Project 1: The nuclear export of unspliced and intron-containing HIV-1 RNA

(Supervising Investigator: Heiner Schaal)

Background: The ability of splice sites to retain mRNAs in the cell nucleus under certain conditions has been demonstrated for papillomavirus constructs [1], the main 5' splice site of EIAV [2], and in HIV 1 env expression vectors [3, 4]. In general, retroviral sequences that negatively affect the nuclear export of RNAs, their stability or their translatability are called INS (instability) or CRS (cis-acting repressor sequences) elements [5-12]. In addition to splice sites, other non-sequence-homologous regions in the HIV-1 gag, pol and env genes have been described as inhibitiors of gene expression [5, 6, 13]. In the case of an HIV-1 gag fragment of less than 300 bp, the activity of the cis-repressive sequence was only inactivated by a large number of point mutations [11], indicatng the complex nature of such CRS elements.

In viral genomes, certain RNA regions form extensive secondary structures, which can counteract the inhibitory effect of such INS/CRS elements. In simple retroviruses, such as the Mason-Pfizer Monkey Virus (MPMV) [14], the Simian retrovirus type 1 (SRV-1) [15], and type 2 (SRV-2) [16], or the Rous Sarcoma Virus (RSV) [17], these RNA regions are called constitutive transport elements (CTE) since they can perform their function without virus-encoded proteins. In the case of complex retroviruses, these RNA regions only show their effect in interaction with virus-encoded regulatory proteins. Corresponding to the regulatory protein, the target sequence is called Rev Responsive Element (RRE) in HIV-1 or Rex Responsive Element RxRE in HTLV I [18].

To date, no sequence similarities between such INS/CRS elements have been identified, which is why the molecular cause of RNA retention in the cell nucleus is still unclear. More recent work is only concerned with the question of how to counteract the retention of such HIV-1 RNAs in the cell nucleus by increasing the functional activity of RRE [19].

Own previous work: In the course of developing a diagnostic tool to predict silent mutations that affect HIV-1 replication fitness (MOI III), we made an observation that opens up a completely new approach to elucidate the retention of intron-containing HIV-1 RNAs in the nucleus. Using the algorithms we developed in recent years to evaluate the intrinsic strength of splice donors [20, 21] to create a hexamer profile that provides information on splice-promoting or splice-reducing properties of viral (MOI II) and cellular sequences [22, 23], we can overlay the described HIV-1 INS/CRS elements with specific target sequences of RNA-binding proteins. The observation that the HIV-1 INS/CRS elements can be superimposed on putative hnRNP "target sites" over remarkably large areas suggests that the long-sought mechanism that retains incompletely processed RNAs in the nucleus is related to the hnRNP occupation of the sequence segments retained in the nucleus.

Aim of the project: As a retrovirus, HIV-1 integrates its proviral DNA into the host DNA. Its genetic information is thus completely subject to the rules of cellular gene expression. According to these rules, the unspliced genomic HIV-1 RNA is retained in the cell nucleus and only the spliced RNA is actively transported into the cytoplasm. For both replication and gag and gag/pol RNA, it is, however, inevitable that the unspliced HIV-1 RNA also reaches the cytoplasm in order to be packaged as genomic RNA into new virus particles on the one hand and translated as mRNA on the other. The mechanism by which the unspliced HIV-1 RNA can be transported from the cell nucleus into the cytoplasm has been the subject of numerous studies. Thus, the CRM1-mediated HIV-1 Rev/RRE export pathway could be elucidated to a

large extent. However, we are still puzzled about the reason why in the absence of HIV-1 Rev the unspliced gag or gag/pol or the intron-containing HIV-1 env RNA is retained in the cell nucleus. In this project we will investigate the molecular cause for the retention of unspliced and intron-containing HIV-1 RNA in the cell nucleus.

Work program: In this project, we will test whether the algorithms we have developed in the past for predicting splice site selection can also be used to identify and narrow down the HIV-1 INS/CRS elements. To this end, we will first of all produce Rev-dependent subgenomic HIV-1 expression vectors, whose transcripts will be made Rev-independent by HEXplorer-based mutagenesis. The mutagenesis experiments will be accompanied by RNA pull-down and/or mass spectrometric analyses to identify the proteins that actually bind to the HIV-1 INS/CRS elements. RNA FISH analyses will be performed to visualize the intracellular nuclear RNA transport. At the same time, the presence of CANc - the trans negatively dominant C-terminal FG-repeat domain of the nuclear pore protein CAN/NUP214 - will block the CRM1-mediated nucleocytoplasmic Rev-dependent nucleocytoplasmic RNA transport in order to test to what extent the HIV-1 RNA retained in the nucleus can be bypassed by the HIV-1 Rev protein.

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