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Breaking the bacterial protein targeting and translocation model: oral organisms as a case in point

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SUMMARY

Insights into the membrane biogenesis of oral and throat bacteria have highlighted key differences in protein localization by the general secretion pathway compared with the well-studied *Escherichia coli* model system. These intriguing novelties have advanced our understanding of both how these microorganisms have adapted to survive and cause disease in the oral cavity, and the field of protein translocation as a whole. This review focuses on findings that highlight where oral bacteria differ from the *E. coli* paradigm, why these differences are biologically important, and what questions remain about the differences in pathway function. The majority of insight into protein translocation in microbes of the oral cavity has come from streptococcal species, which will be the main topic of this review. However, other bacteria will be discussed when relevant. An overview of the *E. coli* model of protein targeting and translocation is provided for comparison.

Keywords

membrane biogenesis; protein secretion; signal recognition particle; targeting; translocation; transport

INTRODUCTION

Proper protein localization is a requirement for all cells. Targeting and compartmentalization allow a cell to organize areas of specialized function and separate disparate molecular machinery. In oral bacteria, proper protein localization is key to many crucial tasks including adhesion (Jiang *et al.*, 2012), stress tolerance (Gutierrez *et al.*, 1999), and virulence (Xiong *et al.*, 2008), among others. Compared with eukaryotic and gram-negative bacterial cells, gram-positive bacteria have fewer non-cytoplasmic compartments - a single cell membrane, cell wall, or extracellular space. However, this lack of potential destinations does not equate to simplified targeting systems. In fact, complex targeting systems are needed for survival in the dynamic oral environment where, compared with the *Escherichia coli* model, added layers of complexity and functional redundancy are often seen.

Proteins destined to leave the cytoplasmic site of synthesis require a means to target and reach their site of function. In all bacteria, the most highly traveled, and well studied, route

for secreted and membrane-inserted proteins is through the Sec translocase. We will first describe the many components and function of the Sec pathway in *E. coli* before discussing the unique aspects of gram-positive protein targeting and translocation (Fig. 1).

***E. COLI*: THE MODEL BACTERIAL SYSTEM**

In bacteria, the vast majority of translocated proteins, both membrane inserted and secreted, travel through the Sec pathway (for review, see Driessen & Nouwen, 2008). At its core, the *E. coli* Sec pathway consists of a transmembrane (TM) channel, the Sec-YEG complex, and a soluble but membrane-associable component, either the motor protein SecA or the ribosome, which threads protein substrates into the pore. However, many accessory components operate within areas of the Sec pathway, providing additional roles for diverse and specialized functions.

Targeting factors

Proteins destined for post-translational translocation across the membrane contain an N-terminal signal peptide (for review, see Driessen & Nouwen, 2008). These tripartate signal sequences are characterized by an N-H-C domain arrangement. The N-terminal N-domain is positively charged, the H-domain is hydrophobic, and the C-domain is more polar. Following translocation, the signal peptide is cleaved off by a signal peptidase. Many translated preproteins encounter two soluble components of the general secretion pathway, SecB and SecA. The SecB chaperone keeps proteins in an unfolded conformation, suitable for secretion, and maintains solubility of the unfolded protein (for review see Bechtluft *et al.*, 2010). Recent evidence suggests that other known chaperones including trigger factor, DnaK and GroEL may also cooperate with or substitute for SecB to facilitate post-translational protein translocation (for review, see Castanie-Cornet *et al.*, 2014). SecB delivers preprotein substrates to SecYEG-associated SecA at the membrane. SecA serves as the motor protein that couples ATP-hydrolysis to the pushing of the unfolded substrate through the translocon in a stepwise manner. Recently in *E. coli*, Sec pathway substrates have been identified that do not require SecB for proper targeting and secretion (Rawat *et al.*, 2014). Further, SecA has been shown to bind ribosome nascent chain (RNC) complexes (Huber *et al.*, 2011). These two findings raise the question of what determines a SecB requirement for secretion, and if SecA can, in some instances, fulfill the chaperone/targeting role by capturing nascent chains directly from the ribosome.

The second route to the SecYEG translocon is co-translational targeting of ribosome nascent chain complexes by the signal recognition particle (SRP) and its membrane-associated receptor, FtsY. The preferred substrates of the SRP contain particularly hydrophobic signal sequences that are recognized as they emerge from the ribosome (for review, see Koch *et al.*, 2003). The SRP-FtsY pathway is conserved across all domains of life, is essential for viability in *E. coli* (Phillips & Silhavy, 1992), and is known to be used for hydrophobic proteins that are co-translationally inserted into the membrane (Powers & Walter, 1997; Ulbrandt *et al.*, 1997). However, some fully translocated secreted proteins may also use the SRP and have cleavable N-terminal signal peptides (Sijbrandi *et al.*, 2003; Palomino & Mellado, 2005; Schneewind & Missiakas, 2014). In all bacteria the signal recognition particle is minimally composed of a protein and RNA component. The protein, known as

Ffh, is a homolog of the 54-kDa eukaryotic SRP protein [54 homolog (Ffh)]. Ribosome-bound Ffh interacts with hydrophobic signal sequences of newly emerging peptides, and the SRP-RNC complex is then targeted to the membrane by an interaction between Ffh and FtsY. The RNC complex is then released to SecYEG via GTP hydrolysis by Ffh and FtsY, which are then recycled for further rounds of targeting (for review, see Akopian *et al.*, 2013; Saraogi & Shan, 2014).

Ffh is composed of a methionine-rich M-domain and a GTPase NG-domain. The M-domain recognizes and binds the signal sequence, as well as the small cytoplasmic RNA (scRNA, 4.5S RNA in *E. coli*) (Romisch *et al.*, 1990; Zopf *et al.*, 1990; Lutcke *et al.*, 1992). The NG-domain interacts with a homologous NG-domain of the FtsY receptor. FtsY does not have an M domain but contains an N-terminal, highly-acidic A-domain, which is responsible for docking at the lipid membrane (Parlitz *et al.*, 2007; Weiche *et al.*, 2008). The 4.5S RNA serves as a structural scaffold along which the Ffh–FtsY GTPase complex travels, first negatively regulated by the translating ribosome and later positively regulated by the SecYEG translocon, so maintaining Ffh and FtsY in appropriate conformations enabling precise timing of molecular events through sequential pathway fidelity checkpoints of the GTP hydrolysis cycle (Zhang *et al.*, 2009; Shen *et al.*, 2012).

Translocase components

The multi-membrane spanning SecY forms the translocation pore of the SecYEG complex (for review, see Driessen & Nouwen, 2008; Mandon *et al.*, 2009; Park & Rapoport, 2012). The ten-TM domain protein is two-lobed in appearance, with TM domains 1–5 composing the N-terminal lobe and TM domains 6–10 forming the C-terminal lobe. The membrane pore is formed between the two domains, which are joined by a periplasmic loop (Van den Berg *et al.*, 2004). In addition to the membrane-spanning pore, SecYEG can also insert TM domains into the bilayer via a lateral gate. SecE assists in the opening and stability of the SecY pore. The non-essential SecG increases translocation efficiency. The SecYEG translocon also associates with a three-protein complex containing SecD, SecF, and YajC. The SecDF complex was shown to exert a pulling force on substrates from the periplasmic side of the membrane in a proton motive force-dependent manner (Tsukazaki *et al.*, 2011). Further, mutants lacking SecD and SecF are cold sensitive and translocation is greatly diminished (Arkowitz & Wickner, 1994; Pogliano & Beckwith, 1994; Nouwen *et al.*, 2005).

The YidC insertase

YidC, an essential protein in *E. coli*, is a member of the Oxa1, Alb3, YidC family of membrane insertases (for review, see Saller *et al.*, 2012; Dalbey & Kuhn, 2014). YidC is found both associated with and independent of SecYEG (Scotti *et al.*, 2000; Nouwen & Driessen, 2002). Integral membrane proteins that exit SecYEG through the lateral gate that opens into the membrane have been shown to interact with YidC (Urbanus *et al.*, 2001). For these proteins, YidC plays an important role, aiding in insertion and proper folding/assembly (Samuelson *et al.*, 2000; Beck *et al.*, 2001; Nagamori *et al.*, 2004; Wagner *et al.*, 2008). YidC can also function as a Sec-independent insertase, generally inserting proteins that span the membrane once or twice (Chen *et al.*, 2002; van der Laan *et al.*, 2004; Serek *et al.*, 2004). The cytoplasmic C-terminus of YidC binds to RNCs (Kohler *et al.*, 2009), and has

also been cross-linked to the SRP protein Ffh (Welte *et al.*, 2012). SRP requirement for this Sec-independent YidC pathway seems to be substrate dependent, but experimental results are conflicting (van Bloois *et al.*, 2004; Yi *et al.*, 2004). A recent report modeled the YidC-RNC membrane complex and produced a mechanism for substrate integration that involves lipid membrane thinning and creation of a hydrophilic environment within the bilayer (Wickles *et al.*, 2014).

ORAL STREPTOCOCCI: BREAKING THE MOLD

The long history of studying protein targeting and translocation in *E. coli* has contributed a wealth of understanding, at ever increasing mechanistic detail, to the field of protein targeting and translocation. However, as the study of transport in other bacteria has grown, the list of important differences has increased.

Protein targeting components

An early paradigm-breaking discovery came from work in the oral pathogen *Streptococcus mutans*. Using a transposon mutagenesis approach to look for mutants sensitive to salt and acid stress, a *Tn917* insertion in a five-gene operon, containing Ffh, was found to decrease acid tolerance (Gutierrez *et al.*, 1999). Follow-up work showed that the signal recognition particle and its receptor were dispensable in *S. mutans* (Crowley *et al.*, 2004; Hasona *et al.*, 2005), a surprising result given the essentiality of the SRP pathway in *E. coli*. Although not lethal, loss of Ffh, scRNA, or FtsY in *S. mutans* resulted in a decreased ability to cope with environmental stressors, such as high salt and low pH. Follow-up work showed similar results in *Streptococcus pyogenes*. Neither loss of Ffh, nor the scRNA were lethal in *S. pyogenes*, but both deletions led to a loss of virulence (Rosch *et al.*, 2008; Trevino *et al.*, 2010).

Unlike the mammalian SRP, which is traditionally composed of six proteins, *E. coli* SRP contains only a single known protein, Ffh. In *Bacillus subtilis*, however, a second protein component, the histone-like protein HBSu, was shown to interact with the scRNA, via the Alu-like domain, and co-purify with Ffh in an scRNA-dependent manner (Nakamura *et al.*, 1999). Further, repressing HBSu increased scRNA production and led to precursor protein accumulation, showing an important function of HBSu in the SRP targeting pathway (Yamazaki *et al.*, 1999). Similarly, an SRP accessory protein was recently identified in *S. mutans* (Williams *et al.*, 2014). The gene encoding YlxM is located directly upstream of *ffh* in *S. mutans*, a conserved gene organization in most gram-positive and some gram-negative bacteria. Further, *ylxM* is often found in close proximity with *ftsY*. The conservation of *ylxM* and SRP gene proximity hints at an SRP-related role. A helix-turn-helix protein, YlxM, was predicted to be a DNA-binding protein and suspected to regulate SRP component transcription/translation (Samuelsson *et al.*, 1997; Oganessian *et al.*, 2004). Surprisingly, functional studies showed no evidence of gene regulatory function, but rather a direct interaction between YlxM and both Ffh and the scRNA (Williams *et al.*, 2014). In addition, YlxM increased GTP hydrolysis of Ffh. Interestingly, YlxM did not interact with FtsY, and diminished GTP hydrolysis when both Ffh and FtsY were present. This points to YlxM serving a regulatory role within the SRP pathway, promoting an unknown cytosolic role for Ffh, while throttling the traditional Ffh–FtsY membrane-localized pathway. Additionally, a

ylxM mutant was severely less competitive in co-culture, and diminished in sorbitol uptake when compared with wild-type – mirroring other SRP pathway mutants (Williams *et al.*, 2014).

Targeting of proteins to transport pathways is orchestrated by signal peptides of varying lengths and hydrophobicities (for review, see Izard & Kendall, 1994). An interesting contribution to the protein targeting picture came from work on the signal peptide of the gram-negative oral bacteria *Aggregatibacter actinomycetemcomitans*. The surface protein EmaA (extracellular matrix protein adhesion A) of *A. actinomycetemcomitans* is critical for collagen binding and both oral and non-oral pathogenesis. EmaA has an extended signal peptide (56 amino acids) due to an N-terminal extension in addition to the canonical NHC regions. Deletions along the elongated signal peptide disrupted different processes, including stability, localization, and assembly of EmaA. Interestingly, a typical signal peptide was sufficient for the protein's secretion and function at 37°C, but localization was impaired at elevated temperatures (Jiang *et al.*, 2011). This hints that the extended signal peptide is needed for stability and stress tolerance in the varied conditions of the oral cavity. This group further showed that the SRP is not involved in secretion of EmaA, but that its transport depends on the SecB chaperone-mediated pathway (Jiang *et al.*, 2012).

Currently, no SecB, or SecB homolog, has been identified in most gram-positive bacteria. Notable exceptions exist outside the oral cavity, including the SecB-like Rv1957 in *Mycobacterium tuberculosis*, which was shown to serve an anti-aggregation role and can functionally replace *E. coli* SecB (Bordes *et al.*, 2011). Additionally, CsaA in *Bacillus subtilis* can suppress a temperature-sensitive SecA mutation in *E. coli*, can bind certain SecA substrates, and shows an affinity for SecA (Muller *et al.*, 2000a,b). However, for most gram-positive bacteria, including the oral streptococci, the extent and mechanism of post-translational transport is largely unknown. Speculation is that general chaperones such as trigger factor, DnaK/J, and GroES/L, or SecA, may substitute for SecB. The previously mentioned finding that SecA can associate with RNC complexes in *E. coli* make it an intriguing candidate to fulfill a hybrid role. Analysis of the extracellular proteome of wild-type compared with *secA*-temperature sensitive and *ffh* conditional mutants of *Bacillus subtilis* suggested that SRP and Sec pathways cooperate to target and translocate most of this organism's extracellular proteins across the cytoplasmic membrane (Hirose *et al.*, 2000).

Accessory secretion systems

A profound divergence from the *E. coli* model is found in some oral bacteria that contain a second secretion pathway. Whereas the classical Sec system is responsible for membrane insertion and secretion of the majority of translocated proteins, the accessory Sec system transports a small number of specific substrates (for review, see Feltcher & Braunstein, 2012; Freudl, 2013; Bensing *et al.*, 2014). Often, the accessory Sec substrates are key virulence factors. Some species have only a second SecA, termed SecA2, while others have a more complete ancillary pathway, composed of SecA2 and SecY2. The SecA2–SecY2 pathway was first discovered in two oral pathogens, *Streptococcus gordonii* (Bensing & Sullam, 2002) and *Streptococcus parasanguinis* (Chen *et al.*, 2004), and also exists in the

oral residents *Streptococcus oralis*, *Streptococcus salivarius*, and *Streptococcus mitis*. Of note, based on currently available genomic sequences, *S. mutans* does not possess this additional pathway. The current understanding of the SecA2–SecY2 pathway is that the accessory systems exist to export large, serine-rich proteins that are heavily glycosylated. The genes for these substrates are usually found in the SecA2–SecY2 pathway locus, which contains the accessory Sec genes, glycosylation factors, and other accessory factors of the pathway. Located between the *secY2* and *secA2* genes are genes encoding a trio of proteins known in *S. gordonii* and others as accessory sec protein (Asp) 1, 2, and 3 (Takamatsu *et al.*, 2005), and called Gap1, 2, and 3 for glycosylation-associated proteins in *S. parasanguinis* (Zhou *et al.*, 2011). Substrate proteins secreted by the SecA2–SecY2 pathway are glycosylated by glucosyltransferases GtfA and GtfB, a process that is necessary to maintain them in a soluble form. Based on co-immunoprecipitation experiments with accessory Sec protein complexes, it is believed that biogenesis of serine-rich repeat glycoprotein substrates may be linked to the canonical Sec system in *S. parasanguinis* (Zhou *et al.*, 2011). Interestingly, *S. gordonii*, also contains *asp4* and *asp5*, which code for predicted membrane proteins, in the same locus. Asp4 and Asp5 have limited, but intriguing homology with SecE and SecG (Takamatsu *et al.*, 2005). It is speculated that in bacteria lacking Asp4 and Asp5, SecY2 may use the traditional SecY helper proteins. SecA2–SecY2 systems are also found in bacteria outside the oral cavity, including *Staphylococcus aureus*, *Streptococcus agalactiae* (Group B streptococcus), and *Bacillus anthracis*, among others (Rigel & Braunstein, 2008).

As mentioned, a subset of bacteria, which reside outside the oral cavity, contain a SecA2-only system. This set includes all mycobacteria, including pathogenic *Mycobacterium tuberculosis* (Braunstein *et al.*, 2001), and other gram-positive bacteria (*Listeria monocytogenes*, *Corynebacterium glutamicum*, and *Clostridium difficile*). SecA2-only systems transport more substrates, most of which are related to survival in a specific niche or pathogenesis, than SecA2–SecY2 pathways. Unlike SecA, loss of SecA2 is not lethal (Braunstein *et al.*, 2001; Lenz & Portnoy, 2002; Gibbons *et al.*, 2007), except in rare cases such as *C. difficile* (Fagan & Fairweather, 2011). However, elimination of SecA2 can have a profound effect on colonization and virulence (Braunstein *et al.*, 2003; Lenz *et al.*, 2003; Kurtz *et al.*, 2006; Sullivan *et al.*, 2012). SecA2 Probably uses the canonical SecYEG translocase for protein export, but it is unclear whether general SecA also participates (Rigel *et al.*, 2009; Fagan & Fairweather, 2011). While SecA2 has demonstrated ATP hydrolysis activity, its cytoplasmic localization (Fagan & Fairweather, 2011) raises the question of whether it functions primarily in the targeting, translocation, or both, of substrates.

Localization of secretion components

One focus of research on gram-positive protein transport has been the physical localization of the Sec-pathway membrane components. In rod-shaped bacteria (*E. coli* and *Bacillus subtilis*) Sec components were localized in helical arrays spiraling the length of the cell (Campo *et al.*, 2004; Shiomi *et al.*, 2006). However, a second study showed even distribution of Sec components over the *E. coli* cell surface (Brandon *et al.*, 2003). Aiming to examine how streptococci organize secretion and folding of proteins, electron microscopy images of fixed *S. pyogenes* probed with gold-labeled antibodies yielded remarkable

findings. Sec-substrate SpeB localized to a single distinct microdomain distal to either cell pole. SecA showed localization to the same microdomain (termed the ExPortal), which the authors speculate is a way for gram-positive bacteria to organize the factors needed for protein secretion and folding (Rosch & Caparon, 2004). That conclusion was strengthened by a follow-up report that identified the surface chaperon HtrA localized at the ExPortal (Rosch & Caparon, 2005), which was shown also to be enriched in anionic lipids (Rosch *et al.*, 2007). This group showed subsequently that the ExPortal is localized at the site of peptidoglycan biogenesis, suggesting that cell wall formation, cell division, and protein secretion may be spatially coordinated (Vega *et al.*, 2013). Additionally, a different group corroborated the ExPortal in *S. mutans*, using antibody labeling and electron microscopy to show co-localization of SecA and the cell wall anchoring enzyme sortase A (Hu *et al.*, 2008). Taken together, these results paint an intriguing picture of the secretion process in oral streptococci, and perhaps other gram-positive bacteria, where secretion, folding, assembly, and cell wall anchoring are all concentrated within a microdomain. However, similar studies in *S. pyogenes* (Carlsson *et al.*, 2006) and *Streptococcus pneumoniae* (Tsui *et al.*, 2011) showed conflicting results, with SecA distributed throughout the cell surface in no discernible microdomains. This has raised the question of whether microdomain localization is a temporal event in response to certain growth or environmental triggers.

The YidC insertases

Unlike the *E. coli* model, *S. mutans* and most gram-positive bacteria possess two YidC paralogs, known as YidC1 and YidC2 (SpoIIIJ and YqjG in *Bacillus*). Multiple paralogs of this protein family are also found in mitochondria (Oxa1 and Cox18) and plant chloroplasts (Alb3 and Alb4). First identified in *S. mutans* (Hasona *et al.*, 2005), these dual insertases have been the subject of much recent work, which has provided interesting, but still mechanistically unclear results.

An early, important finding showed how elimination of *yidC2* resulted in a stress-sensitive phenotype with decreased F₁F₀ ATPase activity, which is important for *S. mutans*' aciduricity. Loss of *yidC1*, however, was significantly more tolerable and phenotypically resembled wild-type (Hasona *et al.*, 2005). A following study showed partial restoration of stress tolerance when complementing the YidC2 mutant with YidC from *E. coli*. Similarly, either YidC1 or YidC2 could restore several functions in an *E. coli* YidC knockout including growth in broth culture, proton motive force generation, F₁F₀ ATPase assembly, and membrane protein insertion (Dong *et al.*, 2008).

A second complementation study in *S. mutans* showed *Saccharomyces cerevisiae* mitochondrial Oxa1, but not Cox18 (Oxa2), could functionally restore ATPase activity and, to a lesser extent, competence, in a YidC2 mutant. Similarly YidC2 could partially restore yeast viability, Cox2 export, and respiration in a Oxa1 mutant. Interestingly, mitochondria lack an SRP pathway and RNCs bind Oxa1 directly through an interaction with its C-terminus. YidC2 partitioned with mitochondrial ribosomes, which lack an SRP. Further, both YidC and Oxa1 appended with the C-terminus of YidC2 partitioned with mitochondrial ribosomes (Funes *et al.*, 2009). These results hint at a potential ribosome-binding function

for the C-terminal tail of YidC2, and such an ability could help to explain the dispensable nature of the SRP pathway in *S. mutans*.

The first characterizations of YidC1/2 function within *S. mutans* showed that both proteins affected protein secretion, albeit in opposite fashion. Deletion of YidC2 led to a decrease in the functional activity of extracellular localized adhesin P1 (Palmer *et al.*, 2012). Surprisingly, deletion of YidC1 showed an increase in immunodetection of the same adhesin on the cell surface. Consistent with those findings was the observation that the *yidC1* mutant showed an increase in biofilm formation and oral colonization in a rat model, while the *yidC2* mutant was diminished on both accounts. Also of note was that the stress tolerance function of YidC2 depended on its C-terminal tail, and that a YidC1 construct in which its C-terminus was replaced with the tail of YidC2 could restore acid and salt tolerance to the *yidC2* mutant. However, not all YidC2-mediated functions require an intact tail. In contrast to deletion of the entire *yidC2* gene, adhesion of *S. mutans* to salivary agglutinin was not affected by elimination of YidC2's C-terminal tail (Palmer *et al.*, 2012). In addition, transformation efficiency of *S. mutans* was diminished by elimination of the C-terminal tail of YidC2, but not to the extent as elimination of the entire *yidC2* gene (Funes *et al.*, 2009).

A more recent study aimed to understand the complex biological interplay of YidC1 and YidC2 at the molecular level (Wu *et al.*, 2013). YidC and its homologs in other organisms are known to bind ribosomes. This interaction has been mapped to the extended C-terminal tails of this protein family. Using surface plasmon resonance, it was shown that the interaction between *E. coli* inverted membrane vesicles and *E. coli* ribosomes was increased when the membranes contained overexpressed levels of either *S. mutans* YidC1 or YidC2. This increase was seen using bare ribosomes, but was more dramatic with stalled RNC complexes. Further, when the overexpressed YidC1 or YidC2 lacked their C-termini, there was no increase in ribosome binding on an *E. coli* background. Similarly, overexpression of either YidC1 or YidC2 increased integration of F₀C in an *E. coli* system (Wu *et al.*, 2013). These results point to a potentially similar mechanism for YidC1 and YidC2 in membrane protein insertion in the heterologous *E. coli* background, which makes the marked biological differences of *S. mutans* YidC1 and YidC2 mutants all the more intriguing.

The molecular understanding of all YidCs took a leap forward with a recent report coming from the gram-positive world but outside the oral cavity. *Bacillus halodurans* YqjG (YidC2) was crystallized, and that structure led to a detailed model of the insertion of single-spanning membrane proteins. A cytoplasmic, two-helix motif located between the YidC TM domains 1 and 2 was identified as the initial binding site of single-spanning substrates having an acidic N-terminal region. The substrate is subsequently moved into the membrane through a hydrophilic groove formed by the YqjG's transmembrane helices (Kumazaki *et al.*, 2014). A conserved arginine (or positively charged) residue at position 72 within TM1 of YqjG is invariant among all YidC homologs of bacteria, mitochondria and chloroplasts despite little sequence conservation elsewhere. The presence of a positively charged residue at this position was necessary for the insertase function of both *B. halodurans* YqjG and SpoIIIJ (Kumazaki *et al.*, 2014) as well as *S. mutans* YidC2, but not of *E. coli* YidC (Chen *et al.*,

2014). How the recent structural model and functional information fit with the previous mechanistic model emphasizing the role of the C-terminus remains to be seen.

CONCLUSIONS AND FUTURE QUESTIONS

More than 30 years of research in protein targeting, transport, and translocation have led to an increasingly detailed understanding of these complex biological processes. Insights ranging from the single molecule to the whole cell have contributed to an ever-evolving field that spans all domains of life. In the bacterial world, the past 10 years have been particularly exciting, as new findings highlight the conservation and uncover key differences between the model *E. coli* and other bacteria. Much of this work has been done in oral bacteria, particularly streptococcal species, which have shown interesting peculiarities compared with the most widely studied bacterium, *E. coli*. These adaptations are often related to survival and virulence within the oral cavity.

Still, many questions, specific to protein transport in oral bacteria, remain unanswered. Are there more SRP accessory proteins and, if so, what are their functions? What determines the spatial organization of secretion components, and is it a static or dynamic arrangement? What is the extent of post-translational compared with co-translational protein translocation and what are the respective substrates of these transport machineries? To what extent does the SRP pathway cooperate with the Sec pathway and/or the multiple YidC homologs? And why do YidC1 and YidC2 have such drastically different effects on the cell's biology when their functional capabilities seem quite similar? More structural and molecular-level data will help to answer these, along with a host of other questions.

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Figure 1.

(Left) The *Escherichia coli* protein targeting and translocation model. In general, signal peptide-bearing secreted proteins are post-translationally chaperoned by SecB to an inner membrane complex of the SecA motor protein and the SecY translocase. Membrane-bound signal peptidase (SPase) cleaves the signal peptides of secreted proteins at the periplasmic side of the membrane. Integral membrane proteins and some fully translocated proteins are co-translationally targeted and translocated by the signal recognition particle (SRP) pathway. The SRP (Ffh protein and scRNA) binds the ribosome nascent chain (RNC) and targets it to the membrane through an interaction with the SRP receptor FtsY. The RNC complex is then passed to SecYEG for membrane protein integration through YidC. YidC also inserts protein into the membrane in a Sec-independent manner. (Right) The oral streptococci targeting and translocation model. Without the aid of SecB, some proteins may be aided by chaperones such as RopA (trigger factor), DnaK/J, and GroEL/ES in the SecA-SecY route for post-translational secretion, but this pathway remains speculative. Five genes annotated as signal peptidases are found in the *Streptococcus mutans* genome. In some species highly repetitive serine-rich substrate proteins are glycosylated by GtfA and GtfB, and secreted by the accessory SecA2-SecY2 system aided by Asp1/2/3 (Gap1/2/3) and possibly Asp4/5, which are thought to substitute for SecE/G. In the co-translational pathway

of *S. mutans*, the newly identified highly conserved SRP accessory protein YlxM modulates the GTPase activities of Ffh and FtsY and contributes to overall competitive fitness. Membrane protein insertion and protein secretion may also involve one of the two YidC insertases (YidC1 and YidC2) working in concert with the SRP and SecY. Either of the two YidCs may also function in a Sec-independent manner. The longer more highly charged tail of YidC2 contributes to some but not all of this homolog's functional activity.