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Emerging Themes in SecA2 Protein Export

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Abstract

The conserved general secretion (Sec) pathway carries out most protein export in bacteria and is powered by the essential SecA ATPase. Interestingly, mycobacteria and some Gram positive bacteria possess two SecA proteins: SecA1 and SecA2. In these species, SecA1 is responsible for exporting the majority of proteins whereas SecA2 exports only a subset of substrates and is implicated in virulence. However, despite the impressive body of knowledge on the canonical SecA (SecA1), less is known concerning SecA2 function. Here, we review our current understanding of the different types of SecA2 systems and outline future directions for SecA2 studies.

Introduction

All bacteria have systems for exporting specific proteins out of the cytoplasm and into the cell envelope or extracellular environment, where they have roles in cell wall synthesis, nutrient acquisition, and other vital physiological processes. In bacterial pathogens, exported proteins also play critical roles in virulence. Most exported proteins are translocated across the cytoplasmic membrane by the conserved general secretion (Sec) pathway[1], which is present in all bacteria and is essential for viability.

The Sec pathway translocates unfolded proteins through a protein complex in the cytoplasmic membrane comprised of SecY, SecE, and SecG proteins. SecY forms the channel through which unfolded proteins pass[2]. SecE is thought to stabilize the open SecY conformation necessary for translocation[3–6] while SecG increases export efficiency[7, 8]. The SecYEG channel is used in two types of Sec export: post-translational and co-translational. In post-translational Sec export, which is the focus of this review, proteins translocate completely across the cytoplasmic membrane through SecYEG. In co-translational export, SecYEG works with the signal recognition particle (SRP) to insert integral membrane proteins into the cytoplasmic membrane[9] or, in some cases, translocate proteins across the cytoplasmic membrane[10]. For integral membrane proteins, SRP recognizes transmembrane domains as they emerge from the ribosome during translation and targets them as ribosome-mRNA-nascent protein complexes to FtsY for delivery to SecYEG[11]. A lateral gate in SecY is thought to then open and allow passage of transmembrane domains into the membrane[12].

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In post-translational Sec export, the proteins destined for translocation across the cytoplasmic membrane are synthesized as preproteins that are distinguished from the larger pool of cytoplasmic proteins by the presence of N-terminal Sec signal peptides. Sec signal peptides have a positively charged N-terminus, hydrophobic core, and polar C-terminus containing a signal peptidase cleavage site[13]. In addition to the signal peptide, another requirement for Sec export is that the mature portion of the preprotein remains unfolded for competent passage through SecY. Some proteins are recognized and kept unfolded by cytoplasmic chaperones, such as SecB[14, 15], although other preproteins may be unfolded as they are translocated[16, 17].

A central component of the post-translational Sec pathway is the cytosolic SecA motor protein[18], which has a vital role in targeting and powering preprotein transport through SecYEG[19, 20]. Since the discovery of SecA in 1981[21], Sec export has been the focus of extensive study. A combination of genetic, structural and biochemical studies including *in vitro* reconstitution have led to a relatively sophisticated understanding of the Sec pathway and SecA function in particular (Fig. 1a). SecA binds preproteins along a cleft that includes the SecA preprotein-crosslinking (PPXD) domain[22–26], and targets them to the SecYEG complex in the membrane through interactions with SecY[27, 28] and membrane phospholipids[29]. After delivery to SecYEG, the signal peptide of the preprotein inserts into SecY to stabilize an open SecY channel conformation[30–32]. SecA then undergoes cycles of conformational changes coupled to ATP-binding and hydrolysis to drive preproteins through the SecY channel[20, 33]. Several models have been proposed to explain how SecA powers preprotein insertion through SecY[18]. Nonetheless, most models propose that portions of SecA, including the IRA-1 (intramolecular regulator of ATP hydrolysis 1) two-helix finger, insert into SecY to promote forward preprotein motion through the channel. During or immediately following export, the signal peptide is removed by signal peptidases[34] and the mature domain of the protein adopts a folded conformation.

SecA, SecY, and SecE all have essential roles in Sec export and are required for cell viabilty[35, 36]. The SecG component of SecYEG is not essential[37] but increases translocation efficiency, possibly by stabilizing the SecY/E complex[7] or assisting the conformational changes of SecA[8]. Other non-essential membrane-bound proteins that increase Sec export efficiency are SecD, SecF and YajC[37–39].

SecA2

For years, it was thought that all bacterial species had a single SecA protein[40]. However, we now know that a number of bacteria possess two SecA homologs: SecA1 and SecA2. The first example of a second SecA was revealed by the *Mycobacterium tuberculosis* genome sequencing project[41, 42]. It is now recognized that two SecA proteins exist in all mycobacteria and a diverse, but small, set of Gram positive bacteria including *Listeria*, *Staphylococcus*, and some *Streptococcus* species[43]. In bacteria with two SecAs, the two proteins are not interchangeable and each SecA has unique functions[42, 44]. SecA1 is the name given to the SecA with higher sequence similarity to the well-studied SecA proteins of *Escherichia coli* and *Bacillus subtilis*. SecA1 is essential and is responsible for canonical Sec export, as described above[42, 45–48]. Unlike SecA1, SecA2 is responsible for exporting a

smaller set of proteins and often dispensable. Notably, proteins exported by SecA2 are linked to virulence in many bacterial pathogens including *M. tuberculosis*[49–51], *Streptococcus gordonii*[52], *Streptococcus parasanguinis*[53], *Staphylococcus aureus*[54], and *Listeria monocytogenes*[55].

Currently, there are two types of SecA2 systems known to exist. Some bacteria with a SecA2 also have an accessory SecY2 protein. As a consequence, these SecA2/SecY2 systems appear to function largely independent of the canonical Sec machinery to export a set of proteins that are highly glycosylated and incompatible with the canonical SecA1/SecYEG[56, 57] (Fig. 1b). There are also SecA2-only systems, so named because they lack a SecY2 or an obvious accessory membrane channel. SecA2-only systems likely function as part of the canonical Sec pathway, utilizing SecYEG[45, 47] (Fig. 1c). Furthermore, the repertoire of proteins exported by SecA2-only systems is more diverse than that of SecA2/SecY2 systems.

While several published crystal structures for SecA (SecA1) proteins exist[56], including those of *M. tuberculosis*[57], *B. subtilis*[58] and *E. coli*[59], there is no structure available for any SecA2 protein. However, sequence alignments and structural modeling predict that most domains, including PPXD and IRA-1 mentioned above, are conserved between SecA (SecA1) and SecA2 (Fig 2). In addition, all SecA2 proteins have two nucleotide-binding domains (NBD1 and NBD2) which together constitute the DEAD (Asp-Glu-Ala-Asp)-like motor domain. The motor domain contains two ATP-binding Walker boxes and is responsible for ATP hydrolysis[58, 60, 61], suggesting that SecA2 proteins are functional ATPases. In fact, SecA2 from *S. gordonii*[44] and *M. tuberculosis*[62] have demonstrated endogenous ATPase activity *in vitro*. Furthermore, SecA2 ATPase activity is shown to be required for accessory SecA2 protein export in mycobacteria and *C. difficile*[45, 47, 62].

Even though SecA2 proteins of SecA2/SecY2 and SecA2-only systems likely function differently, it is interesting that all SecA2 proteins are smaller than their SecA1 counterparts due to a carboxyl-terminal domain (CTD) truncation, although the boundary of this truncation varies (Fig. 2). In *E. coli*, portions of the SecA CTD binds phospholipids[29], SecB[63–67], and Zn^{+}[29, 66]. One area of the CTD missing in all SecA2 proteins is the C-terminal linker (CTL), which lies within the preprotein-binding cleft and in *E. coli*, has been shown to influence substrate binding[23]. In addition to the CTD truncation, the helical wing domain (HWD) is absent in the mycobacterial SecA2 and truncated in other SecA2 proteins. However, even in the canonical SecA1, the function of the HWD is not clear. The significance of the CTD and HWD truncations in SecA2 proteins awaits further studies.

Our understanding of the mechanisms of canonical Sec export is at an impressive level of detail[1], but by comparison our understanding of accessory SecA2 export is limited. Here, we review the current state of knowledge of SecA2 systems and provide models for both the SecA2/SecY2 and SecA2-only pathways. We also discuss gaps in our knowledge of SecA2 export and how these questions can be addressed by extending approaches used previously to study the general Sec pathway.

SecA2/SecY2 systems

Bacteria with SecA2/SecY2 systems share a conserved *secA2/secY2* genomic locus, comprised of a suite of similarly arranged homologous genes (Fig. 3a). In addition to genes encoding SecA2 and SecY2, each locus contains a gene that encodes a large serine-rich glycosylated protein that is exported by the SecA2/SecY2 system, as well as glycosylation factors that modify this substrate, and additional export machinery with unknown functions. The SecA2/SecY2 systems that are found in a subset of Gram positive species include pathogenic *Streptococcus gordonii*[68], *Streptococcus agalactiae*[69], *Streptococcus parasanguinis*[70], *Streptococcus pneuomoniae*[71], and *Staphylococcus aureus*[72], although it should be noted that not all streptococcal species possess SecA2/SecY2 systems[43]. Another notable Gram positive pathogen with a putative SecA2/SecY2 pathway is *Bacillus anthracis*. However, the *B. anthracis secA2* is phylogenetically more distant from those of *Streptococcus* and *Staphylococcus*[43], which is also reflected by the dissimilar organization of the *Bacillus secA2* locus (Fig. 3a). It will be interesting to determine if *Bacillus* SecA2/SecY2 export functions in the same manner as the *Streptococcus* and *Staphylococcus* systems described here.

The current model of SecA2/SecY2 export suggests that these specialized systems exist to export the large serine-rich protein encoded in the *secA2/secY2* locus. The serine-rich substrates have cleavable N-terminal signal peptides that are unusually long and mature domains that are heavily glycosylated[43]. Examples of experimentally confirmed SecA2/SecY2-exported substrates include GspB of *S. gordonii*[68], Fap1 of *S. parasanguinis*[70], Srr1 of *S. agalactiae*[69], and SraP of *S. aureus*[72]. These substrates have roles related to bacterial adhesion to host tissues and/or biofilm formation[53, 54, 68, 69, 73, 74]. Consequently many of these exported glycoproteins, and presumably their respective SecA2/SecY2 systems, are required for virulence[52, 54, 69, 71]. In *S. parasanguinis*, the FimA adhesin is a second protein whose export is reported to depend on SecA2[70]. However, FimA is not a serine-rich glycoprotein and the *fimA* gene is not at the *S. parasanguinis secA2*/*secY2* locus. It is currently unknown whether FimA is a true SecA2/SecY2 substrate.

SecA2 is not essential for growth in these bacteria; however, SecA2 is absolutely required for export of their respective serine-rich substrates, suggesting a lack of functional redundancy with canonical SecA1. Mutations in *secA2* abolish export of the serine-rich glycosylated substrates of *S. gordonii*[68], *S. parasanguinis*[70], *S. agalactiae*[69], and *S. aureus*[72]. Below, we discuss other genes in the *secA2/secY2* loci that have also been analyzed for roles in glycosylation and/or export.

Glycosylation factors of SecA2/SecY2 systems

In export-defective SecA2/SecY2 mutants, the serine-rich substrate retained in the cytoplasm is glycosylated[70, 75] indicating that the protein is modified by cytoplasmic glycosylation factors prior to export[70, 75]. There are two core glycosyltransferases conserved in all SecA2/SecY2 systems, GtfA and GtfB (Gtf1 and Gtf2) (Fig. 3a). These enzymes are shown to be required for substrate glycosylation in *S. gordonii*[76], *S. agalactiae*[69], and *S. parasanguinis*[77, 78], and in *S. parasanguinis* a Gtf1-Gtf2 interaction is required for substrate glycosylation[77]. Some SecA2/SecY2 systems include additional glycosylation factors that

further modify the substrate prior to export, including Gly and Nss of *S. gordonii*[79], Nss (Gtf3) and GalT1-2 of *S. parasanguinis*[80, 81], and the GtfC-GtfH proteins of *S. agalactiae*[69]. While not identical, the carbohydrate modifications on two SecA2/SecY2 exported proteins, Fap1 and GspB, are primarily composed of N-acetylglucosamine and glucose[82, 83]. In *S. parasanguinis*, Fap1 is modified by O-linked glycosylation[83] but it is unclear if this type of linkage extends to the other SecA2/SecY2 substrates. Furthermore, while the sugar composition of the Fap1 and GspB glycoproteins is known, the number of linkage sites and structure of the glycosyl modifications remains unknown.

Export machinery of SecA2/SecY2 systems

In *S. gordonii*[68] and *S. aureus*[72], *secY2* mutations result in a loss of substrate export that is equivalent to the export defect exhibited by *secA2* mutations, demonstrating that SecY2 is essential for accessory SecA2/SecY2 export in these systems. However, in *S. parasanguinis*[78], deletion of *secY2* has only a modest effect on Fap1 export and the residual exported Fap1 species is incorrectly glycosylated[78]. This result suggests that in the absence of SecY2 and full glycosylation, Fap1 export defaults to the canonical SecA1/SecYEG pathway. This result also suggests that in the *S. parasanguinis* SecA2/SecY2 system, export and glycosylation of Fap1 are coupled (as discussed further below).

There are additional proteins encoded by *secA2*/*secY2* loci that are referred to as accessory secretion proteins (Asps) in *S. gordonii* or glycosylation accessory proteins (Gaps) in *S. parasanguinis*. All SecA2/SecY2 systems include the Asp1, Asp2, and Asp3 proteins (Gap1- 3). While Asp1 and Asp3 are predicted cytosolic proteins, Asp2 may be membrane localized[84]. Some organisms, including *S. gordonii*, have the additional Asp4 and Asp5 (Fig. 3a). Asp4 and Asp5 are both required for GspB export in *S. gordonii*[85] and are predicted integral membrane proteins with sequence homology to *B. subtilis* SecE (17% identity) and SecG (15% identity), respectively. This sequence homology is intriguing, albeit limited, and suggests that Asp4 and Asp5 may be accessory components of a SecY2 membrane channel. However, the exact role of Asp4 and Asp5 in export remains to be defined. Also unknown is whether there are functionally equivalent proteins in SecA2/SecY2 systems lacking Asp4 and Asp5.

With regard to the role(s) of Asps 1–3, findings in different bacterial systems are not in complete agreement. In both *S. aureus* and *S. gordonii*, Asps 1–3 are clearly required for export of the respective serine-rich glycoproteins, SraP and GspB[72, 79]. Additionally, in a glycosyltransferase-deficient *S. gordonii* background, export of the non-glycosylated GspB variant is still compromised in *asp1*, *asp2*, and *asp3* mutants, indicating a role in export that is independent glycosylation for these Asp proteins[84]. By contrast, deletion of either *gap1* or *gap3* in *S. parasanguinis* (the *asp1* and *asp3* homologs) has only a modest effect on export of Fap1, and the residual exported Fap1 protein has altered glycosylation[86, 87]. On the basis of this result, the Asp1 and Asp3 homologs of *S. parasanguinis* were named Gap1 and Gap3 to reflect a proposed function in glycosylation. However, a *secY2* deletion in *S. parasanguinis* results in a phenotype similar to that of the *gap1* and *gap*3 mutants – export of an aberrantly glycosylated Fap1[78, 86, 87]. It seems highly unlikely that SecY2 would have a direct role in protein glycosylation. Additionally, the *S. gordonii* and *S. aureus* studies of

Asp1-3 in SecA2/SecY2 export are compelling. It is possible that the discrepancy in *S. parasanguinis* results is because Fap1 glycosylation and export are highly coupled processes. In this case, export defects of *gap1, gap3* and *secY2* mutants[78, 86, 87] would indirectly affect Fap1 glycosylation to such an extent that the resulting altered Fap1 species is then compatible for export by the canonical SecA1/SecYEG system. However, at this time, a more direct role for Gaps in glycosylation cannot be ruled out.

While currently there is no clear understanding of the function of any of the Asp proteins, a network of interactions between Asp1, Asp2, Asp3 and SecA2 has been mapped in *S. gordonii*[84] and *S. parasanguinis*[87–89]. Asp3 may be a central scaffolding protein in this network as it interacts with multiple members of the SecA2/SecY2 system in *S. gordonii* including Asp1, Asp2, SecA2, and itself[84]. Interestingly, the *S. gordonii* Asp2 and Asp3 proteins also bind the GspB substrate prior to its glycosylation, which suggests a possible function for these proteins in delivering the substrate to an export/glycosylation complex[90]. In *S. parasanguinis*, interactions between Gap1 (Asp1), Gap3 (Asp3), and SecA2 have also been identified[87–89]. In addition, it was recently reported that Gap1 of *S. parasanguinis* stabilizes Gap3 to allow efficient Fap1 export[89].

Targeting proteins to the SecA2/SecY2 pathway

The serine-rich glycoproteins exported by SecA2/SecY2 systems have features that not only prevent their routing to the canonical SecA1/SecYEG pathway but promote their targeting to the SecA2/SecY2 pathway (Fig. 4). The characteristic glycosylation of these exported substrates is one such element[69, 70, 75]. In addition to being important for protein stability[69, 91], glycosylation of these proteins also blocks their export by the canonical Sec pathway in both *S. gordonii* and *S. parasanguinis*[91, 92]. For example, in the absence of *secA2*, the canonical SecA1/SecYEG pathway can export a stable, truncated GspB variant that is non-glycosylated. However, a glycosylated GspB protein cannot utilize the canonical Sec pathway and instead requires SecA2/SecY2 for export[91].

As mentioned above, SecA2/SecY2 serine-rich proteins are glycosylated in the cytoplasm prior to export. This is in contrast to many other glycosylated Sec substrates in bacteria[93, 94], as well as the analogous eukaryotic Sec pathway where glycosylation occurs only after proteins are translocated from the cytosol into the endoplasmic reticulum lumen[95]. However, there is evidence that some bacterial proteins in addition to SecA2/SecY2 substrates share the unusual property of being glycosylated prior to Sec export[96–98], such as the HMW1 adhesin of *Haemophilus influenzae*[96]. But, there is likely a limit to the modifications that the SecA1/SecYEG system can handle, as the level and/or type of glycosylation of SecA2/SecY2 substrates appear incompatible with canonical Sec export. It will be interesting in the future to determine the degree and structure of glycosylation modifications that are incompatible with the SecA1/SecYEG. Additionally, it will be important to understand how these modifications are accommodated by SecA2/SecY2 systems. One possibility is that the pore size of the SecY2 channel is larger than that of SecY1[99] to allow passage of proteins with large glycan branches. A structure of SecY2 would be incredibly valuable for addressing these unknowns.

In addition to glycosylation, there are other features of SecA2/SecY2 substrates that dictate export by the accessory SecA2 pathway. The distinctive long signal peptides of GspB, Fap1, and presumably other SecA2/SecY2 substrates, are absolutely required for export[91, 92, 100]. Furthermore, three glycine residues in the hydrophobic core of the GspB signal peptide promote SecA2/SecY2-dependent export[100]. However, these same glycine residues also act along with glycosylation to block export by the canonical SecA1[100]. The mechanisms by which these glycine residues act in preprotein targeting are currently unknown. Interestingly, these glycine residues are conserved in the signal peptides of most SecA2/SecY2 substrates and could represent a SecA2/SecY2-targeting element that is shared among diverse species.

Finally, there is also a region of approximately 20 amino acids at the start of the mature domain of GspB that is required for targeting this protein to the SecA2/SecY2 system[101]. Introduction of glycine residues into this region can disrupt this SecA2/SecY2-targeting domain, suggesting it may form an alpha-helix[101]. Currently, it is not known if this accessory Sec transport (AST) domain is a conserved feature of all SecA2/SecY2 substrates. However, the first 34 amino acids of the Fap1 mature domain are also required for SecA2-dependent export[92]. In addition to targeting to the SecA2/SecY2 translocase, the AST domain may also stabilize an open SecY2 channel conformation[101].

Model for SecA2/SecY2 export

The current model of SecA2/SecY2 export is as follows (Fig. 1b). The distinctive glycosylation of the serine-rich proteins of SecA2/SecY2 systems is incompatible with export via the canonical SecA1/SecYEG pathway and demands a specialized export system. In a signal peptide-dependent manner, the SecA2/SecY2 preproteins are targeted to the SecA2/SecY2 machinery[91, 92, 100]. Features of the mature domain, such as the AST[101], may also be involved in targeting. In addition, Asp2 and Asp3 could contribute to translocase-targeting by binding the unmodified substrate[90].

Analogous to canonical Sec export, SecA2 likely uses cycles of ATP hydrolysis to drive unfolded, glycosylated preproteins through the SecY2 channel. In some bacteria, Asp4 and Asp5 may function like SecE and SecG, whereas SecA2/SecY2 systems lacking Asp4 and Asp5 could utilize the canonical SecE or SecG for export. In fact, there is some genetic evidence that SecY2 and SecG may function together in *S. aureus*[102]. Also akin to canonical Sec export, experiments using a slow-folding model protein suggest that SecA2/SecY2 preproteins must remain unfolded for passage through the SecY2 channel[101].

At this time, there remain many unknowns regarding the mechanism of SecA2/SecY2 export and glycosylation. What is the sequence of preprotein interactions among the SecA2/SecY2 export and glycosylation machinery? Are glycosylation and export coupled? What are the features of SecA2/SecY2 glycosylation that necessitate export by the SecA2/SecY2 system? Does SecA2 function in targeting and/or translocation across the membrane? What is the role of each Asp protein, and do these roles vary among SecA2/SecY2 systems of different bacteria? *In vitro* glycosylation and translocation systems would help address questions about this new type of export system.

SecA2-only systems

Bacteria with SecA2-only systems lack a SecY2 homolog or obvious accessory membrane channel. The emerging model is that the SecA2 proteins of these systems work with the canonical SecA1/SecYEG translocase. Unlike *secA2*/*secY2* loci, there is no conservation of gene content or organization at the *secA2* genomic region for SecA2-only systems (Fig. 3b). In addition, there is a greater variety in the types of proteins exported by SecA2-only systems when compared to the category of glycosylated serine-rich proteins exported by SecA2/SecY2 systems. SecA2-only systems exist in all mycobacteria, including the human pathogen *M. tuberculosis*[42], as well as some Gram positive bacteria such as *L. monocytogenes*[103], *Corynebacterium glutamicum*[48], and *Clostridium difficile*[47].

In mycobacteria[42, 49, 104] and *Listeria*[103, 105–108], SecA2 is not essential for growth in liquid media and *secA2* mutants are defective in the export of specific proteins. However, *secA2* mutants of both *M. tuberculosis* and *L. monocytogenes* are attenuated for growth in infection models, indicating the importance of the respective SecA2 systems for exporting virulence factors[49, 50, 55]. Additionally, *secA2* mutants of both *M. tuberculosis* and *L. monocytogenes* elicit aberrant immune responses during infection, which has led to the use of these mutants in vaccination studies[50, 109–111]. By contrast, SecA2 is essential for growth of *Corynebacterium glutamicum*[48], and *Clostridium difficile*[47].

SecA2-only exported substrates

Proteins exported by SecA2-only systems have been identified in the model organism *M. smegmatis* (a nonpathogenic mycobacterial species), *M. tuberculosis*, *L. monocytogenes*, and *C. difficile*. Some of these proteins have predicted N-terminal Sec signal peptides and some do not. In *M. smegmatis* there are two lipoproteins (Ms1704 and Ms1712) with predicted N-terminal Sec signal peptides that are exported to the cell wall in a SecA2-dependent manner[45, 112]. Ms1704 and Ms1712 are homologous and both are predicted ABC type sugar-binding proteins of putative ABC transporters[112]. However, it is important to note that not all mycobacterial lipoproteins require SecA2 for export[112].

In *M. tuberculosis* and *L. monocytogenes*, several proteins are reduced in exported fractions of *secA2* mutant bacteria analyzed by 2D-PAGE, 3 and 17 respectively[49, 55]. Of these proteins, only a few have been studied further and confirmed to be SecA2-dependent. In *M. tuberculosis*, one of these proteins is superoxide dismutase SodA, which notably lacks a predicted cleavable Sec signal peptide. There are likely additional SecA2-dependent proteins in *M. tuberculosis* because SecA2 is required to block phagosome maturation and the SecA2-dependent effectors of this process are unknown[51].

In *L. monocytogenes* the p60 autolysin, which is a cell wall hydrolase that cleaves peptidoglycan, is a confirmed SecA2 substrate[55]. The gene for p60 is positioned adjacent to *secA2* in the genome (Fig. 3b); although, other SecA2-dependent proteins of *Listeria* are encoded elsewhere. An additional peptidoglycan-hydrolyzing autolysin NamA (MurA) of *Listeria* is also SecA2-dependent[55, 106, 107]. However, unlike p60, NamA lacks a typical Sec signal peptide. MnSod superoxide dismutase is another protein lacking a predicted Sec signal peptide that is exported in a SecA2-dependent manner in *L. monocytogenes*[113]. This

particular finding parallels the SecA2-dependence of SodA export in *M. tuberculosis* and suggests that other similarities may exist between the mycobacteria and *Listeria* SecA2-only systems. Also among the list of SecA2-dependent proteins identified in *L. monocytogenes* are four predicted lipoproteins with Sec signal sequences[55]. One of these lipoproteins is a predicted ABC type sugar-binding protein[55], which is in the same family as the Ms1704 and Ms1712 substrates of *M. smegmatis*[112].

In *C. difficile* the S-layer protein SlpA,[47] which constitutes a proteinaceous lattice structure surrounding the *Clostridium* cell[114] has been identified as being exported in a SecA2-dependent fashion. SlpA is a member of a larger family of 29 clostridial cell wall proteins (Cwp)[115] that are implicated in host-pathogen interactions[116–118]. CwpV is another protein shown to require SecA2 for export[47], suggesting that additional members of this Cwp family may be SecA2-dependent as well. In *C. difficile*, the *secA2* gene is adjacent to *slpA* and the larger *secA2* genomic region includes genes encoding 12 Cwps (Fig. 3b)[47]. However, the gene encoding CwpV is notably located elsewhere in the genome[47]. In *C. difficile*, both of the demonstrated SecA2-dependent proteins (SlpA and CwpV) contain predicted N-terminal Sec signal peptides[115].

Export machinery of SecA2-only systems

SecA2-only systems lack an obvious alternative membrane channel and accessory export factors. An attractive idea is that SecA2 works with the canonical SecA1/SecYEG machinery either through cooperation with SecA1 or by sharing SecYEG. In support of this model, depletion of the essential SecA1 protein in *M. smegmatis* abolishes export of the SecA2 substrate Ms1712[45]. The simplest interpretation of this experiment is that mycobacterial SecA2 export requires the canonical SecA1. However, it remains possible that SecA1 depletion in this experiment has an indirect effect on SecA2 export.

In mycobacteria and *C. difficile*, studies using ATPase-defective dominant negative SecA2 proteins are also consistent with a model where SecA2 works with the canonical Sec machinery[45, 47]. Dominant negative proteins often exert their effect by forming nonfunctional complexes with their normal binding partners. In mycobacteria, overexpression of the dominant negative SecA2 inhibits growth[45]. This result implies an interaction between SecA2 and proteins important to an essential process, with the essential SecA1/SecYEG machinery being a leading candidate. In *C. difficile*, expression of the corresponding dominant negative SecA2 also inhibits growth and over shorter time frames is shown to impact protein export[47]. Importantly, overexpression of a dominant negative SecA1 in *C. difficile* reduces export of SecA2 substrates, possibly by blocking accessibility of the SecA2 substrates to the SecYEG channel[47]. However, unlike in mycobacteria, depletion of SecA1 in *C. difficile* does not influence export of SecA2 substrates[47] suggesting that in *Clostridium* SecA2 works with SecYEG but not SecA1.

There has not been a similar investigation for a relationship between SecA2 and SecA1/SecYEG in *Listeria*, but recently it was shown that secretion of the SecA2-dependent proteins p60 and NamA depends on the DivIVA protein[105]. The DivIVA protein is involved in localizing proteins to the cell poles and septa of bacteria[119]. Interestingly, GFP fusions to DivIVA, SecA2, p60, and NamA all localize to the septum in *Listeria*[105]. Thus, it is possible

that the SecA2-only system is specifically localized and DivIVA is required to either establish that localization pattern or deliver the SecA2-dependent proteins to the SecA2 machinery.

Targeting proteins to the SecA2-only pathway

The features defining exported substrates of SecA2-only systems have not yet received significant attention. The two *M. smegmatis* lipoproteins that require SecA2 for export contain typical N-terminal Sec signal peptides. While these signal peptides are required for export[112], they are not specific for targeting these proteins to SecA2 (Feltcher and Braunstein, unpublished). Therefore, there appears to be one or more features of the mature domains that determine SecA2-dependent export in mycobacteria. The mature domain of these SecA2-dependent substrates could possess post-translational modifications, as seen with the SecA2/SecY2 substrates, although there is currently no evidence for this possibility.

Model for SecA2-only Export

For SecA2-only systems, genetic studies support a model where SecA2 utilizes SecYEG to assist in Sec export of a certain class of preproteins[45, 47](Fig. 1c). These SecA2 substrates must have features, which are not understood at this time, that make them incompatible for Sec export without the assistance of SecA2. SecA2 of both mycobacteria and *Clostridium* primarily localize to the cytoplasm, while much of SecA1 is found at the membrane in these bacteria[45, 47]. Thus, SecA2 could possibly function in the cytoplasm to recognize and target for export a specific subset of proteins that are otherwise overlooked or incompatible with the canonical SecA1. Alternatively, SecA2 could serve as an alternate motor protein that is necessary for translocation of certain proteins through SecYEG. In either case, SecA2 ATPase activity is required for export.

Application of an *in vitro* translocation system would help strengthen this developing model and address basic questions about the operation of SecA2-only systems. Is there a role for SecA1 during SecA2-only export? What prevents the substrates of SecA2-only systems from being exported solely by SecA1/SecYEG system and how does SecA2 recognize these proteins? Does SecA2 function in targeting and/or translocation?

Another unresolved issue is whether preproteins without recognizable Sec signal peptides, like the Sod proteins of *M. tuberculosis* and *L. monocytogenes*, are true SecA2 substrates or if the export of these proteins is indirectly affected by SecA2. For example, SecA2 could export a currently unknown protein with a signal peptide that is itself required for export of proteins like Sod. Still, it is also plausible that proteins without signal peptides are recognized by SecA2 and exported directly through the SecYEG channel. In support of this model, SodA of *Rhizobium leguminosarum* is exported in a SecA-dependent manner despite lacking a recognizable signal peptide[120]. However, similar to *M. smegmatis*[45] and *C. difficile*[47] studies, an indirect role for the Sec system in exporting SodA of *R. leguminosarum* cannot be ruled out.

Future directions

While it is now clear that SecA2 systems are critical for exporting specific proteins, surprisingly little is known about how these export systems function. The canonical Sec pathway is a well characterized process resulting from years of extensive study; however, the acquisition of SecA2 proteins in some bacteria suggests limitations to SecA1 and SecYEG function. The same structural, biochemical and *in vitro* reconstitution analyses used to understand canonical Sec export will surely prove valuable in answering the many mechanistic questions that exist about the SecA2/SecY2 and SecA2-only systems.

Structural regions of difference between SecA1 and SecA2 proteins will be of obvious interest to investigate. It will also be informative to compare SecA2 structures from SecA2/SecY2 systems to those of SecA2-only systems. Surprisingly, the predicted domain organization of SecA2 proteins from SecA2/SecY2 systems and SecA2-only systems is similar, despite the known differences between these two types of SecA2 export pathways. Given the possibility that SecA2 proteins might be specifically adapted to accommodate binding of certain glycosylated preproteins or proteins lacking signal peptides, it will be particularly interesting to compare the architecture of SecA2 preprotein-binding clefts to the corresponding regions of SecA1 proteins. Also of interest will be the SecA2 IRA-1 two-helix finger domain[120, 121]. The amino acid at the tip of the two- helix finger, which contacts the preprotein, is not conserved between SecA1 and SecA2 proteins[121]. This could reflect unique interactions between SecA2 proteins and their respective substrates or interactions with a unique SecY2 channel.

Finally, although there remains controversy about the multimeric state of the canonical SecA during export[56], it will be interesting to establish whether SecA2 forms and functions as dimers. Heterodimers of SecA1 and SecA2 could possibly explain the roles of both these SecA proteins in export of some mycobacterial substrates[45]. In fact, SecA2 can bind SecA1 in *S. parasanguinis*[88], but it is unknown if this interaction has any biological function in export.

Accessory SecA2 systems make significant contributions to protein export in a diverse set of bacteria. Future research should apply the same depth of structural and biochemical studies to SecA2, as done for the canonical Sec pathway. A deeper understanding of accessory SecA2 systems will reveal additional mechanisms used by bacteria to transport proteins across the cytoplasmic membrane, and it is also likely to reveal unappreciated limitations of canonical Sec export.

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Glossary

***In vitro* reconstitution**	A technique for studying biochemical processes *in vitro*. *In vitro* reconstitution of the Sec pathway involves incubation of preprotein and purified SecA with SecYEG-containing inverted membrane vesicles (IMV). Preprotein translocation through SecYEG into the IMV is monitored by the loss of preprotein sensitivity to protease.
Preproteins	Proteins synthesized with N-terminal Sec signal peptides for targeting to the Sec machinery.
Signal peptide	An N-terminal amino acid sequence present on preproteins. The signal peptide helps target specific proteins for export out of the bacterial cytoplasm. Sec signal peptides are comprised of a tripartite structure with a positively charged N-terminus, hydrophobic core, and signal peptidase cleavage site.
Glycosyltransferase	An enzyme that catalyzes the post-translational addition of carbohydrate mono- or oligosaccharides to an acceptor molecule. Some glycosyltransferases attach sugars to proteins, resulting in glycoproteins. The sugar composition and structure of glycosyl modifications can be diverse.
N-linked glycosylation	A type of glycosylation where a saccharide moiety is added to the to the amide nitrogen of asparagine.
O-linked glycosylation	A type of glycosylation where a saccharide moiety is added to the hydroxyl oxygen of serine or threonin.
Lipobox	An amino acid motif found in the signal peptidase cleavage site of bacterial lipoproteins. The lipobox motif contains an invariant cysteine that becomes the first amino acid of the mature protein after signal peptidase cleavage and is the site of diacylglycerol attachment.
Autolysin	Hydrolases that break the peptidoglycan matrix in the bacterial cell wall. Autolysins are important in bacterial cell growth and division.
S-layer	An ordered array of protein subunits that form a lattice structure surrounding the bacterial cell wall. S-layers exist in many Gram-positive and Gram-negative bacteria, as well as Archaea. S-layers serve as scaffolding structures for enzymes, contribute to cell surface adhesion, and act as virulence factors among other functions.
Phagosome maturation	Phagosomes are vacuoles that form around foreign particles or bacteria during phagocytosis by eukaryotic cells. The phagosome undergoes multiple maturation events and ultimately

fuses with lysosomes resulting in a degradative phagolysosomal compartment.

Biographies

Meghan Feltcher

Meghan Feltcher obtained a B.S. in genetic engineering from Cedar Crest College. She is currently a Ph.D. candidate in the Department of Microbiology and Immunology at the University of North Carolina in Chapel Hill. Working in the laboratory of Dr. Miriam Braunstein, her graduate work is focused on defining mechanisms of SecA2 export in mycobacteria and identifying novel SecA2 substrates in *M tuberculosis*.

Miriam Braunstein

Miriam Braunstein is an associate professor in the Department of Microbiology and Immunology at the University of North Carolina at Chapel Hill. She obtained a B.S. in biology from Tufts University and went on to receive M.A. and Ph.D. degrees in molecular biology from Princeton University. Her Ph.D. research was conducted with Dr. James Broach and focused on the role of histone acetylation and the Sir2 deacetylase on gene expression in *Saccharomyces cerevisiae*. Her postdoctoral studies were conducted with Dr. William Jacobs Jr. at the Albert Einstein College of Medicine, where she began studying the protein export systems of *M. tuberculosis*. In 2001, she took an assistant professor position at the University of North Carolina Chapel Hill. Her laboratory studies conserved and specialized protein export systems of *M. tuberculosis*. Her research group is also working to identify *M. tuberculosis* proteins secreted during host infection and determine the functional significance of these proteins in virulence.

References

1. du Plessis DJ, Nouwen N, Driessen AJ. The Sec translocase. Biochim Biophys Acta. 2011; 1808:851–865. [PubMed: 20801097]
2. Van den Berg B, et al. X-ray structure of a protein-conducting channel. Nature. 2004; 427:36–44. [PubMed: 14661030]
3. Taura T, Baba T, Akiyama Y, Ito K. Determinants of the quantity of the stable SecY complex in the *Escherichia coli* cell. J Bacteriol. 1993; 175:7771–7775. [PubMed: 8253665]
4. Harris CR, Silhavy TJ. Mapping an interface of SecY (PrlA) and SecE (PrlG) by using synthetic phenotypes and in vivo cross-linking. J Bacteriol. 1999; 181:3438–3444. [PubMed: 10348856]
5. Tam PC, Maillard AP, Chan KK, Duong F. Investigating the SecY plug movement at the SecYEG translocation channel. EMBO J. 2005; 24:3380–3388. [PubMed: 16148946]
6. Duong F, Wickner W. The PrlA and PrlG phenotypes are caused by a loosened association among the translocase SecYEG subunits. EMBO J. 1999; 18:3263–3270. [PubMed: 10369667]
7. Nishiyama K, Mizushima S, Tokuda H. Preferential interaction of Sec-G with Sec-E stabilizes an unstable Sec-E derivative in the *Escherichia coli* cytoplasmic membrane. Biochem Biophys Res Commun. 1995; 217:217–223. [PubMed: 8526914]
8. Nishiyama K, Suzuki T, Tokuda H. Inversion of the membrane topology of SecG coupled with SecA-dependent preprotein translocation. Cell. 1996; 85:71–81. [PubMed: 8620539]
9. Xie K, Dalbey RE. Inserting proteins into the bacterial cytoplasmic membrane using the Sec and YidC translocases. Nat Rev Microbiol. 2008; 6:234–244. [PubMed: 18246081]

10. Shimohata N, Akiyama Y, Ito K. Peculiar properties of DsbA in its export across the *Escherichia coli* cytoplasmic membrane. J Bacteriol. 2005; 187:3997–4004. [PubMed: 15937162]
11. Valent QA, et al. Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and trigger factor. Mol Microbiol. 1997; 25:53–64. [PubMed: 11902726]
12. Egea PF, Stroud RM. Lateral opening of a translocon upon entry of protein suggests the mechanism of insertion into membranes. Proc Natl Acad Sci U S A. 2010; 107:17182–17187. [PubMed: 20855604]
13. von Heijne G. The signal peptide. J Membr Biol. 1990; 115:195–201. [PubMed: 2197415]
14. Bechtluft P, Nouwen N, Tans SJ, Driessen AJ. SecB- -a chaperone dedicated to protein translocation. Mol Biosyst. 2010; 6:620–627. [PubMed: 20237639]
15. Fisher AC, DeLisa MP. A little help from my friends: quality control of presecretory proteins in bacteria. J Bacteriol. 2004; 186:7467–7473. [PubMed: 15516557]
16. Arkowitz RA, Joly JC, Wickner W. Translocation Can Drive the Unfolding of a Preprotein Domain. Embo Journal. 1993; 12:243–253. [PubMed: 8428582]
17. Nouwen N, Berrelkamp G, Driessen AJ. Bacterial sec-translocase unfolds and translocates a class of folded protein domains. J Mol Biol. 2007; 372:422–433. [PubMed: 17669421]
18. Kusters I, Driessen AJ. SecA, a remarkable nanomachine. Cell Mol Life Sci. 2011; 68:2053–2066. [PubMed: 21479870] **A comprehensive review on the structure and function of SecA proteins**.
19. Brundage L, Hendrick JP, Schiebel E, Driessen AJ, Wickner W. The purified *E. coli* integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation. Cell. 1990; 62:649–657. [PubMed: 2167176]
20. Economou A, Wickner W. SecA promotes preprotein translocation by undergoing ATP-driven cycles of membrane insertion and deinsertion. Cell. 1994; 78:835–843. [PubMed: 8087850]
21. Oliver DB, Beckwith J. *E. coli* mutant pleiotropically defective in the export of secreted proteins. Cell. 1981; 25:765–772. [PubMed: 7026050] **References 19, 20, and 21 are among a large collection of seminal papers describing SecA-dependent protein export**.
22. Papanikou E, et al. Identification of the preprotein binding domain of SecA. J Biol Chem. 2005; 280:43209–43217. [PubMed: 16243836]
23. Gelis I, et al. Structural basis for signal-sequence recognition by the translocase motor SecA as determined by NMR. Cell. 2007; 131:756–769. [PubMed: 18022369]
24. Musial-Siwek M, Rusch SL, Kendall DA. Selective photoaffinity labeling identifies the signal peptide binding domain on SecA. Journal of Molecular Biology. 2007; 365:637–648. [PubMed: 17084862]
25. Auclair SM, et al. Mapping of the signal peptide-binding domain of *Escherichia coli* SecA using Forster resonance energy transfer. Biochemistry. 2010; 49:782–792. [PubMed: 20025247]
26. Kimura E, Akita M, Matsuyama S, Mizushima S. Determination of a region in SecA that interacts with presecretory proteins in *Escherichia coli* . J Biol Chem. 1991; 266:6600–6606. [PubMed: 1826108]
27. Mori H, Ito K. Different modes of SecY-SecA interactions revealed by site-directed in vivo photo-cross-linking. Proc Natl Acad Sci U S A. 2006; 103:16159–16164. [PubMed: 17060619]
28. van der Sluis EO, et al. Identification of two interaction sites in SecY that are important for the functional interaction with SecA. J Mol Biol. 2006; 361:839–849. [PubMed: 16890955]
29. Breukink E, et al. The C terminus of SecA is involved in both lipid binding and SecB binding. J Biol Chem. 1995; 270:7902–7907. [PubMed: 7713885]
30. Li W, et al. The plug domain of the SecY protein stabilizes the closed state of the translocation channel and maintains a membrane seal. Molecular Cell. 2007; 26:511–521. [PubMed: 17531810]
31. Smith MA, Clemons WM Jr, DeMars CJ, Flower AM. Modeling the effects of prl mutations on the *Escherichia coli* SecY complex. J Bacteriol. 2005; 187:6454–6465. [PubMed: 16159779]
32. Gouridis G, Karamanou S, Gelis I, Kalodimos CG, Economou A. Signal peptides are allosteric activators of the protein translocase. Nature. 2009; 462:363–367. [PubMed: 19924216]

33. van der Wolk JP, de Wit JG, Driessen AJ. The catalytic cycle of the *Escherichia coli* SecA ATPase comprises two distinct preprotein translocation events. EMBO J. 1997; 16:7297–7304. [PubMed: 9405359]
34. Paetzel M, Karla A, Strynadka NC, Dalbey RE. Signal peptidases. Chem Rev. 2002; 102:4549–4580. [PubMed: 12475201]
35. Oliver D, Kumamoto C, Quinlan M, Beckwith J. Pleiotropic mutants affecting the secretory apparatus of *Escherichia coli* . Annales De Microbiologie. 1982; A133:105–110. [PubMed: 6803638]
36. Murphy CK, Stewart EJ, Beckwith J. A double counter-selection system for the study of null alleles of essential genes in *Escherichia coli* . Gene. 1995; 155:1–7. [PubMed: 7698651]
37. Nishiyama K, Hanada M, Tokuda H. Disruption of the gene encoding p12 (SecG) reveals the direct involvement and important function of SecG in the protein translocation of *Escherichia coli* at low temperature. EMBO J. 1994; 13:3272–3277. [PubMed: 8045257]
38. Pogliano JA, Beckwith J. SecD and SecF facilitate protein export in *Escherichia coli* . EMBO J. 1994; 13:554–561. [PubMed: 8313900]
39. Duong F, Wickner W. Distinct catalytic roles of the SecYE, SecG and SecDFYajC subunits of preprotein translocase holoenzyme. EMBO J. 1997; 16:2756–2768. [PubMed: 9184221]
40. Economou A. Following the leader: bacterial protein export through the Sec pathway. Trends Microbiol. 1999; 7:315–320. [PubMed: 10431204]
41. Cole ST, et al. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature. 1998; 393:537–544. [PubMed: 9634230]
42. Braunstein M, Brown AM, Kurtz S, Jacobs WR Jr. Two nonredundant SecA homologues function in mycobacteria. J Bacteriol. 2001; 183:6979–6990. [PubMed: 11717254]
43. Rigel NW, Braunstein M. A new twist on an old pathway--accessory Sec systems. Mol Microbiol. 2008; 69:291–302. [PubMed: 18544071] **A review of SecA2 export that includes a phylogenetic tree depicting the evolutionary relationship of SecA2 proteins from both SecA2-only and SecA2/SecY2 systems.**
44. Bensing BA, Sullam PM. Characterization of *Streptococcus gordonii* SecA2 as a paralogue of SecA. J Bacteriol. 2009; 191:3482–3491. [PubMed: 19363114]
45. Rigel NW, et al. The accessory SecA2 system of mycobacteria requires ATP binding and the canonical SecA1. J Biol Chem. 2009; 284:9927–9936. [PubMed: 19240020] **The first demonstration that a SecA2-only system requires one or more components of the canonical Sec pathway, which was later confirmed in *C. difficile* in reference 47**.
46. Guo XV, et al. Silencing *Mycobacterium smegmatis* by using tetracycline repressors. J Bacteriol. 2007; 189:4614–4623. [PubMed: 17483222]
47. Fagan RP, Fairweather NF. *Clostridium difficile* has two parallel and essential Sec secretion systems. J Biol Chem. 2011; 286:27483–27493. [PubMed: 21659510]
48. Caspers M, Freudl R. *Corynebacterium glutamicum* possesses two *secA* homologous genes that are essential for viability. Arch Microbiol. 2008; 189:605–610. [PubMed: 18246326]
49. Braunstein M, Espinosa BJ, Chan J, Belisle JT, Jacobs WR Jr. SecA2 functions in the secretion of superoxide dismutase A and in the virulence of *Mycobacterium tuberculosis* . Mol Microbiol. 2003; 48:453–464. [PubMed: 12675804]
50. Kurtz S, McKinnon KP, Runge MS, Ting JP, Braunstein M. The SecA2 secretion factor of *Mycobacterium tuberculosis* promotes growth in macrophages and inhibits the host immune response. Infect Immun. 2006; 74:6855–6864. [PubMed: 17030572]
51. Sullivan JT, Young EF, McCann JR, Braunstein M. The *Mycobacterium tuberculosis* SecA2 System Subverts Phagosome Maturation to Promote Growth in Macrophages. Infect Immun. 2012
52. Xiong YQ, Bensing BA, Bayer AS, Chambers HF, Sullam PM. Role of the serine-rich surface glycoprotein GspB of *Streptococcus gordonii* in the pathogenesis of infective endocarditis. Microb Pathog. 2008; 45:297–301. [PubMed: 18656529]
53. Wu H, Mintz KP, Ladha M, Fives-Taylor PM. Isolation and characterization of Fap1, a fimbriae-associated adhesin of *Streptococcus parasanguis* FW213. Mol Microbiol. 1998; 28:487–500. [PubMed: 9632253] **References 52 and 53 show that SecA2/SecY2 systems export proteins that are first glycosylated in the cytoplasm**.

54. Siboo IR, Chambers HF, Sullam PM. Role of SraP, a Serine-Rich Surface Protein of *Staphylococcus aureus*, in binding to human platelets. Infect Immun. 2005; 73:2273–2280. [PubMed: 15784571]
55. Lenz LL, Mohammadi S, Geissler A, Portnoy DA. SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. Proc Natl Acad Sci U S A. 2003; 100:12432–12437. [PubMed: 14527997]
56. Sardis MF, Economou A. SecA: a tale of two protomers. Mol Microbiol. 2010; 76:1070–1081. [PubMed: 20444093]
57. Sharma V, et al. Crystal structure of *Mycobacterium tuberculosis* SecA, a preprotein translocating ATPase. Proc Natl Acad Sci U S A. 2003; 100:2243–2248. [PubMed: 12606717]
58. Hunt JF, et al. Nucleotide control of interdomain interactions in the conformational reaction cycle of SecA. Science. 2002; 297:2018–2026. [PubMed: 12242434]
59. Papanikolau Y, et al. Structure of dimeric SecA, the *Escherichia coli* preprotein translocase motor. J Mol Biol. 2007; 366:1545–1557. [PubMed: 17229438]
60. Sianidis G, et al. Cross-talk between catalytic and regulatory elements in a DEAD motor domain is essential for SecA function. EMBO J. 2001; 20:961–970. [PubMed: 11230120]
61. Keramisanou D, et al. Disorder-order folding transitions underlie catalysis in the helicase motor of SecA. Nat Struct Mol Biol. 2006; 13:594–602. [PubMed: 16783375]
62. Hou JM, et al. ATPase activity of *Mycobacterium tuberculosis* SecA1 and SecA2 proteins and its importance for SecA2 function in macrophages. J Bacteriol. 2008; 190:4880–4887. [PubMed: 18487341]
63. Fekkes P, et al. Preprotein transfer to the *Escherichia coli* translocase requires the co-operative binding of SecB and the signal sequence to SecA. Mol Microbiol. 1998; 29:1179–1190. [PubMed: 9767586]
64. Fekkes P, van der Does C, Driessen AJ. The molecular chaperone SecB is released from the carboxy-terminus of SecA during initiation of precursor protein translocation. EMBO J. 1997; 16:6105–6113. [PubMed: 9321390]
65. Zhou J, Xu Z. Structural determinants of SecB recognition by SecA in bacterial protein translocation. Nat Struct Biol. 2003; 10:942–947. [PubMed: 14517549]
66. Fekkes P, de Wit JG, Boorsma A, Friesen RH, Driessen AJ. Zinc stabilizes the SecB binding site of SecA. Biochemistry. 1999; 38:5111–5116. [PubMed: 10213615]
67. van Wely KH, Swaving J, Freudl R, Driessen AJ. Translocation of proteins across the cell envelope of Gram-positive bacteria. FEMS Microbiol Rev. 2001; 25:437–454. [PubMed: 11524133]
68. Bensing BA, Sullam PM. An accessory sec locus of *Streptococcus gordonii* is required for export of the surface protein GspB and for normal levels of binding to human platelets. Mol Microbiol. 2002; 44:1081–1094. [PubMed: 12010500]
69. Mistou MY, Dramsi S, Brega S, Poyart C, Trieu-Cuot P. Molecular dissection of the *secA2* locus of group B Streptococcus reveals that glycosylation of the Srr1 LPXTG protein is required for full virulence. J Bacteriol. 2009; 191:4195–4206. [PubMed: 19395494]
70. Chen Q, Wu H, Fives-Taylor PM. Investigating the role of *secA2* in secretion and glycosylation of a fimbrial adhesin in *Streptococcus parasanguis* FW213. Mol Microbiol. 2004; 53:843–856. [PubMed: 15255897]
71. Obert C, et al. Identification of a candidate *Streptococcus pneumoniae* core genome and regions of diversity correlated with invasive pneumococcal disease. Infect Immun. 2006; 74:4766–4777. [PubMed: 16861665]
72. Siboo IR, Chaffin DO, Rubens CE, Sullam PM. Characterization of the accessory Sec system of *Staphylococcus aureus* . J Bacteriol. 2008; 190:6188–6196. [PubMed: 18621893]
73. Samen U, Eikmanns BJ, Reinscheid DJ, Borges F. The surface protein Srr-1 of *Streptococcus agalactiae* binds human keratin 4 and promotes adherence to epithelial HEp-2 cells. Infect Immun. 2007; 75:5405–5414. [PubMed: 17709412]
74. Wu H, Fives-Taylor PM. Identification of dipeptide repeats and a cell wall sorting signal in the fimbriae-associated adhesin, Fap1, of *Streptococcus parasanguis* . Mol Microbiol. 1999; 34:1070–1081. [PubMed: 10594831]

75. Bensing BA, Gibson BW, Sullam PM. The *Streptococcus gordonii* platelet binding protein GspB undergoes glycosylation independently of export. J Bacteriol. 2004; 186:638–645. [PubMed: 14729688]
76. Takamatsu D, Bensing BA, Sullam PM. Four proteins encoded in the *gspB secY2A2* operon of *Streptococcus gordoni*i mediate the intracellular glycosylation of the platelet-binding protein GspB. J Bacteriol. 2004; 186:7100–7111. [PubMed: 15489421]
77. Bu S, et al. Interaction between two putative glycosyltransferases is required for glycosylation of a serine-rich streptococcal adhesin. J Bacteriol. 2008; 190:1256–1266. [PubMed: 18083807]
78. Wu H, Bu S, Newell P, Chen Q, Fives-Taylor P. Two gene determinants are differentially involved in the biogenesis of Fap1 precursors in *Streptococcus parasanguis* . J Bacteriol. 2007; 189:1390–1398. [PubMed: 16997950]
79. Takamatsu D, Bensing BA, Sullam PM. Genes in the accessory sec locus of *Streptococcus gordonii* have three functionally distinct effects on the expression of the platelet-binding protein GspB. Mol Microbiol. 2004; 52:189–203. [PubMed: 15049820]
80. Zhou M, Zhu F, Dong S, Pritchard DG, Wu H. A novel glucosyltransferase is required for glycosylation of a serine-rich adhesin and biofilm formation by *Streptococcus parasanguinis* . J Biol Chem. 2010; 285:12140–12148. [PubMed: 20164186]
81. Wu H, Zeng M, Fives-Taylor P. The glycan moieties and the N-terminal polypeptide backbone of a fimbria-associated adhesin, Fap1, play distinct roles in the biofilm development of *Streptococcus parasanguinis* . Infect Immun. 2007; 75:2181–2188. [PubMed: 17296746]
82. Bensing BA, Lopez JA, Sullam PM. The *Streptococcus gordonii* surface proteins GspB and Hsa mediate binding to sialylated carbohydrate epitopes on the platelet membrane glycoprotein Ibalpha. Infect Immun. 2004; 72:6528–6537. [PubMed: 15501784]
83. Stephenson AE, et al. The Fap1 fimbrial adhesin is a glycoprotein: antibodies specific for the glycan moiety block the adhesion of *Streptococcus parasanguis* in an in vitro tooth model. Mol Microbiol. 2002; 43:147–157. [PubMed: 11849543]
84. Seepersaud R, Bensing BA, Yen YT, Sullam PM. Asp3 mediates multiple protein-protein interactions within the accessory Sec system of *Streptococcus gordonii*. Mol Microbiol. 2010; 78:490–505. [PubMed: 20807195] **Demonstrates that glycosylation of a SecA2/SecY2 substrate blocks export by the canonical SecA1/SecYEG**.
85. Takamatsu D, Bensing BA, Sullam PM. Two additional components of the accessory sec system mediating export of the *Streptococcus gordonii* platelet-binding protein GspB. J Bacteriol. 2005; 187:3878–3883. [PubMed: 15901716]
86. Peng Z, et al. Role of *gap3* in Fap1 glycosylation, stability, in vitro adhesion, and fimbrial and biofilm formation of *Streptococcus parasanguinis* . Oral Microbiol Immunol. 2008; 23:70–78. [PubMed: 18173801]
87. Li Y, et al. A conserved domain of previously unknown function in Gap1 mediates protein-protein interaction and is required for biogenesis of a serine-rich streptococcal adhesin. Mol Microbiol. 2008; 70:1094–1104. [PubMed: 18826412]
88. Zhou M, Zhang H, Zhu F, Wu H. Canonical SecA associates with an accessory secretory protein complex involved in biogenesis of a streptococcal serine-rich repeat glycoprotein. J Bacteriol. 2011; 193:6560–6566. [PubMed: 21965576]
89. Zhou M, Zhu F, Li Y, Zhang H, Wu H. Gap1 functions as a molecular chaperone to stabilize its interactive partner Gap3 during biogenesis of serine-rich repeat bacterial adhesin. Mol Microbiol. 2012; 83:866–878. [PubMed: 22251284]
90. Yen YT, Seepersaud R, Bensing BA, Sullam PM. Asp2 and Asp3 interact directly with GspB, the export substrate of the *Streptococcus gordonii* accessory Sec System. J Bacteriol. 2011; 193:3165–3174. [PubMed: 21531800]
91. Bensing BA, Takamatsu D, Sullam PM. Determinants of the streptococcal surface glycoprotein GspB that facilitate export by the accessory Sec system. Mol Microbiol. 2005; 58:1468–1481. [PubMed: 16313630]
92. Chen Q, Sun B, Wu H, Peng Z, Fives-Taylor PM. Differential roles of individual domains in selection of secretion route of a *Streptococcus parasanguinis* serine-rich adhesin, Fap1. J Bacteriol. 2007; 189:7610–7617. [PubMed: 17766425]

93. Nita-Lazar M, Wacker M, Schegg B, Amber S, Aebi M. The N-X-S/T consensus sequence is required but not sufficient for bacterial N-linked protein glycosylation. Glycobiology. 2005; 15:361–367. [PubMed: 15574802]

94. VanderVen BC, Harder JD, Crick DC, Belisle JT. Export-mediated assembly of mycobacterial glycoproteins parallels eukaryotic pathways. Science. 2005; 309:941–943. [PubMed: 16081738]

95. Schwarz F, Aebi M. Mechanisms and principles of N-linked protein glycosylation. Curr Opin Struct Biol. 2011; 21:576–582. [PubMed: 21978957]

96. Grass S, et al. The *Haemophilus influenzae* HMW1 adhesin is glycosylated in a process that requires HMW1C and phosphoglucomutase, an enzyme involved in lipooligosaccharide biosynthesis. Mol Microbiol. 2003; 48:737–751. [PubMed: 12694618]

97. Choi KJ, Grass S, Paek S, St Geme JW 3rd, Yeo HJ. The *Actinobacillus pleuropneumoniae* HMW1C–like glycosyltransferase mediates N-linked glycosylation of the *Haemophilus influenzae* HMW1 adhesin. PLoS One. 2010; 5:e15888. [PubMed: 21209858]

98. Charbonneau ME, Mourez M. The *Escherichia coli* AIDA-I autotransporter undergoes cytoplasmic glycosylation independently of export. Res Microbiol. 2008; 159:537–544. [PubMed: 18657609]

99. Bonardi F, et al. Probing the SecYEG translocation pore size with preproteins conjugated with sizable rigid spherical molecules. Proc Natl Acad Sci U S A. 2011; 108:7775–7780. [PubMed: 21518907]

100. Bensing BA, Siboo IR, Sullam PM. Glycine residues in the hydrophobic core of the GspB signal sequence route export toward the accessory Sec pathway. J Bacteriol. 2007; 189:3846–3854. [PubMed: 17369296]

101. Bensing BA, Sullam PM. Transport of preproteins by the accessory Sec system requires a specific domain adjacent to the signal peptide. J Bacteriol. 2010; 192:4223–4232. [PubMed: 20562303]

102. Sibbald MJ, et al. Synthetic effects of secG and secY2 mutations on exoproteome biogenesis in Staphylococcus aureus. J Bacteriol. 2010; 192:3788–3800. [PubMed: 20472795]

103. Lenz LL, Portnoy DA. Identification of a second Listeria *secA* gene associated with protein secretion and the rough phenotype. Mol Microbiol. 2002; 45:1043–1056. [PubMed: 12180923] **First description of SecA2 and an associated exported substrated in Listeria**.

104. Sadagopal S, et al. Reducing the activity and secretion of microbial antioxidants enhances the immunogenicity of BCG. PLoS One. 2009; 4:e5531. [PubMed: 19436730]

105. Halbedel S, Hahn B, Daniel RA, Flieger A. DivIVA affects secretion of virulence-related autolysins in *Listeria monocytogenes* . Mol Microbiol. 2012; 83:821–839. [PubMed: 22353466]

106. Mishra KK, Mendonca M, Aroonnual A, Burkholder KM, Bhunia AK. Genetic organization and molecular characterization of *secA2* locus in *Listeria* species. Gene. 2011; 489:76–85. [PubMed: 21939744]

107. Machata S, Hain T, Rohde M, Chakraborty T. Simultaneous deficiency of both MurA and p60 proteins generates a rough phenotype in *Listeria monocytogenes* . J Bacteriol. 2005; 187:8385–8394. [PubMed: 16321943]

108. Burkholder KM, et al. Expression of LAP, a SecA2-dependent secretory protein, is induced under anaerobic environment. Microbes Infect. 2009; 11:859–867. [PubMed: 19454322]

109. Hinchey J, et al. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis* . J Clin Invest. 2007; 117:2279–2288. [PubMed: 17671656]

110. Hinchey J, et al. Lysine auxotrophy combined with deletion of the *secA2* gene results in a safe and highly immunogenic candidate live attenuated vaccine for tuberculosis. PLoS One. 2011; 6:e15857. [PubMed: 21264335]

111. Rahmoun M, et al. Priming of protective anti-*Listeria monocytogenes* memory CD8+ T cells requires a functional SecA2 secretion system. Infect Immun. 2011; 79:2396–2403. [PubMed: 21402759]

112. Gibbons HS, Wolschendorf F, Abshire M, Niederweis M, Braunstein M. Identification of two *Mycobacterium smegmatis* lipoproteins exported by a SecA2-dependent pathway. J Bacteriol. 2007; 189:5090–5100. [PubMed: 17496088]

113. Archambaud C, Nahori MA, Pizarro-Cerda J, Cossart P, Dussurget O. Control of *Listeria* superoxide dismutase by phosphorylation. J Biol Chem. 2006; 281:31812–31822. [PubMed: 16905535]

114. Calabi E, et al. Molecular characterization of the surface layer proteins from *Clostridium difficile* . Mol Microbiol. 2001; 40:1187–1199. [PubMed: 11401722]
115. Fagan RP, et al. A proposed nomenclature for cell wall proteins of *Clostridium difficile* . J Med Microbiol. 2011; 60:1225–1228. [PubMed: 21252271]
116. Waligora AJ, et al. Characterization of a cell surface protein of *Clostridium difficile* with adhesive properties. Infect Immun. 2001; 69:2144–2153. [PubMed: 11254569]
117. Calabi E, Calabi F, Phillips AD, Fairweather NF. Binding of *Clostridium difficile* surface layer proteins to gastrointestinal tissues. Infect Immun. 2002; 70:5770–5778. [PubMed: 12228307]
118. Janoir C, Pechine S, Grosdidier C, Collignon A. Cwp84, a surface-associated protein of *Clostridium difficile*, is a cysteine protease with degrading activity on extracellular matrix proteins. J Bacteriol. 2007; 189:7174–7180. [PubMed: 17693508]
119. Bramkamp M, van Baarle S. Division site selection in rod-shaped bacteria. Curr Opin Microbiol. 2009; 12:683–688. [PubMed: 19884039]
120. Zimmer J, Nam Y, Rapoport TA. Structure of a complex of the ATPase SecA and the protein-translocation channel. Nature. 2008; 455:936–943. [PubMed: 18923516] **A landmark structural paper showing SecA bound to SecY**.
121. Erlandson KJ, et al. A role for the two-helix finger of the SecA ATPase in protein translocation. Nature. 2008; 455:984–987. [PubMed: 18923526]
122. Mitchell C, Oliver D. Two distinct ATP-binding domains are needed to promote protein export by *Escherichia coli* SecA ATPase. Mol Microbiol. 1993; 10:483–497. [PubMed: 7968527]
123. Ye J, Osborne AR, Groll M, Rapoport TA. RecA-like motor ATPases-lessons from structures. Biochim Biophys Acta. 2004; 1659:1–18. [PubMed: 15511523]
124. Karamanou S, et al. A molecular switch in SecA protein couples ATP hydrolysis to protein translocation. Mol Microbiol. 1999; 34:1133–1145. [PubMed: 10594836]
125. Vrontou E, Karamanou S, Baud C, Sianidis G, Economou A. Global co-ordination of protein translocation by the SecA IRA1 switch. J Biol Chem. 2004; 279:22490–22497. [PubMed: 15007058]

Online Summary

- The post-translational general secretion (Sec) pathway is powered by the essential SecA ATPase, which works with the SecYEG channel to translocate proteins across the cytoplasmic membrane.
- Mycobacteria and some Gram positive bacteria have two non-redundant SecA proteins: SecA1 and SecA2. SecA1 powers the essential canonical Sec pathway, while SecA2 exports of a limited set of proteins.
- SecA2 export systems have diverse and important roles in cell envelope biogenesis and bacterial pathogenesis.
- There are two types of SecA2 systems: SecA2/SecY2 systems and SecA2-only systems.
- SecA2/SecY2 systems appear to operate mostly independent of the canonical Sec machinery as dedicated transporters of serine-rich glycoproteins that function as adhesins. SecA2/SecY2 systems are encoded by a genomic locus that includes open reading frames for glycosylation and export machinery.
- SecA2-only systems do not contain a SecY2 or an obvious accessory membrane channel. Instead, SecA2-only systems appear to use the canonical SecYEG channel for exporting a diverse assortment of proteins.
- It is unknown how SecA2 functions in comparison to the well-studied canonical SecA (SecA1) proteins. The same structural, biochemical and *in vitro* reconstitution analyses used to understand canonical Sec export will surely prove valuable in answering the many mechanistic questions concerning SecA2/SecY2 and SecA2-only systems.

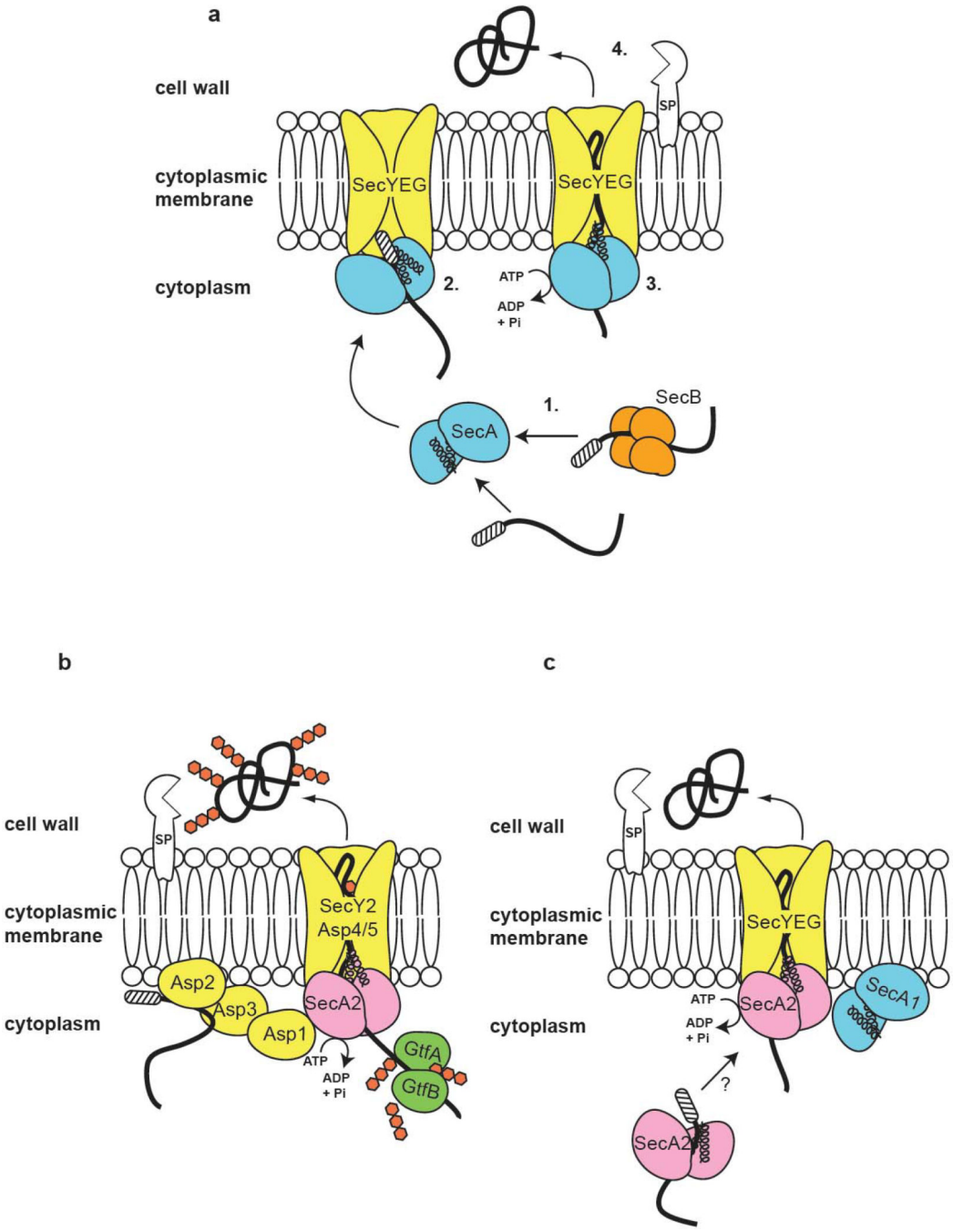

Figure 1. Models of Sec export

a) Post-translational Sec export is powered by the essential SecA ATPase. SecA can be divided into two main structural domains: a motor domain that drives ATP hydrolysis and a specificity domain that interacts with the preprotein destined for export. Step 1, preproteins synthesized with N-terminal Sec signal peptides (hatched) are bound by cytoplasmic SecA along a cleft between the two domains. Cytoplasmic chaperones, such as SecB, aid in keeping some preproteins unfolded prior to export and can directly deliver these preproteins to SecA. Step 2, SecA delivers the preprotein to a membrane-spanning complex composed

of SecY, SecE, and SecG. Here, the signal peptide inserts into SecY to help keep an open channel conformation. Step 3, SecA goes through rounds of conformational changes coupled to ATP hydrolysis to promote forward movement of the unfolded preprotein through the SecY channel. During these cycles, it is proposed that SecA inserts into the SecY channel, specifically by the IRA-1 two-helix finger (two helices). Step 4, during or shortly after translocation the signal peptide is removed by periplasmic signal peptidases (SP) and the protein then adopts its mature, folded conformation. The SecD, SecF, and YajC transmembrane proteins contribute to the efficiency of Sec export but are not shown. After translocation, the exported protein can remain associated with the cell envelope or be fully secreted into the extracellular environment. **b)** Biogenesis of surface glycoproteins by SecA2/SecY2 systems involves both glycosylation factors (green) and export machinery (yellow) that are distinct from the canonical Sec machinery. Serine-rich proteins are synthesized with N-terminal signal peptides (hatched). The accessory Sec proteins (Asps 1–3) promote SecA2/SecY2 export by unknown mechanisms, but could target preproteins to the translocase and/or serve as a scaffold for the export complex. Asp4 and Asp5 are putative accessory components of the SecY2 channel, but they are not present in all SecA2/SecY2 systems. SecA2/SecY2 export and glycosylation are likely coupled processes. As glycosyl groups (orange hexagons) are added to the preprotein by cytoplasmic glycosylation factors, including the core GtfA and GtfB glycosyltransferases, the SecA2 ATPase will energize transport of the unfolded preprotein through a channel formed by SecY2. SecA2/SecY2 substrates also contain a C-terminal cell wall anchoring domain, which targets the exported protein to the cell wall after SecA2/SecY2 export through the membrane. **c)** SecA2-only systems lack a SecY2 channel; therefore, SecA2 likely utilizes the canonical SecYEG channel for export. SecA2 could target a certain subset of preproteins to the Sec translocase that are otherwise overlooked or incompatible with SecA1. There are examples of proteins with N-terminal Sec signal peptides (pictured here) and proteins lacking signal peptides that require SecA2 for export. SecA2 may deliver some preproteins to SecYEG and/or associate with SecYEG as needed to export substrates. The ATPase activity of SecA2 likely drives export of preproteins through the SecYEG channel, possibly in conjunction with SecA1.

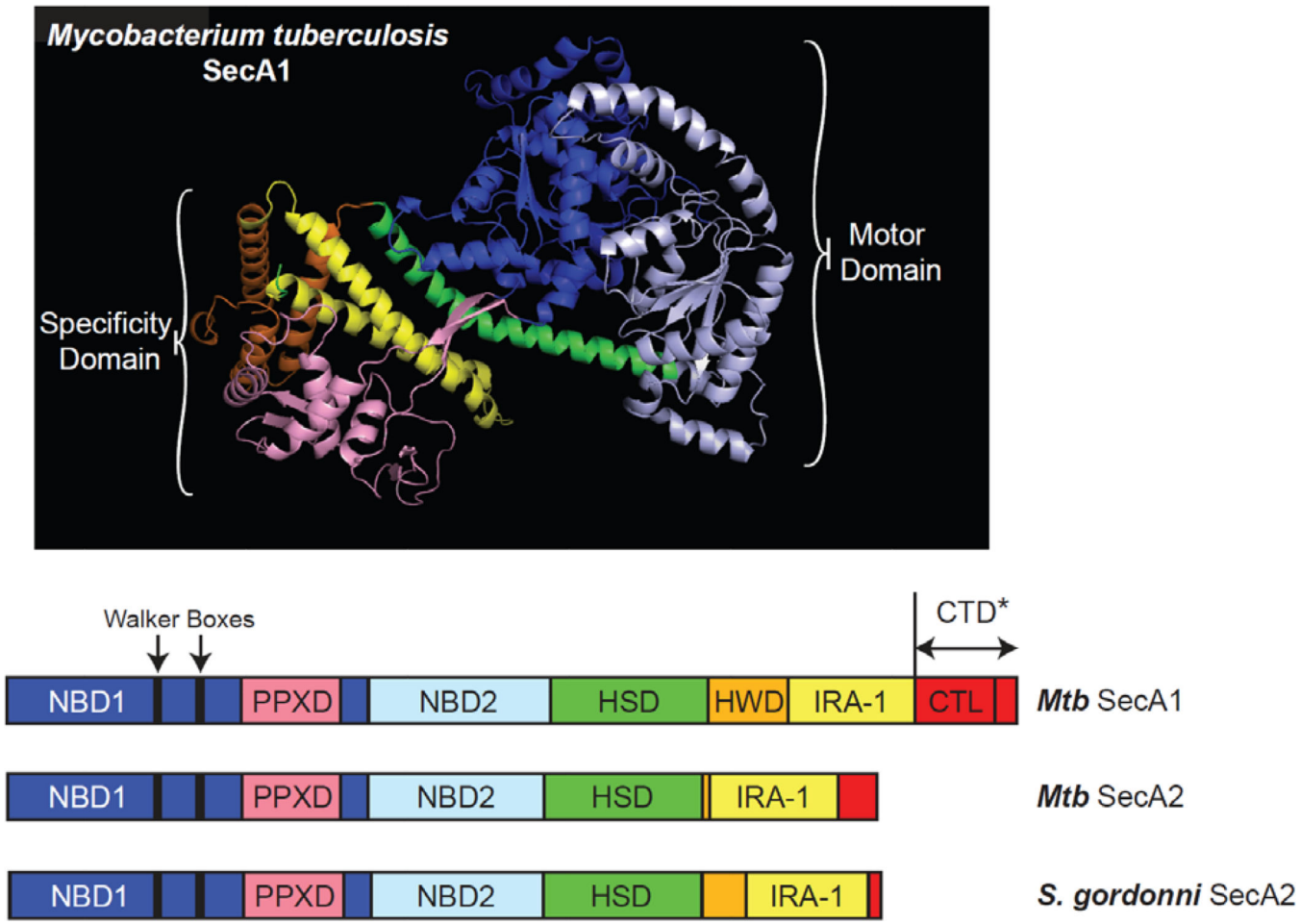

Figure 2. Domain organization of accessory SecA2 proteins

Sequence alignments and structural modeling suggest that most functional domains are conserved between SecA1 and SecA2 proteins. The crystal structure of the *M. tuberculosis* SecA1 protein depicted here represents a typical SecA1 protein with the corresponding colored domains outlined below[57]. *The C-terminal domain (CTD) was not resolved in the *M. tuberculosis* crystal structure but is shown in the domain graphic. For comparison to SecA1, the predicted domain organization of *M. tuberculosis* SecA2 and *Streptococcus gordonii* SecA2 are included. SecA1 can be divided into two main structural domains, which are both composed of several subdomains[18,56]. The DEAD (Asp-Glu-Ala-Asp)-like motor domain is responsible for the ATP hydrolysis[58,60,61] and consists of two nucleotide-binding folds: NBD1 and NBD2. NBD1 contains the two ATP-binding Walker boxes[122,123]. The helical scaffold domain (HSD) connects the motor domain with the rest of the specificity domain, to allow coupling of preprotein-binding with ATP hydrolysis[124,125]. Interactions between SecA1 and the preprotein map along a hydrophobic cleft formed by NBD1, the preprotein crosslinking domain (PPXD)[22–24], and portions of the HSD linker. Within the HSD region is a two-helix finger known as IRA-1, which is thought to assist inserting of the preprotein into the SecY channel during translocation. Conservation of the motor domain between SecA1 and SecA2 proteins confirms the observation that SecA2 proteins are ATPases[44,62]. There are differences between SecA1 and SecA2 in the specificity domain

that could affect substrate specificity and/or function. All SecA2 proteins are smaller than their SecA1 counterparts due to a truncation of the CTD and helical wing domain (HWD). Although not depicted in the *M. tuberculosis* SecA1 structure, the CTD was resolved in an *E. coli* SecA structure[23] and the C-terminal linker (CTL) within the CTD lies along the hydrophobic preprotein-binding cleft.

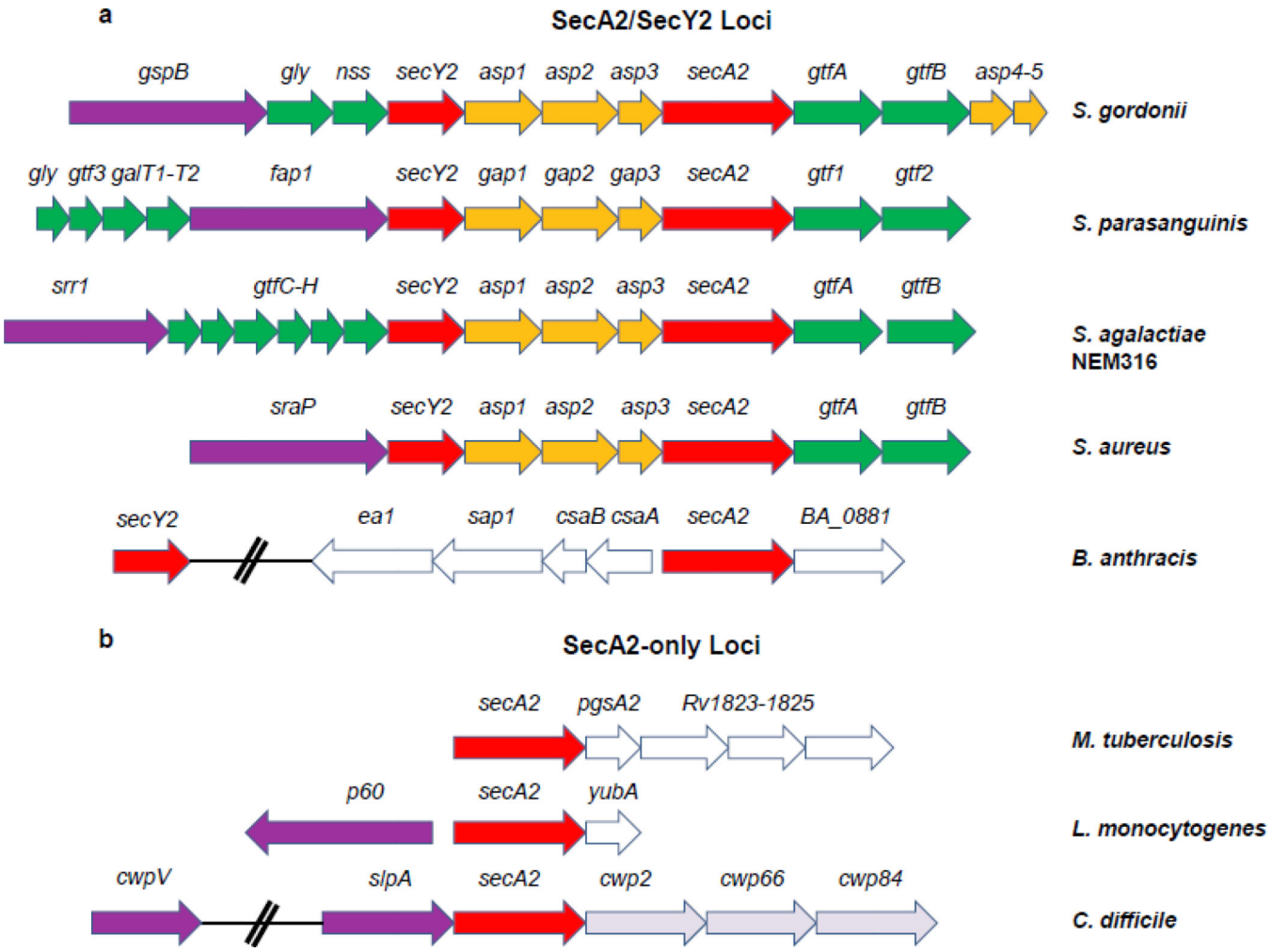

Figure 3. Organization of accessory *secA2* genomic loci

a) Accessory SecA2/SecY2 systems are found in a diverse set of *Streptococcus* and *Staphylococcus* species, where they function in biogenesis of surface glycoproteins (depicted in purple). All of these loci contain the core *secA2* and *secY2* genes shown in red. Some *Bacillus* species, including *B. anthracis*, have putative SecA2/SecY2 systems, although the exported substrates of these systems are unknown and the *secA2* and *secY2* genes are separated in these cases. Genes encoding putative export machinery are shown in yellow while those encoding glycosylation machinery are shown in green. **b)** Examples of SecA2-only systems are found in mycobacteria, *Clostridium*, and *Listeria*. The *secA2* loci of SecA2-only systems are not conserved and export a diverse set of substrates. In some cases, the genes encoding the exported substrates (purple) are found at the *secA2* locus. However, this is not always the case and genes located elsewhere in the genome encoding SecA2-dependent proteins are not depicted. In addition to *slpA*, the *C. difficile secA2* locus contains genes encoding eleven additional cell wall proteins that are putative SecA2 substrates, of which three are shown in light purple.

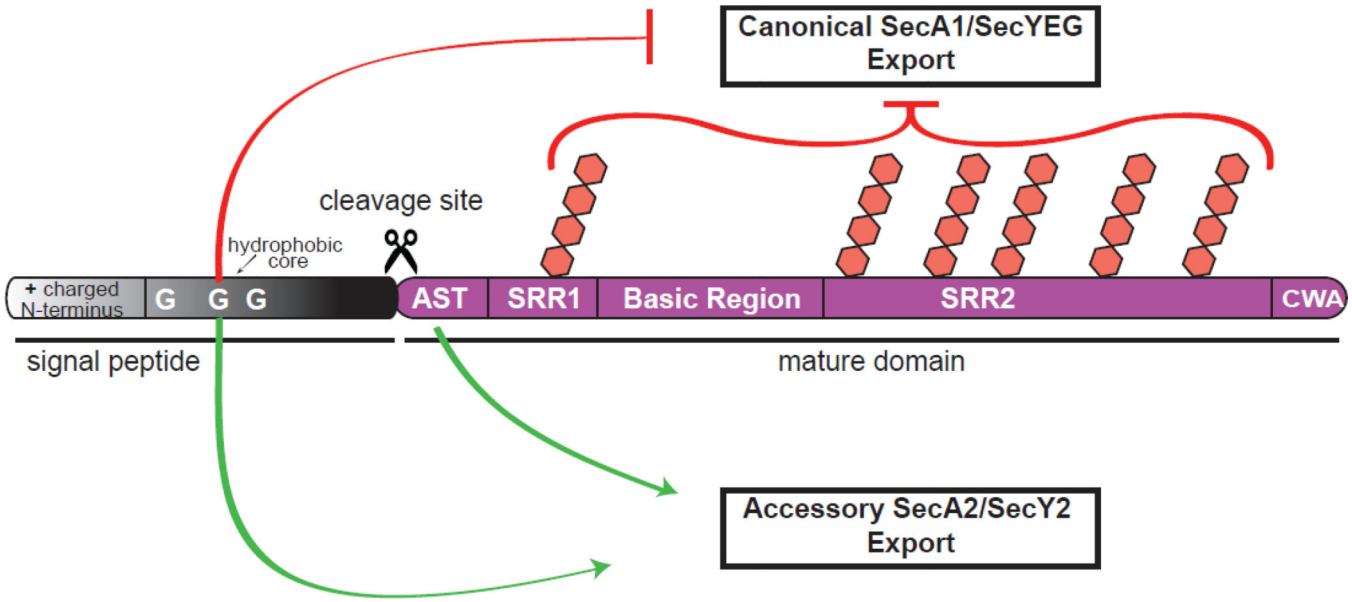

Figure 4. SecA2/SecY2-targeting features

SecA2/SecY2 preproteins have features for both targeting to the SecA2/SecY2 machinery for glycosylation/export, and for blocking export by the canonical Sec pathway. Most of these targeting features have been defined using the *S. gordonii* GspB protein, shown here. The mature domain of GspB can be divided into several domains. Two serine-rich repeats domains (SRR1 and SRR2) are glycosylated in the cytoplasm prior to export and this post-translational modification blocks GspB export by SecA1/SecYEG. GspB, like all SecA2/SecY2 preproteins has an unusually long N-terminal signal peptide that is required for export. The signal peptide has the same tripartite structure of Sec signal peptides including a positively charged N-terminus, hydrophobic core, and cleavage domain. Within the hydrophobic core of the signal peptide are three glycine residues that are required for GspB export though SecA2/SecY2, but these same glycines also inhibit export by SecA1/SecYEG, through unknown mechanisms. The accessory Sec transport (AST) domain is adjacent to the signal peptide and is required for export by SecA2/SecY2. Also depicted in the mature domain are the basic region and C-terminal cell wall anchoring domain (CWA).