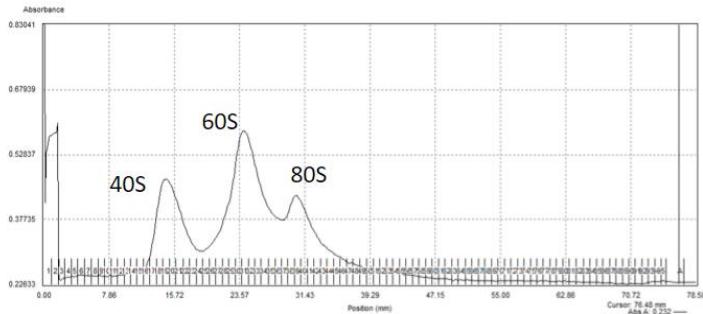
# *In vitro* reconstitution of CTT with vRNAP and ribosomes



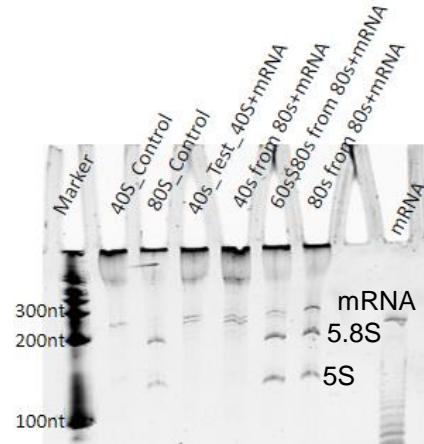
# Binding of vRNAP to the scaffold DNA and RNA



# Binding of the ribosome to the mRNA

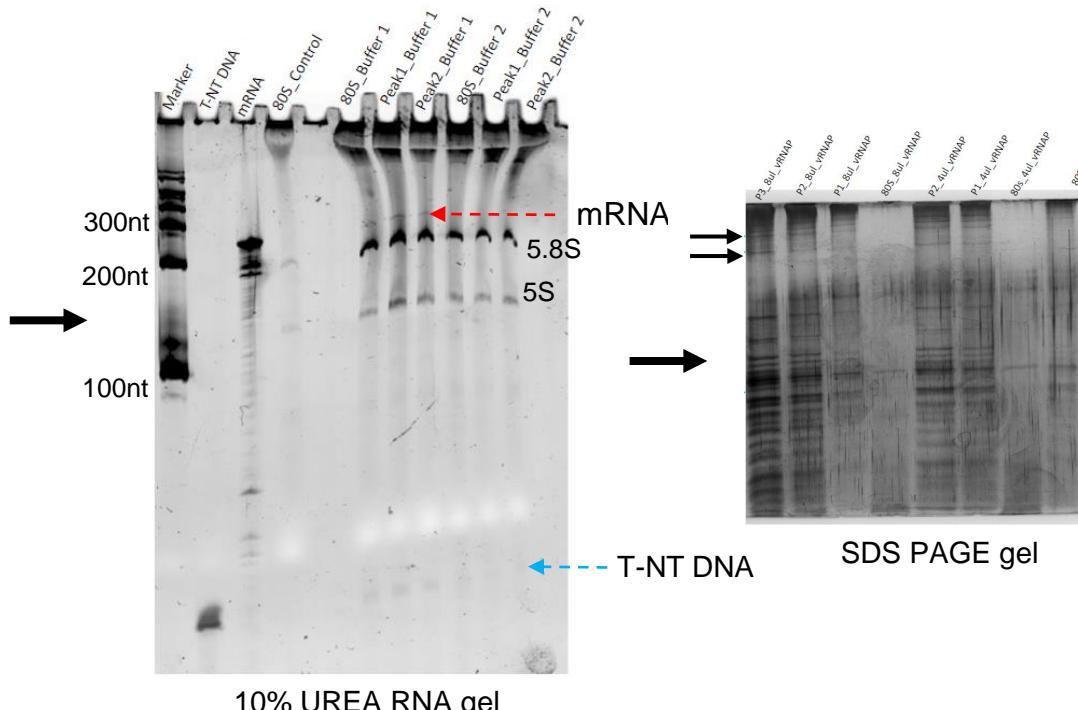
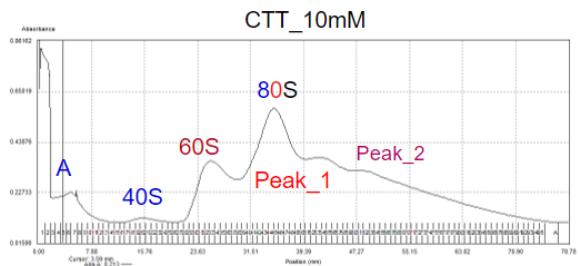


sucrose gradient profile for human ribosomes



10% UREA RNA gel

# *In vitro* reconstitution of CTT with vRNAP and ribosomes



NIVB



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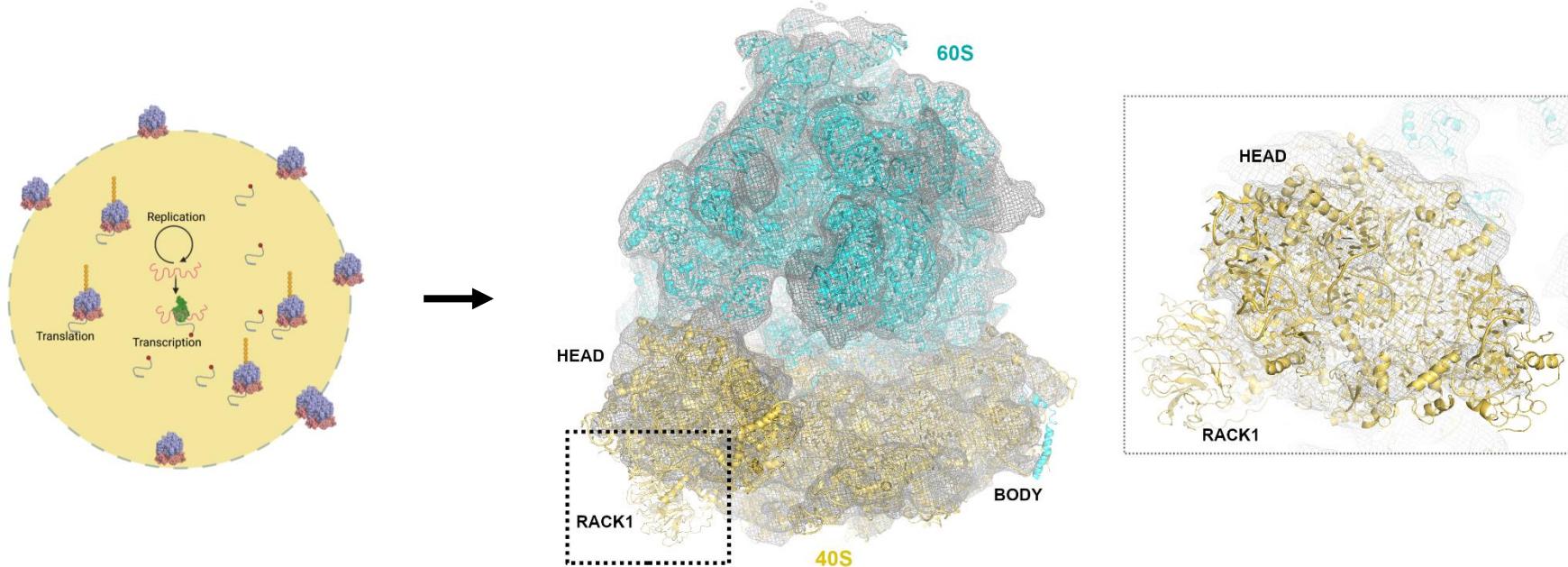
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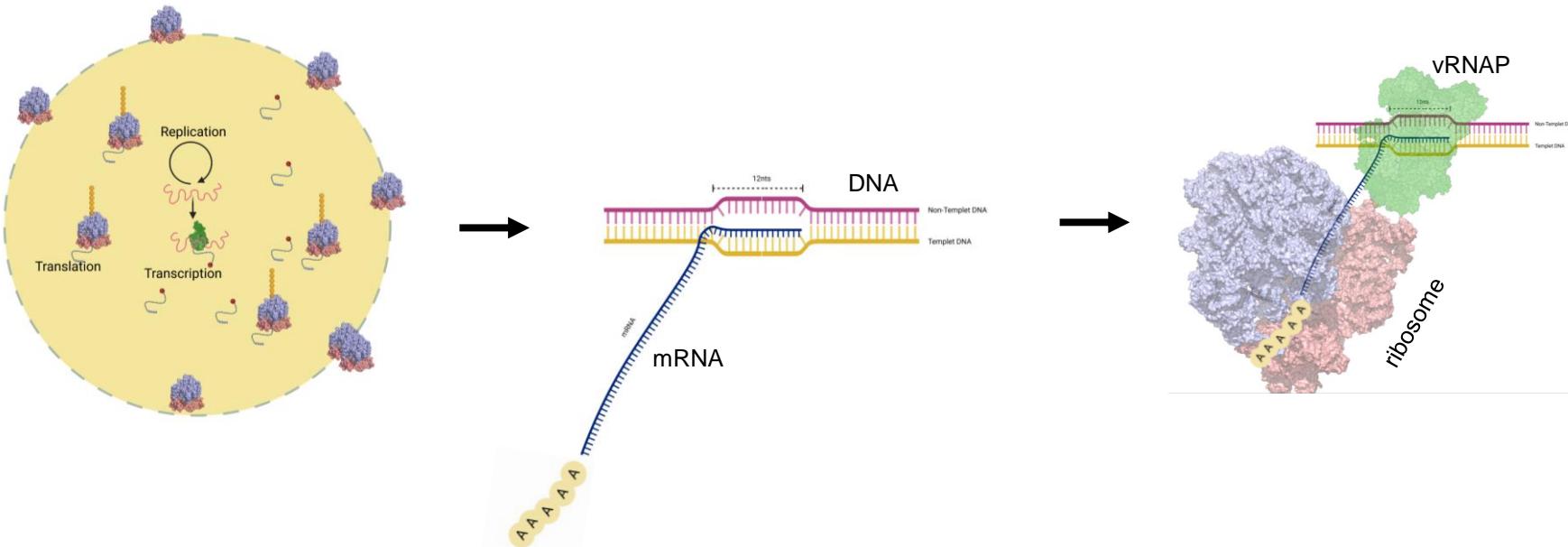
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# Ribosome modifications upon VACV late stage infection



# *In vitro* reconstitution of CTT – future plans



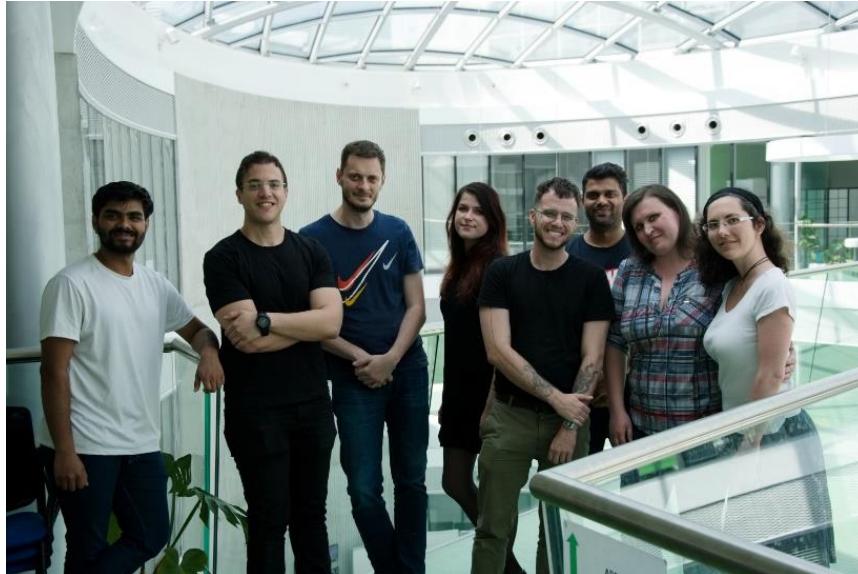
# Acknowledgement

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## Demo lab

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Sylva Brabencova  
Veronika Konopova  
Ludmila Vesela  
Hassan Zafar  
Ahmed Hassan  
Gabriel Soares  
Amiya Das



## Collaborations



## Fischer lab

Utz Fischer  
Julia Bartuli  
Clemens Grimm



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# Thank you!

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