Protein export through the bacterial Sec pathway

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Abstract | The general secretory (Sec) pathway comprises an essential, ubiquitous and universal export machinery for most proteins that integrate into, or translocate through, the plasma membrane. Sec exportome polypeptides are synthesized as pre-proteins that have cleavable signal peptides fused to the exported mature domains. Recent advances have re-evaluated the interaction networks of pre-proteins with chaperones that are involved in pre-protein targeting from the ribosome to the SecYEG channel and have identified conformational signals as checkpoints for high-fidelity targeting and translocation. The recent structural and mechanistic insights into the channel and its ATPase motor SecA are important steps towards the elucidation of the allosteric crosstalk that mediates secretion. In this Review, we discuss recent biochemical, structural and mechanistic insights into the consecutive steps of the Sec pathway — sorting and targeting, translocation and release — in both co-translational and post-translational modes of export. The architecture and conformational dynamics of the SecYEG channel and its regulation by ribosomes, SecA and pre-proteins are highlighted. Moreover, we present conceptual models of the mechanisms and energetics of the Sec-pathway dependent secretion process in bacteria.

Membranome
The portion of the bacterial exportome that is integrated into the plasma membrane (approximately 22% of the total proteome of *Escherichia coli* K12).

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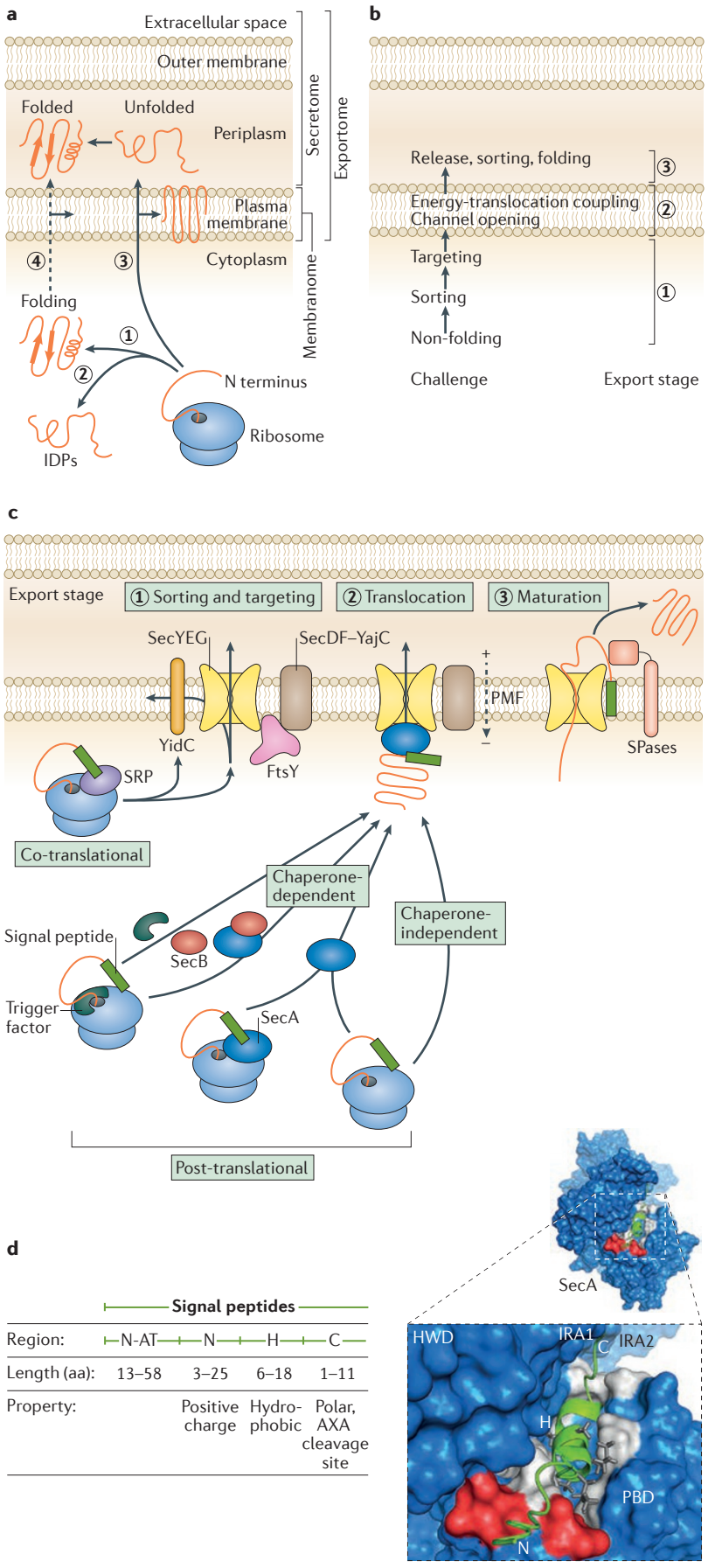
More than one-third of the bacterial proteome that is synthesized on cytoplasmic ribosomes is exported to extracytoplasmic locations, where the polypeptides acquire their native functional states. These proteins are either membrane-embedded (comprising the 'membranome'; FIG. 1a) or are secreted to the *trans* side of the plasma membrane[1]; for example, to the periplasm (comprising the 'secretome'). Typically, export temporally delays protein folding and alters the cellular topology of this process, as most proteins are exported in unfolded states (FIG. 1a).

Protein trafficking to, into and across membranes is an essential, finely tuned process. Exported proteins overcome several challenges[2] (FIG. 1b): they avoid cytoplasmic folding and maintain unfolded, but soluble, states (that is, the 'translocation-competent state'); they become sorted from cytoplasmic proteins; they are targeted to export points at the plasma membrane with high fidelity; they allosterically activate specialized export channels; they translocate laterally into, or to, the *trans* side of the membrane in an energy-dependent manner; and they are sorted to their final destination and/or become folded[3].

Bacteria have evolved several sophisticated export mechanisms for cell envelope and membrane biogenesis (BOX 1; FIG. 1c). Among these, only the general secretory (Sec) pathway is essential for viability and is ubiquitous in all domains of life[4]. The central component of the Sec system is the transmembrane SecYEG channel (also known as the SecYEG translocon; SEC61 in eukaryotes; FIG. 1c), which translocates proteins into, or across, the plasma membrane. Bacteria have an additional essential component, the ATPase motor SecA, that assembles with SecYEG at the plasma membrane to form the translocase holoenzyme, recognizes exported proteins with high affinity (Supplementary information S1 (table)) and mediates chemo–mechanical conversion during transmembrane crossing (FIG. 1c). Some Gram-positive bacteria have additional accessory copies of SecYEG and/or SecA[5], known as the SecY2 and SecA2 paralogues (for example, *Streptococcus* spp. and *Mycobacterium* spp., respectively). Auxiliary components, such as SecDF–YajC[6,7] and YidC[6,8,9], have been shown to enhance translocation efficiency.

Escherichia coli uses the Sec pathway for around 96% of its exportome[1]. These proteins are dissimilar in sequence, and cell envelope topology and concentration (Supplementary information S2 (table)); 60% are plasma membrane proteins[1]. All Sec-dependent secretory proteins, and some plasma membrane proteins, are synthesized as pre-proteins that have cleavable signal



	Signal peptides			
Region:	N-AT	N	H	C
Length (aa):	13–58	3–25	6–18	1–11
Property:		Positive charge	Hydro-phobic	Polar, AXA cleavage site

◀ Figure 1 | **Bacterial protein export. a** | Newly synthesized cytoplasmic proteins fold (pathway 1) during, or immediately following, synthesis, with the exception of intrinsically disordered proteins (IDPs; pathway 2). Almost half of the bacterial proteome is exported to extracytoplasmic locations (exportome) in which polypeptides acquire their native functional states. These proteins are either membrane-embedded (comprising the 'membranome') or secreted to the *trans* side of the plasma membrane; for example, to the periplasm or to, and beyond, the outer membrane (comprising the 'secretome'). Most exported proteins evade cytoplasmic folding (pathway 3), with the exception of a minority of proteins that are exported in a folded state (pathway 4), and are sorted to their final destination and/or become folded[3]. Numbers in this panel represent different pathways and not sequential steps. **b** | Protein export comprises three stages: sorting and targeting (step 1), translocation (step 2), and maturation and release (step 3). Exported proteins face several challenges: they must remain unfolded and soluble, they must be sorted from cytoplasmic residents, they need to be correctly targeted to transmembrane channels and activate channel opening, they use energy to translocate, become released and undergo sorting and/or folding in the cell envelope. **c** | Sec-pathway-dependent export stages. Sorting and targeting (step 1): unfolded proteins (orange) that contain signal peptides (green) and plasma membrane proteins are co-translationally sorted and targeted to the transmembrane SecYEG channel (yellow) by the signal recognition particle (SRP; purple) and its membrane receptor FtsY[11] (pink), or, post-translationally, by SecA (blue)[12]. Chaperones aid post-translational targeting: trigger factor (dark green)[22,24] and the ATPase motor SecA[12,14] bind to pre-proteins on the ribosome or in the cytoplasm; the chaperone SecB (red) binds to them in the cytoplasm[2]. Pre-proteins may be targeted to the SecYEG–SecA translocase in a chaperone-independent manner. Translocation (step 2): pre-proteins translocate through SecYEG to the periplasm or into the plasma membrane[2], the process of which is powered by repeated cycles of ATP binding and hydrolysis by SecA[16,144] and the proton motive force (PMF)[16]. The auxiliary components SecDF–YajC[6] (brown) and YidC[11] (light orange) enhance translocation efficiency. Maturation and release (step 3): signal peptidases (SPases; pale pink) cleave signal peptides and the mature domain is released into the periplasm[21]. **d** | All Sec-dependent secretory proteins, and some plasma membrane proteins, are synthesized as pre-proteins. Pre-proteins contain signal peptides (left panel), which are fused to mature domains at the amino terminus, that act as sorting and targeting tags in the cytoplasm and are proteolytically cleaved at late stages of translocation[10]. Signal peptide properties and regions[2,10] (left panel). The N-terminal region (N) of signal peptides is mostly positively charged. Autotranporters (AT) have an N-terminal extension (N-AT) of varying length. The helical hydrophobic region (H) engages either SRP[11,59,60] or SecA[42] or trigger factor[24]. The mainly polar carboxy-terminal region (C) contains the AXA SPase cleavage motif[21]. Signal peptide binding to the ATPase motor SecA (blue; RCSB Protein Data Bank (PDB) entry 2VDA; right panel)[42]. Signal peptides (green) bind through their H regions to an elongated hydrophobic groove (grey) in SecA that is formed by the pre-protein-binding domain (PBD) and intra-molecular regulator of ATPase 1 (IRA1), and electrostatically, through N-terminal regions, to acidic residues in SecA (red). aa, amino acids; HWD, helical wing domain.

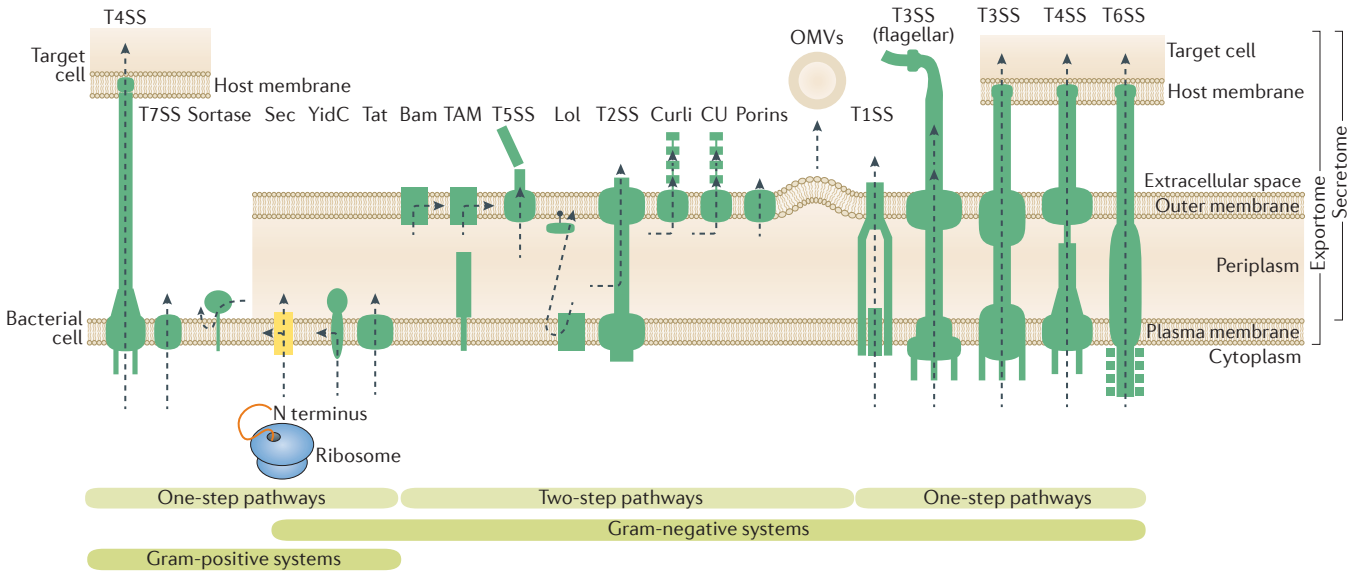
Box 1 | **The remarkable array of bacterial protein export systems**

In bacteria, most exported proteins are transported across the plasma membrane through the Sec pathway[12]. YidC, alone or in complex with SecYEG, functions as an insertase for membrane proteins[153]. A minority of proteins that first fold and/or associate with cytoplasmic cofactors before crossing the plasma membrane are transported through the twin-arginine translocation (Tat) pathway[154].

Gram-negative bacteria have specialized export systems (see the figure, one-step pathways, right): proteins that are involved in pathogenesis and nutrient scavenging are transported by the type 1 secretion system (T1SS)[155]. Flagellum proteins are transported by the flagellar T3SS and toxins are exported by the pathogenic T3SS[156]. Proteins and nucleic acids are injected into other cells, as virulence factors or as a means of genetic exchange, through the T4SS[155]. Pathogenic effectors are injected into a eukaryotic or bacterial target cell through the T6SS[155].

Gram-positive bacteria have three specialized secretion systems (see the figure, one-step pathways, left): virulence proteins cross the cytoplasmic membrane through the T7SS[157]. Sortases recognize, cleave and attach proteins that have a carboxy-terminal sorting signal to the membrane and are responsible for the assembly of pilus structures[157]. Virulence factors or genes are injected into other cells by T4SSs[155].

Once the Gram-negative plasma membrane is crossed, secretory proteins can be diverted to secondary secretion pathways (recently reviewed in REF. 3). Outer membrane proteins are assembled by the β-barrel assembly machine (Bam) complex or the translocation and assembly module (TAM)[158]. The soluble domains of outer membrane proteins can be secreted to the extracellular milieu through the T5SS[155]. Lipoproteins are transported from the plasma membrane to the outer membrane through the localization of lipoproteins (Lol) pathway[159]. Folded periplasmic proteins cross the outer membrane through the T2SS[155]. Outer membrane-anchored structures, such as amyloids and pili, are secreted and/or assembled by the curli secretion machinery or the chaperone–usher (CU) pathway, respectively[155]. Some extracellular proteins use either a complex of porins (for example, YebF)[160] or outer-membrane vesicles (OMVs)[161] to reach the extracellular milieu.



peptides (FIG. 1d). These signal peptides are amino-terminally fused to mature domains and act as sorting and targeting signals[2,10]. Sec-pathway-dependent proteins are exported in unfolded states in three stages (FIG. 1c). First, they are sorted and targeted to the Sec translocase; second, they are translocated into, or across, the plasma membrane; and, finally, they acquire mature forms through the cleavage of their signal peptides and are then released.

Proteins are targeted to the plasma membrane either co-translationally (mainly plasma membrane proteins), by the ribonucleoprotein signal recognition particle (SRP) and its membrane receptor FtsY[11], or post-translationally (secretory pre-proteins; FIG. 1c, step 1)[2]. Export-specific factors, such as SecA and the chaperone SecB, or general chaperones, such as trigger factor, mediate the post-translational targeting of proteins to free cytoplasmic SecA or SecYEG-bound SecA[12,13] at late stages of synthesis or after the completion of translation and ribosomal release (FIG. 1c, step 1, middle; chaperone-dependent). SecA has also been identified in ribosome-bound states[14,15], which highlights the possible role of ribosome-bound factors in determining the fate of emerging nascent polypeptide chains. Alternatively, pre-proteins traffic uncomplexed (chaperone-independent) from ribosomes to SecYEG–SecA. Pre-protein transmembrane crossing (FIG. 1c, step 2) is then energized by either the co-translating ribosome or, post-translationally, by metabolic energy from ATP hydrolysis (through SecA)[16,17] and by the proton motive force (PMF)[16,18,19]. The insertion of proteins that lack long periplasmic segments into the plasma membrane is accomplished solely by SecYEG or by SecYEG in cooperation with the membrane protein insertase YidC[20]. Finally, signal peptides are proteolytically cleaved from translocated mature domains[21] that are released for folding or further trafficking[3] (FIG. 1c, step 3).

The complexity of the Sec pathway and the steps that are involved is apparent and many questions regarding the intricate details of the pathway have remained elusive. How are the numerous unrelated proteins that are destined for export selectively guided to the correct export route? Which 'proof-reading' mechanisms prevent the illicit export of some polypeptides, but

Secretome
The portion of the bacterial exportome that is exported beyond the plasma membrane (that is, to the periplasm, outer membrane, extracellular milieu or a host cell; approximately 13% of the total proteome of *Escherichia coli* K12).

Chemo–mechanical conversion
The conversion of chemical energy, such as the energy that is produced through breaking chemical bonds during ATP hydrolysis, to mechanical work, such as the movement of protein domains or whole proteins.

efficiently translocate hundreds of others? How is the Sec translocase activated and selectively opened for lateral or vectorial translocation? And how does SecA drive successive translocation steps?

Recent scientific breakthroughs have provided mechanistic insights into Sec-dependent protein export and have answered some of these questions. Technological advances have revealed the intermolecular interactions between pre-proteins and their binding partners[22–25]. Structural (Supplementary information S3 (table)) and kinetic studies of ribosome–nascent chain complex (RNC)–SRP–FtsY have defined the 'checkpoints' that mediate selective co-translational targeting[26–31].

Moreover, several structures of SecYEG in complex with RNCs, SecA, pre-proteins, peptide mimics or SecA–pre-proteins (Supplementary information S3 (table)), together with many computational, biochemical and biophysical studies, have improved our understanding of the dynamics of the translocase that govern protein export. In addition, these reports suggest that the conformation of SecYEG, and thus its function, is regulated by distinct interaction partners[32–40]. In parallel, SecA uses its quaternary structure dynamics[41] to carry out its distinct roles during post-translational translocation[23,41–46].

In this Review, we discuss our current understanding of the consecutive steps of the Sec pathway: sorting and targeting, translocation and release for both co-translational and post-translational targeting mechanisms. We focus on the architecture and dynamics of SecYEG and its regulation by ribosomes and SecA. Moreover, we present current models of the mechanisms and energetics of co-translational membrane integration and SecA-dependent post-translational translocation. Independent integration of plasma membrane proteins through YidC (BOX 1) is not discussed (recently reviewed in REF. 20). Most of the findings that are discussed in this Review were obtained through studies on *E. coli*, as it is the best-characterized bacterial system. Nevertheless, thousands of bacterial genomes and biochemical data suggest that most features of the Sec pathway that have been identified in *E. coli* are universal to bacteria. Features that are unique to some bacteria are discussed where appropriate.

Pre-protein sorting and targeting

As signal peptides and mature domains emerge from a ribosome, secretory pre-protein or plasma membrane protein nascent chains are recognized by distinct protein factors (FIG. 1c, step 1). These dynamic interactions and inherent structural features of the signal peptide and mature domain facilitate cytoplasmic sorting, influence the extent of protein folding and the targeting route for export.

During co-translational targeting, highly hydrophobic signal peptides or N-terminal transmembrane helices (TMHs) of plasma membrane proteins are recognized by the SRP at the ribosomal exit tunnel[11], which delivers the RNC to SecYEG or SecYEG–YidC[11,20] (FIG. 1c, step 1). The SRP membrane-associating receptor, FtsY, directly interacts with lipids[47] and SecYEG[48], which leads to the insertion of the nascent chain into the entrance of the SecY channel[28,49].

During post-translational targeting, ribosome-docked chaperones, such as trigger factor[22,50,51] or ribosome-bound SecA[14,15], may interact with emerging secretory polypeptide chains[2,22]. Alternatively, exported proteins leave the ribosome and may bind to cytoplasmic chaperones that have holdase activity, such as SecB[52], that maintain pre-proteins in an unfolded and soluble state[24,53,54]. However, neither trigger factor nor SecB are essential[55–57] and the extent to which these factors are involved in the Sec-dependent protein export pathway remains to be determined. Moreover, in the absence of chaperones, pre-proteins remain unfolded and translocation-competent *in vitro*, owing to their signal peptides that delay the folding of their mature domains[58] and/or to inherent properties of the mature domain[23]. Whether they are released from the ribosome alone or chaperoned, unfolded pre-proteins are subsequently delivered to cytoplasmically diffusing or SecYEG-bound SecA[12,13] (FIG. 1c, step 2).

Signal peptides. Although signal peptides share conserved physicochemical properties, they differ in sequence[10] (FIG. 1d, left). The hydrophobic helical H region of signal peptides could form already at the ribosomal exit tunnel[26] and can engage either SecA[42] (FIG. 1d), SRP[11,59,60] or trigger factor[24] through hydrophobic and electrostatic interactions. Increased hydrophobicity in the signal peptide favours association with SRP and co-translational routing[11]. Owing to the low SRP:ribosome intracellular ratio (Supplementary information S1 (table)), signal peptide recognition has to occur rapdily[61,62]. Non-optimal codons that are found in pre-protein-encoding regions[63,64], mainly in signal peptides[65], might increase the efficiency of this step by delaying translation and folding[66]. The arrest of translational elongation[63], the kinetic enhancement of nascent pre-protein recognition[64] and the prevention of misfolding[67] by avoiding fast aberrant folding may generally contribute to membrane targeting.

Pre-proteins that contain signal peptides with lower hydrophobicity evade SRP surveillance and instead may bind to ribosome-bound trigger factor[68] or SecA[14] (FIG. 1d) through their signal peptides[23,24,42] and/or their mature domains (see below). However, these dynamic processes do not adhere to strict hydrophobicity thresholds and are affected by the concentrations of the ribosome-interacting factors. Examples of escape from SRP[69,70] or unexpected SRP recruitment on nascent chains that lack signal peptides (or TMHs)[71] have been reported, and pre-proteins that preferentially bind to SRP were shown to also use SecA[72] in an unknown manner.

Mature domains. The ribosomal exit tunnel is a narrow, aqueous ante-chamber that enables the initiation of folding of cytoplasmic and transmembrane nascent chains[73] prior to complete folding, which occurs after ribosomal release[74]. Co-translationally exported proteins that are SRP-dependent remain protected from exposure to water and avoid premature folding or aggregation[69], as the nascent chain is released inside the SecY channel.

Exportome
The non-cytoplasmic portion of the bacterial proteome that is integrated into the plasma membrane or exported beyond the plasma membrane (that is, to the periplasm, outer membrane, extra-cellular milieu or a host cell).

Sortases
Cysteine transpeptidases, found mainly in Gram-positive bacteria, that recognize carboxy-terminal signals (cell wall sorting signals) with an LPXTG motif on their substrate proteins (surface proteins), cleave them at the threonyl residue of the motif and then attach them on the cell surface.

Pre-proteins
Exported proteins that are synthesized as pre-forms, that is, containing an amino-terminal signal peptide (also known as a signal sequence or leader peptide) extension that is proteolytically removed during, or after, export.

Chaperones
Proteins (ATP-dependent or independent) that assist in *de novo* protein folding or refolding, or disaggregation or the prevention of aggregation under stress or physiological conditions.

Proton motive force
(PMF). The potential energy stored in the plasma membrane, due to proton and voltage gradients across the membrane, that becomes liberated during proton movement through the membrane plane towards achieving electrochemical equilibrium.

Holdase
A chaperone that does not promote protein folding, but 'holds' onto substrates to prevent misfolding and/or aggregation.

By contrast, post-translationally exported proteins face a major challenge in maintaining their mature domains in an unfolded and translocation-competent state during ribosome–membrane trafficking[2,23]. Three major factors have been implicated in the maintenance of this state: signal peptides delay the folding of fast-folding mature domains, such as those of maltose-binding protein (MBP)[58]; the formation of a complex that consists of the ribosome-released mature domains with either trigger factor[24,75], SecA[14], specialized chaperones (for example, SecB[25,76]; see below) or house-keeping chaperones[51], may stabilize the unfolded state; and protein folding might be prevented in the reducing environment of the cytoplasm, as some structural properties of the mature domains are only acquired in the oxidative environment of the periplasm (for example, disulfides)[23].

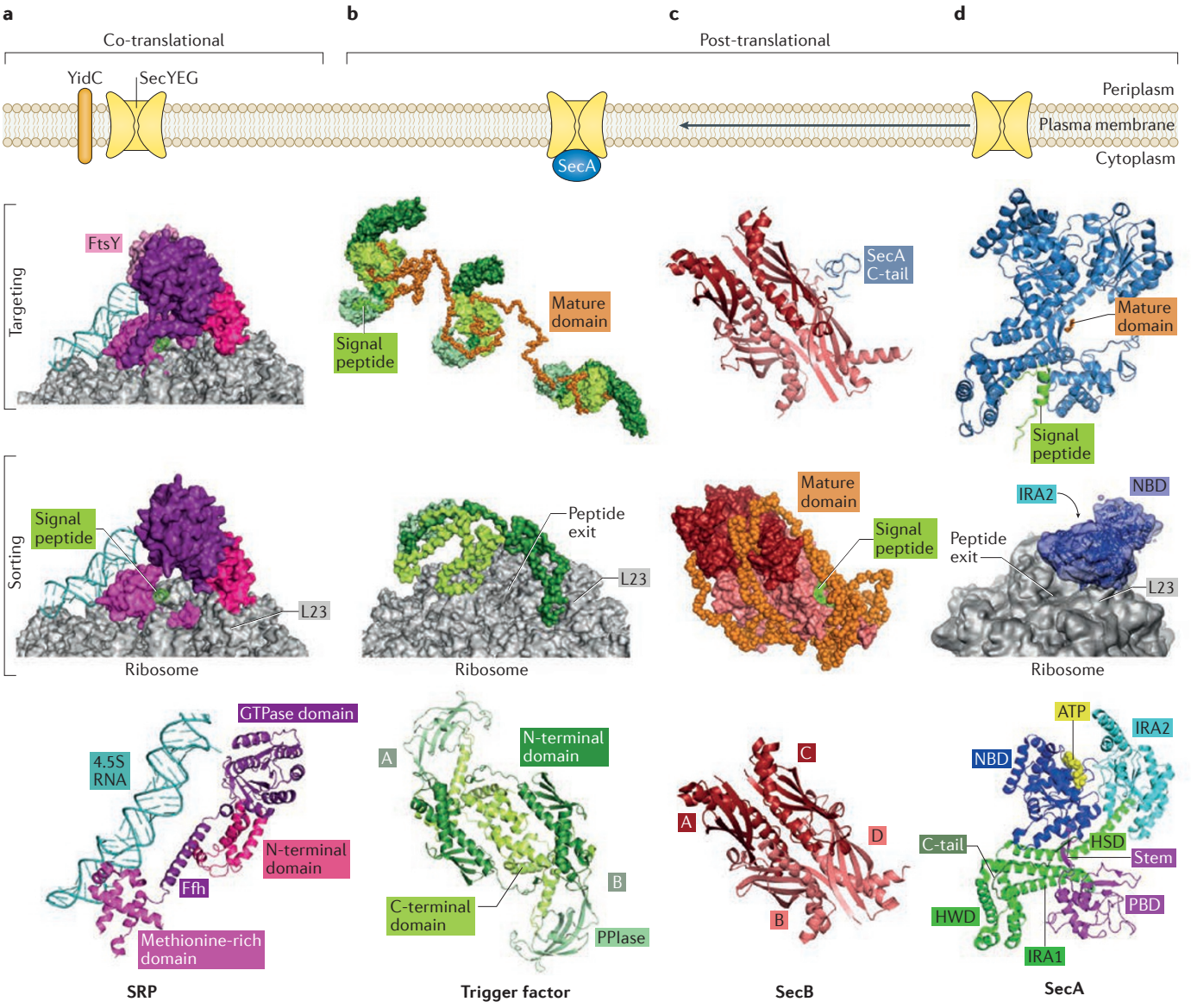
Some unfolded mature domains can be sorted and targeted *in vivo* or *in vitro* in the absence of signal peptides[23,77] or chaperones[23], and they were shown to bind to SecA with high affinity (Supplementary information S1 (table)) at sites that differ from the binding sites of signal peptides[23]. Therefore, unfolded mature domains may contain currently unknown signals that may confer selectivity in chaperone recruitment[25] and SecA-mediated targeting[23].

Signal recognition particle. Bacterial SRP consists of Ffh (an SRP54 homologue) and a hairpin-structured 4.5S RNA[26] (FIG. 2a, bottom). Ffh contains a helical N-terminal domain, a GTPase domain and a methionine-rich domain, and can functionally replace its eukaryotic homologue[78] but lacks the ability to arrest translation. The N-terminal and GTPase domains of Ffh dock onto the L23 and L29 proteins of empty, or translating, ribosomes, whereas the methionine-rich domain inserts into the exit tunnel[26] for signal peptide recognition[27,79] (FIG. 2a, middle). This high-affinity complex (Supplementary information S1 (table)) is kinetically stabilized only by emerging substrates that have appropriately hydrophobic signal peptides or TMHs[29,49,62], and it enables the sub-stoichiometric SRP to rapidly scan several ribosomal exit tunnels. Substrate specificity is further regulated through the recruitment of FtsY. FtsY also contains N-terminal and GTPase domains, and FtsY in *E. coli* additionally has an acidic N-terminal extension. In the presence of TMHs or signal peptides that have a preference for SRP, stable RNC–SRP–FtsY intermediates are formed that are targeted to the plasma membrane[29,31,49] (FIG. 2a, top). Ffh and FtsY interact through their N-terminal and GTPase domains, and this association is mediated by the 4.5S RNA and regulated by GTP hydrolysis[26,28]. Weak complexes that comprise SRP, FtsY and exported nascent chains that have 'incompatible' signal peptides for co-translational export (that is, signal peptides with low hydrophobicity or with high hydrophobicity but extended N-terminal regions), or cytoplasmic nascent chains that randomly interact with SRP at early recognition steps, may dissociate in the cytoplasm[29,49] following premature GTP hydrolysis[29]. By contrast, the stable RNC–SRP–FtsY super-assembly relocates to the membrane-bound translocase. The binding of lipids by FtsY allosterically weakens the affinity of SRP for the ribosome[49,80]. SecYEG triggers GTP hydrolysis and outcompetes SRP for ribosomal binding[28,49], RNCs are unloaded into the channel and SRP is recycled to scan other ribosomes.

Trigger factor. Trigger factor is an ATP-independent chaperone (FIG. 2b, bottom) that is ubiquitously expressed in bacteria[51], but is dispensable for protein export and viability[56,57,81]. The cytoplasmic apoprotein is dimeric, but trigger factor binds to ribosomes as a monomer[50,68]. As its N-terminal domain docks onto L23, the entire body of the trigger factor protein is exposed to the exit tunnel (FIG. 2b, middle) and can engage in hydrophobic and/or polar interactions with nascent polypeptide chains[50,51,68,82]. Some of the cytoplasmic proteins that are recognized by trigger factor are then ushered to folding machines, such as the DnaK–DnaJ and GroEL–GroES complexes[51,82] (FIG. 1a, step 1). Owing to its high intracellular concentration and high ribosomal affinity (Supplementary information S1 (table)) trigger factor is probably the most prominent ribosome-bound chaperone that can outcompete the binding of dimeric SecA[15], but not SRP, to the ribosome[30]. Through 'co-habitation', SRP and trigger factor can screen nascent substrates simultaneously[30,61]. The binding of trigger factor to specific signals that are present on a nascent polypeptide chain[30], which typically comprise extended hydrophobic patches with flanking positively charged residues[83], weakens RNC–SRP interactions and RNC–SRP–FtsY membrane-targeting complexes[30,61]. Thus, 'incompatible' substrates, such as secretory pre-proteins that are targeted through the post-translational route or cytoplasmic proteins, are eliminated from the co-translational SRP pathway. Trigger factor recognizes both signal peptides[24] and mature domains, and commonly binds to nascent polypeptide chains that consist of more than 100 amino acid residues[22]. Binding occurs at several sites in trigger factor that are located on its prolyl isomerase (PPIase), N-terminal and carboxy-terminal domains[24,68] (FIG. 2b, top). Persistent binding during translational elongation[50] may load several trigger factor chaperones onto a single nascent chain[24]. Owing to dynamic, multivalent interactions with aggregation-prone regions[24,82] trigger factor may contribute to pre-protein sorting[22,84,85] and targeting by maintaining pre-proteins in soluble, non-native and translocation-competent states[24,51,75,82,85]. It remains to be determined whether, or how, trigger factor can relay nascent polypeptide chains to SecB or SecA.

SecB. SecB is a non-essential, ATP-independent holdase[52] that assembles into a dimer of dimers ($SecB_4$)[86,87] (FIG. 2c, bottom). Although limited to proteobacteria[2], *secB*-like genes[52] exist in other taxonomic groups. *E. coli* uses SecB for the export of only approximately 4% of its secretome[12], but it is a paradigm of secretion-specific chaperones.

SecB binds to completely or partially synthesized nascent pre-proteins[2] mainly through hydrophobic contacts[88,89], spanning a wide area of its surface[89,90] (FIG. 2c, middle). NMR-derived solution structures of SecB in complex with two pre-proteins revealed that $SecB_4$



slightly re-arranges its structure to extend its hydrophobic surfaces and accommodate longer pre-proteins[89]. Binding is of high affinity (Supplementary information S1 (table)), but broad specificity[90,91], that may include signal peptides[89,92], and may overlap with that of trigger factor[51]. SecB binds to pre-proteins that lack tertiary structure[25,53,76,89], but that may have secondary structure[25,53], and delays or prevents their compaction or aggregation. The prevention or delay of folding by SecB is probably determined by the rates of association of a pre-protein with SecB and of intrinsic folding of the pre-protein[76,89], and the stability of the SecB–substrate complex[88,89].

SecB relays pre-proteins to cytoplasmic or SecYEG-bound SecA (FIG. 1c) through high-affinity interactions between SecA and SecB[13,93] (Supplementary information S1 (table)) that are enhanced by pre-proteins[13]. $SecA_2$–$SecB_4$ complexes share several contact sites[94,95]: a flat SecB β-sheet and its C terminus bind to the zinc-containing C-terminal tail (FIG. 2c, top) and N terminus of SecA, respectively. This asymmetric interaction may facilitate pre-protein release onto SecA[94]. Crosslinking[96] and electron microscopy[97] methods have led to the suggestion of possible SecA–SecB orientations; however, high-resolution structures are still lacking.

SecA. The translocase motor SecA is essential and conserved in bacteria. Its nucleotide-binding domain (NBD; also known as NBD1) and the intra-molecular regulator of ATPase 2 (IRA2; also known as NBD2) sandwich a single nucleotide and form a superfamily 2 helicase motor[98] (FIG. 2d, bottom). The pre-protein-binding domain (PBD), which is rooted in the NBD through a stem, contributes to pre-protein recognition. The C-terminal domain, which is fused to IRA2, docks SecA onto SecY[37,42,99,100]. C-terminal domain regions include the helical scaffold domain (HSD), which physically interconnects all SecA domains, the intra-molecular switch IRA1 (REF. 101) (or 'two-helix finger'), the helical wing domain (HWD) and the C-terminal tail that folds onto the stem.

Figure 2 | **Structures of the sorting and targeting factors.** The signal recognition particle (SRP), the trigger factor dimer (TF_2), tetrameric $SecB_4$ chaperones and a protomer of dimeric $SecA_2$ are depicted in the free (bottom), sorting (middle) and targeting (top) states. **a** | Bacterial SRP consists of Ffh (which comprises a helical amino-terminal domain, a GTPase domain and a methionine-rich domain) and a hairpin-structured 4.5S RNA (bottom; RCSB Protein Data Bank (PDB) entry 2XXA). Bacterial SRP anchors onto the L23 and L29 ribosomal proteins through its N-terminal domain and GTPase domain, and inserts its methionine-rich domain into the ribosomal exit tunnel[26] for the recognition of signal peptides or transmembrane helices (TMHs) in emerging nascent chains[27,79] (middle; PDB entry 5GAF). SRP binds to its receptor FtsY and the formed stable ribosome–nascent chain complex (RNC)–SRP–FtsY intermediates are targeted to the membrane-bound translocase[29,49] (top; PDB entry 5GAD). Ffh and FtsY interact through their N-terminal and GTPase domains, and this association is mediated by the 4.5S RNA and regulated by GTP hydrolysis[26,28]. RNCs are transported to the SecYEG channel or to the membrane protein insertase YidC[11] (light orange). **b** | Trigger factor is an ATP-independent chaperone (bottom; letters represent trigger factor monomers; PDB entry 1T11). Although the cytoplasmic apoprotein is dimeric, trigger factor binds to ribosomes as a monomer through its N-terminal domain[68] (middle; PDB entries 2VRH and 1W2B; Supplementary information S3 (table)), which docks onto L23 near the ribosomal exit tunnel. Emerging signal peptides and mature domains can interact with the entire body of trigger factor through hydrophobic and/or polar interactions[50,68,82]. Binding occurs at several sites on trigger factor that are located on its prolyl isomerase (PPIase), N-terminal and carboxy-terminal domains[24,68] (top; PDB entries 2MLX, 2MLY and 2MLZ). Persistent binding during translational elongation[50] may load several trigger factor proteins onto on a single nascent chain[24]. Trigger factor detaches from the ribosome and may relay pre-proteins to SecB or SecA. **c** | SecB is a non-essential, ATP-independent holdase[52] that assembles into high affinity tetramers ($SecB_4$; bottom, letters represent individual SecB monomers; PDB entry 1QYN)[86,87]. $SecB_4$ uses a large area of its surface[89,90] to bind to completely or partially synthesized nascent pre-proteins (middle; PDB entry 5JTL) mainly through hydrophobic interactions[88,89]. SecB delivers pre-proteins to cytoplasmic, or SecYEG-bound, SecA[13,93]. Among several $SecA_2$–$SecB_4$ contacts[94,95], a flat SecB β-sheet binds to the zinc-containing C-terminal tail (C-tail) of SecA (top; PDB entry 1OZB) and the C terminus of SecB contacts the N terminus of SecA (not shown). **d** | SecA acts as a pre-protein-targeting factor and receptor, and a translocase motor. As a motor, it belongs to the superfamily 2 helicase, owing to the nucleotide-binding domain (NBD) and the intra-molecular regulator of ATPase 2 (IRA2; bottom; PDB entries 1M6N and 2VDA; Supplementary information S3 (table)) that sandwich a single nucleotide[98] (yellow spheres represent ATP). The pre-protein-binding domain (PBD), rooted in the NBD through a stem, and the C-terminal domain, fused to IRA2, facilitate the recognition of the polypeptide and binding to SecY[37,42,99,100]. C-terminal domain regions include the helical scaffold domain (HSD), which physically interconnects all SecA domains, the intra-molecular switch IRA1 (REF. 101) (or 'two-helix finger'), the helical wing domain (HWD) and the C-tail that folds onto the stem. SecA domains are indicated on one $SecA_2$ protomer. The dimeric cytoplasmic SecA may dissociate into monomers following binding to ribosomes for pre-protein recognition, with a single SecA monomer docking onto L23 (REFS 14,15) (middle; EBI Electron Microscopy Data Bank entry 2565 and PDB entry 1M74), or may adopt a ribosome-bound elongated dimeric state[15] (not shown), with the second $SecA_2$ protomer contacting L22 and L24 (not shown). SecA promiscuously recognizes both signal peptides and mature domains (top; PDB entries 3JV2 and 2VDA) through two distinct sites[23]. A tripeptide (orange) indicates a potential mature domain-binding region on SecA[99].

SecA exists in cytoplasmic (free or ribosome-bound[14,15]) and SecYEG-bound states[41], all of which are capable of binding to pre-proteins[14,23,42,102]. Cytoplasmic SecA is dimeric (Supplementary information S1 (table)) and binds to the ribosomal L23 protein as a monomer[14,15] (FIG. 2d, middle) or as an elongated dimer[15], with the second protomer interfacing with L22 and L24. Cryo-electron microscopy (cryo-EM) superpositions suggest that monomeric SecA might scan nascent chains simultaneously with trigger factor, but not with SRP[15].

SecA promiscuously recognizes signal peptides and mature domains with high affinity through two distinct sites[23,42]. Its elongated, hydrophobic signal peptide-binding groove accommodates H regions of signal peptides of different lengths and electrostatically traps their positively charged N-terminal regions through acidic residues[42] (FIG. 1d, right; red). The C-terminal tail of SecA partly caps the groove[42]. How SecA recognizes more than 500 unfolded mature domains that have dissimilar sequence, size, charge, structure or hydrophobicity, and where, remains elusive. Suggested binding sites for mature domains include the PBD[100] and a PBD–IRA2 clamp[99,102] (FIG. 2d, top).

A mechanism for the promiscuity and selectivity of SecA–pre-protein recognition emerges: the conserved biophysical properties of signal peptides ensure their docking onto the same groove of SecA[42]. Acidic residues at the entrance of this groove in SecA[42] might prevent unspecific hydrophobic interactions with non-Sec-dependent exported or cytoplasmic proteins. Mature domains make contact with SecA[23], possibly in a less specific manner, to stabilize signal-peptide-mature domain–SecA complexes. The C-terminal tail partially occludes and auto-inhibits SecA from engaging unspecific substrates in its signal-peptide-binding groove[42], as indicated by the finding that the binding affinity of signal peptides is enhanced in the absence of the SecA C-terminal tail[42]. Some signal peptides may displace the C-terminal tail, whereas others may require SecB for higher-affinity binding to SecA[42]. SecB binds to the C-terminal tail[93,95] and may release it from the signal peptide-binding groove of SecA[42]. Therefore, the SecA C-terminal tail provides a substrate 'proof-reading' mechanism.

Architecture and dynamics of SecYEG

Both co-translational and post-translational targeting pathways converge at SecYEG. SecYEG is a unique channel: it combines both a vectorial and a lateral opening. In addition, its conformation is regulated by ribosomes, SecA and pre-proteins, and it maintains the membrane permeability barrier while markedly dilating to translocate elongated polymers[103].

Architecture of the SecYEG channel. SecY comprises 10 TMHs that transverse the plasma membrane and form the channel pore[4] (FIG. 3a). TMHs 1–5 juxtapose TMHs 6–10 around a hydrophilic pre-protein passage and they are linked by a periplasmic hinge. Six bulky hydrophobic residues form a passage-constricting pore ring in the centre of the channel (FIG. 3a, left; cyan spheres). In its characteristic hourglass-shaped resting state (FIG. 3a, left), the ring restricts channel opening to 5 Å and its periplasmic funnel is sealed by a helical plug. The ring and plug may act as channel gates[103,104]. Parallel to the cytoplasmic funnel, the loose junction of TMH 2 and TMH 3 with TMH 7 and TMH 8 creates a lipid-facing lateral gate (FIG. 3a, bottom). SecE engages SecY externally, diametrically opposite to the lateral gate, and stabilizes the conformation of SecY. The non-essential, poorly conserved SecG makes tight hydrophobic contacts with TMH 3 and TMH 4 of SecY (FIG. 3a), and its cytoplasmic loop seals the *cis* side of the SecY cytoplasmic funnel[105].

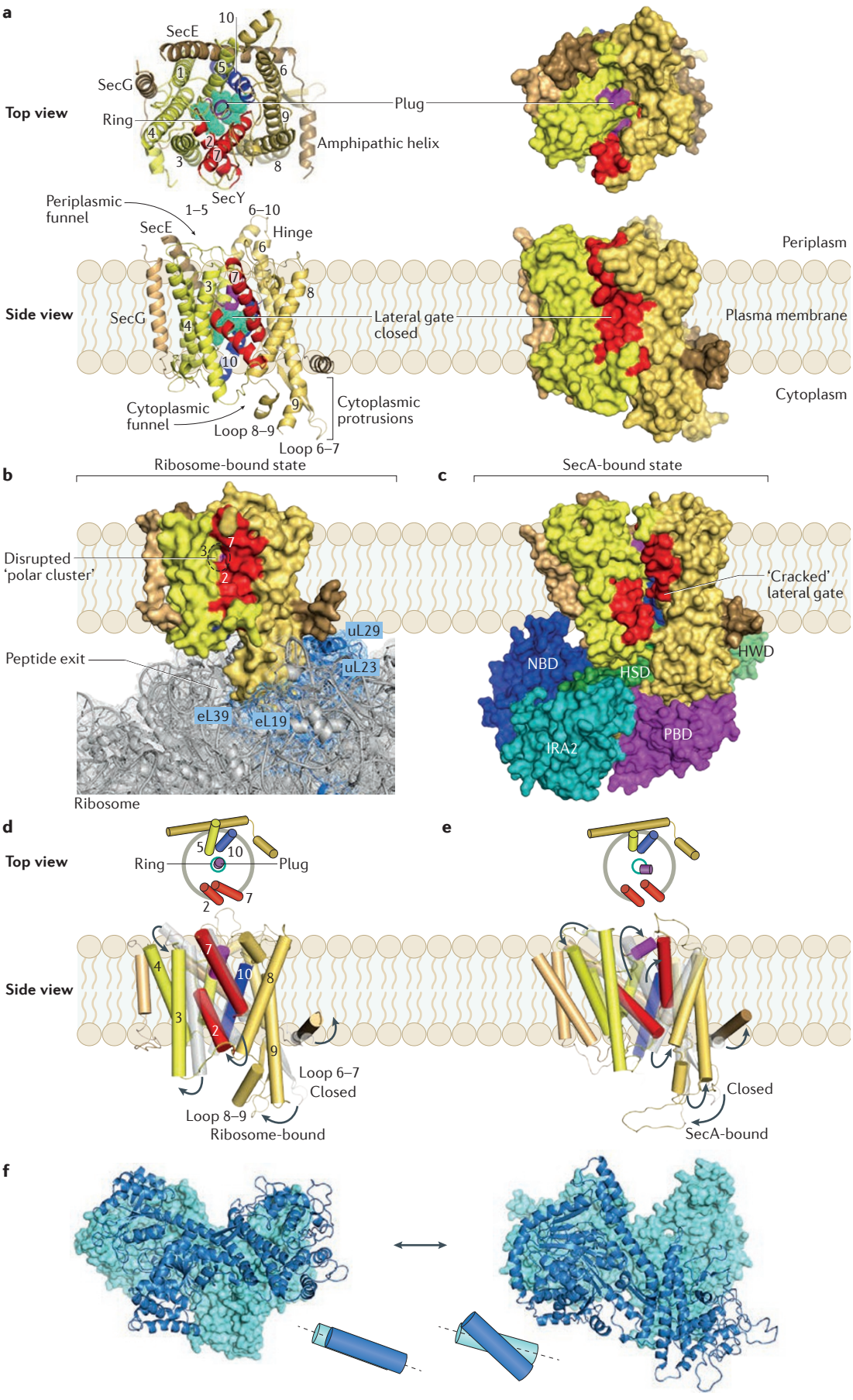


Figure 3 | **Architecture and dynamics of the Sec translocase components.** **a** | Top and side views of a resting SecYEG homologue (SecYEβ from *Methanococcus jannaschii*; RCSB Protein Data Bank (PDB) entry 1RH5)[4]. In the ribbon representation (left panel) the two SecY halves (transmembrane helices (TMHs) 1–5 are shown in yellow and TMHs 6–10 are shown in light yellow), which are connected by a periplasmic hinge, are surrounded by SecE (brown; its amphipathic helix is indicated) and SecG (light brown; *Mj*Secβ). The periplasmic and cytoplasmic SecY funnels are indicated; the closed lateral gate (red), the plug (magenta) and pore ring (cyan spheres; residues from TMH 2, TMH 5, TMH 7 and TMH 10), restrict channel permeability. In the surface representation (right) the laterally (side view) and vectorially (top view) closed channel are shown. **b** | Ribosomes bind to SecYEG near SecY loop 6–7 and loop 8–9, mainly through the universally conserved ribosomal L23 and L29 proteins (uL23 and uL29; the appended 'u' indicates that these proteins are universal) that line the ribosomal peptide exit, creating a continuous conduit with the SecY pore[117]. This interaction slightly alters the conformation of SecYEG, as observed in SEC61, the SecYEG eukaryotic homologue (colours match those used in part **a**; PDB entry 3J7Q), that is 'primed' by a non-translating ribosome[117] (grey; with the exception of SEC61-binding proteins, which are in light blue. Ribosomal proteins that are present only in eukaryotes (eL19 and eL39; 'e' indicates eukaryotic) and also interact with SEC61 are indicated as well). In this state, the network of hydrogen bonds between TMH 2, TMH 3 and TMH 7 of SecY, termed the 'polar cluster', that stabilizes the closed lateral gate is disrupted. **c** | SecA associates with SecYEG to form the translocase holoenzyme. The lateral gate of SecYEG (PDB entry 3DIN) complexed to one SecA–ATP analogue is slightly open (approximately 5 Å)[37]. **d** | A diagrammatic model of ribosome-bound SEC61 (ribosome not shown). Only the important channel elements are indicated (top view); the passage-constricting pore ring (residues from TMH 2, TMH 5, TMH 7 and TMH 10), the periplasmic seal termed the 'plug', and the lateral gate that is proposed as an egress towards the plasma membrane, control channel opening. In the side view (ribbon representation) arrows indicate important motions from the closed (grey; PDB entry 1RH5) to the ribosome-bound (superimposed colours; PDB entry 3J7Q) state: the interaction between the ribosome and loops 6–7 and 8–9 of SecY, shifts TMH 2 and TMH 3 of SecY outwards, which disrupts the 'polar cluster' between TMH 2, TMH 3 and TMH 7 of the lateral gate. **e** | The SecA–ATP-bound SecYEG state[37] (PDB entry 3DIN; SecA is not indicated). The C-terminal half of SecY rotates outwards, which destabilizes the pore ring. The lateral gate slightly opens by outward displacement of TMH 7 of SecY. TMH 9 is also substantially displaced and forces the SecE amphipathic helix outwards to accommodate the more relaxed SecY conformation. The plug moves from the pore ring towards TMH 7 of SecY, but it still provides pore sealing. **f** | Cytoplasmic SecA forms high-affinity interconverting homodimers (PDB entries 1M6N (left) and 1NL3 (right)). The two $SecA_2$ quaternary structures[41] (ribbon structure indicates the front; surface structure indicates the back protomers) share the same dimerization elements, but different inter-protomer orientations[41] (see diagrammatic representation). Numbers that are superimposed on, or next to, helices indicate the respective TMH. HSD, helical scaffold domain; HWD, helical wing domain; IRA2, intra-molecular regulator of ATPase 2; NBD, nucleotide-binding domain; PBD, pre-protein-binding domain.

The functional oligomeric state of SecYEG remains elusive. Detergent-solubilized complexes range from monomers[4,37] to higher oligomers[106–108], which include back-to-back[106,107] and front-to-front[108] SecYEG dimers. Both arrangements were also captured by *in vivo* crosslinking[109]. The back-to-back model provides facile lateral escape of membrane proteins through either one of its lipid-facing lateral gates (see below).

A single SecYEG complex is sufficient for SecA binding, pre-protein engagement[110,111] and translocation[39,112,113]. Moreover, an inserted signal peptide localizes to only one of the two lateral gates of a SecYEG dimer[106] and the structure of SecYEG with a single trapped pre-protein has been reported[36,46]. Dimers may enhance translocation efficiency[110]. The association of SecYEG complexes may indirectly stabilize the translocase, possibly through the binding of SecA, or facilitate its activation[110,111,113].

Regulation of SecYEG conformation by ribosomes and SecA. Translocation of a plasma membrane protein or a secretory pre-protein requires the SecYEG pore to switch from its closed, energetically favoured ground state[114] to an open state. Molecular dynamics simulations have estimated a free-energy penalty of 20 kcal mol^{-1} for translocation or membrane integration through SecYEG without external destabilizing interactions[115]. Both ribosomes and SecA interact with the highly dynamic[116], cytoplasmic protrusions of SecY[37,117]; that is, elongated helices 6, 7, 8 and 9, that extend into the membrane plane (FIG. 3b,c) and facilitate pore opening[34,114,118]. Through these allosteric 'handles' (REFS 33,118), ribosomes or SecA induce long-range conformational changes in the plug, ring and lateral gate domains of SecYEG[34,37,117] (FIG. 3b,c) and stabilize the high energy open state (FIG. 3d,e). The effects can be mimicked by protein localization (*prl*) conformational mutations (for example, *prlA* for SecY, *prlG* for SecE and *prlD* for SecA[119]) that enable signal peptide-independent translocation, possibly by destabilizing the closed state[120,121] or by strategically placed crosslinks[33] (for example, the *E. coli* SecY-I284C-T404C double mutant on a cysteine-free template; the introduced cysteinyl residues crosslink TMHs 7–10 of SecY[33]).

For a compendium of channel dynamics, we integrated conformational snapshots of archaeal, eukaryotic and bacterial SecYEG homologues, based on the high structural and functional evolutionary conservation of the channel.

Ribosomes bind to SecYEG with high affinity (Supplementary information S1 (table)) but exert limited influence on channel conformation. Ribosomes anchor near SecY loop 6–7 and loop 8–9, mainly through the universally conserved ribosomal L23 and L29 proteins[122] that line the ribosomal peptide exit, creating a continuous conduit with the pore[117] (FIG. 3b). Loop 6–7 and loop 8–9 shift inwards towards the channel (FIG. 3d; arrows indicate motions from the closed (grey) to the ribosome-bound (coloured) state). Subsequently, the lateral gate slightly opens through the outward displacement of TMH 2 and TMH 3 (REF. 117) (FIG. 3d). This disrupts a conserved hydrogen bond network (termed the 'polar cluster', which is located between TMH 2, TMH 3 and TMH 7) that stabilizes the lateral gate closed state[36,38] (FIG. 3b). The slightly opened lateral gate enables further opening for the intercalation of the hydrophobic nascent chain, while weakening the interactions between the non-polar patch of the plug and the polar cluster of the lateral gate[38]. Thus, the seemingly unaltered plug[117] (compare FIG. 3d to FIG. 3a, left) might become subtly destabilized, but not fully detached, before plasma membrane proteins or secretory pre-proteins bind to SecYEG[38,40]. Premature plug displacement may alter the electrostatic field inside the pore and affect the channel-engaged plasma membrane protein or secretory pre-protein orientation[123].

In contrast to the ribosome that recognizes SecYEG in transient interactions, SecA is a dedicated, specialized subunit of the translocase holoenzyme, and, together, they form an allosteric ensemble[13,19,41]. SecA exhibits remarkable dynamics and flexibility[12]. Its domains show conformational plasticity and/or move relative to one another[43,44,98,124–127], and these intra-protomeric interactions

are further influenced by its quaternary state. Cytoplasmic SecA forms high-affinity interconverting homodimers[41,128] (for example, compare FIG. 3f, left[98] to right[129]). The dynamics of SecA are regulated by pre-proteins, nucleotides[43,44,98,124–127], SecYEG, lipids and chaperones[12] to exert its function as a substrate receptor, channel modulator, loading pump and processive ATPase motor[23,41]. Nucleotide cycling[39,43,98,130,131] in the helicase motor[44] alters its conformational and quaternary[41] transitions, and these, in turn, affect the conformations of the SecYEG channel and the bound pre-proteins.

The functional quaternary states of SecA when bound to SecYEG are a matter of debate; monomeric[132] or dimeric[32] states and equilibria are shifted either way by ligand-binding[12] or buffer conditions (for a detailed discussion see REF. 133). A recent study bridged the apparent controversies, revealing both dimers and monomers as true and essential intermediates of translocation-engaged SecA[41]. SecA binds to SecYEG as an asymmetric dimer through one protomer and acquires at least three distinct subsequent quaternary conformers, each of which has a unique catalytic role prior to monomerization[41]. The structure of $Sec(YEG)_2$–$SecA_2$ is elusive. The only available high-resolution insight comes from detergent-solubilized, monomeric SecYEG–SecA–ADP-BeFx (BeFx: beryllium fluoride ion; FIG. 3c,e) and is suggestive of the conformational influence of the SecA monomer on the channel in an ATP-pre-hydrolysis state[37]. In this state, TMH 7 of SecY is displaced (FIG. 3e) and the lateral gate stays slightly open (~5 Å) (FIG. 3c). In parallel, the C-terminal SecY TMHs rotate outwards (FIG. 3e), which causes slight destabilization of the pore ring. The substantial shift of TMH 9 (FIG. 3e) is relayed and outwardly displaces the SecE amphipathic helix (FIG. 3e, right arrow). The plug moves from the ring to TMH 7 (FIG. 3e), but still seals the pore (FIG. 3e). Fluorescence and single-molecule fluorescence resonance energy transfer (FRET) analysis showed nucleotide-dependent plug relocation and lateral gate opening of SecA-bound SecYEG in the absence of pre-protein[39,134,135].

Translocation and release

Co-translational membrane integration. Co-translational integration of plasma membrane proteins is mediated by hydrophobic, helical, ~20-residue-long TMHs that are proposed to move through the lateral gate[4,40].

During the early stages of nascent chain engagement, exported TMHs potentially bind to a hydrophobic opening at the cytoplasmic side of the lateral gate[105] (FIG. 4a). Then, according to the 'lateral gate egress' model, TMHs of the nascent chain intercalate into the lateral gate by replacing interactions between TMH 2 and TMH 7 of SecY[40] (FIG. 4b) and thermodynamically partition between lipids and the aqueous pore by sliding sideways through the open lateral gate[136,137] (FIG. 4e). A wide-open lateral gate is stabilized only by peptides that are strongly hydrophobic[34,115]. The helicity of the TMHs from the nascent chain decreases the energy cost of partitioning[138]. Subsequent hydrophilic segments are occluded from lipids and remain inside the hydrophilic pore, inducing minor conformational changes to the lateral gate[40,115] (FIG. 4c).

Structural and bioinformatics data suggest a model for SecYEG conformational changes during the insertion of TMHs. The pore ring may dilate in response to the incoming polypeptide, which leads to plug destabilization[139] (FIG. 4c), or in response to lateral gate opening[106,139] that is induced by the intercalation of the exported TMH (FIG. 4b). The latter notion is supported by studies that show the co-translational engagement of a periplasmic pre-protein[36] (FIG. 4d), the signal peptide of which also intercalates in the lateral gate, which induces pore ring dilation (compare the pore ring in FIG. 4d with FIG. 4b,c). During the insertion of TMHs, pore dilation could be compensated by tighter interactions between the plug and residues from TMH 10 of SecY, close to the pore ring (compare the positions of the plug and TMH 10 in FIG. 4b and FIG. 3a, left), to maintain the membrane permeability barrier[40]. The seemingly distinct responses of the channel to transmembrane nascent chains, hydrophilic nascent chains and secretory pre-proteins (FIG. 4b–d; Supplementary information S3 (table)) imply that engaged chains rearrange the three conformational regulators of the channel (that is, the plug, the ring and the lateral gate) on demand[40]. Thus, translocation swiftly switches between vectorial and lateral.

SecY exerts biphasic pulling forces on exported TMHs during channel insertion and membrane integration[140]. Single-spanning plasma membrane proteins that have strong helical propensity and hydrophobicity slide outwards rapidly[137,140]. The insertion of polytopic plasma membrane proteins is more complicated. Several TMHs are retained temporarily in the dilated pore[35]. Neighbouring TMHs assist in the egress of moderately hydrophobic preceding TMHs[141]. Insertion into lipids and sliding into the bilayer can be sequential or en bloc[35].

When complexed with SecYEG, YidC (BOX 1) is typically located adjacent to the lateral gate and has a role in cooperative membrane integration[8]. SecA is conditionally recruited co-translationally, whenever extended hydrophilic loops of plasma membrane proteins need to be translocated[142]. Cytoplasmic chaperones have also been hypothesized to stochastically 'push' nascent chains towards the channel through SecYEG–ribosome interface gaps[122].

Phospholipid molecules that surround the translocase can influence membrane protein insertion and folding. They function as chaperones for the folding of membrane protein and non-membrane protein domains and are important determinants of the topology of membrane proteins[143].

Post-translational translocation. SecA is a central player in this mode of export. The translocation process, although not yet fully resolved, can be summarized in a working model of sequential steps (FIG. 5a, steps 1–8).

In the first step, $SecA_2$, which is assembled mainly through electrostatic inter-protomer contacts, docks stochastically onto SecYEG or $(SecYEG)_2$ (REF. 41) by only one of its protomers and becomes asymmetric[23]. The SecYEG–SecA holoenzyme is assembled[13,19] and ATP-turnover by SecA is slightly stimulated[17,19].

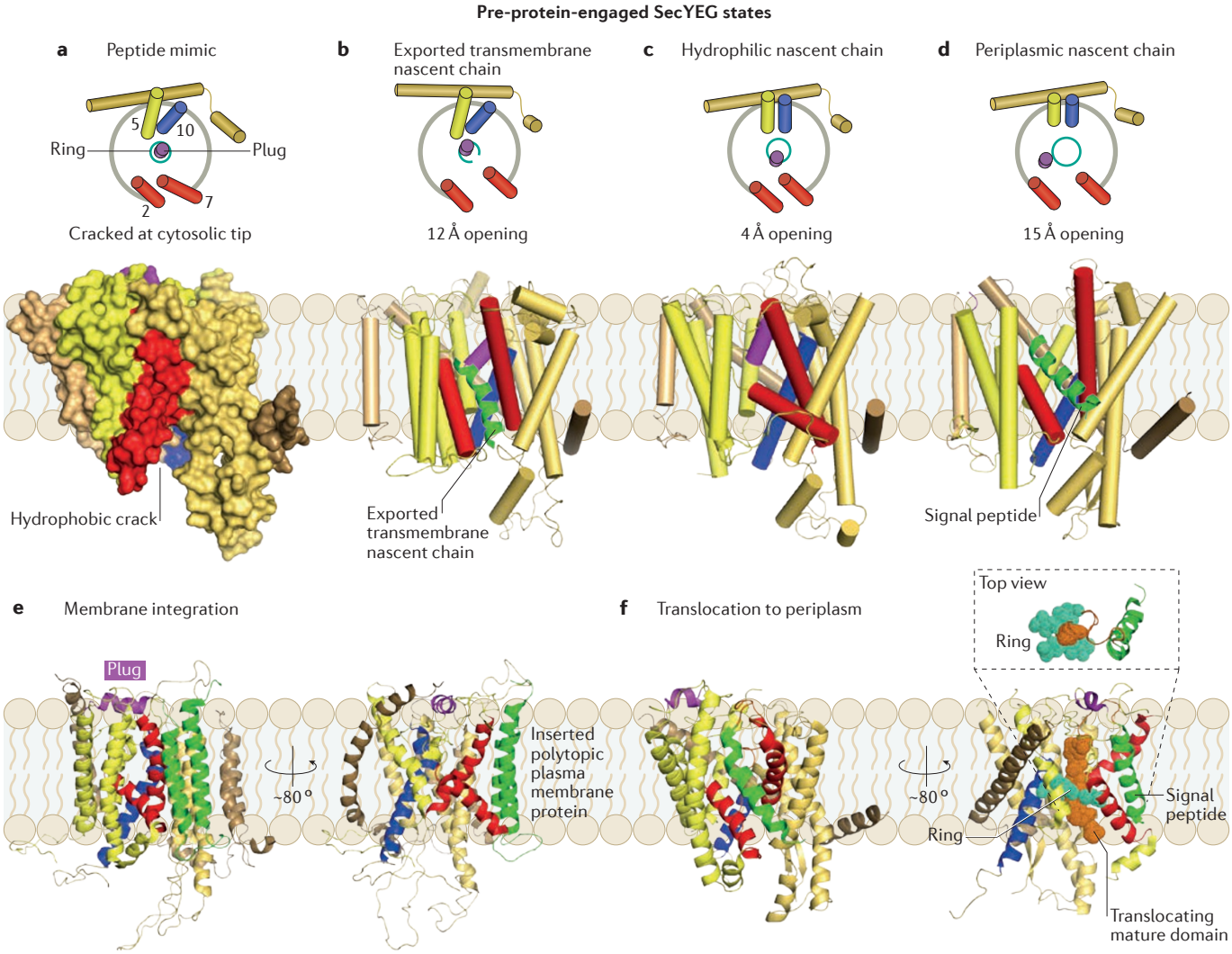


Figure 4 | **Conformational states of the SecYEG channel. a–d** | The activation of SecYEG (Sec61 in eukaryotes; the two halves of the SecY homologue (transmembrane helices (TMHs) 1–5 are shown in yellow and TMHs 6–10 are shown in light yellow) are surrounded by the SecE (brown) and SecG (light brown) homologues) by secretory pre-protein or plasma membrane protein factors. The effects on channel conformation by a peptide mimic[105] (part **a**; RCSB Protein Data Bank (PDB) entry 5CH4), an exported transmembrane nascent chain[40] (part **b**; PDB entry 4CG6), a hydrophilic nascent chain[40] (part **c**; PDB entry 4CG5) and a periplasmic nascent chain[36] (part **d**; PDB entry 3JC2) are shown from a side view (bottom; ribbon structures) or top view (top; diagrammatic representation). A peptide mimic (not indicated) binds to the cytosolic tip of the lateral gate, inducing a 'crack' on this side (part **a,** numbers that are superimposed on, or next to, helices indicate the respective TMH). This could comprise the first interaction site of signal peptides and exported TMHs with the lateral gate[105]. Then, TMHs[40] and signal peptides[36] (parts **b**,**d**; indicated in green) open and intercalate the lateral gate. Hydrophilic peptides are thermodynamically incapable of substantially opening the lateral gate[40,115] (part **c**). The lateral gate opens the channel through rigid body movements. This may be compensated by tighter contacts between TMH 10 of SecY and the plug for lateral insertion of transmembrane proteins[40] (part **b**). Slight pore opening and plug displacement by hydrophilic chains could be adequate for vectorial exit[40] (part **c**). The pre-protein induces maximal channel opening[36] (part **d**). **e** | The conformational state of SecYE during membrane integration of a polytopic plasma membrane protein[162] (green; PDB entry 5ABB). In the rotated view (right structure) TMH 3 and TMH 4 of SecY were removed for visualization. The polytopic plasma membrane protein was captured outside of the SecY lateral gate (red). **f** | SecYE (as in part **e**) is shown engaged with a periplasmic secretory protein for post-translational translocation[46] (PDB entry 5EUL). The signal peptide is depicted in green (ribbon representation) and the mature domain in orange (ribbon and spheres). The zoomed in top view (top inset of right structure) shows the pore ring surrounding the translocating pre-protein mature domain[46].

Next, a single pre-protein[23] binds to the SecYEG-bound protomer of the asymmetric $SecA_2$ (REF. 41) with high affinity; signal peptide and mature domain dock onto distinct sites of SecA[23]. Even in $(SecYEG)_2$ only one pore is active[106,113].

In the third step, signal peptide binding alters the conformation of, elongates and loosens $SecA_2$ (REF. 41). These changes are transmitted to the associated SecYEG complex[39]. The activation energy of the holoenzyme is decreased[17,23,43]. Structure-loosening *prl* mutations in

either of the SecA–SecYEG components can mimic this signal peptide-mediated 'triggering' effect[41,77,119,120]. For all of the downstream steps in the native Sec system, the physical presence of signal peptides is essential[23].

ATP binding[131] leads to a tight association between substantial parts of the $SecA_2$ C-terminal domain and the channel, which was experimentally identified in protease-resistance studies[144]. Concomitantly, short pre-protein segments of 20–30 residues co-insert[16].

The pre-protein stimulates ATP hydrolysis through the triggered SecA protein[17,23,43] in a manner that is dependent on the second 'regulator' SecA protomer[41]. Poorly resolved but crucial steps ensue: the mature domain becomes 'trapped' in the SecA–SecYEG holoenzyme, although it is not deeply inserted into the channel[16], and SecA monomerizes[41,132]. The SecA IRA1 switch, which was previously buried in the dimer interface (FIG. 3f), becomes exposed to, and associates with, the cytoplasmic funnel of SecY and potentially controls chain motions[37,45,145]. Increased IRA1 mobility may not be necessary for pre-protein ushering into the channel, as IRA1 immobilization at the edge of, and inside, the channel does not impede translocation[146].

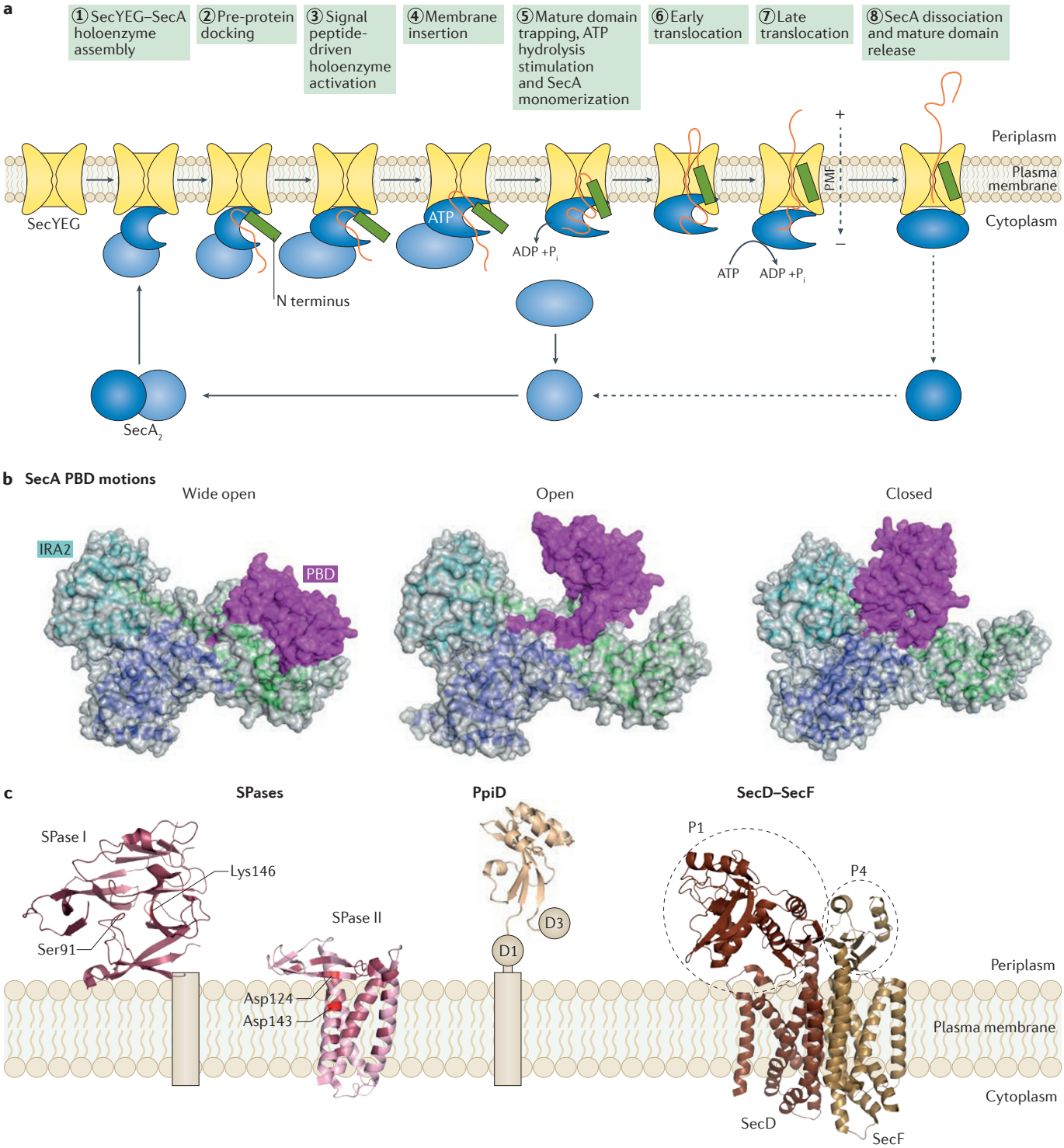
Next, signal peptides relocate to the lateral gate with their N terminus oriented towards the cytoplasm and their C terminus oriented towards the periplasm[46,106] by an unknown mechanism; mature domains become fully threaded along the pore and the plug is shifted outwards[46] (FIG. 4f). The dilated pore ring forms a 'gasket' around the partly extended translocating chain (FIG. 4f, top view); this may be the only tight contact between the mature domain and the channel[46]. An evident lateral gap of the pore ring (FIG. 4f, top view), due to the opened lateral gate, (FIG. 4f) may allow pre-protein exposure to lipids during translocation, which enables the sliding of the hydrophobic signal peptide outside of the lateral gate[46]. The translocating polypeptide loop fills the SecY periplasmic funnel (FIG. 4f), which was previously sealed by the plug in the idle channel state, and the C-terminal signal peptide segment laterally seals the channel from surrounding lipids[46]. Thus, the configuration of the translocating polypeptide as a loop inside of the channel and the hourglass shape of SecYEG may facilitate unimpeded passage through the channel while maintaining the membrane permeability barrier[46].

Cycles of ATP binding and hydrolysis are essential to promote forward segmental translocation of the mature domain[16,144]. PMF promotes the translocation of mature domain segments that have been released from SecA following hydrolysis[16]. SecDF alone (FIG. 5c, right) or SecDF–YajC bound to SecYEG (FIG. 1c), may assist export in a PMF-dependent manner[6,147].

Distinct models and/or their combination could explain the different roles of SecA and how it sustains chain movement and translocation. In a 'piston' model, SecA acts as a monomeric processive motor[37,41]. Extended mature domains slide along a clamp that is formed by the PBD and IRA2 (REFS 102,125,126) (FIG. 5b; see also FIG. 2d, top). The tip of IRA1 (REF. 45) temporarily latches onto the chain and pushes it towards the pore[145].

Figure 5 | **Model of Sec-dependent post-translational translocation and pre-protein maturation and release factors. a** | Overview of post-translational translocation. One $SecA_2$ protomer (dark blue) binds to the membrane-embedded SecYEG channel (one SecYEG is shown), and $SecA_2$ becomes asymmetric (step 1)[41]. The signal peptide (green) and mature domain (orange) of a single pre-protein[23] bind to the SecYEG-bound SecA protomer[41] at distinct sites (step 2)[23]. Binding of the signal peptide conformationally alters SecA[41], induces SecY lateral gate opening (not shown) and stabilizes a more open or loose SecYEG state[39] (not shown), and triggers the ATPase activity of SecA (step 3)[23]. ATP–SecA[144] and the pre-protein partially insert into the SecYEG channel (step 4)[16]. Pre-protein-stimulated ATP hydrolysis[17,43], which is regulated by the second $SecA_2$ protomer[41] (light blue), traps the mature domain in the holoenzyme[16] and SecA monomerizes (step 5)[41,132]. The pre-protein translocates, the process of which is powered by repeated cycles of ATP binding and hydrolysis[16,144] and the proton motive force (PMF; step 6)[16]. SecA–ADP weakly binds to SecYEG[45] (step 7), before it dissociates[144] (step 8), re-dimerizes in the cytoplasm and repeats the translocation cycle. Changes in the shape of SecA indicate conformational changes. **b** | The pre-protein-binding domain (PBD; magenta) of SecA (coloured ribbon representation superimposed on a transparent protomer surface) can swivel from a wide-open state (left; RCSB Protein Data Bank (PDB) entry 1M6N) to an open state (middle; PDB entry 1TF5) or a closed state (right; PDB entry 3DIN)[12]. A clamp that is formed between the PBD and intra-molecular regulator of ATPase 2 (IRA2) may form a mature domain-sliding groove[99,102]. **c** | Structures of proteins that assist in pre-protein maturation and release: Signal peptidase I (SPase I (the *Escherichia coli* SPase I, LepB, is shown; PDB entry 3S04) and SPase II (the *Pseudomonas aeruginosa* SPase II, LspA, is shown; PDB entry 5DIR) cleave the signal peptides of secretory proteins or lipoproteins, respectively, during late steps or after the completion of translocation and release the mature domain at the *trans* side of the plasma membrane. SPase I contains a periplasmic domain and the catalytic Ser-Lys dyad of SPase I is indicated in red. SPase II is membrane-anchored and has a small periplasmic domain that contains the proposed conserved active site (dark purple) and two catalytic aspartic acid residues (red). The SecYEG-associated plasma-membrane-anchored periplasmic chaperone PpiD possibly assists in the release of secretory proteins. PpiD is anchored to the membrane by an unresolved α-helix near SecYEG[151], and has three periplasmic domains (D); one of which has been resolved (PDB entry 2KGJ). The SecD–SecF (dark brown and light brown, respectively; PDB entry 3AQP) complex, which consists of 12 transmembrane helices (TMHs) and two periplasmic domains (P1 and P4), facilitates the translocation of the pre-protein through its P1 domain[147]. N terminus, amino terminus; P_i, inorganic phosphate.

ATP-binding and hydrolysis act as power strokes for the stepwise directional movement of pre-protein[16], by pre-protein binding and release[45,148] and SecA conformational cycles[144]. Another possibility is that SecA acts as an 'allosteric channel regulator': SecA conformations control SecYEG through cycles of ATP hydrolysis. This would facilitate a Brownian ratchet mechanism: an



engaged but freely moving polypeptide can undergo passive diffusion through the dilated channel without the requirement of energy[45,135,149]. Finally, acting as a 'break', SecA prevents chain back-sliding to enable PMF-driven or Brownian forward motion[16,46].

Once most of the mature domain is translocated, SecA loses contact sites[45] and hence ATPase stimulation. SecA–ADP is now peripherally associated with the translocase[16,144].

In the final step, the peripherally bound SecA–ADP protomer dissociates from SecYEG[41,144], re-dimerizes in the cytoplasm and can repeat the translocation cycle.

Pre-protein maturation and release. Signal peptidases (SPase I for secretory proteins and SPase II for lipoproteins; FIG. 5c, left) cleave the signal peptide of a pre-protein that was fully, or at least 80%, translocated through SecYEG and release the mature domain at the

trans side of the plasma membrane[21]. Membrane-anchored SPase I has a *trans* serine protease domain that cleaves the signal peptide after a specific AXA motif[21,150]. The SecYEG-associated plasma-membrane-anchored periplasmic chaperone PpiD[151] (FIG. 5c, middle) and SecDF[147] (right), possibly help in the release of secretory proteins[152]. Following release, proteins either become directed to post-plasma membrane secretion systems or fold in the cell envelope, extracellular milieu or target cell[3] (BOX 1).

Conclusions and outlook

During the past three decades, several advances, including numerous structures (Supplementary information S3 (table)) and detailed biochemical and biophysical profiling of distinct steps, have elucidated basic aspects of the bacterial Sec pathway and revealed a sophisticated process that is finely regulated by multiple factors. Despite substantial progress, several mechanistic aspects remain unresolved. How do exported proteins overcome cytoplasmic folding or aggregation and reach the translocase? Do chaperone relay networks have a role? How are export-destined, unfolded proteins distinguished from cytoplasmic proteins? What are the structural SecA–SecYEG states during catalysis? How does SecA bind to mature domains? What is the route through the body of SecA that pre-proteins follow to enter the channel? What is the role of PMF in translocation? The complexity and dynamic nature of these events require sensitive and dynamic approaches for their elucidation. Single-molecule studies, high-resolution structures of the various complexes and methods that monitor conformational dynamics should be used. Elucidating the mechanisms of protein trafficking in bacteria will benefit the development of biopharmaceuticals and antibiotics and provide insight into this process in higher organisms during physiological and disease states.

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Competing interests statement
The authors declare no competing interests.

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http://www.rcsb.org/pdb/home/home.do
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