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Review

Morphogenesis and functional organization of viral inclusion bodies

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ABSTRACT

Eukaryotic viruses are obligate intracellular parasites that rely on the host cell machinery to carry out their replication cycle. This complex process involves a series of steps, starting with virus entry, followed by genome replication, and ending with virion assembly and release. Negative strand RNA and some DNA viruses have evolved to alter the organization of the host cell interior to create a specialized environment for genome replication, known as IBs, which are precisely orchestrated to ensure efficient viral replication. The biogenesis of IBs requires the cooperation of both viral and host factors. These structures serve multiple functions during infection, including sequestering viral nucleic acids and proteins from innate immune responses, increasing the local concentration of viral and host factors, and spatially coordinating consecutive replication cycle steps. While ultrastructural and functional studies have improved our understanding of IBs, much remains to be learned about the precise mechanisms of IB formation and function. This review aims to summarize the current understanding of how IBs are formed, describe the morphology of these structures, and highlight the mechanism of their functions. Given that the formation of IBs involves complex interactions between the virus and the host cell, the role of both viral and cellular organelles in this process is also discussed.

Main points

Intracellular parasites, such as RNA and DNA viruses, induce the formation of inclusion bodies (IBs) to support their replication process. Some virus IBs may resemble liquid-like, membrane-less organelles, however, other virus IBs are wrapped with cellular membranes. The relationship between viral IBs and cellular membranes is an open question and remain to be finely characterized. The presence of one or more viral proteins mediates IB formation, while host factors supporting this process are currently unknown. IB formation requires the cooperation of membrane transport, lipid metabolism, and other organelles. It is speculated that IBs allow viral genome replication and serve as a site for message exchange and transport of viral mRNA to the cytoplasm. For negative strand RNA and DNA viruses, replication typically occurs inside IBs, and these structures may escape host immune responses by hijacking related factors and inhibiting stress granule generation. However, the topology and molecular mechanism of these processes require further investigation.

Main

Eukaryotic viruses are obligate intracellular parasites that have coevolved with their host cells, and depend on the host to complete their life cycle. The infection cycle begins with viral entry into the host cell, followed by genome replication, and culminates of new virions, these processes occur in close association with cellular structures and organelles (de Castro et al., 2013). In particular, the process of genome synthesis and transcription of eukaryotic viruses occurs in the unique environment that is spatially and temporally separated from the host cell. Negative strand RNA and DNA viruses induce the formation of structures that support genome replication, commonly referred to as inclusion bodies (IBs), viral factories (VFs), viroplasms (VPs), Negri bodies (NBs) or replication organelles (ROs) (Nevers et al., 2020). These structures are formed through the interaction of viral proteins and host factors. IBs perform multiple functions during infection, such as concentrating viral and host factors to enhance replication efficiency, sequestrating viral nucleic acids and proteins from the innate immune responses, and coordinating sequential replication steps in a spatially organized manner

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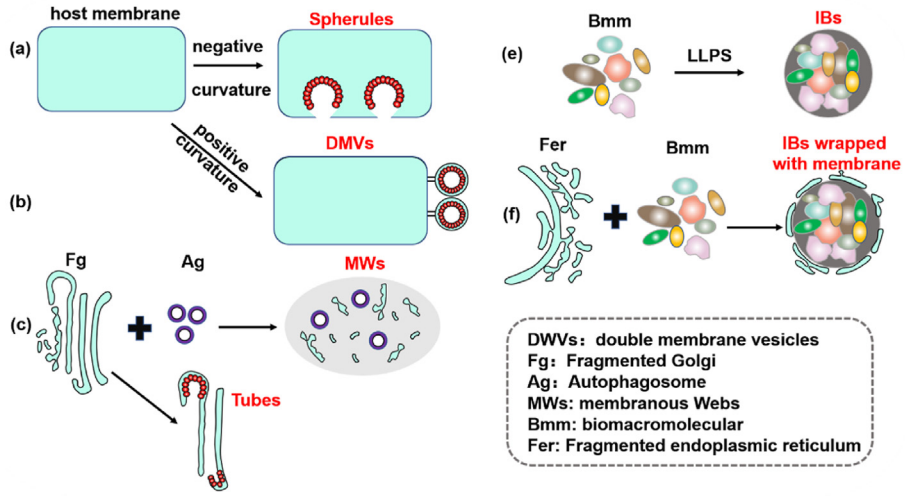

Fig. 1. Diverse Forms of Replication Organelles Induced by Virus infection. (a) Spherules formed by membrane invagination from diverse cellular membranes during virus infection (plasma membrane, mitochondria, endosome, peroxisome and ER). Replicase proteins (indicated as red spheres) are recruited to the cytoplasmic face of membrane-bound organelles. The spherule is connected to the limiting membrane of the organelle and communicates with the cytoplasm through a pore. (b) Formation of double membrane vesicles (DMVs) occurs through membrane protrusion. Replicase proteins are recruited to the inner face of the membrane-bound organelles. (c) Membranous webs (MWs) are replication organelles consisting of complex membrane structures that can vary depending on the virus. MWs contain a variety of components that are involved in the virus replication cycle, and their complexity reflects the diversity of functions required to complete the replication process. (d) Tubes formed by organelle membrane remodeling, primarily found in Golgi apparatus during virus infection. (e) Formation of IBs through liquid-liquid phase separation of biomacromolecules in virus-infected cells. (f) Fragmented ER membrane is hijacked by viruses to wrap around IBs during viral replication.

(de Castro et al., 2013; Netherton & Wileman, 2011; Novoa et al., 2005). The application of ultrastructural and functional analyses has provided valuable insights into the biogenesis of IBs. However, the mechanisms underlying the formation and functions of these structures remain unclear for many viruses. In this review, we discuss the possible mechanisms of IB formation, describe the morphological characteristics of IBs, and investigate the mechanisms underlying their functional roles in viral replication. As the formation of IBs involves a complex interplay between the virus and the host, we also discuss the roles of both viral and cellular organelles in the biogenesis of these structures.

1. Virus-induced replication organelles: Different names, similar functions

Viruses have evolved various mechanisms to achieve genome replication within the cytoplasm or nucleus of the host cell. The morphology of the ROs can vary depending on the virus, and typically manifest in six common forms: spherules formed by membrane invagination, double membrane vesicles (DMVs) formed by membrane protrusion, membranous web containing different membrane structures, tubes formed by organelle membrane remodeling, IBs formed by aggregation of biomacromolecules, and IBs wrapped with fragmented endoplasmic reticulum (ER) membranes (see Fig. 1).

Positive strand RNA viruses induce intracellular membrane remodeling to generate different structures, such as invaginated vesicles or spherules, DMVs, tubes, or MWs (Belov & van Kuppeveld, 2012; Miller & Krijnse-Locker, 2008). As an illustration, within the Flaviviridae family, Zika virus and dengue virus are known to induce the development of invaginated vesicles with negative membrane curvature, which are single-membrane structures that harbor dsRNA replication intermediates as well as viral replicase complex proteins (Cortese et al., 2017; Welsch et al., 2009), Whereas hepatitis C virus (HCV) induces the curvature of the endoplasmic reticulum (ER) membrane to create a DMVs web (Romero-Brey et al., 2012). Marianne D. Hansen et al. have demonstrated that in addition to DMVs, HCV also induces Golgi fragmentation and autophagosome formation to create complex MWs that facilitate replication (Hansen et al., 2017). The formation of DMVs has also been observed in other virus families including *Picornaviridae*, *Arteriviridae* and *Coronaviridae* (Belov et al., 2012; Knoops et al., 2008, 2012; Miller & Krijnse-Locker, 2008). Bunyaviruses, as well as plant viruses such as

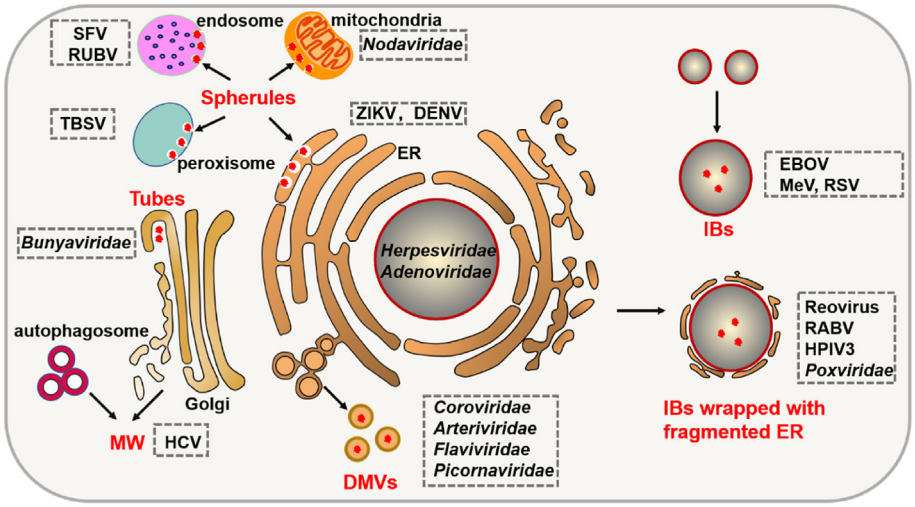

Fig. 2. Genome Replication Sites for Various RNA and DNA Viruses. SFV and RUBV, TBSV, ZIKV, DENV respectively replicate their genome in the spherules of remodeled organelle membrane. Endosome, peroxisome, mitochondria, and ER are represented by different color structures (pink, lake green, orange, brown). *Bunyaviridae* replicate genome in the tubes of remodeled peroxisome membrane (light brown). HCV replicates in MWs consisting of autophagosomes and fragmented Golgi membrane. The replication sites of *Coroviridae, Arteriviridae,* Flaviviridae, *Picornaviridae* are DMVs. Additionally. the replication sites of RABV, EBOV, MeV, RSV, reovirus, HPIV3, *poxviridae* are IBs, which are formed through liquid-liquid phase separation. However, it has been proved that the IBs of reovirus, HPIV3 and poxviridae are wrapped with fragmented ER membrane.

Tombusviruses and Brome mosaic virus, induce host cell membrane rearrangements to form replication structures. Bunyaviruses form tubular replication structures around the Golgi complex throughout their lifecycle.

(Fontana et al., 2008), while Tombusviruses and Brome mosaic virus use the host endomembrane system to create elaborate membranous ROs, that consist of spherules and vesicle-like invaginations (Diaz & Wang, 2014; Nagy & Feng, 2021).

Non-segmented negative strand RNA viruses, which are classified within the order mononegavirales, replicate their genetic material within specialized cytoplasmic structures called IBs, which is different from positive strand RNA viruses. Important human and animal pathogens, including Ebola virus (EBOLV), measles virus (MeV), rabies virus (RABV), respiratory syncytial virus (RSV), and human parainfluenza virus (HPIV), belong to this viral order (Amarasinghe et al., 2018). IBs were first described during RABV infection, and were named Negri bodies. Viral RNAs, including mRNA, genomic and antigenomic RNAs, are synthesized in these dense granules, which have a diameter up to a few micrometers (Lahaye et al., 2009). Similar structures have been observed in cells infected with other negative strand RNA viruses, and these structures serve as histological proof of the virus infection (Hoenen et al., 2012; Kristensson et al., 1996; Rincheval et al., 2017; Zhang, Chen, et al., 2013; Zhou et al., 2019).

IBs are not limited to non-segmented negative strand RNA viruses, as segmented negative strand RNA viruses, such as Influenza A virus, also form discrete IBs in the cytosol. These viral IBs are spatially regulated, assembling near the ER exit site and depending on continuous ER-Golgi vesicular cycling. The formation of viral IBs triggers nucleation of interactions among the different viral segments to assemble a complete genome (Alenquer et al., 2019).

In addition to RNA viruses, some DNA viruses also reshape the host organization to form IBs during infection. Most DNA viruses replicate their genome in the nucleus, and they induce the formation of replication compartments through extensive reorganization of the host nucleus during replication. These structures serve as platforms for the synthesis of viral DNA and the production of DNA-containing capsids, and occupy most of the nuclear space. Such viruses include adenovirus virus and cytomegalovirus (Everett, 2013; Hidalgo & Gonzalez, 2019; Strang, 2015), they replicate their genome within the IBs formed from cytoplasm.

In general, viruses that undergo replication in both the cytoplasm and nucleus are capable of forming specialized ROs within infected cells (refer to Fig. 2). We think that IBs, which form during virus infection, represent a simplified “nucleus” or “virus nucleus”. This specialized structure serves as a “base area” for the viral genetic material within the infected cell and facilitates efficient replication and proliferation of the virus.

2. Are IBs biomolecular condensation or membrane-associated structures?

Drawing inspiration from soft matter physics, liquid phase condensates are emerging paradigm for the intracellular organization of membrane-less organelles, driven by multivalent macromolecular interactions, these structures are formed via liquid-liquid phase separation (LLPS), with assembly/disassembly regulated by an array of physicochemical factors (Alberti & Dormann, 2019; Banani et al., 2017; Milicevic et al., 2022). Examples of such membrane-less and dynamic biomolecular condensates in cells include chromatin (Larson et al., 2017), nucleolus (Brangwynne et al., 2011), cajal bodies (Handwerger et al., 2005), PML bodies (Corpet et al., 2020), P bodies (Kroschwald et al., 2015), and stress granules (SGs) (Molliex et al., 2015). Similarly, the liquid-like nature of spherical IBs has been confirmed through live-cell imaging for RABV, VSV, and MeV. IBs readily fuse and round up into a single larger spherical one, and can reversibly deform when encountering a physical barrier, disappearing when exposed to an osmotic shock. Fluorescence recovery after photobleaching (FRAP) measurements are also in agreement with the liquid nature of IBs (Heinrich et al., 2018; Nikolic et al., 2017; Zhou et al., 2019). Given their morphology and assembly process, many researchers agree that IBs form liquid-like biomolecular condensates that share properties with membrane-less organelles driven by LLPS (Lakdawala et al., 2021; Su et al., 2021).

Emerging evidence suggests that the involvement of membranes in IBs cannot be ignored. In particularly, poxviruses, which carry out DNA replication in the cytoplasm rather than the nucleus, form IBs that are gradually enveloped by rough ER membrane. Recruitment of individual ER cisternae to the replication sites leads to their fusion and the formation of an almost completely sealed envelope that is later dispersed during viral assembly (Schramm & Locker, 2005; Tolonen et al., 2001). Similarly, Negri bodies, as observed by electron microscopy, undergo dynamic formation and can be associated with membranes derived from the ER at later time points of infection (Lahaye et al., 2009; Nikolic et al., 2017, 2019). EBOV IBs form in close proximity to the ER and ribosomes can be found nearby (Nelson et al., 2016); Reovirus-infected cells nucleate IBs with viral nonstructural proteins μNS and δNS, which partition the ER to form the matrix of IBs. The resulting membranous webs likely serve to anchor viral RNA-protein complexes for replication of the reovirus genome and assembly of progeny virions (Becker et al., 2003; Fernandez de Castro et al., 2014; Tenorio et al., 2019).

In light of the information presented, the categorization of IBs as liquid organelles remains subject to debate. The commonly accepted criteria for defining a liquid organelle are that it should be spherical, able to fuse with each other, exhibit reversible deformation when it encounters a physical barrier, and demonstrate recovery from photobleaching (Alberti et al., 2019). However, this may not be a sufficiently rigorous set of criteria. For instance, during vaccinia virus replication, the gradual wrapping of IBs by the ER occurs over a period of approximately 45 min, and the ER envelope disassembles during virion assembly. This explains why previous electron microscopy observations did not reveal the presence of an ER membrane web at the IB site, as these observations were made either too early or too late in infection (Schramm & Locker, 2005; Tolonen et al., 2001). Another example is provided by human parainfluenza virus 3 (HPIV3), in which small IBs (which do not perform replication functions) can fuse with each other to form larger IBs. In this case, HPIV3 remodels the ER membrane, and ER debris is loosely distributed around large IBs to promote virus replication (Li et al., 2019; Zhang et al., 2017).

As a summary, a possible explanation is that IBs are initially small, spherical, and liquid-like structures during the early phase of infection. As infection progresses, they become more heterogeneous in shape, structure, and composition, with the formation of large IBs that are involved in genome replication. These large IBs are gradually coated or filled with membrane, serving to protect the genome from host degradation. However, it is important to acknowledge that different viruses may use distinct mechanisms for IB assembly and display varying characteristics. Therefore, additional research is necessary to reach a consensus on this topic.

3. The formation of IBs is a complex process that involves the interaction and cooperation of both viral and host factors

A transfection method was utilized to investigate the minimal viral components responsible for the formation of IBs in the absence of viral infection for several viruses, including RABV, VSV, MeV, HPIV3, and RSV. The results indicated that the co-expression of N and P proteins in transfected cells was either sufficient or required for the generation of IBs, highlighting the requirement for the association between these proteins in IB formation. Additionally, the dimerization and tetramerization domains of P protein, as well as an amino acid of N protein, were found to play important roles in the formation of IBs for specific viruses (Galloux et al., 2020; Heinrich et al., 2018; Nikolic et al., 2017; Zhang, Chen, et al., 2013; Zhou et al., 2019). For example, the size of MeV IBs

was reduced by inhibiting casein kinase 2 (CK2) or mutating the two major CK2 phosphorylation sites of P protein (Zhou et al., 2019). Furthermore, nonstructural and structural proteins of reovirus were found to be critical for the formation and structural organization of IBs, with the specific interaction between μ2 and μNS required for IB formation and recruitment of viral and host factors involved in viral genome replication and assembly (Becker et al., 2003; Broering et al., 2002; Miller et al., 2010; Parker et al., 2002; Rahman et al., 2020). The phosphorylation of NS2 was also found to be important in the assembly of IBs required for virus replication and genome packaging in certain RNA viruses, including HIV type 1, EBOV, VSV, rubella virus, and hepatitis C virus (Das & Pattnaik, 2004; Hemonnot et al., 2004; Law et al., 2003; Modrof et al., 2002; Ross-Thriepland et al., 2015). These findings suggest that one or more viral proteins can serve as a basic scaffold for IB formation during virus infection, and that the modification of some viral proteins is necessary for IBs formation. Additionally, the role of other viral proteins and RNA in IB formation requires further investigation, although it has been demonstrated that the ability of N protein to bind RNA and/or oligomerize is critical for the formation of RSV and HPIV3 IBs (Galloux et al., 2020; Zhang, Chen, et al., 2013).

3.1. Membrane transport

Similar to some positive strand RNA virus such as poliovirus, Coxsackievirus B3, hepatitis C virus, and red clover necrotic mosaic virus (RCNMV) (Belov et al., 2007; Lanke et al., 2009; Qiu et al., 2012; Sun et al., 2014), membrane transport-associated proteins are also involved in the formation of IBs in both DNA and negative strand RNA viruses. For instance, nonstructural proteins of Grass carp reovirus (GCRV) and Birnavirus recruit ADP ribosylation factor 1 (Arf1) into their IBs, and the small GTP domain of ARF1 plays a crucial role in promoting GCRV replication and infection. Inhibition of ARF1 GTPase activity significantly reduces the number of IBs (Gimenez et al., 2022; Zhang et al., 2022). Cytomegalovirus (CMV) IBs are composed of membranes derived from the endosomal recycling compartment, early endosomes, and the trans-Golgi network, and MCMV infection causes a massive reorganization of the Arf system of host cells, leading to the over-recruitment of Arf proteins onto the membrane of the IBs. Knockdown of Arf1 and Arf6 also abolishes the establishment of infection (Pavisic et al., 2021).

3.2. Lipid and energy metabolism

In addition to the previously described requirement of the ER for the formation of viral IBs, recent studies have demonstrated the involvement of various other organelles and structures. For example, during rotavirus infection, IBs were found to recruit lipid droplets (LDs), and proteins associated with LDs were observed to co-localize with IBs. Interfering with LD formation using chemical compounds resulted in decreased numbers of IBs and inhibited RNA replication, suggesting a crucial role of LDs in IB biogenesis (Cheung et al., 2010). Another example is the enrichment of PI4P on IBs, which highlights the ability of viruses to hijack cellular lipid metabolism for their own replication. However, the specific role of lipids in IBs formation remains to be fully elucidated (Li et al., 2019), and further research is required to investigate the mechanisms underlying LD recruitment to IBs and the function of lipids in IB biogenesis.

An interesting possibility is that that IB formation and maturation may require a high level of ATP. The replication of viruses places a substantial energetic burden on the host cell, potentially competing with its physiological activities. In addition to supporting IB formation, increased local ATP levels may act as a biological hydrotrope to increase protein solubility within IBs, or may be required to maintain the shape of IBs, as observed for stress granules (Patel et al., 2017) (Jain et al., 2016). Recent reports have shown that mitochondria are in close proximity to IBs in the case of RSV, reovirus and African swine fever virus (ASFV), which replicates in the cytoplasm (Fernandez de Castro et al., 2014; Lifland et al., 2012; Rojo et al., 1999). This suggests that ATP may play a role in IB formation and maturation, and raises the question of whether the recruitment of mitochondria to the vicinity of IBs facilitates the delivery of ATP produced in mitochondria to IBs. Additionally, it remains to be investigated whether the fatty acids that generate energy originate from LDs, which presents another avenue for future research.

3.3. Cytoskeleton within the IBs

Reovirus strains are known to produce filamentous IBs, and the underlying mechanism involves the interactions of μ2 with microtubules and the stabilization of the microtubule network. This filamentous distribution of IBs is observed to be colinear with microtubules as revealed by immunofluorescence microscopy, and the intact microtubular network is required for the assembly, maintenance, and dynamics of both filamentous and globular IBs (Parker et al., 2002). The uNS protein also associates with microtubules, facilitating the movement and enlargement of IBs during infection. Additionally, the reorganization of vimentin intermediate filaments during infection is exploited by reovirus to facilitate genome packing. Thus, reovirus hijacks the cellular cytoskeleton to aid in IB formation and function. (Eichwald et al., 2018);. Another example of this phenomenon is observed during HPIV3 infection, where acetylated α-tubulin assists in the fusion of small IBs into larger structures, which promotes efficient viral replication (Zhang et al., 2017).

3.4. Chaperones

In addition to manipulating host membranes, recruiting LDs and mitochondria, and hijacking membrane transport and energy metabolism, certain viruses recruit protein-folding chaperones into their IBs. TRiC/CCT, Hsp70, and Hsp90 are eukaryotic cytosolic chaperonins that bind and stabilize nascent polypeptide chains in an ATP-dependent manner during or after translation (Kaufer et al., 2012; Mayer & Bukau, 2005). Influenza A virus PB2, a subunit of RNA polymerase, has been shown to associate with CCT, and silencing of CCT results in reduced viral replication, indicating a crucial role for CCT in the influenza virus life cycle (Fislová et al., 2010). CCTα, CCTγ, and Hsc70 also relocalize to IBs in RABV-infected cells, and cotransfection of nucleo- and phosphor-proteins of RABV is sufficient to recruit CCTα to the IB structure. Inhibition of CCTα expression reduced the replication and transcription of RABV (Lahaye et al., 2012; Zhang, Chen, et al., 2013, 2014). Additionally, the , chaperonins TCP-1 γ and ε (subunits of TRiC) interact with the Gag polyprotein of retrovirus type D and the nuclear protein EBNA-3 of Epstein-Barr virus, respectively (Hong et al., 2001; Kashuba et al.). Hsp90 and Hsp70 are anchored to IBs via their interaction with the viral protein, suggesting a potential role for viral proteins in concentrating the folding machinery required for assembly. Overall, these findings suggest that chaperonins play a vital role in the formation and function of viral IBs, and will likely emerge as an important area of virus-host interaction research.

4. The functions of IBs extend beyond viral replication

4.1. Sub-compartment within IBs

IB-associated granules (IBAGs) are sub-compartments within RSV IBs, which have been suggested to be liquid organelles (Panas et al., 2016). IBAGs transiently concentrate newly synthesized viral mRNA and the viral transcription anti-terminator M2-1, while excluding viral genomic RNA, nucleoprotein, L polymerase and its cofactor P. This suggests that IBAGs may be involved in sorting viral mRNAs and regulating their translation and stability (Rincheval et al., 2017). Although IBAGs share similarities with SGs in concentrating untranslated viral mRNA with translation initiation factors, they do not exhibit cellular SG markers (G3BP and TIA1), and can be regarded as viral-specific mRNP granules.

Further investigations are required to explore the subtle structure of

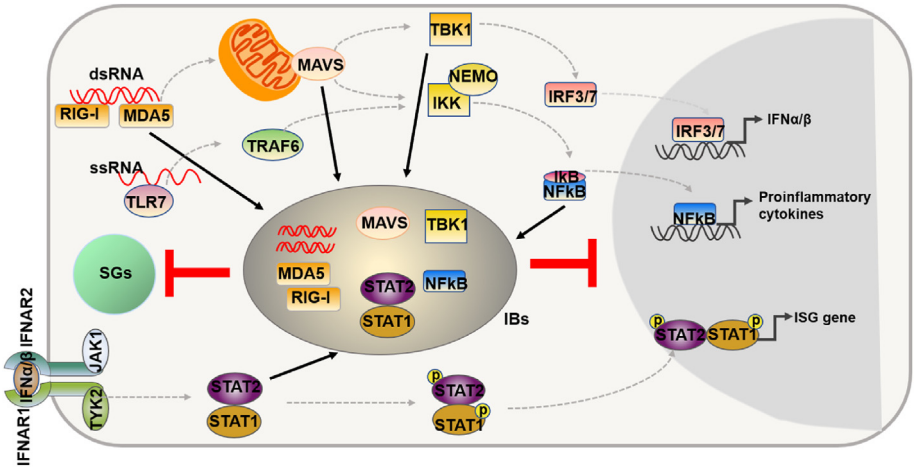

Fig. 3. Strategies of virus resistance to host immune response. Activation of PPRs like RIG-1, MDA5 and TLR7 recognizing cytosolic viral RNA leads to the activation of type 1 interferon and inflammatory responses combating viral infection. Virus imprisons related host factors (MDA5, RIG-I, MAVS, TBK1, NFκB, STAT1 and STAT2) into IBs to escape host immune response. In addition, IBs escape the antiviral effect of SGs by concealing their own newly synthesized viral RNAs.

IBs and the corresponding function. One area of limited understanding is how IBAGs exchange materials with the cytosol. IBAGs embedded within intact IBs contain cytosolic proteins such as eIF4G and PABP, and viral mRNA released from IBAGs must reach the cytosol to be translated. Some positive strand RNA viruses use structures similar to nuclear pores to ensure communication between the ROs and cytosol. For example, Bromovirus RNA is replicated in spherules, vesicle-like invaginations of the outer perinuclear ER membrane that remain connected to the cytoplasm via a neck-like opening (Diaz & Wang, 2014). Recent studies have also shown that MHV and SARS-CoV-2 generate a crown-like pore on ROs, allowing for the exchange of luminal and cytoplasmic material and export of viral RNA (Wolff et al., 2020). In addition, the flavivirus genus forms invaginations into ER cisternae, and each spherical vesicle is connected to the ER membrane by a neck with a pore opening to the cytosol that allows the transit of viral RNA (Cortese et al., 2017; Neufeldt et al., 2018; Welsch et al., 2009). For negative strand RNA viruses, EBOV usurps the nuclear RNA export factor 1 (NXF1) to export viral mRNAs from IBs (Wendt et al., 2022), which implies that IBs may also have a structure similar to the nuclear pore to ensure transport with the cytosol. Therefore, it is important to investigate the structure and working mechanism of IBs, particularly how IBAGs exchange materials with the cytosol, in order to gain a better understanding of viral replication and pathogenesis.

4.2. *Platform switching from replication to assembly in viral infection*

During the evolution of negative strand RNA viruses, viral mRNAs are first transcribed and translated into proteins. The N protein preferentially wraps viral RNA to form N-RNA template, which serves as the basics for further virus assembly. Once the packaging elements (RNA and protein) are sufficient, the virus switches from replication/transcription to assembly/budding. This process is mediated by various factors such as EBOV VP40, which is first recruited to IBs by associating with the N-terminus of N protein. This interaction triggers a conformational change in the N protein C-terminus that exposes its hydrophobic core. Subsequently, the exposed hydrophobic core interacts with VP40, facilitating the incorporation of viral components into virus-like particles (VLPs) and transitioning from RNA synthesis to virus assembly/budding. (Wu et al., 2020). Additionally, after the completion of HPIV3 RNA replication within IBs, vimentin may also play a critical role in preparing for virus assembly and budding by inhibiting the formation of HPIV3 IBs. This occurs through the downregulation of α-tubulin acetylation via enhancing the degradation of α-TAT1 (Liu et al., 2022). Together, these findings suggest that IBs might serve as a platform for the switching from replication/transcription to assembly/budding, with the involvement of various factors depending on the virus type.

4.3. *Host immune evasion strategies employed by IBs*

In certain circumstances, the innate immune system can recognize pathogen-associated molecular patterns (PAMPs), which are evolutionary conserved features of pathogens, through various pattern recognition receptors (PRRs). PRRs such as RIG-1, MDA5, and TLR7 detect cytosolic viral RNA and trigger the activation of type 1 interferon and inflammatory responses to combat viral infection (Bartok & Hartmann, 2020; Chatterjee et al., 2016; Gerlier & Lyles, 2011). However, the formation of viral IBs, which are spatially segregated from the surrounding cytoplasm, can serve as an additional viral escape strategy to evade recognition by recruiting intracellular components of the antiviral defense machinery (see Fig. 3).

IBs formation may prevent the activation of cell-intrinsic defense by sterically exclusion or the concentrated sequestration of antiviral sensors, thereby avoiding the activation of downstream pathways (Wu et al., 2014). The sequestration of immunostimulatory proteins into IBs is a mechanism employed by RSV N protein to counteract innate immune recognition (Jobe et al., 2020; Milicevic et al., 2022). Similarly, sequestering of antiviral factors to viral IBs has been observed in SFTS virus and RABV P protein (Hong et al., 2019; Kitagawa et al., 2018; Ning et al., 2015; Santiago et al., 2014) (Brzozka et al., 2005; Chelbi-Alix et al.; Ning et al., 2014).

Apart from the innate immune response, SGs also act as an additional defense response to combat viral replication. However, IBs can prevent SG formation by shielding viral RNAs, as demonstrated in HPIV3 (Hu et al., 2018). RSV sequesters p38 and OGT into IBs to suppress SG assembly, while EBOV sequesters many SG marker proteins to form SG-like structures inside viral IBs, likely inhibiting the antiviral role of SGs (Fricke et al., 2013; Lindquist et al., 2010) (Nelson et al., 2016). Overall, IBs are not only the sites for virus replication, but also carry various responsibilities, such as escaping innate immune recognition, serving as a platform for switching from replication to assembly, and performing undetermined functions in substructure. Therefore, IBs are structures with multiple functions.

5. Conclusion

Among the various stages of virus infection, genome replication is the most critical and conservative event. In this regard, IBs are specialized structures that play a critical role in promoting productive viral genome

replication and facilitating the assembly of numerous progeny viral particles. Since aspects of this replication mechanism may be common across different virus families, identifying the cellular and viral factors involved in IB formation can have broad implications. By deciphering the roles of these key factors, we can gain insights into how IBs promote viral replication, and potentially uncover new targets for antiviral drug development. Additionally, the role of IBs in innate immunity, how sequestration of cellular antiviral proteins into viral IBs may contribute actively to counteract antiviral activity, and the undetermined function of substructures within IBs will be also of great interest in the next years. As many eukaryotic viruses can cause severe pathologies and require additional antiviral therapeutics, research on viral IBs is relevant to human health. If it was possible to selectively target viral IBs without impacting other physiological cell structures, we may significantly reduce the pathological effects of viral infection. Therefore, the clinical implications behind viral IBs are significant, and hold great promise for future translational research.

Declaration of competing interest

The authors declare no competing financial interests.

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