

## Microreview

# Virus factories: biogenesis and structural design

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### Summary

Replication and assembly of many viruses occur in specific intracellular compartments known as 'virus factories'. Our knowledge of the biogenesis and architecture of these unique structures has increased considerably in the last 10 years, due to technical advances in cellular, molecular and structural biology. We now know that viruses build replication organelles, which recruit cell and viral components in a macrostructure in which viruses assemble and mature. Cell membranes and cytoskeleton participate in the biogenesis of these scaffolds and mitochondria are present in many factories, where they might supply energy and other essential factors. New inter-organelle contacts have been visualized within virus factories, whose structure is very dynamic, as it changes over time. There is increasing interest in identifying the factors involved in their biogenesis and functional architecture, and new microscopy techniques are helping us to understand how these complex entities are built and work. In this review, we summarize recent findings on the cell biology, biogenesis and structure of virus factories.

### Introduction

Viruses are obligate intracellular parasites that depend on cell materials for their multiplication. In every single replication round, new viral genomic sequences are created in infected cells due to the mutation rates of viral RNA and DNA polymerases. Most of these mutations produce non-viable viruses, but some are the origin of new interactions between viral and cell factors, and can generate new

strategies for virus replication (Domingo *et al.*, 2012). In the course of this constant ongoing evolution, the signals that trigger the assembly of viral factories might have been selected because these structures provide clear advantages for the virus. The factory scaffold facilitates adequate spatial coordination of viral genome replication and assembly, with maximum efficiency in the use of cell resources. Factories could also provide sites for viruses to hide from host cell antiviral defences (Novoa *et al.*, 2005; Netherton and Wileman, 2011). Some viruses build factories inside the cell nucleus, although their organization is still poorly characterized. This is most likely due to our limited comprehension of the functional architecture of the cell nucleus (Peng *et al.*, 2010; Chang *et al.*, 2011). Most of our knowledge is derived from factories assembled in the cytosol, where they present two common features: (i) organization by recruitment of viral and cellular components around replication organelles built by viral replicases in cell endomembranes, and (ii) most factories recruit and use mitochondria.

Since the early reviews on virus factories and replication organelles (Mackenzie, 2005; Novoa *et al.*, 2005; Wileman, 2006), there has been considerable progress in their characterization, as shown in several excellent, recently published reviews that cover different aspects of the cell biology of viral replication (Den Boon and Ahlquist 2010; Nagy and Pogany, 2011; Netherton and Wileman, 2011). Progress has been possible thanks to technical advances in genomics, proteomics and microscopy techniques, and to growing interest in understanding how viruses take control of cells. In this review, we will focus on what we have learned in the past few years about the architecture of virus factories and the key cell factors involved in their biogenesis, dynamics and internal macromolecular transport.

### Biogenesis and structure of replication organelles

Early in infection, viral polymerases and cofactors interact with cell membranes, where they build functional replication complexes (RC) and replication organelles (Miller and Krijnse-Locker, 2008). These complexes make multiple copies of the viral genome and transfer them to the assembly sites. Viruses assemble the RC in cell endomembranes; less often, they can use the plasma membrane as

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well. This is the case of alphaviruses and rubella virus (RUBV), two members of the *Togaviridae* family of RNA viruses that use the cytosolic face of the plasma membrane to pre-assemble their RC (Frolova *et al.*, 2010; Spuul *et al.*, 2010; Risco *et al.*, 2012). These complexes are later internalized and transported to lysosomes, where replication continues; it is not known why the togavirus viral replicases must travel to the plasma membrane to pre-assemble the RC. Another peculiar case has been described for vaccinia virus (VV), the best-characterized member of the *Poxviridae*. VV assembles 'mini-nuclei' with rough endoplasmic reticulum (RER) cisternae, where viral replication takes place (Tolonen *et al.*, 2001). Apart from these exceptional cases, most viruses that replicate in the cytosol assemble membranous structures that belong to three main categories: single-membrane spherules, tubulovesicular cubic membranes and planar oligomeric arrays (Fig. 1).

Built by viral non-structural proteins, viral RNA and cell cofactors, spherules are single-membrane vesicles with a narrow opening to the cytosol. They form as invaginations in a variety of organelles such as mitochondria, ER, lysosomes, peroxisomes, Golgi apparatus or chloroplasts (Novoa *et al.*, 2005; Nagy and Pogany, 2011) (Fig. 1A and B). The neck-like connection restricts the entry of undesirable molecules inside the spherule, protects the replicated genome from degradation and also controls exit of the newly synthesized viral genome. Typical spherules are those assembled by the turnip yellow mosaic virus (TYMV) in chloroplasts (Prod'homme *et al.*, 2001) (Fig. 1A), the brome mosaic virus (BMV) in ER-derived membranes (Schwartz *et al.*, 2002), the Semliki Forest virus in modified lysosomes (Zhao *et al.*, 1994) and the flock house virus (FHV) in mitochondria, which were the first replication organelles characterized in three dimensions (3D) by electron tomography (ET) (Kopek *et al.*, 2007).

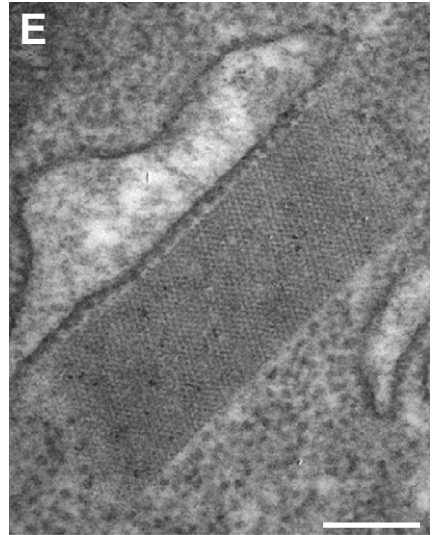
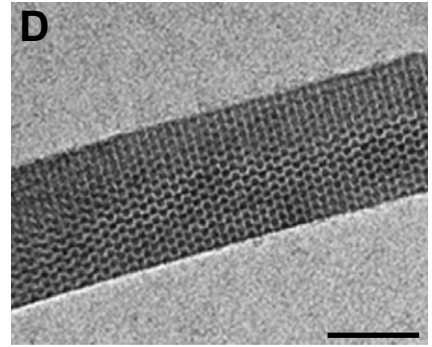
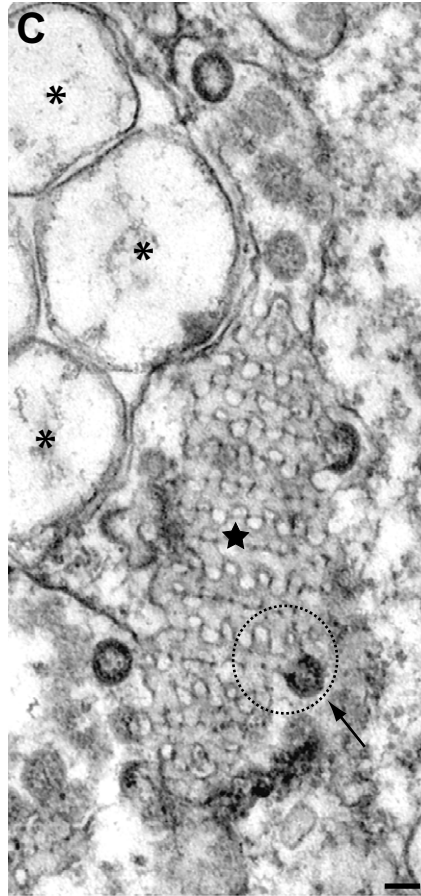
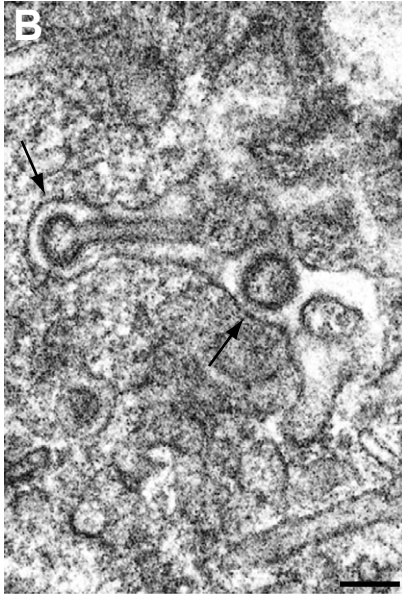
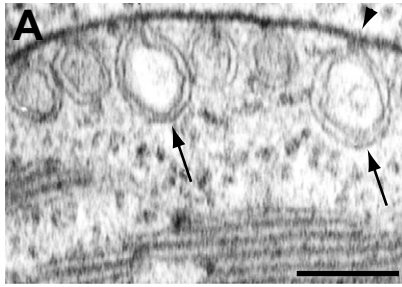
Bunyamwera virus (BUNV) assembles atypical spherules in Golgi membranes. BUNV is an RNA virus of negative sense polarity, and the best-characterized member of the *Bunyaviridae* (Elliott, 1997; Walter and Barr, 2011). The BUNV spherules are often seen attached to a cylindrical structure (Fig. 1B); these tubular elements have openings to the cytosol and grow in length over time. Their structural characterization both *in situ* and *in vitro* suggested that whereas the spherules are the site of viral genome replication, the cylinders might store and protect the replicated viral RNA while awaiting transport to the assembly sites in nearby Golgi membranes (Fontana *et al.*, 2008).

Also frequently detected in virus-infected cells and described in the literature with many different names, the membranous tubulovesicular structures (TBS) are indeed cubic membranes (Fig. 1C). These membranous webs, which most probably originate from the ER, are induced

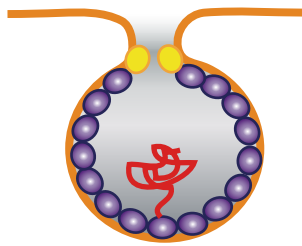
basically by all types of viruses, RNA and DNA. They consist of highly curved, 3D-folded lipid bilayers that build a platform for viral replication and virion assembly. Deng and collaborators studied cubic membranes through mathematical 3D simulations to analyse how these structures can work as a virus factory. They concluded that the interconnected channels of the TBS could facilitate molecule transport from the viral RC to the cytoplasm or the nucleus; the pores of the outer surface would control entry of materials, and the highly curved nature of the TBS might facilitate virus budding (Deng *et al.*, 2010). In cells infected by the SARS CoV (severe acute respiratory syndrome coronavirus), the membranous webs are seen in contact with double membrane vesicles (DMV) and nascent virions (Goldsmith *et al.*, 2004) (Fig. 1C). DMV were first considered potential viral replication sites, but extensive analysis of 3D tomographic reconstructions did not reveal any membrane openings between DMV and the cytoplasm (Knoops *et al.*, 2008). Moreover, ultrastructural analysis of viral replicase and double-stranded RNA (dsRNA), which is an intermediate of RNA replication, suggested that the TBS is the site of viral genome replication, whereas DMV could play a different role such as storage of replicated viral RNA (Knoops *et al.*, 2008; 2012). In the case of polioviruses, the DMV support viral RNA replication, but only at long times post infection (p.i.); during the exponential phase of replication, viral RNA synthesis occurs in single-membrane webs (Belov *et al.*, 2012). DMV structure suggests that they might be autophagosomes, although this is still a matter of debate (Netherton and Wileman, 2011).

Dengue virus, a member of the family *Flaviviridae* and a very important human pathogen, assembles an ER-derived network with spherules that has been characterized by ET (Welsch *et al.*, 2009). The 3D views of these complex webs showed connections between the ER-derived spherules that harbour the RC and the assembly sites in nearby ER membranes. This study showed the potential of ET to illustrate how the different steps of the virus life cycle can be connected inside the factory.

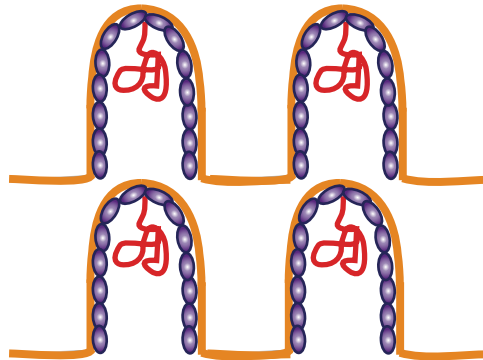
A third class of membranous structures that harbour viral RC are planar 2D oligomeric arrays (Fig. 1D and E). Studies with polioviruses showed that RNA polymerases assemble 2D arrays *in vitro* (Hobson *et al.*, 2001; Lyle *et al.*, 2002), and further analysis indicated that viral polymerase molecules could have both structural and enzymatic roles in replication organelles *in situ* (Spagnolo *et al.*, 2010). Several studies suggested that oligomeric arrays of viral polymerases can assemble in cells. For example, transmission electron microscopy (TEM) of cells infected with coxsackievirus showed intracellular organized lattices (Fig. 1E), very similar to those assembled by the viral RNA polymerase *in vitro* (Kemball *et al.*, 2010). A



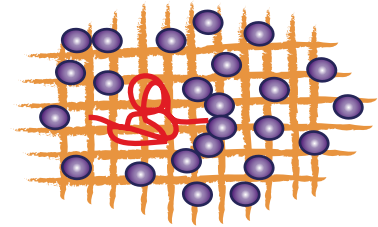
**F** Spherules





Cubic membranes



Oligomeric arrays



 Virus polymerase

 Virus RNA

 Host factors



**Fig. 1.** Transmission electron microscopy of replication organelles.

A. TEM of a plant cell infected with turnip yellow mosaic virus (TYMV). Arrows point to viral spherules, the structures that harbour the replication complex (RC), on the periphery of a chloroplast. The arrowhead indicates the neck-like connection that communicates the spherule with the cytoplasm.

B. BHK-21 cell infected with Bunyamwera virus. Spherules (arrows) are associated with Golgi membranes. The spherule on the left is connected with a tubular structure.

C. Tubuloreticular cubic membranes (star) in contact with double membrane vesicles (DMV; asterisks) in a Vero E6 cell infected with the SARS coronavirus. The dashed circle surrounds a group of curved membranes connected with a budding virus (arrow).

D. Oligomeric lattice assembled *in vitro* by the poliovirus RNA-dependent RNA polymerase in the presence of RNA, visualized by negative staining and TEM.

E. Lattice structure adjacent to the RER in a pancreatic acinar cell infected with coxsackievirus.

Bars, 100 nm.

F. Models showing our interpretation of the structures in the images.

A, C, D and E, reproduced with permission from Prod'homme *et al.* (2001), Goldsmith *et al.* (2004), Lyle *et al.* (2002) and Kemball *et al.* (2010) respectively.

close relationship between self-interaction and replication activity is reported for viral polymerases of other viruses such as FHV (Dye *et al.*, 2005), hepatitis C virus (Qin *et al.*, 2002) and RUBV (Risco *et al.*, 2012). It thus appears that close packing of viral polymerases might be necessary for RC activation and efficient viral replication in cells.

To build their replication organelles, viruses must take control of cell endomembranes, which they do by interfering with lipid metabolism, protein regulation, targeting and transport. The assembly of virus-induced cubic membranes could be linked to changes in cell cholesterol metabolism and trafficking (Deng *et al.*, 2010). Other viruses depend on phosphatidylcholine synthesis, fatty acid synthesis or geranylation (Netherton and Wileman, 2011). For enteroviruses and flaviviruses, a local increase in phosphatidylinositol-4-phosphate (PI4-P) levels is necessary to maintain the integrity of the membranous viral RC. Virus non-structural proteins recruit and activate PI4 kinases; this increases local production of PI4-P, which then mediates the incorporation of more replicase molecules (Hsu *et al.*, 2010; Reiss *et al.*, 2011). Recruitment of PI4 kinase might be mediated by the Arf1 GTPase and its guanine nucleotide exchange factor GBF1, a process modulated by specific viral proteins (Hsu *et al.*, 2010; Nagy and Pogany, 2011).

Other cell factors involved in RC assembly are the ESCRT (endosomal sorting complex required for transport) and the reticulon homology proteins, both involved in membrane bending and remodelling (Barajas *et al.*, 2009; Díaz *et al.*, 2010), SNARE (soluble NSF attachment protein receptor) proteins, mediators of vesicle fusion and involved in ER-to-Golgi transport (Pierini *et al.*, 2009; Liu *et al.*, 2011), chaperones, essential for the folding, stability and insertion of viral replication proteins into cell membranes (Pogany *et al.*, 2008), and prolyl isomerases (PPIases), which affect the folding, processing and stability of RC components (Watashi *et al.*, 2005; Kaul *et al.*, 2009).

Membrane rearrangements are induced by both enveloped and non-enveloped viruses. This suggests that viral

replication relies on the physical support of cell membranes, even for those viruses that do not incorporate membranes in their viral particles.

### Organelle recruitment

Expression of viral polymerases and assembly of replication complexes are usually sufficient for organelle recruitment (Fontana *et al.*, 2007). Some viruses build a single large factory (Novoa *et al.*, 2005) that causes major reorganization of cell content, as visualized by confocal microscopy (Fig. 2A and B). These large structures have a diameter of several microns and can be studied in 3D after serial sectioning, TEM and image processing (Fontana *et al.*, 2008). Early in infection, Bunyamwera virus assembles a large factory, shown by confocal microscopy in Fig. 2B and in 3D reconstruction in Fig. 2C. With TEM resolution, we see that the Golgi complex, RER cisternae and mitochondria have changed their normal shape, size and location and transformed into a rounded network of membranes. Higher magnification views show that the structures harbouring the RC, which are atypical spherules (Fig. 1B), connect Golgi stacks with RER (Fig. 2D), nascent viruses (Fig. 2E) and mitochondria (Fontana *et al.*, 2008). These 'viral tubes' that harbour the RC thus also act as the physical link between the organelles of the factory, probably facilitating interchange of materials. Three-dimensional reconstructions of serial sections and TEM are very useful for studying these large structures, as they show how different components contact each other within the factory scaffold.

Instead of a single large factory, some viruses build several mini-factories by local recruitment of organelles and factors. This is the case of alphaviruses and RUBV, both of which use modified lysosomes, the viral replication organelles of togaviruses (Magliano *et al.*, 1998). Freeze-fracture and TEM showed massive recruitment of organelles such as mitochondria and RER around the RUBV replication organelle (Fig. 2F). Although cell organelles surround most of the periphery of the replication organelle, it maintains communication with the cytosol and

**Fig. 2.** Virus factories in 3D.

A–E. Confocal microscopy and 3D TEM of the large factory assembled by Bunyamwera virus in BHK-21 cells.

A and B. Control (A) and infected (B) cells were labelled with antibodies to protein disulfide isomerase (PDI), a marker of the rough endoplasmic reticulum (RER; red) and the viral glycoprotein (Gc; green). Nuclei were stained with DAPI (blue). Confocal microscopy (B) shows massive recruitment of RER elements around the factory.

C. Factory as visualized by TEM of serial sections, 3D reconstruction and image processing. Mitochondria (red) surround a network of RER (yellow) and Golgi (beige) membranes. Viral tubes, the structures that harbour the viral RC, are grey. N, nucleus.

D and E. Volumes at higher magnification, showing a viral tube attached to Golgi and RER membranes (D), and another near a viral particle (blue) (E).

Bars: A and B, 10 µm; E, 100 nm. C–E, reproduced with permission from Fontana *et al.* (2008).

F–I. Mini-factories of rubella virus in BHK-21 cells.

F. Freeze-fracture, metal replication and TEM shows the recruitment of mitochondria (mi) and RER around the replication organelle (asterisk), which is a modified lysosome. N, nucleus.

G. Three-dimensional model of the factory as visualized by ET. The replication organelle (yellow) is surrounded by RER (green) and presents an opening to the cytosol (arrow). A mitochondrion is coloured red.

H and I. Computational tomographic slices from different tomograms, showing contacts between the RER and the replication organelle (asterisks), seen as closely apposed membranes (arrow in H) and protein bridges (arrow in I).

Bars: F, 200 nm; H and I, 50 nm. F–I, reproduced with permission from Fontana *et al.* (2010).

J–O. Viral factories assembled by the giant mimivirus in amoeba cells.

J. Electron tomographic slice of an early replication factory at 4 h.p.i. (stage 1). Arrows point to viral cores.

K. Surface rendering of the boxed region in J, showing the two viral cores (yellow) surrounded by viral DNA (blue).

L. Thin section of a virus factory at 8 h.p.i. (stage 2) as visualized by TEM, revealing viral particles (arrows) at various assembly stages.

M. Three-dimensional views of a viral factory within an amoeba cell lysed at 8 h.p.i. and visualized by SEM.

N. SEM of a virus factory isolated at 8 h.p.i. The blue arrow indicates an immature virus particle and the white arrow, a mature particle.

O. SEM of a virus factory isolated at 10 h.p.i. (stage 3). Only mature viruses can be detected.

Bars: J, 200 nm; L and O, 500 nm; M, 2 µm; N, 300 nm. J, K, reproduced with permission from Mutsafi *et al.* (2010); L–O, from Zauberman *et al.* (2008).

the endocytic pathway (Fontana *et al.*, 2010). Cells cryo-fixed by high-pressure freezing, a technique that provides outstanding ultrastructural preservation (Studer *et al.*, 2008), were analysed in 3D by ET; these studies revealed the inter-organelle contacts in RUBV factories in considerable detail (Fig. 2G–I). The RER usually surrounds the replication organelle (Fig. 2G), and there is constant interchange of molecules between these two compartments. RER sends newly synthesized viral proteins to the replication organelle, which transfers viral genomic and subgenomic RNA to the RER to continue protein synthesis (Lee and Bowden, 2000). This macromolecule interchange might be mediated by the two types of contacts detected in tomograms, consisting of closely apposed membranes (Fig. 2H) and protein bridges (Fig. 2I). The molecules that create these contacts have not been identified, although their dimensions point to the tethering proteins that function in inter-organelle communication. Curiously, organelle attachment is not accompanied by membrane fusion; this has been observed not only for RER, but also for mitochondria and for the RUBV assembly organelle, the Golgi complex (Fontana *et al.*, 2010).

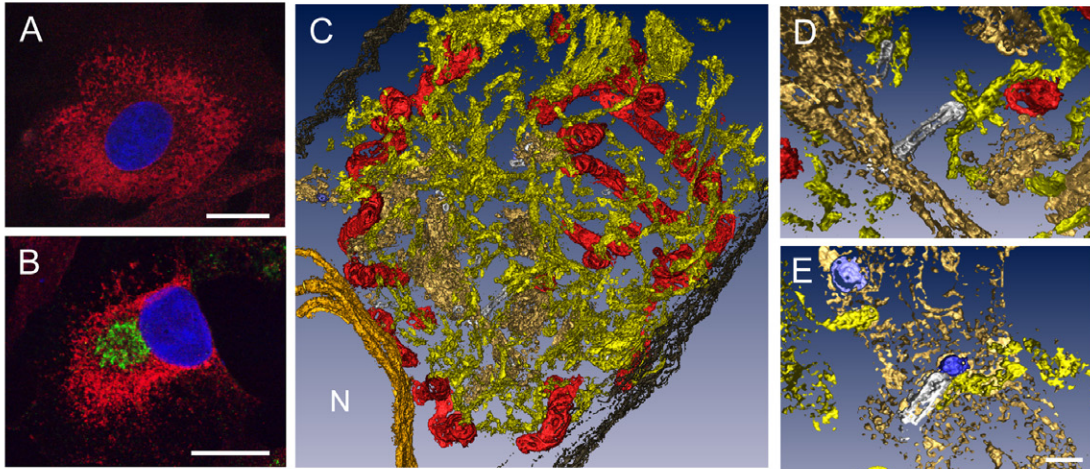
As mentioned above, mitochondria are recruited to many virus factories. Their main role might be to act as an energy source for virus needs. In certain cases, they can also function as a replication organelle (Kopek *et al.*, 2007; Pathak *et al.*, 2008) or for virus assembly (Garzon *et al.*, 1990; Lanman *et al.*, 2008). Recent data show that mitochondria can provide additional host replication factors. Among these, p32, a mitochondrial matrix protein that participates in a number of apoptotic pathways (Itahana and Zhang, 2008), leaves the mitochondria in

RUBV-infected cells and in cells transfected with RUBV replicons, and is incorporated into replication organelles (Fontana *et al.*, 2007; Ilkow *et al.*, 2010). p32 is detected in the internal membranes of the RUBV replication organelle, where viral polymerase and dsRNA molecules are also found (Fontana *et al.*, 2010). p32 participates in mitochondria recruitment (Claus *et al.*, 2011) and binds to the RUBV capsid, an interaction that enhances virus replication by a still unknown mechanism. Transcription of the RUBV subgenomic RNA in fact appears to depend on p32 binding to the RUBV capsid (Mohan *et al.*, 2002; Beatch *et al.*, 2005). p32 interacts with proteins from other viruses, such as the human immunodeficiency virus 1 (HIV-1), herpes simplex virus, adenovirus, Epstein-Barr virus and HCV, known in all these cases to be advantageous for viral replication (Ghebrehiwet *et al.*, 2001). The list of mitochondrial proteins identified as important for tombusvirus replication includes the MSP1 ATPase, as well as MAM33, which participates in oxidative phosphorylation and interacts with the p33 viral replicase, the inner mitochondrial membrane protein MDM38, which binds to viral RNA and stimulates viral replication, and TUM1, which also interacts with p33 (Nagy and Pogany, 2010).

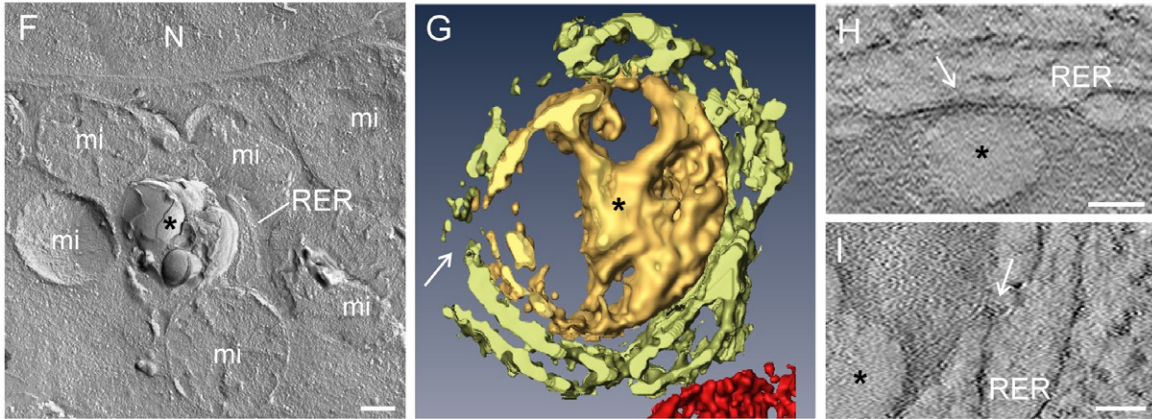
The identification of factors involved in mitochondria recruitment and modification in virus-infected cells can give us important clues to understanding the biogenesis of virus factories. The only signalling pathway involved in factory biogenesis identified so far is that of aggresomes, used by large DNA viruses such as the African swine fever virus (ASFV), the poxviruses and the iridovirus frog virus 3 (Rojo *et al.*, 1998; Novoa *et al.*, 2005). The aggresome



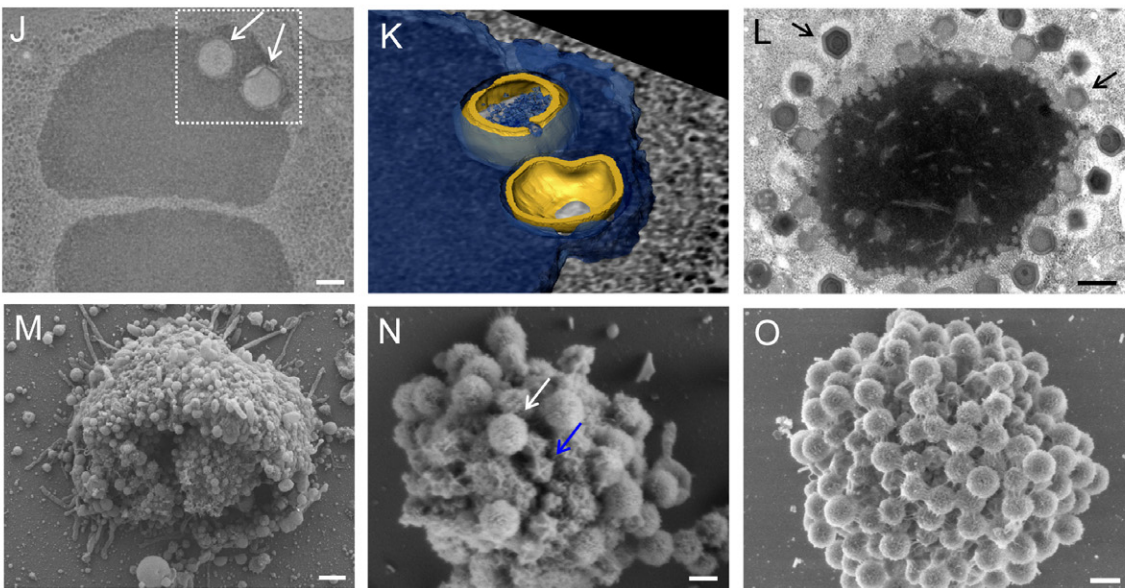
Bunyavirus - ssRNA (-)



Rubella virus - ssRNA(+)



Mimivirus - dsDNA



is a cell response to protein misfolding and aggregation (Kopito, 2000). Virus factories and aggresomes both assemble at the microtubular organizing centre (MTOC), recruit cell chaperones and mitochondria, and build a cage of vimentin filaments, a structure that is maintained by the activity of dynein motors on microtubules (Netherton and Wileman, 2011). Herpesviruses, adenoviruses and simian virus 40 seem to use aggresome-like structures termed promyelocytic leukaemia nuclear bodies (PML-NB) as sites for the assembly of nuclear factories (Maul *et al.*, 2000); however, recent work suggested that additional elements participate in the biogenesis of the nuclear factory of polyomaviruses (Erickson *et al.*, 2012). It is proposed that viruses kidnap the aggresome pathway to concentrate the numerous factors needed for replication and morphogenesis, and to avoid being recognized by cell defences. Alternatively, viral macromolecular complexes could be mistaken for misfolded proteins by the cell, which would then trigger the aggresome response (Netherton and Wileman, 2011).

#### **Structural transformation of viral factories: from viral replication to morphogenesis**

Virus factories have a dynamic structure that changes to accommodate virus needs. Although we do not know how these changes are coordinated, several examples have been studied by EM. Here we describe three of these, the bunyaviruses, poxviruses and mimiviruses. The compact, rounded structure built by BUNV during the replication phase (Fig. 2B and C) changes remarkably later in infection, coinciding with the assembly and maturation of new viral particles. Mitochondria and RER networks are removed from the perinuclear region, and secretory vesicles bearing the virus progeny move towards the cell surface (Fontana *et al.*, 2008). The VV factory changes several times during the course of infection (Novoa *et al.*, 2005). For viral genome replication, the virus first assembles cytoplasmic mini-nuclei with attached mitochondria (Tolonen *et al.*, 2001); virus morphogenesis then starts an aggresome-like structure (Risco *et al.*, 2002), where immature viruses assemble using an atypical membrane remodelling mechanism that has been characterized by ET (Chlanda *et al.*, 2009). Final envelopment and viral maturation takes place in Golgi stacks (Schmelz *et al.*, 1994).

Like poxviruses, mimiviruses undergo their entire life cycle in the infected cell cytoplasm (Mutsafi *et al.*, 2010). The early factories of these giant viruses can be seen at 4 h.p.i. (hours post infection). They are built by viral cores that release the DNA into the cytosol, where replication starts (Mutsafi *et al.*, 2010). Each core seeds its own factory, and ET shows that factories remain around their seeding cores (Fig. 2J and K). When factories expand at

late infection stages, they fuse to form a single factory with a very different appearance, where assembly begins and viruses at various assembly stages can be distinguished (Zauberman *et al.*, 2008) (Fig. 2L). Scanning electron microscopy (SEM) showed the structure of these factories in 3D. Images of factories isolated at 8 h.p.i. have both immature and mature particles (Fig. 2M and N), but 2 h later, only mature viruses are detected (Fig. 2O). Mimiviruses have a core containing a dsDNA genome, an icosahedral capsid and a lipid membrane. The factory is not surrounded by membranes, but the assembly of new viral particles is predicted to require membrane recruitment, as the mature viruses have a lipid envelope (Xiao *et al.*, 2005). The origin of such membranes is an open question for mimiviruses and other nucleocytoplasmic large DNA viruses, including the extensively characterized VV.

#### **Movement of macromolecular complexes**

Inside virus factories, cellular and viral macromolecules must be transported efficiently to concentrate all necessary materials and to connect viral genome replication with assembly and exit of mature virions. Viruses thus need to take control of the cell's trafficking machinery. Returning to the replication organelles and virus factories (Figs 1 and 2), one might wonder how molecules are able to move in and out these structures. Viruses can use the cytoskeleton and associated motor proteins as well as the host endomembrane system for intracellular movement (Boevink and Oparka, 2005; Greber and Way, 2006; Radtke *et al.*, 2006; Harries *et al.*, 2010; Harries and Ding, 2011). Plant viruses encode movement proteins (MP) that transport the viral genome from cell to cell through plasmodesmata (Niehl and Heinlein, 2011). In addition to MP, other categories of viral proteins are involved in intracellular movement of macromolecules in plant cells, including some that work in viral replication (Schoelz *et al.*, 2011). For example, the viral replication complexes of TMV and turnip mosaic virus localize to and traffic along microfilaments (Liu *et al.*, 2005; Harries and Ding, 2011). The movement of these viral components along actin filaments suggests involvement of the myosins, a large superfamily of microfilament-associated molecular motors. For intracellular movement, animal viruses also use vesicular transport, microtubules, microfilaments and associated motors (Greber and Way, 2006; Radtke *et al.*, 2006; Iwasaki and Omura, 2010). Actin and myosin have been detected in the replication organelles of Bunyamwera virus (Fontana *et al.*, 2008). Treatment with drugs that inhibit these proteins suggests that the actin-myosin complex mediates transport of the viral genome out of the RC, although direct demonstration is still needed (Fontana *et al.*, 2008).



Numerous aspects of viral subparticle transport inside cells remain unknown, for example, how the replicated genome escapes from viral polymerases in replication organelles, how viruses move across distinct intracellular compartments, or whether the actomyosin network mediates short-distance movements of viral complexes in cell endomembranes. A case of special complexity is that of herpesviruses, which must connect their factory inside the nucleus with a cytosolic factory by moving large DNA molecules in and out of the nucleus (Chang *et al.*, 2011). The characterization of these processes will increase our understanding of the cellular machinery involved in regulating intracellular trafficking of proteins and nucleic acids.

### Conclusions and perspectives

Recent advances in proteomics, genomics and structural biology are helping us to address very complex interactions such as those that operate during virus factory construction. Although we are beginning to understand how replication organelles are assembled, information is still limited about how cell organelles are recruited, about the mechanisms of macromolecular transport between compartments, and about the signals that regulate the major structural changes in the factory during distinct stages in the virus life cycle. Even before we were able to understand all of these complex processes, scientists made the surprising discovery of the virophages, viruses that parasitize factories built by other viruses (La Scola *et al.*, 2008; Desnues *et al.*, 2012). This myriad of interactions is changing our concept of viruses as inert entities to that of 'live' organisms that have surely played a major role in the evolution of cells (Raoult and Forterre, 2008; Bandea, 2009; Feschotte and Gilbert, 2012).

Functional and mechanistic studies will be necessary to increase our current understanding of the biogenesis and structure of virus factories. Powerful new imaging technologies such as live-cell microscopy, super-resolution light microscopy and correlative light and electron microscopy (CLEM), together with new probes for *in situ* molecular mapping, will have increasing impact on this field (Van Weering *et al.*, 2010; Fu and Johnson, 2011; Huang *et al.*, 2011; Jouvenet *et al.*, 2011; Risco *et al.*, 2012). By deciphering how viruses build their factories, we will not only learn about viruses, but also about how cells position their proteins and regulate organelle shape, size and movement.

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