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Abstract

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ER redox rescues virus from ER trap

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How viruses manage to resist physical and chemical stress and yet open their protective coats during cell infection has been a longstanding, fundamental question. A study with the DNA tumour virus SV40 now shows that protein folding and quality-control factors of the endoplasmic reticulum reshuffle disulfide bonds within the viral capsid, providing a molecular mechanism for the exit of infectious virions from the endoplasmic reticulum.

The concept of a viral replication cycle is exceedingly simple, yet its mechanisms hold many secrets. Upon engulfment of a virus by the host cell, uncoating of the viral genome from the protective capsid is required for the transcriptional activation of viral genes, using the transcriptional machinery of the host cell to drive the synthesis of viral progeny. Newly formed particles exit the cell, infect new cells, and the cycle starts again. On their way into a cell, animal viruses pass through cellular compartments where they encounter triggers that initiate viral coat destabilization. Examples of destabilization events include virus-receptor interactions, the shedding of minor capsid proteins, or the low pH in the digestive tract or in endosomal vesicles activating viral or cellular proteases that catalyse limited proteolysis
1. In the past, therapeutic inhibition of uncoating has been a prominent strategy in the development of anti-viral drugs, particularly for non-enveloped viruses, such as picornaviruses
2. Although in principle successful, the strategy has been largely abandoned
due to the rapid appearance of viral escape mutants in patients treated with such antiviral drugs. Developing therapeutics directed against the host may be an interesting alternative.

A recent paper by Schelhaas et al. reveals new host functions required for the infectious entry of the polyoma virus Simian Virus 40 (SV40). Polyoma viruses are non-enveloped DNA viruses that cause cancer in rodents and traces of their genome can be found in human tumours. Notably, SV40 was accidentally inoculated into millions of humans during vaccinations with chemically-inactivated poliovirus in the 1950s when the vaccine virus was produced in SV40-infected monkey cells. The passage of SV40, mouse polyomavirus (mPy) and human BK virus into cells is intriguing, since these viruses use glycolipids as their cell attachment site (Fig. 1). Polyoma viruses are assembled in the nucleus under reducing conditions, and are oxidized outside the cell. The spatial separation of viral assembly and disassembly in part explains why a virus can be stably assembled in an infected cell, and disassembled on entry into a naïve cell.

The work by Schelhaas et al. shows how a virus uses the protein folding and quality-control apparatus for uncoating and membrane translocation. It provides a molecular explanation of two long standing observations, namely that the incoming SV40 is found in large amounts inside the ER and that infectious particles are present in the cytosol. The SV40 capsid has icosahedral symmetry and is composed of 72 homopentamers of the major capsid protein VP1. Twelve pentamers are five-coordinated, the others are six-coordinated. It is unusually structured because the VP1 proteins are linked together via a network of interchain C9-C9 and C104-C104 disulfide bonds (Fig 1). The C-terminal peptide of VP1 extends to binding sites on VP1 molecules of neighbouring pentamers that are stabilized by calcium ions. In the life cycle of polyoma viruses, progeny capsids are assembled in the nucleus under reducing conditions and oxidized outside the cell. They do not come in contact with the enzymatic redox system in the ER, the site of viral uncoating.
The initial trigger for the research by Schelhaas et al. was the observation that in SDS polyacrylamide gels, treatment of non-reduced virus with alkylating agents yields VP1 multimers indicative of intersubunit disulfide bonds. These are not observed in the absence of alkylation, where free cysteines are available for reshuffling the interchain disulfides of VP1. Moreover, alkylation of virus blocked infection and alkylated virus did not release VP1 during infection, indicative of a requirement for disulfide isomerisation rather than disulfide reduction for productive infection. Depletion of the two ER thiol–disulfide oxidoreductases ERp57 and the closely related protein disulfide isomerase (PDI) by small interfering RNA (siRNA) led to a significant reduction of infection, but only the depletion of ERp57 inhibited VP1 release in vivo, indicating that only ERp57 resolves the interchain disulfide bonds. ERp57 is an essential soluble protein of the ER where it catalyses disulfide reduction, isomerization, and dithiol oxidation in substrate proteins. These findings are nicely complemented by an in vitro assay where both purified ERp57 and PDI were able to induce the loss of VP1 on isolated virus. To find out whether ERp57 and PDI serve as isomerase or reductase, alkylated (non-isomerizing) virus was used. Following treatment with ERp57, alkylated SV40 capsids did not release VP1 monomers although PDI was able to release VP1, suggesting that ERp57 acts as an isomerase and PDI acts as reductase. However, native SV40 released VP1 following ERp57 treatment, supporting the siRNA knock down data. The disulfide isomerization activity of ERp57 is needed to resolve interchain disulfide bonds of the pentamers, yielding the intrachain disulfide bond C9–C104 (Fig. 1). Nevertheless, isomerization of disulfide bonds is not sufficient for the dissociation of the pentamers; the loss of the VP1 associated Ca$^{2+}$ ions is also required. Cryo-electron microscopy of partially uncoated viruses revealed that it was the five-coordinated pentamers that were released, suggesting that they are less tightly associated than the six-coordinated pentamers. Interestingly, the C9–C104 disulfide bond is already in place in native mouse polyoma virus VP1 which has no interchain disulfide bonds and hence does not require the
Erp57 activity for infection\(^9\). The conformational changes around the SV40 VP1 disulfides weaken the capsid such that the myristoylated amino-terminal region of VP2 may swing out, and potentially positions the capsid to the luminal face of the ER membrane. Electron micrographs suggest that SV40 is proximal to the luminal ER membrane\(^6\). A similar principle of conformational change is used by mPy where \textit{in vitro} experiments have shown that the thioredoxin-like Erp29 is required for membrane association of the virion\(^9\).

But how is a nucleo-protein complex of 50\(\times\)nm in diameter translocated through the ER membrane? The observation that proteasome inhibitors block SV40 entry suggests an involvement of ER-associated degradation (ERAD)\(^3\). ERAD requires chaperones that recognize misfolded proteins and the retrotranslocation machinery\(^10\). Cholera toxin, for example, requires reduced PDI for unfolding and presentation to the ERAD machinery. Accordingly, knockdown of \textit{PDI}, \textit{Derlin-1} and \textit{Sel1L} reduced SV40 infection suggesting a role of ERAD in SV40 retrotranslocation. Derlin-1 is a putative pore forming component, while Sel1L has been suggested to be required for substrate recognition. The sensor that recognizes the disulfide-reshuffled virus in the ER has not been identified, but may involve PDI or the signal peptide peptidase, perhaps recognizing VP2\(^10\). This sensor may target the virus to the retrotranslocation complex. The rather low efficiency of SV40 release from the ER into the cytosol (estimated to be 1\%) may explain why it remains unclear whether the translocated virus particles are ubiquitinated, a modification that normally occurs on proteins that are targeted to ERAD. Nevertheless, the translocated virus with isomerized disulfides sheds the VP1 pentamers in the low calcium conditions of the cytosol. This poises the particle for the release of infectious DNA into the nucleus, as shown for other DNA viruses\(^11\).

One can speculate that the co-option of the ERAD machinery by SV40 occurs in conjunction with biosynthetic functions of the ER membranes, such as lipid synthesis, or lipid droplet formation\(^12\). Viral escape from the ER is, however, reminiscent of viral gene products that co-opt the
ERAD machinery to induce rapid degradation of host receptors or of immune surveillance molecules, such as MHC class I proteins\textsuperscript{10}. The fact that the ER redox system contains more than 15 different disulfide isomerasers suggests that other viruses that visit the ER or interfere with ER functions, such as the papilloma viruses or picornaviruses, may use a similar pathway as SV40 — perhaps by engaging different isomerasers. Future studies will uncover additional host factors and these may be useful targets for anti-viral therapies as they are not subject to viral resistance mutations.

**Figure^1** ERp57 and low calcium are involved in SV40 pentamer dissociation. (a) Infectious entry of SV40 into human and monkey cells occurs via caveolin and lipid raft-dependent endocytosis on binding to the GM1 ganglioside receptors at the plasma membrane\textsuperscript{13,14}. From the caveosome the viruses are transported inside vesicles along microtubules to the ER where they accumulate\textsuperscript{15}. A cryo-electron microscopy structure of intact SV40 capsids is shown in blue and reduced and calcium-depleted particles that release the pentavalent pentamers of the major capsid protein VP1 are in yellow\textsuperscript{3}. The ER thiol-disulfide oxidoreductase ERp57 isomerasers SV40 interchain disulfide bonds. PDI, derlin-1 and Sel1L, three proteins involved in ER-associated degradation (ERAD) are then thought to retrotranslocate particles to the cytosol where the low calcium concentration leads to pentamer dissociation. Cytosolic particles then enter the nucleus via the nuclear pore complex where viral transcription and replication takes place. (b) Model of SV40 disulfide isomerisation mediated by ERp57. Monomers of the major capsid protein VP1 of a five-coordinated pentamer (blue) are connected to neighboring six-coordinated pentamers (orange and green) via interchain disulfide bonds C9–C9 and C104–C104 (possible bonds are displayed by dashed lines). During disulfide bond isomerisation, the intrachain disulfide bond C9–C104 is formed for the VP1 molecule of the five-coordinated pentamer and the six-coordinated pentamers are connected by a C104–C104 disulfide bond. This isomerisation reaction uncouples the five-coordinated pentamer from the disulfide bond network of the virus.
capsid which may provide a signal for recognition of the particle by the ERAD machinery for translocation to
the cytosol.