Abstract ID : **19**

Detection and characterisation of influenza virus RNA polymerase dimers using Bimolecular Fluorescence Complementation (BiFC)

Content

Influenza virus encodes a heterotrimeric RNA-dependent RNA polymerase (RdRP), composed of subunits PB1, PB2 and PA. The RdRP carries out both transcription and replication of the viral RNA genome segments in the context of ribonucleoproteins (RNPs). Replication of negative-sense viral RNA is a two-step process, progressing via a positive-sense complementary RNA intermediate. The mechanism of viral genome replication is mostly unknown, though there are multiple reports indicating RdRP-RdRP interactions may be central for the process. Purified RdRPs from human and avian influenza A viruses both form dimers of heterotrimers in solution. Using a combination of X-ray crystallography and SAXS analysis our group has identified the interface involved in RdRP dimerization, which is primarily located on the PA C-terminal domain. We establish a bimolecular fluorescence complementation (BiFC) assay to monitor intermolecular interactions between RdRPs in cells expressing viral RNPs. Using this system we confirm the existence of RdRP dimers in the context of actively replicating RNPs. Mutating amino acid residues at the identified dimer interface causes loss dimerization and inhibition of RNA replication in minigenome assays. These data suggest that dimerisation of RdRP via the PA C-terminal domain is important for replication of the viral RNA genome.

Choose primary session

Viral Replication

Choose secondary Session

Viral Replication

Contribution Type : Paper presentation