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REVIEW ARTICLE
Protein phosphorylation and its role in archaeal signal transduction

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One sentence summary: The authors review the current knowledge about protein phosphorylation in Archaea and its impact on signaling in this organism group.

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ABSTRACT
Reversible protein phosphorylation is the main mechanism of signal transduction that enables cells to rapidly respond to environmental changes by controlling the functional properties of proteins in response to external stimuli. However, whereas signal transduction is well studied in Eukaryotes and Bacteria, the knowledge in Archaea is still rather scarce. Archaea are special with regard to protein phosphorylation, due to the fact that the two best studied phyla, the Euryarchaeota and Crenarchaeota, seem to exhibit fundamental differences in regulatory systems. Euryarchaeota (e.g. halophiles, methanogens, thermophiles), like Bacteria and Eukaryotes, rely on bacterial-type two-component signal transduction systems (phosphorylation on His and Asp), as well as on the protein phosphorylation on Ser, Thr and Tyr by Hanks-type protein kinases. Instead, Crenarchaeota (e.g. acidophiles and (hyper)thermophiles) only depend on Hanks-type protein phosphorylation. In this review, the current knowledge of reversible protein phosphorylation in Archaea is presented. It combines results from identified phosphoproteins, biochemical characterization of protein kinases and protein phosphatases as well as target enzymes and first insights into archaeal signal transduction by biochemical, genetic and polyomic studies.

Keywords: Archaea; Crenarchaeota; Euryarchaeota; reversible protein phosphorylation; protein kinase; protein phosphatase; regulation; signal transduction
INTRODUCTION

Archaea

The third domain of life, Archaea, has been established in the 1970s–1990s by Carl Woese and colleagues (Woese, Kandler and Wheelis 1990) based on their phylogenetic studies on small subunit ribosomal RNA (rRNA). Initially Archaea were categorized as extremophiles, which thrive in hostile environments characterized by extremes of temperature, pH, salt or combinations thereof, or organisms with unique metabolic traits, i.e. methanogens. Today, using environmental molecular biology approaches, it is well accepted that Archaea are ubiquitous and widely distributed in moderate habitats and play a major role in geochemical cycles (Delong 1998; DeLong and Pace 2001). But still, to date, most of the cultivated species are extremophiles.

Today 1237 fully sequenced archaeal genomes are available (as of May 2016) (Genome Online Database [GOLD] [http://genomesonline.org/]) and six major archaeal phyla have been proposed: Euryarchaeota, Crenarchaeota, Nanoarchaeota, Thaumarchaeota, Aigarchaeota (candidate phylum) and Korarchaeota. The largest and first established phyla were the Euryarchaeota and Crenarchaeota and more recently four of them (Thaumarchaeota, Aigarchaeota, Crenarchaeota and Korarchaeota) were combined to the “TACK” superphylum (Guy and Ettema 2011).

At a first glance, Archaea resemble Bacteria, the second prokaryotic lineage, in respect to their unicellular lifestyle, the lack of organelles and nucleus, cell shape and size. Also they possess a similar DNA structure with one circular DNA, plasmids and operon structures are commonly found. In regard to metabolic complexity, Archaea resemble Bacteria and lower Eukaryotes. Absolutely unique for the Archaea is the absence of murein, one of the key features of bacterial cell walls, and their membrane. The archaeal membrane lipids are composed of isoprenoid chains ether-linked to sn-glycerol 1-phosphate head groups (i.e. dibiphytanyltetraethers or biphytanylethers) rather than fatty acids ester-linked to phosphatidylglycerol 3-phosphate, -glycerol 1-phosphate, as found in Bacteria and Eukaryotes (Albers and Meyer 2011).

Intriguingly, related to information processing, the processes involved in transformation of DNA to protein (e.g. replication, transcription, translation, repair) in Archaea resemble their respective eukaryotic counterparts, but are often less complex (Bell and Jackson 1998; Bell, Magill and Jackson 2001; Soppa 2001; Hickey, Conway de Macario and Macario 2002; Geiduschek and Ouhammouch 2005; Grohmann and Werner 2011). In line with this similarity, the recent identification of Lokiaarchaeota, as a missing link between Archaea and Eukaryotes, promotes the direct descent of Eukaryotes from the archaeal TACK superphylum. Thus, a new two domain tree of life on Bacteria with one side and on the other side Archaea with the Eukaryotes emerging as a monophyletic group within the Archaea is currently discussed and is a matter of scientific debate (Guy and Ettema 2011; Raymann, Brochier-Armanet and Gribaldo 2015). In the context of the shared ancestry of Archaea and Eukaryotes and the similarities found in DNA metabolism, Archaea are especially interesting targets to study regulation by post-translational modification (PTM) and mechanisms of signal transduction.

PTMs in Archaea

PTMs enable organisms to respond rapidly to changing environmental conditions, such as depletion of nutrients or changes of abiotic factors such as temperature. This allows cells to change the properties of their current proteome in a way that ensures adaptation and suits their current lifestyle best, without relying on the synthesis of new proteins.

In 2011, Khoury, Baliban and Floudas (2011) performed a study where they curated the SWISS-Prot database regarding PTMs, which were either identified experimentally (via proteomics) or predicted in all three domains of life. They were able to identify more than 300 000 PTMs subcategorized to 28 different types of PTMs across 5605 different organisms. Of these PTMs, phosphorylation was the most abundant modification (~140 000 modifications). Only 305 (0.13%) phosphorylation sites were identified in the 49 archaeal species available at that time. This study illustrates nicely that the investigation of PTMs in Archaea is still in its infancy.

Nevertheless, several different PTMs have been reported in Archaea. Among these are many PTMs that are also present in Bacteria and Eukaryotes like phosphorylation, acetylation, N- and O-glycosylation and methylation (summarized in Table 1). In Archaea, the ubiquitin-like modification SAMylation (small archaeal modifier protein), which targets proteins for the proteosomal degradation, was identified in Haloferax volcanii (Humbard et al. 2010). Recently, the small protein modification, urmylation, which was only reported in Eukaryotes so far, was found in Sulfolobus acidocaldarius (Anjum et al. 2015). Furthermore, Archaea also possess amino acid modifications like hypusination and thiolation (Eichler and Adams 2005).

Generally, PTMs found in Bacteria and Eukaryotes can also be found in Archaea. Many archaeal PTMs are rather similar to their eukaryotic counterpart like the use of a dolichyl pyrophosphate carrier in N-glycosylation and urmylation. However, here we want to focus on phosphorylation and its role in archaeal signal transduction.

Reversible protein phosphorylation

One of the best-studied PTMs in all three domains of life is reversible protein phosphorylation, which plays a major role in signal transduction and is involved in the regulation of nearly all processes within the cell. Protein phosphorylation and dephosphorylation is a covalent, reversible modification of amino acids that involves protein kinases (PKs) and protein phosphatases (PPs). PKs catalyze the phosphorylation, i.e. the transfer of the γ-phosphate group from nucleoside triphosphates (usually adenosine triphosphate (ATP)) to other proteins, whereas PPs remove the covalently linked phosphate group from the phosphorylated protein (phosphoprotein) by hydrolysis (dephosphorylation). Therefore, reversible protein phosphorylation is capable of regulating the properties of proteins rapidly and thus allows for quick responses to external stimuli (Kennelly 2003).

Protein phosphorylation was originally discovered in the 1950s by Krebs and Fischer during their investigation of the rabbit skeletal muscle (Krebs and Fischer 1956). They demonstrated that phosphorylase B is converted to phosphorylase A via autophosphorylation in the presence of [γ-32P]ATP. It took 22 years until PK activity was demonstrated in the Gram-negative bacteria Salmonella typhimurium (Wang and Koshland 1978) and Escherichia coli (Manai and Cozzone 1979). One year later, the identification of the first prokaryotic target protein, isocitrate dehydrogenase, was reported in E. coli (Garnak and Reeves 1979). Only a short time later protein phosphorylation was reported in the Archaea, i.e. the Euryarchaeon Halobacterium salinarum (Spudich and Stoeckenius 1980), leading to the discovery of the first
Table 1. Overview of some important PTM in all three domains of life.

<table>
<thead>
<tr>
<th>PTM</th>
<th>Archaea</th>
<th>Bacteria</th>
<th>Eukarya</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>His/Asp, TCS</td>
<td>Yes (not Crenarchaeota)</td>
<td>Yes</td>
<td>Yes</td>
<td>Ashby (2006); Kobir et al. (2011); Pereira, Goss and Dworkin (2011); Shi et al. (2014); Hanks and Hunter (1995); Kennelly (2014)</td>
</tr>
<tr>
<td>Ser/Thr</td>
<td>Yes (Hanks type)</td>
<td>Yes (Hanks type)</td>
<td>Yes (Hanks type)</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>Yes (Hanks type)</td>
<td>Yes (Walker type)</td>
<td>Yes (Hanks type)</td>
<td></td>
</tr>
<tr>
<td>Glycosylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-linked</td>
<td>Yes (dolichyl mono- or pyrophosphate carrier)</td>
<td>Yes (only in some δ- and ε-proteobacteria, undecaprenyl pyrophosphate carrier)</td>
<td>Yes (dolichyl pyrophosphate carrier)</td>
<td>Dell et al. (2010); Nothaft and Szymanski (2010); Jarrell et al. (2014); Schwarz and Aebi (2011)</td>
</tr>
<tr>
<td>O-linked</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Acetylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-terminal</td>
<td>Yes (rare in methanogens)</td>
<td>Yes</td>
<td>Yes</td>
<td>Soppa (2010); Ouidir, Kentache and Hardouin (2016)</td>
</tr>
<tr>
<td>Protein internal (Lys ε-amino group)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Bedford and Richard (2005); Lanouette et al. (2014)</td>
</tr>
<tr>
<td>Methylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small protein modifications</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Maupin-Furlow (2011, 2013a,b, 2014); Striebel et al. (2014); Anjum et al. (2015); Jüdes et al. (2015)</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Pupylation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>SAMPylation</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Urymylation</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Selected references about the respective PTM are listed in the table, more general reviews related to PTMs in the three domains of life are as follows: Cain, Solis and Cordwell (2014); Eichler and Adams (2005); Maupin-Furlow (2013b); Walsh, Garneau-Tsodikova and Gatto (2005); Mann and Jensen (2003).
and Armitage 2011; Burnside and Rajagopal 2012; Capra and Laub 2012; Shi et al. 2014; Dworkin 2015). Interestingly, comparative genome analyses were unable to identify any His kinases and response regulators (RRs) within the Crenarchaeota and Nanoarchaeota, whereas several homologs were found in the Euryarchaeota (Eichler and Adams 2005; Asby 2006; Galperin et al. 2006, 2010).

In this review, we summarize the current knowledge of protein phosphorylation in Archaea, including the findings from phosphoproteome studies, the description of characterized bacterial-type TCS and Hanks-type PKs (also named eukaryotic-like PKs, ePKs) and PPs. We also present the results of genetic approaches that enabled the analysis of the first archaeal signal transduction cascades.

PHOSPHOPROTEINS AND PHOSPHOPROTEOME STUDIES IN ARCHAEA

Despite phosphoproteins being reported in Archaea already in the 1980s (Spudich and Stoeckenius 1980; Skorok 1984, 1989) and the identification of several phosphoproteins later on (for summary, see Table 2), it took until the 2000s for the first phosphoproteome studies to be carried out in the Archaea. The first genome-wide approach was performed in a Halobacterium salinarum (strain R1) wild-type (WT) and serine/threonine phosphatase (serB) deletion strain (Aivaliotis et al. 2009) using TiO2 enrichment and liquid chromatography/mass spectroscopy. In total, 90 unique phosphopeptides from 69 H. salinarum proteins were identified and 81 phosphorylation sites were determined with a Ser/Thr/Tyr ratio of 86/12/1%. In accordance with the deletion of the serB gene, a 3-fold increase in serine phosphorylation in comparison to WT was observed.

Later on, a precursor acquisition independent of ion count (PACIFIC) approach was used to analyze the phosphoproteins in Sulfolobus solfataricus cells grown on either D-glucose or tryptone (Esser et al. 2012). In this study, a total of 1318 phosphorylation sites located on 690 phosphopeptides from 540 unique proteins were identified. Notably, a high preference on tyrosine phosphorylation was detected with a Ser/Thr/Tyr ratio of 26/21/54%. The identified phosphoproteins belong to almost all functional classes (21 out of 26 archaeal Clusters of Orthologous Genes (arCOGs)) supporting an essential role of protein phosphorylation in most cellular processes in S. solfataricus. The study focused on changes of phosphorylation patterns in the central carbohydrate metabolism in response to the offered carbon source. It revealed a significant role of protein phosphorylation in the control of central carbohydrate metabolism and channeling of the carbon flux in different metabolic pathways.

A second PACIFIC study in S. acidocaldarius enabled further insights into the importance of protein phosphorylation in Archaea (Reimann et al. 2013). In this study, the in vitro and in vivo functions of the only two phosphatases, Saci-PTP and Saci-PP2A, were analyzed by biochemical characterization as well as genetic and polyomics approaches. In S. acidocaldarius (parental strain, MW001) as well as the two HP deletion strains in total 801 unique phosphoproteins (1206 phospho-peptides) were identified again with an unusually high number of phosphorylated Tyr residues (pSer/pThr/pTyr % ratio of 35.6/28.1/36.2). Like in the previous study performed in S. solfataricus, phosphoproteins were identified in almost all arCOGs and 18 transcriptional regulators were found to be phosphorylated, among others ArnR1 the positive regulator of the archaellum operon (see below) as well as 5 of the predicted serine/threonine PKs of S. acidocaldarius (Reimann et al. 2013).

Therefore, as shown within Bacteria and Eukaryotes, a major number of proteins are phosphorylated within Archaea. This highlights the role for reversible protein phosphorylation in this domain of life. The broad distribution in almost all arCOG categories underlines the global regulatory function of protein phosphorylation. Unusually, a high number of tyrosine phosphorylation was identified in Sulfolobus sp. This feature has not been reported for hyperthermophilic Bacteria (i.e. Thermus thermophilus; Takahata et al. 2012) or the mesophilic Euryarchaeon H. salinarum strain R1 (Aivaliotis et al. 2009). Notably the two latter studies used TiO2 enrichment strategies and in both organisms bacterial-type TCS are present in addition to phosphorylation on Ser, Thr and Tyr.

HIS AND ASP PHOSPHORYLATION (TCSs) IN EURYARCHAEOTA

Phosphorylation on His and Asp residues is found in a specific type of regulatory system, the TCS. These systems are present in all three domains of life (Koretke et al. 2000). The classical TCS consists of a His sensor kinase (HisK) and a response regulator (RR). The HisK is usually membrane bound and consists of two domains, a sensor input domain (extracellular) and a His kinase, transmitter domain (intracellular), whereas the RR is usually a cytoplasmic protein. The sensor input domain is stimulated via an environmental signal (e.g. a small molecule ligand), which leads to the activation of the HisK domain. Within the HisK domain, a specific His residue is then autophosphorylated and the phosphoryl group is subsequently transferred to an Asp residue of the RR receiver domain. The phosphorylation of the RR leads to the activation of its output domain, which triggers the corresponding cellular response, i.e. phosphorylation of the target protein (Fig. 1). TCSs were originally discovered in Bacteria by several independent studies in 1985 and 1 year later the name ‘two-component regulatory system’ was introduced by Nixon, Ronson and Ausubel (1986). Today, it is known that TCSs also exist in the genomes of Euryarchaeota and in certain non-animal Eukaryotes (e.g. fungi, plants) (Alex and Simon 1994; Loomis, Shaulesky and Wang 1997; Lohrmann and Harter 2002; Schaller, Shiu and Armitage 2011). As mentioned above, the exception within the Archaea are the Nanoarchaeota and Crenarchaeota (Ashby 2006; Galperin 2006, 2010). Both phyla comprise only (hyper)thermophilic microorganisms and possess no TCSs.

Apart from these classical two-step phosphorylay TCSs, hybrid kinases (four-step phosphorylay) represent a common modification in Prokaryotes and Eukaryotes. In hybrid kinases, a receiver domain of a RR is directly fused to the sensor kinase (HisK) and therefore autophosphorylation and phosphotransfer occur within the same protein. Subsequently, the phosphoryl group is further transferred via a histidine phosphotransferase to the receiver domain of a second RR (Fig. 1). However, to date, there are no phosphorylays studied in the Archaea. Therefore, we will not discuss these further in this review.

Furthermore, there is a third system, the one-component system (OCS). The detailed analysis of 145 prokaryotic genomes by Ulrich, Koonin and Zhiulin (2005) revealed that OCSs (~17 000) are more abundant in the investigated genomes than TCSs (~4000). In OCSs, the sensor input domain is directly fused to the output domain in a single protein. They lack the histidine kinase domain of the HisK and the receiver domain of the RR and thus no protein modification is involved in OCSs. Typical examples are transcriptional regulators that contain a ligand-binding domain and DNA-binding helix-turn-helix (HTH) domain. Analysis of transmembrane regions in this study predicted that OCSs are
<table>
<thead>
<tr>
<th>Accession Nr.</th>
<th>Target protein</th>
<th>Predicted function</th>
<th>Organism</th>
<th>Information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q973R9</td>
<td>ST0829</td>
<td>FHA domain-containing protein</td>
<td>Sulfolobus tokodaii</td>
<td>Phosphorylated by ST1565</td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td>Q76KA7</td>
<td>TK0925</td>
<td>Methyltransferase activating protein</td>
<td>Methanosarcina barkeri</td>
<td>Autophosphorylation with $^{32}$P</td>
<td>Daas et al. (1996)</td>
</tr>
<tr>
<td>Q5L340</td>
<td>TK1400</td>
<td>Phosphomannomutase</td>
<td>Thermococcus kodakarenensis</td>
<td>Phosphorylated on Ser residue</td>
<td>Solow et al. (1998); Ray et al. (2005)</td>
</tr>
<tr>
<td>Q980S1</td>
<td>SSO0207</td>
<td>Hexosephosphate mutase</td>
<td>Sulfolobus solfataricus</td>
<td>Phosphorylated on Ser residue</td>
<td>De Felice et al. (2006)</td>
</tr>
<tr>
<td>D4GY21</td>
<td>HVO1562</td>
<td>$\beta$-Subunit of 205 proteasome</td>
<td>Haloferax volcanii</td>
<td>Phosphorylated with $\gamma$-$^{32}$P ATP and Mg$^{2+}$</td>
<td>Rudolph and Oesterhelt (1996)</td>
</tr>
<tr>
<td>B0R4J9</td>
<td>CheA</td>
<td>Taxis sensor His kinase cheA</td>
<td>Halobacterium salinarum</td>
<td>Phosphorylated with $\gamma$-$^{32}$P ATP and Mg$^{2+}$</td>
<td>Rudolph and Oesterhelt (1996)</td>
</tr>
<tr>
<td>B0R4K1</td>
<td>CheY</td>
<td>Response regulator CheY</td>
<td>Halobacterium salinarum</td>
<td>Phosphorylated on Ser residue</td>
<td>Rudolph and Oesterhelt (1996)</td>
</tr>
<tr>
<td>D9PYR8</td>
<td>MTH1412</td>
<td>Cell division control protein 6 homolog 1</td>
<td>Methanobacterium thermautotrophicum</td>
<td>Autophosphorylation with $^{32}$P</td>
<td>Grabowski and Kelman (2001)</td>
</tr>
<tr>
<td>D9PU97</td>
<td>MTH1599</td>
<td>Cell division control protein 6 homolog 2</td>
<td>Methanobacterium thermautotrophicum</td>
<td>Autophosphorylation with $^{32}$P</td>
<td>Grabowski and Kelman (2001)</td>
</tr>
<tr>
<td>Q8ZYK1</td>
<td>PA0737</td>
<td>Cell division control protein 6</td>
<td>Pyrococcus aerophilum</td>
<td>Phosphorylated on Ser residue</td>
<td>Grabowski and Kelman (2001)</td>
</tr>
<tr>
<td>O8S655</td>
<td>PH0961</td>
<td>Translation initiation factor 2 subunit $\alpha$</td>
<td>Pyrococcus horikoshii</td>
<td>Phosphorylated by hPKR from human</td>
<td>Tahara et al. (2004)</td>
</tr>
<tr>
<td>P9S28</td>
<td>SSO2154</td>
<td>Zn-dependent aminopeptidase</td>
<td>Sulfolobus solfataricus</td>
<td>Phosphorylated on Ser or Thr residue</td>
<td>Condo et al. (1998)</td>
</tr>
<tr>
<td>Q97U27</td>
<td>SSO3198</td>
<td>$\alpha$-Subunit of succinyl-CoA synthetase</td>
<td>Sulfolobus acidocaldarius</td>
<td>$^{32}$P incorporation</td>
<td>Ray et al. (2005)</td>
</tr>
<tr>
<td>Q9BOA0</td>
<td>SSO0417</td>
<td>Phosphoglycerate mutase</td>
<td>Sulfolobus solfataricus</td>
<td>Phosphorylation at Ser$^{59}$</td>
<td>Potters et al. (2003)</td>
</tr>
<tr>
<td>Q9HVL3</td>
<td>TA0122</td>
<td>2-Keto-3-deoxy-glucuronic kinase</td>
<td>Thermoplasma acidophilum</td>
<td>No enzyme activity after incubation with phosphatase</td>
<td>Jung and Lee (2005)</td>
</tr>
<tr>
<td>Q4LH3</td>
<td>ArnB</td>
<td>Regulation of archaellum expression</td>
<td>Sulfolobus acidocaldarius</td>
<td>Phosphorylated in WT, saci,PF2A and sacLPTP deletion mutant, Phosphorylated by two ePKs (SaciL1193 and SacL1694)</td>
<td>Reimann et al. (2012, 2013)</td>
</tr>
<tr>
<td>Q4L1L2</td>
<td>AmR1</td>
<td>Regulation of archaellum expression</td>
<td>Sulfolobus acidocaldarius</td>
<td>Phosphorylated in sacLPTP deletion mutant</td>
<td>Reimann et al. (2012, 2013)</td>
</tr>
</tbody>
</table>

(*) Possible phosphoenzyme intermediate.
cytosolic proteins (e.g. 97% of one-component regulators with HTH motif), whereas most of the sensor histidine kinases of TCSs (73%) were membrane bound. Thus, OCSs and TCSs were postulated to be involved in the detection of intracellular and extracellular signals, respectively (Ulrich, Koonin and Zhulin 2005). From an evolutionary point of view, it was suggested that OCSs are the predecessors of TCSs. This is based on three observations: (i) OCSs have a simpler design than TCSs, (ii) the domain architecture of one-component regulators is more versatile than that of TCSs and (iii) OCSs are more abundant in Prokaryotes compared to TCSs. Further on it was proposed that the last common ancestor of Archaea and Bacteria possessed OCSs, but lacked TCSs and that TCSs are an invention of Bacteria. The invention of TCSs occurred due to the insertion of the HisK domain and the receiver domain into OCSs (Ulrich, Koonin and Zhulin 2005). This theory would fit with the assumption that TCSs in Archaea were acquired via horizontal gene transfer from the Bacteria (Korete et al. 2000).

Only a year after, the study by Ulrich, Koonin and Zhulin (2005), Galperin (2006, 2010) and Ashby (2006) analyzed the distribution, structure and diversity of genes encoding RRs and TCSs in Archaea, respectively. Galperin analyzed 4610 RRs encoded in 200 bacterial and archaeal genomes, and performed a detailed classification according to their domain structure. The study led to the identification of new output domains, which were, in some cases, assigned to established...
protein domain families. Ashby analyzed 23 completely annotated and 3 partially annotated archaeal genomes (21 euryarchaeal and 2 crenarchaeal genomes) via BLASTP and gene category lists (Ashby 2006). This approach led to the identification of 489 putative TCS genes in 14 euryarchaeal genomes and none in crenarchaeal or nanoarchaeal genomes. Furthermore, no TCS genes were identified in the available Thermoplasmatales genomes (Thermoplasmata acidophilum, Tpl. volcanium and Picrophilus torridus), Methanococcoides jannaschii, Methanopyrus kandleri and Pyrococcus furiosus all representing thermocidophlic/hyperthermophilic Euryarchaeota. Only in four euryarchaeal hyperthermophiles (Archaeoglobus fulgidus DSM4304 (OGT 83 C), P. abyssi G5 (OGT 96 C), P. horikoshii OT3 (OGT 98 C), Thermococcus kodakarenosis (OGT 85 C, Ng et al. 2000)) and one thermophile (Methanothermobacter thermautotrophicus Delta H (OGT 65 C)), TCS genes were identified. Members of the Thermococcales (P. abyssi G5, P. horikoshii OT3, T. kodakarenosis) had only three TCS genes (0.146% of the genome), whereas A. fulgidus DSM4304 harbored 31 TCS genes (1.26% of genome) and the thermophilic archaean M. thermautotrophicus 23 TCS genes (1.2% of the genome).

The absence of RR and TCS in Crenarchaota and Nanoarchaota, as well as some members of the Euryarchaeota (e.g. Thermoplasmatales), is discussed in regard to their genome complexity, unique lifestyles (e.g. symbiotic/parasitic for Nanoarchaota) as well as their unchanging and unique habitat (e.g. thermocidophiles) (Galperin 2004, 2005; Ashby 2006). In general, it is proposed that a more complex life style and habitat requires increased genome complexity (number of encoded genes) with more complex regulation at the gene level ‘a higher IQ’, reflected by an increased number of regulators.

In addition, another fact that might play a role is the adaptation to life at high temperature. The P-His and P-Asp bound are relatively high-energy bonds and therefore the thermodynamic and kinetic properties might be affected by temperature. Studies of the chemotaxis TCSs in the hyperthermophilic bacterium Thermotoga maritima (growth optimum at 80 °C) revealed that the phosphorylation site of the RR CheY is only stable for 25 s at 50 °C (150 s at 25 °C) (Swanson, Sanna and Simon 1996). However, there are a few exceptions in Archaea, as outlined above, with A. fulgidus (Euryarchaeota, optimal growth temperature at 83 °C) and 31 TCS genes being the most impressive one. But so far none of the TCS in hyperthermophilic Archaea was analyzed.

Characterized TCSs in Archaea

Despite the fact that TCS genes have been identified in Euryarchaeota, their physiological role has not been studied in great detail (Bourret and Silversmith 2010). Studies so far only include TCS in halophilic (Halobacterium salinarium, Haloferax volcanii) or methanogenic (Methanoseta harundinacea, Methanosarcina bark eri) Archaea. Alam and Oesterhelt (1984) observed that H. salinarum displayed chemotaxis. This process involved stochastic switching of the motility structure, the archaellum (formerly archaelflagellum); Jarrell and Albers 2012; Albers and Jarrell 2015), but the proteins involved in this process were not known. However, it was assumed that homologs of the bacterial chemotactic signaling cascades such as CheA and CheY might be involved. Indeed, almost 10 years later Rudolph and Oesterhelt (1995) identified CheA, which is the HisK of the H. salinarum chemotactic system. A cheA deletion strain lost its chemotactic behavior, as reported for deletion strains of Escherichia coli and Bacillus subtilis (Oosawa, Mutoh and Simon 1988; Fuhrer and Ordal 1991). In the same year, Rudolph et al. (1995) also reported that the recombinant proteins CheA and CheY are active and form a TCS in H. salinarium. CheA was shown to autophosphorylate in presence of Mg2+ and [γ-32P]ATP.

Since the experiments carried out by the Oesterhelt group, it has become clear that the archaeal motility structure is in contrast to the bacterial flagellum related to type IV pili and therefore its motor and protein composition is totally different (Jarrell and Albers 2012; Albers and Jarrell 2015). Therefore, it is surprising that the same chemotactic system is employed in Archaea and Bacteria to control the rotational direction of their motility structure. How this system has been adapted to achieve this will be topic of future studies.

TCS in Methanosarcinales—Recently, the regulation of methanogenesis by the Fill-FilRs TCS in M. harundinacea 6Ac was reported by Li et al. (2014). Before this study, they found that Fill (Mhar_0446) is responsible for the production of signaling molecules (carboxyl-acyl homoserine lactones) in M. harundinacea. It was demonstrated that carboxyl-acyl homoserine lactones control cell morphological transitions and influence the C-flux for CH4 production and biomass formation (Zhu et al. 2012). The M. harundinacea 6Ac genome possesses three HisKs (Mhar_0446 (Fill), Mhar_0936, Mhar_1766) with the HisKA and HATPase domains, but only Fill exhibits the HisK characteristic two transmembrane domains. In addition, five putative RRs were identified (Mhar_0169, Mhar_0445 (FilR1), Mhar_0447 (FilR2), Mhar_1520, Mhar_2042), but only FilR1 and FilR2 possess an REC domain. The genome organization of the three genes (fil, filR1, filR2) suggested that fill and filR2 form an operon, which was verified by RT-PCR. Phosphotransfer studies with Fill and both RRs confirmed that Fill phosphorylates FilR1 and FilR2; however, the addition of the carboxyl-acyl homoserine lactone had no influence on the activity of Fill. Furthermore, ChiP-PCR studies revealed that the RR FilR1 binds to its own as well as to the fill-filR2 promoter, which is common for RRs from bacterial TCSs. Even more interesting was the finding that FilR1 binds to promoters of several genes/operons, encoding proteins essential for methanogenesis (acs1 operon, acs4 gene, mtr operon, fudCABD operon and omp gene). This is the first study in Methanosarcinales indicating a positive regulation of methanogenesis by a TCS.

SER, THR AND TYR PHOSPHORYLATION IN ARCHAEA

Ser/Thr and Tyr phosphorylation in Eukaryotes and Archaea is carried out by specific eukaryotic PKs, called Hanks-type kinases. Today there are seven major clusters of Hanks-type PKs (also named ePKs) characterized. These are called the tyrosine kinase (TK) group; PK A, G and C families (AGC) group; the calcium and calmodulin-regulated PKs (CAMK) group; the tyrosine kinase-like (TKL) group; the cycline-dependent/mitogen-activated/glycogen synthase/cycline-dependent like PK (CMGC) group; homologs of yeast STE7, STE11 and STE20 PKs (STE) group; and the cell kinases (CK1) group (Manning et al. 2002; Taylor and Kornev 2011). All Hanks-type kinases share a conserved catalytic domain containing 12 subdomains (Fig. 2A). The Mg2+-ATP molecule binds usually to the amino-terminal lobe (subdomain I-V) and the hinge region, whereas the substrate binds to the carboxy-terminal lobe (subdomain V-XI). The most important residues for catalytic function are as follows: the Lys (K) residue in subdomain II; the Asp (D) in subdomain VII, which is involved in the orientation and anchoring of the ATP; and the invariant Asp (D) in the subdomain VIB, which is likely to be the
Figure 2. Conserved subdomains of Hanks-type PKs and alignment of Sulfolobus spp. Hanks-type PKs showing the 12 conserved subdomains. (A) The subdomains of Hanks-type Ser/Thr and Tyr kinases are represented with gray boxes and labeled with roman numbers. The boxes contain conserved amino acids important for function of the kinase domain. Locations of special regions within the domain that are of specific importance are labeled (e.g. catalytic loop) in black. (B) Alignment of Hanks-type kinases (eSTKs) of *S. soflataricus*, *S. acidocaldarius* and *S. tokodaii*. Highlighted in blue and labeled with roman numbers are the conserved Hanks-type PK subdomains. The consensus sequence of the motifs is depicted above the respective box. Subdomains without labeling have no conserved consensus sequence and seem to contribute mainly in supporting the structure of the kinase domain. Information about the location of these subdomains as well as important functional residues was obtained by comparing the available data for SSO3207 (Ray et al. 2015), modeled on the structural model of ArnC (Saci1193) and ArnD (Saci1694) as well as available data on the localization of secondary structure elements (Hanks and Hunter 1995; Kannan et al. 2007; Wang et al. 2010; Ray et al. 2015). Residues labeled in red and green have been identified in STK15650 by combined mutational and in vitro studies and revealed to be essential for function or to decrease kinase activity, respectively (Wang et al. 2010). These residues are conserved in Hanks-type kinases (eSTKs) in Sulfolobus spp. The alignment was generated with Clustal Omega, * conserved residues, : strong similarity between residues, . weak similarity between residues. The N-terminal part of all sequences was trimmed and only the kinase domain is shown in the alignment.
catalytic base involved in the phosphotransfer reaction (Hanks
2003) (Fig. 2A).

Interestingly, in Bacteria, Hanks-type Ser/Thr PKs (eSTKs) are omnipresent, whereas Hanks-type Tyr PKs are rare and mainly members of the bacterial protein-tyrosine kinases (BY) family are found. The BY-kinases represent either single membrane proteins with a membrane and cytoplasmic part or are split into two proteins. The cytoplasmic part forms the catalytic site, which is characterized by three Walker A motifs (A, A’ and B) usually found in ATPases and GTPases (Grangeasse, Nessler and Mijakovic 2012; Shi et al. 2014; Dworkin 2015). In addition to the two separate ways of phosphorylation (His/Asp and Ser/Thr/Tyr), more and more evidence for cross-reactions between eSTKs/ BY-kinases and transcriptional regulators emerges in Bacteria. Here, the eSTKs and BY-kinases phosphorylate transcription factors and RRs of TCS, which changes e.g. their DNA-binding behavior (Fig. 1) (Wright and Ulijasz 2014; Kalantari et al. 2015). Today, there are several different examples of Bacteria in which these cross-reactions were observed but no such interaction was described in Archaea, yet.

A detailed classification of ePKs in Archaea has not been performed so far. However, in 2014, it was shown that many Archaea contain at least one ePK and usually two phosphatases in their genome (Kennelly 2014). The most detailed studies regarding the presence of ePKs were performed with species of the Sulfolobales and so far only eSTKs were characterized (see Table 3). Blast searches (BlastP) with typical BY-kinases (PtKa, Bacillus subtilis and Wzc, Escherichia coli) reveal no obvious homologs in Archaea (Shi et al. 2014). Therefore, the PKs involved in Tyr phosphorylation remain to be elucidated in Archaea. As Crenarchaeota possess no TCSs, they solely rely on eSTKs for signal transduction and so they possess several of these kinases. For example, Sulfolobus solfataricus encodes 8 eSTKs and for S. acidocaldarius 11 are predicted based on arCOG functional annotation (Kennelly 2003; Esser et al. 2011). In contrast, euryarchaeal species were reported to have less eSTKs, but encode a variety of TCS (Ponting et al. 1999; Kennelly 2003).

eSTKs in Archaea

The only study concerning Ser, Thr and Tyr protein phosphorylation in methanogens was performed in Methanosarcina barkeri DSM 800 (Daas et al. 1996). The methyltransferase activation protein (MAP) was purified from the crude extract of M. barkeri and autophosphorylation was demonstrated in the presence of [γ-32P]ATP. The phosphate bound was stable under acidic conditions, suggesting that the phosphorylation site is an O-phosphate linkage (Ser, Thr, Tyr residues). Incubation of the p-MAP with the corrinoid-containing methanol-5-hydroxybenzimidazolocobamide methyltransferase (MT1), resulted in activation of MT1. The authors suggest that either p-MAP functions like a PK and phosphorylates MT1, leading to an activated p-MT1; or p-MAP does not covalently change MT1, but affects the MT1 structure and functions similarly to a chaperone. Since then, no studies regarding MAP from M. barkeri have been published, thus this question remains unsolved.

Most of the information available on archaean eSTKs stems from studies in Sulfolobus spp. (see Table 3). Fifteen years ago, the Kennelly group started to characterize the PKs from the crenarchaeal model organism S. solfataricus (see Table 3). Since then, four ePKs and one non-canonical Hanks-type PK (atypical PK, aPK) were analyzed, applying various in vitro techniques in order to determine characteristics of the kinases, such as autophosphorylation behavior, specificity and ion preference (Table 1; for recent review, see Kennelly 2014). All of these ePKs performed phosphorylation on Ser/Thr (eSTKs), while preferring Mn2+ as ion for phosphorylation activity on non-native substrates such as casein, lysozyme or bovine serum albumin (BSA). Interestingly, SSO3184 (Sso-PK4) was proposed to be involved in the phosphorylation of the translation factor eEF2a (Ray et al. 2015). However, to date, information concerning the physiological role of these kinases in S. solfataricus remains scarce. Sulfolobus acidocaldarius, a closely related organism, contains 11 predicted PKs, of which at least 5 contain most or all of the conserved subdomains of eSTKs (compare Fig. 2B). Even though there is biochemical information on some kinases of S. solfataricus available and both organisms belong to the Sulfolobales, the information cannot be directly transferred between these two organisms because the homology between the kinases is often low and only the kinase domain is conserved but not the rest of the protein.

eSTKs in Sulfolobus tokodaii

Based on in silico analysis, eight putative eSTKs were identified in S. tokodaii strain 7 (STK_00364, STK_00686, STK_00778, STK_00810, STK_00899, STK_1565, STK_19960 and STK_24400) (Wang et al. 2010). All of them contained the catalytic loop (DVKPSN), the DFG motif and the conserved residues K166, D287 and D314 known from classical eukaryotic homologs (see Fig. 2B). The putative ePK STK_15650 was chosen for detailed characterization. In addition, Wang and coworkers performed a detailed analysis of forkhead-associated (FHA) domain-containing proteins in S. tokodaii. These proteins are known to be closely linked to pathways involving protein phosphorylation, since the FHA domain can recognize p-Thr epitopes on proteins. They are usually found in Eukaryotes as well as Bacteria and play an important role in the phosphorylation-dependent assembly of protein complexes (Durocher and Jackson 2002). Specific interaction of the kinase STK_15650 and the FHA domain-containing STK_00829 was demonstrated in vitro analysis. The biochemical investigation of STK_15650 revealed that this PK is specific for Mn2+ as cofactor, and mutational studies revealed that the residues K166, D287, D314 and T329 (Fig. 2B, red labeled amino acids) are essential for the activity, resulting in an inactive enzyme when changed to A. The amino acids are located in subdomain II (invariant lysine), Vβ (catalytic loop), VII (DFG motif) and finally, T329 is located inside the activation loop. Kinases often require phosphorylation of a specific residue within the activation loop in order to be active (Taylor and Radzio-Andzelm 1994; Nolen, Taylor and Ghosh 2004; Lochhead 2009). Interestingly, this residue is conserved in all other kinases presented in our alignment (Fig. 2), which suggests a similar mechanism of activation for all these kinases of S. acidocaldarius and S. solfataricus. However, there is currently no information available which proves this point from either one of these organisms. In contrast, the mutation of T326 to A in STK_15650 stimulated the PK activity, suggesting that T326 plays an important regulatory role in this ePK (Fig. 1, green labeled residue). This threonine is again conserved in two other kinases of S. acidocaldarius (SacI_1041 and SacI_1181), which implies that a similar mechanism for regulation like in S. tokodaii is present. However, this has not yet been studied.

STK_15650 was shown to phosphorylate the FHA domain-containing STK_00829, and the mutations of STK_15650 had the same effect on the phosphorylation of STK_00829 as on the autophosphorylation. Interestingly, Mg2+ had a positive effect on the phosphorylation of STK_00829. This was not observed for the autophosphorylation of the eSTK (STK_15650). To
### Table 3. Characterized ePKs and aPKs from *Sulfolobus* spp.

<table>
<thead>
<tr>
<th>ORF Name</th>
<th>Membrane bound</th>
<th>Phosphorylated amino acid on substrate</th>
<th>Substrate</th>
<th>Preferred ion</th>
<th>Inhibitors</th>
<th>Additional information</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sso2291</td>
<td>Sso-PK1</td>
<td>Yes, 3 N-terminal TMDs</td>
<td>Ser/Thr</td>
<td>Casein, RCM-lysozyme (RCML), mixed histones/histone H4, specific peptide</td>
<td>Mg$^{2+}$ (at 25°C) and Mn$^{2+}$ (at 65°C)</td>
<td>PKI peptide, H7, ML-9</td>
<td>GTP, ADP and GDP could be used as cosubstrates, protein is glycosylated</td>
</tr>
<tr>
<td>Sso0433</td>
<td>Sso-PK5</td>
<td>No</td>
<td>Ser</td>
<td>p53, casein, RCML</td>
<td>Mn$^{2+}$</td>
<td>Not tested</td>
<td>aPK, homolog of piD261/Bud32 kinase family, is activated by DNA, ADP-ribose and 5′-AMP</td>
</tr>
<tr>
<td>Sso0469</td>
<td>Sso-PK3</td>
<td>No</td>
<td>Ser (only casein on Ser/Thr)</td>
<td>Casein, BSA, Myelin basic protein (MBP), RCML</td>
<td>Mn$^{2+}$</td>
<td>Tamoxifen</td>
<td>No autophosphorylation detected</td>
</tr>
<tr>
<td>Sso3182</td>
<td>Sso-PK4</td>
<td>Yes, 6 N-terminal TMDs</td>
<td>Ser/Thr</td>
<td>Casein, RCML, MBP, histones</td>
<td>Mn$^{2+}$</td>
<td>3′,5′-cAMP</td>
<td>Proposed homolog of archaeal eIF2α kinase, increased catalytic efficiency in presence of oxidized CoA</td>
</tr>
<tr>
<td>STK15650</td>
<td></td>
<td>No</td>
<td>Not tested</td>
<td>STK0829</td>
<td>Mn$^{2+}$</td>
<td>Not tested</td>
<td>Interacts with and phosphorylates ST0829 in vitro</td>
</tr>
<tr>
<td>Saci1193</td>
<td>AmC</td>
<td>No</td>
<td>Ser/Thr</td>
<td>ArnA, ArnB</td>
<td>Mn$^{2+}$</td>
<td>Not tested</td>
<td>Phosphorylates two negative regulators of motility in <em>S. acidocaldarius</em>, ArnA and ArnB, in vitro</td>
</tr>
<tr>
<td>Saci1694</td>
<td>ArnD</td>
<td>No</td>
<td>Ser/Thr</td>
<td>ArnB</td>
<td>Mn$^{2+}$</td>
<td>Not tested</td>
<td>Phosphorylates only ArnB in vitro</td>
</tr>
</tbody>
</table>
conclude which residues in STK_00829 are important for interaction with the kinase STK_15650, bacterial two-hybrid experiments were performed with point mutations (R276, S278, T290 or N200 changed to A), revealing that all four residues seem to be essential for the interaction of both proteins. Subsequently, it was proposed that the FHA domain-containing protein bound in a phosphorylation-dependent manner to the flaX promoter (Duan and He 2011), which codes for a structural part of the archaellum (archaeal flagellum) assembly apparatus. However, in vivo confirmation has not been reported so far.

**Signal transduction by eSTKs in Sulfolobus acidocaldarius: ‘The archaellum regulatory network’**

The findings obtained from in vitro studies in S. tokodaii were the first results reported on an archaeal signal transduction cascade involving eSTKs. However, the physiological function of this cascade was not elucidated until Reimann et al. (2012) showed that in *S. acidocaldarius* a regulatory system consisting of the homolog of ST0829, ArnA and various other proteins regulates expression of the motility structure, the archaellum. In *S. acidocaldarius*, an operon consisting of seven genes, which are expressed from two promoters encodes for the archaellum (Fig. 3) (Lassak et al. 2012). One promoter is located upstream of the archaellum encoding gene flaB, which is the filament protein of the archaellum. The second one is located upstream of the accessory protein flaX and has low constitutive activity. The main promoter for expression is the one upstream of flaB that was shown to be induced by starvation (Lassak et al. 2012). The expression of the archaellum has to be tightly regulated. Regulation is achieved on the transcriptional and posttranslational level by the archaellum regulatory network. Reimann et al. also showed that two regulatory proteins, ArnA and ArnB, as well as two kinases and one phosphatase are part of the complex archaellum regulatory network. ArnA and ArnB are located in an operon. ArnA contains a zinc-finger (ZnF) and FHA domain, while ArnB possesses a von Willebrand type A domain (vWA). Deletion of either one of the two regulators showed a hypermotile phenotype in vivo and protein levels of the archaellum FlaB were strongly enhanced compared to the WT strain (Reimann et al. 2012). Moreover, ArnA and ArnB interact tightly with each other in a phosphorylation-dependent manner. Here, the highly phosphorylated, flexible C-terminal part of ArnB interacts with the FHA domain of ArnA. Furthermore, ArnB binds to the flaB promoter forming a repression module with ArnA on the DNA (Hoffmann et al. in preparation). In addition to the negative regulators, there are two proteins flanking the archaellum operon, ArnR and ArnR1, identified as activators of archaellum expression (Lassak et al. 2013) (Fig. 3A). Both proteins are membrane-bound transcription factors and therefore considered to represent OCs. They fulfill their function by binding to a specific target region upstream of the flaB promoter under starvation conditions. Even though both proteins consist of the same domains, they are only highly homologous with respect to their DNA-binding region (HTH domain), but differ from each other in the sensory region (HAMP domain and sensory domain close to the membrane anchor). This observation led to the conclusion that ArnR/R1 might be able to sense different signals. Interestingly, only *S. acidocaldarius* has two homologs, ArnR and ArnR1, in contrast to other Sulfolobales, which only encode ArnR (Lassak et al. 2013).

Furthermore, the archaellum regulatory network includes at least two eSTKS, ArnC and ArnD. Both kinases belong to the eSTKs and are able to phosphorylate the negative regulators ArnA and ArnB in vitro. While the kinase ArnC is able to phosphorylate both ArnA and B, ArnD only phosphorylates ArnB (Reimann et al. 2012). Deletion studies of the kinases revealed that they have a different effect; while the ArnC deletion resulted in reduced motility compared to the wt, ArnD-deficient strains showed a hypermotile phenotype (for examples, see Fig. 3B). As a consequence, the assumption is that they fulfill different roles in the archaellum regulatory network. Since both kinases can phosphorylate the negative regulators of archaellum expression, it is assumed that they act on the repressor module (ArnA/B) (Hoffmann et al. submitted).

Finally, yet importantly, the phosphatase Saci_PP2A is another factor in this regulatory cascade. Reimann et al. (2013) showed that deletion of the respective gene resulted in a hypermotile phenotype in vivo (see also below Saci phosphatases). Furthermore, transcriptome analysis as well as qRT-PCR and western blots revealed that a *Δsaci pp2A* strain mimicked the starvation phenotype of *S. acidocaldarius*. Components of the archaellum were strongly expressed and high FlaB protein levels could be detected in the deletion strain, as is usually found under starvation conditions (Reimann et al. 2013). Interestingly, the archaellum regulators ArnR1 and ArnB were also observed to be phosphorylated in vivo in a phospho-proteome study performed with the *saci pp2A* and *saci ptp* deletion mutants.

To date, the archaellum regulatory network represents the first archaeal signal transduction cascade involving eSTKs and PP investigated so far, including regulation at the transcriptional as well as post-translational level.

**NON-CANONICAL HANKS-TYPE PKs (aPKs)**

Apart from ePKs the non-canonical Hanks-type PKs (aPKs) of the ABC1, right open reading frame (RIO), pid261, AQ578 and Pkn2 families were introduced by Leonard, Aravind and Koonin (1998). Their bioinformatics study, based on 12 bacterial, 4 archaean and 2 eukaryotic genomes, revealed that the aPKs are distant members of the ePK superfamily and that with exception of the Pkn2 family, all aPK families are found within the Archaea. Based on the structural comparison, it was evident that aPKs comprise the typical ePK subdomains I, II, VIb and VII with the conserved residues (Fig. 4). Furthermore, the DFG triplet, which chelates Mg2+/Mn2+ ions, is well conserved in the identified pid261/Bud32 and ABC1 members, whereas this triplet differs in the AQ578 and RIO family members. However, the Asp residue in the DFG triplet is conserved in all of them. Interestingly, subdomain VIII (APE consensus sequence), which is usually phosphorylated in ePKs and important for ePK activation, is absent in aPK members. Nevertheless, for the Saccharomyces cerevisiae pid261/Bud32 homolog (YGR262C), which lacks the APE motif, activity was demonstrated suggesting that this motif is not strictly essential for catalytic activity of aPKs (Stocchetto et al. 1997).

**Archaeal pid261/Bud32 family**

In yeast, pid261/Bud32 is, together with the kinase-associated endopeptidase 1 (Kae1), part of the KEOPS (kinase, peptidase and other proteins of small size) protein complex also known under the name EKC (endopeptidase-like and kinase associated to transcribed chromatin) protein complex that is essential for telomere elongation and transcription of essential genes. This complex is composed of four or five subunits (i.e. Pcc1p, Gon7p (unique to fungi), Cgt121p, pid261/Bud32 and Kae1) (Downey...
The current view of the archaellum regulatory network. (A) The archaellum locus flaBXGFHIJ encodes the archaellum, a rotating, type IV pilus-like structure which functions as the motility structure of *S. acidocaldarius*. The encoding genes and the respective proteins in the archaellum are depicted in the same colors. The locus is under the control of two promoters, the main flaB promoter and a second weak flaX promoter (transcription start sites are indicated by arrows). The locus is flanked by the genes encoding the one-component membrane-bound transcription factors ArnR and ArnR1, as well as Saci\_1179 (protein of unknown function). In addition to ArnR and ArnR1, the biofilm regulator AbfR1 (Saci\_0446) has been identified as positive regulator of the flaB promoter (depicted by (+) arrow) (Lassak et al. 2013; Orell et al. 2013). On the other hand, the two repressors, ArnA (Saci\_1210) and ArnB (Saci\_1211), are negatively regulating flaB expression (depicted by (–) arrows). In addition, two eSTKs, ArnC and ArnD, can phosphorylate the repressors ArnA and ArnB. Here, ArnD seems to be involved in repression, too, since its deletion results in hypermotility. Furthermore, the phosphatase PP2A seems to play an important function in the negative control of the motility operon, as well, since a pp2a deletion mutant exhibits a hypermotile phenotype, too. However, the specific target of the PP2A that relays the signal is so far unknown (adjusted from Albers and Jarrell 2015).

(B) Example of swimming motility assays of the *S. acidocaldarius* MW001 (parental strain), and the \(\Delta\)arnB as well as \(\Delta\)arnR/\(\Delta\)arnR1 deletion mutants. The deletion of the negative regulator ArnB results in a hypermotile phenotype and of the positive regulators ArnR and ArnR1 in a non-motile phenotype.

Strikingly, whereas Kae1 is found in all three domains of life, orthologs of the piD261/Bud32 aPKs are only present in Eukaryotes and Archaea (Lopreiato et al. 2004). The diversity of analyzed archaeal piD261/Bud32 members is much greater than that of archaeal ePKs. So far three piD261/Bud32 aPKs from the Euryarchaeota (*Haloflax volcanii*, *Pyrococcus abyssi* and *Methanocaldococcus jannaschii*) and one enzyme from the Crenarchaeota (*Sulfolobus solfataricus*) were characterized (Hecker et al. 2008, 2009; Haile and Kennelly 2011; Naor et al. 2012). Interestingly, in nearly all archaeal genomes, the two genes are found in juxtaposition with each other and in some euryarchaeal
Figure 4. Amino acid sequence comparison of archaeal canonical Hanks-type PKs (eSTKs) and non-canonical Hanks-type PKs (aPKs) of the Rio1, Rio2 and piD261/Bud32 family. In the alignment, the eSTPKs from *S. acidocladarius*, *S. solfataricus* and *S. tokodaii* were included (as shown in Fig. 1), but for size reduction only ArnC (Saci 1193) is depicted. In addition, Rio1 and Rio2 kinases from *A. fulgidus* and *P. abyssi* as well as the piD261/Bud32 family member from *P. abyssi* were included. The conserved subdomains of Hanks-type Ser/Thr and Tyr kinases are indicated by yellow boxes and conserved residues are indicated above the sequence (as shown in Fig. 1). The alignment was generated with Clustal Omega, * conserved residues (black shading), : strong similarity between residues (gray shading), . weak similarity between residues.
piD261/Bud32 functions as P-loop ATPase rather than as bona fide PKs, at least in conjunction with Kae1 in the KEOPS/EKC complex or in species that form single fusion proteins such as M. jannaschii or Haloferax spp. (Perrochia et al. 2013). Whether the piD261/Bud32 aPK might function as PK if it is not bound to Kae1 remains to be elucidated. ATPase function is also proposed for Rio 2 kinases.

Archaeal RIO kinases

Archaeal RIO kinases have been the focus of several studies in the last few years. RIO kinases were originally identified in Archaea and Eukaryotes and a common origin with an ancestral RIO gene has been predicted (Leonard, Aravind and Koonin 1998). Until now, four different subfamilies of RIO kinases were identified: Rio 1, Rio 2, Rio 3 and Rio B kinases (LaRonde-LeBlanc and Wlodawer 2005). Phylogenetic analysis confirmed the distribution of RIO kinases in all three domains of life and proposed the presence of a combination of one Rio 1 kinase and one Rio 2 kinase in less complex species (i.e. Prokaryotes and single cellular Eukaryotes), whereas multicellular Eukaryotes including humans also possess Rio 3 kinases (Manning et al. 2002).

In general, RIO kinases are regarded as a trimmed version of canonical ePKs that lack the subdomains VIII, X and XI (involved in substrate binding) (LaRonde-Leblanc and Wlodawer 2004) (Fig. 6). RIO kinases possess an insertion of 18–23 amino acids between the αC and β3 (flexible loop), which is absent in ePKs. In general, Rio 1 and Rio 2 kinases are very similar regarding their overall fold, despite the N-terminal domain of the Rio 2 family comprising a winged HTH (wHTH) domain, which is absent in all other RIO families. Members of the different RIO families can be distinguished by their specific P-loop (interaction and orientation of the ATP triphosphate moiety) and DFG-loop (metal binding and positioning) sequence (LaRonde-LeBlanc et al. 2005a,b). In contrast to Prokaryotes, the role of RIO kinases in Eukaryotes is less clear and involves a major role in ribosome synthesis, which has been established (for recent review, see LaRonde 2014). While Rio1 fulfills an essential role in chromosome maintenance and cell cycle progression, both Rio kinases are involved in 18s rRNA progression. Therefore, deletion of either one of the two RIO kinases in yeast results in cell death (Vanrobays et al. 2001, 2003; Angermayr, Roidl and Bandlow 2002; Giaever et al. 2002; Geerlings et al. 2003; Schäfer et al. 2003). In humans, RIO kinase activity was shown to be essential in small ribosomal subunit (40S) biogenesis, and recent studies indicate that Rio1, 2 and 3 function as ATPase rather than PK in the maturation of the pre-40S particle (Kiburu et al. 2014; LaRonde 2014). The function of RIO B is still unclear.

A major breakthrough was achieved based on the crystallography of the archaeal Rio 1 and Rio 2 kinases from A. fulgidus (LaRonde-Leblanc and Wlodawer 2004; LaRonde-LeBlanc et al. 2005a). For both Rio 1 and Rio 2 kinase of A. fulgidus, autophosphorylation in the presence of labeled ATP was demonstrated and phosphorylation of common artificial kinase substrates was shown (LaRonde-Leblanc and Wlodawer 2004; LaRonde-LeBlanc et al. 2005a; Hecker et al. 2009). Unusually, in this case, the observation that Af-Rio2 was autophosphorylated in the absence of Me2+ ions and Ser128 was identified as the autophosphorylation site (LaRonde-Leblanc and Wlodawer 2004; Hecker et al. 2009).

Bioinformatic analysis based on genome neighborhood analysis and sequence comparisons revealed that in all archaeal sequenced genomes (121 in August 2012) members of the RIO family are present (Esser and Siebers 2013). All archaeal species analyzed contain at least one (Rio 1 or Rio 2 kinase) but typically two

Figure 5. Structural comparison of archaeal non-canonical Hanks-type PKs (aPKs) of the Rio1 and piD261/Bud32 family. The structure of the Rio1 kinase from A. fulgidus (LaRonde-LeBlanc et al. 2005b) (A) and the piD261/Bud32 family member from M. jannaschii (C-terminal domain of the Kae1-Bud32 fusion protein) (Hecker et al. 2008) (B) as well as an overlay of both structures (C) is depicted.
Figure 6. Amino acid sequence comparison of archaeal non-canonical Hanks-type PKs (aPKs) of the Rio1, Rio2 and piD261/Bud32 family. The Rio1 and Rio2 kinases from *A. fulgidus* and *P. abyssi* as well as the piD261/Bud32 family member from *M. jannaschii* (C-terminal domain of the Kae1-Bud32 fusion protein) were included. The two *A. fulgidus* Rio kinases and the *M. jannaschii* Bud32 are well studied and the crystal structures are available (Laronde-LeBlanc and Wlodawer 2004, 2005; LaRonde-LeBlanc et al. 2005a; Hecker et al. 2008, 2009). The typical Rio kinase domain features (yellow boxes and flexible loop (red), identified sequence features specific for the Rio1 and Rio2 family (sequences shown above the alignment) as well as the Rio2 specific wHTH domain with possible DNA-binding function (green, *A. fulgidus* Rio2) are depicted (Laronde-LeBlanc and Wlodawer 2004, 2005; LaRonde-LeBlanc et al. 2005b). The alignment was generated with Clustal Omega, ∗ conserved residues (black shading), : strong similarity between residues (gray shading), . weak similarity between residues.

The only functional role in Archaea has been reported for the Rio 1 kinase from *H. volcanii*, which is capable of phospho-rylating the α1 protein of the proteasome 20S core particle in vitro (Humbard et al. 2010). In vivo analyses after mutation of the phosphorylation sites of α1 revealed an important role of proteasome phosphorylation for pigmentation and cell viability. Mutation studies of Ser58Ala, Thr147Ala or Thr158Ala reduced the kinase activity of *Hvo*-Rio1 significantly. In addition, there is some structural evidence from comparison to their eukaryotic counterparts that suggests a similar role of archaeal RIO kinases in ribosome biogenesis with ATPase, rather than a kinase function (Laronde 2014). Therefore, the in vivo function of RIO kinases in Archaea is very unclear and requires further study.

copies of either RIO1 and RIO2, Rio B and Rio 2 kinase, or in a few cases two Rio 2 kinases. Interestingly, in all archaeal genomes, a conserved clustering of RIO genes together with genes encoding KH domain proteins (human KH (K homology) domain protein hnrNP (heterogeneous nuclear ribonucleoprotein), aEIF-1A (archaeal translation initiation factor-1A), DUF (domain of unknown function) 460, DEAD/DEAH-box helicases, snRNP (small nuclear ribonucleoproteins) or CbiA (coybrinic acid a,c-diamide synthase)) were observed. In addition, members of the Sulfolobaceae seem to be special in this regard. In those species, a strictly conserved genome neighborhood of nine genes was identified, which are all related to transcription and translation regulation (Esser and Siebers 2013).
PPs IN ARCHAEA

The counterparts of PKs are the PPs, which remove covalently linked phosphate groups. Overall, three families of PPs are known: (1) phosphatases active on pSer and/or pThr, (2) the ones which dephosphorylate pTyr (PTP) and (3) aspartate-based phosphatases such as the TFIIF-associated component of RNA polymerase II carboxy-terminal domain phosphatase (FCP) and the small carboxy-terminal domain phosphatases (SCP) (Shi 2009). Investigations in Eukaryotes also revealed the presence of atypical PPs, which were, so far, not detected in Prokaryotes (Sadatomi et al. 2013).

Ser/Thr PPs can be further classified into two subfamilies, the protein Ser/Thr phosphatases (PPPs) and the Mg<sup>2+</sup>- or Mn<sup>2+</sup>-dependent protein phosphatases (PFM). In Archaea, both subfamilies are present. PPFs are usually responsible for the dephosphorylation of pSer/pThr in Eukaryotes and Prokaryotes, whereas the PPMs are more common in bacteria (Barford 1996; Cohen 1997; Kennedy 2002, 2003). A great difference between members of the PPM and PPP subfamilies is that PPFs usually possess different subunits. The heterodimeric PPFs consist of the catalytic subunit and a scaffold subunit, whereas the heterotrimeric PPs have an additional regulatory subunit. The interplay between the different subunits determines substrate specificity (reviewed in Virshup and Shenolikar 2009). In contrast, PPMs are monomeric and contain sequence motifs and additional domains, which might mimic the presence of regulatory subunits (Lower, Bischoff and Kennedy 2000; Virshup and Shenolikar 2009). The PTP family can be subdivided into three groups, the PTPs, which are specific for pTyr dephosphorylation; the dual-specific PTPs, which can dephosphorylate pSer/pThr as well as pTyr; and the low-molecular-weight PTPs (<18 kDa). They all share one common amino acid motif, CX<sub>5</sub>R (Shi, Potts and Kennedy 1998).

Since the discovery of protein phosphorylation in archaea, four PPs (S. solfataricus, Kennelly et al., 1993; Leng et al., 1995); Methanosarcina thermophila (Oxenriper et al., 1993; Solow, Young 1997); Pyrodictium abyssi (Mai et al., 1998); and Haloferax volcanii (Oxenriper and Kennelly 1993), one PPM (T. vulcanium (Dahche et al. 2009)) and three PTPs (S. solfataricus (Chu and Wang 2007), S. acidocaldarius (Reimann et al. 2013) and T. kodakaraensis (Jeon et al. 2002)) were analyzed (for a review, see Kennelly 2014).

Archaeal PPs

Archael PPs of the PPF group resemble their eukaryotic orthologs regarding their amino acid sequences. However, to date, only homologs of the catalytic subunit have been detected in Archaea and the biochemical properties seem to be different, especially regarding their inhibition behavior. The first archaeal PP which was characterized was the enzyme from the euryarchaeon H. volcanii (Oxenriper and Kennelly 1993). PP activity was demonstrated in soluble extracts with pSer/pThr substrates (i.e. casein, mixed histones and phosphorylase A), whereas no activity could be detected with pTyr substrates (i.e. reduced, carboxymethylated or maleylated lysozyme). Activity was stimulated by Mn<sup>2+</sup> and Cd<sup>2+</sup> ions, but inhibited by NaF, Zn<sup>2+</sup>, vanadate, molybdate, inorganic phosphate/pyrophosphate, p-nitrophenyl phosphate and diethylenepyrocarbonate. The addition of more specific PP inhibitors like okadaic acid and microcystin-LR, which are common inhibitors of eukaryal PP1/2A/2B PPs, had no influence on the Hvo-PP. At the same time, similar studies were performed in S. solfataricus P1. The native PPF from S. solfataricus P1 was isolated from the soluble fraction and was shown to dephosphorylate 32P-labeled target proteins like casein (Kennelly et al. 1993). No PPP activity was detected with pTyr-labeled substrates, revealing that the enzyme is a protein Ser/Thr phosphatase. Furthermore, the Sso-PP revealed a strong Me<sup>2+</sup>-ion dependency, using Mn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup> and Mg<sup>2+</sup> as cofactors. A few years later, the amino acid sequence of the Sso-PP was identified and the highest similarities towards eukaryal members of the PP1/2A/2B super family were observed (Leng et al. 1995). However, whereas eukaryal PP1/2A/2B members are sensitive towards low amounts of okadaic acid, calyculin A and microcystin-LR (Cohen 1991), no inhibition was observed for the S. solfataricus ortholog (Kennelly et al. 1993). In addition, Sso-PP1 was shown to be active as a monomer, showing high similarity towards the known catalytic subunits from the eukaryal heterooligomeric PPFs.

Only recently, the PP from S. acidocaldarius DSM639 was investigated, revealing similar biochemical properties (Reimann et al. 2013). Based on the sequence alone, the S. acidocaldarius ortholog can be assigned as a member of the PP2A branch of the PPP super family, which usually requires metal ions for activity and are involved in a variety of cellular processes like cell cycle control and cell mobility (reviewed in Shi 2009). The Saci-PP2A also requires Me<sup>2+</sup> ions for activity, showing highest activity with Cu<sup>2+</sup>. Activity was only observed with a pThr peptide (RRA(pT)VA), whereas no activity was detected with a pTyr peptide (TEVGKR(pY)RLVGDKN), which was identified during the phosphoproteome study conducted in S. acidocaldarius (see below; Reimann et al. 2013). However, the Saci-PP2A was strongly inhibited by okadaic acid (56% at 10 nM okadaic acid), which showed no inhibitory effect on the Sso-PP1 enzyme. Furthermore, a function in archaeal regulation was suggested. The deletion of pp2a led to hypermotile cells and, therefore, it is likely that PP2A plays a role in repression of archaellum expression (see also above, Reimann et al. 2013).

The analysis of soluble extracts from M. thermophila TM-1 (Oxenriper et al. 1993; Solow, Young andKennelly 1997) revealed, in a similar way to the S. solfataricus enzyme, PP activity with 32P-casein in the presence of Mn<sup>2+</sup>, Ni<sup>2+</sup> and Co<sup>2+</sup>. The addition of Cd<sup>2+</sup>, Cu<sup>2+</sup> or Zn<sup>2+</sup> inhibited the PP activity completely. Activity could be demonstrated with pSer/pThr substrates, whereas no activity was detected with pTyr substrates, suggesting that the Mth-PP is also specific for pSer and pThr. Similar to the results for Saci-PP2A, but in contrast to Sso-PP1, Mth-PP was also very sensitive towards the specific PP1/2A/2B inhibitors okadaic acid, microcystin-LR and calyculin A (μM range). Four years later, the sequence of the Mth-PP was identified, revealing that this PP belongs to the PP1-arch2 branch (Solow, Young and Kennelly 1997). Mth-PP1 was heterologously expressed in Escherichia coli and the previously reported activity from soluble extracts was confirmed, demonstrating that PP1/2A/2B PPs are present in both major phyla of the Archaea, Crenarchaeota and Euryarchaeota.

The PP from the euryarchaeon Pyd. abyssi shows a high similarity to the enzymes of S. solfataricus and M. thermophila (Mai et al. 1998) and belongs to the PP1 branch within the PP1/2A/2B super family. Similar to the previous studies, Fdab-PP1 requires Me<sup>2+</sup> ions and the highest activity was observed with Mn<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup>, decreased activity with Mg<sup>2+</sup> and no activity at all in presence of Ca<sup>2+</sup>. Fdab-PP1 was also inhibited by Cu<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup>, Na<sup>F</sup>, NaK tartarate, DEPC and okadaic acid. Activity was only observed with the pThr-peptide (RRA(pT)VA), which was also used for detailed characterization for Saci-PP2A (Reimann et al. 2013). In vivo analysis of Pyd. abyssi cells grown in the presence of 32P<sub>P</sub> revealed six phosphorylated polypeptides between 30 and...
250 kDa, which, however, could not be dephosphorylated by the P dab-PP (Mai et al. 1998).

Archaeal PPM

The only study concerning archaebacterial PPMs to date was conducted by Dahche et al. (2009), in the euryarchaeon T. volcanium. The identified PPM in the genome of T. volcanium comprises only 218 amino acid residues; however, the comparison with the PPM from Bacillus subtilis revealed that all essential motifs are present. As assumed, this enzyme was Me(2+) dependent and specific for Mn(2+) ions, whereas Mg(2+), Ni(2+), Ca(2+), Cu(2+) or Zn(2+) had no effect or in the case of Zn(2+), inhibited the enzyme. Notably, Tvo-PPM possesses very broad substrate specificity towards pSer/pThr and also pTyr substrates, dephosphorylating all of them with a comparable rate.

Archaeal PTPs

The first archaeal PTP was characterized in 2002 from the hyperthermophilic euryarchaeon T. kodakaraensis KOD1 (Jeon et al. 2002). The biochemical characterization of the heterologously overexpressed PTP and site-directed mutagenesis revealed that the Tko-PTP dephosphorylates pTyr and pSer. However, no activity with pThr was observed. Mutation of the residue A(63), which is considered as general acid/base involved in the hydrolysis of the phosphate monoester (Zhang, Wang and Dixon 1994; Wu and Zhang 1996), resulted in an increased activity, suggesting that A(63) is not the residue involved in the phosphate binding. The residues C(93) and R(99) are both part of the PTP signature motif —CMGGGLGRS— (Fig. 7) and it was shown that the Cys residue is important for the formation of the phosphoryl-Cys intermediate (Denu et al. 1996), whereas the Arg residue is essential for the transition state and the substrate binding in general (Barford 1996). This assumption was confirmed by mutational analysis and in addition, Jeon et al. (2002) were able to identify three in vivo substrates using a trapping approach. They identified three Tyr phosphorylated proteins via immunoblotting with anti-pTyr antibodies corresponding to RtcB (part of the RNA terminal phosphatase cyclase), phenylalanine-tRNA synthetase and phosphomannomutase (PMM).

Two more PTPs were investigated both from the crenarchaeal model organisms S. solfatarius (Chu and Wang 2007) and S. acidocaldarius DSM639 (Reimann et al. 2013). The activity of the Sso-PTP was assayed using the general phosphate substrate para-nitrophenyl phosphate (pNPP) and the pTyr peptides A(pY)R and NK(pY)GN. The latter corresponds to the predicted phosphorylation site of the PMM, which was identified as an interaction partner/substrate for the Tko-PTP (Jeon et al. 2002). Using these substrates, in vitro PTP activity was confirmed. However, PP activity with pSer and pThr was not observed, revealing that the Sso-PTP is a conventional PTP without dual substrate specificity. Furthermore, the crystal structures of the WT Sso-PTP apo-enzyme and the enzyme in combination with phosphate and tungstate as well as the structures of an inactive Sso-PTP(C96S) mutant were solved. The overall structure resembles a compact fold of known PTPs (Cdc45B, KAP1 and VHR) with a five-strand β-sheet and four α-helices, although the loop regions between β2-β3 (box 1, α-helix in Sso-PTP) and β3-β4 (box 2, helix-loop-helix Sso-PTP) are different (Yuvaniyama et al. 1996; Song et al. 2001; Gray et al. 2003) (Fig. 7). The motif Dx(25-41)HxGxxGxxR(T/S) (x stands for any residue) is conserved in PTPs and dual-specific phosphatases (DSPs) and comprises the catalytic Asp and the Cys residue that act as a general acid and a nucleophile in the catalytic mechanism, respectively. Furthermore, the structure of an inactive Sso-PTP(C96S) in the presence of a peptide, NK(pY)YGN, revealed that the phosphate moiety of P-Tyr is bound to the P-Loop and buried in the active site surrounded by the P-loop. Additional binding of the phosphate moiety was observed to Asp69 (D-Loop) and Gln135 (Q-Loop) (Chu and Wang 2007). Gln135 is an important structural feature, since it hinders the entrance of either P-Ser or P-Thr in Sso-PTP. In addition, interactions between Arg136 and the P-loop with the bound substrate were observed. These interactions are shown by red dots in Fig. 7 and were also identified for other PTPs.

Until now, it has not been possible to distinguish between dual-specific or conventional PTPs based on the amino acid sequence alone. However, one general observation derived from available structures is that the active site of a conventional PTP is usually deeper than the active site of a dual-specific PTP for better interaction with the ‘bulky’ pTyr. The in vitro activity measurements already revealed that the Sso-PTP is specific for pTyr. This was also confirmed by the shape of the Sso-PTP active site, which allows for a better interaction with pTyr than with pSer or pThr. Potentially, the most important residue for this interaction is Gln135, which hinders a proper binding of pSer or pThr substrates in the active site (Chu and Wang 2007).

In vivo evidence for phosphatase function in Sulfolobus acidocaldarius

Recently, the PPs from S. acidocaldarius, Saci-PP2A (see above) and Saci-PTP were analyzed by using a combined in vitro and in vivo approach. Deletion mutants were constructed and phenotypically characterized (i.e. growth experiments, cell size, motility assays) in comparison to the parental strain (MW001). In addition, the different S. acidocaldarius strains were analyzed using transcriptomics (RNAseq), phosphoproteomics and both PPs were characterized with respect to their enzymatic properties (Reimann et al. 2013). The characterization of the Saci-PPs was performed with pNPP and three phosphopeptides (NIDAIRA(pS)LNIMSR (Saci_1346), ETTRYERW(pT)TITRER (Saci_1857) and TEV GKIR(pY)R LV G (Saci_1938)) identified during the phosphoproteome analysis. For the Saci-PP2A pSer/pThr phosphatase activity and inhibition by okadaic acid (56% activity in presence of 10 nM of okadaic acid) as well as metal dependence (highest activity with Cu(2+) followed by Mn(2+), Ni(2+), Mg(2+), Cd(2+) and Co(2+); complete inhibition by EDTA) was demonstrated. In contrast, the Saci-PTP exhibited dual substrate specificity (pSer/pThr and pTyr phosphatase) and no metal dependence or inhibition by okadaic acid was observed. The Saci-PTP showed nearly the same Km value for all three tested phosphopeptides; however, the determined Vmax value for the pTyr peptide was 30 to 131-fold higher, compared to the pSer and pThr peptide, suggesting that pTyr is the favored physiological substrate. Notably, even though Sso-PTP and Saci-PTP have 63% sequence identity they do not show the same specificity. Saci-PTP is a DSP, while Sso-PTP is solely active on pTyr residues, despite the fact that both have the essential residues important for specificity and activity (Fig. 7).

Whereas the deletion of Saci-PTP revealed no obvious phenotype, in the Saci-PP2A deletion strain, pronounced alterations in growth, cell shape and cell size were observed. The genome-wide transcriptome and phosphoproteome analysis revealed 155 genes that were differently expressed in the two mutants compared to the parental strain. A total of 801 unique phosphoproteins (1206 phosphopeptides) were identified, with a
Figure 7. Amino acid sequence comparison of PTPs. Structure-based sequence alignment of the investigated PTPs from *S. solfataricus* (SSO2453), *S. acidocaldarius* (Saci0545), *T. kodakarensis* (Tko0241) and *M. janaschii* (MJ0215). Secondary structure elements of the solved Sso-PTP structure are shown above the sequence with β-sheets depicted as orange arrows and α-helices as green boxes (Chu and Wang 2007). The variable region of PTPs and DSPs, box 1 and box 2, are boxed red and the conserved sequence motif (Dx(25-41)HCxxGxxRT/S) is shown underneath the sequence and shown in red in the Sso-PTP sequence. The amino acid residues identified in the Sso-PTP to be involved in binding of the phosphopeptides A-pY-R and N-K-pY-G-N are marked by red dots. The alignment was generated with Clustal Omega, * conserved residues (black shading), : strong similarity between residues (gray shading), . weak similarity between residues.

significant increase in the phosphatase deletion strains. Again an unusually high number of phosphorylated Tyr residues (pSer/pThr/pTyr %‐ratio of 35.6/28.1/36.2) was detected. Regulated phosphoproteins from most functional arCOGs categories were observed, including components of the motility system (archaellum), respiratory chain and regulatory proteins such as many transcriptional regulators and Ser/Thr PKs. In the Saci-PP2A deletion strain, the upregulation at the transcript level as well as the phosphorylation pattern resembled starvation stress response, which led to the upregulation of the archaellum. This observation was supported by the hypermotile phenotype of the mutant (see in section archaellum regulation and Fig. 3B; Reimann et al. 2013).

**CONCLUSION**

This review highlights the essential role of reversible protein phosphorylation in the regulation of basic cellular processes in Archaea and summarizes first insights into archaeal signal transduction. A striking feature in Archaea is the unusual split between the two major archaeal kingdoms, the Euryarchaeota and the Crenarchaeota, with bacterial-type TCS being only present in the Euryarchaeota, whereas protein phosphorylation on Ser, Thr and Tyr residues (via Hanks-type PKs, ePKs) is commonly distributed.

Phosphoproteome studies revealed that in common with Bacteria and Eukaryotes, for Archaea a very high number of phosphoproteins is observed (Schulz et al. 2014). Members of almost all arCOG categories were observed to be phosphorylated underpinning its fundamental role. An unexpected observation (so far) was the high amount of Tyr phosphorylation specifically in Sulfolobus species and seemingly absent in *Halobacterium salinarium*. In Archaea, no bona fide Hanks-type Tyr PKs or BY-kinases were identified so far, raising questions about the kinases involved in Tyr phosphorylation. This is further underpinned by the high number of phosphoproteins and relatively low amount of ePKs and PPs in Archaea, suggesting the presence of other so far unknown enzymes in Archaea.

In general, Tyr phosphorylation is regarded as an invention of Eukaryotes to regulate more complex cellular networks. In the light of the newly proposed two-domain tree of life with the origin of Eukaryotes in the archaeal TACK superphylum, this might represent a shared phylogenetic trait. Therefore, it is of special importance to identify and study more pathways in Archaea in which regulation by reversible protein phosphorylation plays a role and to understand their mechanisms.
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