to the LOV domain of WC-1 and that WC-1 is the fungal blue-light photoreceptor. Interestingly, blue-light photoreceptors in plants carry a chromophore-binding motif that associates with another flavin molecule, flavin mononucleotide (FMN).

In complementary work, Froehlich *et al.* (*4*) arrived at the same conclusion via a different route. They identified two light-responsive DNA fragments in the promoter of the circadian clock gene *frq*. In electrophoretic mobility shift assays, they subsequently showed that both WC-1 and WC-2 were able to bind to these DNA fragments. They observed that one type of WC-1/WC-2 complex was present primarily in the dark, whereas the other prevailed under light conditions. Light intensity and wavelength requirements were similar for the formation of the light-specific WC-1/WC-2 complex in vitro and for the regulation of the circadian clock in vivo, supporting the notion that the light-sensitive WC-1/WC-2 complex contained the blue-light photoreceptor. WC-1, WC-2, and the chromophore FAD were sufficient for the formation of the light-specific complex, but only WC-1 seemed to be capable of binding FAD. Indeed, addition of FAD conferred light sensitivity on the WC-1/WC-2 dark complex. Corroborating the He *et al.* results, Froehlich and colleagues found that FAD but not FMN was required for light perception by *Neurospora*. Like He and colleagues, these authors also concluded that WC-1 is the blue-light photoreceptor responsible for light entrainment of the circadian clock in fungi.

Mutation of conserved amino acids in the WC-1 LOV domain results in a variety of defects in *Neurospora*'s response to light (*10*). These findings indicate that WC-1 is the blue-light photoreceptor of the fungal circadian clock, and is also a central switch that, with help from WC-2 and FAD, directs transcription of light-regulated genes in response to light. However, given that some *wc-1*–deficient *Neurospora* mutants can still respond to light, WC-1 may not be the only blue-light photoreceptor in fungi (*11*). Fungal proteins with the potential to be responsive to blue light include rhodopsin, proteins containing LOV domains, and proteins that resemble the photoreceptors of higher plants.

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PERSPECTIVES: VIROLOGY

CMV Makes a Timely Exit

Veronica Sanchez and Deborah H. Spector

A virus faces many obstacles during its life cycle. As an obligate parasite, it must exploit the machinery of the host cell to ensure its own replication. This is particularly true for large DNA viruses, such as the herpesviruses, that replicate their genomes in the nucleus of the host cell but require both the nucleus and cytoplasm for assembly of mature viral particles (see the figure, next page). Thus, the subviral particles of herpesviruses must exit the nucleus after replication of their DNA, a task made more difficult by the physical barrier of the nuclear envelope. On page 854 of this issue, Muranyi *et al.* (*1*) reveal the tricks that cytomegalovirus (CMV), a β-herpesvirus, plays in order to dissolve the nuclear lamina of the host cell nuclear envelope and gain entry to the cytoplasm.

The herpesvirus virion has a complex structure: The large DNA core is packed into an icosahedral capsid that is surrounded by an amorphous layer called the tegument, which includes at least 15 distinct proteins. The tegument is enclosed in a cell-derived lipid membrane envelope containing virus-encoded glycoproteins that are important for attachment of the virus particle to the host cell (see the figure, next page). Successful assembly of virions requires that the virus overcome the barriers that impede (i) encapsidation of the newly replicated DNA in the host cell nucleus, and (ii) the subsequent envelopment and release of viral progeny from the cytoplasm.

The nuclear envelope provides a major obstacle to movement of herpesvirus virions into the cytoplasm. This structure consists of two concentric lipid bilayers, termed the inner and outer nuclear membranes, which are connected to each other at the nuclear pores (see the figure, next page). The outer nuclear membrane is contiguous with the endoplasmic reticulum, and membrane proteins diffuse freely between the two structures. The inner nuclear membrane has a distinct set of transmembrane proteins, and current models postulate that these proteins are held in place by the nuclear scaffold. Underlying the inner nuclear membrane are intermediate filament-like proteins called lamins, which form a network about 50 nm thick that stabilizes the nuclear envelope [for a review, see (*2*)].

The Muranyi *et al.* study suggests a provocative strategy by which CMV circumvents the physical barrier of the nuclear envelope (*1*). The authors propose that CMV is able to modify and destabilize the nuclear lamina by recruiting a host cell protein kinase C to the inner nuclear membrane. In uninfected cells, solubilization of the nuclear lamina is a prerequisite for dissolution of the nuclear membrane at mitosis. This solubilization is accomplished by phosphorylation of the lamins by protein kinase C or by the Cdk1–cyclin B complex. Accumulation of protein kinase C at the nuclear envelope in CMV-infected cells enables the virus to disrupt the nuclear lamina, allowing virion progeny access to the inner nuclear membrane and initiating the first stage of envelopment.

Herpesviruses replicate their DNA in nuclear domains called viral replication centers. The newly replicated DNA is inserted into preformed capsids in the nucleus [for a review, see (*3*)]. Because the subviral particles are too large to pass through the nuclear pore, they must cross the nuclear lamin meshwork to reach the inner nuclear membrane. The particles acquire some of their tegument proteins and a primary envelope by budding from the inner nuclear membrane, resulting in enveloped particles within the perinuclear space. The next step is still unclear, but most of the available data imply that there is de-envelopment and loss of the primary envelope by fusion of perinuclear particles with the outer nuclear membrane, followed by release of DNA-containing capsids into the cytoplasm. These naked nucleocapsids are ushered to the Golgi complex in the cytoplasm, where they acquire additional tegument proteins and their mature envelope. They then bud into Golgi-derived vesicles that travel to the plasma membrane, where the virions are released from the host cell by exocytosis. (The alternative and less popular model is that the perinuclear virions leave the cell through the secretory pathway.)

Genetic and biochemical studies of herpes simplex virus (HSV) and pseudorabies virus have set the stage for the Muranyi *et*

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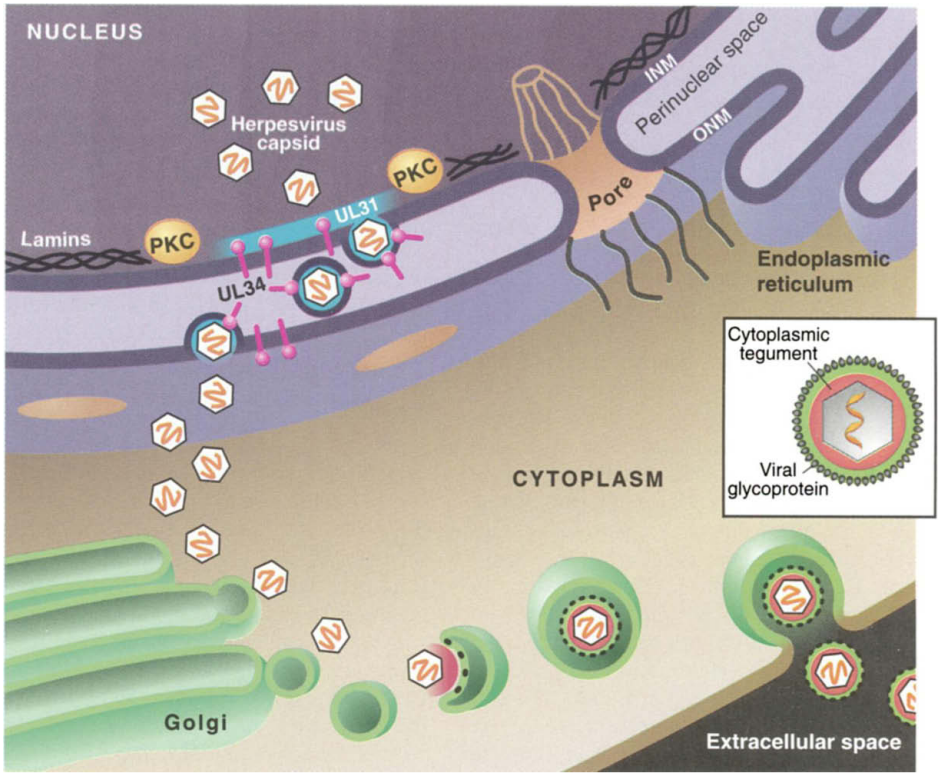
al. work. Two conserved HSV proteins, UL31 and UL34, are essential for the primary envelopment step (*4–8*). UL34 encodes a type II transmembrane protein that is retained at the inner nuclear membrane in infected cells, presumably through its interaction with UL31, a viral protein in the nucleus. Viral mutants that lack either UL34 or UL31 accumulate capsids within the nucleus and exhibit defects in envelopment at the inner nuclear membrane (*4, 6–8*). When coexpressed transiently in cultured host cells in the absence of other viral proteins, UL31 and UL34 colocalize at the nuclear envelope (*5, 9*). Interestingly, UL34 accumulates at the nuclear membrane in the absence of UL31, and also accumulates in the cytoplasm (*4, 5*). In addition, overexpression of UL34 alone is sufficient to cause gross alterations in the lipid leaflets of the nuclear membrane, reminiscent of those seen in infected cells (*10*). When UL31 is expressed alone in normal host cells, it shows a diffuse nuclear pattern rather than the nuclear membrane localization characteristic of infected cells (*4, 5*). Similarly, Dal Monte *et al.* (*11*) recently showed that UL53, the human CMV homolog of UL31, exhibited a diffuse nuclear distribution by immunostaining when expressed alone in cultured cells. In contrast, UL53 colocalized with lamin B and was observed in punctate patches along the nuclear periphery as well as in juxtanuclear cytoplasmic aggregates in cells infected with human CMV.

Taken together, these studies and other data strongly imply that UL31 and UL34 (and their β- and γ-herpesvirus homologs) are promoters of primary envelopment at the nuclear membrane. However, the exact mechanism by which these proteins induce structural changes in the nuclear membrane remains unclear. In the new work, Muranyi *et al.* identify and characterize the murine CMV counterpart of the UL34 protein, called M50/p35. They accomplished this by cloning candidate CMV open reading frames into an expression vector and screening for modifications of the nuclear membrane (such as irregular lamin staining) characteristic of CMV-infected cells. Like those working on UL34, Muranyi and co-workers discovered that expression of M50/p35 alone in normal cultured cells induced alterations in the nuclear envelope that slightly differed from the changes observed in infected cells. Predictably, these investigators discovered that when the murine CMV homolog of UL31 (M53/p38) was coexpressed with M50/p35, the alterations in the nuclear envelope resembled those seen during CMV infection.

The big leap forward made by Muranyi *et al.* is their discovery that during CMV infection M50/p35 recruits host cell protein kinase C (but not Cdk1) to the nuclear membrane, where the kinase increases phosphorylation of the lamins. Lamin phosphorylation may result in partial disruption of the nuclear lamina, thereby facilitating virion envelopment at the inner nuclear membrane. This is consistent with recent data showing that HSV-1 infection leads to increased lamin solubility and altered rates of diffusional mobility of specific inner nuclear membrane proteins, such as the lamin B receptor (*12*). Thus, the ability of herpesviruses to modify the nuclear lamina, and consequently the nuclear envelope, may be a general mechanism that these viruses have evolved to ensure their efficient and timely departure from the nucleus.

Whether protein kinase C is important for the nuclear egress of other herpesviruses remains to be established, but certainly the findings presented here open several new avenues of investigation. In addition, although it is clear that UL31 and UL34 and their homologs are important for nuclear egress, the fact that neither protein is absolutely essential suggests that other viral proteins may be able to compensate for them during herpesvirus infection. The advent of bacterial artificial chromosome technology should facilitate the isolation of viral mutants and the analysis of other viral proteins involved in nuclear egress and virion envelopment.



The great escape. Newly replicated herpesvirus DNA is inserted into preformed capsids in the nucleus of the host cell. To reach the inner nuclear membrane (INM) of the nuclear envelope, the subviral particles must transit through the physical barrier of the nuclear lamin meshwork. The viral protein UL34 (pink) of HSV-1 (the homolog of M50/p35 in murine CMV) is a type II transmembrane protein that is localized at the INM in infected cells via its interaction with UL31 (turquoise), a viral protein in the nucleus. UL34 and UL31 recruit protein kinase C (PKC) to the nuclear membrane, resulting in an increase in phosphorylation of the lamins. This causes partial disruption of the lamin network, thereby facilitating envelopment of subviral particles at the INM. The particles acquire some of their tegument proteins and a primary envelope by budding through the INM. They then lose their primary envelope as they bud through the outer nuclear membrane (ONM) of the nuclear envelope and are released into the cytoplasm. The DNA-containing capsids move to the Golgi complex, where they acquire additional tegument proteins and a mature envelope. Finally, they travel in Golgi-derived vesicles to the plasma membrane, where they are released from the host cell.

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