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Poxvirus membrane biogenesis

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ABSTRACT

Poxviruses differ from most DNA viruses by replicating entirely within the cytoplasm. The first discernible viral structures are crescents and spherical immature virions containing a single lipoprotein membrane bilayer with an external honeycomb lattice. Because this viral membrane displays no obvious continuity with a cellular organelle, a de novo origin was suggested. Nevertheless, transient connections between viral and cellular membranes could be difficult to resolve. Despite the absence of direct evidence, the intermediate compartment (ERGIC) between the endoplasmic reticulum (ER) and Golgi apparatus and the ER itself were considered possible sources of crescent membranes. A break-through in understanding poxvirus membrane biogenesis has come from recent studies of the abortive replication of several vaccinia virus null mutants. Novel images showing continuity between viral crescents and the ER and the accumulation of immature virions in the expanded ER lumen provide the first direct evidence for a cellular origin of this poxvirus membrane.

Contents

Brief introduction to poxviruses

Poxviruses are large DNA viruses that infect vertebrates and invertebrates and include species that cause severe human disease (e.g. smallpox and monkeypox) and others that serve beneficial roles as vectors for vaccines against unrelated infectious agents (Damon, 2013; Moss, 2013a). The ability to reproduce entirely within the cytoplasm is a defining characteristic of the poxvirus family and depends on viral proteins for replication (Moss, 2013b) and transcription (Broyles and Knutson, 2010) of the large DNA genome. Approximately 100 genes, conserved in all chordopoxviruses, are required for reproduction in cultured cells (Upton et al., 2003; Xu et al., 2014); a similar number of less well conserved genes are important for optimal infection of animals (Haller et al., 2014; Smith et al., 2013). The cytoplasmic sites of viral DNA synthesis expand into factories where the intermediate and late stages of transcription and translation occur (Katsafanas and Moss, 2007). Crescent membranes appear within the factories and enlarge to form spherical immature virions (IVs) that condense into dense brick-shaped mature virions (MVs) (Gaylord and Melnick, 1953; Morgan et al., 1954; Morgan and Wyckoff, 1950). Depending on the poxvirus genus, MVs may be enclosed by an additional membrane derived from the trans-Golgi, endosomal cisternae or plasma membrane (Boulanger et al., 2000; Hiller and Weber, 1985; Schmelz et al., 1994; Tooze et al., 1993) to form wrapped virions (WVs) and

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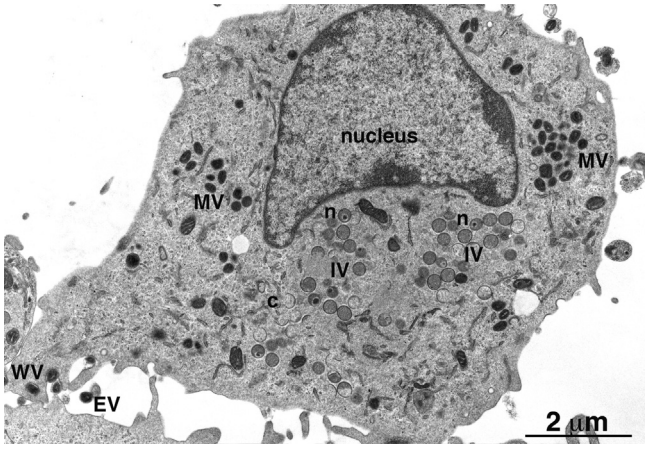

Fig. 1. Transmission electron microscopic image of a HeLa cell infected with VACV. Abbreviations: MV, mature virion; IV, immature virion; WV, wrapped virion; EV, extracellular enveloped virion; n, IV with nucleoid. Scale bar at bottom. Provided by A. Weisberg.

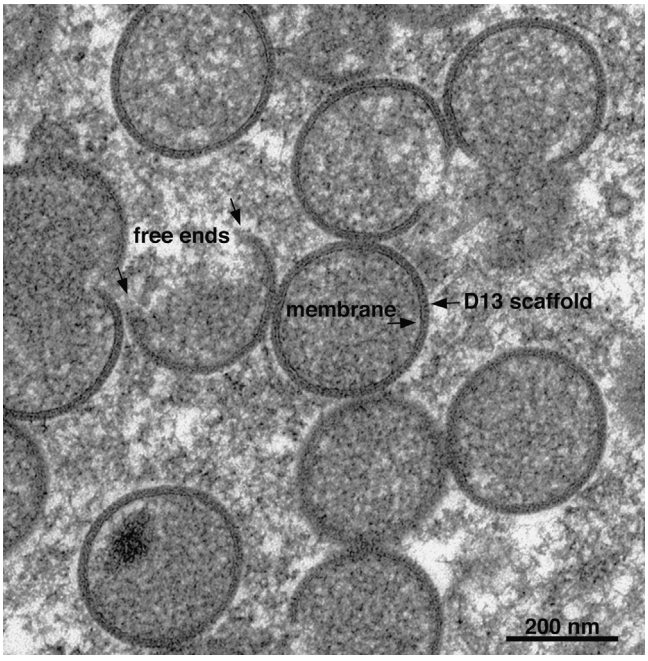

Fig. 2. Transmission electron microscopic image of a cell infected with VACV showing IVs forming within a virus factory. Free ends, lipoprotein membrane and D13 scaffold are labeled with arrows. Scale bar at bottom. Provided by A. Weisberg.

transported to the cell periphery and exocytosed as extracellular enveloped virions (EVs) (Fig. 1).

The structure and origin of the poxviral membrane delimiting crescents and IVs have intrigued virologists for more than half a century. The inability to discern connections between the viral membranes and cellular organelles (Fig. 2) led to the idea that viral membranes form de novo (Dales and Mosbach, 1968). However, the connections could be transient and an origin from cell membranes comprising the intermediate compartment (ERGIC) between the endoplasmic reticulum (ER) and Golgi apparatus (Sodeik and Krijnse-Locker, 2002) and from the ER itself (Husain et al., 2006) have been considered. This review focuses on recent studies that provide evidence for the formation of the crescent and IV membrane from the ER. Although the main features of morphogenesis are similar in all poxviruses, most research has been carried out with vaccinia virus (VACV). Several broad reviews of poxvirus structure and morphogenesis are available (Condit et al., 2006; Liu et al., 2014; Moss, 2013a; Roberts and Smith, 2008).

One membrane or two?

Electron micrographs from the 1950s describe clusters of spherical IVs and dense brick-shaped MVs (Gaylord and Melnick, 1953; Morgan et al., 1954; Morgan and Wyckoff, 1950). In some images, the IVs appear to have a double membrane with an outer dense layer of 4 to 6 nm and an inner of about the same thickness (Higashi et al., 1960). In other images (Dales and Mosbach, 1968) there appears to be a single membrane sheet coated with dense spicules, rather than two lipid membranes. The latter interpretation is supported by studies with the drug rifampicin, which prevents the formation of the spicule layer and provides clear images of a single membrane bilayer (Grimley et al., 1970; Moss et al., 1969; Nagayama et al., 1970). Nevertheless, the concept of a double-membrane was revived in a series of subsequent publications that posit two membranes so tightly apposed as to give the illusion of a single membrane (Krijnse-Locker et al., 1996; Risco et al., 2002; Salmons et al., 1997; Sodeik et al., 1993, 1994, 1995). However, careful measurements of the thickness of the IV membrane (Hollinshead et al., 1999) and freeze-fracture (Heuser, 2005) fully support the prior single lipid membrane model and deep-etch and immunoelectron microscopy demonstrate that the "spicule layer" is a honeycomb lattice comprised of VACV D13 protein trimers external to the single membrane (Heuser, 2005; Szajner et al., 2005). The controversy ended when proponents of the double-membrane and others reported electron tomography images confirming a single membrane (Chichon et al., 2009; Chlanda et al., 2009).

De novo membrane biogenesis or acquisition from host membranes?

Failure to detect continuity between the crescent membrane and cellular organelles led to the conclusion that the open-ended sheets are formed de novo (Dales and Mosbach, 1968). Although seemingly a heretical notion, in view of the formation of all known membranes from pre-existing ones, a de novo viral origin could not be dismissed out of hand since poxviruses are complex and encode proteins for many other functions including genome replication, transcription and disulfide bond formation (Moss, 2013a). Moreover, no viral protein components of the IV or MV are known to have signal peptides or to be glycosylated, which are signatures of trafficking through the secretory pathway of the cell. However, immunogold electron microscopic studies localized some VACV membrane proteins to the rough ER and the ERGIC, suggesting that the latter might contribute to the formation of the viral membrane (Krijnse-Locker et al., 1996; Rodríguez et al., 1996; Salmons et al., 1997; Sodeik et al., 1995; Sodeik and Krijnse-Locker, 2002). Smooth ER membranes labeled with protein disulfide isomerase (PDI) and viral proteins have also been found in close proximity to crescents (Chlanda et al., 2009; Husain et al., 2006). In addition, the association of VACV membrane proteins with microsomes was demonstrated by in vitro translation (Betakova et al., 1999a; Krijnse-Locker et al., 1996). However, analysis of purified MVs failed to detect cellular membrane proteins (Chung et al., 2006; Krauss et al., 2002; Resch et al., 2007) and the above studies only provide circumstantial evidence for participation of the ERGIC or ER in viral membrane formation.

Further studies were intended to discriminate functionally between possible contributions of different cellular organelles to viral membrane assembly. The fungal metabolite brefeldin A did not prevent the formation of IVs and MVs, although wrapping of the latter with Golgi membranes was impaired (Ulaeto et al.,

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