Journal of Bioenergetics and Biomembranes, Vol. 36, No. 1, February 2004 (© 2004)

Extreme Secretion: Protein Translocation Across the Archaeal Plasma Membrane

Gabriela Ring[1] and Jerry Eichler[1,2]

In all three domains of life, extracytoplasmic proteins must overcome the hurdle presented by hydrophobic, lipid-based membranes. While numerous aspects of the protein translocation process have been well studied in bacteria and eukarya, little is known about how proteins cross the membranes of archaea. Analysis to date suggests that archaeal protein translocation is a mosaic of bacterial, eukaryal, and archaeal features, as indeed is much of archaeal biology. Archaea encode homologues of selected elements of the bacterial and eukaryal translocation machines, yet lack other important components of these two systems. Other aspects of the archaeal translocation process appear specific to this domain, possibly related to the extreme environmental conditions in which archaea thrive. In the following, current understanding of archaeal protein translocation is reviewed, as is recent progess in reconstitution of the archaeal translocation process in vitro.

KEY WORDS: Archaea; inverted membrane vesicles; protein secretion; protein translation; protein translocation; signal peptidase; translocon.

Exposed to the outside world, the archaeal plasma membrane must not only withstand the drastic conditions encountered in the extreme habitats in which archaea are often found, but must also assume a central role in a variety of biological activities, such as nutrient uptake, cell division, bioenenergy production, and protein secretion. In archaea, numerous proteins, including various enzymes and components of the protein-based surface layer, must escape the confines of the cell. However, in contrast to our advanced understanding of how proteins translocate across membranes in bacteria and eukarya, little is known of how archaeal proteins destined to reside beyond the confines of the cytoplasm are translocated into and across the plasma membrane. Understanding how protein translocation is realized in archaea would thus not only further our understanding of the molecular strategies employed by these microorganisms in overcoming the physical challenges of their environments, but could also shed new light on the processes itself.

As in bacteria and eukarya, translocation of secretory proteins in archaea requires that such proteins first be distinguished from the pool of cytoplasmic proteins, that they then be targeted to membranous translocation sites, and ultimately, that they be delivered to the cell exterior. Archaea, like bacteria and eukarya, make use of the general secretory (Sec) pathway for protein export and, accordingly, contain many of the same Sec pathway components found in the other two domains of life. Archaeal protein secretion may also occur via the Sec-independent, twin arginine transport (Tat) translocation pathway. In this review, recent developments in our understanding of the archaeal protein translocation process will be presented, as will the current state of efforts aimed at in vitro reconstitution of the translocation event. For a detailed description of the recognition and targetting phases of the translocation event, the reader is directed to the article by Moll in this issue.

ARCHAEAL SIGNAL PEPTIDES

In archaea, as in bacteria and eukarya, secreted proteins are synthesized as preproteins, with an N-terminal

[1] Department of Life Sciences, Ben Gurion University, P.O. Box 653, Beersheva 84105, Beersheva, Israel.
[2] To whom correspondence should be addressed; e-mail: jeichler@bgumail.bgu.ac.il.

0145-479X/04/0200-0035/0 © 2004 Plenum Publishing Corporation

signal peptide that serves to target the protein to the membrane-embedded export machinery, yet which is not part of the mature secreted product (von Heijne, 1990a,b). Several recent papers have addressed the presence and nature of signal peptides in a variety of archaeal species using computer-based prediction tools (Albers and Driessen, 2002; Bolhuis, 2002; Dilks *et al.*, 2003; Nielsen *et al.*, 1999; Rose *et al.*, 2002). Experimental verification of these predictions is, however, presently available in only a limited number of cases. Archaeal secretory proteins may contain Sec-type signal peptide, composed of a basic n-region, a hydrophobic h-region, and a c-region terminating in the signal peptidase cleavage site (von Heijne, 1990a,b). Alternatively, archaea also encode preproteins that contain signal peptides recognized by the Tat pathway (Bolhuis, 2002; Dilks *et al.*, 2003; Rose *et al.*, 2002). Archaeal flagellin proteins also contain characteristic signal peptides (Faguy *et al.*, 1994).

In bacteria and eukarya, Sec signal peptides are similar and often interchangeable (von Heijne, 1990a,b). On the basis of bioinformatic predictions and supported experimentally by the processing of archaeal preproteins heterologously expressed in bacteria and eukarya (Duffner *et al.*, 2000; Horlacher *et al.*, 1998; Jorgensen *et al.*, 1997; Smith and Robinson, 2002), it seems that this group also includes archaeal Sec signal peptides. Still, archaea-specific traits may exist. Analysis of the *Methanococcus jannaschii* genome suggests, in this organism, that Sec signal peptides may combine a eukarya-like cleavage site, a bacteria-like charge distribution and a unique, archaea-specific hydrophobic region composition (Nielsen *et al.*, 1999). These rules apparently also apply to *Sulfolobus solfataricus* Sec signal peptides (Albers and Driessen, 2002). In other species, however, other rules may apply (Bardy, *et al.*, 2003).

Archaea also encode for proteins bearing Tat pathway signal peptides. Such signals can be distinguished from Sec-type signal peptides by the presence of twin arginine residues in the n-region and a less hydrophobic h-region. Tat signal peptides also bear a Sec-system avoidance signal in the c-region and tend to be longer than Sec signal peptides (Berks, 1996; Bogsch *et al.*, 1997; Cristobal *et al.*, 1999). While Sec-type signal peptides are predicted to predominate in those species examined at the genome level thus far (Dilks *et al.*, 2003; Rose *et al.*, 2002), studies addressing the secretome of *Halobacterium* sp. NRC-1 have suggested that the Tat system is the major secretion system in this species (Bolhuis, 2002; Rose *et al.*, 2002) (see below).

It should be stressed, however, that predictions on the nature and distribution of archaeal signal peptides are based on the similarities of these sequences to their counterparts in eukarya and bacteria. As such, the existence of signal peptides typical of archaea, not detectable by current screening approaches, cannot be discounted.

THE ARCHAEAL TRANSLOCON AND OTHER TRANSLOCATION-RELATED PROTEINS

As in the other two domains of life, passage of archaeal secretory proteins across the membrane occurs through a dedicated membrane protein complex, the translocon. In bacteria, the translocon is based on the SecYEG complex (Brundage *et al.*, 1990), although a minimal complex formed of only SecYE is competent for translocation (Akimaru *et al.*, 1991; Duong and Wickner, 1997a). In eukarya, the Sec61$\alpha\beta\gamma$ complex is embedded in the membrane of the endoplasmic reticulum (ER) and serves as the entrance to the secretory pathway (Rapoport *et al.*, 1996). In addition, the bacterial and eukaryal translocons contain addition subunits that also participate in the translocation event. Examination of completed archaeal genomes as well as isolation of translocon-related genes or their products in other species reveals an archaeal translocation apparatus of hybrid character, incorporating both bacterial and eukaryal traits in addition to archaeal-specific features (Fig. 1; Table I).

SecY/Sec61α

The archaeal version of SecY has been detected in all sequenced archaeal genomes to date. In addition, *secY*-encoding genes have also been cloned in several other strains (Arndt, 1992; Auer *et al.*, 1991; Irihimovitch

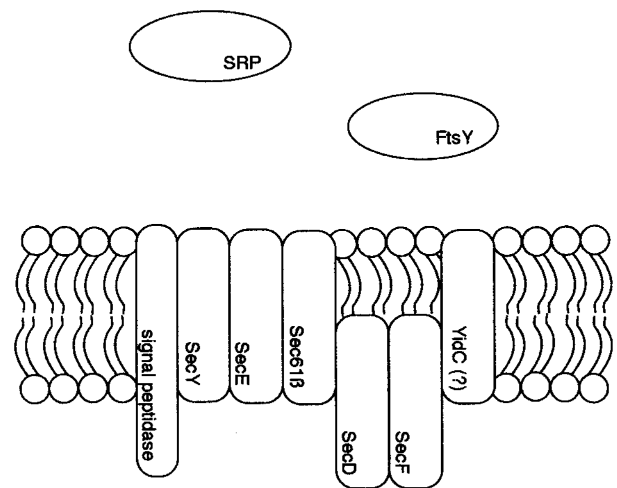

Fig. 1. Currently known components of the Sec translocation pathway in archaea.

Table I. A Current Overview of archaeal Protein Translocation

Aspect of Translocation		Comments[a]
Relation to translation		
Secretion	Posttranslational	Shown with chimeras
Membrane insertion	Cotranslational	Shown with bacterioopsin
Signal peptides	Sec	As in eukarya, bacteria
	Tat	May predominate in haloarchaea
Protein targeting		
SRP	7S RNA	Eukarya-like secondary structure
	SRP54	
	SRP19	Archaea-specific traits
SRP receptor	FtsY	Bacteria-like; no integral receptor
Translocon	SecYE	Eukarya-like
	Sec61β	Eukarya-like
	SecDF	Bacteria-like
	YidC (?)	
Driving force	???	No SecA
Signal peptidase		
Sequence	Boxes A–E	Bacteria-like domain II in some species
Oligomeric state	Single subunit	As in bacteria
Mechanism	eukarya-like (?)	Contains eukaryal SH, not bacterial SK
Membrane lipids	Ether-based	Archaea-specific
		Tetraether monolayer in hyperthermoarchaea

[a] See text for details.

et al., 2003; Kath and Schäfer, 1995). Although termed according to prokaryotic nomenclature, phylogenetic analysis reveals that archaeal SecY proteins are more reminiscent of eukaryal Sec61α proteins than of their bacterial counterparts (Cao and Saier, 2003; Rensing and Maier, 1994). Like other SecY/Sec61α proteins, archaeal SecY proteins are predicted to span the membrane 10 times (Auer *et al.*, 1991; Cao and Saier, 2003; Irihimovitch *et al.*, 2003; Kath and Schäfer, 1995), and as such are likely to form the translocation pore through which translocating proteins cross the membrane, as proposed in bacteria and eukarya (Joly and Wickner, 1993; Mothes *et al.*, 1994).

Apart from such sequence-based comparisons, little data on archaeal SecY at the protein level has been reported to date. Complementation of a temperature-sensitive *secY Escherichia coli* mutant with the *Methanococcus vanielii* SecY-encoding gene resulted in the ability of the mutant strain to grow at the nonpermissive temperature (Auer *et al.*, 1991). The finding suggests not only that an archaeal SecY can functionally replace its bacterial counterpart, but that the archaeal protein is active even in the absence of the unique ether-based phospholipids that comprise the archaeal plasma membrane (see below). In the haloarchaea *Haloferax volcanii*, the salt-insensitive interaction between a cellulose binding domain and cellulose has been exploited for purification of a chimera containing the SecY protein. In these studies, the SecY-containing protein was shown to be stably expressed and exclusively localized to the membrane (Irihimovitch *et al.*, 2003).

SecE/Sec61γ

Like archaeal SecY, phylogenetic analysis reveals that archaeal SecE is also more similar to the eukaryal version of the protein, i.e., Sec61γ (Cao and Saier, 2003). Indeed, the homology between bacterial SecE and its eukaryal counterpart was first shown by comparing each with the archaeal *S. solfataricus* SecE gene (Hartmann *et al.*, 1994). Like other SecE/Sec61γ proteins (with the exception of *E. coli* SecE (Murphy and Beckwith, 1994)), archaeal SecE is thought to possess a single membrane-spanning domain near the C terminus of the protein (Cao and Saier, 2003). The only study of archaeal SecE at the protein level to date came with purification of a cellulose binding domain-SecE chimera from transformed *H. volcanii*. As with the corresponding SecY chimera, the SecE-incorporating protein was also shown to be stably expressed and restricted to the plasma membrane (Irihimovitch *et al.*, 2003).

Sec61β

In bacteria, the core SecYE complex is accompanied by a third component, SecG (Brundage *et al.*, 1992;

Douville *et al.*, 1994; Nishiyama *et al.*, 1993), while in eukarya, Sec61β exists in complex with the Sec61α and γ subunits (Gorlich and Rapoport, 1993). In contrast to the homology of SecYE and Sec61$\alpha\gamma$, respectively, SecG and Sec61β do not resemble each other (Cao and Saier, 2003; Matlack *et al.*, 1998). Accordingly, these latter two translocon components apparently serve distinct functions, possibly reflecting differences in the bacterial and eukaryal translocation processes. SecG serves to increase the efficiency of translocation (Hanada *et al.*, 1994; Nishiyama *et al.*, 1994) yet can be functionally replaced by SecDF (Duong and Wickner, 1997a), reflecting its auxilliary role in translocation. Sec61β, thought to kinetically facilitate cotranslational translocation by participating in the insertion of a nascent polypeptide chain into the translocon and to interact with signal peptidase, the enzyme responsible for signal sequence cleavage, is also not essential for translocation (Kalies *et al.*, 1998).

Whereas archaeal homologues of SecY/61α and SecE/61γ were readily detected in archaea, archaeal versions of either SecG or Sec61β were not originally reported (Eichler, 2000; Pohlschroder *et al.*, 1997). More recently, an archaeal version of Sec61β, revealed through PSI-BLAST searches, has been proposed (Kinch *et al.*, 2002). Experimental verification of the expression and function of this protein, however, remains to be presented. Still, the finding that all archaeal genomes examined thus far encode for homologue of the eukaryal Sec61β (as well as Sec61α and Sec61γ) suggests similarities in protein translocation in these two domains of life.

SecDF

In bacteria, SecYEG can exist as part of a higher order complex including SecDF (Duong and Wickner, 1997a). Whereas the absence of SecDF affects the efficiency of protein translocation in vivo (Pogliano and Beckwith, 1994), SecDF are not required for the reconstitution of efficient protein translocation in vitro (Duong and Wickner, 1997a; Matsuyama *et al.*, 1992). While SecDF have been implicated in maintaining the proton motive force that exists across the plasma membrane (Arkowitz and Wickner, 1994; see Nouwen *et al.*, 2001, for a counterview), in modulating the translocation-related, membrane-associated behavior of SecA (the ATPase component of the bacterial protein translocation apparatus) in vitro (Duong and Wickner, 1997b; Economou *et al.*, 1995), in protein release following translocation (Matsuyama *et al.*, 1993), and in signal sequence clearance (Bolhuis *et al.*, 1998), the true function(s) of SecDF remain to be defined.

Analysis of completed archaeal genomes reveals the existence of SecDF homologues in some, but not all, species (Eichler, 2003; Tseng *et al.*, 1999). Indeed, some bacterial strains also lack SecDF-encoding genes (Tseng *et al.*, 1999). The significance of this observation is not clear, although it remains a possibility that SecDF-lacking strains express structural homologues of these proteins, not detectable by sequence-based tools, or that other members of the resistance-nodulation-cell division permease superfamily of transporter proteins to which SecDF belongs may assume the role of SecDF in these strains (Tseng *et al.*, 1999).

Comparison of bacterial and archaeal sequences reveals that both SecDF populations present similar membrane topologies and positioning of conserved sequence elements. However, closer examination reveals that the makeup of these conserved elements can be divided along bacteria–archaea lines (Eichler, 2003). This difference is most obvious in SecD domain 2, situated in the large extracytoplasmic loop of the protein. It is tempting to speculate that such sequence differences reflect function distinctions between bacterial and archaeal SecDF, and as such, between the translocation processes themselves. One such difference could be related to the interaction of SecDF with SecA. In *E. coli*, SecDF serve to modulate the membrane-association of SecA during translocation (Duong and Wickner, 1997b; Economou *et al.*, 1995). It is unlikely that archaeal SecDF assumes a similar role, given the apparent absence of SecA in archaea (Eichler, 2000; Pohlschroder *et al.*, 1997).

YidC

Along with SecDF, SecYEG can be coisolated with other components, including YidC (Scotti *et al.*, 2000), the bacterial homologue of Oxa1 and Alb3, elements involved in insertion of selected proteins into the membranes of mitochondria and thylakoids, respectively (Hell *et al.*, 2001; Moore *et al.*, 2000). As such, YidC also participates in the insertion of certain proteins into the bacterial plasma membrane (Samuelson *et al.*, 2000). Moreover, YidC has been shown to catalyze membrane protein insertion in a Sec-independent manner (Chen *et al.*, 2002). Recent phylogenetic analysis of the YidC/Oxa1/Alb3 family has proposed the existence of related proteins in archaea (Luirink *et al.*, 2001; Yen *et al.*, 2001). The putative archaeal YidC proteins display a topology like that of their bacterial counterparts (Luirink *et al.*, 2001), yet are generally smaller (Yen *et al.*, 2002), as is thought to be generally the case with archaeal transport proteins (Chung *et al.*, 2001). However, as the homology of the archaeal proteins and other YidC family members is low, these bioinformatic prediction awaits experimental confirmation.

Signal Peptidase

Signal peptidase (type I) is responsible for the removal of the signal sequence following protein translocation (Dalbey *et al.*, 1997; Paetzel *et al.*, 2000). As with other facets of the archaeal translocation machinery, the archaeal signal peptidase presents a mosaic of bacterial, eukaryal, and archaeal properties. Like its bacterial and eukaryal counterparts, the archaeal enzyme also encompasses five regions of sequence homology termed boxes A–E (Eichler, 2002; Paetzel *et al.*, 2000; Tjalsma *et al.*, 1998). However, in the archaeal enzyme (as in eukaryal signal peptidases), the lysine residue of the strictly conserved Box B Ser90-Box D Lys145 pair found in bacterial signal peptidases (*E. coli* numbering), proposed to serve as the catalytic dyad of the enzyme, is replaced by a histidine residue (Eichler, 2002; Paetzel *et al.*, 2000; Tjalsma *et al.*, 1998). Hence, as is the case with the eukaryal enzyme, the catalytic mechanism of archaeal signal peptidase remains unknown. However, in contrast to the eukaryal enzyme, which functions as part of a multisubunit complex (YaDeau *et al.*, 1991), archaeal signal peptidase appears to function independently, like its bacterial counterpart.

Examination of available archaeal signal peptidase sequences also reveals that variations exist within this kingdom. Comparison of archaeal signal peptidase sequences with that of the *E. coli* enzyme, for which 3D information is available, reveals that some, but not all, archaea encode for domain II (Eichler, 2002). Domain II corresponds to a stretch of amino acids situated between boxes D and E that folds into a large structure on top of the catalytic core of the bacterial enzyme formed by boxes B–E (Paetzel *et al.*, 1998). The role assumed by domain II and the reason for its absence in certain archaeal signal peptidases remains open to speculation. The finding that signal peptidases of some archaea (e.g., *Thermoplasma* species) contain a bacteria-like domain II, whereas others do not, could suggest that primitive archaeal signal peptidases originally contained this region, yet during subsequent diversification, this domain was lost.

THE Tat PATHWAY IN ARCHAEA

Archaea also encode for components of the Sec-independent Tat pathway, as observed in bacteria, chloroplasts, and mitochondria (Bogsch *et al.*, 1998). In bacteria, the Tat pathway is largely responsible for the translocation of folded proteins, sometimes in complex with cofactors (Berks *et al.*, 2000; Robinson and Bolhuis, 2001). Current understanding of the role of the Tat pathway in archaea is largely based on surveys of completed genomes that predict differing extents of usage of this translocation system (Bolhuis, 2002; Dilks *et al.*, 2003; Rose *et al.*, 2002; Yen *et al.*, 2002). Interestingly, such analyses predict that the Tat pathway is the predominant mode of translocation employed by halophilic archaea, possibly to overcome potential dangers of protein misfolding in the highly saline cytoplasm of these species (Bolhuis, 2002; Rose *et al.*, 2002). As elsewhere, however, molecular aspects of the archaeal Tat pathway are not well-characterized. Archaeal TatA, TatB, or TatC homologues, proteins that mediate translocation via the Tat pathway, can be detected in some, but not all, species (Dilks *et al.*, 2003; Eichler, 2000; Yen *et al.*, 2002). Specifically, all sequenced crenarchaeotes encode for Tat components, whereas only some euryarchaeotes contain such genes. Different species, furthermore, may contain differing numbers of Tat component homologues. Indeed, in some species, such as *Methanopyrus kandleri* AV19, despite the predicted absence of Tat system substrates, the presence of at least one Tat component is suggested (Dilks *et al.*, 2003).

ARCHAEAL PROTEIN TRANSLOCATION: A CO- OR POSTTRANSLATIONAL EVENT?

Whereas the bacterial and eukaryal protein translocation machineries include many homologous components, the two systems differ in terms of the temporal relationship between the translation and translocation of secretory proteins. In bacteria, signal-peptide-bearing precursors of secreted proteins are translocated posttranslationally, i.e., once most, if not all, of the protein has been translated in the cytoplasm (Koshland and Botstein, 1982; Randall, 1983). In contrast, the translocation of secretory proteins across the membrane of the eukaryal ER, the topological homologue of the bacterial plasma membrane, is coupled to protein translation (Blobel and Dobberstein, 1975), although eukaryal posttranslational translocational has been reported in yeast (Rapoport *et al.*, 1999). In archaea, the relation of protein translation to protein export remains to be elucidated. On the one hand, archaea contain a signal recognition particle (SRP), the agent responsible for linking translation and translocation in eukarya (Keenan *et al.*, 2001), reminiscent of it eukaryal counterpart (Eichler and Moll, 2001). On the other hand, many archaea encode for SecDF (Eichler, 2003; Tseng *et al.*, 1999), proteins which in bacteria serve to modulate the membrane association of SecA, the ATPase that drives posttranslational translocation. As noted above, however, searches of completed archaeal genomes have thus far failed to reveal an archaeal SecA homologue (Eichler, 2000; Pohlschroder *et al.*, 1997).

Of the limited number of studies addressing the relationship between archaeal protein translation and translocation to date, the majority have focused on the biosynthesis of bacterioopsin, the *Halobacterium salinarum* multispanning membrane protein that serves as the apoprotein of bacteriorhodopsin. Early work showing cosedimentation of 7S RNA and bacterioopsin mRNA with membrane-bound polysomes, together with puromycin-induced release of the 7S RNA from the polysomes, lead to the conclusion that a cotranslational mode of insertion was at play (Gropp *et al.*, 1992). More recent in vivo kinetic labelling experiments revealed the cotranslational insertion of protein's N-terminal region, but posttranslational insertion of the more C-terminal portion of the the protein (Dale *et al.*, 2000; Dale and Krebs, 1999). In contrast, studies following the membrane insertion of a chimeric version of bacterioopsin heterologously expressed in *H. volcanii* reported that expression of the seventh and final transmembrane domain was required for membrane insertion, pointing at a wholly posttranslational mode of insertion (Ortenberg and Mevarech, 2000). Studies relying on newly synthesized bacterioopsin as a reporter of the relation between translation and translocation in archaea may not, however, reflect the general situation, given that bacterioopsin is synthesized with an unusually short, 13 residue cleavable signal peptide lacking a hydrophobic core and containing negatively charged glutamate residues (Gropp *et al.*, 1992; Seehra and Khorana, 1984). In most signal peptides, a positively charged region is followed by a hydrophobic core and a region containing the cleavage site (von Heijne, 1990a,b). Moreover, given the central role of bacterioopsin in the generation of "purple membranes" (cf. Krebs and Isenbarger, 2000), it is possible that a dedicated system for bacterioopsin membrane insertion exists.

The interaction between translation and translocation of a membrane protein may not necessarily reflect the relation between protein translation and secretion. In experiments designed to elucidate the temporal relation between translation and translocation during archaeal protein secretion, *H. volcanii* cells were transformed to heterologously express chimeric preproteins containing the signal peptide of the surface layer glycoprotein, the major exported protein in this species (Irihimovitch and Eichler, 2003). The molecular composition of the 34 amino acid residue-long surface layer glycoprotein signal peptide is similar to those found in secretory preproteins in the other two domains of life (von Heijne, 1990a,b) and includes a cleavage site recognized by type I signal peptidases (Sumper *et al.*, 1990). Kinetic radiolabeling experiments revealed that secretion took place only after the preproteins had been translated in the cytosol of transformed *H. volcanii* cells. Furthermore, arrest of continued protein translation failed to prevent secretion of previously pulse radiolabeled preprotein (Irihimovitch and Eichler, 2003). Finally, as in bacteria and chloroplasts, the archaeal Tat system may also translocate proteins in a posttranslocational manner (Rose *et al.*, 2002).

Despite the limited amount of data available in support of such a claim, it is tempting to speculate that in archaea, protein secretion occurs posttranslationally while membrane insertion occurs in an SRP-dependent, cotranslational manner. Not all archaeal membrane proteins need, however, rely on SRP for their insertion, as possibly exemplified by studies addressing the homologous (Dale *et al.*, 2000; Dale and Krebs, 1999; Gropp *et al.*, 1992) and heterologous (Ortenberg and Mevarech, 2000) expression of bacterioopsin. Indeed, the SRP pathway is involved in the insertion of only a subset of bacterial membrane proteins (Ulbrandt *et al.*, 1997).

THE DRIVING FORCE OF ARCHAEAL PROTEIN TRANSLOCATION

One of the biggest challenges to understanding archaeal protein translocation is related to the driving force of the process. Examination of different protein translocation systems reveals that a variety of energetic sources are employed in transporting proteins across membranes. At the ER membrane, GTP energy is spent in coupling a translating ribosome to the translocon, where subsequent elongation threads the polypeptide chain across the membrane (Keenan *et al.*, 2001; Rapoport *et al.*, 1996). In posttranslational translocation across the yeast ER membrane, the resident ER chaperone BiP uses ATP energy to pull the transiting preprotein into the ER lumen (Rapoport *et al.*, 1999). Similarly, import of preproteins into the mitochondrial matrix relies on an ATP-driven reaction mediated by the matrix-localized member of the hsp70 family (Ungermann *et al.*, 1994). Furthermore, ATP energy is also employed in the targeting phase of these posttranslational translocation processes (Ngosuwan *et al.*, 2003; Young *et al.*, 2003). In bacteria, SecA relies on ATP hydrolysis to drive the insertion of a translocating preprotein into the plasma membrane at SecYEG sites (Manting and Driessen, 2000; Mori and Ito, 2001). In contrast, the Tat translocation system found in bacteria and chloroplasts does not employ ATP, but does requires a membrane potential (Brock *et al.*, 1995; Cline *et al.*, 1992; Yahr and Wickner, 2001). Finally, the Ran GTPase plays a central role in the shuttling strategy employed as part of nuclear protein import (Macara, 2001).

It is conceivable that a cotranslational mode of translocation, similar to that detected at the ER membrane, could be employed in archaea. Indeed, the SRP pathway, responsible for coupling translation to translocation into the ER lumen, exists in archaea (Eichler and Moll, 2001; Eichler and Zwieb, 2002; Moll, 2004). However, studies examining archaeal protein secretion (see above) reveal that in at least some cases, a posttranslational mode of translocation is employed. In the case of posttranslational archaeal translocation, the nature of the driving force is more difficult to predict. Given the improbability of sufficiently high concentrations of ATP accumulating at the outer cell surface, it is unlikely that archaea rely on chaperones like BiP or mitochondrial hsp70 to pull polypeptides out of the cytoplasm. Moreover, many archaeal species do not encode for hsp70 (Macario *et al.*, 1999), a chaperone involved in the posttranslational delivery of proteins to translocation sites in a variety of membranes (Harano *et al.*, 2001; Ngosuwan *et al.*, 2003; Qi *et al.*, 2002; Rial *et al.*, 2000; Wild *et al.*, 1992; Young *et al.*, 2003). Considering the high degree of conservation amongst bacterial and chloroplast SecA sequences, the failure of genomic searches to reveal an archaeal SecA homologue would argue against the existence of a bacteria-like process in archaea, although the existence of an archaeal structural homologue of SecA, not detectable through sequence-based search tools, cannot yet be discounted.

ARCHAEAL PHOSPHOLIPIDS

One of the distinguishing traits of archaea is the unique chemical composition of the phospholipids that comprise the plasma membrane. Unlike bacterial and eukaryal phospholipids, in which acyl groups are ester-linked to the *sn*-1,2 positions of glycerol, archaeal phospholipids contain repeating isoprenyl units linking to the *sn*-2,3 positions of glycerol through ether bonds (Kates, 1993; Koga *et al.*, 1993; Sprott, 1992). Moreover, while archaeal ether-based phospholipids form bilayers as do phospholipids in eukarya and bacteria, hyperthermophilic archaea (i.e., archaea found at $>85^{\circ}$C) may be surrounded by a monolayer formed from membrane-spanning tetraetheric phospholipids (De Rosa and Gambacorta, 1988). A monolayer configuration would serve to reduce membrane fluidity that could be a danger at such high temperatures. Indeed, the presence of ether linkages in archaeal phospholipids is believed to be an adaptation to harsh environments encountered by these microorganisms, endowing archaeal membranes with resistance to the physical stresses associated with extreme surroundings. Accordingly, liposomes formed from archaeal phospholipids have been shown to be less permeant to protons, sodium ions, and other solutes than liposomes formed from bacterial phospholipids (Elferink *et al.*, 1994; Komatsu and Chong, 1988; van der Vossenberg *et al.*, 1999; Yamauchi *et al.*, 1992, 1993).

It is unknown how, or even if, the unique chemistry of archaeal membrane phospholipids would affect protein translocation. It is conceivable that these lipid species could modulate the behavior of various membrane-associated components of the translocation process, such as FtsY, the translocon, and signal peptidase. While archaeal membrane proteins have been functionally reconstituted into proteoliposomes prepared with nonarchaeal phospholipids (Bogomolni *et al.*, 1984; Huang *et al.*, 1980; Ninio and Schuldiner, 2003) and nonarchaeal membrane proteins have been reconstituted into archaeal liposomes (Elferink *et al.*, 1992, 1993; In't Veld *et al.*, 1992), existing evidence suggests that the activities of archaeal membrane proteins are optimal when embedded in proteoliposomes prepared from archaeal phospholipids (Gleissner *et al.*, 1994; Hojeberg *et al.*, 1982), pointing to specific interactions between archaeal membrane proteins and archaeal membrane lipids. It also remains to be shown whether the process of membrane protein insertion into ether-based membranes also differs from insertion into bacterial and eukaryal ester-based membranes. Finally, just as various phospholipid species have been shown to modulate the translocation process in bacteria (de Vrije *et al.*, 1988; Hendrick and Wickner, 1991; Kusters *et al.*, 1991, 1994; Lill *et al.*, 1990; Matsumoto, 2001; Rietveld *et al.*, 1995), individual lipid subclasses may also play a role in archaeal protein translocation.

IN VITRO RECONSTITUTION OF ARCHAEAL PROTEIN TRANSLOCATION

The current advanced state of understanding of bacterial and eukaryal protein translocation is due, in a large part, to the availability of reconstituted translocation systems composed of purified protein components, native and reassembled ribonucleoprotein complexes as well as various membrane preparations. Work towards the developments of an in vitro archaeal protein translocation system is progressing, with preparation of several of the molecular tools central to such efforts having been reported of late.

Ribosomes

The availability of purified archaeal ribosomes has been an important element in recent advances in

understanding ribosomal function (Ban *et al.*, 2000; Nissen *et al.*, 2000; Yonath, 2002). Purified and functional archaeal ribosomes capable of performing in vitro translation reactions will also serve as a cornerstone for in vitro studies of cotranslational protein translocation in archaea. To date, however, in vitro translation systems based on archaeal ribosomes have achieved only limited success. Systems for the poly(U)-dependent synthesis of polyphenylalanine using ribosomes from various halophilic archaea including *Halobacterium cutiruburm, H. salinarum*, *Haloferax mediterranei*, and *Haloarcula marismortui* have been available for many years (Bayley and Griffith, 1968; Sanz *et al.*, 1988; Saruyama and Nierhaus, 1985). Similarly, poly(U)-dependent poly-phenylalanine translation systems based on ribosomes from the thermophilic archaea *Desulfurococcus mobilis*, *S. solfataricus*, *Thermococcus celer*, *Thermoplasma acidophilum*, and *Thermoproteus tenax* (Cammarano *et al.*, 1982; Klink *et al.*, 1983; Londei *et al.*, 1986) and for use with ribosome-containing extracts of the methanoarchaea *M. vannielii*, *Methanobacterium formicicum*, and *Methanosarcina barkeri* (Elhardt and Bock, 1982) also exist. Indeed, in several of these systems, poly-phenylalanine synthesis was achieved using archaeal ribosomes, together with additional factors obtained from nonarchaeal sources, suggesting that development of a general archaeal in vitro translation system would be relatively simple. Unfortunately, none of these systems have been reported to be capable of translating exogenously added mRNA, clearly a requirement for inclusion in any in vitro archaeal protein translation system.

Later attempts at developing an in vitro archaeal system capable of translating added mRNA have been more successful. Employing *H. salinarum* ribosomes, a system able to translate proteins upon addition of total RNA was reported, although the efficiency of such translation was 10- to 20-fold lower than achieved by the poly(U)-dependent poly-phenylalanine biosynthesis assay developed for this species (Gropp and Oesterhelt, 1989). Moreover, this system failed to translate added bacterioopsin mRNA. More recently, translation of added *S. solfataricus* mRNA up to 800 bp in length has been achieved using an unfractionated *S. solfataricus* cell extract (Condo *et al.*, 1999; Ruggero *et al.*, 1993). Furthermore, this system was also shown capable of translating nonarchaeal mRNA (Grill *et al.*, 2000).

SRP

As discussed elsewhere in this issue (see the contribution by Moll), archaea express components of the SRP targeting pathway. The recent reconstitution of archaeal SRP from *Archaeoglobus fulgidus* (Bhuiyan *et al.*, 2000) and *H. volcanii* (Tozik *et al.*, 2002), of SRP RNA-SRP54 complexes from *Pyrococcus furiosus* (Maeshima *et al.*, 2001) and of SRP RNA-SRP19 complexes from *A. fulgidus* (Diener and Wilson., 2000), *P. furiosus* (Maeshima *et al.*, 2001), and *M. jannaschii* (Hainzl *et al.*, 2002; Oubridge *et al.*, 2002) will allow for eventual incorporation of these targeting factors into any reconstituted archaeal translocation system.

Inverted Membrane Vesicles (IMVs)

Efforts in analyzing the functional behavior of proteins involved in archaeal protein translocation have been hampered by an inavailability of functional archaeal membrane preparations of known orientation, such as IMVs. Difficulties in obtaining IMVs from the vast majority of archaeal species where vesicle preparation was tried is thought to be related to the protein-based rigid surface layer associated with the plasma membrane (Schafer *et al.*, 1999). For instance, preparation of IMVs from *Methanosarcina mazei*, one of the few archaeal strains from which IMVs are currently available, involves prior proteolytic digestion of surface structures (Becher and Muller, 1994). The effects of such digestion on the translocational competence of such vesicles is not known. More recently, despite the presence of a glycoprotein-based surface layer (Kessel *et al.*, 1988; Sumper *et al.*, 1990), large-scale amounts of IMVs from the halophilic archaeon *H. volcanii* have been prepared (Ring and Eichler, 2001). On the basis of the outward exposure of enzyme activities which face the cytoplasm in the intact cell, protease protection assays, electron microscopy, lectin accessibility as well as ATP-synthesizing ability, the *H. volcanii* membrane preparation was shown to contain inverted, sealed, and biologically functional membrane vesicles. Indeed, enzymatic and electron microscopy analyses reveal that 70–90% of the obtained membranous structures are in the form of IMVs. These IMVs are currently being tested for their ability to translocate signal-peptide-containing preproteins.

An alternative approach for the preparation of translocation-competent membrane vesicles involves the introduction of purified archaeal SecY, SecE, and Sec61β into liposomes prepared from archaeal membrane phospholipids to yield proteoliposomes containing a reconstituted archaeal Sec-based translocation complex. Such an approach, involving individually purified bacterial SecY, SecE, and SecG proteins, has been successfully employed for the preparation of translocation-competent

proteoliposomes (Hanada *et al.*, 1994). Similar efforts in the archaeal system may be facilitated by the recent purification of *H. volcanii* SecY and SecE fused to the cellulose binding domain of the *Clostridium thermocellum* cellulosome (Irihimovitch *et al.*, 2003), exploiting the salt-insensitive interaction of *C. thermocellum* cellulose binding domain with cellulose (Morag *et al.*, 1995). In addition, efforts aimed at capturing *H. volcanii* SecYE-containing complexes via the CBD-cellulose affinity purification system are under-way. Such studies are also addressing the use of engineered protease cleavage sites for removal of the CBD tag following purification, in high salt concentrations.

CONCLUSIONS

At this time, preliminary steps have been made towards a detailed description of the biochemistry, cell and structural biology of archaeal protein translocation. Such efforts will, in future, be facilitated by the upcoming release of additional completed archaeal genome sequences and by advances in understanding other facets of archaeal biology. Indeed, as novel and improved molecular tools for working with a wide range of archaeal strains are becoming available, significant strides towards the developing of techniques necessary for in vitro reconstitution of archaeal protein translocation will be realized. Having a clearer picture of archaeal protein translocation carries, however, implications beyond better understanding of a central biological question, i.e., how proteins cross biological membranes. A enhanced grasp of the process of archaeal protein translocation will also help decipher the molecular strategies adopted by extremophilic organisms in overcoming environmental challenges. Moreover, realizing the enormous commerical potential of large-scale production of industrially useful archaeal enzymes will be hastened by a better characterization of archaeal protein export.

ACKNOWLEDGMENT

Our work is supported by the Israel Science Foundation (Grant 291/99).

REFERENCES

Akimaru, J., Matsuyama, S., Tokuda, H., and Mizushima, S. (1991). *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6545–6549.
Albers, S. V., and Driessen, A. M. (2002). *Arch. Microbiol.* **177**, 209–216.
Arkowitz, R. A., and Wickner, W. (1994). *EMBO J.* **13**, 954–963.
Arndt, E. (1992). *Biochim. Biophys. Acta* **1130**, 113–116.
Auer, J., Spicker, G., and Bock, A. (1991). *Biochimie* **73**, 683–688.
Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A. (2000). *Science* **289**, 905–920.
Brady, S. L., Eichler, J., and Jarrell, K. F. (2003). *Protein Sci.* **12**, 1833–1843.
Bayley, S. T., and Griffiths, E. (1968). *Biochemistry* **7**, 2249–2256.
Becher, B., and Muller, V. (1994). *J. Bacteriol.* **176**, 2543–2550.
Berks, B. C. (1996). *Mol. Microbiol.* **22**, 393–404.
Berks, B. C., Sargent, F., De Leeuw, E., Hinsley, A. P., Stanley, N. R., Jack, R. L., Buchanan, G., and Palmer, T. (2000). *Biochim. Biophys. Acta* **1459**, 325–330.
Bhuiyan, S. H., Gowda, K., Hotokezaka, H., and Zwieb, C. (2000). *Nucleic Acids Res.* **28**, 1365–1373.
Blobel, G., and Dobberstein, B. (1975). *J. Cell Biol.* **67**, 835–851.
Bogomolni, R. A., Taylor, M. E., and Stoeckenius, W. (1984). *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5408–5411.
Bogsch, E., Brink, S., and Robinson, C. (1997). *EMBO J.* **16**, 3851–3859.
Bogsch, E. G., Sargent, F., Stanley, N. R., Berks, B. C., Robinson, C., and Palmer, T. (1998). *J. Biol. Chem.* **273**, 18003–18006.
Bolhuis, A. (2002). *Microbiology* **148**, 3335–3346.
Bolhuis, A., Broekhuizen, C. P., Sorokin, A., van Roosmalen, M. L., Venema, G., Bron, S., Quax, W. J., and van Dijl, J. M. (1998). *J. Biol. Chem.* **273**, 21217–21224.
Brock, I. W., Mills, J. D., Robinson, D., and Robinson, C. (1995). *J. Biol. Chem.* **270**, 1657–1662.
Brundage, L., Fimmel, C. J., Mizushima, S., and Wickner, W. (1992). *J. Biol. Chem.* **267**, 4166–4170.
Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J., and Wickner, W. (1990). *Cell* **62**, 649–657.
Cammarano, P., Teichner, A., Chinali, G., Londei, P., de Rosa, M., Gambacorta, A., and Nicolaus, B. (1982). *FEBS Lett.* **148**, 255–259.
Cao, T. B., and Saier, M. H., Jr. (2003). *Biochim. Biophys. Acta* **1609**, 115–125.
Chen, M., Samuelson, J. C., Jiang, F., Muller, M., Kuhn, A., and Dalbey, R. E. (2002). *J. Biol. Chem.* **277**, 7670–7675.
Chung, Y. J., Krueger, C., Metzgar, D., and Saier, M. H., Jr. (2001). *J. Bacteriol.* **183**, 1012–1021.
Cline, K., Ettinger, W. F., and Theg, S. M. (1992). *J. Biol. Chem.* **267**, 2688–2696.
Condo, I., Ciammaruconi, A., Benelli, D., Ruggero, D., and Londei, P. (1999). *Mol. Microbiol.* **34**, 377–384.
Cristobal, S., de Gier, J. W., Nielsen, H., and von Heijne, G. (1999). *EMBO J.* **18**, 2982–2990.
Dalbey, R. E., Lively, M. O., Bron, S., and van Dijl, J. M. (1997). *Protein Sci.* **6**, 1129–1138.
Dale, H., Angevine, C. M., and Krebs, M. P. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7847–7852.
Dale, H., and Krebs, M. P. (1999). *J. Biol. Chem.* **274**, 22693–22698.
De Rosa, M., and Gambacorta, A. (1988). *Prog. Lipid Res.* **27**, 153–175.
de Vrije, T., de Swart, R. L., Dowhan, W., Tommassen, J., and de Kruijff, B. (1988). *Nature* **334**, 173–175.
Diener, J. L., and Wilson, C. (2000). *Biochemistry* **39**, 12862–12874.
Dilks, K., Rose, R. W., Hartmann, E., and Pohlschroder, M. (2003). *J. Bacteriol.* **185**, 1478–1483.
Douville, K., Leonard, M., Brundage, L., Nishiyama, K., Tokuda, H., Mizushima, S., and Wickner, W. (1994). *J. Biol. Chem.* **269**, 18705–18707.
Duffner, F., Bertoldo, C., Andersen, J. T., Wagner, K., and Antranikian, G. (2000). *J. Bacteriol.* **182**, 6331–6338.
Duong, F., and Wickner, W. (1997a). *EMBO J.* **16**, 2756–2768.
Duong, F., and Wickner, W. (1997b). *EMBO J.* **16**, 4871–4879.
Economou, A., Pogliano, J. A., Beckwith, J., Oliver, D. B., and Wickner, W. (1995). *Cell* **83**, 1171–1181.
Eichler, J. (2000). *Eur. J. Biochem.* **267**, 3402–3412.
Eichler, J. (2002). *J. Mol. Evol.* **54**, 411–415.
Eichler, J. (2003). *Mol. Phylogenet. Evol.* **27**, 504–509.
Eichler, J., and Moll, R. (2001). *Trends Microbiol.* **9**, 130–136.
Eichler, J., and Zwieb, C. (2002). *Archaea* **1**, 27–34.

Elferink, M. G., de Wit, J. G., Demel, R., Driessen, A. J., and Konings, W. N. (1992). *J. Biol. Chem*. **267**, 1375–1381.
Elferink, M. G., de Wit, J. G., Driessen, A. J., and Konings, W. N. (1993). *Eur. J. Biochem*. **214**, 917–925.
Elferink, M. G., de Wit, J. G., Driessen, A. J., and Konings, W. N. (1994). *Biochim. Biophys. Acta* **1193**, 247–254.
Elhardt, D., and Bock, A. (1982). *Mol. Gen. Genet.* **188**, 128–134.
Faguy, D. M., Jarrell, K. F., Kuzio, J., and Kalmokoff, M. L. (1994). *Can. J. Microbiol.* **40**, 67–71.
Gleissner, M., Elferink, M. G., Driessen, A. J., Konings, W. N., Anemuller, S., and Schafer, G. (1994). *Eur. J. Biochem*. **224**, 983–990.
Gorlich, D., and Rapoport, T. A. (1993). *Cell* **75**, 615–630.
Grill, S., Gualerzi, C. O., Londei, P., and Blasi, U. (2000). *EMBO J.* **19**, 4101–4110.
Gropp, R., Gropp, F., and Betlach, M. C. (1992). *Proc. Natl. Acad. Sci. U.S.A*. **89**, 1204–1208.
Gropp, R., and Oesterhelt, D. (1989). *FEBS Lett.* **259**, 5–9.
Hainzl, T., Huang, S., and Sauer-Eriksson, A. E. (2002). *Nature* **417**, 767–771.
Hanada, M., Nishiyama, K. I., Mizushima, S., and Tokuda, H. (1994). *J. Biol. Chem*. **269**, 23625–23631.
Harano, T., Nose, S., Uezu, R., Shimizu, N., and Fujiki, Y. (2001). *Biochem. J*. **1**, 157–165.
Hartmann, E., Sommer, T., Prehn, S., Gorlich, D., Jentsch, S., and Rapoport, T. A. (1994). *Nature* **367**, 654–657.
Hell, K., Neupert, W., and Stuart, R. A. (2001). *EMBO J.* **20**, 1281–1288.
Hendrick, J. P., and Wickner, W. (1991). *J. Biol. Chem*. **266**, 24596–24600.
Hojeberg, B., Lind, C., and Khorana, H. G. (1982). *J. Biol. Chem*. **257**, 1690–1694.
Horlacher, R., Xavier, K. B., Santos, H., DiRuggiero, J., Kossmann, M., and Boos, W. (1998). J. *Bacteriol.* **180**, 680–689.
Huang, K. S., Bayley, H., and Khorana, H. G. (1980). *Proc. Natl. Acad. Sci. U.S.A*. **77**, 323–327.
In't Veld, G., Elferink, M. G., Driessen, A. J., and Konings, W. N. (1992). *Biochemistry* **31**, 12493–12499.
Irihimovitch, V., and Eichler, J. (2003). *J. Biol. Chem*. **278**, 12881–12887.
Irihimovitch, V., Ring, G., Elkayam, T., Konrad, Z., and Eichler, J. (2003). *Extremophiles* **7**, 71–77.
Joly, J. C., and Wickner, W. (1993). *EMBO J*. **12**, 255–263.
Jorgensen, S., Vorgias, C. E., and Antranikian, G. (1997). *J. Biol. Chem*. **272**, 16335–16342.
Kalies, K. U., Rapoport, T. A., and Hartmann, E. (1998). *J. Cell Biol*. **141**, 887–894.
Kates, M. (1993). *Experientia* **49**, 1027–1036.
Kath, T., and Schäfer, G. (1995). *Biochim. Biophys. Acta* **1264**, 155–158.
Keenan, R. J., Freymann, D. M., Stroud, R. M., and Walter, P. (2001). *Annu. Rev. Biochem*. **70**, 755–775.
Kessel, M., Buhle, E. L., Jr., Cohen, S., and Aebi, U. (1988). *J. Ultrastruct. Mol. Struct. Res*. **100**, 94–106.
Kinch, L. N., Saier, M. H., Jr., and Grishin, N. V. (2002). *Trends Biochem. Sci*. **27**, 170–171.
Klink, F., Schumann, H., and Thomsen, A. (1983). *FEBS Lett*. **155**, 173–177.
Koga, Y., Nishihara, M., Morii, H., and Akagawa-Matsushita, M. (1993). *Microbiol. Rev.* **57**, 164–182.
Komatsu, H., and Chong, P. L. (1998). *Biochemistry* **37**, 107–115.
Koshland, D., and Botstein, D. (1982). *Cell* **30**, 893–902.
Krebs, M. P., and Isenbarger, T. A. (2000). *Biochim. Biophys. Acta* **1460**, 15–26.
Kusters, R., Breukink, E., Gallusser, A., Kuhn, A., and de Kruijff, B. (1994). *J. Biol. Chem*. **269**, 1560–1563.
Kusters, R., Dowhan, W., and de Kruijff, B. (1991). *J. Biol. Chem.* **266**, 8659–8662.
Lill, R., Dowhan, W., and Wickner, W. (1990). *Cell* **60**, 271–280.
Londei, P., Altamura, S., Cammarano, P., and Petrucci, L. (1986). *Eur. J. Biochem*. **157**, 455–462.
Luirink, J., Samuelsson, T., and de Gier, J. W. (2001). *FEBS Lett.* **50**, 1–5.
Macara, I. G. (2001). *Microbiol. Mol. Biol. Rev*. **65**, 570–594.
Macario, A. J., Lange, M., Ahring, B. K., and de Macario, E. C. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 923–967.
Maeshima, H., Okuno, E., Aimi, T., Morinaga, T., and Itoh, T. (2001). *FEBS Lett*. **507**, 336–340.
Manting, E. H., and Driessen, A. J. (2000). *Mol. Microbiol*. **37**, 226–238.
Matlack, K. E., Mothes, W., and Rapoport, T. A. (1998). *Cell* **92**, 381–390.
Matsumoto, K. (2001). *Mol. Microbiol.* **39**, 1427–1433.
Matsuyama, S., Fujita, Y., and Mizushima, S. (1993). *EMBO J.* **12**, 265–270.
Matsuyama, S., Fujita, Y., Sagara, K., and Mizushima, S. (1992). *Biochim. Biophys. Acta* **1122**, 77–84.
Moll, R. G. (2004). *J. Bioenerg. Biomembr*. **36**(1), 47–53.
Moore, M., Harrison, M. S., Peterson, E. C., and Henry, R. (2000). *J. Biol. Chem*. **275**, 1529–1532.
Morag, E., Lapidot, A., Govorko, D., Lamed, R., Wilchek, M., Bayer, E. A., and Shoham, Y. (1995). *Appl. Environ. Microbiol*. **61**, 1980–1986.
Mori, H., and Ito, K. (2001). *Trends Microbiol.* **9**, 494–500.
Mothes, W., Prehn, S., and Rapoport, T. A. (1994). *EMBO J*. **13**, 3973–3982.
Murphy, C. K., and Beckwith, J. (1994). *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2557–2561.
Ngosuwan, J., Wang, N. M., Fung, K. L., and Chirico, W. J. (2003). *J. Biol. Chem*. **278**, 7034–7042.
Nielsen, H., Brunak, S., and von Heijne, G. (1999). *Protein Eng*. **12**, 3–9.
Ninio, S., and Schuldiner, S. (2003). *J. Biol. Chem*. **278**, 12000–12005.
Nishiyama, K., Hanada, M., and Tokuda, H. (1994). *EMBO J*. **13**, 3272–3277.
Nishiyama, K., Mizushima, S., and Tokuda, H. (1993). *EMBO J*. **12**, 3409–3415.
Nissen, P., Hansen, J., Ban, N., Moore, P. B., and Steitz, T. A. (2000). *Science* **289**, 920–930.
Nouwen, N., van der Laan, M., and Driessen, A. J. (2001). *FEBS Lett*. **508**, 103–106.
Ortenberg, R., and Mevarech, M. (2000). *J. Biol. Chem*. **275**, 22839–22846.
Oubridge, C., Kuglstatter, A., Jovine, L., and Nagai, K. (2002). *Mol. Cell* **9**, 1251–1261.
Paetzel, M., Dalbey, R. E., and Strynadka, N. C. (1998). *Nature* **396**, 186–190.
Paetzel, M., Dalbey, R. E., and Strynadka, N. C. (2000). *Pharmacol. Ther*. **87**, 27–49.
Pogliano, J. A., and Beckwith, J. (1994). *EMBO J*. **13**, 554–561.
Pohlschroder, M., Prinz, W. A., Hartmann, E., and Beckwith, J. (1997). *Cell* **91**, 563–566.
Qi, H. Y., Hyndman, J. B., and Bernstein, H. D. (2002). *J. Biol. Chem.* **277**, 51077–51083.
Randall, L. L. (1983). *Cell***33**, 231–240.
Rapoport, T. A., Jungnickel, B., and Kutay, U. (1996). *Annu. Rev. Biochem.* **65**, 271–303.
Rapoport, T. A., Matlack, K. E., Plath, K., Misselwitz, B., and Staeck, O. (1999). *Biol. Chem*. **380**, 1143–1150.
Rensing, S. A., and Maier, U. G. (1994). *Mol. Phylogenet. Evol*. **3**, 187–191.
Rial, D. V., Arakaki, A. K., and Ceccarelli, E. A. (2000). *Eur. J. Biochem*. **267**, 6239–6248.
Rietveld, A. G., Koorengevel, M. C., and de Kruijff, B. (1995). *EMBO J*. **14**, 5506–5513.
Ring, G., and Eichler, J. (2001). *J. Membr. Biol*. **183**, 195–204.
Robinson, C., and Bolhuis, A. (2001). *Nat. Rev. Mol. Cell Biol*. **2**, 350–356.
Rose, R. W., Bruser, T., Kissinger, J. C., and Pohlschroder, M. (2002). *Mol. Microbiol*. **45**, 943–950.
Ruggero, D., Creti, R., and Londei, P. (1993). *FEMS Microbiol. Lett.* **107**, 89–94.

Samuelson, J. C., Chen, M., Jiang, F., Moller, I., Wiedmann, M., Kuhn, A., Phillips, G. J., and Dalbey, R. E. (2000). *Nature* **406**, 637–641.

Sanz, J. L., Marín, I., Balboa, M. A., Ureña, D., and Amils, R. (1988). *Biochemistry* **27**, 8194–8199.

Saruyama, H., and Nierhaus, K. (1985). *FEBS Lett.* **183**, 390–394.

Schafer, G., Engelhard, M., and Muller, V. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 570–620.

Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W., von Heijne, G., van der Does, C., Driessen, A. J., Oudega, B., and Luirink, J. (2000). *EMBO J*. **19**, 542–549.

Seehra, J. S., and Khorana, H. G. (1984). *J. Biol. Chem*. **259**, 4187–4193.

Smith, J. D., and Robinson, A. S. (2002). *Biotechnol. Bioeng*. **79**, 713–723.

Sprott, G. D. (1992). *J. Bioenerg. Biomembr*. **24**, 555–566.

Sumper, M., Berg, E., Mengele, R., and Strobel, I. (1990). *J. Bacteriol*. **172**, 7111–7118.

Tjalsma, H., Bolhuis, A., van Roosmalen, M. L., Wiegert, T., Schumann, W., Broekhuizen, C. P., Quax, W. J., Venema, G., Bron, S., and van Dijl, J. M. (1998). *Genes Dev*. **12**, 2318–2331.

Tozik, I., Huang, Q., Zwieb, C., and Eichler, J. (2002). *Nucleic Acids Res*. **30**, 4166–4175.

Tseng, T. T., Gratwick, K. S., Kollman, J., Park, D., Nies, D. H., Goffeau, A., and Saier, M. H., Jr. (1999). *J. Mol. Microbiol. Biotechnol.* **1**, 107–125.

Ulbrandt, N. D., Newitt, J. A., and Bernstein, H. D. (1997). *Cell* **88**, 187–196.

Ungermann, C., Neupert, W., and Cyr, D. M. (1994). *Science* **266**, 1250–1253.

van de Vossenberg, J. L., Driessen, A. J., Grant, W. D., and Konings, W. N. (1999). *Extremophiles* **3**, 253–257.

von Heijne, G. (1990a). *Curr. Opin. Cell Biol.* **2**, 604–608.

von Heijne, G. (1990b). *J. Membr. Biol.* **115**, 195–201.

Wild, J., Altman, E., Yura, T., and Gross, C. A. (1992). *Genes Dev*. **6**, 1165–1172.

YaDeau, J. T., Klein, C., and Blobel, G. (1991). *Proc. Natl. Acad. Sci. U.S.A*. **88**, 517–521.

Yahr, T. L., and Wickner, W. T. (2001). *EMBO J*. **20**, 2472–2479.

Yamauchi, K., Doi, K., Kinoshita, M., Kii, F., and Fukuda, H. (1992). *Biochim. Biophys. Acta* **1110**, 171–177.

Yamauchi, K., Doi, K., Yoshida, Y., and Kinoshita, M. (1993). *Biochim. Biophys. Acta* **1146**, 178–182.

Yen, M. R., Harley, K. T., Tseng, Y. H., and Saier, M. H., Jr. (2001). *FEMS Microbiol. Lett*. **204**, 223–231.

Yen, M. R., Tseng, Y. H., Nguyen, E. H., Wu, L. F., and Saier, M. H., Jr. (2002). *Arch. Microbiol.* **177**, 441–450.

Yonath, A. (2002). *Annu. Rev. Biophys. Biomol. Struct*. **31**, 257–273.

Young, J. C., Hoogenraad, N. J., and Hartl, F. U. (2003). *Cell* **112**, 41–50.