MOI Project 1

Comparative studies of regulatory sequence elements for RNA processing in HIV-1 and Influenza A

(Supervising Investigator: Dr. Lisa Müller, Institute for Virology, HHU Düsseldorf)

The investigation of RNA processing mechanisms plays a pivotal role in understanding viral pathogenesis. RNA viruses have a limited genome size and have therefore developed common mechanisms to efficiently utilize their genetic information to generate all components required for efficient replication. RNA viruses such as HIV-1 and Influenza A, despite their different genome structure and nuclear replication, share alternative RNA splicing as such a common mechanism. Interestingly, there are molecular intersections between alternative splicing and the subsequent nuclear export of viral mRNA that is regulated by a common set of protein interaction partners. Cis-regulatory RNA sequences provide binding sites for these regulatory proteins, with two protein families, the SR and hnRNP proteins, being the main interaction partners. Both protein families can act in a position dependent manner on the recognition and usage of splice sites. Their association with nuclear export is based on the almost exclusive localization of hnRNPs in nuclear fractions and their involvement in retention of transcripts in the nucleus, members of the SR protein family such as SRSF1, SRSF3 and SRSF7 have been identified as positive factors in nuclear export as they can shuttle into the cytoplasm. Both protein families regulate the recognition and utilization of splice sites and have also been shown to play a role in nuclear mRNA export. Thus, these regulatory elements are considered to have a dual function in RNA processing. Therefore, their detailed characterization is of particular interest.

The aim of this project is the bioinformatic and experimental mapping of regulatory RNA sequence motifs in a comparative between the HIV-1 and Influenza A genome. Since such regulatory sequence elements and their protein binding partners can have both splicing and export regulatory functions, the functional analysis of the sequence elements will consider the influence on both processes.

The project requires a detailed comparative bioinformatic analysis of the HIV-1 and Influenza A genomes with a focus on spliced transcripts in to map potential regulatory sequence elements. The identified elements will be characterized in a set of subgenomic reporters with different readout options to test their involvement in RNA processing. To further characterize the sequence elements, corresponding protein binding partners will be identified to understand their mode of regulation. In addition, the natural occurrence of mutations in the identified sequence elements will be investigated by analysing sequencing results of different isolates available at the Institute of Virology. Both, wild type and variant version of the regulatory elements will be analysed in the presence of splicing and export inhibitors in transfection and infection experiments to analyse their influence on susceptibility towards potential inhibitory substances.

Overarching goal of the project is the identification of regulatory sequence elements that are shared between RNA viruses and commonly influence RNA processing. Characterization of the mode of action of such sequence elements might pave the way for the identification of inhibitory substance that interfere with viral RNA processing.