Might a vanguard of mRNAs prepare cells for the arrival of herpes simplex virus?

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Sciortino *et al.* (1) report in a previous issue of PNAS that herpes simplex virus 1 (HSV1) particles contain three RNA-binding proteins. They searched for these proteins to probe the mechanism by which mRNAs are incorporated into herpes virus particles (2–4). As yet, it is not clear whether one of more of these RNA-binding proteins deliver mRNAs to virions, but the identity of one of them led Sciortino *et al.* to an experiment with an intriguing result. VP22 is the HSV1 RNA-binding protein at issue. It is a major component (≈2,000 copies per virion) of the tegument, a structure residing between the capsid and envelope of herpes virus particles. VP22 has been reported to traffic between cells, transiting the plasma membrane of target cells (5). Sciortino *et al.* show that VP22 can bind and ferry an mRNA encoding a green fluorescent fusion protein between cells in an RNase-sensitive state, and, importantly, their assay demonstrates that the mRNA is translated within the recipient cell. Directed intercellular transfer of naked mRNA is a new concept in virology.

A variety of proteins, such as some bacterial toxins (6) and growth factors (7), are designed to enter cells by binding to cell-surface receptors and then entering the cell by endocytosis. VP22 is a member of a small set of proteins that seem to enter at least some cell types in a receptor- and energy-independent fashion. Besides VP22, these proteins include the HIV Tat protein (8) and the *Drosophila* Antennapedia (Antp) transcription factor (9). The ability of these proteins to transit membranes has been mapped to short, positively charged domains (9, 10). The complete membrane transit protein, the basic transit domain from one of these proteins, or an artificial basic transit domain has been reported to deliver fusion proteins into target cells (e.g., refs. 11–13). The mechanism by which these proteins transit membranes is not entirely clear. In some cases, internalization might result from interaction with cell-surface proteins followed by endocytosis. In other cases, the mechanism seems to be different. Transport domains synthesized from D- or L-amino acids, as well as peptides with reversed amino acid sequences, transit into cells with similar efficiencies (14, 15), arguing that a receptor interaction is not involved. The Antp basic peptide has been shown to accumulate within vesicles bounded by a pure lipid bilayer (16), further arguing that membrane proteins are not required for entry. The transport mediated by these basic domains also occurs at both 37 and 4°C (5, 10, 14, 15), suggesting that entry does not require the expenditure of energy. Receptor-independent entry has the potential to mediate more efficient delivery than a receptor-mediated process because the membrane-transiting protein and its cargo are not sequestered within an endosome after entry.

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It is conceivable, however, that the membrane transit domain serves only to nonspecifically bind these proteins to the cell surface and does not mediate translocation through the membrane. The positively charged transit domains bind heparin and presumably interact with cell-surface heparin (ref. 17 and references therein), as do many growth factors. It has been argued that the apparent membrane penetration and internalization of VP22 and other membrane transit proteins is an artifact of the immunofluorescence assays that are generally used to monitor intercellular protein movement (e.g., refs. 18 and 19). In these assays, fixation disrupts membranes, potentially allowing the protein to reach the nucleus, where its positive charge would promote its interaction with DNA. Thus, it would seem that the protein had entered the cell and moved to the nucleus, when, in fact, it had only been bound to the cell surface. This view is supported by the failure of a fusion protein comprised of the diphtheria toxin A-fragment plus the membrane transit domain from Tat or the entire VP22 protein to kill cultured cells, even though the fusion protein bound to the cell surface (17).

In counterpoint to the suggestion that a fixation artifact might confound the interpretation of some assays, VP22, Tat, and Antp have been reported to deliver fusion proteins that mediate physiological consequences within living recipient cells (e.g., refs. 12 and 13). The demonstration by Sciortino *et al.* (1) that VP22 can deliver a bound RNA molecule that is subsequently translated within the recipient cell strongly supports the conclusion that VP22 can, indeed, move between cells. It is likely that fixation artifacts have lent confusion to this field, but the experiment of Sciortino *et al.* did not employ fixation; FACS analysis was used to demonstrate expression of a reporter RNA. The mechanism underlying the intercellular transfer remains a mystery and the efficiency is uncertain, but VP22 has clearly sponsored the intercellular transfer of mRNA molecules.

Normand *et al.* (20) have previously shown that the C-terminal region of VP22 can mediate the delivery of DNA and RNA oligonucleotides into cells. When the VP22 fragment was mixed with oligonucleotide, it formed fairly large aggregates that were internalized by cultured cells. Sciortino *et al.* (1) dramatically extend this earlier result, showing that VP22 can deliver a complete mRNA that is translated within the recipient cell.

There are numerous implications of this result. The most obvious is that VP22 has the potential to deliver mRNAs to uninfected cells in the vicinity of infected cells. This transfer could deliver a set of viral mRNAs whose products prepare neighboring cells for efficient infection or for infection without alerting the immune system. For example, the virus might send an mRNA encoding a product that blocks the ability of MHC class I to present antigens

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on the cell surface before the arrival of infecting virus. Alternatively, the mRNA might encode a protein that antagonizes the antiviral functions of an approaching natural killer cell or cytotoxic T lymphocyte. Delivering mRNAs as opposed to proteins has potential advantages. Because one mRNA molecule can code for the production of multiple copies of its cognate protein, fewer mRNA molecules would need to reach the target cell than would be the case if protein were delivered. Further, because proteins with signal sequences must be cotranslationally inserted into the endoplasmic reticulum, mRNA delivery provides an opportunity to introduce and properly localize membrane and secreted proteins.

The potential for an intercellular mRNA transfer process raises many questions. Is there specificity to the transfer? So far, there is no indication that VP22 is selective in its interactions with mRNAs. If there is no selectivity, perhaps the protein delivers a random selection of the mRNAs that accumulate within an infected cell, a random set that would include mRNAs encoding functions that favor viral replication and spread in neighboring uninfected cells. Could mRNAs survive in the extracellular environment when a single cleavage will generally destroy mRNA function, and is the affinity of the VP22–mRNA interaction sufficient to mediate efficient transfer? Perhaps the transfer process involves cells that are very close or in contact with each other. And the really big question: is the transfer physiologically relevant to viral replication, spread, or pathogenesis?

If the process is physiologically relevant to HSV1 biology, it is likely that other viruses practice intercellular RNA transfer as well. As Sciortino *et al.* (1) point out, the HIV1 Tat protein is a case in point. Tat, like VP22, binds to HIV RNAs through a tar motif (reviewed in ref. 21) and transits membranes. Consequently, it has the potential to move viral RNAs between cells.

Viruses are notorious mimics of their host cells. They adopt or modify cellular functions to achieve their goals. If viruses do, indeed, facilitate their replication and spread by transferring mRNAs between cells, they have almost certainly learned this trick from the organisms they inhabit.

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