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The archaeal Sec-dependent protein translocation pathway

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Over the past three decades, transport of proteins across cellular membranes has been studied extensively in various model systems. One of the major transport routes, the so-called Sec pathway, is conserved in all domains of life. Very little is known about this pathway in the third domain of life, archaea. The core components of the archaeal, bacterial and eucaryal Sec machinery are similar, although the archaeal components appear more closely related to their eucaryal counterparts. Interestingly, the accessory factors of the translocation machinery are similar to bacterial components, which indicates a unique hybrid nature of the archaeal translocase complex. The mechanism of protein translocation in archaea is completely unknown. Based on genomic sequencing data, the most likely system for archaeal protein translocation is similar to the eucaryal co-translational translocation pathway for protein import into the endoplasmic reticulum, in which a protein is pushed across the translocation channel by the ribosome. However, other models can also be envisaged, such as a bacterial-like system in which a protein is translocated post-translationally with the aid of a motor protein analogous to the bacterial ATPase SecA. This review discusses the different models. Furthermore, an overview is given of some of the other components that may be involved in the protein translocation process, such as those required for protein targeting, folding and post-translational modification.

Keywords: archaea; protein targeting; protein translocation; Sec pathway; membrane

1. INTRODUCTION

Prokaryotes can be divided into two lineages: bacteria and archaea. The latter were recognized only in the 1970s as a group of organisms that are evolutionarily completely distinct from bacteria (Woese & Fox 1977), and it is now generally accepted that life can be divided into three domains: eucarya, bacteria and archaea (Woese *et al.* 1990).

Archaea share a number of features with the other domains of life. Typical bacterial features found in archaea are a similar morphology and cell size, the absence of membrane-surrounded organelles, the presence of a small circular chromosome and the organization of genes in operons. Furthermore, several aspects of the metabolism of archaea are related to those of bacteria. By contrast, many features relating to processes such as transcription and translation are of the eucaryal type. Archaea are not sensitive to various bacterial antibiotics, such as those that block transcription (e.g. rifampicin) or translation (e.g. kanamycin). Other typical eucaryal features found in archaea are the presence of introns (albeit mostly in tRNA genes) and N-linked glycosylated proteins. Notably, archaea contain O-linked glycosylated proteins as well (Lechner & Sumper 1987), but such proteins are found in all domains of life.

A characteristic feature that really sets the archaea apart from the other domains of life is the chemical composition of their cytoplasmic membrane. Bacterial and eucaryal membranes contain mostly phospholipids that are derived from fatty acids linked to glycerol via an ester bond. By contrast, archaeal lipids are composed of saturated phytanyl chains that are linked to glycerol via an ether bond. Some archaea, in particular thermophiles and acidophiles, have tetraether lipids that span the entire membrane and form a monolayer instead of a bilayer. Archaeal lipids are very stable and probably play an important role in the extreme environments in which many archaea are found (van den Vossenberg *et al.* 1998). These environments vary enormously, as archaea have been found in conditions with very high temperatures, extreme pH values or hypersalinity. Notably, archaea are also present in more 'normal' environments, such as oceans, and form a major part of the biomass on Earth (Karner *et al.* 2001).

The archaea can be divided into two major kingdoms—the Euryarchaeota and the Crenarchaeota. The kingdom Euryarcheota comprises the methanogens, extreme halophiles, thermophilic sulphate reducers, the *Thermoplasma* group and the *Thermococcus–Pyrococcus* group. Initially, it was thought that the kingdom crenarchaeota contained only sulphur-metabolizing hyperthermophiles, but now several other archaea, including mesophilic organisms, are included in this group (DeLong *et al.* 1994). Two other kingdoms have been proposed: the Korarchaeota, which have not been cultured and are known only from DNA sequences (Barns *et al.* 1996); and the recently discovered Nanoarchaeota. The latter kingdom contains, at present, only one species, *Nanoarchaeum equitans*, which is a nano-sized thermophile that lives in symbiosis with a crenarchaeon (Huber *et al.* 2002).

Relatively little is known about the cell biology of archaea. This is partly due to the difficulty in culturing

many archaea. Furthermore, research has also been hampered by the lack of genetic tools. In recent years, however, a lot of information has become available through genome sequencing projects, although it is important to stress that a significant proportion of open reading frames in archaeal genomes do not have any homologues in the other domains of life. One important aspect of the cell biology of all living organisms is the trafficking of proteins. This subject has been studied extensively ever since the discovery that translocated proteins are synthesized as precursor proteins (preproteins) with amino-terminal signal peptides (Devillers-Thiery *et al.* 1975). These signal peptides have a characteristic tripartite structure: a charged N domain, a hydrophobic H domain and a more polar C domain, which contains the cleavage site for signal peptidase (SPase).

The number of model systems in which protein transport was studied has always been quite limited. For bacteria, most information is derived from the Gram-negative bacterium *Escherichia coli* and, to a lesser extent, the Gram-positive bacterium *Bacillus subtilis*. In eucarya most of the research was limited to *Saccharomyces cerevisiae* and mammalian cells. None of the organisms studied belonged to the domain of archaea. Using genomic sequencing data several of the main components for protein translocation in bacteria or eucarya have been identified in archaea (for reviews, see Pohlschröder *et al.* 1997; Eichler 2000; Bolhuis 2002). Sequence information shows clear similarities between the Sec systems of bacteria, eucarya and archaea. However, some fundamental differences also exist, in particular in the driving force of the systems. This review gives an overview of the Sec systems in bacteria and eucarya, and compares them with the archaeal system using information derived from genomic sequences and some experimental data that have been obtained in recent years.

2. PROTEIN TRANSPORT IN EUCARYA

In most eucarya, proteins are transported co-translationally into the lumen of the endoplasmic reticulum (ER). In this mode (figure 1*a*), a signal peptide that emerges from the ribosome is bound by the signal recognition particle (SRP), which is a complex comprising a 7S RNA molecule and, in mammalian cells, six proteins: SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72 (for a review, see Keenan *et al.* 2001). Binding of SRP to the signal peptide arrests protein translation. Next, the entire ribosome–nascent chain–SRP complex is targeted to the membrane with the aid of the SRP receptors SRα and SRβ (also called docking protein (DP)α and DPβ). At the ER membrane, the ribosome docks onto the protein translocation channel and SRP is released from the nascent chain. Translation of the nascent chain resumes, and synthesis of the protein by the ribosome pushes the nascent chain through the translocation channel. This channel is formed by the Sec61 complex, which consists of three subunits, Sec61α, Sec61γ and Sec61β (for a review, see Johnson & Van Waes 1999).

In addition to the core components of the translocation channel, a number of accessory factors are required for efficient protein translocation, such as Sec63 (Young *et al.* 2001), TRAM (Voigt *et al.* 1996) and TRAP (Fons *et al.* 2003). During or shortly after translocation, two other complexes modify the precursor protein. These are the signal peptidase complex (SPC) (Evans *et al.* 1986), which removes the signal peptide, and the oligosaccharyl transferase complex (OST), which is required for the glycosylation of proteins (Kaplan *et al.* 1987). In the ER lumen the protein folds into its native conformation with the aid of various chaperones (for a review see Fewell *et al.* 2001), such as Hsp70s/Hsp40s, disulphide oxidoreductases, which catalyse the formation of disulphide bonds, and peptidyl prolyl isomerases (PPIases), which catalyse the *cis–trans* isomerization of peptidyl–prolyl bonds. Some chaperones, in particular calreticulin and calnexin, are specifically involved in the folding of N-glycosylated proteins (Helenius & Aebi 2001).

Proteins in eucarya can also be translocated post-translationally. *Saccharomyces cerevisiae*, in particular, makes use of this pathway; post-translational translocation plays only a minor role in mammals. In the post-translational mode, cytosolic Hsp70/Hsp40s are involved in targeting the ER membrane (Chirico *et al.* 1988; Deshaies *et al.* 1988). Preproteins are targeted to a translocation channel comprising again Sec61α, Sec61β and Sec61γ, and four additional factors: Sec62, Sec63, Sec71 and Sec72 (Panzner *et al.* 1995). Protein transport is driven by a pulling action from the ER luminal protein BiP (Kar2 in yeast), which belongs to the family of Hsp70s. After translocation the signal peptide is removed by the SPC complex and the protein is released into the lumen.

3. PROTEIN TRANSLOCATION IN BACTERIA

In bacteria, the main mode of protein transport is post-translational (figure 1*b*). After synthesis, proteins are kept in a translocation-competent conformation and targeted to the membrane with the aid of cytosolic chaperones such as SecB (Randall & Hardy 2002). Next, the precursor is delivered to the peripheral membrane protein SecA, an essential ATPase that provides the driving force by pushing the precursor into the translocation channel (for a review, see Manting & Driessen 2000). Complete translocation of a precursor requires several cycles of SecA binding, membrane insertion and membrane deinsertion (Economou *et al.* 1995). The efficiency of the translocation process is increased by the protonmotive force (PMF) (Nishiyama *et al.* 1999).

The core components of the bacterial translocation channel are SecY, SecE, and SecG. SecY is a homologue of the eucaryal Sec61α and SecE is a homologue of Sec61γ. At first sight SecG is not homologous to Sec61β. However, a consensus sequence shared by these proteins has been found (Cao & Saier 2003), suggesting a common ancestry. These proteins probably fulfil a similar role in protein translocation.

SecD, SecF and YajC are accessory factors that form a trimeric complex that interacts with SecYEG. These proteins have been proposed to play a role in the membrane cycling of SecA (Duong & Wickner 1997), but other functions have also been suggested. These include assembly of the translocase (Pohlschröder *et al.* 1997) or removal of signal peptides or misfolded proteins from the translocase (Bolhuis *et al.* 1998). This clearing function is consistent with the classification of SecD/F as a member of the RND

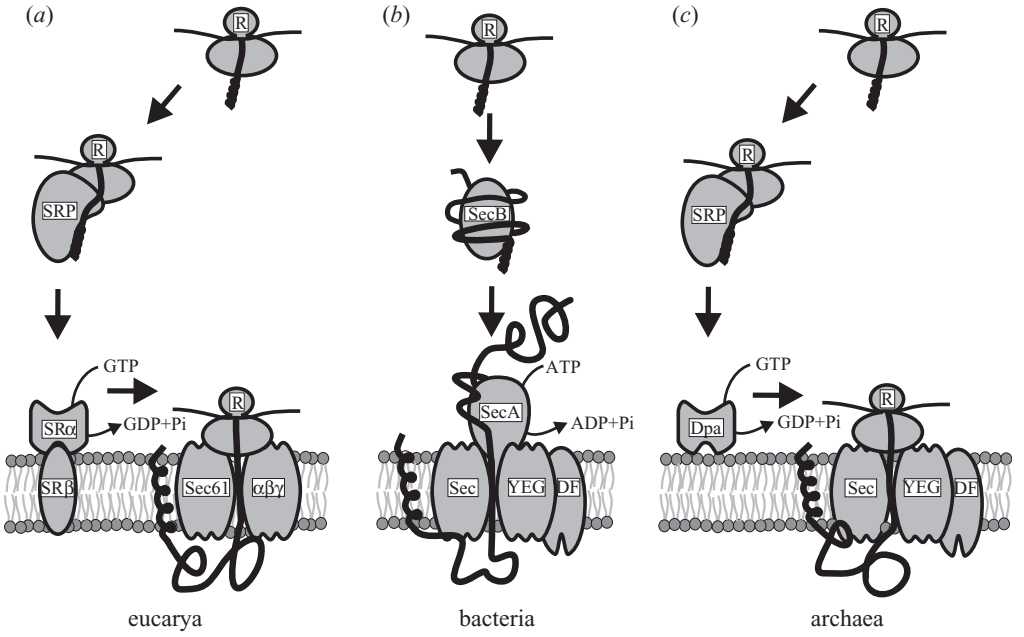

Figure 1. Simplified models of the Sec-translocation pathway of (*a*) eucarya, (*b*) bacteria and (*c*) archaea. For bacteria and eucarya, only the main routes are shown. For archaea, only the most likely system of co-translocational translocation is shown. See §§ 2, 3 and 4 for details. R, ribosome.

superfamily of PMF-driven transporters (Bolhuis *et al.* 1998; Tseng *et al.* 1999).

Bacteria also contain an SRP pathway, albeit in a simplified form when compared with eucarya. Bacterial SRP comprises 4.5S RNA, which is similar to the eucaryal 7S RNA, and Ffh (fifty-fourth homologue), which is a homologue of the eucaryal SRP54. Targeting is assisted by a homologue of the eucaryal SRα called FtsY. Components that are involved in the translational arrest in eucarya (SRP9, SRP14) and a homologue of SRβ (the membrane-bound subunit of the SRP receptor) are absent in bacteria. Several studies have shown that the bacterial SRP targeting route is mainly involved in targeting of membrane proteins (Seluanov & Bibi 1997; Ulbrandt *et al.* 1997).

YidC is an essential *E. coli* membrane protein involved in the biogenesis of membrane proteins (Froderberg *et al.* 2003), and it partly associates with the Sec translocase through interaction with the SecD/SecF/YajC complex (Scotti *et al.* 2000; Nouwen *et al.* 2001). YidC is also involved in the biogenesis of Sec-independent membrane proteins. A recent study demonstrated that these membrane proteins are delivered by the SRP pathway, which indicates that two routes for inner membrane proteins exist: an SRP–SecYEG route for Sec-dependent membrane proteins and SRP–YidC route for Sec-independent membrane proteins (Froderberg *et al.* 2003). The Gram-positive bacterium *B. subtilis* contains two homologues of the YidC protein, which have different but overlapping functions (Tjalsma *et al.* 2003). It was shown that in this organism depletion of both YidC homologues affected not only the biogenesis of membrane proteins but also the stability of secreted proteins. The latter may, however, be an indirect effect.

Shortly after translocation the signal peptide is removed by an SPase, and the mature protein folds into its active conformation. Folding is assisted by several factors, such as thiol-disulphide oxidoreductases, PPIases and other extracytoplasmic chaperones (for reviews, see Wulfing & Pluckthun 1994; Tjalsma *et al.* 2000; Ritz & Beckwith 2001).

4. ARCHAEAL PROTEIN TRANSLOCATION

(a) *Protein targeting*

The only area in which a substantial progress has been made in the field of archaeal protein translocation is the SRP pathway (for a review, see Eichler & Moll 2001). Archaea contain homologues of SRP54, SRP19 (which is absent in bacteria) and 7S RNA. It has recently been shown that archaeal SRP is required for viability, and that SRP54, SRP19 and 7S RNA interact *in vivo* (Rose & Pohlschröder 2002). Reconstitution of purified archaeal SRP components showed that SRP19 promotes the binding of SRP54 to 7S RNA (Bhuiyan *et al.* 2000; Diener & Wilson 2000; Tozik *et al.* 2002). Furthermore, the crystal structures of archaeal SRP19 (Pakhomova *et al.* 2002), SRP19-7S RNA complex (Hainzl *et al.* 2002; Oubridge *et al.* 2002) and SRP54 (Montoya *et al.* 2000) have been determined. A homologue of FtsY/SRα is also present in all archaea (in most archaea denoted as Dpa), but like in bacteria, SRβ is absent. The best characterized membrane protein in archaea is the light-driven proton pump bacteriorhodopsin from *Halobacterium salinarum*, and a number of studies showed that this protein is inserted into the membrane co-translationally in, most probably, an SRP-dependent fashion (Gropp *et al.* 1992; Dale *et al.* 2000).

It is also conceivable that Sec-dependent proteins are transported post-translationally in archaea. If that is the case, which factors could be involved in the targeting? SecB is absent in archaea, but that is not very unusual since Gram-positive bacteria also lack SecB. It has been suggested that *B. subtilis* uses SRP for post-translational targeting (Bunai *et al.* 1999), and archaea may use SRP in a similar fashion. Another protein that could be involved in protein targeting in *B. subtilis* is CsaA (Tjalsma

et al. 2000; Van Wely *et al.* 2001). This protein, which was identified as a suppressor of an *E. coli* SecA(ts) mutant (Müller *et al.* 1992), has chaperone activity (Müller *et al.* 2000*a*) and interacts with SecA and a precursor protein (Müller *et al.* 2000*b*). Most archaea (with the exception of possibly the methanogens) contain a homologue of CsaA, and it is conceivable that it does play a role in archaeal protein transport.

In bacteria, some of the general housekeeping chaperones, such as DnaK (Hsp70) and GroEL/ES, can be involved in protein translocation (Kusukawa *et al.* 1989; Altman *et al.* 1991; Wild *et al.* 1992). Interestingly, however, not all archaea contain DnaK, whereas the group I chaperonins GroEL/ES are completely absent (Gribaldo *et al.* 1999). Archaea do, however, contain proteins that are homologous to eucaryal chaperones. First, archaea contain the group II chaperonins, which are homologous to the eucaryal chaperonin containing TCP-1 (CCT) (also called TriC). In eucarya, CCT is mostly involved in folding of the cytoskeletal proteins actin and tubilin (Yaffe *et al.* 1992; Gao *et al.* 1992). Archaeal CCT proteins (also termed thermosome or TF55) have weak ATPase activity and function in binding, prevention of aggregation and folding of denatured polypeptides (Trent *et al.* 1991; Guagliardi *et al.* 1994, 1995; Waldmann *et al.* 1995). A second type of chaperone found in archaea is GimC (prefoldin). Its eucaryal counterpart promotes folding of actin and tubulin by transferring these proteins unfolded to CCT (Vainberg *et al.* 1998; Siegers *et al.* 1999). Archaeal GimC can stabilize unfolded proteins (Leroux *et al.* 1999), and it was proposed that it fulfils a similar role to DnaK in bacteria. Like in eucarya, archaeal GimC can transfer an unfolded protein to CCT (Okochi *et al.* 2002). In summary, archaea do contain a number of chaperones that may be involved in keeping precursor proteins in a translocation-competent (i.e. largely unfolded) conformation. It remains to be seen, however, whether these chaperones are also involved in the targeting of proteins to the membrane, or whether there are any novel factors involved in this process.

(b) *Translocation motor*

The most remarkable feature of archaeal protein translocation is the absence of the bacterial translocation motor SecA, which strongly suggests that the archaeal Sec pathway uses a mechanism that is similar to the eucaryal SRP-dependent co-translocation mode of protein translocation (figure 1*c*). Interestingly, it was recently shown that fusion proteins containing the main cell-surface glycoprotein (Csg) signal peptide of the halophilic archaeon *Haloferax volcanii* were translocated post-translationally (Irihimovitch *et al.* 2003). There are a number of explanations on how a protein could be translocated post-translationally in an archaeon. The first option is that Csg is translocated Sec dependently using a bacterial-like system. This option does make sense since the Csg signal peptide is predicted to be Sec dependent. The second option is that Csg depends on the Twin-arginine translocation (Tat) pathway. This post-translational translocation system appears to be the main transport system in halophilic archaea (Bolhuis 2002; Rose *et al.* 2002). The Csg signal peptide does not contain the RR motif characteristic for Tat substrates, but it cannot be excluded that this protein is Tat dependent in *H. volcanii*. The final option is that Csg is translocated by a completely novel transport system.

If Csg is Sec dependent, components for post-translational targeting and translocation must be present. There are various factors that could be involved in post-translational targeting (see § 4a), but it is completely unclear how the transport process would be driven. A system similar to the eucaryal post-translational translocation, in which the pulling of BiP provides the driving force, is quite unlikely owing to the absence of ATP on the *trans* side of the membrane. The most likely option, therefore, seems to be the presence of a completely novel component with an analogous function to SecA. Such a protein is, most probably, an ATPase that is possibly membrane bound, but it will be very difficult to identify such a protein by sequence gazing only. Therefore, biochemical and/or genetic techniques will have to be developed to determine whether a SecA analogue exists in archaea.

(c) *Translocation channel*

The core components of the translocation channel are conserved in all domains of life. All archaea contain the proteins SecY/Sec61α and SecE/Secγ. A clear SecG homologue is not present, but homologues of Sec61β have been reported (Kinch *et al.* 2002). Very recently, the first crystal structure of a Sec complex was reported from the archaeon *Methanococcus jannaschii*, which showed the presence of all three core components in the archaeal Sec complex (van den Berg *et al.* 2004), including the Sec61β homologue (denoted Secβ). Phylogenetic analysis suggests that the core components proteins are more closely related to their eucaryal counterparts (Cao & Saier 2003). In view of the closer relationship of the components of the translocation channel with the eucaryal Sec61 complex and the absence of SecA, it is surprising to find that of the accessory factors only homologues of the bacterial SecD and SecF proteins are present. As discussed in § 3, bacterial SecDF was proposed to play a role in the cycling of SecA. This indicates that archaeal SecDF has a function different from bacterial SecDF, or that bacterial SecDF is only indirectly involved in SecA cycling. The latter makes in fact a stronger case for the alternative functions that have been proposed for the role of SecD and SecF in protein translocation (see § 3). Notably, SecDF is absent in crenarchaea and organisms belonging to the *Thermoplasma* group (at least in those which have been fully sequenced).

All other accessory factors found in bacteria or eucarya (e.g. YajC, Sec62/63, Sec71/72, TRAM) are apparently absent in archaea. Notably, the crystal structure of the Sec complex of *M. jannaschii* did not reveal any accessory factors, since this complex was purified from an *E. coli* strain that contained a plasmid with only the genes encoding the core components (van den Berg *et al.* 2004). As mentioned in § 3, YidC is required for biogenesis of inner membrane proteins. A recent report suggested that this protein is conserved in all domains of life (Yen *et al.* 2001). However, the similarity of YidC to the archaeal proteins described in this report is very weak. The highest level of similarity was found with HtlB from *Halobacterium* sp. NRC-1, and in that case the number of identical

residues was still only 13%. Therefore, it remains to be seen whether YidC is really conserved in all domains of life.

(d) *Bioenergetics of the archaeal Sec system*

The key questions for archaeal Sec-dependent protein translocation are:

(i) is the Sec system is co- or post-translocational; and
(ii) what is the driving force of the archaeal Sec system?

If protein transport in archaea is co-translational, the system will require GTP for the cycling of SRP. It becomes more complicated, however, if the archaeal Sec system functions in a bacterial-like manner and translocates (some) proteins post-translationally. The bacterial Sec system requires ATP, but its efficiency also depends on the PMF (Driessen 1992). Owing to the extreme environments in which many archaea live, the contribution of the chemical and electrical gradient to the PMF varies enormously. For example, acidophiles such as *Thermoplasma acidophilum* have a reversed membrane potential, and the PMF depends largely on the ΔpH, whereas in alkaliphiles (e.g. *Natronobacterium pharaonis*) the PMF depends almost entirely on the membrane potential. Also, many archaea use a sodium motive force instead of a PMF (for a review on the bioenergetics of archaea, see Schäfer *et al.* (1999)). Thus, depending on the environment in which a particular archaeon lives, different sources of energy may be used for protein translocation (and other cellular processes).

(e) *Post-translational modification*

During or shortly after translocation, the signal peptide is removed from the mature protein by a type I SPase. Bacterial type I SPases (and those of mitochondria and chloroplasts) are characterized by a serine–lysine catalytic dyad, whereas in eucaryal type I SPases the lysine is replaced by a histidine (Tjalsma *et al.* 1998). Interestingly, all archaea contain a eucaryal SPase. Bacteria also contain a type II SPase that is specifically involved in the processing of lipomodified preproteins. The signal peptides of these proteins are reminiscent of normal signal peptides, but they are characterized by a diagnostic L(A/G)(G/A)C motif in their C domain (Von Heijne 1989). The cysteine residue is lipomodified by a diacylglyceryl transferase and becomes the first residue of the mature protein after cleavage by SPase II. Lipomodified proteins remain anchored to the membrane. Archaea contain several proteins that are predicted to be lipomodified. For instance, a genomic survey of *Halobacterium* sp. NRC-1 revealed the presence of *ca*. 50 putative lipoproteins, which is almost 50% of the total number of predicted extracytoplasmic proteins in this organism (Bolhuis 2002). Furthermore, a lipomodified N-terminal cysteine (containing a diphytanyl glycerol diether group) was found in a protein from the haloalkaliphic archaeon *Natronobacterium pharaonis* (Mattar *et al*. 1994). Surprisingly, homologues of the bacterial enzymes involved in lipoprotein modification and processing are absent from archaea, and it is therefore likely that archaea contain a novel pathway for the modification and processing of lipoproteins (Bolhuis 2002).

Apart from removal of the signal peptide, several other post-translocational modifications can occur. Best studied

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Sto (Q970K7)        VGAEWCPYCAAERWAL
Sso (Q97X81)        VGAEWCPYCGAERWAL
Mac2 (Q8TIA1)       VGAEWCGPCQQMKPIL
Mma (Q8PY73)        FGAEWCGPCQQMKPIL
Mac1 (Q8TJS5)       MGSKWCPDCRSMKPIL
Mth (O27777)        FSASWCPACQKLESET
Pab (Q9UZR1)        FGVNTCPHCRRMKELL
Afu1 (O29547)       FYSDSCPHCREVKPYV
Afu2 (O28917)       FSNYACGHCADFAIET
Hal (Q9HN25)        FSDPSCPHCQDFEADV
Pae (Q8ZTF0)        LYDLHCPFCAIAHERL
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Figure 2. Alignment of the region containing the CxxC motif of archaeal proteins that may be involved in disulphide bond formation on the *trans* side of the cytoplasmic membrane. The SwissProt accession numbers are shown in parentheses. Sto, *Sulpholobus tokodaii*; Sso, *Sulpholobus solfataricus*; Mac, *Methanosarcina acetivorans*; Mma, *Methanosarcina mazei*; Pab, *Pyrococcus abyssi*; Afu, *Archaeoglobus fulgidus*; Hal, *Halobacterium* sp. NRC-1; Pae, *Pyrobaculum aerophilum*.

are the S-layer proteins from halophilic archaea. First of all, these cell-surface proteins are usually glycosylated. In eucarya, the OST complex is associated with the translocation channel (Wang & Dobberstein 1999). This complex contains several subunits, and the most conserved subunit in this complex, STT3, is also found in archaea. This protein plays an essential role in peptide recognition and/or catalytic activity of the eucaryal OST complex (Yan & Lennarz 2002). In *Archaeoglobus fulgidus*, the *stt3* gene is in a cluster with genes encoding glycosyl transferases and a dolichyl-*P*-glucose synthetase (Burda & Aebi 1999), making it very likely that archaeal STT3 is involved in N-linked glycosylation. Archaea also appear to use the same Asn–X–Ser/Thr motif used by the eucaryal OST complex (Lechner & Wieland 1989), and it has been speculated that N-linked glycosylation in eucarya is actually derived from archaea (Burda & Aebi 1999). As in eucarya, the glycosylation event takes place after translocation (Lechner & Wieland 1989; Eichler 2001).

Cell-surface glycoproteins of haloarchaea are also modified with isoprene-derived lipids (Kikuchi *et al.* 1999; Konrad & Eichler 2002). Like glycosylation, this modification takes place after translocation (Konrad & Eichler 2002). Another type of lipid modification is found in the archaeon *Sulpholobus acidocaldaricus*. This organism contains at least one glycosylphosphatidylinositol (GPI)-anchored protein (Kobayashi *et al.* 1997), which is rather surprising since GPI-anchored proteins were previously thought to be present only in eucarya.

(f) *Protein folding*

After translocation, a protein has to fold into its active conformation. In bacteria and eucarya PPIases and thiol-disulphide oxidoreductases play an important role in the folding of proteins. Archaea do contain cytoplasmic PPIases (Maruyama & Furutani 2000), but extracytoplasmic or membrane-bound PPIases have not yet been identified. Archaea do, however, contain putative extracytoplasmic proteins with a CxxC-containing domain that is characteristic for thiol-disulphide oxidoreductases (figure 2). All of these have a hydrophobic N-terminal domain that could serve as a membrane anchor or signal peptide. In fact,

several (Mac1, Mac2, Mma, Hal and possibly Pab) are predicted to be lipoproteins and may be anchored to the membrane via a lipomodified cysteine. Studies on the disulphide oxidoreductase DsbA from *E. coli* revealed that the dipeptide between the two active-site cysteines are important determinants for the redox potential of the protein (Grauschopf *et al.* 1995). Based upon the dipeptide only, it would be predicted that Pab and Afu1 are highly oxidizing (same dipeptide as DsbA in *E. coli*), that Afu2 is mildly oxidizing (same dipeptide as PDI in the ER lumen), and that Mac2, Mma, Sso and Sto are more reducing (same dipeptides as glutaredoxin or thioredoxin). However, such predictions may not apply to these organisms, owing to the unusual environments in which they live. *Methanosarcina acetivorans* and *Methanosarcina mazei* are strictly anaerobic, whereas *Sulpholobus solfataricus* and *Sulpholobus tokodaii* live at high temperatures (*ca*. 80 °C) and a very low pH (around pH 3).

A number of hyperthermophiles, and in particular the crenarchaea *Pyrobaculum aerophilum* and *Aeropyrum pernix*, contain several cytoplasmic proteins with disulphide bonds (Mallick *et al.* 2002). These disulphide bonds may be required for stabilization of thermostable proteins, and point to a more oxidizing cytoplasm when compared with other organisms that very rarely have intracellular proteins with disulphide bonds. If these organisms do indeed secrete proteins with disulphide bonds, it is an intriguing question how these would escape the formation of disulphide bonds in the cytoplasm, since (partly) folded proteins are usually incompatible with the Sec pathway. The simplest explanation is that these proteins are secreted co-translationally, but other explanations can also be envisaged. These include unfolding at the membrane shortly before or during translocation, or export via the Sec-independent Twin-arginine translocase, which is able to transport fully folded proteins (Robinson & Bolhuis 2001).

5. CONCLUDING REMARKS

There are many open questions concerning the archaeal protein transport, in particular concerning the mechanics and energetics of the system. Furthermore, bearing in mind the large number of archaeal open reading frames without homologues in bacteria or eucarya, the absence of components that are essential for protein transport in bacteria or eucarya and the extreme environments in which many archaea thrive, it is quite likely that novel components will be involved in the protein transport process. The reported crystal structure of the *M. jannaschii* Sec complex (van den Berg *et al.* 2004) did not address these issues, as this complex was isolated and purified from *E. coli* without any further biochemical characterization.

Several tools need to be developed to analyse archaeal protein translocation. The most important one is an *in vitro* translocation assay using inverted membrane vesicles or, even better, a fully reconstituted system using purified components. Such assays can answer many questions, such as the following.

(i) Is archaeal Sec-dependent transport co- or post-translational?
(ii) Are any cytoplasmic factors involved?
(iii) What are the energetic requirements of the archaeal Sec system?

A reconstituted system can also be used to address the individual roles of the different components. If, however, novel unknown components are involved in the archaeal Sec pathway, such a strategy will be difficult to realize. Novel components can be isolated using biochemical techniques such as co-purification or chemical cross-linking. Alternatively, novel components can be identified using genetic methods. In *E. coli* a number of powerful genetic screens resulted in the identification of nearly all Sec components (for a review, see Schatz & Beckwith 1990). These screens involved the isolation of suppressor mutants of secretory proteins with defective signal peptides and isolation of mutant strains that do not channel fusions of β-galactosidase with secretory proteins to the translocase (which is lethal as it blocks the translocase). Similar methods can be employed for archaea, but the lack of genetic amenability of most archaea is a major problem. Halophilic archaea are readily transformable with plasmid DNA and offer therefore the best possibilities. However, genetic tools such as inducible promoters (for the analysis of essential genes) are not yet available, and a further development of such genetic tools for halophilic and other archaea is most certainly required.

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