Project 2: Interaction of the Hepatitis C virus protein NS5A with tyrosine kinase SRC in the context of the viral replication complex

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Background: Hepatitis C virus (HCV) is a human pathogenic flavivirus, which accounts for a large proportion of patients with chronic hepatitis [1]. The virus-encoded nonstructural protein 5A (NS5A) plays an important role both in the replication of viral RNA and in the production of new virions. In fact, NS5A represents the target structure of several modern direct-acting antivirals (DAAs), although its molecular mechanism is still poorly understood [2]. In addition to an N-terminal amphipathic α-helix, three domains have been described in NS5A, only the first of which (D1) features a stable tertiary structure. The protein occurs in a basally phosphorylated and a hyperphosphorylated form, which differ in terms of function. While serine/threonine phosphorylation of NS5A is relatively well characterized, data on tyrosine phosphorylation and its physiological relevance is still scarce.

Own previous work: We have shown previously that replication of viral RNA in human hepatoma cells requires an interaction of NS5A and NS5B with SRC, a host cell tyrosine kinase [3]. Specifically, association with NS5A depends on an intact SH2 domain of SRC, which displays significant affinity for tyrosine phosphorylated NS5A-D1. Binding studies using synthetic peptides as well as modified D1 constructs uncovered Y93 as the critical phosphorylation site. The relevance of Y93 for the function of NS5A could be confirmed in cell culture systems [4].

Aim of the project: Following up on previous studies, we intend to investigate the architecture of the HCV replication complex in detail.

Work program: (i) *In vitro* tyrosine phosphorylation of NS5A using heterologously expressed tyrosine kinases, e.g. human SRC. The toxicity of active tyrosine kinases for bacterial expression hosts can be alleviated by fusion to a tyrosine phosphatase, which can be cleaved off later during the purification process. (ii) Biophysical characterisation of the interaction of *in vitro* phosphorylated NS5A with SRC (e.g. using BLI, SPR, ITC), possibly including DAAs like daclatasvir. (iii) Determination of the 3D structure of SRC:NS5A, primarily via X-ray crystallography. For crystallisation various constructs can be envisaged, including synthetic peptides in case of NS5A. (iv) Structural characterisation of a minimal replication complex containing NS5B and the viral RNA template, in addition to NS5A and SRC. For this purpose cryo-electron microscopy will be pursued in parallel to X-ray crystallography. (v) Visualisation of the localisation of NS5A and SRC in a cell culture system using, amongst others, superresolution fluorescence microscopy.

[1] Webster DP et al, Lancet 385, 1124–1135 (2015).
[2] Ross-Thriepland D and Harris M, J Gen Virol 96, 727–738 (2015).
[3] Pfannkuche A et al, Hepatology 53, 1127–1136 (2011).
[4] Klinker S et al, J Biol Chem 294, 7388–7402 (2019).