Funded PhD project in Integrated Structural & Cell Biology in Grenoble, France (2024-2027)

Title of the PhD project: Molecular mechanism of avian influenza virus inhibition by the human restriction factor MxA

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Host team: Viral Replication Machines - Team Crépin
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Project summary:
The interferon induced myxovirus resistance (Mx) proteins are powerful factors restricting a broad range of viruses. Human MxA specifically inhibits avian Influenza A viruses, preventing zoonotic infections into the human population through interaction with the viral nucleoprotein. The aim of this project is to elucidate the molecular mechanism of influenza virus inhibition by MxA. The current models suggest that (i) MxA oligomerizes in ring-like structures around the viral ribonucleoprotein (vRNP) preventing them to enter the nucleus, and (ii) disassembled MxA captures newly synthetized nucleoprotein (NP) in the cytoplasm, impeding the formation of progeny vRNPs. We have developed a reconstituted nucleocapsid from recombinant NP and synthetic RNA (RNP-like), and an engineered short homogeneous vRNP (shRNP), these tools are far better suited for biochemical and structural characterization than the natural vRNP (little amount of material from infected cells, heterogenous sample, etc.). In this project, we intend to combine biochemical, biophysics and electron microscopy techniques to characterize MxA reversible oligomerization in vitro, and to characterize the interaction between MxA and NP/RNP, using the RNP-like and shRNP tools, as well as RNA-free NP. We expect this work will help better understanding the risks of Influenza virus evolving to escape human innate immunity upon inter-species transmission, and may open-up novel antiviral strategies that mimic the natural restriction factor mechanism.

Preferred skills: Biochemistry and structural biology

Student role: The PhD candidate will be in charge of the operational aspect of the project. They will produce the proteins of interest expressed in bacteria or in the baculovirus-insect cells system (MxA). They will also use molecular biology and conceive the necessary tools to generate mutations and express the corresponding recombinant proteins. They will run the different biochemical and biophysical assay to assess the oligomeric states and to investigate the interaction between the protein or nucleoprotein of interest with the support of the ISBG facilities. They will be trained with EM techniques (from negative staining to image analysis collected on a cryoEM microscope), they will carry out EM experiments and generate 3D reconstructions. Finally, they will design functional mutations to be tested by our collaborator at the Institut Pasteur. Additionally, they will be involved in the writing of the publication(s) and oral presentation during meetings and conferences.

Keywords: influenza virus, restriction factor, antiviral activity, viral escape, cryoEM

Relevant publications of the team:

1. *Cryo-EM structure of influenza helical nucleocapsid reveals NP-NP and NP-RNA interactions as a model for the genome encapsidation.* Chenavier F, Estrozi LF, Teulon JM, Zarkadas E, Freslon LL, Pellequer JL, Ruigrok RWH, Schoehn G, **Ballandras-Colas A**, **Crépin T**. (2023) *Sci Adv*. Dec 15;9(50):eadj9974.
2. *X-ray structure of the human karyopherin RanBP5, an essential factor for influenza polymerase nuclear trafficking.* Swale C, Da Costa B, Sedano L, Garzoni F, McCarthy AA, Berger I, Bieniossek C, Ruigrok RWH, Delmas B and **Crépin T.** (2020) *J. Mol. Biol.* 432(10):3353-3359.
3. *Differential behaviours and preferential bindings of influenza nucleoproteins on importins-α.* Donchet A, Vassal-Stermann E, Gérard FCA, Ruigrok RWH and **Crépin T** (2020) *Viruses*, 12, 834.
4. *Destabilisation of the human RED-SMU1 splicing complex as a basis for host-directed anti-influenza strategy.* Ashraf U, Tengo L, Le Corre L, Fournier G, Busca P, McCarthy AA, Rameix-Welti M-A, Gravier-Pelletier C, Ruigrok RW, Jacob Y, Vidalain P-O, Pietrancosta N, **Crépin T** and Naffakh N (2019) *Proc Natl Acad Sci USA*, 116:10968-10977.
5. *The structure of the nucleoprotein of Influenza D shows that all Orthomyxoviridae nucleoproteins have a similar NP$_{CORE}$, with or without a NP$_{TAIL}$ for nuclear transport.* Donchet A, Oliva J, Labaronne A, Tengo L, Miloudi M, Gérard FCA, Mas C, Schoehn G, Ruigrok RW, Ducatez M and **Crépin T** (2019) *Sci Rep*, 9:600.

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