Single-Molecule Studies of HIV-1 Rev-RRE Interactions

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HIV/AIDS continues to be a major health problem. The current generation of anti-HIV drugs target viral enzymes, but their efficacy is compromised by the ability of the virus to rapidly mutate and become resistant to the drugs. A promising alternative strategy is to target proteins of the host cell that are essential for viral replication but are less prone to mutation. This proposal focuses on host proteins that interact with the HIV-1 Rev protein and it's cognate RNA, the Rev Responsive Element (RRE). The goal of the research proposed here is to understand how two host proteins, the DEAD box protein DDX1 and the transport protein CRM1, contribute to Rev function. The Rev protein (Regulator of Expression of Virion proteins) mediates the export of unspliced and singly spliced viral mRNA transcripts from the nucleus to the cytoplasm of an infected cell. The unspliced RNA encodes the viral RNA genome and the genes encoding viral structural proteins. Since the structural proteins and RNA genome are both needed to form new viral particles, the RNA export activity of Rev is a key step in the viral replication cycle. To promote nuclear export, Rev must bind to and oligomerize on the RRE, a conserved element found in unspliced and singly spliced viral RNA transcripts. Rev must also recruit CRM1 to form a functional export complex. DDX1 also enhances Rev-mediated export of unspliced and partially spliced transcripts and is essential for efficient viral replication. One goal of this project is to understand in mechanistic detail how DDX1 promotes oligomerization of Rev on the RRE. Another goal is to determine how CRM1 is recruited to the Rev-RRE complex and how many CRM1 molecules are incorporated. To answer these questions, novel fluorescence spectroscopic methods will be used to visualize Rev and DDX1 (or Rev and CRM1) assembling together on the same RRE molecule, under defined conditions in vitro. New methods will also be developed to observe the Rev-RRE-host complexes within mammalian cells and to determine their stoichiometry before and after export from the nucleus to the cytoplasm. The performance of these studies will be enhanced through interactions with the CHEETAH center (Cellular Host Elements in Egress, Trafficking and Assembly of HIV), an interdisciplinary center devoted to the study of HIV-host interactions. The project will fill a gap in our knowledge of Rev biology by revealing how specific host proteins contribute to Rev function by promoting formation of the Rev-RRE nuclear export complex. This new information will lead to a detailed picture of the entire assembly pathway, which should ultimately help in the design of small molecules that interfere with specific steps in the pathway and thereby inhibit HIV-1 replication.