REVIEW

Protein Translocation Across Biological Membranes

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Subcellular compartments have unique protein compositions, yet protein synthesis only occurs in the cytosol and in mitochondria and chloroplasts. How do proteins get where they need to go? The first steps are targeting to an organelle and efficient translocation across its limiting membrane. Given that most transport systems are exquisitely substrate specific, how are diverse protein sequences recognized for translocation? Are they translocated as linear polypeptide chains or after folding? During translocation, how are diverse amino acyl side chains accommodated? What are the proteins and the lipid environment that catalyze transport and couple it to energy? How is translocation coordinated with protein synthesis and folding, and how are partially translocated transmembrane proteins released into the lipid bilayer? We review here the marked progress of the past 35 years and salient questions for future work.

Emerging technologies have enabled progress in understanding protein translocation. In vitro protein synthesis led to the discovery that the mRNAs for secreted proteins are attached to membranes, whereas cytosolic proteins are made on free polysomes (*1*–*3*). The junction between endoplasmic reticulum (ER)–bound polysomes and the membrane is tight enough to exclude protease (*4*), and nascent chains are discharged directly into the lumen (*5*). Mouse myeloma mRNA was found to encode a polypeptide 1.5 kD larger than mature immunoglobulin light chain (*6*). The larger form was postulated to be a precursor with an N-terminal extension that specifies secretion, a research direction that culminated in the signal hypothesis (*7*). "Signal sequences" were deciphered for proteins that crossed a membrane, including the ER, mitochondria, chloroplast, and bacterial envelope. Armed with a signal sequence, mature domains merely had to be susceptible to unfolding in order to translocate (*8*, *9*). The signal sequences of each organelle have shared motifs of polarity and structure but no sequence conservation. For example, bacterial or ER export signals are basic at the N terminus, followed by a stretch of 8 to 14 apolar residues and a short cleavage motif that is recognized by a dedicated peptidase. Bacterial *sec* (secretion) genes, encoding proteins that support translocation, were identified either as suppressors of signal sequence mutants (*10*) or as temperature-sensitive mutants that failed to initiate secretion at the nonpermissive temperature (*11*). The yeast Sec61p (*12*) is homologous to bacterial SecY, establishing that the membrane-embedded portion of these translocons is conserved. In vitro translocation reactions were developed by adding isolated organelles to protein synthesis extracts for ER (*7*), mitochondria (*13*, *14*), chloroplasts (*15*), and bacterial plasma membrane (*16*, *17*). These in vitro systems were the basis for discovering energy requirements, determining whether translocation needed chaperones or ongoing protein synthesis, and initiating enzymological dissection of the process. In most cases, preproteins engage with chaperones or ribosomes, bind to a membrane receptor (which may also serve as a motor), transfer to a membrane-embedded translocon, and are released laterally into the lipid bilayer or completely cross the membrane with the aid of chaperones on the trans surface (Fig. 1A).

The Sec Translocase

Although ER and bacterial proteins share the same signal sequences, early studies suggested that their translocation mechanisms are very different. Bacterial translocation requires both adenosine 5′-triphosphate (ATP) and the membrane electrochemical potential, whereas ER translocation only needs nucleotides. Only short nascent preproteins can initiate translocation into canine ER (*18*). Signal recognition particle (SRP), a complex of 7*S* RNA and six polypeptides, coordinates translation and translocation (Fig. 1B) through binding to emerging signal sequences and slowing chain growth (*19*, *20*). Translocation resumes when the complex of polysome, nascent chain, and SRP reaches its ER-bound receptor (*21*–*23*). In contrast, preprotein translocation across the bacterial plasma membrane is not coupled to translation, either in vivo (*24*, *25*) or in vitro (*26*). Specific proteins provide the mechanistic basis for this dichotomy; some full-length preproteins that have left the ribosome bind to the SecB chaperone and then engage the SecA protein as their membrane receptor (Fig. 2) (*27*). SecA is activated to bind and hydrolyze ATP for powering translocation by its associations with the signal sequence and mature domain of a preprotein and with the SecYEG translocon, the membrane receptor for SecA (*27*, *28*). By the mid-1980s, it appeared that ER and bacterial translocation were fundamentally different, although this distinction soon blurred.

The membrane-embedded proteins needed for translocation were isolated by solubilizing membranes in detergent, fractionating the mixed micellar extracts, and assaying for proteins needed to reconstitute translocation-competent proteoliposomes upon detergent removal (*29*–*31*). In bacteria, three membrane-embedded proteins—SecY, SecE, and SecG—are tightly associated as a complex termed SecYEG (Fig. 2). Proteoliposomes bearing SecA:SecYEG will efficiently translocate pure preprotein, driven by ATP and a membrane potential (*29*). About 20 amino acyl residues are translocated for each ATP that is bound and hydrolyzed by SecA (*32*) in a cycle accompanied by substantial SecA conformational change (*33*). Each SecA translocates a preprotein through a single SecYEG (*34*, *35*), and the translocation pathway appears, by crystallography (*36*) and cross-linking (*37*), to pass through the center of SecY. The structure of SecYEG (*36*) shows a narrow constriction through which a polypeptide chain may move, rather than a large opening that would leak small molecules, and has provided the first molecular model of how apolar domains may be laterally released into the lipid bilayer. Cocrystals of SecYEG with SecA and with preprotein substrate may lead to further molecular understanding of the translocation cycle.

Fractionated detergent extracts of eukaryotic ER also yield a membrane-embedded heterotrimeric complex, termed Sec61αβγ, with marked similarity to SecYEG (*30*). Proteoliposomes bearing this Sec61 complex and the SRP receptor will translocate nascent chains initiated in the presence of SRP. The complex of polysome, nascent preprotein, and SRP binds to the SRP receptor. Upon the binding of guanosine 5′-triphosphate (GTP) to both the SRP and its receptor (*38*, *39*), the polysome and nascent chain are transferred to the Sec61 complex, allowing translation and trans-

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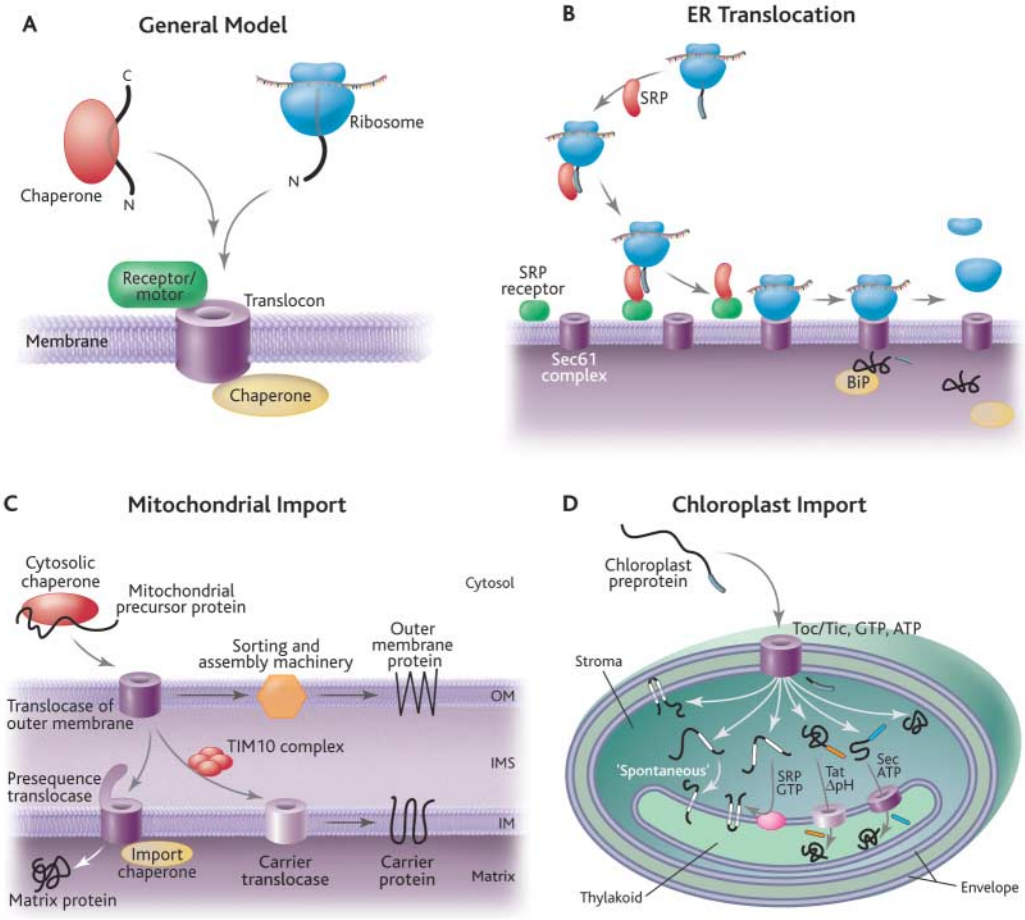

Fig. 1. Conserved translocation themes. (**A**) Either chaperones (red) or occupancy of the C-terminal region of nascent chains in the ribosomal exit tunnel (blue) prevents premature stable folding of preproteins. Membrane-bound receptors (green) bind preproteins and transfer them into the translocon (purple), which can conduct them across the bilayer or release apolar regions laterally into the bilayer. Motors and chaperones (yellow) on the trans surface of the membrane complete the transport task. (**B**) Cotranslational translocation into the ER. (**C**) Mitochondrial protein import [adapted from Wiedemann *et al.* (*94*)]. OM, outer membrane, IMS, intermembrane space, IM, inner membrane. (**D**) Chloroplast protein import [adapted from Jarvis and Robinson (*56*)].

location to resume (Fig. 1B). Preproteins translocate through the Sec61 complex or SecYEG translocons in the N-to-C direction. When ~20 largely apolar amino acyl residues enter the translocon, they are released sideways into the lipid bilayer.

It is possible that the chaperone systems for bacterial and eukaryotic ER translocation are not really so different. SRP-mediated translational arrest is not required for translocation (*40*, *41*); SRP and the ribosome (*42*) may be viewed as targeting chaperones. Yeast prepro-α factor and prepro-carboxypeptidase Y can translocate into the ER posttranslationally (*43*–*45*), and *Saccharomyces cerevisiae* can grow, albeit slowly, without SRP or its receptor (*46*, *47*). Bacterial SRP and SRP receptor are required for the assembly of many hydrophobic proteins into the membrane (Fig. 2) (*48*) but generally not for export to the periplasm or outer membrane (*49*). After import across the two envelope membranes, chloroplasts use SRP and its conserved receptor to assemble proteins into the thylakoid membrane (Fig. 1D) (*50*). This SRP-mediated thylakoid insertion is clearly posttranslational, emphasizing the receptor and chaperone roles of SRP. Targeting and chaperone functions are at the core of both cotranslational and posttranslational translocation pathways; irreversible folding or the aggregation of apolar membrane anchors can be prevented by engaging nascent polypeptides with the translocase as soon as its signal sequence emerges from the ribosome, whereas posttranslational translocation uses chaperones to target proteins and maintain translocation competence.

Import to the Mitochondrial Matrix

Mitochondrial protein uptake (Fig. 1C) is largely posttranslational, in vivo and in a reconstituted in vitro reaction (*13*, *14*, *51*, *52*). Mitochondria import 99% of their proteins posttranslationally from the cytosol, including integral membrane proteins of both the inner and outer membrane. Mitochondrial matrix preproteins have a distinct signal sequence, an amphipathic α-helical rod. Matrix preproteins bind to receptors on the surface of the mitochondrial outer membrane (*53*). They then enter a common translocation channel [the translocase of the outer membrane (called TOM)] to the intermembrane space. Preproteins translocate across the outer membrane in an unfolded state in an N-to-C direction. The membrane protonmotive force drives the initial stage of transport across the inner mitochondrial membrane through a distinct multisubunit complex, the translocase of inner membrane (TIM). As proteins enter the matrix, they are captured by mHsp70, a TIM-associated ATP-driven chaperone that binds each segment of the chain as it enters, thereby restricting net movement to import (*54*). During import, mitochondrial proteins may span both membranes with large C-terminal folded domains still exposed to the cytoplasm and their N terminus in the matrix; the matrix ATP-driven chaperones can then actually drive a net unfolding of the C-terminal domains (*55*).

The Diversity of Transport Systems

Mitochondria, gram-negative bacteria, and chloroplasts (*56*) have multiple, and branched, translocation pathways. Upon reaching the intermembrane space, mitochondrial preproteins enter divergent pathways (Fig. 1C): β-barrel proteins integrate into the outer membrane by means of a specialized outer membrane translocase (*57*, *58*); some proteins remain in the intermembrane space; and proteins with matrix-targeting presequences use the TIM translocase, exploiting two energy sources, the membrane potential ΔΨ and the mHsp70 adenosine triphosphatase (ATPase). Apolar inner membrane proteins use a separate inner membrane translocase system that needs ΔΨ but not

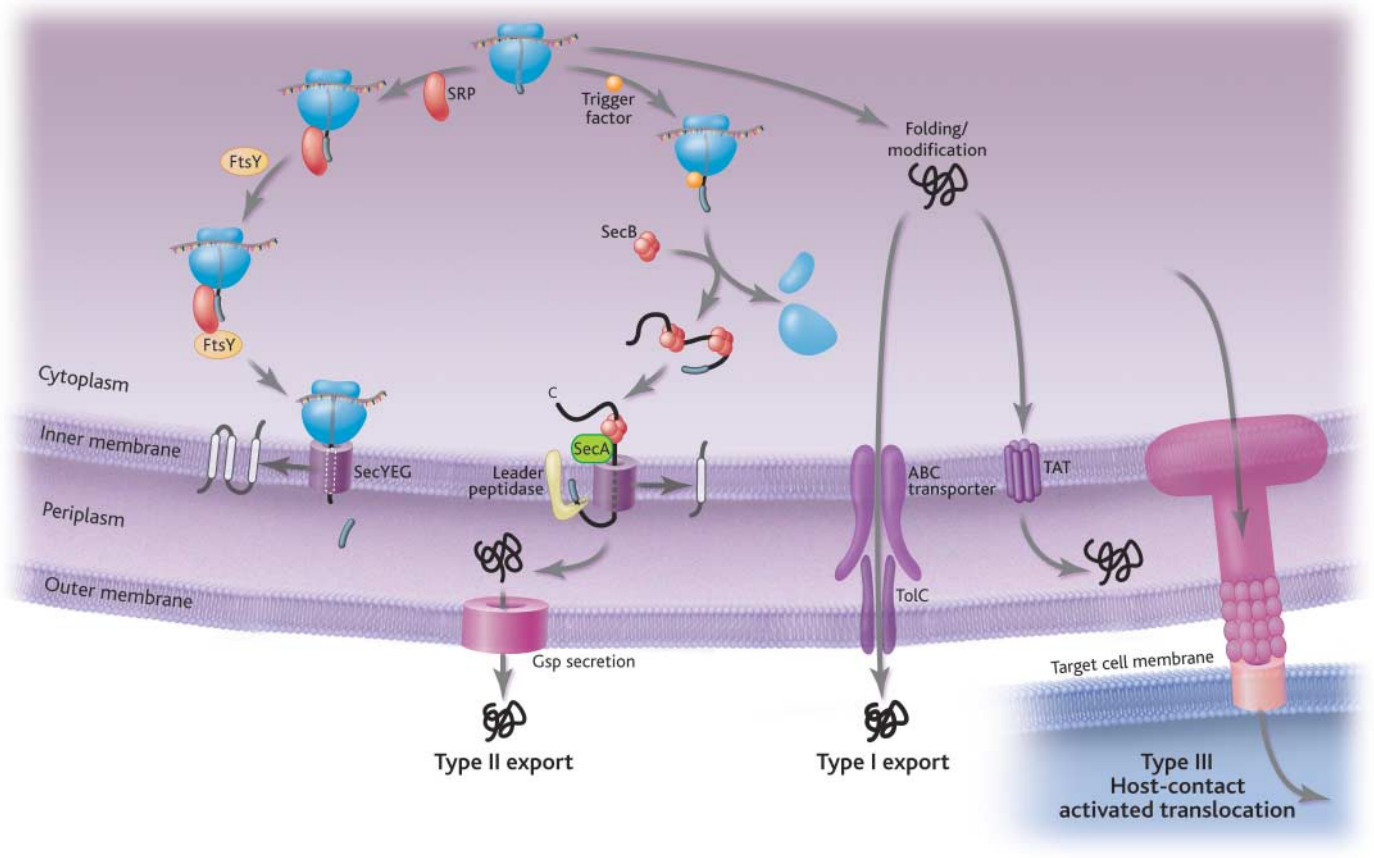

Fig. 2. Diversity of bacterial translocation pathways. [Adapted from Luirink and Sinning (*95*)]

ATP (*59*). Several particularly apolar proteins, encoded by the mitochondrial genome, are synthesized in the matrix and inserted into the inner membrane with the help of an inner membrane protein, Oxa1p (*60*). Other multimembrane systems are just as complex. Bacteria have the Sec translocase, TAT translocase, and YidC [homologous to Oxa1p (*61*)] for proteins entering the plasma membrane or periplasm, five transport systems for crossing the outer membrane, and distinct transport systems for coordinated transport across the inner membrane, outer membrane, and a target-cell membrane (Fig. 2). Chloroplasts (Fig. 1D) import proteins across two membranes and into the stroma by a coupled outer membrane and inner membrane transit pathway (*56*). From the stroma, import pathways into the thylakoid membrane and lumen are varied but markedly similar to bacterial protein export.

Not all protein translocation is dependent on an unfolded conformation. The TAT bacterial translocase exports proteins bearing a unique twin-arginine motif (*62*). Not only is this translocation uncoupled from ongoing protein synthesis, but it accommodates fully folded proteins that remain folded during membrane transit. The proteins translocated by TAT can be oligomeric, with some subunit(s) providing the TAT recognition motif, whereas others are translocated "piggyback," solely by virtue of their association with the TAT-motif–tagged subunit (*63*). TAT translocase subunits can oligomerize, suggesting a means for providing large transport pores (*64*). Oligomeric folded proteins are also imported into the peroxisome. Translocation is not limited to a single membrane or two membranes; chloroplasts have three distinct membrane layers, with unique aqueous spaces between each, and the type III transport systems of pathogenic bacteria can inject proteins across both bacterial envelope bilayers as well as across the target-cell plasma membrane.

Peroxisome Assembly

Peroxisomes display a curious patchwork of translocation mechanisms that resemble aspects of bacterial, mitochondrial, and nuclear translocation. Import of peroxisomal proteins is posttranslational and requires a short C-terminal signal sequence (-SKL is common) that is decoded by a receptor, the Pex5 protein (*65*, *66*). Most peroxisomal proteins are imported as an uncleaved mature species (*67*); certain proteins are even imported as oligomeric complexes (*68*). Soluble peroxisomal precursors may combine with a signal-specific receptor in the cytoplasm, enter the peroxisome with this receptor, and then release, allowing receptor recycling to the cytoplasm (*69*). Peroxisomal proteins, similar to nuclear proteins, fold in the cytoplasm and retain a native conformation during import. Though nuclear proteins traverse a large nuclear pore, no such morphological feature has been described for the peroxisome. Despite a rich list of genes (PEX) and proteins (peroxins) involved in peroxisome biogenesis, the translocation channel remains elusive. Several integral membrane proteins, including Pex3, Pex10, Pex15, and Pex19, are candidates (*70*). Although most peroxisomal matrix and membrane proteins are assembled into a mature organelle, the true origin of the peroxisomal membrane and at least one peroxin, Pex3, has remained controversial.

Until recently, the prevailing view has been that peroxisomes are self-renewing autonomous organelles. Blocks in secretion appear to have no effect on peroxisome proliferation (*71*). Many peroxisome assembly mutants produce ghostlike membrane remnants that may explain how PEX mutants restore normal peroxisome function on reintroduction of the missing peroxin (*70*). However, other results suggest a role for some other organelle as the origin of peroxisomal membrane. Peroxisomes in *S. lipolytica* contain glycoproteins, suggesting origins in the

early secretory pathway (*72*). *S. cerevisiae* pex3 null mutant cells have no apparent vestige of peroxisomal membrane, yet expression of Pex3 in this strain induces peroxisome proliferation (*73*).

This conundrum has apparently been resolved by the observation that Pex3 inserts into the ER membrane and then is diverted into vesicles that join by homotypic fusion or fuse with existing peroxisomes (*73*). Thus, Pex3 may exploit the secretory pathway to form new peroxisomes. The role of the ER Sec61 channel and of ER vesicle budding proteins in this process remains to be established. Activation of protein import in newly minted peroxisomes also remains an unexplored question.

Protein Dislocation

Eukaryotic cells use the cytoplasmic proteosome to degrade misfolded secretory glycoproteins, implying a retrograde translocation (or dislocation) of glycoproteins from the lumen of the ER to the cytosol (*74*–*76*). A number of proteins linked to this ER-associated degradation (ERAD) process have been identified in yeast and mammals (*77*). Dislocation requires luminal chaperone-like molecules that recognize and prepare misfolded proteins for export, integral membrane proteins that convey the misfolded proteins through the ER membrane, and cytoplasmic proteins that withdraw and covalently modify the substrates for degradation. Substrates include mutant and misfolded secretory and membrane proteins, bacterial toxins that penetrate to the cytoplasm and evade degradation, major histocompatibility complex (MHC) class I antigen that is diverted from the ER in cytomegalovirus-infected cells, metabolically regulated ER membrane enzymes such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and possibly even encapsulated virus particles that escape from the ER to replicate in the nucleus.

Although dislocation bears superficial similarity to translocation, the export pathway is not simply a reversal of import. Nevertheless, translocation and dislocation share some catalysts. Lumenal chaperones such as the hsp70-like BiP and protein disulfide isomerase recognize and may even initiate unfolding of dislocation substrates (*78*, *79*). Certain substrate molecules must acquire a specific glycan structure to be recognized for dislocation, and two glycan-binding proteins have been implicated in carbohydrate recognition (*80*–*82*).

Three distinct integral ER membrane proteins divert proteins for dislocation. One unique class, the cytomegalovirus gene products US2 and US11, binds to newly synthesized MHC class I receptor molecules in the ER (*83*, *84*). A complex between the class I protein and US11 associates with another ER protein called Derlin 1, initiating the diversion of class I protein from the ER. Derlin was first discovered as a yeast ER membrane protein, Der1. DER1 mutants block the ERAD of mutant secretory proteins but have no effect on the degradation of most membrane proteins targeted for destruction (*85*). There is also evidence that the ER protein import channel, Sec61, dislocates certain ERAD substrates (*86*,*87*). Indeed, there may be substantial differences in the mechanism of dislocation of membrane and soluble lumenal proteins, although we do not know enough to draw categorical distinctions. The actual channel for dislocation remains elusive.

Other proteins withdraw dislocating polypeptides from the ER membrane. First contact appears to be made by an AAA ATPase called p97 in mammals and Cdc48 in yeast. This complex may bridge Derlin 1 with the proteosome and a ubiquitination complex that covalently tags dislocating chains (*84*). Dislocation may be driven by ATP hydrolysis by p97, although not all substrates require the intervention of this chaperone. ERAD of unglycosylated α-factor precursor requires neither ubiquitin conjugation nor Cdc48. Indeed, ERAD of this substrate has been reconstituted with isolated ER membranes and the pure, intact proteosome complex (*87*). This reaction may be mediated by direct contact between the proteosome and the cytoplasmic face of the Sec61 channel (*88*).

Solutions to the Translocation Puzzles

How has nature answered each of the puzzling problems of translocation? Targeting is largely by recognition of small linear polypeptide domains, the ''signals.'' Many transport systems are designed only for unfolded polypeptide chains. For these translocons, preproteins either use chaperones to retain their capacity to be unfolded or couple their translocation to ongoing translation. Other translocases can carry even folded proteins across a membrane without lethal ion leakage, though TAT-dependent transport into thylakoids may leak as many as 30,000 protons (*89*). Translocons such as SecYEG or Sec61 complex can use either full-length proteins delivered by chaperones or nascent chains emerging from a polysome for which SRP and its receptor coordinate translation and translocation. This decision is probably made early in each protein's lifetime through a competition of ribosome-bound chaperones such as SRP or trigger factor for association with the emerging nascent chain (*90*). The energetics of transport are varied. ATP can power either SecA to ''push'' the translocation of ~20 amino acyl residues at a time or conserved Hsp70 ATPases which ''ratchet'' chains across the mitochondrial or ER membrane (*54*, *91*). The membrane electrochemical potential can directly act on transiting protein, ''electrophoresing'' its movement, and can promote the functional cycle of translocase proteins per se (*92*). Guanosine triphosphatases (GTPases) coordinate nascent preprotein delivery to the Sec61 complex (*38*) and regulate protein synthesis. Exploration of other membranes and organisms will likely reveal additional diverse translocation mechanisms.

Future Prospects

Structural biology may reveal how a motor such as SecA couples the movement of a preprotein segment to the energy of binding ATP or how SecYEG permits lateral exit of an apolar polypeptide segment into the bilayer (*93*). How do mitochondrial preproteins move so far into the organelle before the energy of the inner membrane potential can capture them for further steps of import, and are the import proteins of the two membranes aligned to facilitate import? How are whole, large folded proteins ''swallowed'' by the TAT or peroxisome transport systems? Finally, dislocation and translocation require recognition, delivery, and energy coupling that work from the opposite sides of the membrane. Our understanding of retrotranslocation is still at an early stage.

Note added in proof: For more on SecYEG structure, see K. Mitra *et al*., *Nature* **438**, 318 (2005).

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REVIEW

The Ins and Outs of DNA Transfer in Bacteria

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Transformation and conjugation permit the passage of DNA through the bacterial membranes and represent dominant modes for the transfer of genetic information between bacterial cells or between bacterial and eukaryotic cells. As such, they are responsible for the spread of fitness-enhancing traits, including antibiotic resistance. Both processes usually involve the recognition of double-stranded DNA, followed by the transfer of single strands. Elaborate molecular machines are responsible for negotiating the passage of macromolecular DNA through the layers of the cell surface. All or nearly all the machine components involved in transformation and conjugation have been identified, and here we present models for their roles in DNA transport.

In bacteria, transformation and conjugation usually mediate the transport of single-stranded DNA (ssDNA) across one or more membranes. Transformation involves the uptake of environmental DNA, whereas conjugation permits the direct transfer of DNA between cells (Fig. 1). Other DNA-transport phenomena in bacteria, such as the passage of DNA through the bacterial division septa and those carried out by many bacteriophages (*1*), involve the movement of double-stranded DNA (dsDNA) and will not be discussed here. Transformation and conjugation probably evolved for the acquisition of fitness-enhancing genetic information, but other mutually nonexclusive theories posit that transformation might have evolved to provide templates for DNA repair or to supply nutrition for bacteria (*2*). Today, both processes are recognized as important mechanisms for horizontal gene transfer and genome plasticity over evolutionary history, and they are largely responsible for the rapid spread of antibiotic resistance among pathogenic bacteria (*3*, *4*).

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Bacterial Transformation

Naturally transformable bacteria acquire a physiological state known as "competence" through the regulated expression of genes for protein components of the uptake machinery. Natural transformation has been most studied in *Bacillus subtilis*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, and *Haemophilus influenzae*. These and other competent bacteria use similar proteins for DNA uptake, with few differences between species. An interesting exception is *Helicobacter pylori*, which uses a conjugation-like system for transformation (*5*). Here, we will discuss the DNA uptake systems of *B. subtilis* and *N. gonorrhoeae* as representative of those in Gram-positive and -negative bacteria, respectively (Fig. 1A). The main distinction between these cell types is that Gram-negative bacteria are enclosed by cytoplas-