Synopsis

Rotavirus Viroplasm structure (VS): The first insights into the architectural assembly of the viral and host factors in the VS"

Rotavirus is a major cause of acute gastroenteritis in infants and young children and responsible for approximately 453,000 infantile deaths per year. Rotaviruses are non-enveloped RNA viruses belonging to the *Reoviridae* family. The rotavirus genome is composed of 11 segments of double-stranded RNA (dsRNA), enclosed in an icosahedral triple-layered protein capsid, and it encodes six structural proteins (VP) and six non-structural proteins (NSPs). Removal of the outer capsid from the triple-layered particle (TLP) during virus entry into the cell activates the synthesis and extrusion of the viral mRNAs from the double-layered particle (DLP) into the cytoplasm. The viral genome replication and assembly of immature DLPs occurs in specialized virus-induced electron-dense cytoplasmic inclusions called viroplasms (VMs), nucleated by two essential non-structural proteins NSP2 and NSP5, and the inner capsid protein VP2. NSP5 is crucial for recruitment of the viroplasmic proteins and the architectural assembly of VMs. VMs are dynamic entities that undergo fusion and localize at the perinuclear region in the infected cells. This study aims to identify the host factors interacting with the VM proteins NSP5 and NSP2, their cytoplasmic relocalization, sequestration in VMs, and the assembly of viral and cellular proteins in the viroplasmic structures.

Part I: Identification of the cellular proteins interacting with viroplasmic proteins NSP5 and NSP2

NSP5 and NSP2-interacting proteins have been affinity purified in a pull-down assay and the protein complexes were analysed using LC-MS/MS. Mass spectrometry data revealed the presence of several cellular proteins including hnRNPs, ARE-BPs and others. These results were further validated by both immunoblotting of the pull down complexes, and co-immunoprecipitation.

Part II: Cytoplasmic relocalization of nuclear proteins, their sequestration by the viroplasmic proteins and their biological significance in virus infection

Rotavirus replication occurs in the cytoplasm, and none of the viral proteins are known to selectively translocate to the nucleus in infected cells. The finding in this study that a large number of hnRNPs and other proteins interact with NSP5 and NSP2 suggested the likely

cytoplasmic relocalization of the host nuclear proteins and their interaction with viroplasmic proteins. The cytoplasmic redistribution of some nuclear proteins has been reported in several other viruses such as Poliovirus, HIV, JEV, MHV, and Enteroviruses, but their large-scale relocalization has not been reported.

Confocal microscopy studies revealed that several hnRNPs and ARE-BPs relocalized to the cytoplasm and colocalized with VMs in the infected cells to form viroplasm structures (VSs). The basis for this large-scale cytoplasmic relocalization and sequestration of majority of the nuclear proteins and nuclear transport proteins in the viroplasm was explored. The results suggest that selective inhibition of nuclear import pathways occurring during rotavirus infection primarily contributes to the cytoplasmic accumulation of nuclear proteins, but inhibition of the importin α/β pathway, affecting the nuclear accumulation of PABPC1, severely affects rotavirus growth. Knockdown and overexpression studies of some of the relocalized cellular proteins revealed their differential influence on viral infection. Altogether, this study redefines the existing concept that 'only virus components are present in the VMs'.

Part III: Modulation of stress granules (SGs) and processing bodies (PBs) during rotaviral infection

In common with many viruses, rotaviruses have been reported to block SG assembly (Montero *et al.,* 2008) and cause dispersion of PBs (Bhowmick *et al.,* 2015), however these results are based on the analysis of only two or three markers of SGs and PBs. Careful examination of these results suggest that in both studies, erroneous conclusions were drawn by the authors despite perfect colocalization of TIA1 and Dcp1a with the viroplasms, probably due to the prevailing concept that VMs consist only of viral components. The present study employing multiple methods, and analysing a large number of SG and PB proteins, unequivocally demonstrated that rotavirus remodels SGs and PBs by inducing selective dissociation of a few components and sequestration of the remodelled granules containing the majority of the SG and PB components into the viroplasms, forming the viroplasm structures (VSs).

Part IV: Studies on the structural assembly of viroplasm structures (VSs)

VMs are dynamic structures and considered to contain only viral components. In the previous part, it was demonstrated that VMs, consisting of NSP5 and NSP2, associate with other viral viroplasmic proteins and several host proteins to form VSs. Using High-resolution confocal

microscopy, the structural organization the viral and cellular proteins in the VS, and the sizes of different VSs inside the infected cells was investigated. These studies revealed that the VSs are more complex than that is currently perceived with viral and cellular proteins being organised in a specific order with the cellular proteins localized in the VS based on their direct or indirect interaction with the viroplasmic proteins. This study provides the first insights into the spatial organization of viral and cellular proteins in the VS.

Overall, the present study conclusively demonstrates that the composition of the VS is more complex than that is currently perceived, and provides the first insights into the unknown spatial organization of the viral proteins NSP5, NSP2, VP1 and VP6 as well as cellular proteins within the VS. These results lay the foundation for future studies in understanding the detailed structural organization of different proteins in the VS and the spatial and temporal assembly of viral and cellular proteins.