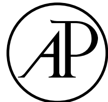

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Evolution of the prokaryotic protein translocation complex: a comparison of archaeal and bacterial versions of SecDF

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Abstract

Protein translocation across the prokaryotic plasma membrane occurs at the translocon, an evolutionarily conserved membrane-embedded proteinaceous complex. Together with the core components SecYE, prokaryotic translocons also contain auxilliary proteins, such as SecDF. Alignment of bacterial and archaeal SecDF protein sequences reveals the presence of a similar number of homologous regions within each protein. Moreover, the conserved sequence domains in the archaeal proteins are located in similar positions as their bacterial counterparts. When these domains are, however, compared along Bacteria–Archaea lines, a much lower degree of similarity is detected. In Bacteria, SecDF are thought to modulate the membrane association of SecA, the ATPase that provides the driving force for bacterial protein secretion. As no archaeal version of SecA has been detected, the sequence differences reported here may reflect functional differences between bacterial and archaeal SecDF proteins, and by extension, between the bacterial and archaeal protein translocation processes. Moreover, the apparent absence of SecDF in several completed archaeal genomes suggests that differences may exist in the process of protein translocation within the archaeal domain itself.
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Keywords: Archaea; Bacteria; Protein export; Protein translocation; SecA; SecDF; Translocon

1. Introduction

Prokaryotic protein export involves the translocation of selected polypeptides across the plasma membrane at proteinaceous sites. Based on the evolutionarily conserved SecY and SecE subunits (Eichler, 2000; Hartmann et al., 1994; Pohlschröder et al., 1997), the bacterial (i.e., *Escherichia coli*) protein translocation complex also incorporates additional subunits (Duong and Wickner, 1997a). The presence of SecG in the SecYEG trimer serves to increase the efficiency of translocation (Hanada et al., 1994; Nishiyama et al., 1994), while YidC is involved in membrane protein insertion (Samuelson et al., 2000; Scotti et al., 2000). In addition, SecD, SecF, and yajC can be co-purified with SecYEG (Duong and Wickner, 1997a) or with YidC (Nouwen and Driessen, 2002). While the absence of SecDF affects the efficiency of protein translocation in vivo (Pogliano and Beckwith, 1994a), SecDF are not required for the reconstitution of efficient protein translocation in vitro (Duong and Wickner, 1997a; Matsuyama et al., 1992). Despite advances in understanding the behaviour of SecDF, the true role(s) played by these components in bacterial protein translocation remains elusive. SecDF have been implicated in maintaining the proton motive force that exists across the bacterial plasma membrane (Arkowitz and Wickner, 1994), although this concept has recently been called into question (Nouwen et al., 2001). SecDF presumably modulate the translocation-related membrane-associated behaviour of SecA, the ATPase component of the bacterial protein translocation apparatus, in vitro (Duong and Wickner, 1997b; Economou et al., 1995). Finally, it has been proposed that SecD plays a role in protein release following the translocation event (Matsuyama et al., 1993).

SecDF are not, however, restricted to Bacteria (Tseng et al., 1999). Examination of the completed genome sequences of the Archaea *Halobacterium* sp. NRC-1,

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Methanococcus jannaschii, *Methanobacterium thermoautotrophicum*, *Pyrococcus abyssi*, and *Pyrococcus horikoshii* has revealed the existence of archaeal SecDF homologues. Comparison of bacterial and archaeal SecDF protein sequences reveals that, while SecDF in both prokaryal kingdoms contain similarly positioned domains of homology, the bacterial and archaeal proteins can clearly be distinguished by the kingdom-specific characters of these domains. In the following, the potential biological significance of this observation, as well as other evolutionary aspects of prokaryal SecDF are considered.

2. Materials and methods

Sequence alignment of SecD from *E. coli* (Accession No. P19673), *Haemophilus influenzae* (P44591), *Halobacterium* sp. NRC-1 (NP_280680), *M. thermoautotrophicum ΔH* (AAB85347), *M. jannaschii* (NP_247075), *Neisseria meningitidis* MC58 (NP_273651), *Pseudomonas aeruginosa* (NP_252510), *P. abyssi* (NP_127445), *P. horikoshii* (PH1985), *Vibrio cholerae* (AAF93908) and *Yersinia pestis* (CAC92424) and of SecF from *E. coli* (P19674), *H. influenzae* (P44590), *Halobacterium* sp. NRC-1 (NP_280681), *M. thermoautotrophicum ΔH* (AAB85346), *M. jannaschii* (NP_248249), *N. meningitidis* MC58 (NP_273652), *P. aeruginosa* (NP_252509), *P. abyssi* (NP_127446), *P. horikoshii* (PH1986), *V. cholerae* (AAF93909), and *Y. pestis* (CAC92423) were performed with ClustalW version 1.81 (http://clustalw.genome.ad.jp) (Thompson et al., 1994). Phylogenetic trees were constructed using the Phylip 3.6a3 programs SEQBOOT, PROTDIST, and NEIGHBOUR (http://bioweb.pasteur.fr)(Felsenstein, 1993) and drawn using NJPlot (Perrière and Gouy, 1996). Predicted transmembrane domains were identified by the TopPred II program (Claros and von Heijne, 1994).

3. Results and discussion

To identify archaeal homologues of *E. coli* SecDF, membrane proteins involved in the bacterial translocation process (Gardel et al., 1990; Pogliano and Beckwith, 1994a), the bacterial sequences were used in a BLAST search against the available archaeal genomes currently listed in the NCBI database (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/genom_table_cgi). Such analyses revealed the presence of SecDF homologues in several archaeal strains, including *Halobacterium* sp. NRC-1, *M. thermoautotrophicum ΔH*, *M. jannaschii*, *P. abyssi*, and *P. horikoshii*, with E values ranging from 2e-14 to 8e-8.

Bacterial and archaeal SecDF proteins were compared in terms of membrane topology. Previous studies, relying on alkaline phosphate fusion mapping, protease accessibility and computer modeling, have predicted that bacterial SecD and SecF each span the membrane six times (Bolhuis et al., 1998; Pogliano and Beckwith, 1994b). In both proteins, a large externally oriented loop is present between the first and second transmembrane segments. The external loop in SecD is longer than in SecF, and the variability observed in the lengths of the various SecDF proteins are mainly the result of differences in the sizes of these loops. In both SecD and SecF, the remaining four membrane-spanning regions are linked by relatively short amino acid stretches. Computer-based predictions revealed that archaeal SecDF proteins assume a membrane topology virtually identical to that of their bacterial counterparts (Fig. 1).

A closer examination of aligned bacterial and archaeal SecDF amino acid sequences was next performed. In agreement with earlier studies (Bolhuis et al., 1998; Gardel et al., 1990), alignment of SecD sequences from a series of randomly selected bacterial strains (*E. coli*, *H. influenzae*, *N. meningitidis* MC58, *P. aeruginosa*, *V. cholerae*, and *Y. pestis*) revealed the presence of six domains of homology (Fig. 2A), while alignment of SecF sequences revealed the presence of four such regions (Fig. 2B). SecD domains 1–3 and SecF domain 1 lie in the large externally oriented loops found between the first pair of transmembrane stretches while the additional domains (SecD domains 4–6 and SecF domains

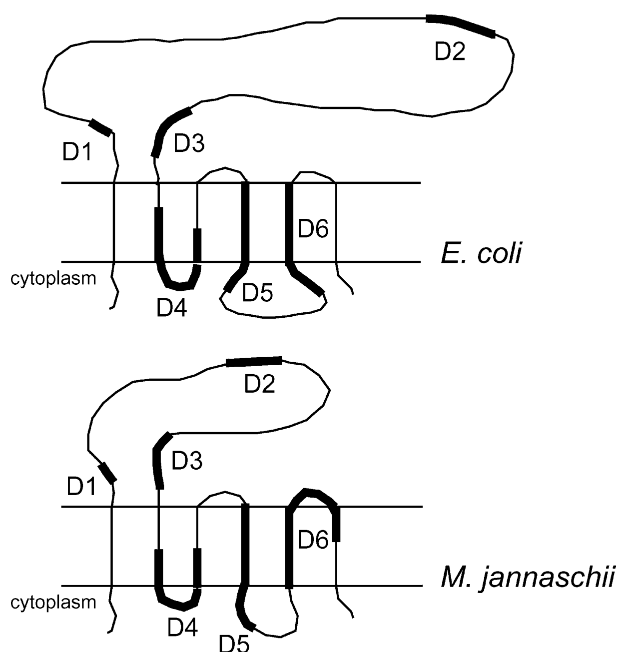

Fig. 1. The membrane topologies of representative bacterial and archaeal SecD proteins. The membrane topologies of *E. coli* and *M. jannaschii* SecD are schematically represented. The topology of *E. coli* SecD has been determined experimentally (Pogliano and Beckwith, 1994b), while that of *M. jannaschii* SecD was predicted by the TopPred II computer program (Claros and von Heijne, 1994). The six conserved sequence domains, D1-6 are depicted in bold.

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(A)                 DOMAIN 1            DOMAIN 2                      DOMAIN 3                          DOMAIN 4
BACTERIA
E.col        124 GLDLRGG   234 LRNRVNQLGVAEPVVQRQG   424 LRAGALIAPIQIVEERTIGPTLG   460 GLLVSILFMIIFYK-KFGLIATSAL
H.inf        124 GLDLRGG   234 LRNRVAELGVAEAVIQRQG   425 LKSGALIAPIQIVEERTIGPSLG   461 GLVAVIAFMLFYYK-MFGVIASFAL
N.men        126 GLDLRGG   233 LHNRVNELGVAEPVIQQSG   412 LRAGSLAAPMQIVEERTIGPSLG   448 GFAIVAAFMVVYYR-LMGFFSTIAL
P.aer        125 GLDLSGG   237 VRNRVNELGVSEPLVQRQG   430 LRAGGLAAPMYFAEERTIGPSLG   466 GMLFVSLFIIVIYR-FFGVIATVAL
V.cho        123 GLDLRGG   236 LRNRVNELGVAEPLVQRQG   426 LRAGALIAPISIVEERTIGPSMG   462 GMVAVMLFTVLYYR-KFGMIANIAL
Y.pes        124 GLDLRGG   234 LRNRVNQLGVAEPLVQRQG   424 LRAGALIAPIQIVEERTIGPTLG   460 GLAVSILFMVVYYR-KFGVIASTAL

ARCHAEA
H.NRC         51 GLELSGG   147 IGSKIDATGLGG-AEVKDI   326 LQAGALPAEITVQNSYYVAPSFA   363 AAIAVAIMVLLRYGEPKIAIPMVFT
M.jan         34 GIDLSGG   142 VPFELTLEGAKKFAEVAKG   217 LKSGALPVKLDIEYISTISPEFG   253 AFIAVGIIVSIRYKQPKIAIPILIT
M.the         36 GLDLQGG   139 VPFRLSTDGARKFAEAARG   213 LKSGSLPVKVKIVGVSSVSPELG   249 AVLAIAVILIVRYRSPILVLPIFFT
P.aby         34 GLDISGG   142 VPFRLSKDAAEKFAKLALG   318 LRSGSLPVKLSIERIDYISPKLG   354 ALLVVGAIVYLHYRKLKIAIPVMFT
P.hor         35 GLDISGG   142 VPFRLSKEAAEKFAKLALG   318 LRSGSLPIKLSIERIDYISPKLG   353 ALLVVGGIVYLHYRKLRIAIPVMLT

                              DOMAIN 5                                   DOMAIN 6
BACTERIA
E.col        500 ATLSMPGIAGIVLTLAVAVDANVLINERIKEEL   549 AFSSIFDANITTLIKVIILYAVGTGAIKGFAIT
H.inf        501 ATLSMPGIAGIVLTLGMSVDANVLIFERIKEEI   550 AFTSIFDANLTTILTAIILYAVGTGPIQGFAIT
N.men        487 ATLTLPGMAALALTLGMAIDSNVLINERIREEL   536 AWATIVDSNLTSLIAGIALLVFGSGPVRGFAVV
P.aer        505 ATLTLPGIAGIVLTMGMAVDANVLIFSRIREEL   554 AFTAILDANLTSLLVGGILYAMGTGPVKGFAVT
V.cho        502 ATMTLPGIAGIVLTVGMAVDANVLIFERIREEL   551 AFSTIADANITTLITAIILFAVGTGAIKGFAVT
Y.pes        500 ATLTMPGIAGIVLTLAVAVDANVLINERIKEEY   549 AFSSIVDANITTLITAIILYAVGTGSIKGFAIT

ARCHAEA
H.NRC        403 LALDLSHLAGFIAVIGTGVDDLVIIADEVMTEQ   449 ALWVIGAAAATTILAMSPLAVLSLGDLRGFALV
M.jan        294 WKLDLPSIAGIIAAVGTGVDNQIVITDEALKRG   337 AFFIIFASAATSIAAMLPLFVLGVGMLKGFAIT
M.the        290 WNIDLAAIAGILAAIGTGVDDQIIITDEVLSGE   342 SAGTLIAAMLPLAYIGFSRGATGIGLLAGFAFT
P.aby        395 WNLDLPSIAGIIAAIGTGVDQQIVITDELLGDV   447 AFFIILASASTTIVAMSFLFKFFVGGLRGFAFT
P.hor        395 WNLDLPSIAGIIAAIGTGVDQQIVITDELLGET   447 AFFIILASASTTIVAMSFLFKFFVGGLRGFAFT

(B)                DOMAIN 1                 DOMAIN 2                      DOMAIN 3              DOMAIN 4
BACTERIA
E.col         49 GLDFTGGT   200 VASLMSVIGYSLNDSIVVSDRIREN   244 TLHRTLITSGTTLMVI   284 GTASSIYVAS
H.inf         59 GLDFTGGV   206 VAAILSVVGYSINDSIVVFDRVREN   250 TLSRTIITSVTTLVVV   290 GTYSSIFVAI
N.men         42 SVEFTGGT   191 LAGILAVLGYSVNESVVVFDRIREN   237 TMSRTIIITHGSTEAMV   277 GIYSSVLVAS
P.aer         40 GLDFTGGT   187 LAAVLAVVGYSLNDTIVIFDRVREN   231 TLLRTIATSVSTLLAI   271 GTYSSIYIAN
V.cho         43 GLDFTGGT   193 VAALLTVVGYSLNDTIVVFDRIREN   237 TLSRTLITSGTTLFVV   277 GTYSSIYVAS
Y.pes         49 GLDFTGGT   199 IASLMSVIGYSLNDSIVVSDRIREN   243 TLSRTIMTSATTLMVV   283 GTVSSIYVAS

ARCHAEA
H.NRC1        48 GIEFTGGS   183 VAALLMLIGYSVDSDILLNNHVLRR   224 TMTLTSIAAMVVMTIV   262 DLMNTYMLNV
M.jan         31 SIDITGGT   176 IAALLMVIGYSVDSDILLTTRVLKR   218 TMTLTTITAMLILLIV   258 DIINTWLLNA
M.the         14 VVDLKGGA   155 VGAILMLIGYSVDTDILLTTRVLKR   197 TMSIAAIASMAALYLV   237 DILTTWLMNL
P.aby         64 GIELKGGS   208 IAALLMLIGYSVDSNILLTTRLLKR   250 TMSTTTLGALISLWLF   285 DFMNTWILNA
P.hor         49 GIELKGGS   193 IAALLMLIGYSVDSNILLTTRLLRR   235 TMSTTTLGALASLWIF   270 DFMNTWIFNA
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Fig. 2. Alignments of the conserved sequence domains of bacterial and archaeal SecD and SecF. The six SecD domains (A) and four SecF domains (B) of conserved amino acid sequence are depicted. Where at least four positions are conserved in the groups of bacterial or archaeal sequence clusters, the residues are highlighted in gray. The bacterial strains examined were *Escherichia coli* (E.col), *Haemophilius influenzae* (H.inf), *Neisseria meningitidis* (N.men), *Pseudomonas aeruginosa* (P.aer), *Vibrio cholerae* (V.cho), and *Yersinia pestis* (Y.pes). The archaeal strains examined were *Halobacterium* sp. NRC-1 (H.NRC), *Methanococcus jannaschii* (M.jan), *Methanobacterium thermoautotrophicum* (M.the), *Pyrococcus abyssi* (P.aby), and *Pyrococcus horikoshii* (P.hor). The numbers at the start of each domain reflect the position of the domain in the amino acid sequence.

2–4) are found either within or linking the more C-terminal transmembrane spanning portions of each protein. Within the archaeal SecDF proteins, the same number and relative positions of the homologous domains also exists (Fig. 1).

Whereas homology of these domains is evident in both the bacterial and archaeal groups of SecD and SecF proteins, sequence similarity is far less pronounced when the sequences of the homologous domains of the bacterial and archaeal proteins are compared with each other (Table 1). With the exception of SecD domain 1, where the proteins from Bacteria and Archaea strongly resemble each other, the sequences of the other conserved SecD domains tend to greatly diverge across kingdom lines. For example, in SecD domain 3, the bacterial proteins addressed are 48% identical (83% similar), while the archaeal proteins considered share 43% identity and 74% similarity. In contrast, comparison of domain 3 sequences between the groups of archaeal and bacterial SecD proteins reveals only 13% identity (43% similarity). Similar trends are observed upon examination of SecD domains 5 and 6 amino acid sequences. The loss in similarity along Bacteria–Archaea lines is most pronounced in SecD domains 2 and 4, where the archaeal sequences differ substantially from their bacterial counterparts, despite the substantial homology of these regions within the respective kingdoms. In the case of SecF, homology is detected in domains 1 and 2 across both kingdoms. In contrast, SecF domain 3 sequences are only 6% identical (25% similar) across kingdom lines, while domain 4 sequences are only 10% similar across Bacteria–Archaea lines, despite being

Table 1
Homology of SecDF domains in Bacteria and Archaea

Domain	Bacteria[a]		Archaea[b]		Bacteria–Archaea	
	% Identity	% Similarity	% Identity	% Similarity	% Identity	% Similarity
SecD						
Domain 1	86	86	43	86	43	86
Domain 2	42	74	53[c]	63[c]	5	16
Domain 3	48	83	43	74	13	43
Domain 4	25	58	14	56	4	13
Domain 5	42	82	39	70	9	33
Domain 6	32	76	23	46	15	35
SecF						
Domain 1	63	88	25	75	25	63
Domain 2	52	92	56	88	12	48
Domain 3	31	56	13	69	6	25
Domain 4	40	60	30	80	0	10

[a] Strains addressed: *E. coli*, *H. influenzae*, *N. meningitidis*, *P. aeruginosa*, *V. cholerae*, and *Y. pestis*.
[b] Strains addressed: *H.* NRC-1, *M. jannaschii*, *M. thermoautotrophicum*, *P. abyssi*, and *P. horikoshii*.
[c] excluding *H.* NRC-1.

highly similar within the representatives of each kingdom.

The analogous membrane topologies of bacterial and archaeal SecD and SecF, together with the adjacent positioning of the encoding genes in both kingdoms (with the apparent exception of *M. jannaschii*), suggests that the *secD* and *secF* genes arose prior to the separation of the two prokaryotic domains. In time, however, the archaeal and bacterial SecDF proteins, regardless of their origin, began to diverge from each other, as reflected in the inter-kingdom differences in the amino acid sequences of the homologous domains of these proteins. Indeed, phylogenetic analysis of SecD and SecF sequences supports the distinct natures of bacterial and archaeal SecD (Fig. 3) and SecF proteins (not shown).

Sequence differences may offer insight into the roles of SecDF in prokaryotic protein translocation as well as highlight differences between the bacterial and archaeal translocation processes. For instance, the striking differences in the amino acid sequences of domain 2 between the bacterial and archaeal SecD proteins could serve to elucidate structure–function relations in the former. Bacterial SecDF have been shown to interact with SecA, serving to coordinate the membrane association of this ATPase component of the bacterial protein translocation complex with the forward movement of the translocating protein (Duong and Wickner, 1997b; Economou et al., 1995). During the so-called 'SecA insertion cycle,' SecA is proposed to insert into the plane of the membrane, becoming accessible from the periplasmic face of the membrane (Economou and Wickner, 1994; Eichler and Wickner, 1998; Kim et al., 1994). As no archaeal homologues of SecA have been described (Eichler, 2000; Pohlschröder et al., 1997), it seems unlikely that archaeal SecDF would be required to fulfil this particular role assumed by their bacterial counterparts. Thus, it is tempting to speculate that SecD domain 2, conserved in Bacteria and Archaea but differing across kingdom lines, may reflect functional differences of the protein in each form of life, such as interaction with the bacterial-specific SecA protein.

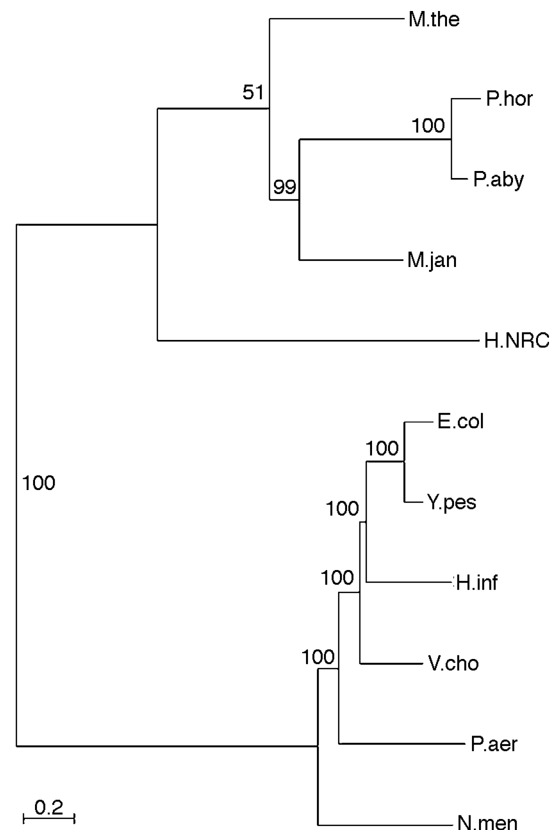

Fig. 3. Phylogenetic analysis of SecD proteins. A neighbour-joining tree was constructed from the aligned SecD sequences analyzed in this study. Numbers attached to internal nodes are bootstrap correspondence levels based on 100 bootstrap replicates of the original alignment. The scale bar represents 0.2 amino acid substitutions per site. Abbreviations are as given in Fig. 2.

More intriguing than the sequence differences between the bacterial and archaeal proteins, however, is the apparent absence of SecDF in some archaeal species. While SecDF have been detected in several completed archaeal genomes, BLAST searches of the completed genomes of other archaeal species, i.e., *Aeropyrum pernix*, *Archeoglobus fulgidus*, *Sulfolobus solfataricus*, and *Sulfolobus tokodaii*, have failed to describe genes encoding for SecDF. A SecF-encoding gene has been reported in the genome of *Thermoplasma volcanium* (TVG1016708), however sequence comparisons raise doubt as to whether this gene, or its *Thermoplasma acidophilum* homologue (TA0863), indeed encode for SecF proteins. Moreover, no SecD-encoding genes were reported in either strain. At present, the physiological significance of the apparent absence of SecDF in some archaeal strains is unclear. As SecDF interact with SecY, the central component of the translocation complex (Nouwen and Driessen, 2002; Sagara et al., 1994), it is reasonable to assume that SecY would differ in SecDF-containing and SecDF-lacking archaeal strains. A comparison of archaeal SecY sequences from various archaeal species, however, fails to reveal any striking sequence differences. Thus, description of the sites of SecY–SecDF interactions may have to wait for high resolution structural data. Alternatively, it is possible that those strains that seemingly lack SecDF in fact express structural homologues of these proteins. Such putative proteins would not be readily identified in sequence-based searches.

Significant insight into the process of protein translocation has been gained by comparison of the amino acid sequences of archaeal translocation apparatus components with their better characterized eukaryal or bacterial counterparts. For example, the similarity between bacterial SecE and eukaryal Sec61γ only became evident upon comparison of each subunit with an archaeal SecE sequence (Hartmann et al., 1994). More recently, analysis of archaeal signal peptidases, responsible for removal of the signal sequence presumably at a late phase of the translocation event, revealed that certain archaeal enzymes possess sequence elements previously thought to be restricted to the bacterial enzyme, while apparently relying on a eukarya-like catalytic mechanism (Eichler, 2002). In this light, further comparison of bacterial and archaeal SecDF proteins could serve to better elucidate the roles played by these proteins in prokaryotic protein translocation.

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