Assembly and function of cell surface structures of the thermoacidophilic archaeon Sulfolobus solfataricus
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Introduction to Archaea

Archaea and Ecology of extreme conditions
Organisms are highly flexible to adapt to a variety of ecological conditions on earth, leading to an impressive branched diversity of species. This is also true for the archaea, representing the third domain of life. So far, they have predominantly been isolated from diverse extreme natural and man-made environments of high or low temperatures, highly acidic or alkaline pH or high saline environments. The ability of archaea to adapt to extreme environments has inspired researchers to understand the molecular mechanisms that allow them to survive in such unusual places in nature.

Archaea can be distinguished from bacteria based on a large number of differences at the genetic and biochemical level. Initially, they were considered as bacterial strains based on analysis of their morphology and the fact that they do not have a nucleus. In 1977, Carl Richard Woese and co-workers introduced a new taxonomy of organisms which was divided into three domains: eukaryotes, bacteria and archaea (Figure 1) (Woese & Fox, 1977, Woese et al., 1990). Their method was based on the sequence comparison of the small ribosomal RNA subunit, an essential component for protein synthesis from all living organisms. However, the idea of archaea as a third domain of life remained rather challenging and unaccepted by many biologists during the following years. Further studies on the protein composition of the DNA-dependent RNA polymerase (RNAP) from Halobacterium halobium, Sulfolobus acidocaldarius and Thermoplasma acidophilum by Wolfram Zillig and his co-workers demonstrated that these proteins are more closely related to the systems employed in eukaryotes (RNAP II) than in bacteria (RNAP) (Zillig et al., 1980).

Figure 1. The domain of bacteria, archaea and eukarya.
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Zillig strongly supported the idea that these species are unique and he provided the first biochemical evidence that archaea followed their own evolutionary pathway and therefore represent the third domain of life. During the following years more evidence was gathered that showed that the archaeal ribosomes and other biological systems such as protein secretion and glycosylation, membrane lipids, pili and flagellum assembly systems are different from those in bacteria.

The composition of the archaeal cell membrane and cell wall structure is unique. The cytoplasmic membrane is composed of isoprenoid chains that are connected to the polar head groups with ether-bond, while in bacteria and eukaryotes the fatty acid and head groups are connected with ester-linkages (De Rosa et al., 1991). Compared to the ester-linked lipids, the ether linkage is more thermal resistant and stable in acidic and alkaline conditions. Membranes from hyperthermophiles and acidophiles contain lipids with a C$_{40-44}$ chain length which are connected to the polar head groups at each end forming a tetra-ether lipid monolayer (Figure 2 and Figure 3) (van de Vossenberg et al., 1998). The archaeal cell wall is mainly composed of 2D crystalline assembly of surface layer proteins called the S-layer (discussed below). These structural adaptations are essential for the stability of cell morphology and likely fulfill some protective role against the extreme environmental conditions.

Archaea are divided into two sub-domains: the Euryarchaeota, representing the methanogens, halophiles and some hyperthermophiles (Pyrococcus), and the Crenarchaeota representing most of the hyperthermoacidophilic archaea. The latter group also contains organisms that have been identified in marine and soil environments. Thus, archaea are per definition not the same as extremophiles.

![Figure 2](image-url)  
**Figure 2.** Lipids from archaea and bacteria. The fatty acid composition of cytoplasmic membrane from bacteria and eukaryots (A). The tetra-ether monolayer lipid composition of cytoplasmic membrane from hyperthermophilic archaea (B) and cyclopentane ring containing lipids from *S. solfataricus* (C).
Introduction

Overall they represent 14% of microorganisms from marine environments and soil (Conrad et al., 2006, Cavicchioli, 2006, Chaban et al., 2006a, DeLong, 2005). In recent years several of the mesophilic archaea were proposed to form a separate sub-domain, the Thaumarchaeota (Brochier-Armanet et al., 2008). The Crenarchaeote Sulfolobus is used in many laboratories as a model organism for understanding the genetic and biochemical aspects of hyperthermocaicidophiles. Sulfolobales have been isolated from hot acidic springs in volcanic areas (Brock et al., 1972, Zillig et al., 1986). S. acidocaldarius was initially isolated in Yellowstone National Park (USA) and was the first isolated hyperthermophile (Brock et al., 1972). S. solfataricus was isolated from volcanic areas near Naples, Italy (Zillig et al., 1986). They grow optimally at pH 3 and 80 °C on a range of different carbon sources, such as glucose, maltose, tryptone and non-carbon based sources like sulfur, pyrite crystal particles are utilized as substrate (Grogan, 1989, Zillig et al., 1986). Here we will discuss the various cells surface associated archaeal organelles, the cell envelope structure and the post-translational modifications that have been a topic of research during the described PhD studies.

The archaeal cell envelope

The surface layer is the main component of the archaeal cell wall and it is composed of glycoproteins. These S-layer proteins are tightly associated with the cytoplasmic membrane (Figure 3), and arranged into a two dimensional crystalline layer. This layer is believed to arise from a self-assembly process resulting in the complete cover of the cell. The S-layer proteins form a stable layer structure around the cells and presumably maintain the cell shape at extreme conditions although this has not been established firmly experimentally. On the other hand, the S-layer is highly dynamic and its structure needs to accommodate cell appendages and other cell surface structures. The symmetry of the S-layer lattice arrangement is hexagonal for most archaea, e.g. p6 symmetry (Koval & Jarrell, 1987, Deatherage et al., 1983, Trachtenberg et al., 2000).

Figure 3. Schematic representation of the Sulfolobales cell envelope composed of S-layer, cytoplasmic membrane and a periplasmic space between the two layers (Albers et al., 2006c).
Other reported arrangements of the S-layer lattice are p4, p3 and p2 symmetries (Figure 4) (Sleytr et al., 2007, Mark et al., 2006). The crystalline layer structure and the proteins are highly stable and resistant to many harsh conditions. The layer can be extracted from the cells with SDS without disrupting its crystalline structure and the proteins are usually only denatured by treatment at high pH and temperature (pH 9 and 60°C) (Grogan, 1996, Peters et al., 1995). It is widely believed that the electrostatic interactions within the S-layer protein are essential for their stability. The number of charged amino acids in the S-layer proteins of methanogens, Methanocaldococcus voltae (optimal growth at 37°C), M. thermolithotrophicus (optimal growth at 65°C) and M. jannaschii (optimal growth at 85°C) increases with the optimal growth temperature (Erol et al., 2002). However, from the analysis of their primary and secondary structures, no domains were found that would hint to the thermal adaptation. The S-layer proteins of halophilic archaea also contain large numbers of charged residues but are especially enriched in acidic amino acids allowing an extensive interactions with the surrounding salt ions through salt bridges that are likely important for S-layer stability (Sumper et al., 1990). The extent of hydrophobic interactions within the S-layer is unknown and S-layer proteins appear to be devoid of hydrophobic regions (Erol et al., 2002, Claus et al., 2002). The S-layers proteins from Sulfolobales, Halobacterium salinarum and H. volcanii have been reported to be N-glycosylated (Grogan, 1996, Voisin et al., 2005, Sumper et al., 1990, Engelhardt & Peters, 1998, Chaban et al., 2006b, Shams-Eldin et al., 2008) and it is expected that this protein modification is a general stabilization factor of the archaeal cell-wall structure. The N-glycosylation pathway of M. voltae and H. volcanii has been studied and appears to follow similar mechanisms as known from eukaryotic N-glycosylation (Chaban et al., 2006, Shams-Eldin et al., 2008, Abu-Qarn & Eichler, 2006). The structure of an S-layer protein has not yet been resolved at atomic resolution.
S-surface layer proteins rearrange into 2D crystalline layer on any physical surfaces during their extraction and purification, which makes the preparation of mono-dispersed proteins for x-ray crystallography studies difficult. The homology among S-layer proteins is in general very poor which makes the identification of the genes encoding the S-layer proteins by BLAST searches difficult (Veith et al., 2009).

It is generally accepted that the S-layer proteins have a crucial structural role and therefore their tertiary and quaternary structure appears to be highly similar despite the low conservation at the primary amino acid sequence level. This is even the case for the S-layer proteins of Sulfolobales showing a low amino acid sequence similarity but a very similar structure. The crystalline lattices are arranged with similar symmetry and appear to include trimeric and hexagonal pores with a repeating distance of 21 nm. The space between the S-layer and cytoplasmic membrane is about 25 nm. The S-layer gene operon of *S. solfataricus*, *Acidianus ambivalens* and *M. Sedula* contains the *slaA* and *slaB* genes, which encode the structural components of the S-layer. A similar operon composition is found in *S. acidocaldarius* and *S. tokodaii* (Baumeister & Lembcke, 1992, Taylor et al., 1982, Deatherage et al., 1983, Veith et al., 2009). The *slaA* codes for the major component of the S-layer, an extracellular glycoprotein with variable size specific for each Sulfolobales species. The hypothetical molecular mass of SlaA in *S. solfataricus*, *S. acidocaldarius* and *S. tokodaii* is around 130 kDa. The homology between the three proteins is high in the C-terminal region, but low at the N-terminal region. The *slaB* codes for a membrane protein with a large extracellular domain. SlaB is anchored to the cytoplasmic membrane with a C-terminal transmembrane segment. It associates with SlaA through its extracellular domain (chapter 4, Figure 8) (Veith et al., 2009, Grogan, 1996).

*Ignicoccus* species are the only archaea that possess an outer membrane instead of an S-layer (Rachel et al., 2002). The outer membrane contains archaeal lipids of unknown
chemical structure and it is separated from the cytoplasmic membrane by a large periplasmic space with an average width of 25 to 400 nm (Figure 5). Cryo-sectioning and transmission electron microscopy (TEM) analysis showed extensive vesicle formation from the cytoplasmic membrane and these vesicles seem to fuse with the outer membrane. From the outer membrane, pore forming proteins were isolated (Rachel et al., 2002, Burghardt et al., 2007). Recently, however, it was demonstrated that also the outer membrane contains an ATP synthase and H$_2$:sulfur oxidoreductase complexes implying that the outer membrane must be an energy transducing membrane (Küper et al., 2010). How this can be reconciled with the presence of pores in the outer membrane is presently unclear, nor is it known whether there are mechanisms that allow energy transduction from the ‘periplasm’ into the cytoplasmic membrane, for instance the presence of an ATP/ADP translocase.

In conclusion, archaeal S-layers face a variety of extreme ecological conditions. They are highly stable thereby likely providing structural rigidity and protection of the archaeal cells against the extreme conditions. Since atomic details on the S-layer structure are missing, the molecular basis for the S-layer stability is unknown.

Protein secretion and assembly of flagella and pili
Prokaryotes possess various kinds of cell surface structures serving a variety of biological roles such as motility and interaction with the environment of the organism. These include different types of filaments such as flagella and type IV pili. Because of the efficient application of electron microscopy for the visualization of microorganisms at high magnifications, these flagella and pili filaments appear highly abundant on many different types of microbial cells. The formation of these structures involves fascinating molecular machineries for the secretion of their components across the cell envelope in a precisely coordinated manner for proper assembly. There are various kinds of protein secretion systems and in addition to the general secretion system (Sec system) and the twin arginine translocase (Tat system), distinct type I to VI secretion systems are found in bacteria, and in particular Gram negative bacteria. In the following section, we will briefly discuss different secretion systems of Gram-negative bacteria that are relevant for the biogenesis of cell surface structures. These are compared with the systems found in Archaea.

Protein translocation across the cytoplasmic membrane Sec and Tat system in Bacteria
The Sec (secretion) pathway is the universally conserved protein-translocation pathway found in the cytoplasmic membrane of bacteria, archaea and the endoplasmic reticulum (ER) and thylakoid membranes of eukaryotes (Albers et al., 2006c). In bacteria, it consists
of a protein-targeting and translocation pathway that mediates the export of unfolded proteins across, and the insertion of membrane proteins into the cytoplasmic membrane. The main component of the Sec system is the translocon channel in the cytoplasmic membrane and it is composed of SecY, SecE and SecG proteins (Veenendaal et al., 2004, Driessen & Nouwen, 2008). The secretory protein (i.e., preprotein) is delivered from the ribosome to the SecB chaperone and transferred to the SecA ATPase bound at the SecYEG channel. The SecA motor pushes the preprotein through the SecYEG channel by cycles of ATP binding and hydrolysis. This process is termed post-translational translocation. In the eukaryotic ER, protein translocation is mostly co-translational and coupled to the synthesis of the preprotein at the ribosome. The energy for translocation is delivered by polypeptide chain elongation at the ribosome. Substrates of the Sec pathway contain an N-terminal signal peptide for recognition and targeting (Albers & Driessen, 2002). The signal peptide has a tripartite structure with a positively charged N-domain, a very hydrophobic H-domain and a C-terminal polar domain containing the signal peptide cleavage site. The signal peptide is removed by a membrane-bound signal peptidase once translocation has been initiated. The Sec system is also the major pathway for the insertion of membrane proteins. During the their translation and insertion into the SecYEG channel, the transmembrane domains are recognized and leave the translocon laterally to insert into the lipid bilayer (Veenendaal et al., 2004, Driessen & Nouwen, 2008).

The Tat system (twin arginine translocation) translocates fully folded proteins across the cytoplasmic membrane (Berks et al., 2005). Substrates of the Tat pathway are often co-factor dependent proteins involved in oxidation-reduction pathways in the periplasm. The Tat system is found in bacteria, archaea and in the thylakoid membrane of chloroplasts from plants (Berks et al., 2005, Bolhuis, 2002, Palmer et al., 2005, Robinson & Bolhuis, 2001). The targeting signal peptide of the Tat system resembles the typical Sec signal peptides except that they usually contain a double arginine motif in the N-domain hence the name of the Tat system (Albers & Driessen, 2002). The main components of the Tat system are TatA, TatB and TatC membrane proteins which form a channel in the inner membrane required for translocation of the Tat substrates though the membrane (Berks et al., 2005). The Tat system in Bacteria and eukaryotes is driven by proton motive force (Robinson & Bolhuis, 2001). The Tat components have been purified from *E. coli* inner membrane and demonstrated that TadBC recognizes the twin-arginine signal sequence of the substrate and initiates the translocation (Sargent et al., 2001). Purified TatA forms ring-shaped oligomers of different sizes and it has been suggested that TatA is recruited by TatBC and to form a custom-sized translocation pore for the translocation of the substrate protein across the cytoplasmic membrane (Gohlke et al., 2005). TatA and TaB are homologous proteins. Interestingly, despite the fact that TatB is essential for the Tat secretion pathway in *E. coli*, this component is missing in many organisms suggesting that TatA can fulfill the role of TatB in these organisms.
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**Type I and type II secretion systems**

Gram-negative bacteria utilize type I secretion to secrete proteins in one step across the inner and outer membrane (Holland *et al.*, 2005, Cescau *et al.*, 2007). Type I secretion systems consists of three components: a membrane-associated ATP-binding cassette (ABC) type transporter, a periplasmic adaptor protein and an outer membrane pore (Figure 6). The ABC component recognizes the substrates in the cytosol and energizes the entry of the substrate into the outer membrane pore via the adaptor domain. The adaptor protein forms a channel of extensive \( \beta \)-folded sheets and connects the membrane transporter to the outer membrane pore (Cescau *et al.*, 2007). Here, the substrate is transported across the channel from the cytosol directly into the external medium without entering the periplasmic space.

Type II secretion systems secrete folded proteins substrates from the periplasmic space of Gram-negative bacteria into the external medium (Possot *et al.*, 2000, Bally *et al.*, 1992, Sandkvist, 2001) (Figure 6). These proteins are first translocated across the cytoplasmic membrane into the periplasm by the Sec or Tat system. Type II secretion systems consist of large complexes encoded by a gene operon with about 12-15 genes. The subunits localize to the cytoplasmic membrane, periplasmic space and outer membrane (Durand *et al.*, 2005, Py *et al.*, 2001). The core component of type II secretion are several membrane proteins, small proteins known as pseudo-pilins and a cytoplasmic ATPase, GspE, which is associated with the membrane protein and that energizes the secretion process (Possot *et al.*, 2000). The GspE ATPase is homologous to the ATPase components of archaeal flagella and pili assembly systems and bacterial type-IV pili assembly systems (Peabody *et al.*, 2003, Albers & Driessen, 2005). The exact mechanism of type II secretion is not known, but it is has been suggested that the cytoplasmic membrane components assemble a pilus-like structure in the periplasm that acts as a piston to push the protein substrate across the outer membrane. The pseudo-pili are structural components of the pilus, and are structurally similar to bacterial type-IV pilins, and archaeal flagellins and pilins (Hansen & Forest, 2006, Patenge *et al.*, 2001) (discussed below). Substrates of type II secretion systems are toxins, polysaccharide hydrolyzing enzymes, proteases and lipases (Sandkvist, 2001).

**Bacterial flagellum assembly and type III secretion systems**

The bacterial flagellum is a complex biological nanomachine, which provides efficient and directed swimming motility for cells. The flagellum structure is assembled from 30 different types of proteins (Macnab, 2003). The main architecture of the flagellum is divided into three domains: the filament, the basal body and the hook structure (Macnab, 2004) (Figure 6). The flagellum filament is a wave-shaped structure of about 2-10 \( \mu \)m in length and a diameter of 20 nm.
Figure 6. Main secretion systems of Gram-negative bacteria.
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The filament is hollow from inside with an inner diameter of 5 nm, and composed of a large number of flagellins that are translocated across the inner tube of the filament in unfolded state. These flagellins self-assemble at the tip of the filament. The flagellin, FlIC, has an approximate size of about 25 kDa and is often O-glycosylated (Logan, 2006). Translocation of FlIC and its assembly into the flagellum is dependent on a type III secretion mechanism (Macnab, 2003) which is an intrinsic part of the flagellum structure. The first 50 nm of the flagellum is called the hook (Samatey et al., 2004). The hook connects the flagellum filament with the basal body at the cell envelope. Its symmetrical arrangement is similar to the flagellum filament but appears as bended-shape on the cell envelope. The hook is very elastic and while it is bended, it efficiently transfers the angular momentum of the basal body rotation (in physics known as the torque) to the filament of the flagellum leading to the fast rotation of the entire filament. The basal body functions as the engine for the rotation of the flagellum by utilizing the proton motive force across the cytoplasmic membrane as the energy source. It is a large protein complex expanding from the cytoplasm to the outer membrane and its main structure is composed of several ring structures located at the cytoplasmic, cytoplasmic membrane and in the periplasmic space. The assembly mechanism of the bacterial flagellum shares many similarities with type III secretion systems of pathogenic bacteria that instead of a flagellum, contain a needle complex that transport toxins and effector molecules directly into the host (eukaryote host cells) prior to its invasion (Cornelis, 2006). When the tip of the needle complex contacts the surface of the host cell, it inserts a channel protein into the host cell plasma membrane whereupon it secretes virulent factors into the cell host to interfere with defense systems and to enable host invasion. The injectisome complex is much simpler than the flagellum and it lacks many components necessary for the rotation of the flagellar basal body. It is believed that the flagellum has evolved from the injectisome. Type III secretion systems do not exist in archaea. The archaeal flagellum will be discussed in a later section as it has a very different ultrastructure compared to bacterial flagella, and they depend on an assembly system that is similar to bacterial systems involved in type IV pili assembly.

Bacterial type IV pili
Apart from flagella dependent swimming motility, bacteria are able to move on surfaces by mechanical motion called twitching or gliding motility. Early genome analysis of mainly Pseudomonas revealed the major components required for this sort of motility. The pil locus controls twitching motility via the assembly of type IV pili on the cell surface (Burrows, 2004, Nudleman & Kaiser, 2004, Wu et al., 1997, Whitchurch et al., 1991). Type IV pili are retractable fibers that can be assembled and disassembled with “stored” pilin subunits in a membrane pool (Wolfgang et al., 2000, Nudleman & Kaiser, 2004) (Figure 6). The dynamics of pilus assembly and disassembly creates a forward driving
motion of the cells on surfaces. Another well studied type IV pili system is the Tad system (surface adherence system) of Actinobacillus actinomycetemcomitans (Kachlany et al., 2000). These are non-retractable type IV pili involved in attachment to and colonization of diverse surfaces by cells. Type IV pili systems have been identified in many bacteria and are involved in a multitude of processes such as motility, cell conjugation, and DNA transport.

The biogenesis and function of type IV pili is controlled by a large number of genes (40 genes for pil locus in P. aeruginosa) (Mattick, 2002, Spangenberg et al., 1995). Type IV pili are typically 5–7 nm in diameter, and are thus thinner than the flagellum. They are composed of pilin subunits that are synthesized with class III signal peptides (type IV pilin signal peptide) that are processed by a dedicated signal peptidase PilD (Strom & Lory, 1992). PilD cleaves between the positively charged N-domain and the H-domain. The H-domain that remains attached to the pilin subunit functions as an assembly scaffold for the generation of the pilus structure (Craig et al., 2006, Craig et al., 2003). In addition to the cleavage of the signal peptide, PilD also catalyses the N-methylation of amino-terminal phenylalanine at the N-terminus of the mature pilin using S-adenosyl-L-methionine as methyl donor (Pepe & Lory, 1998, Strom et al., 1993, Strom & Lory, 1992). The N-methyltransferase activity is uncoupled from the signal sequence cleavage activity as the mutation of the catalytic residue (Gly95) in PilD only results in loss of the N-methyltransferase activity (Pepe & Lory, 1998). Interestingly, the loss of N-methylation has no effect on pilus assembly and function of type IV pili (Pepe & Lory, 1998). Type IV pilins and the pseudo pilins of type II secretion systems contain similar types of signal sequences and share a common mechanism of signal sequence cleavage and assembly (Hansen & Forest, 2006). The core components of the type IV pilus assembly system include a cytoplasmic ATPase which functions as motor for assembly of the pilus and a membrane protein that functions as an anchoring site for the ATPase. The pili assembly ATPase in Pseudomonas aeruginosa and Myxococcus xanthus is PilB which is homologous to for instance the HP0525 secretion ATPase of the Cag system of Helicobacter pylori, TadA of A. actinomycetemcomitans and VirB11 of the Ti-transfer system of Agrobacterium tumefaciens (Peabody et al., 2003). These proteins form a large family of secretion ATPases which includes the GspE ATPase of type II secretion systems (Planet et al., 2001). Electron microscopy and size exclusion chromatography of VirB11 and other homologues showed that these proteins form a hexametric ring complex (Savvides et al., 2003, Krause et al., 2000). The crystal structures of VirB11 of Helicobacter pylori and EpsE of Vibrio cholerae were resolved at atomic resolution and these proteins were shown to consist of two large domains, i.e., the N- and C-domain that together form the ATP binding and hydrolysis site (Yeo et al., 2000, Robien et al., 2003). Molecular modeling suggested that the C-domain is involved in the hexametric oligomerization forming the inner ring of the complex while the N-domain forms the outer ring.
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Figure 7. The hexameric complex of GspEII ATPase from hypertheromophilic archaeon Archaeoglobus fulgidus (A) (reprinted from the ref (Yamagata & Tainer, 2007) with permission of the publisher) and asymmetric conformation of the PilT ATPase from hyperthermophilic bacterium Aquifex aeolicus (B) (reprinted from the ref (Satyshur et al., 2007) with permission of the publisher). The subunits A and D are in open conformation.

The archaeal secretion ATPase GspE of Archaeoglobus fulgidus was crystallized as a complete hexametric complex (Figure 7a) (Yamagata & Tainer, 2007). This protein exhibits the same domain architecture as bacterial secretion ATPases. During twitching motility, another motor protein known as PilT ATPase is responsible for the retraction of the pilus (Whitchurch et al., 1991, Wu et al., 1997, Planet et al., 2001). ATP hydrolysis by PilT drives the disassembly of the pilus resulting in the disposition of the pilin subunits into the cytoplasmic membrane. This retraction phenomenon creates a pulling motion of the cells across the interacting surface. PilT is a member of the secretion ATPase family and homologous to PilB, but more similar to type II secretion ATPases. PilT has only been found in type IV pili systems involved in retraction motility. The structure of the PilT ATPase from Aquifex aeolicus has been solved as a hexameric ring (Satyshur et al., 2007). The domain architecture of PilT is very similar to that of the other secretion ATPases. The hexamer PilT ring, however, showed an asymmetric occupancy of its nucleotide binding sites, with 4 subunits in closed, ATP bound state and two subunits in an open non-nucleotide bound state (Satyshur et al., 2007) (Figure 7B). It has been postulated that asymmetric hexamerization leads to a large domain movement of the subunits and that this drives pilus retraction. The ATPase components are associated with the membrane through their interaction with the main membrane protein components of the core complex. A mechanistic understanding of the ATPase cycle causing the assembly and disassembly of the pilus in conjunction with the membrane protein complex remains unknown.
Archaeal protein secretion systems

Similar to bacteria, archaea need to secrete proteins into their surrounding environment, a process that is essential for viability. However, little is known about archaeal secretion systems and most information has been deduced based on comparative genomics such as *in silico* analysis of proteins with putative secretion signal sequences (Albers & Driessen, 2002, Rose *et al.*, 2002, Bolhuis, 2002, Hutcheon & Bolhuis, 2003).

The Sec translocon channel in all kingdoms of life is conserved. The crystal structure of SecYE complex from *Methanocaldococcus jannaschii* was resolved at 3.2 Å (Van den Berg *et al.*, 2005). This structure fits with the electron density map of the *E. coli* SecYEG complex which was determined by cryo electron microscopy of 2D crystals at 8 Å resolution (Breyton *et al.*, 2002, Van den Berg *et al.*, 2005). In archaea, no homolog of the translocation motor SecA has been found and it has been postulated that the protein translocation pathway either resembles the eukaryotic ER system that depends on a co-translational mechanism involving the ribosome, or that it involves a unique motor protein whose identity has not yet been resolved (Albers *et al.*, 2006c).

The Tat pathway is distributed in several archaeal species but not all. The general function of the Tat system is similar to bacteria, e.g. translocation of co-factor dependent proteins such as alkaline phosphatase from *Thermus thermophilus* (Angelini *et al.*, 2001, Hutcheon & Bolhuis, 2003). The Tat system is the main route for protein secretion in halophilic archaea (Rose *et al.*, 2002). Many secreted proteins of halophilic archaea do not have co-factors, but instead it is argued that due to extreme high salt concentrations their proteins fold rapidly before they are translocated necessitating the Tat system for secretion (Rose *et al.*, 2002, Dilks *et al.*, 2005). A bioinformatics analysis on *Halobacterium* sp. NRC-1 genome identified 27 cofactor independent proteins and 7 redox proteins with Tat signal sequence (Bolhuis, 2002). The extracellular protease from haloalkaliphilic archaeon *Natrialba magadii* contains a Tat secretory signal peptide and requires Tat dependent translocation (De Castro *et al.*, 2008). Recently, the Tat dependent translocation of AmyH from the haloarchaeon *Haloarcula hispanica* was studied *in vivo* and *in vitro* and it was shown that AmyH requires the sodium motive force instead of the proton motive force for translocation (Kwan *et al.*, 2008). Homologs of TatA and TatC have been found in archaea but no TatB (Hutcheon & Bolhuis, 2003). Possibly, the archaeal Tat system contains other sofar unidentified components, although TatB appears no be absent in many bacteria suggesting that the TatAC system might be a minimal functional entity.

Novel archaeal secretion systems

Since archaea generally do not have an outer membrane with the exception to *Ignicoccus* species, the canonical type I and II secretion systems are absent. However, the genome of *Sulfolobales* contains ORFs coding for putative ABC-type secretion systems of unknown function (Zolghadr *et al.*, 2010). These genes have certain similarities with
bacterial ABC-type multi drug resistant transporters. At this stage, no experimental data is available to determine as to whether Archaea contain type I secretion systems, although such systems might be expected for polypeptides and and/or solutes such as glycan polymers. Another interesting example are the proteins in the outer membrane of Ignicoccus that is separated in space from the cytoplasmic membrane. The mechanism of outer membrane biogenesis in this organism is entirely unknown, but a novel type of trafficking system for proteins to the outer membrane through vesicles has been suggested (Rachel et al., 2002). Also, proteins that are secreted by Ignicoccus need to pass the outer membrane. This likely involves some novel type of mechanism and possibly the pore-like structures that have been observed to be present in the outer membrane are related to this.

Archaeal flagella: structure and function

Archaeal flagella have been studied at the genetic, structural and functional level for several archaea. Early observations of these pill-like filaments at the archaeal cell surface by electron microscopy led to the suggestion that they are functionally analogous to bacterial flagella performing similar tasks in swimming motility and biofilm formation. Cell motility by flagella has been demonstrated for the archaea *H. salinarium, M. voltae, S. acidocaldarius* and *S. solfataricus* (Thomas et al., 2002, Szabo et al., 2007a, Bardy et al., 2002, Nutsch et al., 2003). In *H. salinarium*, the bi-directional rotation of the flagellum creates a motion to forward or reverse direction by instant switching of the flagellum rotation which appears to be similar to the rotation of bacterial flagellum (Nutsch et al., 2003). Such a rotational motion has not yet been observed for other archaeal flagella. The flagella are also essential for surface attachment and colonization as demonstrated for *Pyrococcus furiosus, Methanopyrus kandlerii* and *S. solfataricus* (Nather et al., 2006, Schopf et al., 2008, Zolghadr et al., 2010). The archaeal flagellum system has evolved by a different mechanism than the bacterial flagellum. The subunit composition, structure and assembly mechanism is very different from that of the bacterial flagellum (Ng et al., 2006, Albers & Pohlschroder, 2009b). The archaeal flagellum has a left-handed helical subunit packaging with a diameter of approximate 14 nm which is much thinner than bacterial flagella (Szabo et al., 2007a, Trachtenberg & Cohen-Krausz, 2006). The flagellum is not hollow from the inside and the inner space is most probably formed by coiled-coil interaction of the N-terminal hydrophobic domains of the flagellins. In this respect, the archaeal flagellum resembles a pilus more than the bacterial flagellum that is hollow on the inside. Moreover, recent studies suggest that the energy required for the rotation of *H. salinarium* flagellum is directly gained from ATP hydrolysis and not from the proton motive force. Therefore, the mechanism of the *H. salinarum* flagellum rotation is fundamentally different from that of the bacterial system (Streif et al., 2008).
The archaeal flagellum is encoded by the *fla* operon, a single locus of 8-10 genes present in many Crenarchaeota and Euryarchaeota (Figure 8). The overall composition of the *fla*-operon shares homology with bacterial type-IV pili assembly and type II secretion systems (Thomas *et al.*, 2001, Szabo *et al.*, 2007a, Albers & Driessen, 2005, Patenge *et al.*, 2001, Thomas & Jarrell, 2001). FlaA and FlaB are the subunits of the flagellum and they contain a class III signal peptide that is necessary for their membrane insertion and assembly into the flagellum. Processing involves the membrane peptidase FlaK (or PibD) (Bardy & Jarrell, 2002, Szabo *et al.*, 2006), and these enzymes are homologous to the bacterial PilD but do not catalyse the N-methylation of the newly formed N-terminus of the flagellin subunit. The remaining H-domain likely folds into an extended hydrophobic α-helix and participates in coiled-coil interactions between subunits within the inner core of the flagellum.
Reconstruction studies of the *H. salinarum* and *S. shibatae* flagella suggests that the H-domains constitute of a central hydrophobic core similar to that of type-IV pili, but there is no direct evidence for a structural role of H-domain (Cohen-Krausz & Trachtenberg, 2002). Archaeal flagella differ in the composition of the flagellin subunits. The fla operon of *M. voltae* contains 4 structural flagellin genes: flaA, flaB1, flaB2 and flaB3 (Kalmokoff & Jarrell, 1991). FlaB1 and FlaB2 are the major components of the flagellum and the deletion of their corresponding gene results in flagellum deficiency. FlaA is distributed throughout the flagellum as a minor component and deletion of flaA result in flagellated but less motile mutants (Bardy et al., 2002). FlaB3 is localized proximal to the cell surface forming a curved shape structure with similarity to the bacterial hook structure. Deletion of flaB3 results in flagellated but non-motile mutants (Bardy et al., 2002). The similarity between this suggestive archaeal hook structure and the hook domain of bacterial flagella may indicate that a similar torque-driven motion is generated by the *M. voltae* flagellum. However, the mechanism of *M. voltae* motility is unknown and the role of the archaeal hook in rotation of the flagellum has not been demonstrated. In *H. salinarum*, five fla genes in two loci (*flaA1, flaA2* and *flaB1, flaB2 flaB3*) encode flagellum subunits (Beznosov et al., 2007, Gerl & Sumper, 1988). The *flaA1* and *flaA2* genes encode the major components of the flagellum. The *Sulfolobales* and *Thermoproteales* fla operons contain only one structural flagellin gene, FlaB (Szabo et al., 2007a, Faguy et al., 1996). In *P. furiosus*, FlaB1 is the main component of the flagellum, but the fla operon contains a second flagellin subunit (FlaB2) with unknown function (Nather et al., 2006). FlaI is encoded by the fla operon and is homologous to the bacterial type IV pili assembly and II secretion ATPases (PilB and GspE). This suggests a conserved mechanism for assembly of the archaeal flagellum and bacterial type IV pili assembly/type II secretion systems (Patenge et al., 2001, Thomas & Jarrell, 2001, Albers & Driessen, 2005, Trachtenberg & Cohen-Krausz, 2006). ATPase activity was demonstrated for the *S. solfataricus* and *S. acidocaldarius* FlaI proteins expressed and purified from overexpressing strains of *E. coli*.
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Albers & Driessen, 2005, Albers et al., 2006a, A. Ghosh unpublished results). So far, FlaI is the only identified ATPase component of the flagellum core complex and although its role in flagellation has been demonstrated by deletion analysis, it remains unclear if the FlaI is also involved in energizing the motility of the cells. FlaJ is the only integral membrane component of the flagellar assembly system (Thomas et al., 2002, Szabo et al., 2007a, Chaban et al., 2007). FlaJ contains 9 transmembrane segments and two large cytoplasmic domains of about 25 and 15 kDa, respectively. These polar domains are thought to function as the interaction site for FlaI as shown for the membrane anchoring proteins of bacterial type II secretion systems. Structural analysis with the interacting domains of the EpsE/EpsN system of Vibrio cholerae indicate that hydrophobic interactions and salt bridges are responsible for the interaction (Abendroth et al., 2005). The function of FlaJ in flagella assembly has not been examined. Although the flagellum of S. solfataricus is essential for motility on surfaces (Szabo et al., 2007a), a rotational motion and a hook-like structure in the flagellum filament remain to be demonstrated. Overall, the mechanism for swimming motility by means of the archaeal flagellum is poorly understood.

Novel archaeal surface structures

Archaea exhibit a wide variety of cell surface appendages some of which are exotic biological structures and functions. These appear to be highly specialized due to the specific adaptation of the microorganisms to their hostile habitats. The cannulae network of Pyrodictium abyssi is an example of such a structure (Nickell et al., 2003, Rieger et al., 1995) (Figure 10a). P. abyssi has been isolated from hydrothermal marine environments and their optimal growth temperatures range from 80 up to 100°C (Horn et al., 1999, Stetter et al., 1983). The cannulae network seems crucial for cell survival as it is highly abundant in the cell colonies. Cannulae tubes have an outside diameter of 25 nm and they consist of at least three different, but homologous, glycoprotein subunits with identical N-termini but with different molecular masses (i.e., 20, 22 and 24 kDa). These proteins are highly resistant to denaturing conditions such as exposure to temperatures up to 140°C. From the 3D reconstruction of the cannulae-cell connections, it appears that cannulae enter the periplasmic space but not the cytoplasm forming an intercellular connection of the periplasmic spaces between cells (Nickell et al., 2003). These connection are formed when cells divide where upon the cells stay connected through the growing cannulae (Horn et al., 1999). The function of the cannulae network is still unclear. It might act to anchor cells to each other or function as a means of communication, mediate nutrients exchange or even transport of genetic material (Ng et al., 2006). It is also not known which system(s) is (are) involved in the assembly of the cannulae network.
Another unusual archaeal cell surface appendage is the “hamus” (Moissl et al., 2005, Ng et al., 2006) (Figure 10b). This structure represents a novel filamentous cell appendage of unexpectedly high complexity. Archaeal cells bearing these structures are found in macroscopically visible string-of-pearls-like arrangements which also entangle bacterial cells mainly *Thiothrix* (SM) or IMB1 proteobacterium (IM) that grow in cold (10°C) sulfidic springs (Rudolph et al., 2001). The archaeal cells are coccoids of approximately 0.6 μm in diameter with about 100 filamentous hamus attached to each cell. Hamus pili are 1 to 3 μm in length and 7 to 8 nm in diameter and have a helical structure with three prickles (each 4 nm in diameter) emanating from the filament at periodic distances of 46 nm. The end of the filament is formed by a tripartite, barbed grappling hamus-like hook. The hamus is composed mainly of a 120-kDa protein. They are stable over a broad temperature and pH ranges (0 to 70°C; pH 0.5 to 11.5) and mediate strong cellular adhesion for the archaeal cells to surfaces of different chemical compositions. It is proposed that the hamus function in surface attachment and biofilm initiation, much like flagella and pili, but in addition provide a strong means of anchoring. *S. solfataricus* contains an UV-induced pili system (Froels et al., 2008) (Figure 11). This system is encoded by the *ups* operon and present in all *Sulfolobales* genomes (Albers & Driessen, 2005). This operon is strongly induced when *S. solfataricus* is exposed to UV light; subsequently the cells assemble pili at their surface and form large cellular aggregates (Froels et al., 2008) (Figure 10b). The Ups pili are much shorter than the wave shaped flagellum of *S. solfataricus* and are relatively thin with a diameter of 7 nm (Szabo et al., 2007a, Froels et al., 2008). They show a right-handed helical symmetry similar to flagellum. Mutants lacking *upsE* gene that encodes the ATPase of the *ups* operon, are deficient in pili formation and cell aggregation. UpsE shares strong
homology with FlaI and other assembly ATPases, and it likely energizes the assembly of the Ups pili. The upsF gene encodes the transmembrane protein of the assembly system and is it highly homologous to FlaJ. Another gene in the operon is upsX. UpsX shows no homology with any other protein and its function is unknown. The ups operon contains two genes that encode pilins, UpsA and UpsB. Both proteins contain a class III signal peptide and are processed by the general class III signal peptidase PibD. Overexpression of UpsA in S. solfataricus results in the formation of unusual long pili. The Ups pili system has a general function in formation of large communities of S. solfataricus and colonization of surfaces (Zolghadr et al., 2010). The Ups system and flagellum can initiate the attachment of S. solfataricus to different surfaces and recent studies on Sulfolobales biofilm formation reveal the Ups system is essential for lateral biofilm formation (Koerdt and Albers, submitted). Recent studies on the flagella and novel pili structures promoted an initiative to broaden the view of archaeal pili biogenesis through a bioinformatics analysis of large number of sequenced archaeal genomes (Szabo et al., 2007b). The Fla-find program was developed to search for proteins containing class III signal sequences. The in silico analysis identified 388 putative class III signal sequence containing proteins in 22 archaeal genomes, from which 102 proteins were annotated with a function: 44 flagellin subunits and 33 as substrate binding proteins. Also extra cellular proteases and redox proteins were present on this list. 120 of these proteins were found connected to an operon similar to bacterial type IV pilus assembly system containing homologues of TadA, TadC and type IV pilin signal peptidases. The Fla-find hits where analyzed for short and highly conserved motifs. Also eight additional SBP and 19 euryarchaeal proteins containing a QXSXEXXXL motif with unknown function were identified (DUF361). In the DUF361 domain, the Q residue was at +1 from the cleavage site. Several of these proteins were identified in an operon together with a novel type IV signal peptidase called EppA from the euryarchaeal Methanocaldococcus maripaludis (Szabo et al., 2007b). Experiments showed that EppA specifically processes proteins belonging to the DUF361 group. The cleavage was tested by co-expressing a DUF361-containing protein with FlaK and EppA. It is
probable that the DUF361 proteins are functionally and structurally different than the well known flagella and pilin proteins due to the requirement of a homologue but yet different type IV signal peptidase for the cleavage of their signal peptide. Recently, the structure of the \textit{M. maripaludis} pilus has been resolved by cryo electron microscopy and revealed a novel structure assembled from two subunit packaging (Wang \textit{et al.}, 2008). A one-start helical symmetry filament and a ring structure of 4 subunits were combined in the same filament. Another novel archaeal pilus assembly system is the bindosome assembly system (Bas) in \textit{S. solfataricus} which is involved in assembly of sugar binding proteins into the bindosome, a structure that is expected to be localized close to the cytoplasmic membrane or integrated within the S-layer (Zolghadr \textit{et al.}, 2007) (Figure 12). The main evidence in support of the presence of this hypothesized structure is that the proposed structural components, the substrate binding proteins (SBPs), contain class III signal peptide sequences, a feature typical for proteins which are well known to form oligomeric structures in both archaea and bacteria. The oligomerization of sugar binding proteins was studied with purified proteins from the membrane of \textit{S. solfataricus} on size exclusion chromatography (chapter 2). Previous studies demonstrated that these sugar binding proteins are substrates for PibD, the archaeal type IV signal peptidase (Szabo \textit{et al.}, 2006, Albers \textit{et al.}, 2003). The oligomerized complex is proposed to play a role in facilitating sugar uptake, a function that enables \textit{S. solfataricus} to grow on a broad variety of substrates. The bas system is rather unique and it has only been identified in \textit{S. solfataricus}.

**Figure 12.** Assembly of flagellum, Ups and Bindosome on the cell surface of \textit{S. solfataricus} (reprinted from the ref (Ng \textit{et al.}, 2008) with permission of the publisher).
The Bas (bindosome assembly system) operon contains five genes that are organized into 2 smaller operons: the *basEF* encoding the main components of the assembly system which are homologues of FlaI/FlaJ of the archaeal flagellum assembly system and UpsE/F from the Ups system of *Sulfolobales* strains (Albers & Driessen, 2005) and a second part that encompasses *basABC*, three small genes that encode small pili-like proteins with class III signal peptides. The BasABC are unique and have only been identified in *S. solfataricus*. Previous studies showed that they are continuously expressed in various conditions but EM images did not reveal any pili structure assembled by BasABC. *BasEF* deletion mutants showed that the uptake and transport of glucose by the cells was strongly inhibited and the growth rate of Δ*basEF* on various substrates was decreased. The deletion of *basABC* only moderately affected the growth rate and sugar uptake. These results suggested that the Bas system is a novel assembly system involved in correct localization of sugar binding proteins to the cell envelope which have a pilin signal peptide. The BasEF forms the core of the assembly machinery in the membrane while the BasABC assists the assembly of the binding proteins by a mechanism which has not been fully understood yet.

**N-Glycosylation in Archaea**

Protein glycosylation is a complex enzymatic process whereby highly branched sugar units are attached to a target protein. Protein glycosylation is involved in diverse cellular functions such as protein folding, protein stability, intracellular protein targeting, transport and intercellular recognition. In archaea, the secreted proteins such as S-layer proteins, pilins and flagellins are glycosylated and this protein modification is considered to be essential for their functionality and the stability of the cell surface structure. Protein glycosylation was initially identified in eukaryotes and due to the complex pathway, it was believed to be too sophisticated for simple prokaryotes. The S-layer protein of *H. salinarum* was the first identified non-eukaryotic glycoprotein and since then, many glycoproteins from bacteria and archaea have been identified and it is now considered as a common protein modification in all domains of life (Sumper et al., 1990, Ng et al., 2006).

In eukaryotes, two glycosylation pathways are known: the N- and O-glycosylation. In the N-glycosylation pathway, the glycan branch is synthesized at the ER on a lipid carrier system. The glycan branch is then translocated across the ER membrane to the lumen of the ER by Rft, a bidirectional secondary transporter, and transferred to the target protein. In the O-glycosylation pathway, the sugar subunits are added to the target protein step by step and the process takes place in the cytoplasm by a cytoplasmic glycotransferase complex. Bacteria and archaea have developed different systems for glycosylation of their flagellins and pilins (Ng et al., 2006). The O-glycosylation is the dominant pathway in bacteria for modification of their S-layer proteins, flagellins and pilins and only *Campylobacter jejuni* strains are known to possess a N-glycosylation pathway while the N-
glycosylation is dominant in archaea. The current focus of archaeal glycosylation is on the AGL system of *Methanococcus voltae* (Ng et al., 2008, Chaban *et al.*, 2006) (Figure 13). In both organisms, the *alg* operon codes for the enzymes of N-glycosylation pathway. In *M. voltae*, the assembly of a trisaccharide is initiated when AglH delivers the GlcNAc as the first sugar unit to the dodichol carrier at the cytoplasmic side of the cytoplasmic membrane. The second and third sugar units (a glucuronic acid and mannuronic acid) are assembled into the glycan branch by AglC and AglA. The *agl* systems of both strains are analogous to the N-glycosylation pathway of eukaryotic system. It was demonstrated that AglH of *M. voltae* complements its homologue system in ∆*alg7* mutants in *Saccharomyces cerevisiae* yeast strain and AglH performed the same glycosyltransferase activity in yeast (Shams-Eldin *et al.*, 2008). In *H. volcanii*, the genes involved in synthesis of glycan branch are identified as AglG, F, I, E and D (Abu-Qarn & Eichler, 2006). They carry out the assembly of a pentasaccharide from two hexose monomers, two hexonic acids and a 190 Da saccharide with unknown composition. When the glycan branch is assembled, it is translocated across the membrane by a ‘flippase activity’ of an unknown system. It was mentioned earlier that in eukaryotes, the glycan branch is transported across the ER membrane by the Rft1 transporter. So far, no homologues of the Rft1 transporter system have been identified in *M. voltae* and *H. volcanii* and an archaeal glycan branch translocation system is still unknown. At the external side of cytoplasmic membrane, the glycan branch is linked to the target protein by AlgB glycotransferase. This protein is homologues to STT3 of eukaryotes, a subunit of a larger protein complex.

![Figure 13. The N-glycosylation pathway in *Methanococcus voltae* for the post-translational modification of the flagellum](image)

*Figure 13.* The N-glycosylation pathway in *Methanococcus voltae* for the post-translational modification of the flagellum (reprinted from the refs (Ng et al., 2008, Jarrell & McBride, 2008) with permission of the publisher).
**Introduction**

The glycan branch is attached to the asparagine residue of the tri-peptide sequence Asn-X-Ser or Asn-X-Thr, and X is any amino acid except proline, which is similar as in eukaryotes. In *M. voltae*, the flagellum and S-layer contain similar trisaccharide branches and both structures are modified by the same N-glycosylation pathway (Voisin et al., 2005). Gene deletion studies on the *agl* operon in *M. voltae* showed that N-glycosylation is necessary for the stability and function of the flagellum but not for its assembly (Chaban et al., 2006). The flagella of deletion mutants were weakly assembled and they appeared to be short and broken compared to the wild-type cells. The cells lacked motility on surface as consequence of weakened flagella structures. The S-layer proteins lacked the glycosyl modification supporting previous evidence that flagellins and S-layer are N-glycosylated by the same system in *M. voltae*. In archaea, the enzymes catalyzing the activation of sugar residues for assembly of the glycan branch are similar to the O-glycosylation system of bacteria. The proteins MMP0705 and MMP0706 from *M. maripaludis* catalyzing the synthesis of UDP-GlcNAc and UDP-ManNAc are homologue to WecB and WecC from *E. coli* (VanDyke et al., 2008).

**Extracellular polysaccharides**

Bacteria secrete glycosylated proteins and exopolymere substances (EPS) into the medium for the synthesis of extracellular structures and biofilm. EPS formation, not to be confused with protein glycosylation, is the assembly of long sugar polymers from diverse monosaccharides glucose, mannose and fructose. EPS is in most cases produced as a capsule surrounding the cell surface and increasing the adhesion to a certain surfaces or cell aggregates from a biofilm (Rodrigues & Elimelech, 2009a, Laue et al., 2006, Tsuneda et al., 2003). Other roles of EPS within biofilms are mainly providing stability for the structures of the biofilm and protection against different contaminants in media like heavy metals and toxic organic compounds. EPS production is in general increased when cells are exposed to contaminants. EPS and biofilm formation by archaea is a new research area. In chapter 3 we have reported archaeal EPS produced by *S. solfataricus*. Using fluorescently conjugated lectins, it was demonstrated that surface attached *S. solfataricus* cells produced EPS containing a variety of different sugars (glucose, mannose, galactose and N-acetylglucosamine). Interestingly, the extracellular network produced by PBL2025, a deletion strain, appeared different to the wild-type strain *S. solfataricus* P2 strain (chapter 3, Figure 4). PBL2025 lacks a set of 50 genes which are by BLAST-search analysis predicted to be involved in sugar metabolism/catabolism and transport of solutes across the cytoplasmic membrane. The disruption of these genes has led to the overproduction of EPS and an analysis of the expression pattern of these genes in P2 demonstrated that they are upregulated during surface attachment of the on mica (Zolghadr et al., 2010), identifying the first archaeal genes involved in modulation of secreted polysaccharides.
Chapter 1

Scope of the thesis

The natural environment of *Sulfolobus solfataricus* is relatively poor in substrates and therefore these organisms rely on an efficient mechanism for substrate uptake. Sugar transport in *S. solfataricus* is mediated by ABC transport systems that consist of an extracellular sugar binding protein, a membrane-embedded permease component and cytosolic ABC-type ATPases. Two well-characterized sugar binding proteins are GlcS which is specific for glucose and galactose and AraS with specificity for arabinose, fructose, and xylose. The genomic organization of ABC transporters in *S. solfataricus* is similar to bacterial ABC transporters and the various subunits of the transporters are found in gene operons that often localize in the vicinity of genes encoding sugar-metabolizing proteins. The signal sequence of a subset of these sugar binding proteins is rather atypical and resemble class III pilin-like signal sequence similar to archaeal flagellin and bacterial type IV pili. Such signal peptides are used for protein substrates that assemble into macromolecular structures outside of the cell. Indeed, the signal peptide of the sugar binding proteins GlcS and AraS are processed by the archaeal type IV signal peptidase PibD that is also involved in the processing of flagellin subunit, FlaB. Processing results in the removal of the positively charged N-terminus that functions as block on secretion and assembly. Upon processing, the substrate can be translocated whereby the H-domain acts as a scaffold for assembly as for instance in the formation of the archaeal flagellum. Therefore, it is hypothesized that the sugar binding proteins with a class III signal peptide also assemble into a large complex, tentatively named the “bindosome”. This process involves an assembly system that contains at least two components, i.e., a cytosolic ATPase GspE and a membrane protein. Using the GspE signature motif, five potential assembly devices have been detected in *S. solfataricus*. One of these systems encoded by the *fla* operon is responsible for the assembly of flagellum whereas the *ups* operon encodes an assembly system for the formation of UV induced pili. The above studies are discussed in the introductory chapter 1 within the broader scope of protein secretion and cell envelope assembly. Chapter 2 describes studies that identified the system responsible for the assembly of the bindosome assembly system, termed Bas. The inactivation of an operon encoding a potential assembly system revealed that cells were specifically defective in the uptake of sugars and consequently growth on these sugars that require a binding protein with a class III signal peptide. It is suggested that the Bas system is involved in the correct localization of these sugar binding proteins in connection to the S-layer and by a so far unknown mechanism that would ensure optimal interaction between binding proteins and extracellular substrates. Chapter 3 describes a further characterization of the Bas assembly system. By overexpressing a BasE mutant incapable of ATP hydrolysis demonstrated this process is essential for the functional surface expression of the binding proteins. We expressed the ATPase BasE together with the membrane domain BasF that contained a C-terminal His tag. The system was purified as complex and partially characterized. Ultra-thin sections and freeze-etching of the S-layer
showed that the morphology of ΔbasEF strains is changed in comparison with the wild type. In Chapter 4, the role of flagella and Ups pili of *S. solfataricus* in surface adherence and colonization was studied. The interaction of hyperthermophiles with their surrounding environment is a largely unresolved area of research. In a previous study it was demonstrated that the flagellum of *S. solfataricus* is essential for motility on surfaces. Strains lacking either the flagella or the UV pili were not able to adhere to several examined surfaces. During surface colonization, cells produced a large amount of extracellular polysaccharides/substances. It is suggested that the flagella are needed for the initial stages of surface adherence, whereas the pili are required for the actual surface association. In chapter 5, the components of the S-layer from three different *Sulfolobales* strains, *Acidianus ambivalens*, *S. solfataricus* and *Metallosphaera sedula* were analyzed in more details. The SlaA and SlaB were identified as the two main components of the S-layer wherein SlaB anchors the S-layer structure to the membrane while SlaA forms the outer surface layer. The thesis is completed with a summary and outlook for further research.