Human Metapneumovirus Phosphoprotein Independently Drives Phase Separation and Recruits Nucleoprotein to Liquid-Like Bodies

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Abstract

Human metapneumovirus (HMPV) inclusion bodies (IBs) are dynamic structures required for efficient viral replication and transcription. The minimum components needed to form IB-like structures in cells are the nucleoprotein (N) and the tetrameric phosphoprotein (P). HMPV P binds to the following two versions of the N protein in infected cells: N-terminal P residues interact with monomeric N (N^0) to maintain a pool of protein to encapsidate new RNA and C-terminal P residues interact with oligomeric, RNA-bound N (N-RNA). Recent work on other negative-strand viruses has suggested that IBs are, at least in part, liquid-like phase-separated membraneless organelles. Here, HMPV IBs in infected or transfected cells were shown to possess liquid organelle properties, such as fusion and fission. Recombinant versions of HMPV N and P proteins were purified to analyze the interactions required to drive phase separation in vitro. Purified HMPV P was shown to form liquid droplets in isolation. This observation is distinct from other viral systems that also form IBs. Partial removal of nucleic acid from purified P altered phase-separation dynamics, suggesting that nucleic acid interactions play a role in IB formation. HMPV P also recruits monomeric N (N^0-P) and N-RNA to droplets in vitro. These findings suggest that HMPV P may also act as a scaffold protein to mediate multivalent interactions with monomeric and oligomeric N, as well as RNA, to promote phase separation of IBs. Together, these findings highlight an additional layer of regulation in HMPV replication by the viral P and N proteins.

Author keywords

inclusion bodies; KEYWORDS HMPV; phase separation; pneumovirus