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Fondation Innovations en Infectiologie

Investigating the Molecular Basis of Paramyxoviral Replication

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The aim of this project is to decipher the molecular mechanisms underlying RNA replication of negative strand RNA viruses form the Mononegavirales order. Viruses of this order have a single negative sense RNA molecule as genome and include numerous human pathogens such as measles virus, rabies virus or respiratory synctical virus. In this project, we propose to work on measles virus, that kills nearly half a million people each year, as well as Sendai virus a model virus of the same family. Although viruses from the Mononegavirales order have different modes of infection and are responsible for very different pathologies, they share a similar genome organization and a similar RNA replication machinery. By studying the RNA replication machinery we hope to gain detailed information that will help in the design of novel antiviral strategies.

In both viral and infected cells, the genomic RNA is protected by multiple copies of the nucleoprotein (N) that forms a helical nucelocapsid.[1] RNA replication and transcription are performed by a viral RNA-dependent RNA polymerase that uses this nucelocapsid as a template. The polymerase is composed of the large (L) protein) and of its essential cofactor the phosphoprotein (P). The P protein plays a key role in RNA synthesis, tethering the polymerase to the nucleocapsid template, while the L protein carries the polymerase activity.

The molecular processes involved in the replication of measles and Sendai viruses are however extremely complex. N is composed of a highly conserved core region (residues 1-400), necessary for the interaction with RNA, and a flexible C-terminal domain (NTAIL, amino acids 400-525). Protein P is a tetrameric modular protein comprising both folded and unfolded domains. P interacts with N via a highly dynamic interaction involving a flexible domain of P and NTAIL, an interaction that is critical for positioning of the polymerase complex on the N-RNA matrix. Both domains belong to the family of intrinsically disordered proteins (IDPs) whose importance is becoming increasingly clear for a vast range of cellular processes.[2] While the inherent flexibility of IDPs obviates the use of X-ray crystallography, nuclear magnetic resonance (NMR) has evolved into one of the most powerful techniques for studying the conformational properties of this family of proteins. NMR provides an amino acid specific description that can be powerfully combined with small angle scattering (SAS) to develop a complete description of the molecular behaviour of the unfolded state.[3] In addition to characterising the proteins in their free forms, we will use approaches developed in the coordinating group to map the interaction between the phosphoprotein and the nucleoprotein. NTAIL is thought to fold only upon binding to the polymerase, and NMR and SAS will be used to follow the conformational changes accompanying this interaction, to identify the role played by the disordered regions of the proteins in the molecular recognition process. This interaction will be studied in situ, in the context of the intact nucleocapsid.

[1] Schoehn, G., Mavrakis, M., Albertini, A., Wade, R., Hoenger, A., Ruigrok, R.W.H. (2004). *J.Mol.Biol.* 339, 301

[2] Fink, A.L. *Curr Opin Struct Biol.* (2005) 15, 35

[3] Bernado, P., Blanchard, L., Timmins, P., Marion, D., Ruigrok, R., Blackledge M. (2005) *Proc. Natl. Acad. Sci.* 102, 17002

The structure of the folded domain of N protein of measles virus is unknown, and has resisted crystallization. Remarkable progress in solid state (SS) NMR in recent years has raised the prospect of studying the structure and dynamics of proteins in a supramolecular
environment such as the nucleocapsid helix. Using techniques pioneered in the laboratory of partner 3,4 we therefore plan to study the structure and dynamics of the N protein in the context of the entire nucleocapsid. This project combines state-of-the-art methodology to develop a complete description of the activity of the replication machinery in conditions approaching the physiological environment.

[4] Pintacuda G, Giraud N, Pierattelli R, Böckmann A, Bertini I, Emsley L. *(*2007) *Angew Chem Int Ed* 46,1079.