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Mini-review

Sugar transport in (hyper)thermophilic archaea

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Abstract

Hyperthermophilic archaea show important metabolic adaptations for growth on carbohydrates under hostile conditions. For carbohydrate uptake so far only ABC-type transporters have been described that are equipped with a uniquely high affinity as compared to mesophilic bacterial systems. This allows these organisms to efficiently scavenge all available carbohydrates from the extreme environment. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Archaea; Hyperthermophily; Sugar transport

1. Introduction

In 1990, archaea (formerly called archaebacteria), on the basis of their 16S rRNA, were identified as belonging to a third domain of life next to eukarya and bacteria [24]. Archaea are prokaryotes, like bacteria, but they also share features with eukarya. The archaeal domain is divided into two subdomains: crenarachaeota and euryarchaeota. 16S rRNA analysis on environmental samples indicates that some archaea belong to a third subdomain, the korarchaeota. However, none of these organisms has been successfully isolated and grown under laboratory conditions. Archaea are usually found in environments in which the physical conditions are extreme with respect to the environments which humans usually encounter. Members of the crenarchaeota are mostly thermophilic (heat-loving) organisms while the euryarchaeota consist of thermophilic, methanogenic and halophilic (salt-loving) organisms.

The genomes of several archaea have been sequenced and annotated (Table 1). Surprisingly, most open reading frames (ORFs) found show homology either to bacterial or eukaryal genes and only a small subset appears to be unique to archaea. Hyperthermophiles are found not only in the archaeal domain, but also in the bacterial domain. These or-

ganisms are placed relatively close to the root of the phylogenetic tree. The hyperthermophilic bacteria *Thermotoga maritima* [19] and *Aquifex aeolicus* [5] contain a number of genes that are uniquely homologous to archaeal genes. It was proposed that due to adaptation to hyperthermophily, lateral gene transfer exists between hyperthermophilic bacteria and archaea [20]. The highest maximal growth temperature so far has been reported for the archaeon *Pyrolobus fumarii* [3] that grows between 90 and 113 °C. For bacteria, this limit lies at 90 °C as reported for *A. aeolicus* [5].

Several archaea are able to grow on saccharides as sole carbon source. In particular, carbohydrate hydrolysing enzymes from hyperthermophilic archaea have been well characterized as they are of interest for industrial applications. Only a few hyperthermophilic archaea can grow on monosaccharides like glucose, but most do grow on di- and polysaccharides. Polysaccharides first have to be cleaved extracellularly into smaller units before they can be transported into the cell. Once inside, these smaller units are hydrolyzed further to glucose. In hyperthermophilic organisms, glucose is converted into pyruvate via modified Embden-Meyerhof (EM) or Entner-Doudoroff (ED) pathways.

Transport of carbohydrates has been studied extensively in bacteria and eukarya, while until recently, little was known about the uptake in archaea. This review discusses the recent advances in our understanding of sugar transport in hyperthermophilic archaea.

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Table 1
Sequenced archaea

Type	Organism	Genome size (Mb)	Database
Crenarchaeote	*Aeropyrum pernix* K1	1.67	http://www.bio.nite.go.jp/
	Pyrobaculum aerophilum	2.22	http://www.tree.caltech.edu/#20
	Sulfolobus solfataricus P2	2.99	http://www-archbac.u-psud.fr/projects/sulfolobus/sulfolobus.html
	Sulfolobus tokodaii	2.69	http://www.bio.nite.go.jp/
Euryarchaeote	*Archaeoglobus fulgidus*	2.18	http://www.tigr.org
	Halobacterium NRC-1	2.57	http://zdna2.umbi.umd.edu/~haloweb/
	Methanobacterium thermoautotrophicum ΔH	1.75	http://www.biosci.ohio-state.edu/~genomes/mthermo/
	Methanococcus jannaschii	1.66	http://www.tigr.org/
	Pyrococcus abyssi GE5	1.80	http://www.genoscope.cns.fr/
	Pyrococcus furiosus	2.10	http://www.genome.utah.edu/
	Pyrococcus horikoshii OT3	1.80	http://www.bio.nite.go.jp/
	Thermoplasma acidophilum	1.56	http://www.biochem.mpg.de/baumeister/genome/home.html
	Thermoplasma volcanium GSS1	1.58	http://www.aist.go.jp/RIODB/archaic/

2. Carbohydrate transport

2.1. Solute uptake mechanisms

In mesophilic bacteria, three main classes of transporters are found for the uptake of sugars (Fig. 1), namely:

(i) secondary transport, in which the sugar is transported over the membrane in symport with protons or sodium ions, thus utilizing the electrochemical gradient of protons or sodium ions;

(ii) phospho*enol*pyruvate (PEP)-dependent phosphotransferase systems (PTS), in which sugar transport is coupled to phosphorylation of the substrate at the expense of PEP;

(iii) ATP binding cassette (ABC) transport, where the substrate is first bound by a binding protein bound to the cytoplasmic membrane. The substrate is then transferred to the transporter domain in the membrane whereupon uptake can take place at the expense of ATP.

Biochemical studies and analysis of the genome sequences suggest that archaea are devoid of PTS systems. These systems are also not found in the genomes of thermophilic bacteria like *T. maritima* and *A. aeolicus*. Although secondary transporters appear abundant, none of these systems has so far been implicated in the uptake of sugars. Rather they seem to be involved in uptake of anorganic substrates (Table 2).

2.2. Binding-protein-dependent ABC-type transporters

The sugar transporters characterized in hyperthermophilic archaea are of the ABC-type (Table 3). ABC importers belong to two main families: the sugar ABC transport-, or carbohydrate uptake transporter (CUT)-family, and the di/oligopeptide transport-, or Opp-family [22]. These two families differ not only in substrate specificity but also in the architecture of the transport complex. Members of the bacterial CUT-family are involved in the uptake of glycerol-phosphate, mono- and oligosaccharides [22]. The CUT1 subfamily transports mainly di/oligo saccharides and glycerol-phosphate, while the CUT2 subfamily is involved in monosaccharide transport. The CUT1 transporters consist of an extracellular binding protein, two membrane proteins that form the translocation path, and a single ATP binding subunit that is thought to act as a homodimer. The best characterized transporter of CUT1 is the maltose/maltodextrin transporter of *Escherichia coli*. The *malE*, *malF*, *malG* and *malK* genes encode the binding protein, two membrane domains and the ATP binding domain, respectively. In the CUT2 subfamily, only one membrane domain is present that presumably forms a homodimer, while the two ATPase domains are fused together. In the genomes of archaea, members of both families are found.

Members of the di/oligopeptide transport family are mainly involved in the uptake of di- and oligopeptides, nickel, heme and substituted sugars. These transporters usually consist of an extracellular binding protein, two membrane domains and two ATPase domains that likely function as a heterodimer. This subfamily of transporters is abundantly present in the genomes of archaea.

Surprisingly, archaeal ABC sugar transporters are found in both the CUT and di/oligopeptide transport families, where these transporters share homology on both primary sequence level and domain composition. The CUT1 family
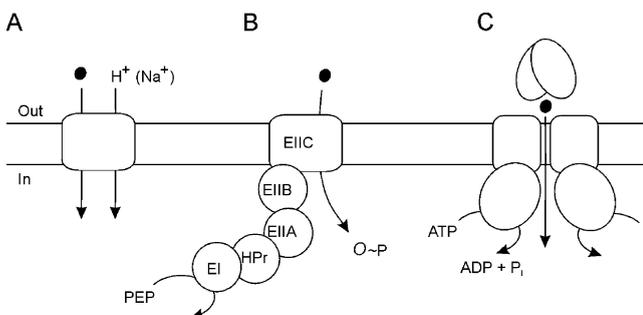

Fig. 1. Schematic overview of the different transport classes, namely, secondary (A), PTS (B), and ABC (C).

Table 2
Distribution of primary and secondary transporters in the genome sequences of extremophiles

Organism	Number of predicted transporters	
	Secondary	ABC-type
Thermatoga maritima	25 (10)[a]	55
Aquifex aeolicus	26 (3)	14
Methanococcus jannaschii	24 (2)	14
Methanobacterium thermoautotrophicum	19 (3)	15
Pyrococcus horikoshii OT3	34 (12)	23
Archaeoglobus fulgidus	38 (17)	25
Escherichia coli	194 (180)	74

[a] In parenthesis are indicated the number of putative secondary organic transporters out of the total number of predicted secondary transporters. *E. coli* is a mesophilic bacterium that is included as a reference.

Table 3
Characteristics of described hyperthermophilic archaeal carbohydrate transporters and their binding proteins

Organism	Substrate	K_m for uptake (nM)	Binding protein	K_d for solute binding (nM)	Ref.
P. furiosus	β-gluco-oligomers (e.g., cellobiose)	175	CbtA	45	[16]
	Maltodextrins (e.g., maltotriose)	–	MDBP	–	[17]
	Trehalose/maltose	–	TMBP	–	[17]
S. solfataricus	Arabinose	–	AraS	130	[9]
	Cellobiose	–	CelB	–	[9]
	Glucose	2000	GlcS	480	[1]
	Maltose	–	MalE	–	[9]
	Trehalose	–	TreS	–	[9]
T. litoralis	Trehalose/maltose	22/17	TMBP	160	[14,25]

–, not determined.

members are homologous to the ABC sugar transporters of mesophilic bacteria. Archaeal examples are the transporters for trehalose/maltose of *Thermococcus litoralis* and *Pyrococcus furiosus*, maltodextrin of *P. furiosus* [14,17], and trehalose of *S. solfataricus* [9]. CUT2 subfamily members present in the archaeal genomes have not been characterized. The transport systems for arabinose and glucose of *S. solfataricus* [9] exhibit a domain composition that is typical of the CUT1 subfamily. Therefore, a subdivision of the CUT family on the basis of the transported substrates seems arbitrary.

The second family of archaeal carbohydrate ABC-type transporters is homologous to the di/oligopeptide transport family of mesophilic bacteria. These include the cellobiose/β-gluco-oligomer transport system of *P. furiosus* [16] and the maltose/maltodextrin and cellobiose/cello-oligomer transporters of *S. solfataricus* [9]. This family of transporters is particularly abundant in the genomes of hyperthermophilic organisms, and the genes are often located in the vicinity of genes encoding enzymes involved in sugar metabolism. In the bacterium *T. maritima*, these transporters have been implicated in peptide transport rather than sugar transport and it was suggested that sugar and peptide metabolism are coordinately regulated [19]. However, since similar systems in *P. furiosus* and *S. solfataricus* are involved in sugar transport, it seems more likely that many of these transporters in *T. maritima* are involved in the uptake of sugars.

ABC transporters of the CUT1 family usually transport only a few structurally or size-related compounds. Carbohydrate transporters of the di/oligotransport family, however, have a much broader substrate specificity. For instance, the cellobiose transporter of *P. furiosus* accepts not only cello-oligomers but also other β-gluco-oligomers, which differ substantially in structure [16]. Like the di- and oligopeptide transporters, the binding sites of these transporters may be versatile allowing the binding of various substrates [16].

Studies in intact cells have demonstrated that hyperthermophiles mediate sugar uptake with an extremely high affinity. K_m values have been reported between 20 nM for the trehalose/maltose transporter of *T. litoralis* and 2 µM for the glucose transporter of *S. solfataricus* (Table 3). In *E. coli*, maltose transport occurs with a relatively high affinity of about 1 µM [4]. Substrate affinities of ABC transporters in the nanomolar range are usually observed only for substrates such as vitamins and iron which are found in very low concentrations in the environment. Uptake of substrates by hyperthermophilic archaea is usually optimal around the growth temperature.

2.3. Binding protein

The binding protein captures the substrate from the medium and delivers it to the membrane domains of the transporter. In bacteria, these proteins either float free in
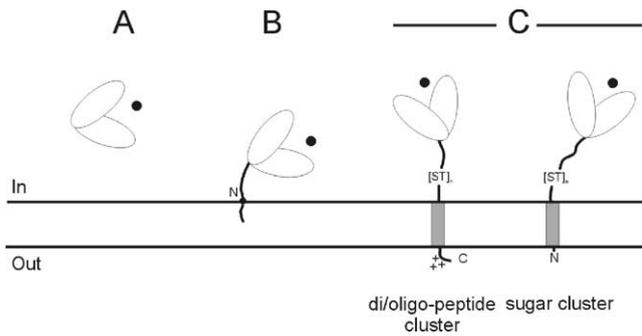
Fig. 2. Modes of membrane anchoring of binding proteins. (A) Gram-negative bacteria; (B) Gram-positive bacteria; (C) archaea. See text for further explanation.

Fig. 3. Domain organization of the two archaeal sugar binding classes. Sugar cluster (A) and di-/oligopeptide cluster (B). SS, signal sequence; S/T-rich, hydroxylated amino-acid-rich region.
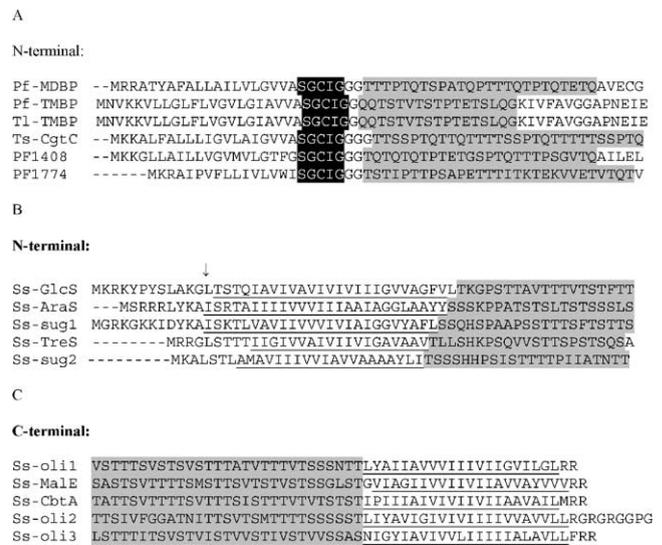

Fig. 4. Amino acid alignment of membrane-bound proteins of *Pyrococcus* and *Thermococcus* species (A), and *Sulfolobus solfataricus* (B and C) showing the putative lipid modification site (black) and the membrane anchoring domain (underlined) with serine/threonine-rich linker (shaded). The arrow indicates the processing site. PF, *P. furiosus*; Ts-CgtC, *Thermococcus* sp. B1001 cyclodextrin binding.
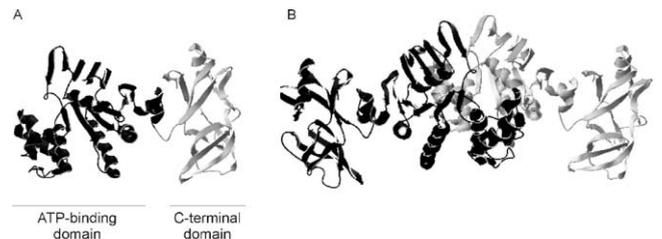

Fig. 5. Ribbon presentation of Tl-MalK (PDB entry: 1G29), showing the monomer, with ATP binding (black) and carboxyl-terminal domain (grey) (A) and the dimer (B). Adapted from [6].

the periplasm (Gram-negative) or are attached to the membrane via a fatty acid modification of the amino-terminus (Gram-positive) (Fig. 2). In archaea, binding proteins are membrane-bound by means of a hydrophobic transmembrane segment that can be present at either the N-terminus or C-terminus. Sequence and hydropathy analysis of the carbohydrate binding proteins reveals the presence of two classes, which coincide with the two transport families (Fig. 3). One class shows homology to MalE of *E. coli*, and therefore belongs to the CUT1 family, while the other class is homologous to oligopeptide binding proteins (OppA).

Archaeal binding proteins of the CUT1 family contain at their amino-terminus a short stretch of positively charged amino acids followed by a hydrophobic region that is sufficiently long to anchor the protein to the membrane. In the binding proteins for glucose, trehalose, and arabinose of *S. solfataricus*, this charged amino-terminus is processed (Fig. 4b) at a site that is normally cleaved by a bacterial type IV pilin signal sequence peptidase [2,9]. The latter enzyme is involved in flagellin subunit processing. Upon the removal of the cytosolically localized positively charged amino-terminus, the remaining hydrophobic domain functions as a scare fold to allow the flagellin subunits to assemble into the growing flagella. In vitro processing and site-directed mutagenesis studies suggest that the precursors of archaeal flagellins and archaeal binding proteins of the CUT1 family are processed by the same membrane-bound peptidase (S.-V. Albers, personal communication). Although signal sequence prediction programs predict cleavage directly following the

hydrophobic domain, this site is apparently not used. The role of this processing step for the binding proteins remains to be elucidated.

It has been postulated that TMBP of *T. litoralis* (Tl-TMBP) is membrane-anchored via a lipid anchor since the amino-terminus contains a putative lipid modification site (consensus motif: SGCIG) [14]. The exact signal-sequence cleavage site could not be determined as the amino-terminus seems to be blocked. The same motif is found in a number of other binding proteins and sugar-hydrolyzing enzymes of Thermococcales (Fig. 4a), but not in other archaea. There is no experimental evidence that lipid-modification does not take place in these organisms, nor has a homologue of lipoprotein signal peptidases been found in archaea. Electron mass spectrometric analysis suggested a lipid modification of halocyanin of the halophile *Natronobacterium pharaonis* [18].

The amino-terminal membrane-anchoring domain is followed by a region rich in hydroxylated amino acids such as serine and threonine. This region may function as a flexible linker that connects the catalytic domain to the membrane-

anchoring region. Archaeal binding proteins studied so far are glycosylated, and it seems plausible that the flexible linker region is the site of glycosylation as demonstrated for the S-layer protein of *Haloferax volcanii* [23]. The *P. furiosus* binding proteins contain glucose moieties [17] (unpublished results), whereas mannose, glucose, galactose, *N*-acetyl glucosamine and most likely 6-sulfoquinovose moieties are found in binding proteins of *S. solfataricus* [9]. Due to the glycosylation, binding proteins can bind to concanavalin A (ConA) affinity columns [1,9,12,16,17], which allows the convenient and rapid enrichment of S-layer and binding proteins from a solubilized membrane fraction. Glycosylation is, however, not essential for substrate recognition, as binding proteins can be expressed actively in *E. coli* in a nonglycosylated form [14,16,17].

The mature carbohydrate binding proteins belonging to the di/oligopeptide transport family show basically a mirror domain organization of the other class of binding proteins (Fig. 3, 4b and 4c). In these proteins, a typical bacterial-like signal sequence is present at the amino-terminus [9,16]. This signal sequence is removed after insertion of the protein into the membrane. The catalytic domain of the binding protein is membrane anchored via a carboxyl-terminal transmembrane segment that connects via the serine/threonine-rich flexible linker [16]. The extreme carboxyl-terminus following the transmembrane segment contains positively charged amino acids that presumably function as a topogenic signal for membrane anchoring.

Although binding proteins in general exhibit a low primary amino acid sequence homology, the overall structure is usually very similar. The substrate binding pocket is formed by two domains that connect via a flexible hinge. Each lobe binds to one of the two membrane domains (reviewed in [4]). The substrate binds according to a so-called "venus flytrap mechanism". In the open state, the substrate can interact with the binding site whereupon the two domains come together forming a closed state in which the substrate is occluded. The three-dimensional structure of the catalytic site of both Tl-TMBP (PDB-entry: 1EU8) and Pf-MDBP (PDB-entry: 1ELJ) has been solved with bound substrate [7,10]. Although both proteins are structurally similar to the *E. coli* MalE, some differences can be observed. Tl-TMBP cannot accommodate carbohydrate oligomers other than maltose or trehalose, while Pf-MDBP only binds the oligosaccharides maltotriose and higher malto-oligomers.

Thermostability of both archaeal proteins is achieved in a different manner. Tl-TMBP is thought to be thermostable by the presence of an additional α-helix and the elongation of most other α-helices present in the binding protein. Increased hydrophobic interactions, an increased number of salt-bridges and the presence of proline residues in key positions also contribute to the thermostability. Furthermore, in Tl-TMBP a decreased number of cavities is found and this results in a lower empty cavity volume and an increase of van der Waals energy. In Pf-MDBP, the total volume of the cavities is similar to that in *E. coli* MalE (Ec-MalE).

The substrate is bound in the binding pocket by hydrophobic interactions. In the case of Ec-MalE and Pf-MDBP, these hydrogen bonds are formed by hydrophobic stacking of aromatic rings in the binding pocket. The aromatic rings interact with the glucopyranosyl rings of the substrate. In the case of Tl-TMBP, where the glucose moieties of the substrates have different angles, hydrophobic stacking is difficult. In this case, increased hydrogen bonding holds the substrate in the binding pocket. Another interesting feature is that the substrate maltotriose is more extensively coordinated in the binding pocket of Pf-MDBP as compared to *E. coli* MalE. In the latter case, the third glucopyranose ring only loosely interacts with the protein. Also the binding cleft of Pf-MDBP is shallower than in Ec-MalE, suggesting that Pf-MDBP is less flexible than Ec-MalE. This latter feature could also contribute to the thermostability of Pf-MDBP.

Homologues of the membrane domain MalF in Gram-negative bacteria contain a large periplasmic loop which is thought to be involved in docking of MalE. This loop is not present in Gram-positive bacteria and archaea (reviewed in [4]). Binding proteins scavenge the environment for substrates and bind these with high affinity. The dissociation constants vary between 40 and 500 nM [1,16] (Table 3). With Tl-TMBP, substrate binding appears to be fast, whereas substrate dissociation is slow and temperature dependent [7,14]. These latter features might be due to the more excessive interactions of the substrate with the binding protein. The kinetics of substrate binding of the glucose binding protein of *S. solfataricus* was found to be extremely pH-dependent. At low pH (1–2), substrate binding was fast, while at higher pH values, substrate binding became invariantly slow [6].

2.4. ATP binding protein

The ATP binding subunits of the ABC transporters couple the binding and hydrolysis of ATP to transport of the substrate through the permease domains. ATP hydrolysis presumably causes a conformational change in the ATP binding domain that propagates the membrane domains to open a channel along which the substrate can pass the membrane. Bacterial and archaeal ATP binding domains are highly homologous, in particular the Walker A and B regions that form the nucleotide binding site. Also, these domains contain the ABC signature motif LSGGQ [4].

The ATP binding proteins of the trehalose/maltose transporter of *T. litoralis* (MalK) [6] and the glucose transporter of *S. solfataricus* (GlcV) (Verdon, Albers, Driessen and Thunnissen, submitted) have been characterized biochemically and crystallized. ATP hydrolysis by the isolated domains occurs with a K_m of 150–300 μM, and is optimal at the respective growth temperatures. Tl-MalK crystalizes in a dimeric form. The monomer structures of Tl-MalK and Ss-GlcV are remarkably similar showing two domains, i.e., the ATP binding domain which is also involved in the hydrolysis of ATP and the interaction with the membrane domains, and

a carboxyl-terminal domain [6]. The ATP binding domains LolD (MJ0796) and LivG (MJ1267) of *Methanococcus jannaschii* lack this second domain [26].

The dimer organization in the crystal structure of Tl-MalK differs from those shown in the crystal structures of MJ0796, MJ1267, HisP [15], a subunit of the histidine transporter of *Salmonella enterica* serovar Typhimurium, and Rad50cd, a DNA repair protein which shows ATP binding domains similar to the domains found in ABC-transporters [13]. Only the Rad50cd structure suggests a role for the ABC signature motif in the dimer organization, where it interacts with the ATP bound to the opposite molecule. In a signature motif mutant of Rad50cd, ATP-induced Rad50cd association was abolished. In contrast to the other proposed dimer organizations, the Rad50cd dimer model explains the results obtained from the mutational analysis in different ATP binding proteins [26]. It is also surprising that Tl-MalK crystalizes in a dimeric form, because the protein has been shown with several techniques to be a monomer in solution [11]. It is possible that for correct dimerization the contact with the membrane components of the complex is necessary.

The carboxyl-terminal domains of both Tl-MalK and Ss-GlcV consist mainly of β-sheets and part of it shows structural homology with the oligonucleotide/oligosaccharide binding fold. This domain is possibly also present in MalK of *E. coli* and involved in the binding of MalT, a transcriptional activator that regulates the expression of the *mal* operon [21]. In the absence of substrate, MalT remains bound to MalK but as soon as maltose or another inducer enters the cell, MalT is released in order to activate the expression of the genes of the *mal* operon. Such transcriptional activators have not yet been described for archaea.

3. Physiology

Hyperthermophilic archaea live in very hostile environments in which organic substrates are usually available at low concentrations. Due to their high affinity, binding-protein-dependent transport systems are optimally suited for these conditions. The cells have to respond quickly to environmental changes. It is therefore not surprising that the expression of ABC transporters is, in most organisms, strongly regulated. Regulation can be at different levels, namely transcriptional, translational and at the protein level. At the first level, transcription of genes is induced after binding or release of regulator proteins. When transcription is constitutive, translation of the mRNA can be regulated by mRNA stability. At the third level, the proteins can be specifically degraded or (in-)activated by phosphorylation or other modifications.

In *S. solfataricus*, the glucose and trehalose binding proteins seem to be constitutively expressed, as these proteins are actively present under a large variety of growth conditions [9]. Other binding proteins, such as those for cellobiose and maltose, are slightly upregulated when cells are grown in

the presence of these substrates. The arabinose binding protein is strongly induced by growth on arabinose. Induction of the sugar binding proteins of *P. furiosus* has been studied both at the transcriptional and protein level. Expression of the binding proteins appears more tightly regulated than in *S. solfataricus*. This possibly relates to a difference in environmental conditions and growth rates. The fast-growing *P. furiosus* is found in the sea where substrates are quickly washed away, while the slower growing *S. solfataricus* is found in acidic solfataric lakes.

Pf-CbtA and Pf-TMBP are only present when *P. furiosus* is grown on a sugar substrate that is transported by the respective systems [16]. The protein levels and binding activities correlate with the mRNA levels as analyzed by Northern blot analysis [16,17].

The trehalose/maltose transporters of *P. furiosus* and *T. litoralis* are almost identical at the amino acid sequence level. The genes encoding this transporter are located on a 16-kb DNA fragment that is flanked by inverted repeats that contains a putative transposase [8]. It is highly possible that this fragment was recently obtained via lateral gene transfer from a yet unknown host. If this is indeed the case, the genes involved in regulation must also be present on this fragment, as the transporter is not expressed constitutively [8,17]. Expression is observed when cells are grown on maltose, trehalose and yeast extract (which contains trehalose) [14]. Expression is also observed upon growth on other substrates containing α-glucosides such as maltotriose and starch, even though these substrates are not recognized by the binding protein. These substrates are most likely hydrolyzed by extracellular α-amylases and amylopullulanases that release glucose, short maltodextrins and the inducer maltose [17]. Pf-MDBP is expressed under identical conditions as Pf-TMBP, except that the latter is not induced by trehalose [8,17]. On the basis of these expression studies, it has been postulated that *P. furiosus* contains two maltose transporters. However, Pf-MDBP does not bind maltose [17]. The discrepancy between the functional and expression studies might be due to the presence of a contaminating maltotriose in the commercial preparations of maltose that are used for the growth studies. Pf-MDBP crystallized in the presence of maltose contains maltotriose in its binding pocket [10]. This further demonstrates that these binding proteins are equipped with a very high binding affinity for their substrate.

4. Conclusions

Since some (hyper)thermophilic archaea are equipped with novel sugar metabolic routes, elucidation of the transport mechanism is of particular importance in understanding the mechanism of energy generation. Although a number of transporters have been described biochemically, and subunits have been analyzed structurally, detailed information on the energetics of uptake is lacking. Reconstitution

of the activity in isolated membrane vesicles or proteoliposomes would enable answering some of these questions. A striking observation is that many archaeal carbohydrate transporters belong to the oligopeptide family of ABC transporters, in particular, systems that are involved in the uptake of oligosaccharides. The wealth of genomic information also demonstrates the presence of secondary transport systems that belong to the major facilitator superfamily. It will be of interest to determine whether secondary transporters are involved in carbohydrate transport or whether this is exclusively restricted to ABC transporters in (hyper)thermophilic archaea.

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