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The Sec-dependent pathway

Jon Beckwith
Department of Microbiology and Immunobiology,Harvard Medical School, HIM Building, Room 1047,77 Avenue Louis Pasteur,Boston, MA 02115, USA

Abstract

The Sec pathway for export of proteins across the cytoplasmic membrane to the bacterial periplasm and outer membrane was the first secretion pathway to be discovered in bacteria. A combination of bacterial genetics, development of an in vitro membrane vesicle system and the concurrent elaboration of the signal hypothesis from studies on eukaryotes led to the identification and characterization of two pathways leading to protein export through the SecYEG cytoplasmic membrane translocon. The Sec pathway is also required for assembly of proteins into the cytoplasmic membrane. Since the membrane translocon for Sec pathways is conserved across the three domains of life, the history of research progress in eukaryotes and bacteria was facilitated by the close interaction between those studying both classes of organisms.

Keywords

SRP; SecYEG; Post-translational secretion; Co-translational secretion; Gene fusions

1. From molecular biology to cell biology of bacteria

Advances in many areas of biology fueled the beginnings of molecular biology in the 1950's and its subsequent expansion in the 1960's. These advances included X-ray crystallography, bacterial and bacteriophage genetics, the identification of DNA as the genetic material and the accumulation of substantial amounts of information on bacterial metabolism resulting from preceding decades of work of biochemists and bacterial physiologists. Geneticists, microbiologists, biochemists, structural biologists and biophysicists progressively self-assembled into a field that they named molecular biology, taking a multi-pronged approach to biological problems. The outcome was a remarkable accumulation of foundational concepts in a short period of time. These included the nature of DNA structure, replication, repair and recombination, the nature and origin of mutations, the defining of the genetic code, the elaboration of gene regulatory mechanisms and the nature of information transfer in cells from transcription of DNA into RNA and translation of RNA into protein. Given the tools developed, the foundational concepts arrived at during this period and the government and private funding that they attracted, ever increasing numbers of young researchers entered molecular biology. They sought to work out the mechanistic details of gene regulation and of the "central dogma" which focused on the transfer of information from

jbeckwith@hms.harvard.edu.

DNA to RNA to protein. For the most part, they continued to use the bacterium *Escherichia coli* as the organism of choice.

However, by the mid 1960's, for many of those who had pioneered molecular biology, studies with bacteria had served their purpose of generating insights into fundamental mechanisms and providing general approaches to molecular biological questions. It was time to apply these insights and approaches and explore, for example, the more complex developmental issues in multicellular organisms. These pioneers and founders of molecular biology became pioneers once again. When the techniques for gene cloning were developed in the 1970's they were given more remarkable tools that made the study of higher organisms even more feasible. There was a feeling, perhaps expressed most strongly by Francois Jacob, that those who continued to work with organisms such as *E. coli* were "rehashing the same questions" and "needed the courage…. to turn to new problems and study them with more suitable organisms." (Jacob, 1998)

Certainly, the successful approaches and methodologies of bacterial molecular biology had provided important incentive to apply them to the more complex eukaryotic cell and to human biology. And it may well have been that those of us who were new recruits to molecular biology were examining "the same questions" as suggested by Jacob. Yet, I think that there was a fundamental error here. The choices were not just to move to higher organisms, even though such a move certainly made sense for many. First of all, the continued studies in bacteria, which are still the organisms most easily manipulable genetically, have contributed enormously to those who study eukaryotic organisms. To mention a few instances, the characterization of specific bacterial DNA repair mechanisms that were then shown to be altered in some human cancers or the discovery of positive control in bacteria at a time when eukaryotic researchers mainly were positing negative control mechanisms. But, more importantly for the purposes of this chapter, there was a narrowness among us in the kinds of problems that we chose to attack. To explain what I mean by narrowness, I will classify two kinds (there are more) of biological problems that one might want to study. (I don't mean to imply an invidious comparison between the two approaches.) The first class comprises sequential linear processes such as the replication or transcription of DNA and most of the major topics that we pursued during the early years of molecular biology. The second comprises processes that are three-dimensional. These include formation of subcellular compartments, substructures, membranes and phage particles, cell movement, cell division, movement of proteins within the cell, out of the cell and between subcellular compartments.

But it is the case that very few biologists were studying three-dimensional processes in bacteria in the 1960's. To the extent that molecular biology chugged along productively for years but, in this sense, limited in its scope, Jacob's concern had some validity. Yet the implication that bacteria were not the "suitable" organisms for approaching some of these three-dimensional problems was not necessarily valid. To the contrary, although it took a while, the powerful genetic techniques that had been developed during this period and their success in allowing the biological community to establish the foundations of a molecular biology ultimately opened our minds to more comprehensive uses of these techniques for studying cell biology.

I do not ignore that there were instances where molecular biologists at least made attempts to explore questions in this three-dimensional domain. Phage particle assembly was one of the early projects of molecular biologists that was considered initially an exciting area (Wood and Edgar, 1967). However, the more readily accessible, more linear problems lured many people away from this endeavor. Other examples include the initiation of studies on chemotaxis in bacteria (Adler, 1965), an attempt to study cell division through the isolation

of a collection of cell division-defective mutants of *Escherichia coli* (Hirota et al., 1968), efforts to understand protein secretion in bacteria (Bissell et al., 1971) and the beginnings of genetic studies on bacterial sporulation (Losick and Sonenshein, 1969). The latter studies focused initially on regulation of gene expression involved in sporulation of *Bacillus subtilis*, using the organism as a means of studying developmental processes. Ultimately, the power of bacterial genetics along with the use of fluorescent microscopy has recently allowed a detailed description of the movement of cellular components necessary for this process to take place. All of these were pioneering efforts themselves that focused on biological processes that involved considerable movement of cells, movement of macromolecules within cells or movement in and out of cells.

2. Protein secretion studies open up in the 1970's: Identification and characterization of bacterial signal sequences

A mention of progress in the field of eukaryotic cell biology, which also occurred in the 1950's, is important background to a discussion of studies of protein export in bacteria. Largely due to the extraordinary fecundity of the collaboration between George Palade and Philip Siekevitz in the 1950's, there emerged a more detailed description of the organelles that made up the cell (Siekevitz and Palade, 1958). In particular, these researchers identified which of these organelles were involved in the protein secretory pathway. But a parallel examination in bacteria of questions such as the mechanism of protein secretion was being approached by very few biologists during this time.

In Gram-negative bacteria such as *E. coli*, 25% of the cell's proteins are located in the cell envelope and periplasmic space. These proteins, which are made on cytoplasmic ribosomes, must be translocated through the cytoplasmic membrane in order for them to reach the periplasm and outer membrane. By the mid-1970's, several laboratories had begun projects to study the mechanism by which this translocation takes place. However, the first major insights into how this process works came from studies of the eukaryotic secretory pathway that followed on the work of Palade and Siekevitz. Milstein and his colleagues had noted in 1972 that the secreted immunoglobulins were made in precursor form with an amino-terminal hydrophobic sequence that was cleaved to the mature form of the protein (Milstein et al., 1972). While this finding was seen as potentially providing insights into how secreted proteins are recognized, it was not until a few years later that Blobel and Dobberstein (Blobel and Dobberstein, 1975) presented substantial and persuasive evidence for their signal sequence hypothesis. According to their model, proteins destined to enter the secretory pathway were recognized as such by the presence of a hydrophobic amino-terminal signal sequence that was cleaved during the translocation process.

At about the same time that the signal hypothesis was presented, microbiologists obtained evidence indicating that proteins exported from the *E. coli* cytoplasm were synthesized as precursors with small cleavable domains. The first evidence for proteins with cleavable signal sequences in bacteria came from studies of the f1 and M13 phage coat proteins which, during phage maturation in bacteria, assembled first in the cytoplasmic membrane (Sugimoto et al., 1977;Wickner et al., 1978). Also, cleavable sequences were detected in precursors for the periplasmic protein alkaline phosphatase (Inouye and Beckwith, 1977) and for three outer membrane proteins (Inouye et al., 1977;Sekizawa et al., 1977). Subsequently, Randall and coworkers identified three more cell envelope proteins that were made with amino-terminal cleavable signal sequences (Randall et al., 1978). Randall's work also showed that proteins destined for export across the cytoplasmic membrane were synthesized on membrane-bound polysomes.

The use of gene fusions in the study of protein secretion in *E. coli* proved to be a powerful tool for obtaining direct evidence for the signal sequence hypothesis. Furthermore, such fusions provided a means of identifying those protein components of the cell that comprised the machinery required for translocating proteins out of the cytoplasm. The fusions used for these purposes consisted of an amino-terminal portion deriving from an exported protein and a carboxy-terminal portion consisting of nearly the entire cytoplasmic protein β-galactosidase. The β-galactosidase portion of the hybrid protein, missing only a few amino acids from its amino-terminus, had been shown previously to be sufficient to exhibit normal enzymatic activity when it was fused to another cytoplasmic protein. However, in these fusion proteins, the signal sequence initiated export of a protein whose normal residence was the cytoplasm. When expressed at high levels, the translocation of these unwieldy hybrid proteins was toxic to the cell, jamming the membrane translocon apparatus (Bassford and Beckwith, 1979;Emr et al., 1978).

Since the toxicity of high level expression of the fusion proteins was dependent on the functionality of the signal sequence, a selection for mutants that eliminated the toxicity yielded fusion proteins with altered signal sequences (Bassford and Beckwith, 1979;Emr et al., 1978). These mutant signal sequences were no longer recognized by the secretory machinery and the hybrid protein remained cytoplasmic. From these genetic selections a collection of mutants were obtained in which hydrophobic amino acids were, for the most part, replaced by hydrophilic or charged amino acids (Bedouelle et al., 1980;Emr et al., 1980). These mutations provided direct evidence for the essential role of signal sequences, and particularly for their hydrophobic nature, in promoting translocation of proteins across the cytoplasmic membrane. Gene fusions to two periplasmic proteins, maltose binding protein and alkaline phosphatase, and to an outer membrane protein, LamB, yielded a large collection of signal sequence mutants with similar characteristics (Bedouelle et al., 1980;Emr et al., 1980;Michaelis et al., 1986).

3. Defining the bacterial Sec machinery for signal sequence recognition and protein export

For the remainder of this review, I will refer to those proteins that are dependent, for their translocation across the bacterial cytoplasmic membrane, on the SecYEG cytoplasmic membrane complex as Sec-dependent proteins. I will concentrate mainly on the set of proteins (and an RNA) that were identified as components of Sec export pathways and broadly on what roles each of those components play in the export process. Except for the SecG protein and components of the SRP pathway (see below), the remainder of the Sec proteins were identified via selection for mutants that altered their activity. In the case of SRP components and the SRP membrane receptor, the genes for them were discovered by noting that they encoded homologues or analogues of eukaryotic proteins involved in the secretory pathway. SecG was discovered by its association with SecY. The complete set of genes and their products were identified between 1981and 1993. The Sec system is also involved in assembly of proteins into the cytoplasmic membrane. There will be only brief mention of this subject, as the focus of this volume is mainly on soluble secreted proteins.

Mutations that relieved the toxicity of the β-galactosidase fusions described above caused defects in the signal sequences of the various proteins that were components of the fusions. However, genetic selection did not yield mutations that affected the activity of components of the cell’s secretion machinery. In fact, it was highly unlikely that the latter class of such mutations would be obtained by this genetic selection, since a mutation significantly defective in a component of the machinery would interfere with the export of all signal-sequence-containing proteins. Such pleiotropic mutations were likely to be as toxic as the original fusion.

However, the properties of the signal sequence mutants themselves provided a means of obtaining mutants that altered components of the secretion machinery. Emr et al. obtained a signal sequence mutant of LamB, an outer membrane protein required for maltodextrin accumulation in the cell, that had much of the hydrophobic domain of the signal sequence deleted. Cells expressing this mutant protein were no longer able to export the LamB protein to the outer membrane, resulting in their inability to use maltodextrins as a carbon source. The researchers then selected for suppressor mutations that restored the ability to utilize maltodextrins but were not linked to the *lamB* gene (Emr et al., 1981). The mutants obtained mapped to a gene they named *prlA*; mutations in it restored export not only to *lamB* signal sequence mutants, but also to signal sequence mutants in other exported proteins. Mutagenesis of this gene was subsequently shown to yield temperature-sensitive mutations that exhibited strong export defects at the non-permissive temperature (Ito et al., 1983). Thus, mutations were obtained in the same gene with both positive and negative effects on protein export. The gene, which was renamed *secY*, encoded an integral cytoplasmic membrane protein; the membrane translocon protein SecY was eventually found to be conserved throughout nature from *E. coli* to humans.

A different approach to genetically defining components of the secretion machinery was provided by a second property of the fusions of β-galactosidase to exported proteins. Apparently, the jamming of the hybrid proteins in the membrane that resulted in toxicity at high levels of expression also resulted in an inactive β-galactosidase even at low levels of expression. Selection for Lac^+ derivatives of the fusion of β-galactosidase to the maltose binding protein yielded mutations in another new gene, SecA (Oliver and Beckwith, 1981). SecA is a cytoplasmic protein that is important for the transfer of secretory proteins to the membrane-embedded translocon. A combination of *prl* suppressors of signal sequence mutants, the Lac^+ selection with certain fusions and selection for mutants that caused derepression of the *secA* gene, yielded mutations that defined many of the genes for components of the secretory machinery that were involved in the major protein export pathway (Gardel et al., 1987;Gardel et al., 1990;Kumamoto and Beckwith, 1983;Riggs et al., 1988;Stader et al., 1989). These included *secA, secB, secD, SecE* and *secF*. However, one of the Sec proteins, SecG, was initially detected in the in vitro system (Nishiyama et al., 1993), although subsequently, *prl* mutations were found in the *secG* gene (Bost and Belin, 1997). The genes for the protein SecG and for the signal peptidase, *lep*, were identified by working back from the sequence of the protein to the DNA sequence (Date and Wickner, 1981;Nishiyama et al., 1994).

The genetic studies of protein export via the Sec proteins were complemented by the development of an in vitro system for assaying protein translocation into cytoplasmic membrane vesicles. Wickner and coworkers (Wickner et al., 1978) established an in vitro system for studying the assembly of the signal-sequence-containing M13 phage protein into the *E. coli* cytoplasmic membrane. While the system was developed for studies on the M13 coat protein, it was subsequently used to study the in vitro translocation of periplasmic proteins and outer membrane proteins into the interior of the membrane vesicles. These in vitro studies verified the role of many of the Sec proteins, demonstrated subcomplexes of proteins (SecYEG and SecDFYajC) and revealed the role of the membrane electrochemical potential gradient in protein translocation (Date et al., 1980;Wickner and Leonard, 1996). (The YajC protein is in an operon with SecD and F and purifies with them, but it is not clear that it plays a role in protein export.) Wickner's system and the studies of Chang et al. also allowed the detection and subsequent studies on the bacterial signal peptidase (Chang et al., 1978;Mandel and Wickner, 1979)

The combination of these early genetic and biochemical studies led to a picture of the role of most of the Sec proteins in the export process.

4. Parallel and contrasting findings in eukaryotic systems: post-translational and co-translational protein export by the SRP pathway

During the period in which the genetics of protein export in bacteria was being investigated, Blobel and his colleagues were identifying the components of the secretory apparatus in eukaryotic cells, reconstituting a membrane vesicle system for assaying protein translocation across dog pancreas microsomal vesicles. They first identified a eukaryotic signal peptidase (Jackson and Blobel, 1977). Further studies led to the identification of membrane components that constituted the machinery for transferring proteins across the ER membrane or incorporation of proteins into it (Walter et al., 1979) and to the discovery of the cytosolic signal recognition particle (SRP) (Walter et al., 1981). The similarities between the eukaryotic and bacterial systems were emphasized by the finding that the mammalian system could translocate bacterial proteins into the ER lumen with their quite comparable signal sequences. Furthermore, the actual membrane translocon complex of proteins through which proteins passed, SecYEG and Sec 61αβγ, appeared to be not only quite similar, but the sequences of the protein SecY, Sec61α and the archaeal SecY were evolutionarily related. It appeared that mechanism had been conserved across the three domains of life (Pohlschroder et al., 1997).

Despite the remarkable similarities in mechanisms to those of the eukaryotic system, the early studies of protein export in bacteria suggested some significant differences. The most apparent of these was related to the connection between protein synthesis and protein translocation. Proteins were translocated into the lumen of the ER in a co-translational fashion (Walter and Blobel, 1981). That is, almost as soon as the signal sequences were synthesized and emerged from the ribosomes, they were recognized by SRP and taken to the ER membrane where they immediately began to be translocated. In contrast, Linda Randall and her coworkers showed that, in bacteria, the export of each protein they studied exhibited mixed co-and post-translational mechanisms (Josefsson and Randall, 1981). That is, substantial lengths (the amount varying from protein to protein) of the polypeptide chains were synthesized and remained in the cytoplasm before protein translocation across the membrane began (Josefsson and Randall, 1981;Kadokura and Beckwith, 2009;Schierle et al., 2003). There was a significant post-translational component to the export process. Furthermore, the genetic studies in bacteria had not revealed any bacterial components of the secretory apparatus that resembled the components of the eukaryotic SRP.

These studies were reconciled when, first, Peter Walter and colleagues identified genes in *E. coli* that were homologous to a protein subunit of eukaryotic SRP (*ffh)*, encoded a 4.5S RNA (*ffs*) that might correspond to the 7S RNA of eukaryotic SRP and encoded a homologue of the SRP receptor (*fts Y*) in the ER membrane (summarized in (Bernstein and Hyndman, 2001b)). Second, later studies revealed that a significant minority of *E. coli* proteins did, in fact, depend on a bacterial SRP system for their export to the periplasm or outer membrane (Huber et al., 2005). Similarly, studies in *Saccharomyces cerevisiae* revealed that secretion by the signal sequence pathway in that organism involved both post- and co-translational pathways (Ng et al., 1996). The choice of pathway was determined by differences between the signal sequences of the two classes of proteins. The proteins exported by a post-translational pathway generally had signal sequences that were less hydrophobic that those that proceed through the SRP pathway.

Many of those working on the bacterial system, including myself, at first resisted the suggestion of a bacterial SRP-based export proposed by Walter and colleagues, as it was based largely on the finding of homologues of eukaryotic SRP components in bacteria (Bassford et al., 1991). Despite this contretemps, the parallel histories of the studies on the secretory pathway in eukaryotes and those on bacteria were mutually reinforcing efforts

with much interaction and intermingling of researchers from both areas who often attended meetings together. Ultimately, it became clear that a major role of the SRP pathway is to co-translationally initiate the membrane incorporation of cytoplasmic membrane proteins. This process occurs by interaction of an SRP carrying a nascent chain with the SRP receptor in the membrane and subsequent transfer to the SecYEG complex for proper membrane localization of the proteins. For a subset of these membrane proteins, an additional membrane component essential for their localization is the YidC protein, a cytoplasmic membrane protein itself (Gray et al., 2012;Samuelson et al., 2000).

5. The roles of gene products constituting the Sec pathway

There is considerable evidence on the structure of most of the components of the Sec pathway. I will not describe those studies in detail, but rather refer to them in connection with certain functions of these proteins.

5.1. SecB

Preventing the folding of a protein in the cytoplasm is important to the successful initiation of protein export for most Sec-dependent proteins. If the protein folds, it can no longer pass through or even reach the SecYEG complex. Prevention of folding can occur in several ways: 1)The chaperone SecB interacts with nascent chains of certain proteins, preventing their folding (Weiss et al., 1988). The SecB-nascent chain complex interacts with the next protein in the pathway, SecA. There is considerable information on the portions of SecB that interact with nascent chains and those of the nascent chains themselves that interact with SecB (Bechtluft et al., 2010;Randall and Hardy, 2002). 2) Many proteins exported to the cell envelope are dependent on protein disulfide bonds for their proper folding. This class of proteins may require no chaperone such as SecB, since disulfide bonds can only form once these proteins have reached the periplasm. However, such proteins may become SecB-dependent when the bacteria are grown at temperatures lower than the usual temperature (e.g. lower than 37° in the case of *E. coli*)(Kusukawa et al., 1989). This may be because the higher temperatures can destabilize interactions within the protein that result in more stable structures at the lower temperature. 3)Signal sequences themselves can interact with portions of the nascent chain to prevent its folding (Park et al., 1988).

Trigger factor, another chaperone that was thought initially to facilitate protein export in the Sec pathway, is now considered to be a more general chaperone which may also influence folding properties of exportable proteins (Crooke and Wickner, 1987;Hoffmann et al., 2012).

5.2.SecA

The SecA protein is an ATPase that interacts with SecB as well as with the SecYEG complex. If a protein does not require SecB to prevent its folding, then the protein will be recognized by SecA and transferred during its synthesis to the SecYEG complex. Evidence suggests that those nascent chains that bind SecB will be eventually transferred to SecA. SecA is not only designed to move the nascent chains to the SecYEG complex, it is also essential for the translocation through that complex, using ATP as a source of energy. SecA remains bound to SecYEG during the translocation process and is released when export is complete. Thus, the protein can be found in both membrane and soluble fractions in cell extracts (Sardis and Economou, 2010).

5.3.SecYEG

These three proteins form a stable complex in the cytoplasmic membrane. However, this translocon is still partly functional in vivo in the complete absence of SecG (a protein with

two transmembrane segments), although export defects are seen at low temperature in a *secG* null mutant. Much of the work on structure and function has been done in vitro with just the SecYE complex (or SecA-SecYE). SecE is an essential gene with three transmembrane segments which is suggested to be responsible for helping maintain the structure of the complex. The component of the complex that actually forms the gated pore in the membrane is SecY, a 10-transmembrane segment protein with inverted symmetry. Interactions of the SecYEG complex with a nascent chain bound to SecA, for instance, triggers the opening of the gate and the ATP-dependent translocation of a protein (Erlandson et al., 2008;Osborne et al., 2005;Zimmer et al., 2008).

5.4.Mysterious SecDF

Studies in vivo on the Sec export pathway indicate that it is highly dependent on the SecDF cytoplasmic membrane proteins, although *secDF* null mutants of *E. coli* grow, albeit very slowly, at 37° and essentially not at all at low temperatures (Pogliano and Beckwith, 1994). SecD and SecF each contain 6 transmembrane segments and substantial hydrophilic periplasmic domains. Several explanations for SecDF function have been proposed (Duong and Wickner, 1997), including a recent suggestion that SecDF functions as a chaperone which uses the proton motive force to power, from the periplasmic side, the movement of a translocating polypeptide through the membrane (Tsukazaki et al., 2011).

Early studies showed that there is a third gene (*yajC*) encoding a membrane protein in the SecDF operon and that the YajC protein co-purifies with SecDF. It is not clear what role, if any, YajC plays. Further, YajC also forms a complex with the *E. coli* AcrB protein. AcrB is a membrane protein with similarities to the SecDF complex, but itself pumps small molecules such as acridines out of the cytoplasm (Fang and Wei, 2011).

5.5.The SRP pathway

Initial experiments to distinguish those *E. coli* proteins that utilize the co-translational SRP export pathway from those that use a post-translational pathway suggested that roughly 25% of exported proteins may contain signal sequences that are recognized by SRP (Huber et al., 2005). The SRP-dependent proteins presumably do not require the SecB protein, as they do not have the opportunity to fold because translocation through the membrane occurs co-translationally. They do, however, still pass through the SecYEG translocon, presumably utilizing the energy of protein synthesis rather than SecA's ATPase activity. The *E. coli* SRP is composed of two components, a 54 kilodalton homologue of a subunit of eukaryotic SRP and a 4.5S RNA. Early in the translation process, a complex is formed between SRP and a nascent polypeptide. The complex then interacts with the FtsY SRP-receptor protein in the cytoplasmic protein. This membrane localization of the nascent chain allows delivery to SecYEG (Bernstein and Hyndman, 2001a).

6. Summary and future research

The beginnings of studies on protein export in bacteria were in progress when the catalytic presentation of the signal sequence hypothesis by Blobel and Dobberstein appeared. The overlap in interests of those cell biologists studying the secretory pathway of eukaryotes and those exploring the export of proteins to the bacterial cell envelope led to an unusual, at that time, joining of forces. As the fields evolved, the finding that the pathways shared both SRPs and SecYEG (Sec61) complexes drew the two groups closer together. In particular, a number of researchers went back and forth in their research between the bacterial and eukaryotic systems.

While a picture of the pathways that utilize Sec components for protein export in bacteria can be drawn in broad outlines, there are a host of interesting questions that are being

explored. Continued investigation of these questions by standard approaches, and particularly by advances in biophysical techniques, are rapidly revealing fascinating details. In particular, studies on the structure of the SecYE complex, its interaction with SecA and the actual movement of exportable proteins through the membrane pore are consistently yielding novel insights. To fully understand both the post-translational and co-translational pathways will still require many more years of work.

There are also new questions arising as the studies proceed. Just to mention a few: Since we now know a lot about the Sec protein export pathways in bacteria and in eukaryotes, how do archaea export proteins? Genes encoding homologues of some, but not all Sec proteins have been found in some archaea (Pohlschroder et al., 2005). Recently, discoveries of more than one Sec pathway in certain bacteria has suggested that distinct Sec pathways (these bacteria may have two SecAs or two SecYs or both) may be necessary for organisms to export proteins with unusual structures for bacteria, e.g. glycosylated proteins (Feltcher and Braunstein, 2012). A recent finding of a complex between SecA and a specific ribosomal protein goes against the current view of the role of SecA and the timing of its interaction with nascent polypeptides (Huber et al., 2011). Finally, recent studies on the function of the SecDF complex and the role of YajC in protein export require further exploration (Fang and Wei, 2011;Tsukazaki et al., 2011).

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