HIV-1 REV BOUND TO HUMAN IMPORTIN BETA : AN INTEGRATIVE STUDY OF A PROTEIN COMPLEX

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HIV-1 Rev is a nucleocytoplasmic shuttling protein that mediates the nuclear export of intron-containing viral RNA transcripts and is essential for viral replication. Rev is synthesized in the early phase of infection and imported into the nucleus by the host cell protein importin beta (ImpB), but how Rev associates with ImpB is poorly understood.

In this study, we characterized the ImpB/Rev complex through a combination of biochemical, biophysical and structural approaches. Size-exclusion chromatography coupled to multi-angle light scattering (SEC/MALLS), native mass spectrometry and isothermal titration calorimetry (ITC) show that ImpB binds up to two monomers of Rev through a high- and a low-affinity binding site. The two Rev binding sites were identified within the N- and C-terminal regions of ImpB by cross-linking mass spectrometry (XL-MS). NMR and a peptide scanning binding assay identify the N-terminal helical hairpin domain of Rev as a major ImpB binding epitope. Fluorescence polarization (FP) assays performed on charge-reversal mutants highlight specific residues involved in complex stability.

Based on these findings, small-angle X-ray scattering (SAXS) data and molecular docking analyses, we propose a structural model of ImpB bound to Rev.

This study sheds light on the molecular basis of Rev nuclear import while underscoring the value of an integrative approach for investigating macromolecular structures resistant to traditional experimental methods.