Titre du stage: Structural and functional characterization of Pestivirus non-structural protein 2, 3 and 4

Résumé:
Introduction

Pestiviruses infect a wide range of cloven-hoofed animals, wild and domestic, causing serious disease. The most studied are the classical swine fever virus (CSFV) (1) and the bovine viral diarrhea virus (BVDV), which impose important economic losses to the livestock industry worldwide (2). They form a genus within the Flaviviridae family of single-stranded RNA viruses, which also includes medically important pathogens in the flavi- and hepacivirus genera. The pestivirus genome is a single mRNA molecule of about 12.3 kb with a single large open reading frame (ORF) coding for a polyprotein precursor of about 3,900 residues. This long ORF is flanked by 5’ and 3’ untranslated regions with cis-active elements essential for virus translation and replication (2). The polyprotein is co- and post-translationally processed by cellular and viral proteases to yield the individual mature viral proteins. The nonstructural proteins NS2 through NS5B are present only in infected cells and are essential for virus replication. Among them, NS2 (a cysteine protease), NS5B (the RNA dependent RNA polymerase), and NS3 have enzymatic activities. NS3 has a molecular mass of about 76 kDa and features two main functional domains, each with different enzymatic activities. The N-terminal domain is a chymotrypsin-like serine protease (NS3p), which is responsible for most of the maturation cleavages of the polyprotein precursor in the cytosolic side of the endoplasmic reticulum membrane; the exception is the NS2/NS3 junction. The C-terminal domain, about two-thirds of NS3, is a helicase belonging to superfamily 2 (SF2) that displays characteristic sequence motifs that constitute the SF2 signature. NS3 helicase domain (NS3h) features two conserved RecA-like domains (D1 and D2) with ATPase activity, plus a third domain (D3) that is important for unwinding nucleic acid duplexes. Despite the fact that several NS3 enzymes have been characterized both structurally and functionally for different members of Flaviviridae, i.e., the hepatitis C virus (HCV) and dengue virus (DENV), important questions remain. For instance, how do positive-strand RNA viruses switch from RNA replication to virion morphogenesis, and what is the exact role of the non-structural viral proteins in virion morphogenesis.

Project
This project follows on our recent results, where we have functionally and structurally characterized the helicase domain from the pestivirus CSFV (pNS3h) (3), as well as the full-length enzyme, containing also the protease domain and the NS4A cofactor (manuscript in preparation). In collaboration with Norbert Tautz (Lübeck, Germany), we are now characterizing the NS2/NS3 interaction interface. For pestiviruses, and in contrast to the related HCV, uncleaved NS2-3 represents an essential factor for virion morphogenesis while free NS3 is an essential component of the viral replicase. Our collaborators recently discovered that pestiviruses can adapt to virion morphogenesis in the absence of uncleaved NS2-3 by just two amino acid exchanges, one in NS2 and the other in NS3 protease (4). Analysis of our structure, indicate that in NS3, the mutation is located in NS3 protease domain within NS3-NS4A interface (data not shown). Together with functional data designed upon our structure, we have preliminary results that suggests that a segment in NS4A domain acts as a dynamic regulator of the NS3-NS4A interaction and could represent a molecular switch that controls RNA replication and virion morphogenesis (data not shown). The student will participate in the characterization of the NS2/NS3 complex, and in its crystallization.
References


