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REVIEW ARTICLE

Archaeal protein translocation

Crossing membranes in the third domain of life

Jerry Eichler[1,2]

[1]*Department of Life Sciences, and* [2]*The Doris and Bertie Black Center for Bioenergetics in Life Sciences, Ben Gurion University, Beersheva, Israel*

Proper cell function relies on correct protein localization. As a first step in the delivery of extracytoplasmic proteins to their ultimate destinations, the hydrophobic barrier presented by lipid-based membranes must be overcome. In contrast to the well-defined bacterial and eukaryotic protein translocation systems, little is known about how proteins cross the membranes of archaea, the third and most recently described domain of life. In bacteria and eukaryotes, protein translocation occurs at proteinaceous sites comprised of evolutionarily conserved core components acting in concert with other, domain-specific elements. Examination of available archaeal genomes as well as cloning of individual genes from other archaeal strains reveals the presence of homologues to selected elements of the bacterial or eukaryotic translocation machines. Archaeal genomic searches, however, also reveal an apparent absence of other, important components of these two systems. Archaeal translocation may therefore represent a hybrid of the bacterial and eukaryotic models yet may also rely on components or themes particular to this domain of life. Indeed, considering the unique chemical composition of the archaeal membrane as well as the extreme conditions in which archaea thrive, the involvement of archaeal-specific translocation elements could be expected. Thus, understanding archaeal protein translocation could reveal the universal nature of certain features of protein translocation which, in some cases, may not be readily obvious from current comparisons of bacterial and eukaryotic systems. Alternatively, elucidation of archaeal translocation could uncover facets of the translocation process either not yet identified in bacteria or eukaryotes, or which are unique to archaea. In the following, the current status of our understanding of protein translocation in archaea is reviewed.

Keywords: archaea; protein translocation; plasma membrane; protein export; secretion; membrane proteins; signal sequence; signal recognition particle; extremophiles.

Of the major changes which biology has experienced in the last few decades, amongst the most far-reaching have taken place in microbiology. This is due in part to the creation of a universal phylogenetic tree of life containing three separate branches: the eucarya (eukaryotes) and the two prokaryotic domains, the bacteria and the archaea [1,2]. Although archaea and bacteria share a common cellular organization and morphology, they are not related evolutionarily and are distinctly different from each other. Indeed, examination of recently completed genome sequences (*Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, *Archaeglobus fulgidus*, *Aeropyrum pernix*, *Pyrococcus horikoshii*, and *Pyrococcus abyssi*) as well as partially completed sequences (*Pyrococcus furiosus* and *Sulfolobus solfataricus*) has confirmed that the archaea are distinct from both the bacteria and the eukaryotes [3–10]. Archaea are best known as extremophiles, thriving in excesses in temperature, salinity and pH, growing in sulfur-based environments or metabolizing single carbon sources [11]. More recently, however, these microorganisms have been detected as major constituents of more common and less extreme environments, such as forest soil and ocean surfaces, indicating that archaea are far more prevalent than originally thought [12]. Despite their seeming omnipresence, relatively little is known about the physiology, genetics or biochemistry of the organisms that inhabit this most recently described domain of life.

Although evolutionary distinct, aspects of archaeal biology recall their bacterial or eukaryotic counterparts. As prokaryotes, archaea share many of the morphological and organizational traits of bacteria. Cells of both groups are surrounded by a plasma membrane and cell envelope and contain no internal organelles [13]. In addition, many of the components and pathways involved in bacterial metabolism find close parallels in archaea [14]. In contrast, archaeal information processing is reminiscent of the eukaryotes. Numerous aspects of archaeal replication, DNA packing, transcription, tRNA splicing and translation are eukaryote-like in nature [15]. Still, as reflected by the fact that no bacterial or eukaryotic counterparts exist for a large proportion of the ORFs in archaeal genomes sequenced thus far (25–68%, depending on the method of comparison [16]), archaea clearly possess distinct and defining traits. In many cases, these are related to the ability of archaea to survive in drastic conditions. Indeed, given the harsh environments in which they can exist, archaea have developed diverse strategies with which to overcome the challenges provided by their surroundings. Haloarchaea can contain internal salt concentrations as high as 5 M [17] and possess an entire biochemistry that functions in this nearly-saturated salty milieu [11]. The

Correspondence to J. Eichler, Department of Life Sciences, Ben Gurion University, PO Box 653, Beersheva 84105, Israel. Fax: + 972 7647 2890, Tel.: + 972 7646 1343, E-mail: jeichler@bgumail.bgu.ac.il

Abbreviations: SRP, signal recognition particle; SRI, sensory rhodopsin I; Tat, twin arginine translocation; TRAM, translocating-chain associated membrane protein.

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hyperthermophiles can be detected at temperatures as high as 113 °C, the current limit of life [18], yet avoid problems of protein denaturation which normally occur at elevated temperatures [19]. Acidophiles and alkaliphiles, microorganisms that thrive at the extremes of the pH scale, have developed strategies to maintain a neutral intracellular pH [20,21].

In direct contact with the outside world, the archaeal cell surface must withstand the drastic conditions of these extreme habitats while fulfilling a variety of membrane-related functions, including protein translocation. In contrast to the well-studied phenomena of protein translocation across the bacterial plasma membrane [22] or across the eukaryotic ER membrane (the topological homologue of the prokaryotic plasma membrane) [23], very little is understood of how proteins cross the plasma membrane of archaea. In archaea, a variety of extracytoplasmic proteins must be translocated into and across the plasma membrane. These include membrane proteins, secreted enzymes and the components of the protein-based surface layer found in numerous archaeal species [11]. At present, most of our knowledge of archaeal protein translocation comes from analysis of archaeal genomes [24] as well as from isolation, cloning and heterologous expression of a limited number of archaeal homologues of proteins involved in eukaryotic or bacterial translocation. Such studies suggest that in some instances, protein transport across the archaeal plasma membrane is reminiscent of the bacterial process, whereas in other aspects of archaeal protein translocation, eukaryote-like processes are involved. Protein translocation in archaea may therefore represent a hybrid of the bacterial and eukaryotic systems. On the other hand, however, facets of archaeal protein translocation show no clear parallels to either the bacterial or eukaryotic processes.

In the following review, current understanding of the various processes, components and mechanisms involved in protein translocation within the domain of the archaea will be discussed. For a comparison of the themes in protein translocation across membranes in the three domains of life, the reader is directed to earlier review by Pohlschröder *et al*. [24].

SIGNAL SEQUENCES OF ARCHAEA

Originally proposed in the early 1970s by Blobel and colleagues (and most recently recognized with a 1999 Nobel prize) [25], it is now clear that proteins destined to reside beyond the confines of the cytoplasm are synthesized as precursors, or preproteins, containing a cleavable N-terminal extension, referred to as the signal sequence. Rather than relying on conserved amino acid sequences, signal sequences are composed of approximately 20–30 amino acid residues organized into three distinct regions: a positively charged n-region, a hydrophobic h-region and a short c-region terminating in the signal sequence cleavage site [26,27]. Bacterial and eukaryotic signal sequences are sufficiently similar to each other as to often be interchangeable [28]. An examination of genes encoding a wide range of archaeal secreted and membrane proteins reveals that these proteins are also synthesized with N-terminal extensions. Archaeal signal sequences are sufficiently similar to their bacterial counterparts, so that heterologous expression of extracytoplasmic archaeal preproteins in bacterial hosts leads to proper targetting of the archaeal proteins [29,30]. Using a neural network-based method for prediction of signal sequences, Nielsen *et al*. [31] examined the genes of 34 putative signal sequence-containing

Fig. 1. Examples of different archaeal signal sequences. 1. *H. volcanii* S-layer glycoprotein; 2. *H. salinarum* bacterioopsin; 3. *M. voltae* S-layer protein; 4. *H. mediterranei* halocin H4; 5. *S. solfataricus* glucose binding protein. References for each signal sequence are given in the text. In each sequence, the n-region, generally defined by an excess of positive charges, is shown on the left, the h-regions are enclosed within boxes while the c-regions terminate in the signal sequence cleavage site, denoted by the arrow.

proteins in *M. jannaschii*, the first completely sequenced archaeon [3]. Such analysis revealed that the archaeal signal sequences contain a bacteria-like charge distribution, a eukaryotic cleavage site and an h-region of unique composition. As is the case with bacterial preproteins, the signal sequences of the examined *M. jannaschii* preproteins have a positively charged n-region. Unlike the bacterial n-region, however, in which lysine and arginine residues are present at similar levels, lysine residues are responsible for the bulk of the positive charge of the archaeal n-region. In further comparison to their bacterial counterparts, the archaeal h-regions contained a high isoleucine content. Finally, tyrosine residues were often detected around the archaeal signal sequence cleavage site. It should be noted that the method used in this study to identify archaeal signal sequences relies on similarities to bacterial and eukaryotic signal sequence properties. Thus, the presence of archaeal-specific motifs in *M. jannaschii* could have been overlooked. Moreover, before the general nature of these observations can be concluded, it will be first necessary to subject additional archaeal genomes to similar analysis.

While the overall structural composition of signal sequences appears to be conserved across evolution, archaea also express proteins containing unusual signal sequences. One such example is bacterioopsin, the well-studied *Halobacterium salinarum* membrane protein which in its rhodopsin-conjugated form, bacteriorhodopsin, converts light energy into a proton gradient. The signal sequence of bacterioopsin is comprised of only 13 amino-acid residues, does not contain a hydrophobic core and has replaced the positively charged residues found in the n-region of standard signal sequences, such as that of the extracellular *Haloferax volcanii* surface-layer glycoprotein [32], with a negatively charged glutamic acid residue [33] (Fig. 1). While this unusual signal sequence is also shared by five other haloarchaeal rhodopsin-incorporating integral membrane proteins [34–38], it remains unclear whether such signals are responsible for proper protein targetting or correct protein folding. Although the signal sequence coding region is required for bacterioopsin mRNA stability and membrane insertion of the protein, re-introduction of the signal sequence's putative ribosome binding site into the coding region of the mature protein in a bacterioopsin signal sequence deletion mutant strain restored mRNA stability and some protein insertion [39].

In addition to bacterioopsin and other rhodopsin-incorporating proteins, additional archaeal extracytoplasmic

proteins also contain 'nonclassical' or unusual signal sequences. Unlike the standard signal sequences of surface layer proteins found in other methanoarchaeal species such as *Methanothermus fervidus* or *Methanothermus sociabilis* [40], the signal sequence of the *Methanococcus voltae* surface layer protein, the only demonstrated signal sequence-bearing protein in this species, is only 12 amino-acids long, contains a hydrophobic core yet does not include any charged residues [41]. The signal sequence of *Haloferax mediterranei* R4 halocin H4 is unusual in that it contains a long and highly charged n-region (+6, 18 amino acids) as well as an atypically long c-region (14 amino acids) [42]. A similar n-region is found in the signal sequence of halolysin, an alkaline serine protease from an unidentified haloarchaeal strain [43]. Albers *et al*. [44] reported the existence of a novel signal peptide in a membrane-anchored glucose-binding protein from *S. solfataricus*. This signal is also 12 amino-acid residues long and appears to be cleaved at a glycine-leucine bond ahead of the putative transmembrane anchor of the mature protein. A search of archaeal genomes revealed the presence of a similar motif in a variety of archaeal proteins, although it remains to be shown in these cases that cleavage of a signal sequence occurs at the predicted site.

ARCHAEAL PREPROTEIN TARGETTING

While extracytoplasmic archaeal proteins are synthesized as precursors that generally contain signal sequences similar to those found in bacterial and eukaryotic preproteins, the manner by which archaeal preproteins are targetted to the translocation machinery is unknown. Targetting mechanisms can be divided into two classes according to their temporal relation to protein translation and translocation. In a cotranslational system, translation, targetting and translocation occur in a highly concerted manner. In the posttranslational model, targetting and translocation occur once translation has been fully or largely completed.

SRP pathway components in archaea

In higher eukaryotes, ribosomes in the process of translating signal sequence bearing polypeptides are targetted to the ER membrane via the signal recognition particle (SRP) [23,45]. This ribonucleic acid–protein complex of six polypeptides and 7S RNA binds to the emerging signal sequence of a nascent polypeptide in a GTP-dependent manner. Binding of SRP to the translating ribosome temporarily arrests further protein translation. The ribosome-nascent chain-SRP complex is then delivered to the membrane largely via the affinity of SRP for its membrane-bound receptor, but also through the affinity of the ribosome for the translocation complex. Upon interaction with its receptor, SRP is released from the complex and protein translation is resumed. Bacteria contain a much simpler version of SRP, comprised of Ffh (a homologue of the SRP 54 kDa subunit, SRP54) and a smaller 4.5S RNA fragment, as well as FtsY, a homologue of the SRP receptor α subunit, SRα [46]. In bacteria, the SRP system appears to be involved in the translocation of only a select group of membrane proteins [47]. Furthermore, it is unclear whether the bacterial SRP system functions in a co- or post-translational manner. Although an archaeal signal recognition particle has yet to be demonstrated, current annotation of completely and incompletely sequenced genomes suggests the existence of archaeal versions of components involved in the SRP protein targetting pathway [48]. Several of these have been isolated from a variety of strains and biochemically characterized.

SRP54 is responsible for binding to the emerging signal sequence of a nascent polypeptide chain via the methionine-rich domain of the subunit [49]. The various archaeal versions of SRP54 are highly homologous, being 50–60% identical to each other. The SRP54 homologue of *Acidianus ambivalens* has been cloned and overproduced in *E. coli*, allowing its function to be analyzed [50]. Like its eukaryotic and bacterial versions [46,51], the archaeal SRP54 displayed GTPase activity (optimal at the elevated temperatures in which this archaea lives) and bound 7S RNA. More recently, bacterially expressed *A. fulgidus* SRP54 was shown to interact with the signal sequence of *in vitro* translated bovine preprolactin [52].

In addition to SRP54, archaea also encode for the SRP19 subunit. In contrast to SRP54, which exists in bacteria as the homologous Ffh protein, SRP19 is only found in eukaryotic signal recognition particles, where it interacts with SRP 7S RNA [53]. Homologues of other proteinaceous components of the mammalian SRP (i.e. SRP9, SRP14, SRP68 and SRP72) [45] have not been detected in archaea [48]. In spite of their apparent absence, archaeal 7S RNA has been proposed to contain binding sites for SRP9, SRP14 and SRP68 [52].

Examination of 7S RNA from a variety of different archaea reveals that in terms of secondary structure, archaeal 7S RNAs display strong similarity to their eukaryotic counterparts [54,55], although archaea contain a region termed helix 1 not found in eukaryotic 7S RNA [56,57]. In terms of primary sequence, conservation is limited to a specific structural domain of the molecule shown in eukaryotes to associate with SRP19 [53]. This domain is well-conserved evolutionarily, being present in bacterial 4.5S RNA as well [55], suggesting an ancient origin for the SRP RNA fragment. Indeed, the archaeal 7S RNA gene from *M. voltae*, *Pyrococcus occultum* and *S. solfataricus* can functionally replace the *E. coli* 4.5S RNA gene [58].

Recently, genes encoding *A. fulgidus* SRP54 and SRP19 have been expressed in a bacterial host, purified and reconstituted into a recombinant SRP together with either *A. fulgidus* or *M. jannaschii*. 7S RNA [52]. These results, together with the ability of human SRP19 and SRP54 to bind to archaeal 7S RNA as well as the binding of archaeal SRP19 and SRP54 to human 7S RNA, suggest structural and functional similarities between archaeal and eukaryotic SRPs.

The SRα subunit is responsible for binding ribosome-SRP-nascent chain complexes [45]. In mammals, the SRα subunit in turn binds to the ER membrane via the membrane-embedded SRβ subunit [59]. The nature of the membrane association of FtsY, the bacterial SRα homologue, remains unclear [60]. ORFs designated as archaeal SRP receptors are present in all sequenced genomes and have also been studied in five additional archaeal strains [48]. Sequence alignments reveal a high degree of homology of the archaeal proteins to each other, supporting the concept of archaea being a monophyletic domain [61], as well as to eukaryotic SRα and bacterial FtsY. The highest degree of similarity in the proteins across the three domains of life can be detected at the X-domain (a region of unknown function), at the four GTP binding motifs and at the putative guanine nucleotide dissociation stimulator site, located in the C-terminal of the protein [55,62]. Archaeal SRα is generally shorter than *E. coli* FtsY and eukaryotic SRα, partly due to the replacement of a small N-terminal region in place of the much longer and bulkier domains found in the bacterial and eukaryotic proteins [63].

Responsible for membrane association, the N-terminal region of mammalian SRα contains two hydrophobic stretches and a basic region, while that of bacterial FtsY contains a large number of acidic amino-acid residues [59,60]. Hydropathic analysis of the *S. solfataricus* SRα failed to reveal any significant hydrophobic stretches in its N-terminal domain [64]. This domain also contains a significant number of charged residues, although similar amounts of acidic and basic amino acids are present. Similarly, the N-terminal domain of SRα from *Thermococcus* AN1 does not contain any putative membrane-spanning α-helices or SRβ binding site and is enriched in charged residues [62]. The deduced primary structure of *A. ambivalens* SRα predicts an N-terminal domain that also possesses only a small charge given the balance between acidic and basic side-chains [63]. Antibodies raised against *A. ambivalens* and *S. acidocaldarius* SRα recognized the protein in the cytoplasm but not in the membrane fraction of the cells in western blotting experiments [63,65]. Archaeal SRα therefore does not appear to directly associate with the membrane. As in bacteria, no SRβ homologue or other putative archaeal SRα/FtsY membrane receptor has been described. Membrane binding of archaeal SRα could thus involve a putative mediator which in turn binds to a novel membrane receptor or directly to membrane phospholipids. Finally, isolation of the gene encoding *A. ambivalens* SRα and subsequent expression of the protein in *E. coli* revealed the intrinsic (and in this case, thermophilic) GTPase activity of archaeal SRα [63]. The GTPase activity was, however, not stimulated in the presence of the *A. ambivalens* SRP54 homologue, unlike the enhanced nucleotide hydrolysis obtained upon pairing of eukaryotic SRP54 and SRα, or bacterial Ffh/4.5S RNA and FtsY [46,51].

Translocation-related chaperones in archaea

In contrast to the cotranslational, SRP-dependent mode of translocation which exists in mammals and other eukaryotes, the bulk of preprotein translocation across the bacterial plasma membrane occurs posttranslationally [22]. Targetting of certain proteins to the membrane-embedded translocation apparatus in the yeast ER also follows translation in the cytoplasm. In both cases, molecular chaperones are involved in maintaining the nascent proteins in translocation-competent conformations and escorting them to translocation sites found in the membrane. The major bacterial chaperone involved in protein translocation is SecB [66]. Through their affinity for SecA, a component of the bacterial translocation apparatus, SecB-preprotein complexes are delivered to translocation sites [67]. In the case of posttranslational translocation in yeast, the cytosolic chaperone Hsp70 is employed for delivery of protein to translocation sites in the ER membrane [23]. To date, no archaeal versions of SecB has been detected, although a possible candidate has been suggested in *M. jannaschii* [68]. In contrast, Hsp70 exists in some, but not all archaea [69]. Hsp70-encoding genes are absent in some methanogens, like *M. jannaschii*, and in hyperthermophiles like *A. fulgidus* or *Pyrococcus* species. Halophilic archaea examined to date contain *hsp70* genes, as do several methanoarchaea, such as *M. thermoautotrophicum and Methanosarcina mazei* S-6. It appears therefore that molecular chaperones are not involved in archaeal protein translocation, or alternatively, that other, presently unrecognized proteins serve in the role of chaperones in targetting preproteins to the archaeal plasma membrane. HtrI could represent one such protein. The membrane-associated HtrI protein serves as a transducer through which sensory rhodopsin I (SRI), the phototactic receptor of *H. salinarum*, transmits its signal to the flagellar motor of the cell [70]. It has been shown that in the absence of HtrI, only negligible quantities of membrane-inserted SRI can be detected [71]. Conjugation of the 13 amino-acid signal sequence of bacterioopsin together with the first eight residues of the mature bacterioopsin protein to the N-terminus of the SRI apoprotein restores SRI membrane insertion, eliminating the need for HtrI in SRI biosynthesis. These results suggest a chaperone-like role for HtrI, assisting in the membrane insertion of SRI.

Protein translation and translocation in archaea

Whereas elements of the SRP targetting pathway, responsible for the cotranslational nature of eukaryotic protein translocation, exist in archaea, the relation between archaeal protein translation and translocation remains an open question. Most of the data addressing the relation between archaeal protein translation and translocation has focused on the biosynthesis of the multispanning *H. salinarum* membrane protein bacterioopsin. Based on cosedimentation of 7S RNA with translating, membrane-bound ribosomes, Gropp *et al.* [33] proposed a cotranslational mode of bacterioopsin insertion. Relying on the kinetics of *in vivo* labelling of engineered, outwardly oriented cysteine residues with a membrane-impermeant reagent, Dale and Krebs [72] also concluded that bacterioopsin inserts in a cotranslational manner. In contrast, heterologous expression of bacterioopsin in *H. volcanii* suggests that membrane insertion occurs in a posttranslational manner. In studies using a chimera comprised of bacterioopsin and dihydrofolate reductase, it was shown that the fusion protein is first found in the cytoplasm and only later inserts into the membrane [73]. Furthermore, deletion of the seventh and final transmembrane domain of the bacterioopsin prevented membrane insertion of the fusion protein. Studies which rely on the biogenesis of bacterioopsin to address the relation between archaeal protein translation and translocation must, however, be considered with caution. Bacterioopsin contains an unusual signal sequence unlike those found in the majority of bacterial, eukaryotic or other exported/membrane archaeal proteins and as such may rely on a unique and possibly dedicated translocation system [33]. Support for the concept that bacterioopsin insertion may reflect a specialized case of protein translation comes with the observation that the presence of the bacterioopsin signal sequence can overcome the need for HtrI as a putative molecular chaperone involved in the membrane insertion of SRI [71]. Thus, before the conclusions regarding the relation between translation and translocation using bacterioopsin as a reporter protein can be drawn, the choice of this protein as a prototypic extracytoplasmic protein marker must be justified. Moreover, secreted and membrane-inserted proteins may require different degrees of coupling between preprotein translation and translocation in bacteria, and possibly in archaea as well. The interaction between the translation and translocation of secreted archaeal proteins has not been addressed.

THE TRANSLOCATION APPARATUS

Protein translocation across the eukaryotic ER and bacterial plasma membranes occurs at Sec61αβγ and SecYEG, respectively [74,75]. The core components of these complexes, Sec61αγ and SecYE are homologous [76,77]. Sec61α and SecY, each spanning the membrane 10 times [78,79], are

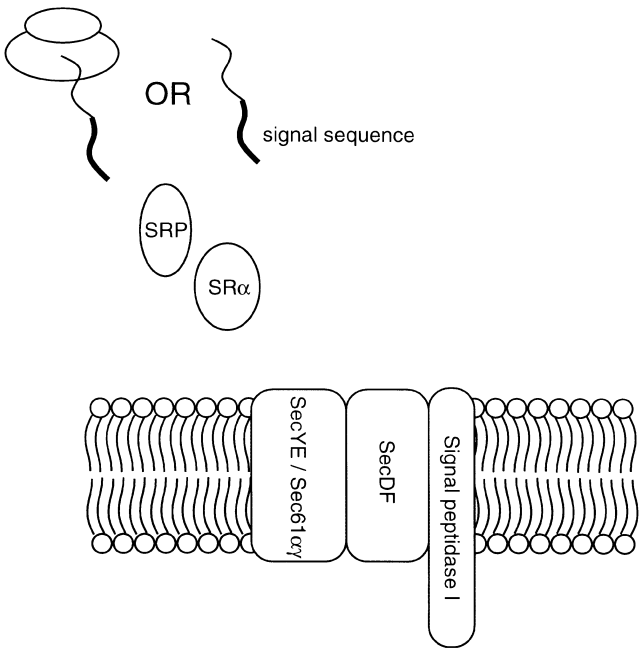

Fig. 2. Currently identified components of the archaeal Sec translocation system. The particular arrangement of the components given in the figure represents one possible combination. Interaction between the SRP-targetting pathway and the membrane-embedded Sec proteins has yet to be shown, as has the post or cotranslational nature of archaeal translocation.

thought to form the actual pore through which the translocating protein passes [80,81]. Genes encoding for archaeal homologues of SecY/Sec61α have been detected in several different archaea. The proposed *secY* gene of *S. acidocaldarius* encodes for a 50.2-kDa protein proposed to span the membrane 10 times [82]. *Haloarcula marismortui* encodes for a proposed SecY protein of 52.6 kDa [83], while *Methanococcus vannielii* contains a secY gene coding for a 47.6-kDa protein also proposed to span the membrane 10 times [84]. Analysis of completed archaeal genomes also reveals the presence of ORFs encoding putative SecY/Sec61α homologues. Although in all cases the putative archaeal SecY proteins are more eukaryote-like than bacterial in sequence, transformation of a temperature-sensitive *secY E. coli* mutant with a plasmid encoding *M. vanielii* SecY resulted in an ability of the mutant strain to grow at the nonpermissive temperature [84]. The finding that an archaeal SecY can replace its bacterial counterpart is striking when one considers the membrane phospholipids in which archaeal SecY is normally embedded. In archaea, membrane phospholipids are comprised of repeating isoprenyl groups linked to a glycerol backbone through an ether linkage, a characteristic feature of this domain of life [85]. In contrast, bacterial and eukaryotic phospholipids contain fatty acyl groups linked to a glycerol backbone via ester bonds. It thus appears that archaeal integral membrane proteins such as SecY are able to function in the presence of bacterial phospholipids.

The second essential component of the bacterial and eukaryotic translocation apparatuses, SecE/Sec61γ, also can be detected in archaea. In *E. coli*, SecE traverses the membrane three times, with the third C-terminal transmembrane domain being essential for translocation function [86]. In all other bacterial and eukaryotic systems, SecE/Sec61γ cross the membrane only once, with the membrane-spanning domain being homologous to the third transmembrane domain of *E. coli* SecE [76]. Analysis of completed genomes as well as a variety of other strains reveals the presence of genes encoding putative SecE/Sec61γ archaeal homologues, which, in all cases, appear to traverse the membrane only once. Sequence analysis of the nine archaeal SecE/Sec61γ genes currently available (six completed genomes, *H. mediterranei* [87], *S. acidocaldarius* [88], *A. ambivalens* [63]) reveals that although the putative archaeal SecE proteins display a relatively low level of similarity (as compared to SecY), they can be roughly divided into two groups, corresponding to the two subdomains of the archaeal kingdom, the euryarchaea and the crenarchaea [2].

The third components of the bacterial and eukaryotic translocation apparatus core complexes, SecG and Sec61β, respectively, do not resemble each other [76]. None of the completed or uncompleted archaeal genomes contain ORFs encoding homologues of *E. coli* SecG or of YvaL, a SecG-homologue present in the Gram-positive bacteria *Bacillus subtilis* [89]. Similarly, no Sec61β homologue has been detected, although *M. jannaschii* contains an ORF that displays some weak similarity. Thus, archaea may rely on a simpler translocation core complex composed of SecYE/Sec61αγ, or may involve a third, as yet unidentified component (Fig. 2).

In addition to SecYEG/Sec61αβγ, membrane-embedded translocation machineries include several auxiliary proteins. ORFs encoding for archaeal SecDF homologues can be detected in four of the six completed archaeal genomes, being absent in *A. fulgidus* and *A. pernix*. In bacteria, SecDF serves to modulate the membrane association of SecA, the essential ATPase component of the translocation apparatus [90,91]. During bacterial translocation, ATP-dependent cycles of SecA membrane insertion/deinsertion are coupled to the forward movement of preprotein across the plasma membrane [22]. Searches of archaeal primary sequences have failed to reveal an archaeal version of SecA, suggesting that archaeal SecDF may be playing a novel role in protein translocation. This concept is supported by the failure of genomic searches to detect an archaeal version of yajC, a small protein which coprecipitates with bacterial SecDF [92]. In the absence of SecDF, bacterial cells are unable to maintain a proton motive force [93]. A similar relation between SecDF and the proton motive force could exist in archaea. Alternatively, the existence of an archaeal protein which corresponds to a functional homologue of SecA remains a possibility. In this scenario, archaeal SecDF could serve a parallel function as in bacteria. Bacterial SecDF also help confer directionality to the translocation process [91], a role which could also be played by their archaeal counterparts.

While the majority of bacterial proteins which cross the plasma membrane rely on the Sec system [94], a novel Sec-independent pathway has been identified. This pathway, referred to as the twin arginine translocation (Tat) pathway due to the presence of twin arginines in the signal sequence of proteins relying on this route, is not only found in bacteria but is also involved in the ΔpH-dependent route of protein translocation across the plant thylakoid membrane [95]. Unlike the Sec system, which delivers loosely folded proteins across the membrane, the Tat pathway is capable of transferring large, cofactor-containing folded proteins across the membrane [96]. Homologues of the Tat pathway components TatC and Hcf106 are found in *A. pernix* and possibly in *A. fulgidus*. The Tat pathway is apparently not universally distributed in archaea, as homologues of these proteins have yet to be detected in any other archaeal strain. Indeed, *M. jannaschii* does not encode proteins bearing twin arginine-containing signal sequences. Interestingly, examination of completed archaeal genomes

reveals that a given strain encodes for either homologues of SecDF or of TatC and Hcf106, components of the Tat pathway, but not both.

In bacteria and eukaryotes, the insertion of certain membrane proteins requires the participation of additional components of the translocation machinery. In the ER, the translocating-chain associated membrane protein (TRAM) is associated with transmembrane domains of integral proteins during their membrane insertion at Sec61αβγ sites [97]. Most recently, the signal anchor of the inner membrane protein FtsQ was shown to contact a novel SecYEG-associated protein, YidC [98]. YidC does not resemble TRAM, although it is homologous to Oxa1, a *Saccharamyces cerevisiae* protein involved in mitochondrial inner membrane protein insertion [99]. Yeast mitochondria, however, do not contain a Sec translocation machinery resembling that of bacteria [100], suggesting that YidC and Oxa1 exist in different environments. Thus, the mechanism of membrane protein integration may differ from membrane to membrane. Given the failure of genomic searches to reveal archaeal homologues of TRAM, YidC or Oxa1, it seems reasonable to predict that archaea also rely on a unique membrane protein insertion machinery.

Finally, during, or shortly after a preprotein is translocated across the membrane, the signal sequence is released through the action of type I signal peptidase by a still not fully understood mechanism [101]. Whereas bacterial and eukaryotic signal peptidases display similar substrate specificities, they differ in molecular composition. In bacteria, signal peptidase exists as a single protein whereas in eukaryotes, a multimeric protein complex is required, with the (yeast) Sec11-like subunit containing the peptidase active site. As determined by genomic analysis, the signal peptidases of archaea appear to consist of a single protein and are more similar in sequence to the eukaryotic Sec11-like ER signal peptidase subunit than to the bacterial version [102]. Type II signal peptidases are found in bacteria, where they are responsible for removing signal sequences from translocated lipoproteins [103]. Although current genome annotation efforts have not identified an archaeal type II signal peptidase (or archaeal lipoproteins for that matter), the existence of signal sequence-cleaved archaeal proteins such as halocyanin from the haloalkaliphilic archaeon *Nantronobacterium pharaonis* [104] suggests the existence of such an enzyme in archaea. Halocyanin, a blue copper protein, contains the classic lipoprotein signal sequence [105] including the presence of a cysteine residue immediately following the cleavage site which serves as the site for covalent attachment of a lipid moiety. While the presence of an attached lipid at the N-terminus of halocyanin was not directly shown, mass spectroscopy supports the sequence prediction of lipid modification. An archaeal homologue of signal peptidase II may, however, not be responsible for removal of the signal sequence of the proposed lipoprotein as other enzymes capable of removing lipoprotein signal sequences have been reported. Type II signal peptidase mutants of *B. subtilis* are capable of removing the signal sequence of PrsA, the major lipoprotein in this species, albeit at an alternative cleavage site [103].

ENERGETIC CONSIDERATIONS

The driving force of translocation across the archaeal plasma membrane remains an open question (Fig. 3). Should archaeal protein translocation occur cotranslationally, then elongation of the growing nascent preprotein from the ribosome could feed the nascent preprotein across the membrane independent of additional driving forces, as occurs across the eukaryotic ER membrane [75]. Should, however, archaeal translocation take place posttranslationally, various driving forces could be implicated. In many systems, posttranslational translocation into and/or across the membrane relies on ATP hydrolysis [106]. In bacteria, SecA 'stuffs' preprotein across the plasma membrane during its ATP-dependent membrane insertion/deinsertion cycle [22]. In yeast, the chaperone BiP 'pulls' the protein into the ER lumen in an ATP-dependent manner [107]. Neither of these approaches is apparently used by archaea. As discussed above, no archaeal version of the highly conserved SecA protein has been detected, although the existence of a functional homologue cannot yet be discounted. The presence of an outwardly oriented, possibly membrane-associated protein which acts to advance a translocating archaeal preprotein in a 'ratchet-like' manner is also plausible [108], although its extracellular localization would argue against an ATP-driven mechanism.

Instead of relying on ATPases, posttranslational archaeal translocation could rely on a charge-related phenomena, such as a proton motive force or electrostatic forces. A proton motive force is involved in the Sec-mediated translocation of proteins in bacteria [109], while the Tat pathway across bacterial and plant thylakoid membranes relies on a pH gradient [96]. The spontaneous insertion of proteins into bacterial and thylakoid membranes relies on hydrophobic forces and charge distribution [110]. Electrostatic forces also participate in delivering preproteins across the mitochondrial outer membrane [111]. Similar forces, often resulting from the unique environments in which they exist, could be responsible for driving protein translocation in archaea. Found in the presence of H^+-ion

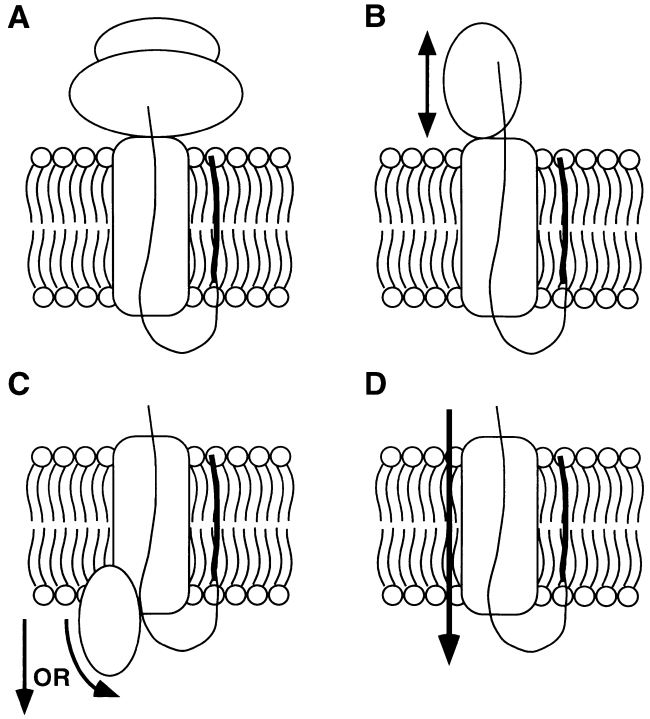

Fig. 3. Putative driving forces of archaeal translocation. (A) During cotranslational translocation, elongation of the growing polypeptide chain feeds the preprotein across the membrane. (B) A protein component on the cytoplasmic face of the membrane could 'pump' the preprotein across the membrane. (C) A protein component on the external face of the membrane could either pull or 'ratchet' the protein across the membrane. (D) A force based on proton or cation gradients, electrostatic conditions, hydrophobicity or otherwise related to charge could drive translocation.

concentrations in some cases even greater than 1 M [112], acidophiles maintain their cytoplasm closer to neutrality [20] and as such possess large pH gradients and corresponding proton motive forces [113]. Many halophilic and methanogenic archaeal strains maintain significant proton and Na^+ gradient which supply the energetic requirements for various cellular processes [113].

The ability of archaea to thrive under extreme conditions could have other implications for the translocation process. For example, as a result of the enormous ΔpH across their plasma membrane, acidophiles such as *S. acidocaldarius* possess a transmembrane electrical potential (Δψ) of reversed polarity (i.e. inside positive instead of negative) relative to other organisms [114]. The Δψ is cited as the major effector of the 'positive-inside rule' of membrane protein topology, which states that an inside negative polarity prevents translocation of positively charged protein domains while facilitating that of negatively charged regions [27,115]. As such, an inverted membrane topology of acidoarchaeal membrane proteins would be expected. Sequence-alignment comparisons of bacterial and eukaryotic membrane proteins of known topology (i.e. SecY, SecE and cytochrome *c* oxidase) with homologues from haloarchaea, methanoarchaea and acidoarchaea revealed, however, a similar orientation within the plane of the membrane for these proteins in all three domains of life and in all archaeal phenotypes [116]. Thus, either the positive-inside rule may not rely on Δψ in the case of acidoarchaea or the contribution of Δψ to the topology of membrane-spanning domains may act through the translocation machinery.

A ROLE FOR ARCHAEAL PHOSPHOLIPIDS?

To fully understand protein translocation in archaea, it will be essential to consider the contribution of archaeal-specific factors, such as the ether-based phospholipids of the archaeal membrane, to the translocation process. Unlike eukaryotes and bacteria, where phospholipids are composed of fatty acyl groups linked through ester bonds to a glycerol backbone, archaeal phospholipids consist of repeating isoprenyl subunits linked to a glycerol backbone through an ether bond [85]. In hyperthermophilic archaea, the isoprenyl groups can span the membrane, thereby connecting the two faces of the membrane and creating a monolayer structure. Ether-based phospholipids are believed to contribute to the ability of archaeal membranes to support the extremes of their environments [117]. In bacteria, the presence and character of membrane and nonmembrane lipids affect the translocation process [118,119]. Whether archaeal membrane lipids also participate in or effect the translocation process remains unknown. Interestingly, it has been shown that liposomes formed from archaeal lipids display higher rigidity and stability, higher salt tolerance and lower proton permeability than their bacterial counterparts [120–122]. Low proton permeability could enhance the efficiency of proton motive force-driven processes, possibly including translocation, in archaea.

OTHER TRANSLOCATION-RELATED SYSTEMS IN ARCHAEA

In Gram-negative bacteria, secreted and cell surface-associated proteins must not only cross the plasma membrane, but also the outer membrane. To do so, a variety of different secretory systems are employed. Type II secretion relies on the general secretory pathway in which signal sequence-bearing proteins first cross the plasma membrane via the Sec-system discussed above. The vast majority of periplasmic intermediates then use the main terminal branch of the general secretory pathway to translocate across the outer membrane and reach the exterior of the cell [123]. While the periplasmic and outer membrane proteins that direct traffic along the main terminal branch tend to be specific for each secreted protein, they can be organized into homologous protein families, referred to as Gsp proteins [124]. Many Gsp proteins show significant sequence homology to Pil proteins involved in the biogenesis of the type IV class of pili, rod-like appendages that protrude from the outer membrane of a number of bacteria including *Pseudomonas aeruginosa* [125]. Examination of archaeal genomes reveals the presence of ORFs which resemble members of the Pil superfamily of proteins involved in pili formation, protein secretion and DNA uptake [126]. ORFs homologous to the ATPase PilB have been detected in all completed genomes as well as *M. voltae* and *P. furiosus*. *S. solfataricus* contains a homologue of VirB, a PilB-like protein from *Agrobacterium tumefaciens*. Interestingly, archaeal flagella from a variety of species also contain proteins homologous to the type IV pili Pil proteins [127]. Moreover, archaeal flagellins, the single protein component of flagellar filaments, possess signal sequences highly similar to those recognized by PilD or prepilin peptidase [127,128]. Searches of current archaeal genomes have, however, failed to reveal an archaeal PilD homologue [126]. Based on proposed similarities in signal sequences, it was speculated that the processing and translocation of certain extracytoplasmic archaeal proteins rely on a mechanism similar to that employed by archaeal flagellins [44], although this concept has been recently called into question [129].

CONCLUSIONS

At present, an examination of protein translocation in archaea raises more questions than provides answers. The most pressing concern the relation of protein translation to protein translocation, the composition of the archaeal translocation machinery and the driving force of archaeal translocation. It is clear that advances on several of these fronts can be expected soon. The upcoming release of several addition complete archaeal genomes from a variety of phenotypic groups will help draw a consensus of the known translocation-related components found in this domain of life. Improved annotation techniques may reveal the existence of archaeal homologues of translocation-related components not presently recognized. Furthermore, efforts to molecularly dissect and recreate archaeal protein translocation *in vitro* are currently in progress in a number of laboratories. It is expected that such studies will reveal similarities to certain aspects of known translocation systems, whereas in other facets of the translocation process, unique solutions, designed to overcome the environment-related challenges faced by archaea, will be described. In both cases, the development of new molecular biology and biochemical tools for working with the various archaeal phenotypes will be of paramount importance.

A comprehension of archaeal protein translocation carries important implications for several reasons, foremost of these is better understanding of a central biological question, i.e. how proteins cross biological membranes. Deciphering the mechanism of archaeal protein translocation and in turn, presenting a clearer picture of archaeal cell surface physiology, will also enhance our understanding of how archaea survive the varied and often drastic conditions of their environments. Examining archaeal protein translocation will contribute to

better understanding of protein phylogeny and the relation between eukaryotes, bacteria and archaea. Finally, understanding protein translocation in archaea will also stimulate the realization of the enormous commercial potential of these microorganisms: large-scale production of extremophilic proteins genetically targeted for secretion will be made more efficient through exploitation of a well-characterized protein export machinery.

The first structure of an archeal SRP pathway component, that of the evolutionarily conserved GTPase domain of *A. ambivalens* SRP54 has recently been solved [130].

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