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The Archaeal Signal Recognition Particle: Steps Toward Membrane Binding

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Signal recognition particles and their receptors target ribosome nascent chain complexes of preproteins toward the protein translocation apparatus of the cell. The discovery of essential SRP components in the third urkingdom of the phylogenetic tree, the archaea (Woese, C. R., and Fox, G. E. (1977). *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5088–5090) raises questions concerning the structure and composition of the archaeal signal recognition particle as well as the functions that route nascent prepolypeptide chains to the membrane. Investigations of the archaeal SRP pathway could therefore identify novel aspects of this process not previously reported or unique to archaea when compared with the respective eukaryal and bacterial systems.

KEY WORDS: SRP; SRP receptor; archaea; SRP RNA; SRP54; Ffh; receptor FtsY; protein targeting.

INTRODUCTION

Protein targeting is an essential cascade of reactions directing ribosomal nascent preproteins toward membranes of the endoplasmic reticulum in higher eukaryal cells or towards plasma membranes in bacterial cells (Keenan *et al.*, 2001; Lütcke, 1995; Stroud and Walter, 1999; Walter and Johnson, 1994). This process is catalyzed by the signal recognition particle (SRP) which binds to the signal peptide of a polypeptide emerging on the ribosome (Fig. 1). Subsequent steps require the interaction of the ternary ribosome-nascent chain-SRP complex with a membrane-bound signal recognition particle receptor (SR) adressing the ribosome nascent chain complex (RNC) to the translocation machinery embedded in the phospholipid bilayer of the respective membrane system. After several GTP-hydrolyzing steps, SRP and its receptor disassemble in order to initiate a new targeting reaction (Connolly *et al.*, 1991; Connolly and Gilmore, 1989, 1993). Since all living organisms feature pacemaking reactions coordinating ribosomal protein synthesis and protein translocation either co- or posttranslationally, key regulatory components are highly conserved within different signal recognition particles (Althoff *et al.*, 1994; Eichler and Moll, 2001; Pohlschröder *et al.*, 1997; Zwieb and Eichler, 2002). These regulate both the binding of the signal sequence to the SRP and binding and release of the RNC to the membrane, the latter steps accompanied by intrinsic functions of the SR.

SIGNAL RECOGNITION PARTICLES AND RECEPTORS IN EUKARYA AND BACTERIA

Eukarya

SRPs in the eukaryal domain of the phylogenetic tree are the protein targeting mediation structures which have been most intensively investigated and the functions of which are best known. In higher eukarya this complex consists of six protein components (SRP54, SRP19, and the SRP68/72 and SRP9/14 heterodimers) intimately contacting a 7S RNA molecule (Lütcke, 1995). The entire complex possesses a rod-like contour with 240 × 60 A in which the electron-dense RNA acts as a frame for SRP protein components (Czarnota *et al.*, 1994). This RNA is composed of seven helices distributed in four RNA domains I–IV. Nascent polypeptides being synthezised by the ribosome are recognized via their N-terminal signal sequences by the SRP54 component which performs main functions in the SRP cycle (Bernstein *et al.*, 1989). Remarkably,

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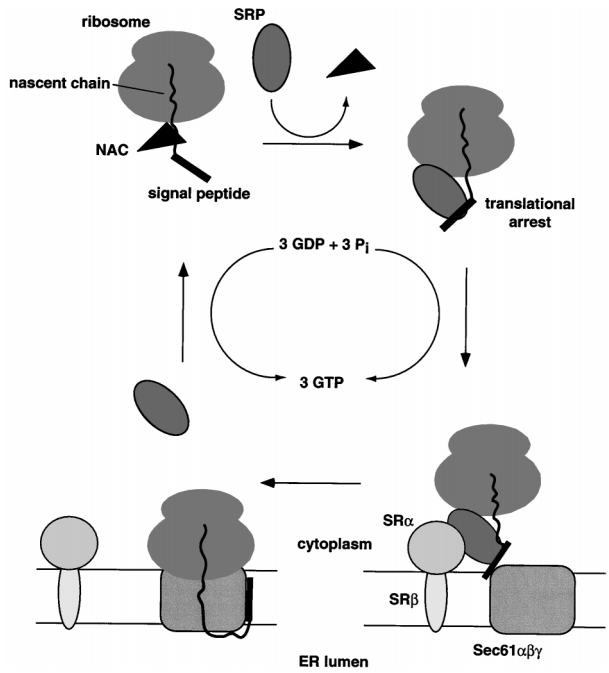

Fig. 1. The signal recognition particle pathway of higher eukarya. The chaperone NAC (nascent chain associated complex) binds first to the nascent polypeptide chain after emerging from the ribosome. The signal recognition particle (SRP) comes in and associates with the signal peptide of the preprotein located at the N-terminal via the methionine-rich C-terminal domain of the SRP54 subunit. Binding of the SRP induces translational pausing or retardation. Subsequently, the ternary complex consisting of ribosome, nascent chain, and SRP interacts with the ER membrane on the cytoplasmic face via the SRP receptor subunit α(SRα) which is mainly attached to the membrane by interactions with the integral receptor subunit SRα. A cycle of GTP-binding and hydrolysis catalyzed by SRP54, SRα, and SRβ regulates the delivery of the ribosome-nascent chain complex to the translocon Sec61$\alpha\beta\gamma$ and the dissociation of the SR/SRP complex. The translation is reinitiated and the growing polypeptide chain is fed cotranslationally in the protein translocation apparatus.

this multidomain protein comprised an N-terminal (N-) domain employed in signal sequence binding (Newitt and Bernstein, 1997; Römisch *et al.*, 1990; Stroud and Walter, 1999), a G-domain responsible for GTP-binding and hydrolysis, and a C-terminal methionine-rich M-domain with signal peptide and RNA-binding functions (Römisch *et al.*, 1990; Zheng and Gierasch, 1997). During protein targeting, it is suggested that this subunit also contacts the SRP receptor.

The eukaryal heterodimeric SRP receptor is composed of two subunits α and β (Tajima *et al.*, 1986), in which SRα contacts SRP54. The N- and G-domain of SRα are homologous to the respective SRP54 domains, suggesting a common evolutionary origin. Actually, as shown for SRP54, SRα is also a GTP-binding and hydrolyzing protein belonging to the SRP GTPase superfamily. It has been suggested that the large N-terminal domain of SRα may be involved in membrane binding (Young *et al.*, 1995). Because of the intense contact between SRα and the membrane-integral SRβ subunit, the whole receptor complex is tightly bound to the ER membrane. SRβ, although featuring GTP hydrolyzing activity, offers stronger sequence similarity to ARF-like GTPases than to SRP GTPases (Miller *et al.*, 1995).

Bacteria

While eukaryal SRPs and SRs are the most complex structures due to the multidomain composition of the RNA and the variety of protein components, the SRP pathway of bacteria is mediated by only three (*E. coli*, *Mycoplasma mycoides* (Macao *et al.*, 1997; Samuelsson, 1992)) or four (*B. subtilis*) components (Dobberstein, 1994; Hershkowitz *et al.*, 2000; Luirink and Dobberstein, 1994). In the case of gram-negative bacteria, the SRP consists of a single protein named Ffh (fifty-four homologue), a 4.5S RNA, and the SRα-homologous receptor component FtsY. The 4.5S RNA only comprises the binding site for the Ffh protein with a highly conserved nucleotide motif in helix 8. In gram-positive species like *Bacillus subtilis*, the RNA is more reminiscent of the eukaryal SRP RNA, though lacking helix 6 which is the binding partner for the SRP19 protein in eukaryal organisms. At least Ffh and the HBsu protein similiar to DNA-binding proteins are functional components of the *Bacillus* SRP (Nakamura *et al.*, 1999). Investigations of *E. coli* clearly demonstrated that the SRP pathway is essential for the insertion of some polytopic membrane proteins, while several *E. coli* preproteins can be targeted via the SecA/SecB secretory pathway (Beck *et al.*, 2000; de Gier *et al.*, 1996; Seluanov and Bibi, 1997; Ulbrandt *et al.*, 1997). Remarkably, both pathways converge at the translocon in *E. coli* cells (Valent *et al.*, 1998). Accordingly, proteomic analysis of *B. subtilis* secA and ffh mutants indicate that most extracellular proteins target on and translocate across the cytoplasmic membrane by cooperation between the two secretory pathways (Hirose *et al.*, 2000). The bacterial SRα homologous FtsY is present as soluble, cytoplasmically localized and as membranous protein (Hershkowits *et al.*, 2000; Luirink *et al.*, 1994). In all bacterial organisms examined to date, SRβ homologous components are lacking.

THE ARCHAEAL SIGNAL RECOGNITION PARTICLE AND ITS RECEPTOR

While SRPs and their receptors in bacteria and eukarya are structurally and functionally well characterized,

much less is known about SRP components in the third domain of life, the archaea (Eichler and Moll, 2001). Considerable interest in these factors has arisen, since conserved SRP components—SRP54/Ffh, SRP19-homologous protein, eukaryal-like SRP-RNA, and SRα/FtsY receptors—have been detected in nearly all archaeal genomes. Their overall structures strongly resemble bacterial as well as eukaryal homologues, underlining the evolutionary conservation and biological importance of SRP-dependent protein targeting in the third urkingdom (Eichler and Moll, 2001; Koch *et al.*, 2003; Pohlschröder *et al.*, 1997; Zwieb and Eichler, 2002). Since SecA/SecB-homologous components have not been detected up to now and are not evident in any of the archaeal genomes investigated to date, the necessity of SRP targeting in routing preproteins to archaeal membranes becomes strikingly obvious. Apart from protein targeting based on the SRP, only the TAT pathway (Berks *et al.*, 2000; Stephens, 1998) may be functional at least in some euryarchaea as deduced from the frequency of the twin-arginine motif in genome-derived signal sequences (Dilks *et al.*, 2003; Rose *et al.*, 2002). Strikingly, the majority of the haloarchaeal preproteins predicted to be targeted via the TAT pathway are nonredox proteins.

Most of the studies already conducted have addressed the in vitro reconstitution of heterologously overexpressed archaeal SRP components and in vitro transcribed SRP RNA in order to prove the functionality of the reconstituted archaeal particle in terms of signal sequence binding, RNA binding, and GTP hydrolyzing activity. To date, several archaeal ribonucleoprotein particles have been reconstituted in vitro: one from an acidophilic and hyperthermophilic crenarchaeal cell (*Acidianus ambivalens*; Moll *et al.*, 1999) and all the others from thermophilic or halophilic euryarchaeal cells (*Archaeoglobus fulgidus* (Bhuiyan *et al.*, 2000), *Pyrococcus furiosus* (Maeshima *et al.*, 2001), *Methanococcus jannaschii* (Hainzl *et al.*, 2002), *Haloferax volcanii* (Tozik *et al.*, 2002)). On the basis of these investigations, the minimal structure of the archaeal SRP is composed of SRP54/Ffh, the SRP 19 homologous component and the 7S-like SRP RNA, as shown in Fig. 2. The structure and composition of the archaeal SRP RNA are discussed in greater detail in an excellent review published recently (Zwieb and Eichler, 2002). Cellular archaeal SRP RNAs coimmunoprecipitate together with the respective Ffh proteins of the hyperthermoacidophilic archaeon *Acidianus ambivalens* (Moll, 2003) and the halophilic euryarchaeon *Haloferax volcanii* (Rose and Pohlschröder, 2002), supporting the view that a highly conserved SRP core exists in all three domains of life. The biological importance of the particle has clearly been shown for the genetically

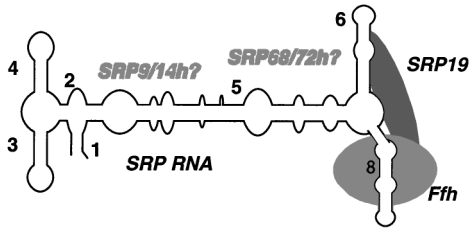

Fig. 2. Composition and hypothetical structure of the archaeal signal recognition particle. The SRP RNA of RNA is displayed by seven helices 1–8, lacking helix 7 (grey shadowed) which is present in the eukaryal SRP. Helices are formed by Watson–Crick base pairing, unpaired regions are represented by bulges and loops. The archaeal Ffh probably binds with a conserved RNA-binding sequence element to form a conserved nucleotide motif in helix 8. The archaeal SRP 19 component—apparently not encoded in *the Thermoplasma acidophilum* genome—interacts with helix 6, which is assumed to strengthen the binding of Ffh to the SRP RNA. SRP9/14- and SRP68/72 homologous (h) protein components (shawdowed in grey) have not been detected yet. If present, they must be archaea-specific. The figure is modified after Eichler and Moll (2001).

tractable organism *Haloferax volcanii* in which deletion of the genomic *ffh* gene results in loss of cell viability (Rose and Pohlschröder, 2002). This valuable genetic system therefore allows the screening of further potential archaeal targeting components under in vivo conditions.

In addition, all the archaeal organisms examined encode the SRα/FtsY receptor component (Eichler and Moll, 2001). As proved for the bacterial and eukaryal receptor, it is suggested that the receptor associates with the SRP/ribosome/nascent chain complex and directs it to the archaeal membrane. Examination of all sequenced archaeal *ftsY* genes reveals a high degree of sequence conservation within the carboxy-terminal N- and G-domains with their bacterial and eukaryal counterparts (Eichler and Moll, 2001). The function of the former protein domains in Mg^{2+}-dependent GTP-hydrolysis has been experimentally demonstrated (Moll *et al.*, 1997). In contrast to the conserved N- and G-domain, only minor conservation was observed in the A-domain, the hydrophilic aminoterminal region of these proteins. There is no obvious sequence similarity between A domains in the three domains of life and the A domain members largely differ in size. Therefore, the function of the A domain is less clear, but it has been suggested that positive charge clusters in the A domain support the binding of soluble *E. coli* FtsY to anionic phospholipids in the plasma membrane (Leeuw *et al.*, 1997, 2000). Clusters of positive charges at the mostly N-terminal end are evident in most archaea, though they are not strongly conserved (Fig. 3). Interspersed among these charge motifs are hydrophobic residues which are

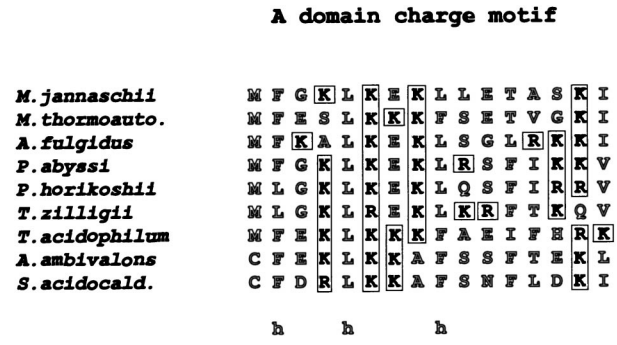

Fig. 3. K/R-positive charge clusters at the extreme N-terminal end of archaeal FtsY sequences. Basic amino acid residues are displayed in boxes. *M. jannaschii: Methanococcus jannaschii; M. thermoauto: Methanobacterium thermoautotrophicum; A. fulgidus: Archaeoglobus fulgidus; P. abyssii: Pyrococcus abyssii; P. horikoshii: Pyrococcus horikoshii; T. zilligii: Thermoproteus zilligii; T. acidophilum: Thermoplasma acidophilum; A. ambivalens: Acidianus ambivalens; S. acidocaldarius: Sulfolobus acidocaldarius*; h: conservation of hydrophobic residues.

assumed to interact with the hydrophobic core of the membrane. Strikingly, the SRβ receptor homologous subunit is lacking in the archaea and in the bacteria investigated to date in contrast to the ubiquitous SRα/FtsY component (Eichler, 2000). It is therefore assumed that a novel binding mode of the receptor to the plasma membrane is acquired during evolution, compensating for the absence of the integral receptor subunit. In addition, the A domains of prokaryotic FtsY act as intrinsic GTP-hydrolysis inactivating proteins, since their deletion enhances the GTPase activity of the NG-domains (Leeuw *et al.*, 1997; Moll *et al.*, 1997).

MEMBRANE-BINDING ACTIVITIES OF ARCHAEAL SRP COMPONENTS

Intrinsic affinities of at least one SRP component are the first prerequisite for the membrane-mediated targeting of nascent polypeptide chains also in archaea. In this connection, attention has focussed on the receptor FtsY, as it is generally considered that this protein combines the cytosolic targeting of preproteins with the translocon-mediated reactions of the membrane in all three urkingdoms (Eichler, 2000; Pohlschröder *et al.*, 1997). In this respect, one of the first archaeal FtsY proteins to be investigated was the homologous protein of the hyperthermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* (Moll *et al.*, 1995, 1996; Ramirez and Matheson, 1991). This 41-kD large receptor component (p41) was identified under normal growth conditions using polyclonal antisera. At first glance, the *S. acidocaldarius* protein was found to be located exclusively in the cytosolic supernatant after ultrasonification of cells at pH 5.5 (Moll *et al.*, 1996). This cytosolic compartmentation was later confirmed for the corresponding protein p51 of the related hyperthermoacidophilic archaeon *Acidianus ambivalens*, although in this case small amounts were also discovered in the membrane fraction (Moll *et al.*, 1997). However, as in the case of *S. acidocaldarius*, the archaeal p51 was mainly detected as a soluble component. On the basis of these results, it was suggested that crenarchaeal FtsY proteins were soluble targeting factors preferentially detected in cytosolic cell fractions under in vitro conditions. A closer reexamination became possible with the application of density-gradient-based flotation assays first described for Ffh-mediated targeting in *E. coli* (Valent *et al.*, 1998) and later adapted for *E. coli* in vitro studies testing the affinity of recombinant FtsY with phospholipid vesicles (Leeuw *et al.*, 2000). Modified flotation assays performed with vesicles composed of enriched tetraether phospholipids clearly detected recombinant *Acidianus ambivalens* FtsY in the floated membrane fraction at the top of the density gradient after ultracentrifugation (Moll, 2003). For this reason, it is suggested that crenarchaeal FtsY has intrinsic affinities for archaeal phospholipids. In the light of the in vitro reconstitution, the distribution of the *S. acidocaldarius* FtsY protein in cytosolic and membrane fractions was reinvestigated using different pH values during cell disruption. Strikingly, the authentic *S. acidocaldarius* FtsY protein is clearly detected in the plasma membrane fraction under pH conditions closer to the external pH of the natural acidic habitats where these hyperthermoacidophilic cells thrive (Fig. 4). Although the internal pH of *S. acidocaldarius* is between 6 and 7 (Moll and Schäfer, 1988), the outside acidic pH is assumed to influence the structure of the membranous compartment. Thus FtsY binding on the cytosolic face of the plasma membrane probably depends indirectly on the extracellular pH, which determines the structure of the lipid phase under in vivo conditions. Taken together, these results strongly suggest that the FtsY of crenarchaeal organisms (i) is soluble, (ii) is distributed between membrane and cytosol to an extent that is as yet unknown under in vivo conditions, and (iii) possesses intrinsic affinities to tetraether phospholipids, making further integral SRβ-like components redundant for the targeting reaction. Despite the membrane-binding capacity of archaeal FtsY proteins, which correlates with their bacterial counterparts but not with their eukaryal homologues, the necessity of FtsY for archaeal protein targeting and export has not yet been demonstrated.

While the crenarchaeal FtsY was difficult to detect in membrane fractions under normal and normalized

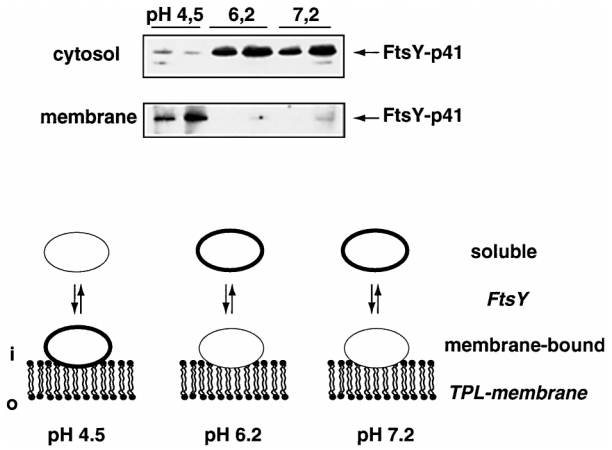

Fig. 4. In vitro distribution of the SRP receptor FtsY (protein p41) of the hyperthermophilic and acidophilic *Sulfolobus acidocaldarius*. The distribution of FtsY between the cytosolic and plasma membrane fractions was measured by varying the buffer pH during cell disruption. The cytosolic supernatant and the plasma membrane fraction derived after two ultracentrifugation steps were subjected to Western blotting, immunodecorated with anti-*S. acidocaldarius* FtsY antisera and detected with a chemiluminescent, alkaline phosphatase coupled reaction. FtsY in the lower part is schematically displayed by an oval, which is drawn with a bold line when FtsY is present predominantly in the respective cell fraction. TPL: tetraether phospholipid.

conditions of cell disruption, the Ffh component was readily detected under the same conditions in the membranous compartment (Moll, 2003; Moll *et al.*, 1999). Anti-Ffh antisera strongly reacted with cellular Ffh in the cytosolic and plasma membrane fractions of *A. ambivalens*. The cellular Ffh migrates with an apparent molecular mass of 46 kDa (p46) in both fractions. Ffh is an unusual protein lacking any membrane spanning domains. Membrane-bound Ffh was substantially extracted with urea, but was resistant to increasing ionic strength or to alkaline conditions. For this reason, the membrane-bound Ffh is assumed not to interact electrostatically with the membrane, but to associate more strongly than suggested for normal peripheral membrane proteins. To address the quality of membrane binding in more detail, the intrinsic affinity of heterologously expressed *A. ambivalens* Ffh to tetraether phospholipid vesicles was investigated by in vitro reconstitution assays and flotation studies in density gradients. Ffh floated to the top of the gradient, indicating its association with phospholipid vesicles, but remained in the bottom fraction in the absence of vesicles. Ffh protein tends to float with a stronger efficiency than FtsY, because higher amounts of floated Ffh were detected using the same concentrations of vesicles. In contrast to the crenarchaeal Ffh *H. volcanii*, Ffh is found in the cytosolic fraction, where it is associated with the ribosomal fraction of the cell (Rose and Pohlschröder, 2002; Tozik *et al.*, 2002).

IS THERE A BASIC BINDING REACTION BETWEEN THE FtsY AND THE Ffh PROTEIN IN ARCHAEA?

Ffh and FtsY build a heterodimeric complex during protein targeting that dissociates after release of the nascent chain to the translocon and after GTP hydrolysis (Connolly *et al.*, 1991; Connolly and Gilmore, 1989, 1993). Therefore a second presumption of SRP-like targeting in archaea has to be confirmed by the interaction of Ffh and FtsY. Although the data available with reference to this central question are preliminary, there are first indications of a receptor function of FtsY for Ffh or vice versa, since nucleotide-independent complex formation was observed between the two proteins (Moll, 2003). While *A. ambivalens* Ffh/RNA and FtsY were detected uncomplexed in cytosolic supernatants as measured by immunoprecipitation, in vitro binding was observed with recombinant Ffh and FtsY after prolonged incubation overnight, suggesting an intrinsic affinity between the two components. This low-affinity binding did not depend on the presence of nucleotides under in vitro conditions, suggesting that nucleotide-free forms of the two archaeal proteins are able to interact.

The direct association of the two components agrees with the structural model proposed previously in which complex formation was deduced from the known protein structures of *A. ambivalens* Ffh-NG and *E. coli* FtsY-NG (Montoya *et al.*, 1997, 2000). In this model, both Ffh and FtsY interact in a head-to-head conformation in which switch regions of the G-domains are involved. However, GTP binding presumably closes a structural gap between the interacting surfaces of the two proteins after conformational changes involving an insertional sequence element specific to all SRP-GTPases (Montoya *et al.*, 1997; Moser *et al.*, 1997). The Ffh-FtsY interaction displays a transient state in protein targeting which depends on other cellular factors such as nucleotide binding, binding of the nascent polypeptide chain, and binding to phospholipids of the membrane. Despite these factors, Ffh and FtsY interact with low affinity. This low-affinity binding is demanded by the concerted-switch model in which empty-site and nucleotide-bound forms of both SRP-GTPases are present in the heterodimeric complex transiently formed during the SRP cycle (Millman and Andrews, 1997; Rapiejko and Gilmore, 1997). In addition, biochemical data on the interaction of bacterial FtsY-NG and Ffh-NG complexed with a nonhydrolyzable GTP-analogue showed slow kinetics

of association and dissociation consistent with a structural union between both components in which the FtsY-N domain participitates directly (Shepotinovskaya and Freymann, 2002).

ARE RIBOSOME-NASCENT CHAIN COMPLEXES TARGETED ON MEMBRANES BY SRP-DEPENDENT EVENTS IN ARCHAEA?

To date, it is not known whether ribosome-nascent chain complexes are recognized by the signal sequence binding domain of the archaeal Ffh protein. At least for the reconstituted *Archaeoglobus* particle, the binding of an eukaryal presequence translated in vitro has been demonstrated. Furthermore it remains to be clarified whether archaeal SRP components act together in order to target preproteins post- or cotranslationally. In *H. salinarum*, cosedimentation of archaeal SRP RNA and bacterioopsin mRNA was observed, pointing to a cotranslational mode of SRP targeting (Gropp *et al.*, 1992). Moreover, more recent in vivo kinetic labeling studies of the membrane insertion of halobacterial opsin support a cotranslational translocation (Dale *et al.*, 2000). Consequently, the archaeal ribosome may possess intrinsic affinity for the translocon as shown for the respective eukaryal system (Kalies *et al.*, 1994; Prinz *et al.*, 2000). Moreover, in *E. coli* cells depleted of Ffh and the translocon subunit SecE, membrane-bound FtsY-ribosomal complexes are captured, inducing the formation of intracellular membranes (Herskovits *et al.*, 2002). Remarkably, the *ftsy* and *sece* genes of hyperthermophilic archaea are neighboring and genomically adjacent to ribosomal genes (Moll *et al.*, 1995, 1997), arguing for common functions in the targeting process. A hypothetical model of the SRP pathway in hyperthermoacidophilic archaeal cells is proposed in Fig. 5. In the light of this model, intrinsic affinities of FtsY *and* Ffh for archaeal phospholipids of the membrane bring the RNC in close contact with the membrane-embedded translocon or pull the RNC to the membrane as a result of the lipid-binding capabilities of FtsY and Ffh. Although the biological importance and the structural fundamentals of these lipid–protein binding processes still need to be resolved—and may not even function in all archaea—they may in this case represent a necessary interface of protein targeting as a primarily cytosolic reaction and protein translocation as a membrane-mediated process. In view of the membrane localization of the crenarchaeal Ffh protein, the primary targeting event of nascent archaeal preproteins may occur both at the membrane *and* in the cytosol, mediated by dimeric FtsY-Ffh complexes. Therefore further investigations of the archaeal SRP pathway will be needed to resolve the fundamentals of targeting reactions which are unique to the third urkingdom of life.

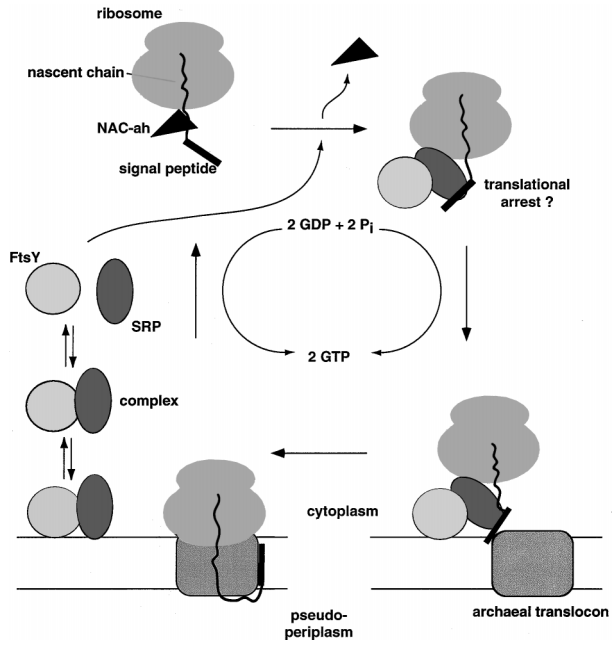

Fig. 5. Hypothetical model of the archaeal SRP cycle. In this model, the archaeal SRP pathway occurs in a modified version of its eukaryal counterpart depicted in Fig. 1. Probably an archaeal monomeric chaperone homologue (ah) of eukaryal NAC (Leroux, 2001) interacts in the early steps with the emerging polypeptide chain. The archaeal SRP cycle obtains new facets due to the significant distribution of the FtsY receptor and the SRP54-homologous component Ffh between cytosol and tetraether phospholipid membrane.

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Note

During publication of this manuscript the crystal structure of the *S. solfataricus* SRP core particle was resolved at 4 Å resolution (Rosendahl, K. R., Wild, K., Montoya, G., and Sinning, I. (2003). *Proc. Natl. Acad. Sci. USA 100*, 14701–14706).

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