

**POSTER 36****MOLECULAR CHARACTERIZATION OF CPSF6 DOMAINS REQUIRED FOR ANTIVIRAL FUNCTION**

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HIV-1 cofactor requirements after entry and prior to provirus establishment are poorly understood. We have characterized truncated forms of cleavage and polyadenylation factor 6 (CPSF6) which interfere with HIV-1 infection after completion of reverse transcription to better understand the HIV-1 postentry pathway. Although depletion of endogenous CPSF6 does not appear to reduce HIV-1 infection, CPSF6 encoding the first 358 residues (CPSF6-358) is sufficient to block HIV-1 infection greater than 100-fold in nondividing cells. Notably, HIV-1 with an N74D substitution in CA evades the CPSF6-358 restriction. We thus investigated whether CPSF6-358 interfered with HIV-1 infection through direct interaction with CA. Affinity tagged forms of CPSF6-358 and cyclophilin A (CypA) were expressed in cells. Tagged CPSF6-358 but not tagged CypA restricted HIV-1 infection. However lysis of cells soon after HIV-1 infection revealed HIV-1 CA to be interacting with CypA and not CPSF6-358, suggesting CPSF6-358 may target other cellular factors required by HIV-1 for infection. These findings were corroborated by experiments using cells expressing a CPSF6-358-CypA fusion protein. Stable expression of CPSF6-358-CypA restricted wild-type HIV-1, but failed to restrict infection by virions carrying N74D mutant CA. By contrast, N74D HIV-1 potentially restricted by TRIM-Cyp. These data indicate that targeting of CPSF6-358 to incoming virions was insufficient for restriction. To better understand the mechanism of CPSF6-358 antiviral function, we performed a deletion-mutagenesis scan along the entire length of the CPSF6-358 protein. These deletional analyses suggest that two regions within CPSF6-358, one that encompasses the RRM domain and another at the C-terminal end of this protein are required for antiviral activity. Tandem tagged wild-type and mutant CPSF6-358 have been produced and affinity purified. Mass spectrometric analysis of proteins associated with tagged restrictive and nonrestrictive forms of CPSF6-358 should help elucidate cell factors that participate in the restriction and that may interact with HIV-1 after entry.