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Affinity-purification of RABV Polymerase Complex and Identification of Cellular Binding Partners

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Rabies Virus (RABV) is a neurotropic negative strand RNA virus that replicates in the CNS and causes acute encephalitis. The large polymerase L represents the catalytic subunit of the RABV polymerase complex and is essentially involved in virus replication. It is unknown whether cellular factors bind to L and whether virus-host interactions may influence host- or cell-type dependent RABV replication. To identify cellular proteins interacting with the viral polymerase complex, a recombinant RABV expressing N-terminally tagged L protein was successfully generated, L protein complexes were isolated from virus infected cells by affinity purification and analysed by nano-LC/MS analysis. One candidate interactor that reliably co-purified with the polymerase complex was Argininosuccinate Synthetase 1 (ASS1). As argininosuccinate synthesis can be a limiting step in antiviral NO production, interaction of viral polymerase complex with ASS1 may represent a novel mechanism of viral interference with NO synthesis. Further data indicate that RABV polymerase is also associated with the tubulin cytoskeleton. Notably, autofluorescent L protein N-terminally tagged with mCherry co-localized with and re-arranged tubulin filaments. Whereas ASS1 co-purification and tubulin co-localization suggested specific interactions with these cellular proteins, the number of cellular proteins that were specifically co-purified was remarkably low.

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