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Poliovirus-induced changes in cellular membranes throughout infection

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The membrane landscape of a cell often changes drastically upon infection by a virus. In the case of the well-studied positive strand RNA virus poliovirus, the short infection cycle induces vesicles and tubular structures early in infection, and double-membraned vesicles late in infection. In this review, the current understanding of membrane changes in a PV-infected cell, the host and viral factors that facilitate these changes, and how these changes may promote virus replication will be discussed. Host factors involved in membrane rearrangement during infection include components of the COPI and COPII secretory pathways, lipid kinases, and the autophagy pathway. The roles of cellular membranes include acting as a scaffold for the RNA replication complex and roles in exit of mature virus. Finally, recent studies suggesting that not all picornaviruses are truly 'non-enveloped' are discussed in the context of the field, raising the possibility that cell-derived membranes play a role in delivering poliovirus particles to the extracellular space.

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Introduction

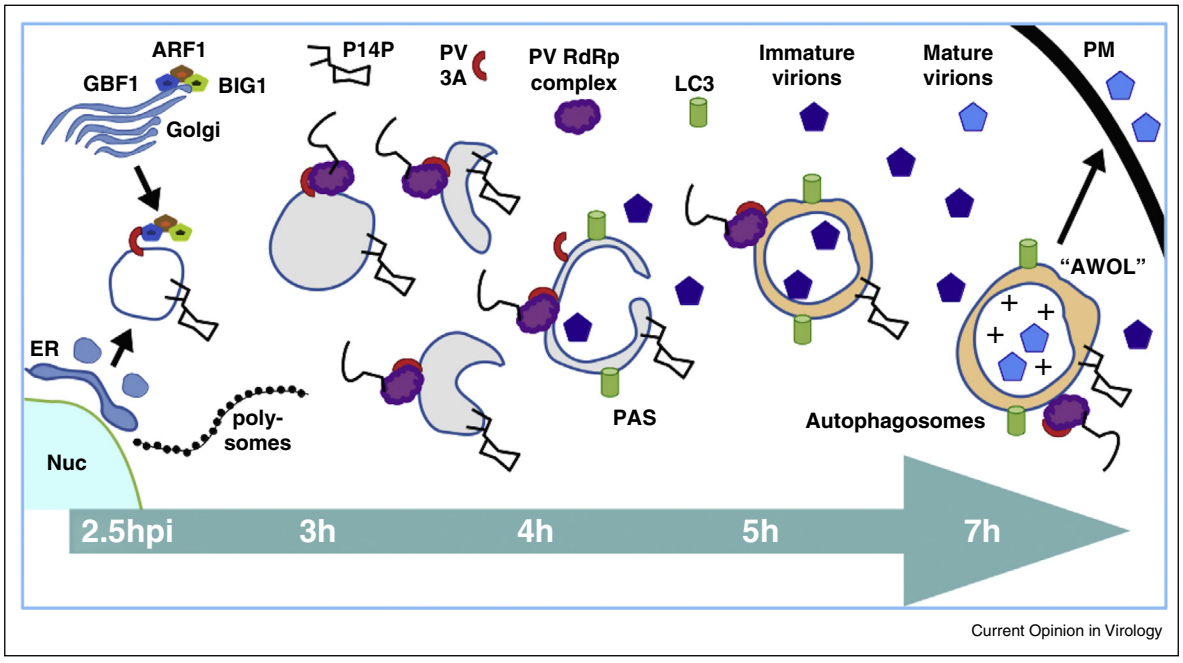
Poliovirus (PV), the causative agent of poliomyelitis, is a picornavirus, traditionally described as a non-enveloped particle containing a short positive-sense RNA genome. Like all positive-strand RNA viruses, PV physically alters the organelle composition of host cells to support its replication. Models of nucleic acid replication tend to describe virtually all steps taking place 'in solution.' For the RNA-dependent RNA replication of viruses such as poliovirus, however, this is not the case. PV RNA replication complexes are membrane associated, although we do not understand the benefit this affords to the virus [1,2]. The membrane association is achieved through multiple interactions. The small poliovirus 3A protein has a transmembrane domain, and the vast majority of the protein is oriented toward the cytosol [3]. Many other poliovirus proteins, including 2BC and the 3D polymerase, are associated with membranes, presumably through interactions with transmembrane proteins or, perhaps, in the case of 3D, specific classes of lipid [3–11].

The scope and focus of this review will be to describe what is known about how poliovirus effects changes in cellular membrane composition and organization. The limited space makes it difficult to incorporate all data from other picornaviruses; however, the review would not be complete without comparing and contrasting some of what has been learned about other picornaviruses, especially, in recent years, coxsackievirus B3 (CVB3). Several key questions have been raised by the literature, and answers are beginning to be found. First, how are the proliferating membranes in PV-infected cells generated? Are they primarily new synthesis, or are they derived from existing membranes? More importantly, why does the cell need to be so dramatically rearranged to support RNA virus replication?

Ultrastructural studies

Electron microscopy remains the most reliable way to study cellular ultrastructure. One early electron microscopy study of fragments from PV-infected cells was carried out in 1959. Although the study is largely focused on the virions themselves, bounding membranes around the virions are frequently observed and commented upon by the authors [12]. In 1965 Dales and Palade performed a seminal study focused on the effect of poliovirus infection on intact HeLa cells, revealing a dramatically altered cytoplasm [13••]. At three hours post-infection, large numbers of polysomes are observed near the ER and nucleus, and some vesicles, with an interior density consistent with cytoplasm, are observed. This time point roughly correlates with the peak of viral RNA replication [14]. Viral particles, some of which are identified as empty capsids, are observed near and within these vesicles by five hours post-infection. Images taken at seven hours post-infection reveal vesicles delineated by two lipid bilayers, many of which have virus within the interior membrane. In or near these double-membraned vesicles are large groups of viral particles. The dramatic and synchronous rearrangements observed at distinct time points post-infection have been a consistent feature of PV membrane studies, and have sometimes led to confusion as different groups compare studies at different stages of the virus infection cycle.

Figure 1



Model timeline of PV membrane-alteration events. Infection proceeds from left to right. At 2.5 hpi, novel organelles form with contributions from COPII vesicles leaving ER exit sites and the Golgi COPI complex Arf1/BIG1/GBF1, recruited by PV 3A and 3CD proteins. The presence of PI4KIIIβ at the newly forming membranes enriches them for PI4P. Large numbers of polysomes can be observed near these vesicles, as viral translation proceeds, using the vast majority of the available ribosomal machinery. By three hpi Arf1/GBF1/BIG1 are no longer directly associated with the membranes, which are often tubular and convoluted. RNA replication is associated with these membranes at this time, which is the peak of RNA replication. By four hpi more invaginations can be seen in these vesicles as they begin to resemble pre-autophagosomal structures (PAS) and attract the lipidated form of the autophagy marker LC3. By 5 h double-membraned autophagosome-like vesicles predominate, often containing virions. These vesicles remain associated with active RNA replication. Maturation of autophagosomes through acquisition of endosomal VTPase causes vesicle acidification (+) and promotes maturation of encapsulated virions. The autophagy pathway promotes exit of these virions though **a**utophagosome-mediated exit **w**ith **o**ut **l**ysis (AWOL) at the plasma membrane (PM).

Almost fifty years after the Dales and Palade paper, the Ehrenfeld group performed similar studies using modern EM tomography, examining cells at three, four, and seven hours post-infection (hpi) [15••]. The 3D-reconstructions reveal that at least some of the early 'vesicles' observed in two dimensions are in fact convoluted tubular structures at both three and four hpi, with the structures appearing more invaginated and convoluted at four hpi. At seven hpi, double-membraned structures, similar to those seen by Dales and Palade, are observed. Evidence of active RNA replication can be observed on both early single-membraned and late double-membraned structures. These data, and images of membrane loops that appear to be near-fusion (as illustrated in Figure 1, 'PAS') lead the authors to suggest that the early structures may develop into the later multi-lamellar structures.

Individual expression of PV proteins has been investigated as a way to understand the roles of the proteins in cellular changes during infection. Expression of PV 2B, for example, leads to Golgi complex disassembly in Vero, NRK, and COS-7 cells, a phenomenon also observed during infection of a variety of cells [7,16]. Expression of 2C or its precursor, 2BC, induces a variety of dramatic changes in the cytosol of HeLa and COS-1 cells, including formation of smooth single-membraned vesicles with electron-light contents [10,17]. 2C expression also induces changes to the ER, resulting in densely packed, anastomotic regions [6]. Double-membraned vesicles resembling those formed during infection can be induced by co-expression of PV 3A and 2BC, indicating that these vesicles are unlikely to be a cellular response to infection but specifically induced by the presence of virus proteins [17]. The wide variety of observations, dependent upon the time point post-infection, technique used, and viral proteins being expressed, indicate that the host–virus interactions leading to membrane rearrangements are complex and dynamic.

PV and COPII-like membranes

Poliovirus efficiently inhibits ER-to-Golgi trafficking. This led to a hypothesis that PV might divert anterograde COPII-dependent trafficking vesicles for use as sites of RNA replication. A light microscopy approach was taken