PERSPECTIVES: VIROLOGY

Hole-istic Medicine

Joshua Zimmerberg

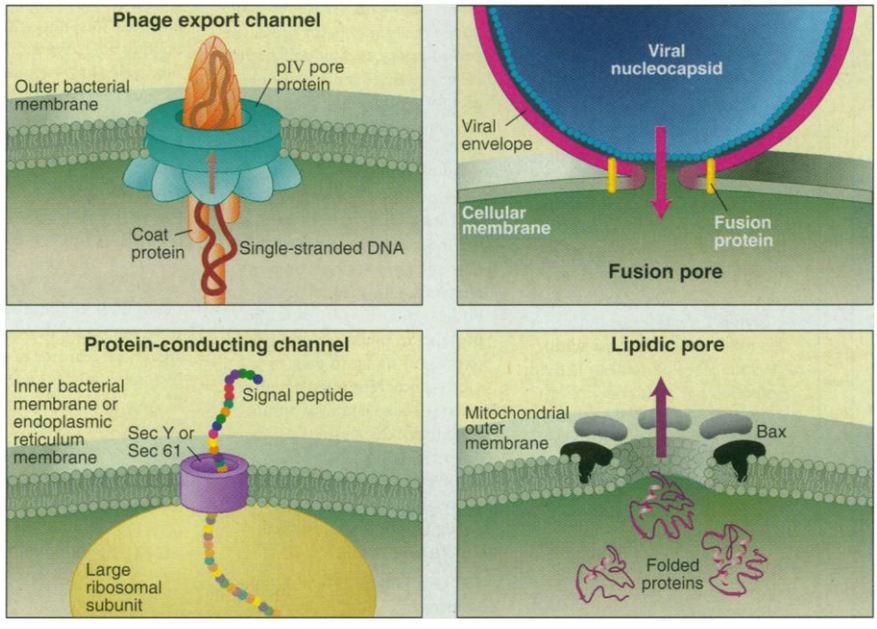
Biological membranes can create a tight barrier that prevents the passive diffusion of ions out of the cell. Paradoxically, bigger entities such as proteins must be able to move freely across membranes. When an enveloped virus infects a cell, the nucleocapsid enters the host cell largely intact and new virions exit the cell fully formed. For lytic viruses, the host cell lyses to release whole virus particles. Yet many viruses do not lyse their host cell but rather instruct it to secrete their infectious progeny. One example of such a virus is f1, a filamentous phage that infects bacteria. Virions of f1 are produced by infected bacteria that continue to grow and divide despite continuously releasing their parasitic cargo. How can this be? The answer may reside in a report on page 1516 of this issue by Marciano *et al.* (*1*). They show that a phage-encoded protein, pIV, which is not a structural element of f1, forms a tightly gated channel in the outer bacterial membrane that exports phage from the host bacterium.

Secretion of f1 virions occurs as the virus particles are being assembled by the bacterium, with the amphipathic viral coat proteins attaching to the emergent loop of single-stranded DNA as it passes through the bacterial inner membrane. The pIV protein mediates the final step in the assembly and passage of f1 virions through the bacterial outer membrane (see the figure). This 45-kD protein forms a cylindrical multimeric channel composed of ~14 subunits with an inner diameter of 7 to 8 nm (*2*). Using osmotically driven fusion of proteoliposomes (*3*), Marciano *et al.* reconstituted both wild-type and mutant pIV into planar phospholipid bilayer membranes. They show that pIV forms the largest voltage-gated channel yet recorded, with a conductance of up to 1.2 nS in 150 mM KCl. This channel is about 100-fold more permeable to ions than the potassium-selective channels of nerve cells. The gating properties of wild-type and mutant pIV in the planar membranes correlate with the permeability and osmotic fragility of the bacterial outer membrane in vivo—the less tightly gated mutant channel increases the permeability and osmotic fragility of the bacterial outer membrane compared with the more tightly gated wild-type pIV.

Proteins homologous to pIV have been found in the outer membranes of a wide variety of bacteria (*4*). These bacterially encoded proteins mediate the transport of toxins and other virulence factors from such villains as *Yersinia pestis*, *Vibrio cholerae*, *Salmonella typhi*, and *Shigella flexneri* (*4*). Even emerging pathogens, such as *Escherichia coli* O157:H7, and plant pathogens have similar proteins (*4*). Marciano and colleagues are the first to show that a member of this family forms a gated channel.

In the world of eukaryotic host cells, enveloped viruses, such as influenza and human immunodeficiency virus (HIV), use another mechanism—fusion—to overcome the eukaryotic membrane barrier during infection. Fusion between the viral envelope and a cellular membrane results in a fusion pore, which differs from channels composed of protein in that it is at least partially, if not totally, composed of lipid (see the figure). Fusion pores, induced to form by fusogenic viral proteins, are lipid-dependent and respond to changes in lipid composition (they are devoid of fixed conductance) (*5*). They are less dependent on the amino acid sequence of the membrane-spanning domains of fusogenic proteins (*6*). Once formed, the fusion pore expands to allow the passage of objects as large as the influenza virus nucleocapsid from the endosome into the cytoplasm.

It is not only pathogens that need to cross biological membranes. Most physiological processes involve the translocation of substances across cellular membranes. The granddaddy of pores, the nuclear pore (80 nm in diameter), allows the passage of nucleic acids, hormone-receptor complexes, and ribosomes that are 25 nm in diameter! Sugars and metabolites are able to pass through bacterial outer-membrane channels (porins) and the voltage-dependent anion channel of the outer mitochondrial membrane (2 to 4 nm in diameter) (*7*). Mem-



Crossing membranes. The pIV protein encoded by f1 phage (a virus that infects bacteria) is synthesized and assembled by the bacterial host into a multimeric channel that becomes inserted into the bacterial outer membrane (**top left**). This gated channel enables virions to complete assembly and to exit the bacterium without lysing the host. The loop of single-stranded DNA picks up copies of the amphipathic coat protein, and the phage emerges from the lumen of the pIV channel fully formed. A protein-conducting channel mediates the translocation of proteins with signal peptides across the bacterial inner membrane and the membranes of organelles such as the endoplasmic reticulum (**lower left**). Very different pores called fusion pores develop during the infection of eukaryotic cells by enveloped viruses (**upper right**). These pores must expand to allow the transfer of viral genetic material (the nucleocapsid) into the cytoplasm of the host cell. They are composed in part (perhaps totally) of lipid and are induced to form by fusogenic viral proteins. Other pores, such as those proposed to explain the membrane activity of the pro-apoptotic protein Bax, may be composed of either lipid or lipid-protein complexes (**lower right**).

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The author is in the Laboratory of Cellular and Molecular Biophysics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892–1855, USA. E-mail: joshz@helix.nih.gov

branes that regulate ionic permeability (such as the inner bacterial membrane and endoplasmic reticulum membrane) cannot withstand any measure of ionic leakage. As with pIV, which should only open as the phage are being assembled for export, tightly coupling the opening of ionic permeability channels to substrate transport is the solution to the leakiness problem. The protein-conducting channels of the endoplasmic reticulum and bacterial inner membrane (*8*) are gated open by the signal peptide of the translocating protein (see the figure). In the absence of protein, these channels do not conduct, and there is no danger of ions leaking out. In this way, proton gradients are maintained across the bacterial inner membrane and calcium gradients across the endoplasmic reticulum.

Can the lipid-protein complexes envisioned for fusion pores also mediate the transport of folded proteins across single membranes? During apoptosis, permeation of proteins sequestered in the space between the inner and outer mitochondrial membranes is considered to be the crucial commitment step in the cell death pathway (see the figure). Pro-apoptotic proteins, such as Bax, may promote the formation of pores that are at least partly composed of lipid (*9*). Pore size is dynamically determined by a balance of forces between those that discourage the formation of new edges (linear tension) and those that pull the pore open (for example, mitochondrial swelling). Thus, these putative pores can easily accommodate folded proteins and can even initiate the lysis of a membrane. Alternatively, Bax may open some pIV-like channel in the outer mitochondrial membrane.

The latest mystery of protein translocation involves the HIV transcription factor Tat (and the *Drosophila* protein ANT). Chimeric proteins containing Tat, when misfolded, will move across the plasma membrane directly into the cytoplasm (*10*). Because Tat is highly charged, it is unable to traverse the lipid bilayer of the cell membrane without going through some aqueous or proteinaceous pathway; the dielectric constant of the lipid membrane poses a formidable barrier to the entry of any charged moiety. Perhaps Tat is able to trigger the opening of a hitherto unnoticed megachannel in the eukaryotic plasma membrane. Alternatively, Tat may induce the bending of lipids into transient, localized pores that allow the passage of proteins; the lipids can then seal behind the protein as it passes through.

Regardless of the mechanism of Bax activity and Tat translocation, the Marciano study has now expanded our view of how protein channels in living membranes can allow passage of macromolecular complexes. Now what? We do not know how f1 (and other nonenveloped viruses such as poliovirus) enter cells. Does negatively charged f1 phage DNA induce a pore in the inner bacterial membrane in order to reach pIV in the outer membrane? Are lipidic pores, clearly seen in purely phospholipid membranes, regulated by proteins in biological systems? To paraphrase Hamlet: There are more things in membranes than are dreamt of in our textbooks.

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PERSPECTIVES: HOMOGENEOUS CATALYSIS

Toward Greener Chemistry

R. Thomas Baker and William Tumas

The "greening" of global chemical manufacturing by minimizing energy consumption and waste production has become a major concern for the chemical industry (*1*). New catalyst systems that allow for rapid, selective chemical transformations as well as effect catalyst and product recovery will have a significant impact. Homogeneous catalysts, where the catalyst is in the same phase as the reactants, have some advantages for optimizing catalytic systems, because they can easily be modified by ligand design and their structure and reaction pathways can be characterized in detail by a range of spectroscopic techniques. They are used in a number of commercial applications, but the difficulty of separating the catalyst from the product creates increasing economic and environmental barriers to their broader application.

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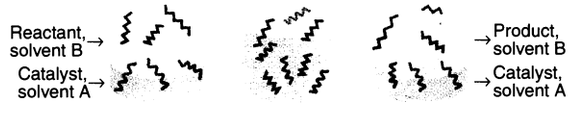
Recently, new strategies have been advanced in which the catalyst is immobilized in a liquid phase that can be separated from the product-containing phase. These novel approaches in so-called biphasic catalysis (*2*) involve designing ligands or engineered soluble supports for catalyst metal centers for high solubility in certain solvents. Solvent systems include fluorocarbons, special mixtures of organic solvents, supercritical CO_2, and ionic liquids. Enhanced aqueous phase systems have also been developed. These fundamental advances in facilitating separation have now sparked renewed interest in developing homogeneous catalysts for efficient synthesis of a wide range of chemicals in the pharmaceutical and chemical industries (*2*).

In an ideal phase-separable or biphasic catalysis system (see the figure on this page), the catalyst and associated ligands would be dissolved in one phase and the reactants and products would be completely soluble in a second phase, which can be removed after reaction and the catalyst phase recycled for further use. To capture the attributes of both a biphasic system and a homogeneous single-phase system, the ideal system would allow for excellent mixing, efficient transfer between phases, or complete miscibility of the phases under the reaction conditions to achieve high reaction rates.

Two approaches have recently been advanced for realizing a totally miscible (one phase) reaction medium under reaction conditions and achieving subsequent efficient separation of phases by cooling the reaction mixture. Both use at least two solvents



Biphasic catalysis. (Left) A homogeneous catalyst is tailored to dissolve in solvent A, while the reactant is dissolved in solvent B. **(Middle)** At the reaction temperature, catalyst, reactant, and solvents A and B form a single phase in which the reaction takes place. **(Right)** After the reaction is completed, the system is cooled down, resulting in phase separation. The catalyst and product are in separate phases, facilitating separation.

The authors are in the Los Alamos Catalysis Initiative and the Chemical Science and Technology Division at Los Alamos National Laboratory, Los Alamos, NM 87545, USA. E-mail: weg@lanl.gov and tumas@lanl.gov