

Move it on over: getting proteins across biological membranes

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Summary

The translocation of proteins across membranes is a central problem in biology. Regardless of the system in question, delivering proteins across a given membrane relies on many of the same basic themes. At the same time, however, each membrane translocation system, be it signal-gated or signal-assembled, makes use of components unique to that system. The latest findings on protein translocation across a variety of biological membranes have been presented in a recent review article.⁽¹⁾

BioEssays 25:1154–1157, 2003.

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Introduction

Given that across evolution the biosynthesis of proteins begins on cytoplasmically localized ribosomes, a protein destined to reside beyond the cytoplasm must first traverse either the membrane separating the cell from the outside world or membranes surrounding the different intracellular organelles, depending on where that protein is to be ultimately situated. While a combination of genetic, biochemical and microscopic approaches have all served to reveal that protein translocation across different membranes invokes elements unique to a given pathway, it is also true that Nature relies on a common set of themes for protein translocation across all membranes, as originally suggested some 20 years ago in the signal hypothesis.⁽²⁾ This concept, which earned its author Gunther Blobel the 1999 Nobel Prize in Medicine, postulates that proteins fated to live outside the cytoplasm are originally synthesized with membrane-specific targeting motifs. These sequences are recognized by targeting receptors which in turn deliver their extracytoplasm-bound protein cargo to multi-subunit membrane protein complexes, or translocons, responsible for mediating the passage of such protein into and across the membrane. Lately, however, it has become clear that the translocation machinery is far more versatile than

originally thought. Translocons are capable of not only transferring proteins across membranes, but also of inserting membrane proteins directly into the lipid bilayer. Long-accepted to be unidirectional, it is now thought that deportation of selected ER proteins to the cytoplasm relies on the same translocon as involved in delivering proteins into the ER lumen. Moreover, the relationship between protein translocation and various protein-processing events such as protein folding is now beginning to be understood. In this article, recently reviewed⁽¹⁾ insights into these and other aspects of the protein translocation process across a variety of membranes are highlighted.

The translocon—a tunnel through the membrane

To fulfill their jobs, translocons must meet several criteria.⁽³⁾ At their cytoplasmic faces, they must be able to receive proteins destined for translocation. They must be able to selectively allow those proteins to traverse the membrane. Finally, they must be coupled to agents that drive translocation. According to Schnell and Hebert,⁽¹⁾ two classes of translocon have evolved that meet these criteria: the signal-gated translocons and the signal-assembled translocons. In signal-gated translocons, signal-bearing, newly synthesized polypeptides are maintained in loose conformations and fed across the membrane through a protein-conducting channel.⁽⁴⁾ In signal-assembled translocons, folded proteins or higher-ordered complexes are delivered across the membrane at transiently formed sites in a manner that does not compromise the membrane permeability barrier.^(5–7)

Made for export

The SecYEG complex in the bacterial plasma membrane and the homologous Sec61 complex in the ER membrane lie at the heart of classic examples of signal-gated translocons. In both of these protein export machines, microscopic, biochemical and biophysical studies have shown the translocon complexes to be arranged around a central cavity estimated to range between 9 and 60 Å, depending on the translocation status of the channel.^(8–11) To maintain the permeability barrier provided by the membrane, passage through pores of such large diameter must be controlled. The mode of regulation is dependent on whether translocation is coupled to protein translation or rather occurs following the translation event. In co-translational translocation, as occurs across the ER

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Funding agency: The Israel Science Foundation (#433/03).

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DOI 10.1002/bies.10382

Published online in Wiley InterScience (www.interscience.wiley.com).

membrane, the translating ribosome sits on the Sec61 complex in such a manner as to prevent free exchange between the cytoplasm and ER lumen.⁽¹²⁾ In post-translational translocation in yeast, where the Sec61 complex is joined by a second oligomer, the Sec62/63 complex, the ER luminal molecular chaperone BiP acts to prevent the flow of ions across the membrane by binding to the luminal face of the channel.⁽¹³⁾ Post-translational translocation also takes place in bacteria, where the SecA ATPase binds to the cytoplasmic side of SecYEG. As a result of ATP-induced conformational changes, bound SecA apparently enters the plane of the membrane to push discrete segments of translocating protein across the membrane.⁽¹⁴⁾ Translocating proteins arrive at Sec translocons via different targeting pathways, again chosen according to the temporal relationship between protein translation and translocation in a given system. In co-translational translocation, the signal recognition particle (SRP), a ribonucleoprotein composed of six polypeptides and 7S RNA in higher Eukarya, serves to deliver the ribosome-nascent polypeptide chain complex to the Sec61 complex in a GTP-dependent manner via the affinity of SRP for its membrane-bound receptor.⁽¹⁵⁾ In post-translational translocation, molecular chaperones serve as targeting factors.^(16,17)

Imported goods

Signal-gated translocons also mediate the import of cytoplasmically expressed proteins across mitochondrial and chloroplast membranes. Given the multiple membranes of these organelles, however, different translocons are responsible for navigating the passage of protein across each different membrane. At the mitochondrial outer membrane, mitochondrially targeted proteins bearing one of two types of targeting signals are captured at the Tom40-based TOM translocon via interaction with Tom20 or other TOM subunits.⁽¹⁸⁾ Access to the 20 Å channel through the TOM complex, capable of transferring cargo bearing limited secondary structure, is controlled by Tom22.⁽¹⁹⁾ The enticement for the energy-independent translocation of protein through the TOM translocon is thought to come from the interaction of the targeting sequence with binding sites of increasing affinity on the *cis* and *trans* faces of the translocon. In contrast, translocation across the chloroplast outer membrane occurs at the trimeric Toc75-based TOC translocon, in concert with the homologous GTPases Toc159 and Toc34.⁽²⁰⁾ It is thought that Toc159 acts as a soluble receptor, delivering its translocation-destined cargo to the translocon through interaction with the membrane associated Toc34 in a manner analogous to the binding of SRP to its receptor at the ER membrane.^(21,22)

At the inner mitochondrial membrane, a translocating protein must choose between two translocons. Based on the signal contained within the translocating protein, the TOM translocon associates either with the Tim23-based TIM translocon, which concerns itself with proteins bearing N-terminal

targeting sequences, or with the Tim22-based TIM translocon, involved in the integration of proteins into the inner membrane. In the first of these translocons, Tim23 forms a narrow channel across the inner membrane wide enough to allow passage of a single unfolded polypeptide, in a process energized by the membrane potential and the ATP-regulated actions of matrix-localized mitochondrial hsp70.^(23–25) In the Tim22-based TIM translocon, polytopic membrane proteins are transferred through a Tim23-sized channel formed by Tim22,⁽²⁶⁾ integrating each successive transmembrane domain that it encounters into the membrane⁽²⁷⁾ by a mechanism that relies on the membrane potential alone. Proteins destined to cross the chloroplast inner membrane travel through the TIC translocon, although the working of this translocon are presently not well understood.⁽²⁰⁾

Translocon helpers

In addition to the core components that make up the various signal-gated translocons, additional elements help these translocons do their job. Molecular chaperones fulfill a variety of translocation-related functions, ranging from helping to maintain cargo proteins in unfolded conformations and acting as targeting factors on the cytoplasmic face of the translocon, to providing the driving force for translocation on the *trans* side of the translocon. In their status as energizers of translocation, molecular chaperones such as mitochondrial hsp70 or BiP in the ER exploit their ATPase ability, in association with translocon complex elements, to pull proteins across the membrane according to either a 'Brownian ratchet' or 'molecular motor' model.⁽²⁸⁾ To realize membrane protein insertion, a process involving lateral opening of the translocons to the membrane interior, the core translocon components are often joined by auxiliary subunits, such as TRAM, YidC, Oxa1, or albino3 in the ER membrane, the bacterial plasma membrane, the mitochondrial inner membrane and the chloroplast thylakoid membrane, respectively.^(29,30) Some of these proteins may even function as independent translocons dedicated to membrane protein insertion.

Translocons for the whole

In addition to the signal-gated Sec, TOM, TIM, TOC and TIC translocons, protein translocation can also be achieved via signal-assembled translocons such as the bacterial and thylakoid TAT/ Δ pH translocons and the translocon of the peroxisome translocation system. Although structurally and evolutionarily distinct, both versions of signal-assembled translocons share the ability to deliver large, fully folded proteins, often as part of higher-ordered complexes, across the membrane.^(5,31) To achieve this feat without compromising the permeability barrier of the membrane, it is currently thought that the translocon subunits transiently assemble to create a translocation pathway of a size determined by the size of the transiting cargo. Following translocation, such

translocons would disassemble, thus maintaining the permeability barrier.⁽⁶⁾

In the peroxisome, 23 Pex proteins have been implicated in the translocation process, although for the most part, their functions remain unknown. Some information is available on those Pex proteins serving as target receptors for proteins bearing one of the two targeting signals employed by this system.⁽³²⁾ Pex5 serves as a soluble receptor for protein containing peroxisomal targeting signal 1, defined by a C-terminal tripeptide. Protein containing the less-widely used peroxisomal targeting signal 2, based on a nine-residue sequence found at or near the N terminus of the protein, are recognized by Pex7. Both Pex5 and Pex7 encounter their cargoes in the cytosol and then shuttle them to the same membrane-associated Pex13-, Pex14- and Pex17-based complex, after which the receptor proteins return to the cytosol.⁽³³⁾ From this point on, little of peroxisomal translocation, including the composition of the translocon, is understood. Indeed, the same can be said of the TAT/ Δ pH translocation pathway. In bacteria, this pathway, relying on a translocon based on the TatA-E subunits (or their thylakoid homologues in plants), is employed by substrates marked by targeting sequences containing twin arginine residues. It is thought that, upon binding of the TAT signal by the stable membrane complex formed by TatB and TatC, the oligomeric TatA tightly associates with the TatBC pair to yield a functional translocon that translocates proteins in a process dependent on the membrane potential.

When things go wrong

The involvement of the translocon in the life of a polypeptide can extend beyond the translocation event itself. With segments of a translocating polypeptide still within the confines of the Sec61 translocon, other segments that have already entered the ER lumen may experience protein folding, glycosylation, disulfide formation, or controlled proteolysis with the assistance of a variety of translocon-associated proteins.^(34,35) Unfortunately, however, not all proteins in the ER mature correctly and so to protect against the dangers associated with an accumulation of misfolded or aggregated proteins, the cell has established a quality control system culminating in the destruction of such undesirable products. The site of destruction is the proteasome, located in the cytosol. Hence, damaged proteins already translocated into the ER lumen must rely on ER-associated protein degradation (ERAD) for their transfer back to the cytoplasm.⁽³⁶⁾ The Sec61 translocon, involved in co- and post-translational translocation into the ER is also responsible for such retrograde translocation.⁽³⁷⁾ As in translocation into the ER, retrograde translocation also requires that cargo proteins be targeted to the translocon and that a driving force ensures the unidirectionality of the process. The manner by which aberrant proteins are targeted to the *trans* face of the translocon is unknown,

although marking a nascent polypeptide for destruction during the time that it first enters the ER yet while still attached to the ribosome on the *cis* side of the translocon would overcome the need for a distinct targeting step.⁽³⁸⁾ Questions related to the directionality of retrograde translocation are better understood. Polyubiquitin-tagging of retrograde translocated protein regions protruding from the translocon at its cytosolic face⁽³⁹⁾ serves to ensure the unidirectionality of the process. Such modification acts as a 'molecular ratchet' to prevent backslipping of the substrate into the ER lumen, and possibly to recruit molecular machines either directly involved in protein degradation, such as the proteasome, or that serve as mediators between the translocon and the proteasome, such as the p97-Ufd1-Npl4 complex.⁽⁴⁰⁾

The future

While great advances have been made in elucidating the process of protein translocation across biological membranes, Schnell and Hebert note that much is still unknown.⁽¹⁾ In all systems, it remains a mystery how translocation is initiated. How do signal-receptor complexes trigger translocon gating or assembly, and how is the cargo protein transferred from the receptor to the translocon? How is the permeability barrier maintained during the translocation event? How are retrograde translocation substrates recognized, targeted and ultimately driven through the translocon? Perhaps answers to these and other pressing questions will emerge from recent advances in describing translocon structure. By combining structural approaches with biochemical analysis, it may be soon possible to visualize the molecular events that take place at various steps of the protein translocation event. Finally, future efforts may provide insight into the relationship between translocation and cell development, physiology and specialization,⁽²⁰⁾ allowing for further integration of protein translocation research into studies on the biogenesis of membrane-enclosed compartments.

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