PERSPECTIVES: MICROBIOLOGY

Redefining Virology

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Human cytomegalovirus (HCMV), a DNA herpesvirus with more than 200 genes, is a giant among viruses. Its DNA is infectious by itself, and the additional proteins that the virus brings into the host cell enhance the efficiency of infection. If herpes simplex virus (HSV), its more modest cousin with only 84 known genes, serves as a guide, HCMV brings into the host cell all that should be necessary to enable its genes to be expressed. Gene expression results in production of lots of infectious progeny and curtailment of any inimical response on the part of the host cell. Yet it seems that this is not quite enough. On page 2373 of this issue, Bresnahan and Shenk (*1*) report that HCMV particles contain not only DNA (as would be expected of a DNA virus) but also four species of mRNA.

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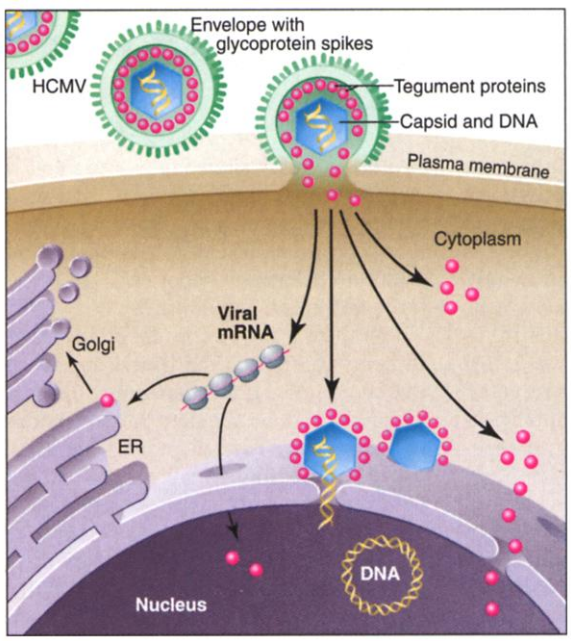
These four mRNA species are transcribed from one immediate-early gene, two early genes, and one late gene. On entering a host cell, the viral capsid is transported to the nuclear pore where its DNA load is released into the nucleus (see the figure). The four mRNAs—which are most likely localized in the viral tegument (*2*), a layer of protein between the capsid and the envelope—remain in the cytoplasm where they are translated into proteins in the absence of newly made viral gene products. This is unexpected—the current dogma is that in cells infected with DNA viruses, all newly synthesized proteins arise from mRNA transcribed from the virus genome after infection.

The functions of the four proteins produced ahead of schedule are not known. The authors, however, provide a plausible explanation for why the virus carries the mRNA of one of them (UL21.5) rather than the protein that it encodes. The UL21.5 protein is associated with the endoplasmic reticulum and the Golgi stacks of the host cell. To become localized in the endoplasmic reticulum, the protein requires a signal sequence, which is then cleaved off. If this protein were to be incorporated in the virus particle, its signal sequence would be cleaved off in transit from the endoplasmic reticulum to the site of assembly of the virus. The investigators point out that the four mRNAs could provide a structural framework for the packaging of tegument proteins in virus progeny.

The tegument is a structure unique to herpesviruses (*2*). It consists of an amorphous layer of protein located between the capsid containing the DNA and the envelope, the membrane that forms the outer layer of the virion (see the figure). Herpesviruses acquire the tegument just before or during envelopment and, in the case of some of them, the tegument is either exchanged or is modified after addition of the envelope (*3*). Of the 84 known viral proteins of HSV, at least 12 are components of the tegument. HCMV, with more than 200 open reading frames, may have twice this number of tegument proteins. After entry of the virus into the host cell by fusion of the viral envelope with the plasma membrane, the capsid (with some associated tegument proteins) is transported to the nuclear pore where the DNA is released (see the figure). Other tegument proteins remain in the cytoplasm or are transported to the nucleus independently of the capsid. Many HSV tegument proteins are dispensable for viral replication in cultured cells but turn out to be crucial for viral pathogenesis in experimental animal systems.

Tegument proteins create an intracellular environment within the host cell that enables the invading pathogen to take over the cell machinery for production of virion progeny. The major tegument proteins of HSV are VP1 and VP2, which are critical for the release of viral DNA into the nucleus. Other tegument proteins include VP16, which together with Oct1 and HC1 activates transcription of immediate-early (alpha) genes (the first set of viral genes to be expressed after infection). There is also UL41, a tegument protein that causes degradation of host mRNA present at the time of infection, thereby facilitating the selective synthesis of viral proteins. Less well understood is US11, a protein present in 2000 copies per virus particle, which binds RNA in both a sequence- and conformation-specific fashion. In newly infected cells it becomes localized in polyribosomes in the host cell cytoplasm (*4*, *5*). If HSV also packages mRNAs in its tegument in the same way as HCMV, then US11 may be the protein that carries mRNAs into the host cell, a hypothesis that remains to be tested.



A viral invader. Early events in the infection of host cells by HCMV. After fusion of the viral envelope with the plasma membrane, the capsid (with some associated tegument proteins) is transported to the nuclear pore where viral DNA is released into the nucleus. There, the DNA forms a circle and is transcribed by the cellular transcription machinery. Other tegument proteins remain in the cytoplasm or are independently transported to the nucleus. Viral mRNAs carried into the host cell with the capsid are translated in the cytoplasm. At least one of the proteins encoded by the viral mRNAs is associated with the endoplasmic reticulum (ER)–Golgi network.

Like most seminal reports, the Bresnahan-Shenk paper raises far more puzzles than it solves. Whether the virion mRNAs provide the structural framework of the tegument or are merely other components, they must interact with tegument or capsid proteins during packaging of the virions. A central question is whether the mRNAs share either sequence similarity or some common aspect of their structure enabling them to bind to the same tegument protein, or whether at least four diverse tegument proteins would be required to package the four mRNAs. An abundance of RNA binding proteins in purified virions should not be difficult to detect. Another issue that remains to be resolved is whether the packaged mRNAs are made at different times after infection. Intriguingly, they are still present in abundant

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amounts late in infection, at the time when assembly of virus particles takes place. If the function of the proteins encoded by the four mRNAs is to facilitate the expression of viral genes early during infection and, ultimately, to enable the efficient takeover of the infected cell, these proteins should not be required later unless they also perform other functions. Multifunctional proteins are rapidly becoming the rule rather than the exception in herpesviruses.

A question also arises as to where these mRNAs are packaged. Are they captured in the nucleus during tegument assembly, or do they remain in the cytoplasm? A contentious issue among those studying herpesviruses is whether the capsids of the viral progeny receive their envelopes at the nuclear membrane, are de-enveloped in the cytoplasm and then re-enveloped by Golgi-derived membranes. Evidence that the mRNAs are captured in the cytoplasm and are, for example, absent from virions accumulating in the space between the inner and outer membranes of the host cell would tremendously bolster the hypothesis that herpesviruses exchange envelopes as they transit through the cytoplasm.

If one were to attempt to predict the function of the HCMV proteins encoded by the four mRNAs, the answer most likely would be that they are factors essential for efficient transcription of viral genes, or for transport of capsid-tegument structures to the nuclear pore. The need for transcription factors is less evident, but herpesviruses do seem to encode them and to transport them into the newly infected cell. The transport proteins would have to differentiate between anterograde transport of de-enveloped capsid-tegument structures, retrograde transport of capsid-tegument structures to the nucleus early in infection, and anterograde transport of virions or capsid-tegument structures through the Golgi apparatus to the extracellular space. It seems rather curious that HCMV has evolved a mechanism to synthesize an endoplasmic reticulum–Golgi protein (UL21.5) independently, before the synthesis of proteins from mRNAs transcribed from viral DNA after infection. This implies that very soon after infection, HCMV needs to modify the translational machinery of host cells for efficient translation or posttranslational processing of viral proteins.

The Bresnahan-Shenk report impinges on yet another key aspect of virology. On the basis of their nucleic acid content, viruses have been classified as either RNA or DNA viruses. The definition reads that a virus can belong to one class or the other, but not to both (*6*). The original definition was designed to differentiate between obligate intracellular parasites containing both types of nucleic acid, and viruses that hitherto were known to contain either RNA or DNA. HCMV is the first DNA virus to be found with DNA and RNA coexisting before transcription of the viral genome. Although the amount and nature of the mRNAs in HCMV readily differentiate this virus from intracellular parasites, the definition of viruses has just lost its key discriminator.

References

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PERSPECTIVES: SIGNAL TRANSDUCTION

FasL Binds Preassembled Fas

Pierre Golstein

One way in which cells perceive their surroundings is through the binding of extracellular ligands to their cell surface receptors. This process is perhaps more complex than one would think. How does binding of a ligand to its receptor translate into a cascade of intracellular signals? The usual answer is that as soon as the ligand engages its receptor, it induces either conformational modification of the receptor or assembly of separate receptor subunits into a complex; downstream activation events then ensue.

Fas (CD95) is a cell surface receptor which, when engaged by its ligand (FasL, CD95L), causes death of the cell that bears it. It is believed that FasL, itself a homotrimer (composed of three identical monomers), engages three Fas monomers, leading to assembly of a trimeric Fas receptor (see the figure). This ligand-induced trimerization brings together "death domains" present in the cytoplasmic region of each Fas monomer (*1, 2*), leading to a downstream signaling cascade that instructs the cell to die. However, reports by Siegel *et al.* (*3*) on page 2354 and Chan *et al.* (*4*) on page 2351 of this issue, together with another recent report (*5*), propose a quite different, upside-down viewpoint. They show that Fas is assembled into trimers even before the ligand appears on the scene (*3, 5*). Furthermore, this preassembly may be required for the binding of FasL to the Fas receptor (*4*), and thus for the triggering of cell death. The new data modify the order of events and the causality chain, and thus call for a reassessment of how much receptors do by themselves and what they leave for their ligands to do. These results suggest that Fas-FasL signaling may be regulated at the level of receptor assembly.

The new reports show that assembly of Fas receptor monomers into trimers in the absence of FasL (*3, 5*) is mediated by a pre-ligand assembly domain (PLAD) in the extracellular, amino-terminal region of Fas (see the figure). Sophisticated fluorescence technology confirms that Fas trimers preassemble in otherwise untreated living cells (*3*). The PLAD domain ensures the assembly of normal Fas monomers into trimers. But sometimes it also mediates assembly of "crippled" Fas monomers: those with mutations in the death domain or normal splice variants that result in Fas receptors with no death domain (*5*). This integration of death domain–crippled monomers into trimers shows that the death domain is not required for preassembly and results in an assembled receptor that is unable to signal. This accounts for the "dominant negative" effect of these death domain mutations in heterozygotes (*6*) and the regulation of Fas signaling by splice variants in normal individuals (*5*). The PLAD domain and the site of interaction with FasL seem to constitute two distinct and apparently structurally independent domains of Fas.

Siegel *et al.* found that mutations that impair FasL binding do not impair Fas preassembly (*3*), implying that Fas preassembly is independent of FasL. Interestingly, FasL too preassembles into a trimer, and the FasL sites required for its trimerization are distinct from those required for its binding to Fas (*7*). Moreover, Chan *et al.* (*4*) found that mutations that impair Fas preassembly also impair subsequent FasL binding, which suggests that preassembly of Fas is required for FasL binding. The possibility remains that a mutation in the PLAD domain may directly or indirectly affect the ligand binding site. Indeed, al-

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