The ribosome and YidC

New insights into the biogenesis of *Escherichia coli* inner membrane proteins

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In the bacterium *Escherichia coli*, inner membrane proteins (IMPs) are generally targeted through the signal recognition particle pathway to the Sec translocon, which is capable of both linear transport into the periplasm and lateral transport into the lipid bilayer. Lateral transport seems to be assisted by the IMP YidC. In this article, we discuss recent observations that point to a key role for the ribosome in IMP targeting and to the diverse roles of YidC in IMP assembly.

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Introduction

The *Escherichia coli* targeting factor signal recognition particle (SRP) consists of a 48-kDa subunit called fifty-four homologue (Ffh) and 4.5S RNA, which are homologous to the eukaryotic SRP54 and the 7S RNA, respectively (Herskovits *et al.*, 2000). The latter two form part of the larger eukaryotic SRP, which functions in the co-translational targeting of both membrane and secretory proteins, through the SRP receptor, to the Sec translocon in the membrane of the endoplasmic reticulum (ER).

The *E. coli* SRP is specialized in the co-translational targeting of inner membrane proteins (IMPs), whereas secreted *E. coli* proteins follow a post-translational pathway that is mediated by the chaperone SecB (Herskovits *et al.*, 2000). The SRP has been shown to interact specifically with the predominantly hydrophobic targeting signal at the amino termini of nascent IMPs at an early stage in IMP biosynthesis, when the signal sequence is not yet fully exposed outside the ribosome (Uillers *et al.*, 2003; Valent *et al.*, 1997). Unlike its eukaryotic counterpart, the *E. coli* SRP is not thought to arrest translation after it binds to the targeting signal (Herskovits *et al.*, 2000), although this view has recently been challenged

(Avdeeva *et al.*, 2002). Once formed, the ribosome-nascent chain (RNC)–SRP complex is targeted to the Sec translocon through a specific interaction between the SRP and its receptor, FtsY (Fig. 1C). In contrast to the membrane-bound mammalian SRP receptor, FtsY is only transiently associated with the plasma membrane and might contact the RNC–SRP complex in the cytoplasm (Herskovits *et al.*, 2000). FtsY is also thought to deliver ribosomes to the membrane in an SRP-independent manner (Herskovits *et al.*, 2001), and the subsequent synthesis of a membrane protein by these targeted ribosomes has been proposed to trigger SRP binding. Irrespective of the preceding steps, the transfer of the RNC to the Sec translocon is coordinated by the concerted action of Ffh and FtsY, both of which are GTPases, although the mechanistic details of this step are unclear.

In vitro crosslinking experiments suggest that in *E. coli*, the RNCs contact the SRP in the cytoplasm and then contact the Sec-translocon components when the nascent protein is approximately the same length (Uillers *et al.*, 2003). This suggests that membrane insertion occurs co-translationally, as is the case in eukaryotes. Consistent with this model, the affinity of ribosomes for the Sec translocon is similar in *E. coli* and eukaryotic cells (Prinz *et al.*, 2000). During IMP assembly, transmembrane domains (TMs) are arrested in the Sec translocon and subsequently move out into the lipid bilayer (Chen *et al.*, 2002; De Gier & Luirink, 2001). YidC, which is required for viability, is thought to assist in this transfer (De Gier & Luirink, 2001). In addition, YidC mediates the membrane insertion of SRP/Sec-translocon-independent bacteriophage proteins (Fig. 1A; Chen *et al.*, 2002).

Here, we discuss recent observations that point to a key role for the ribosome itself in IMP targeting and to diverse roles of YidC in IMP assembly.

A regulatory role for ribosomal proteins in IMP targeting?

Recent evidence suggests that the ribosome has an important regulatory role at several stages of SRP-mediated targeting. The eukaryotic ribosomal subunits L23a and L35, which are close to the nascent-chain exit site, have been shown to constitute the ribosome attachment site for SRP54 (Pool *et al.*, 2002). This strategically positions SRP54 to scan emerging polypeptides for the

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Fig. 1 | Model of the roles that the ribosome and YidC might have in the targeting and insertion of membrane proteins in *Escherichia coli*. Two inner membrane protein (IMP) assembly pathways are known to exist in *E. coli*: (A) the YidC pathway and (C) the SRP–Sec translocon–YidC pathway. In addition, an SRP–YidC pathway (B) might be operational. The ribosome seems to have a key role in the targeting of at least SRP–Sec translocon–YidC-dependent IMPs. FtsY, SRP-receptor; L23, L23 component of the large subunit of the ribosome; L29, L29 component of the large subunit of the ribosome; RNC, ribosome nascent chain complex; SRP, signal recognition particle; TF, trigger factor.

presence of a signal sequence. On targeting of the RNC–SRP complex to the ER for co-translational membrane insertion, the L23a/L35 site is one of the main connections between the ribosome and the translocon (Beckmann *et al.*, 2001; Morgan *et al.*, 2002). As the interactions of L23a/L35 with SRP54 and the Sec translocon seem to be mutually exclusive, SRP54 must be released from L23a/L35 before the establishment of the ribosome–translocon junction. The SRP receptor in the ER membrane might be involved in this step, as it triggers the evacuation of SRP54 from L23a (Pool *et al.*, 2002).

In *E. coli*, L23 (a homologue of L23a) seems to have an even more intricate role. It functions as an attachment site not only for the SRP (Gu *et al.*, 2003; Ullers *et al.*, 2003), but also for the chaperone and prolyl isomerase trigger factor (TF; Kramer *et al.*, 2002). In addition, TF has been shown to be associated with L29 (a homologue of L35). The role of TF in protein targeting and folding is unclear. Crosslinking studies have identified TF as the first chaperone to interact generically with nascent polypeptides (Hestkamp *et al.*, 1996; Valent *et al.*, 1995), and recent evidence points to its cooperation with another chaperone, DnaK, in co-translational folding (Deuerling *et al.*, 1999, 2003; Teter *et al.*, 1999). It has been suggested that TF also has a discriminatory role in targeting by interacting specifically with the early mature

region of secretory proteins, thereby reducing the affinity of their mildly hydrophobic signal peptide for the SRP (Beck *et al.*, 2000). This would select secretory proteins for subsequent interaction with the targeting factor SecB, which delivers them to SecA at the Sec translocon by a mechanism that is essentially post-translational. However, other studies suggest that TF binds to the nascent chain by default and that only the SRP can confer targeting specificity because of its high affinity for newly synthesized, strongly hydrophobic targeting sequences in nascent IMPs (Lee & Bernstein, 2001; Valent *et al.*, 1997). In either of these mechanisms, L23 might coordinate the positioning of TF and SRP on the ribosome, perhaps in response to specific structural features of the synthesized polypeptide.

Interestingly, it has recently been shown that the eukaryotic SRP has a higher affinity for active, translating ribosomes than for those that are inactive, even when the nascent chain is still entirely located in the ribosomal exit tunnel (Flanagan *et al.*, 2003). Possibly, the peptide that is buried in the exit tunnel causes a change in conformation of the L23 region on the ribosome surface, thereby influencing its affinity for SRP and TF. In this scenario, the function of TF might be to prevent the inadvertent association of ribosomes that do not carry a nascent IMP with the Sec translocon by covering the L23/L29 component of the ribosome–translocon junction site. In support of this idea, inactivation of the *TF* gene has been shown to accelerate the translocation of secretory proteins and to suppress their dependence on SecB, whereas overproduction of TF retards their translocation (Lee & Bernstein, 2002).

The Sec translocon

Before discussing the role of YidC in IMP assembly it is necessary to give a more detailed introduction to the Sec translocon. Its core consists of the integral IMPs SecY, SecE and SecG, and the peripheral subunit SecA. Together, SecY, SecE and SecG form a protein-conducting channel, and the peripheral subunit SecA pushes proteins into and through this channel (Manting & Driessen, 2000). SecA is not required for SRP-mediated targeting and the initial membrane insertion of a nascent IMP (Scotti *et al.*, 1999), but is necessary for the translocation of large periplasmic loops (Andersson & von Heijne, 1993; Fröderberg *et al.*, 2003).

The higher order oligomeric form of the Sec translocon is a matter of debate. It is clear that SecY, SecE and SecG form a heterotrimer (Manting & Driessen, 2000). Electron microscopic examination of purified translocons indicates that dimeric SecA catalyses the assembly of four SecYEG heterotrimers into what might be one large protein-conducting channel (Manting & Driessen, 2000). However, fluorescence resonance energy transfer analysis of SecYE heterodimers suggests that they form constitutive oligomers (Mori *et al.*, 2003). Further data, from two-dimensional crystallography, analytical ultracentrifugation and native-gel electrophoresis experiments, have led to the recent proposal that the active Sec translocon is comprised of two SecYEG heterotrimers (Bessonneau *et al.*, 2002; Breyton *et al.*, 2002). However, it should be noted that SecA was not present in the Sec-translocon preparations used in this last set of experiments. Finally, co-immunoprecipitation and crosslinking experiments indicate that even single SecYEG heterotrimers can form protein-conducting channels (Yahr & Wickner, 2000). However, this last study does not exclude the possibility that the SecYEG

heterotrimers form higher order oligomers. In other words, protein-conducting channels could be grouped in the membrane and this might be necessary in particular situations, such as for the assembly of IMP complexes.

The oligomeric states of other components of the complex are less debated. SecA itself forms stable dimers, which may dissociate during protein translocation (Or *et al.*, 2002). SecD, SecE and YajC are nonessential Sec-translocon subunits that facilitate the translocation reaction and form an auxiliary subcomplex that can be copurified with the SecYEG complex under certain conditions. Whether this SecDFYajC complex has any role in IMP insertion and/or assembly is not yet clear (De Gier & Luijck, 2001). It is known that during IMP assembly, TMs are arrested in the Sec translocon and subsequently move out into the lipid bilayer (Chen *et al.*, 2002; De Gier & Luijck, 2001), and the protein YidC is thought to assist in this transfer (De Gier & Luijck, 2001).

YidC and assembly of SRP/Sec-translocon-dependent IMPs

YidC is a relatively abundant IMP that exists in two physical states: one that is associated with the Sec translocon and another that is not (Chen *et al.*, 2002; De Gier & Luijck, 2001). The Sec-associated form has been implicated in the integration of IMPs that are targeted through the SRP pathway to the Sec translocon (Fig. 1C). Depletion of YidC has a relatively mild effect on the integration of this class of proteins into the membrane as judged by the accessibility of their periplasmic domains to proteases (for example, see Fröderberg *et al.*, 2003; Samuelson *et al.*, 2000; Urbanus *et al.*, 2001). These data indicate that YidC influences the efficiency and kinetics of Sec-dependent membrane integration without being essential for this process.

Little is known about the oligomeric organization of YidC-containing Sec translocons. *In vitro* reconstitution and crosslinking studies indicate that YidC and SecYEG are in close proximity, at least during the initial stages of IMP insertion (Urbanus *et al.*, 2001; van der Laan *et al.*, 2001; Beck *et al.*, 2001; Houben *et al.*, 2002). Consistent with these observations, YidC can be copurified with the core SecYEG translocon, and overexpression of Sec-translocon components positively affects YidC expression, which suggests a functional relationship (Scotti *et al.*, 2000). In addition, it has been shown that the chloroplast homologue of YidC, Albino3 (Alb3), interacts directly with the chloroplast homologue of SecY, cpSecY (Klostermann *et al.*, 2002). These observations indicate that YidC interacts with SecYEG, possibly through the SecY subunit, although it has also been reported that YidC interacts with SecD and SecE to form a heterotetrameric SecDFYajCYidC accessory complex (Nouwen & Driessen, 2002). Whether the association of YidC with the Sec translocon is static or dynamic is an issue that remains to be addressed. If the process is dynamic, YidC might be recruited from the 'free' pool on demand.

The function of YidC in Sec-dependent IMP integration is uncertain. *In vitro* crosslinking studies to monitor the molecular environment of nascent IMPs—'integration intermediates'—revealed prominent contacts with YidC (for example, see Beck *et al.*, 2001; Scotti *et al.*, 2000; Urbanus *et al.*, 2001). These contacts are specific to TM regions and are made only in the context of the translating ribosome. Hence, it has been suggested that YidC facilitates the partitioning of TM segments into the lipid bilayer after their co-translational insertion into the Sec translocon (Scotti *et al.*, 2000). Interestingly, YidC was found to be crosslinked to different TMs in the

same nascent polytopic IMP MtIA (Beck *et al.*, 2001). This observation may reflect a role for YidC in the packing and assembly of TMs in or near the Sec translocon before their coordinated release into the lipid bilayer.

Crosslinking analysis of nascent IMPs of differing lengths that are derived from the bitopic IMP FtsQ showed that its TM first contacts SecY and then YidC (Urbanus *et al.*, 2001). However, in a more extensive study using the polytopic model IMP Lep, different segments in the first TM could be crosslinked to both SecY and YidC, even when the nascent chains were very short and the first TM was not yet fully exposed outside the ribosome (Houben *et al.*, 2002). This suggests that YidC also has an early role in the reception and recognition of TMs, at a stage when the translating ribosome must be in close proximity to the Sec translocon. It is possible that YidC assists in the docking of specifically those ribosomes that synthesize IMPs to allow co-translational insertion.

It should be noted that only a few model IMPs were used in these studies. Clearly, more information is necessary to assess fully the functions of YidC in the insertion and assembly of Sec-dependent IMPs of different complexity and topology.

YidC and assembly of Sec-translocon-independent IMPs

Whereas YidC depletion has only a moderate effect on the assembly of SRP/Sec-translocon-dependent IMPs, it almost completely blocks the integration of small phage-coat proteins that require neither the SRP pathway nor the Sec translocon for insertion into the membrane (for the pathway, see Fig. 1A). Nothing is known about the Sec-independent structure of YidC, but the homologue of this protein in the fungus *Neurospora crassa*, which has neither an SRP pathway nor a Sec translocon, forms homo-oligomeric complexes that are thought to constitute translocons (Nargang *et al.*, 2002). For YidC, it will be crucial to determine whether it functions in a homo- or a hetero-oligomeric complex during the assembly of SRP/Sec-independent phage-coat proteins.

Considering that YidC is not required for the assembly of the SRP/Sec-dependent IMPs (many of which are essential cellular proteins), and that phage-coat proteins, which do require YidC, are obviously not essential for growth, why is YidC essential for viability? A recent study has shown that the loss of YidC rapidly leads to defects in the functional assembly of cytochrome *o* oxidase and the F_0F_1 -ATPase complex, which is reminiscent of the effects of mutations in the *yidC* homologue *OXA1* in the yeast mitochondrial inner membrane (van der Laan *et al.*, 2003). Specifically, the integration of CyoA (subunit II of the cytochrome *o* oxidase) and F_0c (membrane subunit of the F_0F_1 -ATPase) into their respective complexes are exceptionally sensitive to the depletion of YidC. Both of these proteins are rather small integral membrane proteins with two closely spaced TMs, and are components of multisubunit oligomeric complexes. It is therefore possible that they are natural substrates of the YidC-dependent/Sec-independent membrane protein insertion pathway that is also used by the phage-coat proteins in *E. coli*. Alternatively, it is possible that YidC is involved in the assembly of IMP complexes, as has been suggested for its mitochondrial homologue Oxa1 (Stuart, 2002). Therefore, the decrease of CyoA and F_0c on YidC depletion could also be explained by the rapid degradation of CyoA molecules that are not part of cytochrome *o* oxidase complexes, and of F_0c molecules that are not part of F_0F_1 -ATPase complexes.

A third IMP assembly pathway in *Escherichia coli*?

Membrane protein assembly studies in chloroplasts gave the first hints towards a possible third IMP assembly pathway in *E. coli* (Fig. 1B). In chloroplasts, the cpSRP/Alb3 pathway is involved in the assembly of many thylakoid membrane proteins (Eichacker & Henry, 2001), and cpSRP and Alb3 are the chloroplast homologues of *E. coli* SRP and YidC, respectively. Recently, it was also shown that, in *E. coli*, engineered model IMPs were assembled into the inner membrane in an SRP/YidC-dependent and Sec-translocon-independent fashion (Fröderberg *et al.*, 2003). It is thus conceivable that an SRP/YidC pathway similar to the cpSRP/Alb3 pathway in chloroplasts, is operational in *E. coli*.

Conclusions and perspectives

In *E. coli*, the large ribosomal subunit component L23 constitutes an overlapping binding site for TF and SRP near the nascent-chain exit site (Fig. 1). We propose that, by virtue of this property, the ribosome might have a more active role in the selection of targeting pathways than had previously been anticipated. Future binding studies are likely to reveal how the binding of TF and SRP to the ribosome is regulated and how this influences the interactions of these factors with the nascent substrate. Decisions that are made at this stage will also affect downstream stages in the targeting process and so not only the binding of SRP and TF to, but also their release from, the ribosome is of interest. Indeed, it seems likely, on the basis of extrapolation of structural data from the ribosome-translocon junction in yeast (Morgan *et al.*, 2002), that L23 must be cleared of binding partners to allow its direct interaction with the Sec translocon during co-translational membrane insertion. At this stage, YidC is near the ribosome-associated SRP/Sec-dependent IMP, but whether it has any role in ribosome docking remains to be investigated. A function for YidC in the reception of ribosomes that synthesize Sec-independent IMPs, as anticipated for the mitochondrial YidC homologue Oxa1 (Hell *et al.*, 2001), is an interesting possibility that warrants further investigation.

In *E. coli* there are at least two IMP assembly pathways, the SRP-Sec-translocon-YidC pathway and the YidC pathway, in which the role of YidC is distinct. In addition, a third IMP assembly pathway, the SRP-YidC pathway, may be operational in this organism (Fig. 1). Some insight into the substrates of the targeting and assembly pathways in which YidC is involved has been obtained. However, the main challenges for the future will be to establish how this protein operates in different complexes so that it executes various tasks in IMP assembly, exactly what these tasks are and how they relate to the structural characteristics of YidC. In a general sense, YidC may be considered as an integral membrane chaperone that interacts transiently with membrane proteins during their biogenesis and stimulates their correct assembly. More specific functions have been proposed on the basis of limited experimental data of a mainly biochemical nature. YidC might facilitate the lipid partitioning of TM segments of Sec-independent IMPs (without being absolutely essential for this process) and stimulate the translocation of their periplasmic domains (Samuelson *et al.*, 2001). For Sec-dependent IMPs, YidC might recognize individual TMs in the context of the Sec translocon early during biogenesis and facilitate their folding and, finally, their partitioning into the lipids (De Gier & Lührink, 2001; Houben *et al.*, 2002). YidC might also collect together different

TMs of polytopic IMPs to catalyse their assembly and coordinated release into the lipid bilayer (Beck *et al.*, 2001). As mentioned before, YidC might even function in the assembly of oligomeric IMP complexes. Finally, YidC might also have a role in the quality control of IMPs, although there is no evidence as yet for a link with proteolytic enzymes.

It is clear that a full appreciation of the versatile roles of both the ribosome and YidC in IMP biogenesis in *E. coli* will require a combined biochemical, genetic and structural effort.

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