Soutenance

THESE

Mardi 23 Mai 2017 à 14h

Salle des séminaires

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Biochemical and mass spectrometry analysis of an HIV-1 ribonucleoprotein export complex

Thèse de Doctorat de l'Université de Grenoble

An important step in the life cycle of human immunodeficiency virus (HIV) is the nuclear export of incompletely spliced viral transcripts, including the replicated viral RNA genome. This process is mediated by the viral RNA-binding protein Rev. In the nucleus, Rev recognizes unspliced and partially spliced viral transcripts by multimerizing on a 350-nucleotide intron sequence, the Rev-response element (RRE). Rev then recruits the host cell export factor CRM1 and the small GTPase Ran to form the RRE/Rev/CRM1/Ran export complex. Knowledge of the 3D architecture of this ribonucleoprotein complex would provide important insights into how unspliced viral RNA export is achieved. However, the molecular details of this complex are poorly understood. In particular, the stoichiometry of Rev and CRM1 molecules bound to the RRE is under debate.

My Ph.D. project aims to investigate the architecture of the RRE/Rev/CRM1/Ran complex. As part of this work, I used biochemical and cell-based assays to characterize the interactions between CRM1 and Rev and between Rev and the RRE. The majority of my efforts focused on investigating these interactions by native mass spectrometry (MS), a powerful method for determining the stoichiometry of macromolecular complexes. I set up protocols for the large-scale preparation of a 66-nucleotide RRE fragment (IIABC) bearing a high-affinity Rev binding site, and adapted these for compatibility with native MS analysis. Because Rev tends to aggregate and precipitate in solution, I engineered a mutant form of Rev (Rev*) to overcome this problem. Analysis of IIABC/Rev* complexes by native gel electrophoresis confirms multimerization of Rev on the RNA. After extensive optimization, I obtained high-quality native MS spectra of these complexes, revealing that IIABC binds up to 6 Rev* monomers. Furthermore, I reconstituted a 4-species complex, IIABC/Rev*/CRM1/Ran, and succeeded in determining its mass and stoichiometry by native MS – a technically challenging task. Additional efforts at analyzing the intact RRE and complexes with wild-type Rev have also yielded informative spectra, while analysis of the intact RRE/Rev/CRM1/Ran holo-complex has had more limited success. These results illustrate the strengths and limitations of native mass spectrometry and its potential for future development as a tool for analyzing ribonucleoprotein complexes.