mitter release is more affected than spontaneous release by the absence of VAMP2 is consistent with the idea that the SNARE complex is already at least partially assembled and awaits the arrival of the calcium trigger to facilitate full assembly and the completion of membrane fusion (*12*, *13*). In contrast, spontaneous release (minis) and sucrose-evoked release, reflecting non– (or low–) calcium-dependent fusion events, are less severely affected by the loss of SNARE catalysis. Notably, 90% of this fusion was eliminated in the absence of VAMP2, indicating that the preferred mechanism of fusion does actually depend on the SNARE complex. Whether calcium ions directly affect the SNARE complex or instead work through a calcium-sensing protein remains to be determined.

The strongest candidate for the calcium-sensing protein is synaptotagmin. This protein was initially dubbed a "fusion clamp" because it was thought to bind to SNAREs, preventing their fusion activity until the arrival of calcium ions. However, because synaptotagmin also oligomerizes and interacts with plasma membrane phospholipids upon binding calcium ions, it could act alternatively as a positive regulator of fusion by becoming inserted into the membrane or by interacting with the fusion pore itself (*14*). The latter model is supported by Wang *et al.*'s (*3*) amperometry experiments. In these experiments, the investigators directly measured release of catecholamine neurotransmitters from PC12 cell dense-core vesicles as a current formed by the oxidation of the neurotransmitter on a carbon-fiber electrode. Their analysis centers on the small initial signal, or "foot," seen at the leading edge of the main spike of neurotransmitter-induced current (see the figure). The foot most likely reflects the flux of a tiny amount of neurotransmitter from the vesicle through the fusion pore as soon as the lipids fully merge, whereas the main spike reflects the expulsion of the remaining neurotransmitter when the fusion pore dilates. Overexpression of synaptotagmin I increases foot duration, whereas overexpression of synaptotagmin IV decreases foot duration. One interpretation of these data is that synaptotagmins directly interact with the fusion pore and regulate its formation and expansion. It remains unclear how this might occur mechanistically and why the two synaptotagmin isoforms have opposite effects. Thus, although the precise physiological function of synaptotagmin is still not understood, the Wang *et al.* study suggests that it should no longer be viewed as a fusion clamp but instead more like a valve, regulating the diameter and open time of the fusion pore by stabilizing the pore lipids. However, like VAMP2, synaptotagmin is unlikely to be absolutely essential because rapid calcium-dependent fusion is not completely abolished in synaptotagmin-deficient cells (*15*). Biochemical mysteries surrounding synaptotagmin also abound, as it is not yet clear whether synaptotagmin acts by becoming inserted into the membrane upon receipt of the calcium trigger, or through altered interactions with SNARE proteins.

It has been widely assumed that the SNARE complex is essential for the merger of membranes and thus might control the rate of a fusion event. In contrast, synaptotagmin is a favorite candidate for the calcium sensor. But it may be that VAMP2 along with the other SNAREs is critical for efficient membrane fusion, particularly in response to calcium ions, whereas the rate of a fusion event itself could be controlled by the synaptotagmins. These data support earlier biochemical studies (*14*) showing that the job of synaptotagmin is intimately tied to that of the SNAREs. Molecular elucidation of the nature of the fusion pore will no doubt shed more light on the individual parts played by the proteins involved. New physical methods, including biochemically reduced preparations that accurately reflect in vivo events, need to be developed in order to address these complex physiological processes in molecular detail (*16*).

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PERSPECTIVES: VIROLOGY

HIV—Breaking the Rules for Nuclear Entry

Miriam Segura-Totten and Katherine L. Wilson

The human immunodeficiency virus (HIV) gains access to human chromosomes by sabotaging the nuclear structure of the host cell. This clever ploy may explain how HIV can infect nondividing host cells such as macrophages (*1*). In nondividing cells, access to the nucleus is limited to proteins or protein complexes that (i) have a nuclear localization signal, and (ii) do not exceed the size limit (~25-nm diameter) of the nuclear pores (*2*). The preintegration complex (PIC) of HIV, which contains a double-stranded DNA copy of the viral genome, forms in the cytoplasm and must enter the nucleus so that the viral DNA can be inserted into a host cell chromosome. However, with a diameter of ~56 nm (*3*), the intact PIC is much too large to pass through the pores in the nuclear membrane by any known mechanism.

Vpr is one of three HIV proteins thought to mediate nuclear entry of the PIC. Vpr is a small protein (11.7 kD, 96 residues) that shuttles between the cytoplasm and nucleus of the host cell (*4*) and can arrest proliferating cells in late G_2 phase of the cell cycle (*5*). On page 1105 of this issue, de Noronha and colleagues (*6*) report that the expression of wild-type Vpr protein in HeLa cells is sufficient to induce transient herniations in the nuclear envelope of these cells. Sporadically and spectacularly, these herniations burst, releasing soluble nuclear proteins into the cytoplasm and presumably also allowing cytoplasmic proteins to freely enter the nucleus (see the figure). Equally remarkable, the broken sections of the nuclear envelope apparently reseal within minutes. De Noronha *et al.* on page 1105 (*6*) propose that these transient ruptures of the nuclear envelope may provide an unconventional route for nuclear entry that bypasses the size-restricted nuclear pore complexes.

Bursting of the Vpr-induced nuclear herniations releases key cell cycle regulators—which include the kinase Wee1, the phosphatase Cdc25C, and cyclin B—into the cytoplasm of the host cell. These proteins regulate the transition from G_2 to mitosis (*7*), and their repeated release into the cytoplasm might explain how Vpr causes G_2 arrest. Consistent with this idea, Vpr mutants that fail to cause herniations also fail to cause G_2 arrest (*6*).

It is remarkable that a single protein, Vpr, can so profoundly disrupt nuclear en-

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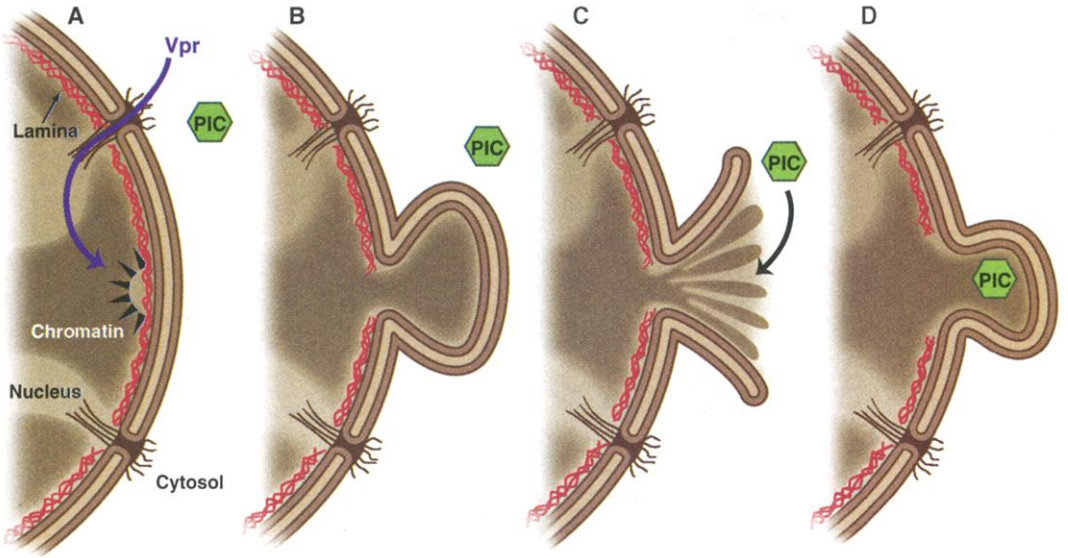

PIC and choose. Herniation, bursting, and re-sealing of the nuclear envelope induced by the HIV protein, Vpr. **(A)** Vpr enters the nucleus through the nuclear pore complexes of the host cell. This HIV protein becomes distributed throughout the nucleus and, in an unknown manner, causes the nuclear envelope to weaken at a few sites. **(B)** The nuclear membranes herniate at sites where structural filaments (lamins) are disrupted. These herniated sites fill with chromatin. Typically, chromatin near the nuclear envelope is structurally compressed, blocking gene expression. It is not known whether herniation involves decondensation of chromatin. **(C)** The nuclear membranes break down, allowing proteins that were previously compartmentalized inside the nucleus to leak out, and allowing viral pre-integration complexes (PICs) to enter, along with soluble cytoplasmic proteins. **(D)** The broken ends of the nuclear membranes fuse together, re-sealing the herniations and enclosing the PICs inside the host cell nucleus.

velope structure. The nuclear envelope has many components, including two membranes (outer and inner), their enclosed lumenal space, and an underlying network of filaments formed by nuclear intermediate filament proteins called lamins (*8*). Lamin filaments are attached to the inner membrane by a large number of integral membrane proteins that collectively form a stable, suprafilamentous structure known as the nuclear lamina (*9*). Importantly, the chromosomes also attach to the lamina through direct and indirect contacts among chromatin, lamins, and inner membrane proteins. One large family of nuclear membrane proteins includes lamina-associated polypeptide 2 (LAP2), emerin, and others, all of which share a 40-residue motif termed the LEM domain (*10*). Emerin and two isoforms of LAP2 bind specifically to lamins (*9*). However, their shared LEM domain (*11, 12*) mediates binding to a small (10 kD, 89 residue) conserved DNA bridging protein called barrier-to-autointegration factor (BAF) (*13–15*). The binding of BAF to LEM proteins may be important for the attachment of chromatin to the inner membrane and lamina of the nuclear envelope.

Nuclear structure is stable but is remarkably plastic. For example, the nuclear envelope can deform, break, and reseal in cells that produce Vpr, and can reversibly assemble and disassemble during mitosis (*8, 9*). Lamins and most lamin-binding proteins are phosphoproteins. Their dynamics during interphase and mitosis are regulated by the site-specific addition and removal of phosphate groups (phosphorylation and dephosphorylation) by kinases and phosphatases, respectively. In at least some cases, the regulatory kinases and phosphatases are recruited to, or resident at, the nuclear envelope [see (*16*)]. An important question raised by de Noronha *et al.* (*6*) is exactly how Vpr causes the nuclear envelope to herniate and break. The Vpr-induced nuclear herniations appear to lack nuclear pore complexes, and they apparently form at sites where there are visible and highly abnormal gaps in lamin A staining. Interestingly, these herniations are filled with chromatin. Vpr itself is not preferentially localized at herniations but is distributed over the entire nucleus and nuclear envelope (*6*). Vpr does not appear to bind directly to either A- or B-type lamins, suggesting that Vpr indirectly weakens the nuclear lamina. For example, Vpr might locally disrupt nuclear proteins required for lamin assembly or stability, or it may trigger the local disassembly or degradation of nuclear lamins. We assume either that these effects do not cause long-term damage or that Vpr is later down-regulated, because the virus needs the host cell to remain viable. Vpr is effective at very low concentrations, because cells can be arrested in G_2 solely by the amount of Vpr found in incoming HIV virions (*6, 17*). This result argues that Vpr might act catalytically. Interestingly, nuclear herniations are also seen in cells that lack lamins (*18, 19*). However, so far there is no evidence that the loss of lamins per se causes the nuclear envelope to rupture. The driving force behind the bursting of Vpr-induced nuclear herniations remains unknown, but potential mechanisms include local changes in chromatin structure.

Many questions remain regarding Vpr and its effects on the nuclear envelope. Paramount among these are the nuclear proteins, if any, with which Vpr might directly interact, and the mechanisms by which the integrity of the nuclear periphery is destroyed. It is worth noting that Vpr-induced herniation and bursting may not be the only way in which the HIV PIC gains entry to the nucleus, because HIV vectors that lack Vpr can replicate in at least some nondividing cells (*6, 20*). Further work on HIV and other viruses—including herpes simplex virus, which disrupts the nuclear lamina (*21*)—will undoubtedly yield new breakthroughs. Viruses have contributed fundamentally to our understanding of the mechanisms of growth control, signaling, and oncogenesis in mammalian cells. We envision that HIV and other viruses will soon yield new molecular insights into the structure and function of the nucleus.

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