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Strategy for Identification and Validation of Cellular Cofactors of Retroviral replication

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The Human Immunodeficiency Virus (HIV)/Acquired Immonodeficiency Syndrome (AIDS) epidemic poses one of the biggest medical challenges of the coming decades. In order to better understand retroviral infection and to identify novel drug targets, it is very important to understand the underlying interactions of retroviruses with their host cells. In our laboratory, we focus on integrase, an essential protein in the integration step of the retroviral life cycle. Therefore we developed a strategy to identify and validate new cofactors.

To identify new interaction partners of integrase, several methods have been used by various laboratories. Yeast-two-hybrid screens are commonly used, but pose the disadvantage of many false positive hits. Recently, several siRNA screens for HIV cofactors have been published [1,2,3]. However, the value of these screens is limited when siRNA's are not controlled for efficacy and off-target effects. We therefore prefer to use high throughput co-immunoprecipitation with the viral protein of interest followed by nanoLC/Q-TOFF-mediated identification to identify new interaction partners. This method comes closest to mimicking the *in vivo* situation and ensures an interaction of the two proteins in the host cell. After identification, validation of a cofactor is based on RNAi mediated knockdown and/or transdominant overexpression to show an effect on retroviral replication. The identification of interaction mutants is also included. When a genuine cofactor has been validated as target for drug discovery, the cofactor can be further characterized and a screening assay established to identify small molecule inhibitors of the protein-protein interaction.. Using this method, we were able to validate two cofactors of HIV integrase: LEDGF/p75 [4] and Transportin SR-2 [5], while invalidating others, such as INI-1, BolA, VBP-1 and MCM7.

References:

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