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Sec-dependent protein translocation across biological membranes: evolutionary conservation of an essential protein transport pathway (Review)

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Abstract

All living organisms, no matter how simple or complex, possess the ability to translocate proteins across biological membranes and into different cellular compartments. Although a range of membrane transport processes exist, the major pathway used to translocate proteins across the bacterial cytoplasmic membrane or the eukaryotic endoplasmic reticulum membrane is conserved and is known as the Sec or Sec61 pathway, respectively. Over the past two decades the Sec and Sec61 pathways have been studied extensively and are well characterised at the genetic and biochemical levels. However, it is only now with the recent structural determination of a number of the key elements of the pathways that the translocation complex is beginning to give up its secrets in exquisite molecular detail. This article will focus on the routes of Sec- and Sec61-dependent membrane targeting and the nature of the translocation channel in bacteria and eukaryotes.

Keywords: *Protein secretion, translocon, SecYEG, Sec61αγ/β, membrane targeting*

Introduction

Not all proteins exert their effects in the cellular compartment in which they are synthesized. Accurate targeting and efficient delivery of proteins to the appropriate cellular location underpins all biological processes and is critical for proper cell function and viability. All cells are composed of at least one compartment (and often more) surrounded by a semi-permeable membrane structure. No matter how simple or complex all living organisms possess the ability to actively translocate proteins into and across lipid membranes to move proteins between cellular compartments; this is an essential biological process. Translocation of proteins across lipid bilayers is mediated by specific multimeric protein complexes that form channels in the membrane to facilitate the passage of secretory proteins. At the same time these channels need to maintain the integrity of the membrane to prevent the exchange of ions or small molecules between compartments that may have detrimental consequences for the cell.

In bacteria secretory precursor proteins are translocated from the cytosol across the cytoplasmic membrane whereas in eukaryotic cells a large number of secretory precursors are translocated through the endoplasmic reticulum (ER) membrane

and into the lumen. Although a number of protein translocation pathways are recognised in bacteria [1], the major route for the translocation of secretory proteins across the cytoplasmic membrane is known as the general secretory (Sec) pathway. Prokaryotic Sec-dependent protein secretion has been most extensively studied in *Escherichia coli* and to a lesser extent in *Bacillus subtilis*, which are model representatives of the Gram-negative and Gram-positive groups of bacteria, respectively. In eukaryotes the ER membrane is a major site for protein translocation and the process is mediated by the Sec61 complex [2]. Proteins destined for secretion are guided into these pathways by the presence of amino-terminal extensions known as signal peptides [3]. The Sec and Sec61 pathways can also translocate proteins that remain associated with membrane as integral membrane proteins (IMPs) or lipoproteins. The core of the Sec and Sec61 pathways are composed of heterotrimeric protein complexes, SecYEG and Sec61αγβ, respectively.

Following passage through the Sec or Sec61 complexes in the membrane a range of post-translocational events can influence the fate, activity and final location of the secretory protein. For bacterial cells the potential final locations of secretory

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proteins are determined largely by cell physiology and architecture of the cell envelope. Nevertheless, following Sec-mediated translocation secretory proteins can remain cell-associated in the extracytosolic compartment provided by the cell envelope structure, or they can traverse the envelope completely to be released into the external environment. Proteins translocated across the ER membrane by the Sec61 complex may be modified and can remain in the lumen or undergo sorting to their final locations by a complex transport system that connects the ER with the other elements of the endomembrane system, the Golgi complex and the plasma membrane. This article will discuss the current understanding of protein translocation pathways in bacteria and eukaryotes mediated by Sec and Sec61 complexes and will focus on the modes of secretory protein targeting to the translocation complex, or translocon, and the nature of the protein conducting channel in the membrane.

Targeting to the Sec/Sec61 translocation machinery

Secretory protein precursors are synthesized in the cytoplasm and need to be targeted to the appropriate export sites in the membrane. In all organisms targeting is achieved by specific properties of the precursors themselves, and also by distinct cellular machineries that ensure the precursors are presented at the correct translocation site in the membrane in a translocation-competent conformation.

Signal peptides distinguish secretory proteins from cytosolic proteins

A number of motifs and features of secretory proteins are known to influence membrane translocation, targeting and sorting in all organisms. In prokaryotes and eukaryotes the presence of an amino-terminal signal peptide is one key feature that distinguishes proteins destined for membrane translocation from cytosolic proteins. Signal peptides mediate targeting and entry of nascent and completed secretory precursors into secretory pathways and also retard the folding of precursors in the cytosol [4]. Signal peptides are generally proteolytically cleaved from the precursor by signal peptidases (SPases) during or shortly after translocation.

Signal peptides are characterized by the presence of three distinct domains, termed N-, H- and C-. The most amino-terminal region, the N-domain, is polar and generally has an overall positive charge with at least one lysine or arginine residue; these basic residues are thought to promote interactions with negatively charged phospholipids and the

translocation apparatus [5,6]. The hydrophobic H-domain is possibly the most characteristic feature of signal peptides from all organisms and is critical for targeting and membrane insertion and forms a helical hairpin that facilitates membrane insertion. The most carboxyl-terminal region of signal peptides, the C-domain, is polar in nature and contains the SPase cleavage site. This cleavage site conforms to the -1, -3 rule proposed by von Heijne and typically has small uncharged residues such as alanine in these positions [7,8]. SPases are IMPs with their catalytic domains positioned on the *trans* side of the membrane so that signal peptide cleavage occurs at the outer leaflet of the cytoplasmic membrane or on the luminal side of the ER membrane. Although signal peptides display considerable heterogeneity in length and sequence, the main structural and physiochemical features are well conserved in all organisms [9]. Signal peptides are frequently interchangeable between species and can direct the translocation of secretory precursors in non-native organisms [10,11].

Targeting factors

Two modes of Sec/Sec61-dependent protein translocation can be recognized. These pathways differ in the temporal relationship between translation and translocation and influence the way in which precursors are targeted to the translocon (Figure 1). In co-translational targeting, elongation of the polypeptide chain at the ribosome is arrested and then directly coupled to translocation through the channel in the membrane. In contrast, in post-translational targeting the synthesis of the precursor is completed (or nearly completed) in the cytosol. Specific cytosolic chaperones prevent the precursor attaining its native conformation and target the precursor to the translocon. No matter which mode is employed, both pathways converge at the Sec/Sec61 complex and have mechanisms to ensure that secretory precursors are presented to the translocon in an unfolded or loosely folded conformation that is compatible with passage through the pore.

Eukaryotic targeting factors

In eukaryotes most proteins are co-translationally translocated across the ER membrane and the mechanisms are well characterized (reviewed in [12,13]). As the signal peptide of a nascent precursor emerges from the ribosome it is recognized and bound by a ribonucleoprotein complex known as the signal recognition particle (SRP, Figure 1). Mammalian SRP is a complex of the 7S RNA
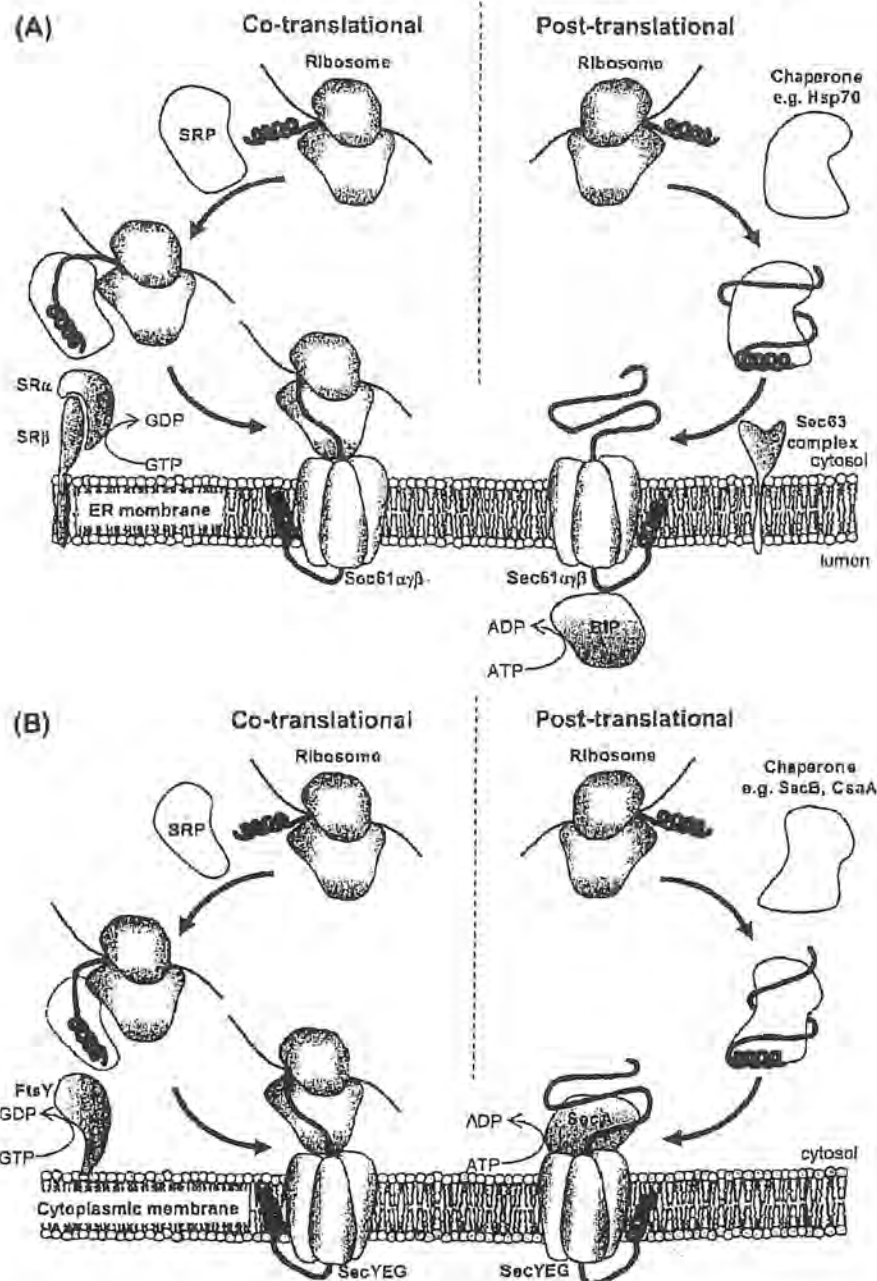
Figure 1. Co-translational versus post-translational targeting of secretory precursors to the Sec61αγβ/SecYEG translocon. Only the main elements of the co- and post-translational targeting pathways to the (A) eukaryotic ER membrane and (B) bacterial cytoplasmic membrane are shown. In the co-translational targeting pathway to the ER membrane translation of the secretory precursor is stalled at the ribosome by the interaction of the SRP with signal peptide of the nascent polypeptide chain. SRP then targets the ribosome/nascent polypeptide complex to Sec61αγβ in the ER membrane via the SRα and SRβ GTPases, which are peripheral and integral membrane proteins, respectively. In the post-translational targeting pathway to the ER membrane translation of the precursor is completed (or nearly completed) and the precursor is maintained in a translocation-competent conformation by cytosolic chaperones. Targeting of the chaperone/precursor complex to Sec61αγβ in the ER membrane in achieved via the Sec63 complex (a multi-protein assembly of Sec63, Sec61, Sec71 and Sec72). The Bip protein in the ER lumen acts as an ATP-dependent molecular ratchet to pull the precursor through the pore. In the bacterial co-translational pathway the SRP in less complex but membrane targeting proceeds through a similar mechanism to the ER pathway. Co-translational targeting to the membrane is achieved by the interaction of the SRP/ribosome/nascent polypeptide chain with the FtsY GTPase (the SRα homologue). FtsY exists in soluble and membrane-associated forms that most likely perform functions analogous to SRα and SRβ, respectively. In the post-translational targeting pathway to the bacterial cytoplasmic membrane cytosolic chaperones prevent folding and present the precursor to the SecA molecular motor in a translocation-competent conformation. SecA binds to the precursor and inserts into the SecYEG pore. Successive rounds of ATP binding, membrane insertion, ATP hydrolysis and membrane deinsertion drive the precursor through the pore in a step-wise manner.

molecule and six proteins, SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72. Binding of SRP to the signal peptide is mediated by the SRP54 GTPase resulting in elongation arrest. Targeting of the SRP/ribosome/nascent polypeptide complex to the ER membrane is achieved by the GTP-dependent interaction of SRP with its integral membrane receptor, SR. SR is a heterodimer of the SRα and SRβ proteins, that are peripheral and intergral membrane GTPases, respectively. SR catalyses the transfer of the ribosome/nascent polypeptide complex from SRP to the Sec61 translocon. This releases the translation arrest and chain elongation pushes the growing peptide through the pore and into the ER lumen.

Eukaryotes can also translocate some proteins across the ER membrane post-translationally [14] (Figure 1), although this pathway may be largely restricted to lower eukayotes such as *Saccharomyces cerevisiae*. In this targeting pathway precursors interact with cytosolic chaperones such as Hsp70 and the TriC/CCT complex which prevent folding into a conformation incompatible with translocation [15]. The Sec63 complex is involved in targeting to the membrane and is thought to act as the membrane receptor for signal peptides of post-translationally translocated proteins [16,17]. This complex is composed of, Sec63, Sec62, Sec71 and Sec72, and homologues of the Sec63 complex are not present in bacteria. Luminal BiP/Kar2 (a member of the HSP70 family) can act as an ATP-dependent molecular ratchet that pulls the protein through the pore [18]. Recent evidence indicates that the SRP may also be involved in post-translational targeting in eukaryotes [19].

Bacterial targeting factors

Bacteria have both co- and post-translational targeting pathways that deliver translocation substrates to the Sec complex in the cytoplasmic membrane (Figure 1). Co-translational translocation in bacteria occurs via a similar mechanism as the SRP pathway in eukaryotes, but the pathway appears considerably less complex (very recently reviewed in [20]). The *E. coli* SRP pathway is thought to be largely involved in the targeting of IMPs and very hydrophobic secretory proteins. *E. coli* SRP is composed of a 4.5S RNA molecule and the Ffh protein, which is a GTPase and the bacterial homologue of SRP54. Ffh recognizes and interacts specifically with the signal peptide of nascent precursors and the trans-membrane segments (TMSs) of IMPs [21]. Targeting to the membrane is achieved by the FtsY protein, the SRα homologue, which also has GTPase activity and exists in soluble and membrane-associated forms

[22]. The membrane-associated form most likely links the SRP complex to the translocon but the function of the soluble form is not clear. The bacterial version of SRβ has not been identified and it is thought that differentially localised forms of FtsY perform the function of both SRα and SRβ. The interaction between Ffh and FtsY stimulates GTP hydrolysis and the ribosome/nascent polypeptide complex is released from SRP and transferred to the Sec translocon [23]. In *B. subtilis* SRP is composed of a small cytoplasmic RNA molecule (scRNA), Ffh and a histone-like protein HBsu [24,25]. As in *E. coli*, FtsY is the SRα homologue that targets the SRP/ribosome/nascent polypeptide complex to the *B. subtilis* translocon.

Although indirect evidence of translation arrest in *E. coli* exists [26], the SRP in this organism lacks SRP9, SRP14 and the 5' and 3' domains of the SRP RNA that are required to halt chain elongation. The fact that the prerequisite 5' and 3' domains are present in *B. subtilis* scRNA clouds this matter even further. It has been suggested that the faster translocation rates and shorter traffic distances reduces the requirement for elongation arrest in the bacterial SRP pathway [27,28]. Therefore, the occurrence of SRP-mediated translational arrest in bacteria is open to speculation and may even depend on the species concerned.

Bacteria also target secretory precursors to the Sec translocon post-translationally (Figure 1). In *E. coli* SecB is a molecular chaperone for secretory precursors that is involved in post-translational targeting (reviewed in [29]). After most of the polypeptide has been synthesised, SecB preferentially interacts with secretory precursors in the cytosol to prevent folding or aggregation and targets them to the translocon by binding to the SecA sub-unit. It has been widely accepted that SecB is a dedicated chaperone for secretory proteins however, recent evidence suggests that SecB may also function as a general chaperone for non-secretory proteins [30]. Notwithstanding this, SecB is still regarded as a key targeting factor in post-translational translocation in *E. coli* but it should be noted that SecB homologues are not found in all bacterial genomes and not all secretory proteins are SecB-dependent. In contrast to SRP, SecB recognizes and binds to long stretches of the mature regions of precursors [31]. SecB interacts with multiple sites on precursors but its substrates share no significant sequence homology, although a minimal motif for SecB binding consists of a stretch of nine amino acids consisting of mainly charged and aromatic residues [32]. The mechanism of substrate differentiation by SecB remains to be elucidated.

The structure of SecB from *E. coli* has been determined [33], as has the structure of SecB from *Haemophillus influenzae* both on its own and in complex with the carboxyl-terminal end of SecA [34,35]. SecB functions as a homotetramer composed of a dimer of dimers. Each tetramer has two proposed precursor binding grooves which are channels 70 Å in length and located on either side of the tetrameric molecule. As one SecB tetramer binds one precursor protein molecule, it is assumed that the substrate wraps itself around SecB to occupy both channels that are located on opposite sides of the tetramer. SecB targets precursors to the translocon by interacting with membrane-associated SecA.

SecA is the peripheral membrane receptor for post-translationally targeted secretory proteins and functions as an ATP-dependent molecular motor to provide the energy for translocation [36]. SecA is essential for viability and translocation and plays a central role in Sec-dependent protein export in all bacteria. SecA exists as cytosolic and membrane-associated forms and interacts with SecYEG, acidic phospholipids and with both signal peptides and mature regions of secretory precursors [37,38].

The catalytic cycle of SecA has been studied in detail (recently reviewed in [39]). Briefly, SecA interacts with high (nM) affinity with SecYEG in the cytoplasmic membrane and this association primes the translocase for translocation. Secretory precursor are transferred from cytosolic targeting factors (such as SecB) to SecA and ATP binding by SecA results in the insertion of the carboxyl-terminal domain of SecA through the SecYEG pore and the translocation of a small section (20–30 amino acids) of the precursor. ATP hydrolysis by SecA then leads to the deinsertion of SecA from the membrane and the release of the precursor. Subsequent cycles of ATP binding, insertion, ATP hydrolysis and deinsertion processively translocate the precursor through SecYEG in a hand-over-hand manner in stretches of 20–30 amino acids. When a significant proportion of the precursor has been translocated the proton motive force (pmf) can finish the translocation reaction, even in the absence of SecA and ATP. Therefore, the pmf may be involved in both SecA deinsertion and the forward movement of the precursor across the membrane.

The structure of full length SecA has been solved [40–42] and also that of the isolated Zn-binding carboxyl-terminal domain [43,44]. Although SecA is known to exist as a homodimer, recent data provides strong evidence that the active form of SecA is actually monomeric [45–48]. The SecB/precursor complex is targeted to the translocon by the interaction of SecB with SecYEG-bound SecA. SecB binds to SecA via the major SecB-binding site at the extreme carboxyl-terminus of SecA [49,50]. This interaction causes the release of SecB and stimulates the ATPase activity of SecA which drives the precursor through the SecYEG pore.

The absence of a recognizable SecB targeting factor is one of the most striking differences between Sec-dependent protein export in *E. coli* and *B. subtilis*. It is possible that other targeting factors, analogous by function but not sequence, are responsible for post-translational targeting to the translocon in *B. subtilis*. One protein that may perform this role is CsaA, an export-related chaperone that binds to mature regions of secretory precursors and can be found in association with SecA [51,52]. Furthermore, *B. subtilis* Ffh is known to interact with SecA during translocation and it has been suggested that the SRP pathway may also play a role in post-translational targeting to the Sec translocon in *B. subtilis* [53,54].

Archaeal targeting factors

Although archaea are evolutionary distinct from bacteria and eukaryotes [55], they also use the Sec/Sec61 pathway to transport proteins across the cytoplasmic membrane [56,57]. To date, protein targeting and translocation in archaeal species has been less extensively studied than in other prokaryotic organisms. Archaea have a SRP targeting pathway that is essential for viability and is composed of SRP54, SRP19 and a 7S RNA molecule [58]. Archaea also possess a FtsY/SRα homologue that is involved in membrane targeting [59] but in a manner analogous to bacteria, SRβ does not appear to be present. An obvious homologue of SecA is missing from archaeal genomes. However, a functional homologue of SecB has been identified in *Methanococcus jannaschii* [60] and a homologue of *B. subtilis* CsaA can be identified in most archaea [56] that could potentially be involved in post-translational targeting to the membrane.

The protein-conducting channel

In the bacterial cytoplasmic membrane

Most elements of the Sec pathway were originally identified in *E. coli* by genetic analysis [61,62] and most of what is known about bacterial protein translocation has come from studies on this organism. In *E. coli* the core of the translocon is a proteinaceous channel formed by the IMPs SecY and SecE, which are associated with an additional IMP, SecG, in a heterotrimeric complex [63]. SecY and SecE are both essential for viability and protein translocation. The SecYEG complex, along with

SecA, is the minimum unit required to translocate proteins across the membrane. SecY interacts with both SecE and SecG and these interactions stabilize SecY in the membrane [64].

SecYEG interacts with another heterotrimeric complex made up of SecD, SecF and YajC [65], to produce a large multidomain assembly for translocation through the membrane. The SecDF-YajC complex plays an accessory role in translocation and is thought to be involved in several aspects of the process including, membrane cycling of SecA, clearing of misfolded proteins/cleaved signal peptides and the proper assembly of the translocon [66,67]. SecY performs a central and important role in the functioning of the translocon as it interacts with SecF and YajC and most likely mediates the interaction of the SecYEG and SecDF-YajC heterotrimers [68].

Recent evidence suggests that an additional *E. coli* membrane protein, YidC, interacts with SecD and SecF to form a heterotetrameric complex, SecDF-YajC-YidC [69]. YidC is a homologue of the mitochondrial Oxa1p protein and the chloroplast Alb1 protein [70], both of which are involved in membrane insertion of eukaryotic IMPs. Although YidC can be cross-linked to the TMSs of nascent IMPs, *in vivo* depletion of YidC has only a slight influence on the membrane insertion of Sec-dependent membrane proteins in *E. coli* [71]. It now appears that YidC can function both independently of, and in conjunction with, the Sec translocon to insert different sub-sets of IMPs into the *E. coli* membrane [72,73]. YidC is required for the membrane insertion of the F_1F_0 ATP synthtase sub-unit c and influences the assembly of the whole F_0 complex [74,75]; this most likely explains why YidC is essential for viability.

The core of the Sec pathways of *B. subtilis* and other related Gram-positive bacteria are generally equivalent to the *E. coli* system yet some notable differences are apparent. *B. subtilis* possesses recognisable homologues of SecY, SecE, SecG, SecD, SecF, YajC and YidC [54]. SecY of *B. subtilis* has the same general domain structure of *E. coli* SecY and both proteins are predicted to have ten TMSs. In contrast, *B. subtilis* SecE is considerably smaller than its *E. coli* homologue and spans the membrane once, compared with the three TMSs of *E. coli* SecE. *B. subtilis* SecE can complement a secretion-deficient mutant of *E. coli secE* [76], suggesting that *B. subtilis* has retained only the part of the protein that is important for SecE function. Although SecG is a central component of the *B. subtilis* translocon, it is not essential for viability or translocation but seems to be more important for translocation efficiency [77]. Bacterial SecG proteins have two TMSs and

SecG proteins from Gram-negatives tend to longer than their Gram-positive counterparts. The SecG proteins are not truly interchangeable between bacterial species [77,78] and it is likely that some species-specific functional differences exist.

Genes encoding Sec components are generally distributed throughout bacterial chromosomes. However, in *E. coli yajC* is immediately upstream of, and co-transcribed with, discrete *secD* and *secF* genes. In *B. subtilis* the SecD and SecF proteins are fused into a single polypeptide, termed SecDF, which is the product of a single gene. The SecDF protein is required for efficient translocation during hypersecretion conditions but is not essential for viability [67]. The gene encoding the *B. subtilis* YajC homologue, *yrbF*, is in close proximity to *secDF* on the *B. subtilis* chromosome [67]. Furthermore, *B. subtilis* has two homologues of Oxa1p/YidC, termed SpoIIIJ and YqjG, that are involved in membrane protein biosynthesis and translocation [79]. Although both the *spoIIIJ* and *yqjG* genes can be inactivated individually, a *spoIIIJ-yqjG* double mutant is lethal. SpoIIIJ and YqjG have distinct but overlapping functions and the *B. subtilis* cell requires the combined activities of both of these proteins to maintain viability [79].

In the ER membrane

The presence of membrane-bound organelles is a defining feature of eukaryotic cells. The membranes separate the contents, and therefore the functions, of distinct aqueous compartments. The membrane of the ER is the topological equivalent of the bacterial cytoplasmic membrane. Translocation across the ER membrane represents the first stage in the journey of proteins through the eukaryotic secretory pathway to their ultimate destination. Translocation of signal peptide-containing precursors across the ER membrane is mediated by a dedicated protein complex that forms an aqueous channel in the membrane through which translocation substrates can pass.

The core of the Sec61 translocon in the mammalian ER membrane is composed of a heterotrimeric complex of three proteins, Sec61α, Sec61β and Sec61γ. Sec61α and Sec61γ correspond to SecY and SecE, respectively [80]. Sec61α shares a high degree of sequence conservation with bacterial SecY and these proteins exhibit the same domain organisation with ten TMSs; this topology is conserved in all members of the SecY/Sec61α family [80]. Not surprisingly, like bacterial SecY, Sec61α is the major component of the aqueous pore of the ER translocon [81]. Sec61γ plays a critical role in translocation and has a single TMS. The domain organisation of Sec61γ is identical to that of *B. subtilis* SecE and

this organization is also apparent in all other SecE/Sec61γ homologues that can be identified in sequenced genomes [80]. The final core component of the ER translocon, Sec61β, has a stimulatory effect on translocation but is not strictly required for the process and is not essential for viability [82].

The Sec61αγβ complex is the central element of the ER translocon but is insufficient for translocation on its own and other factors are also required. Sec61, together with SRP, SR and the translocating-chain-associating membrane (TRAM) protein, are the minimum components required for translocation [12]. TRAM is important for efficient translocation of many secretory proteins and its interaction with nascent precursors is dependent upon features of the signal peptide. Unlike the Sec61 complex, which is distributed throughout all eukaryotes, the presence of TRAM is restricted to higher eukaryotes. Other accessory factors required for efficient translocation include the translocon-associated protein (TRAP) complex [83] and Sec63 [84]. Despite the conservation of SecYE/Sec61αγ, homologues of the bacterial SecDF-YajC complex have not been found in any eukaryote [80], suggesting that this accessory complex may be required for some aspect of the translocation process that is specific to bacterial cells.

In the archaeal cytoplasmic membrane

Archaea have SecY/Sec61α and SecE/Sec61γ components but in both cases the proteins are more closely related to the eukaryotic translocon sub-units than those found in bacteria [57,80]. Archaea also possess a Sec61β sub-unit which is not related to SecG [85]. Given the similarities of the archaeal translocon core to Sec61αγβ, it is therefore somewhat surprising to find that certain archaea have homologues of bacterial SecDF [86].

The structure of the protein-conducting channel

Recently the molecular details of the structure and function of the conserved Sec translocon have been illuminated by the high resolution (3.2 Å) structure of the Secαγβ complex from the archaeon *M. jannaschii* [87]. This structure is in agreement with the lower resolution structure of *E. coli* SecYEG [88] and is thought to be representative of the complex in all organisms. The putative translocon pore is organized such that the α sub-unit is divided into two halves, made up of TMSs 1–5 and 6–10. The α sub-unit TMSs form the channel through the membrane and the two halves are connected at the back of the molecule by an external loop and clamped together by the γ sub-unit. The β sub-

unit is located on the periphery of αγ complex and makes only limited contact with the α sub-unit, possibly explaining why Sec61β or SecG are not essential components of their respective translocons.

When viewed through a cross-section of the membrane the putative channel resembles an 'hour glass' with two funnel-shaped chambers that open at the cytosolic and external faces of the membrane and a central constriction that most likely closes the channel to polypeptides and small molecules. At the cytosolic side of the membrane the aqueous chamber is 20–25 Å in diameter and this narrows to 5–8 Å at the constricted region. A small loop from the α sub-unit in the constricted region separates the funnel-shaped chambers on the cytosolic and external leaflets of the membrane and presumably acts as a plug to restrict passage through the channel. This loop is most likely destabilised and displaced by the signal peptide-mediated insertion of the precursor into the channel. The internal walls of the putative channel are largely made up of uncharged amino acids which may serve to minimise interactions during the passage of substrates through the pore. At its narrowest point the pore is lined by a ring of hydrophobic residues that are suggested to form a gasket-like seal around translocation substrates to maintain the barrier function of the membrane during translocation. As well as opening up across the membrane, the channel can also open laterally to allow TMSs of Sec-dependent IMPs to gain access to the lipid phase during membrane insertion; this bi-directionality distinguishes the Sec/Sec61 pathway from many other membrane transport systems.

The functional Sec translocation channel exists as a large oligomeric assembly of SecYEG heterotrimers containing between two and four individual heterotrimer complexes [89,90]. Until recently, it has been generally assumed that a large pore forms at the interface of multiple heterotrimer complexes. However, the *M. jannaschii* structure suggests that a single αγβ heterotrimer may be sufficient to function as an active pore [87]. The structure of the putative pore indicates that the basic functional unit is a back-to-back dimer of two αγβ heterotrimers with the lateral exit sites on opposites faces of the dimer [87,88]. In this situation two dimers may also associate side-by-side to form a large assembly containing four heterotrimers but the evidence implies that only one heterotrimer complex participates in translocation at any given time. If this holds true, the role of oligomerization of SecYEG/Sec61αγβ complexes in the translocation process and the mechanism(s) of regulation of individual heterotrimers within those oligomeric assemblies will be exciting areas of future research. It should be noted that X-ray crystallography relies on highly

organized and static proteins within the crystal lattice, whereas the *in vivo* functioning of the translocon is a highly dynamic process. Consequently, it is important that all structural data is viewed and interpreted in combination with the genetic and biochemical approaches that have been so successful for the analysis of protein translocation in the past.

Protein folding and quality control

Translocation substrates need to be in an unfolded or loosely folded conformation for passage through the translocon pore. During or immediately after translocation, the signal peptide is cleaved by SPases and the mature domain folds into the native conformation. Folding on the *trans* side of the cytoplasmic membrane or in the ER lumen is assisted by a range of chaperones and folding catalysts that ensure proteins fold efficiently and into the proper conformation [91,92]. The ER lumen has a range of folding effectors from different chaperone families including HSP40, HSP70 (e.g., Bip/Kar2), HSP90, the lectin chaperones calnexin and calreticulin, peptidyl-prolyl *cis/trans* isomerases (PPIases) and thiol-disulphide oxidoreductases. Although these folding effectors can have a number of different biochemical activities there are two important classes that are common to both bacteria and eukaryotes, namely the PPIases and thiol-disulphide oxidoreductases.

PPIases catalyse the isomerization of *cis*-proline residues. Members of the two main PPIase families, FK506-binding proteins and cyclophilins, are found in the ER. In bacteria members of a third PPIase family, the parvulins, are also found in the extracytosolic compartment and include *E. coli* SurA [93] or *B. subtilis* PrsA [94]. Despite catalysing the *in vitro* isomerization of prolyl peptide bonds, the physiological role of the PPIase activity of many of these proteins is unclear and the PPIase activity is even dispensable for *in vivo* function in some cases [95,96].

Many proteins, of both prokaryotic and eukaryotic origin, require the presence of disulphide bonds for structural integrity and activity. Thiol-disulphide oxidoreductases catalyse the oxidation, reduction or isomerization of disulphide bonds and are found in the ER lumen and in the extracytosolic compartment of bacterial cells, where the redox conditions are suitably oxidizing for disulphide bond formation. In the ER lumen a range of thiol-disulphide oxidoreductases are present and include PDI, PDIR, PDIp, P5, ERp57 and ERp72 (recently reviewed in [97]). In *E. coli* at least six proteins, DsbA, DsbB, DsbC, DsbD, DsbE and DsbG, are involved in

disulphide bond formation on the *trans* side of membrane [98] and *B. subtilis* has four thiol-disulphide oxidoreductases, BdbA, BdbB, BdbC and BdbD [99,100].

The physico-chemical nature of the microenvironment into which secretory proteins are translocated can also be a critical factor for folding and stability and this environment must be optimal for both of these processes. For example, the presence of divalent cations such as Ca^{2+} can have a dramatic influence on folding at the outer surface of the bacterial cytoplasmic membrane or in the ER lumen [92,101–108].

Proteins that do not fold correctly, or that do not achieve their native folded state fast enough, are cleared from the bacterial cell envelope or ER lumen. The quality control mechanisms that perform this clearing function avoid potentially lethal blockages of the translocon and ensure that only proteins in the proper conformations survive to reach their final destinations. In the ER lumen aberrantly folded proteins are identified, retro-translocated through the Sec61 complex to the cytosol and degraded by proteasomes in a ubiquitin-dependent manner, a process known as ER-associated degradation (ERAD, recently reviewed in [109]). Likewise in bacteria, fidelity is achieved through the activity of proteolytic enzymes such as *E. coli* HtrA (DegP) [110] and *B. subtilis* WprA [105,106] on misfolded substrates, but the quality control mechanisms operate in the extracytosolic cell envelope compartment [91,111]. Some of these extracytosolic quality control proteins, such as the HtrA-like proteases, can have dual chaperone/protease activities so that that proof-reading, repair or removal occurs more or less simultaneously [110,112].

Conclusions

Despite the obvious evolutionary diversity between bacteria and eukaryotes, the core elements of the bacterial Sec and eukaryotic Sec61 translocons are well conserved and seem to have functionally equivalent roles in the translocation process. This conservation also extends into the third domain of life, the evolutionary distinct archaea, where the translocon is a mosaic of elements of the bacterial and eukaryotic translocons [56,57]. The inescapable conclusion is that nature has ensured that all living organisms depend on the Sec/Sec61 translocation complex for the membrane translocation (and insertion) of a significant proportion of their secretory protein inventory.

The vectorial movement of macromolecules across membranes is an active process and irrespective of the organism concerned, co- and post-

translational targeting mechanisms vary in the nature of the driving force. In co-translational translocation there is a strict requirement for GTP in the cycling of SRP and the central elements of this pathway, SRP54, Ffh, SRαβ, and FtsY, have specific GTPase activities that are involved in the fidelity of the targeting reaction. Post-translational translocation generally requires energy from ATP hydrolysis to fuel motor proteins that either push (SecA) or pull (BiP/Kar2) the precursor through the channel. The pmf is also important for bacterial Sec-dependent translocation [113].

It is convenient to consider co- and post-translational targeting as discrete and independent pathways but the distinctions between the two modes may not be so clear-cut, particularly in prokaryotic cells. For example, SecA and SRP cooperate during co-translational membrane insertion of certain *E. coli* IMPs [114] and SecA and Ffh interact in *B. subtilis* [53,54]. However, the precise role of SecA in co-translational translocation is not clear and at present, the involvement of the SRP pathway in post-translational targeting in any bacterial species cannot be ruled out. Nevertheless, no matter which targeting route or driving force is used, the pathways converge at the Sec/Sec61 translocon, which is then used to deliver the precursor protein across (or into) the cytoplasmic or ER membrane.

Our understanding of this conserved protein translocation apparatus has increased significantly over the last decade. Recently, the availability of structures for a number of elements of the Sec and Sec61 pathways, notably that of the putative translocon pore, are starting to reveal the secrets of translocon function in fine detail. More structures will follow that may prove even more insightful and reveal in molecular detail exactly how secretory proteins cross biological membranes. In particular the structures of the translocon core in complex with other elements of the complex (such as SecA), and also the structure of the pore in association with a translocation substrate, are eagerly anticipated.

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