ANRAGE – MIME 2007

BIOLOGIE & SANTÉ 2011

Structural dynamics of the rabies virus replication complex: search for new antiviral targets

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Background: Negative sense RNA viruses as human pathogens

Rabies virus causes a human brain disease, which rapidly leads to death if untreated. A vaccine and a post-infection treatment are available, but no treatment exists once the disease is declared. Because of efficient vaccination campaigns of dogs and wild animals, rabies is currently under control in Europe and North America. However, it still kills more than 50,000 people every year mainly in Africa and Asia. Developing a drug that blocks the replication of the virus would provide a useful complement to the current therapy.

Rabies virus, together with vesicular stomatitis virus, is prototype of the *Rhabdoviridae*. Members of this large family of viruses cause diseases of varying nature and gravity in animals and plants. Their genome is made of a single molecule of negative sense RNA. The Rhabdoviridae are are regrouped into the order *Monegavirales* with three other families which also include major human pathogens, the *Paramyxoviridae* (measles virus, RSV, Nipah virus,…), the Filoviridae (Ebola virus, Marburg virus) and the Bornaviridae (Borna disease virus). Vaccines are also available against some of these viruses, but no drug.

Although their interactions with host cells lead to very different pathologies, all viruses of the MNV share a common genome organization, encode a similar machinery for their transcription and replication, and share common mechanisms of transcription and replication. Therefore, identifying molecular targets or inhibitors that could block the replication of RAV could also prove useful for other viruses of the MNV.

Goals of the project

Our project aimed - at characterizing the molecular organization of the transcription/replication machinery of rabies virus (RAV) and vesicular stomatitis virus (VSV),
- at identifying molecular targets for developing inhibitors and
- at searching peptides or small molecules that inhibit viral replication

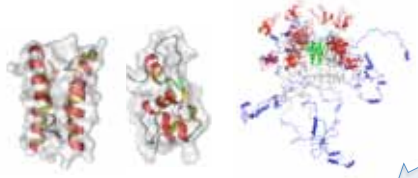
One part of the project was focused on studying the interactions between two proteins from the viral replication machinery of rabies virus and of vesicular stomatitis virus, the nucleoprotein (N) and the phosphoprotein (P). These proteins interact with each other at different stages of the replication process and they are amenable to biophysical and structural studies. In this work, we study the structure, the assembly and the dynamical remodeling of the phosphoprotein alone, of the complex formed between the N-RNA template and P, which plays a central role in the attachment of the polymerase on its actual template and of the RNA-free N°-P complex, in which P chaperones N and maintains it soluble and monomeric to encapsidate newly synthesized RNA genomes.

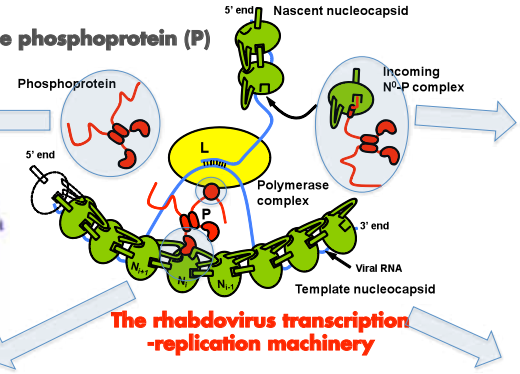
In the second part of the project, we sought to identify peptides or small organic molecules that interfere with these molecular processes.

Results

Structure and dynamical properties of the phosphoprotein (P)

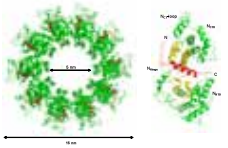
- ❑ P is dimeric protein (article 1)
- ❑ P is a modular protein (article 3)
- ❑ The N-terminal region of P is disordered but contains unstable α-helices (article 7)
- ❑ Structure of the central dimerization domain of RAV P (article 6) and of the C-terminal domain of VSV P (article 2)
- ❑ P is an intrinsically disordered protein





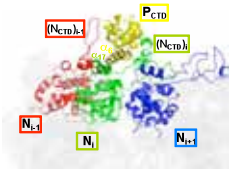
Structure of the N⁰-P complex

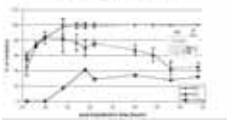
- ❑ Mimics of the N⁰-P complex can be reconstituted from purified components (article 8)
- ❑ The N-terminal region of P is a molecular recognition element (MoRE) which folds upon binding to N (articles 7 et 8)
- ❑ The crystal structure of the complex reveals how P prevents both RNA binding and assembly of N
- ❑ Solution studies show that the flanking regions of the MoRE of P remain flexible in the complex



Structure of the N-RNA-P complex

- ❑ RAV P or its C-terminal domain binds to circular N-RNA in a non-cooperative process
- ❑ Stoichiometry 2 P dimer/ ring of 10 N subunits
- ❑ Computational model by flexible docking of the complex
- ❑ The binding site of P on the N-RNA template is made of two adjacent subunits of N (article 4)
- ❑ Fluorescent test in 96 well-plate for measuring the binding of P on N-RNA complex





Peptides of P inhibit viral replication

- ❑ Localization of the L-binding region in P (article 5)
- ❑ Peptides containing the N-terminal MoRE of P (P_{57}, P_{60}) inhibits the transcription/replication of a rabies minigenome encoding luciferas in BHK-21-T7 cells (article 5)
- ❑ Inhibition of infection in BHK-21-T7 and in neuronal cells
- ❑ *Phage display was used for selecting peptides that binds to N-RNA (article 9)*

Conclusions

The work carried out in this research project led to:

- ❑ a detailled structural characterization of the components and complexes involved in the replication complex of the rhabdoviruses, which provides new insights on the mechanisms of RNA synthesis and open new routes for studying these mechanisms.
- ❑ the structural characterization of two different complexes formed between the nucleoprotein (N) and the phosphoprotein (P) that are essential for viral replication and can thus serve as targets for designing inhibitors
- ❑ the discovery that peptides encompassing the MoRE of P inhibit transcription of a minigenome as well as the replication of the virus in infected cells

Publications

Articles

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3.Modular Organization of Rabies Virus Phosphoprotein. Gerard F.C.A., Ribeiro E.A., Leyrat C., Ivanov I., Blondel D., Longhi S., Ruigrok R.W. and Jamin M. (2009) J. Mol. Biol. 388, 978-996.

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5.Peptides that mimick the amino terminal end of the rabies virus phosphoprotein have antiviral activity. Castel G, Chtéoui M, Caignard G, Prehaud C, Méhouas S, Réal E, Jallet C, Jacob Y, Ruigrok R and Tordo N. (2009) J Virol. **83**, 10808-20.

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10. Damnacanthal: new potential antibodies compounds. Chtéoui M, Belloti A, Castel G, Jallet C, Hani K & Tordo N. (2011) en revision.

Reviews

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R2. Le Phage Display: une nouvelle arme pour la recherche antivirale. Castel G, Heyd B & Tordo N. (2009) Virologie. **13**, 93-102.

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R5. Rabies virus transcription and replication. Albertini AA, Ruigrok RW, Blondel D. Adv Virus Res. (2011) **79**, 1-22.

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