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.

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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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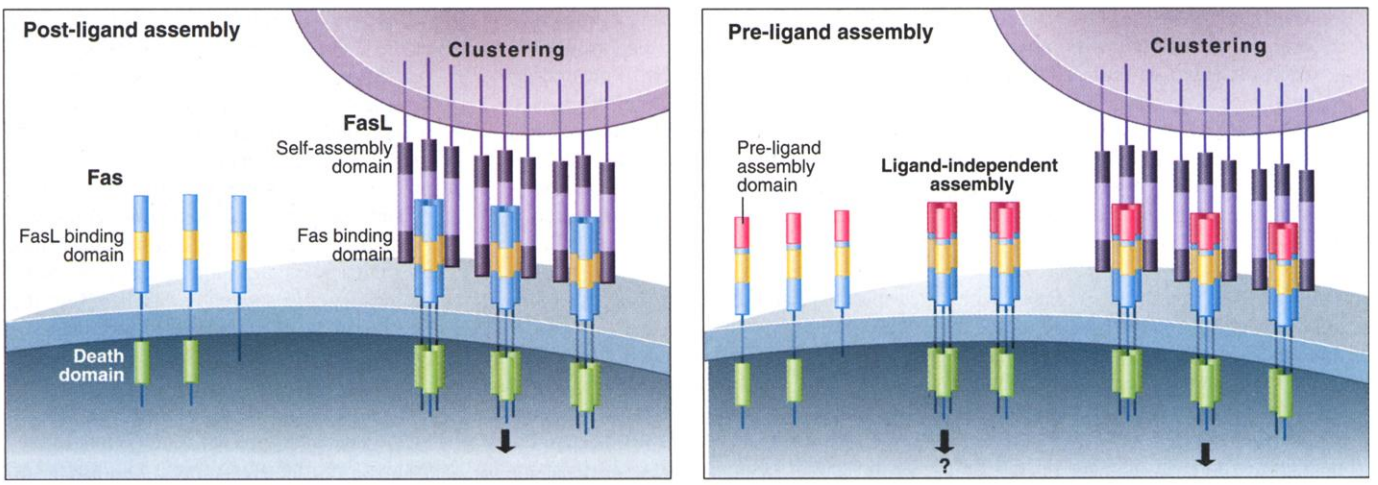
though most of the residues in Fas that contribute to FasL binding are located far from the Fas amino terminus, some are scattered throughout the extracellular domain (*8*). However, an antibody to the principal FasL binding site binds to Fas PLAD mutants as efficiently as it does to wild-type Fas. A requirement that the receptor preassemble before binding of the ligand has also been demonstrated for homologs of Fas (such as the tumor necrosis factor receptors TNFR1 and TNFR2) using similar methods and mutant-based approaches (*4, 9*). Overall, two main features distinguish the "pre-ligand assembly" model from the favored "post-ligand assembly" model: the presence at the cell surface of assembled Fas trimers in the absence of ligand, and the possible requirement for Fas trimerization before the ligand binds (see the figure).

In the absence of FasL, is a preassembled Fas trimer functionally neutral, or does it activate a signal? If it does, then this signal may be different from the signal sent by the same receptor after ligand engagement. This is reminiscent of "addiction receptors" such as DCC (deleted in colorectal cancer), which induces cell death when unoccupied by ligand and directs axon guidance when bound to ligand (*10*). It could be that preassembled Fas in the absence of FasL does not transduce a cell death signal but does transduce other signals that set in motion alternative functions of Fas (*11*). Interestingly, in the presence of FasL, a Fas complex that includes the adaptor molecule FADD and caspase-8 is required for downstream cell death signaling. In contrast, in the absence of FasL, Fas complexes of surface-crosslinked cells show moderate recruitment of FADD but not of caspase-8 (*3*). However, if Fas were to be active in the absence of FasL, then mutations leading to either the absence of Fas or the absence of FasL should present different phenotypes, which is currently believed not to be the case.

As FasL is not required for Fas trimer preassembly, it is unclear what role is left for FasL. In what other ways can binding of FasL to Fas trigger a cell death signal? FasL-Fas engagement may somehow induce conformational changes in Fas. Preassembled erythropoietin receptor dimers exist in a conformation that prevents downstream signaling; however, when bound by their ligand, they undergo a conformation change that initiates the signaling cascade (*12*). Alternatively, FasL may induce clustering of Fas trimers—FasL is itself present as multiple trimers at cell surfaces, and its engagement with Fas may promote clustering of Fas trimers. This is in line with the common observation that soluble FasL or antibodies to Fas cause cell death more efficiently if polymerized, for instance by adsorption on plastic or by molecular crosslinking (*13*). More generally, assembly into trimers, followed by clustering of these trimers, leads to a high local surface density of Fas and as a consequence a high local density of death domains (which send the cell death signal).

Fas preassembly may ultimately control FasL-induced cell death. In that case, what regulates Fas preassembly besides the PLAD domain? If preassembled trimers form at the cell membrane (which is not formally established), this would require that the monomers are mobile in the membrane. Mobility depends on local membrane composition and hence on specialized membrane domains (such as membrane rafts). Trimerization might also be regulated by other extracellular or cytoplasmic molecules. For instance, the binding of SODD (silencer of death domains) to TNFR1 death domains is thought to prevent the trimerization of these death domains and thus to inhibit downstream signaling (*14*). Moreover, the addition of sialic acid residues to Fas itself is thought to prevent assembly of the trimeric receptor (*15*). Although little is known about the normal regulation of Fas trimerization, it may be worth considering ways to therapeutically interfere with this process (*4*), for example, by masking the PLAD domain.



Dressed to meet in PLAD. Two models for the assembly of the Fas receptor trimer. FasL and Fas are shown here at the surface of two different cells. In the post-ligand assembly model (**left**), the Fas receptor monomers assemble into trimers only after FasL engages its binding site (yellow). In the pre-ligand assembly model (**right**), Fas trimers are preassembled. This preassembly may be required for engagement of FasL, which in turn causes the trimers to cluster. FasL bears in its extracellular region a Fas binding domain and a self-assembly motif (*7*). (FasL monomers and their assembly into trimers are not shown.) Fas bears in its extracellular region pre-ligand assembly domain (PLAD) (pink) and a FasL binding domain (yellow) (*3, 5*). Some Fas monomers (such as splice variants) are crippled but may be included in assembled trimers. The proportion of trimers within clusters that have at least one crippled monomer may condition signaling. Arrows indicate signal transduction pathways.

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