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REVIEW

Metabolism of halophilic archaea

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Abstract In spite of their common hypersaline environment, halophilic archaea are surprisingly different in their nutritional demands and metabolic pathways. The metabolic diversity of halophilic archaea was investigated at the genomic level through systematic metabolic reconstruction and comparative analysis of four completely sequenced species: *Halobacterium salinarum*, *Haloarcula marismortui*, *Haloquadratum walsbyi*, and the haloalkaliphile *Natronomonas pharaonis*. The comparative study reveals different sets of enzyme genes amongst halophilic archaea, e.g. in glycerol degradation, pentose metabolism, and folate synthesis. The carefully assessed metabolic data represent a reliable resource for future system biology approaches as it also links to current experimental data on (halo)archaea from the literature.

Keywords Metabolism · Archaea · Haloarchaea · *Halobacterium salinarum* · Pathway database · Metabolic pathways · Enzymes · Comparative genomics

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Abbreviations

DAP	Diaminopimelate pathway
DHA(P)	Dihydroxyacetone (phosphate)
DKFP	6-Deoxy-5-ketofructose-1-phosphate
ED	Entner-Douderoff pathway
EM	Embden-Meyerhoff pathway
IPP	Isopentenyl diphosphate (isopentenyl pyrophosphate)
KD(P)G	2-Dehydro-3-deoxy-(6-phospho)gluconate (2-keto-3-deoxy-(6-phospho)gluconate)
PAB	*p*-Aminobenzoate
PP	Pentose-phosphate pathway
PRPP	5-Phospho-D-ribosyl-1-pyrophosphate
RuBisCO	Ribulose-bisphosphate carboxylase
RuMP	Ribulose monophosphate pathway
TCA	Tricarboxylic acid cycle
THF	5,6,7,8-Tetrahydrofolate

Introduction

Extremely halophilic archaea are a diverse group of euryarchaeota that inhabit hypersaline environments (3–5 M) such as salt lakes, salt ponds, and marine salterns. They are often referred to as “*halobacteria*,” named after the model organism *Halobacterium salinarum*, whose proton pump bacteriorhodopsin is one of the best-studied membrane proteins. Although haloarchaea share certain features in order to adapt to their extreme environment, i.e. acidic protein machineries, respiratory chains and rhodopsins, their metabolism is considerably different from each other. Although there are carbohydrate-utilizing species such as *Haloferax mediterranei*, *Haloarcula marismortui*, and *Halococcus saccharolyticus*, which catabolize hexoses

(glucose, fructose), pentoses (arabinose, xylulose), sucrose, and lactose (Rawal et al. 1988; Altekar and Rangaswamy 1992; Johnsen et al. 2001; Johnsen and Schonheit 2004), other haloarchaea like *H. salinarum* are not capable of sugar degradation (Rawal et al. 1988). Instead, non-carbohydrate-utilizing species thrive on amino acids and typical compounds of hypersaline habitats. *Haloferax volcanii*, for example, is able to grow on glycerol and organic acids (Kauri et al. 1990) excreted by primary halophilic producers *Dunaliella salina* (Elevi Bardavid et al. 2006) and *Microcoleus chthonoplastes* (Zviagintseva et al. 1995), respectively. Haloarchaea differ not only in their catabolic pathways but also in their nutritional requirements. While simple growth media were described for *Haloferax volcanii* (Kauri et al. 1990) and *Natronomonas pharaonis* (Falb et al. 2005), *H. salinarum* exhibits complex nutritional demands. Growth of *Halobacterium* cells is often limited in synthetic media (Oesterhelt and Krippahl 1973; Grey and Fitt 1976), in spite of rich amino acid (at least 10 amino acids) and cofactor supplements (folate, biotin, thiamine).

The metabolic diversity of halophilic archaea has not yet been investigated at the genomic level by metabolic reconstruction and comparative analysis. The absence of enzyme genes for numerous metabolic reactions in archaeal genomes has limited reconstruction of metabolic pathways so far. However, many of these pathway gaps have been elucidated recently with the discovery of novel non-orthologous enzymes in archaea, e.g. for the de novo synthesis of mevalonate (Barkley et al. 2004; Grochowski et al. 2006b), purines (Graupner et al. 2002; Ownby et al. 2005), and cobamide (Woodson et al. 2003; Woodson and Escalante-Semerena 2004; Zayas et al. 2006). Archaea also employ novel enzymes and precursors for pentose formation (Grochowski et al. 2005) and aromatic amino acid synthesis (White 2004; Porat et al. 2006) circumventing absent enzymes of the classical pentose-phosphate pathway.

For this detailed review of haloarchaeal metabolism, metabolic pathways of halophilic archaea were systematically reconstructed. Currently, genome sequences of four diverse haloarchaeal species are available for comparative analysis (Table 1), namely that of the model organism *H. salinarum* (Ng et al. 2000; Pfeiffer et al. 2008; http://www.halolex.mpg.de), the metabolic-versatile *H. marismortui* (Baliga et al. 2004), the haloalkaliphile *N. pharaonis* (Falb et al. 2005), and the square-shaped *Haloquadratum walsbyi* (Bolhuis et al. 2006). The presented metabolic data will be a valuable resource for future system biology approaches, as each metabolic reaction has been carefully assessed and linked to experimental data from the literature.

Materials and methods

Metabolic reconstruction procedure

Metabolic pathways of *H. salinarum* were reconstructed in a two-step procedure. At first, relevant reactions that take part in a given metabolic pathway were chosen from the complete set of reference reactions downloaded from the KEGG ligand database (Kanehisa et al. 2004). In the second step, organism-specific metabolic pathways were

Table 1 Overview of the currently sequenced haloarchaea

	H. salinarum strains NRC-1/R1[a]	*H. marismortui*	*H. walsbyi*	*N. pharaonis*
Gene identifier	VNG/OE	rrn, pNG	HQ	NP
Salt optimum [M]	4–5 M	4.5 M	3.3 M	3.5 M (pH 8.5)
Isolation	Salted fish	Dead Sea (Israel)	Solar saltern (Spain)	Soda lake (Egypt)
Main research interests	Rhodopsins, signal transduction	Versatile nitrogen metabolism	Square-shaped cells, halomucin	Haloalkaliphilicity, respiratory chain
Genome size [Mb]	2.61/2.72	4.37	3.24	2.80
# Plasmids	2/4	8 (incl. CHRII)	1	2
%GC chromosome	68.0	62.4	47.9	63.4
rRNA operons	1	3	2	1
Fla genes (motility)	Yes	Yes	No	Yes
# Transducer genes	18	21 (18)	0	19
# Rhodopsin genes (bop, hop, sop)	4 (1,1,2)	6 (3,1,2)	3 (2,1,0)	2 (0,1,1)

[a] Two strains of *H. salinarum* have been sequenced, strains NRC-1 (Ng et al. 2000) and R1 (Pfeiffer et al. 2008; http://www.halolex.mpg.de). These are virtually identical and differ only in their distribution of insertion elements and their plasmid arrangements. Unless mentioned otherwise, the two strains are not distinguished in this review, because they exhibit analogous sets of enzyme encoding genes. Gene identifiers of *H. salinarum* strain R1 (e.g. OE1001F) will be used throughout the text

reconstructed by flagging each reaction existent or non-existent in *H. salinarum* with a certain confidence value. The enzyme gene predicted to catalyze this reaction in *H. salinarum* was linked to the reaction. In case experimental data is available from the literature, a comment was linked to the reaction or enzyme gene, which includes the PubMed identifier of the external reference. Each reaction was manually assessed based on automatic enzyme assignments derived from various similarity searches (i.e. blast search with UniProt enzymes, COG assignments, Pfam search), genome data (http://www.halolex.mpg.de), and experimental literature data for *H. salinarum*. In case, a reaction or enzyme is confirmed by experiments but no genetic evidence was found in the genome (indicating novel yet unknown enzyme variants), the reaction was marked existent and the conflict was labeled. The metabolic data for *H. salinarum* given in Supplementary Material S1 was internally stored in a MySQL database and managed via a Web-interface.

Comparative analysis of the complete haloarchaeal gene sets

For comparison of haloarchaeal gene sets, protein databases (fasta format) of the four completely sequenced haloarchaeal genomes, *H. salinarum* strain R1, *N. pharaonis, H. walsbyi*, and *H. marismortui*, were blasted against each other and against the non-redundant (nr) database. For example, each predicted protein sequence of *N. pharaonis* (species 1) was searched against the *H. salinarum* database (species 2) and the nr database (all species), then the difference between the best blast score for the general database (nr) and for the related species *H. salinarum* was calculated. Listing *N. pharaonis* sequences by these differences in descending order detects genes present in *N. pharaonis* but absent in *H. salinarum*. In few cases, the *N. pharaonis* gene had a close homolog in the general database but only a more distant homolog in *H. salinarum*. Such a gene is considered present in *H. salinarum* when the blast hit is highly significant (*E*-value better than e^{-20}) were considered. The differential blast analysis routine was applied to all combinations of the set of four haloarchaeal genomes. A list of genes present in only a subset of genomes was obtained and filtered for metabolic function (Supplementary Material S2).

Results and discussion

The metabolism of *H. salinarum* was reconstructed by evaluating metabolic reactions of biochemical reference pathways from KEGG (Supplementary Material S1). Enzyme annotations from an automatic assignment routine and from curated genome data (http://www.halolex.mpg.de) were carefully assessed in order to avoid misassignments due to domain rearrangements (e.g. purine biosynthesis, Fig. 2) or overannotation of the exact substrate specifity (e.g. 2-oxoacid dehydrogenase complex). Novel enzyme variants closing former pathway gaps in archaea as well as previously published experimental data of *H. salinarum* were also considered throughout the reconstruction process. Comparative analysis of the currently sequenced haloarchaea, *H. marismortui*, *H. walsbyi, N. pharaonis*, and *H. salinarum*, disclosed major metabolic differences, i.e. alternative enzymes and metabolic pathways employed by haloarchaea (Supplementary Material S2).

In the following, reconstructed metabolic pathways of halophilic archaea will be reviewed in detail with emphasis on different enzyme genes and metabolic pathways, which lead to the diverse catabolic and anabolic capabilities of halophilic archaea. In case an enzyme gene is present in all four haloarchaeal species, only the *N. pharaonis* identifier (e.g. NP1002A) will be exemplarily mentioned.

Glycolytic pathways

Major differences in sugar degradation capabilities were identified amongst halophilic archaea that reflect the previous classification of halophilic archaea into carbohydrate- and non-carbohydrate utilizers (Fig. 1, Supplementary Material S2). *H. salinarum*, *H. marismortui*, and *H. walsbyi* possess all required enzyme genes for the semi-phosphorylated Entner-Douderoff (ED) pathway previously described for carbohydrate-utilizing haloarchaea (Rawal et al. 1988; Danson and Hough 1992; Verhees et al. 2003). However, only *H. marismortui* and *H. walsbyi* encode a classical KDPG aldolase, which is the key enzyme of the pathway, in addition to the novel archaeal KD(P)G aldolase recently described for thermophilic archaea (Ahmed et al. 2005). Thus, operation of different ED pathway variants between *H. salinarum* and the other two species is indicated. It should further be noted that neither the ED pathway nor growth on glucose as the sole carbon source has been established for *Halobacterium* so far (Gochnauer and Kushner 1969; Rawal et al. 1988). *N. pharaonis* is likely to be incapable of glucose degradation, because it completely lacks all enzyme genes of the ED pathway.

The carbohydrate-utilizing *H. marismortui* has acquired a wide range of enzyme genes involved in the uptake and degradation for various sugars. Fructose and sucrose are likely degraded via the modified Embden-Meyerhof (EM) pathway, which has been described for haloarchaea (Altekar and Rangaswamy 1992). The pathway involves

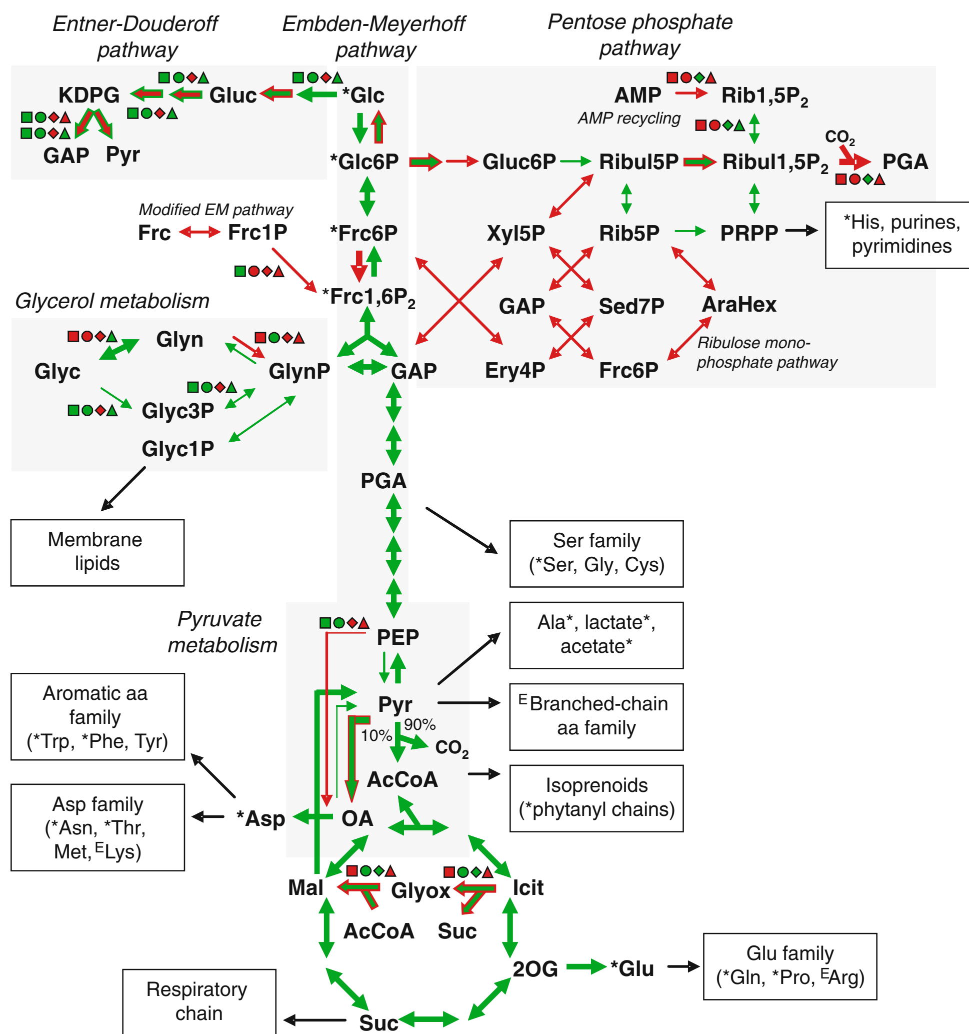

Fig. 1 The central intermediary metabolism in halophilic archaea. The *reaction arrows* depict the reconstructed metabolism of the reference species *H. salinarum* (*green* reaction exists, *red* reaction absent). The four geometric symbols illustrate differences in enzyme gene sets between the four sequenced haloarchaea (square: *H. marismortui*, circle: *H. walsbyi*, diamond: *N. pharaonis*, triangle: *H. salinarum*, *green* gene exists, *red* gene absent). Reactions that have been investigated experimentally through NMR studies or enzyme activity tests in *H. salinarum* are highlighted by *bold arrows* (*green* reaction exists, *red* reaction absent). For some of the experimentally verified reactions, there are currently no genetic evidences in the *H. salinarum* genome (*green arrows with red border*). Vice versa, some reactions have been experimentally excluded, but probable enzyme genes are present in the *H. salinarum* genome (*red arrows with green border*). Compounds that have been identified through labeling studies are marked by *asterisks*. Proposed essential amino acids for *H. salinarum* are indicated ([E]). Compounds: AraHex—D-arabino-3-hexulose-6P, Ery4P—erythrose-4P, Frc—fructose, GAP—glyceraldehyde-3P, Glc—glucose, Gluc—gluconate, Glyn—glycerone, Glyc—glycerol, Glyox—glyoxylate, Icit—isocitrate, KDPG—2-dehydro-3-deoxy-6-phosphogluconate, Mal—malate, OA—oxalacetate, 2-OG—2-oxoglutarate, PEP—phosphoenolpyruvate, PGA—3-phosphoglycerate, Pyr—pyruvate, Rib5P—ribose-5P, Ribul5P—ribulose-5P, Suc—succinate, Xyl5P—xylulose-5P, Sed7P—sedoheptulose 7-phosphate, AcCoA—acetyl-CoA

the key enzyme 1-phosphofructokinase (EC 2.7.1.56, rrnAC0342) and sucrose 6-phosphate hydrolase (EC 3.2.1.26, rrnAC1479). *Haloarcula* also contains probable gene clusters for maltose uptake (maltose ABC transporter, rrnAC2346-rrnAC2349) and metabolism (several α-glucosidases, e.g. rrnAC0224) as well as a gene cluster for D-xylulose oxidation (rrnAC3032–rrnAC3039). The latter encodes the previously characterized D-xylulose dehydrogenase (rrnAC3034) (Johnsen and Schonheit 2004).

Embden-Meyerhof pathway and gluconeogenesis

Consistent with the previous biochemical findings (Rawal et al. 1988; Altekar and Rangaswamy 1992), genes for 6-phosphofructokinase (EC 2.7.1.11), the key enzyme of the classical Embden-Meyerhof pathway, are absent in all four haloarchaeal genomes. Alternative archaeal types of 6-phosphofructokinases that depend on ADP (*Thermococcus, Pyrococcus*) or PP(i) (*Thermoproteus*) (Kengen et al. 1994; Selig et al. 1997) as co-substrates have not been found

either. However, a 1-phosphofructokinase gene for fructose degradation via the haloarchaeal variant of the EM pathway (Altekar and Rangaswamy 1992; Rangaswamy and Altekar 1994a, b) is present in *H. marismortui* (rrnAC0342). Although the upper, hexose part of the classical EM pathway is missing in haloarchaea, the lower, triose part of the pathway that leads to pyruvate is likely to be functional in all species, as required enzyme genes are encoded in their genomes. In fact, several enzyme activities have already been proven in *H. salinarum* (Rawal et al. 1988). The final glycolysis step from phosphoenolpyruvate to pyruvate might be catalyzed by pyruvate kinase (EC 2.7.1.40, e.g. NP1746A) and pyruvate, water dikinase (EC 2.7.9.2, e.g. NP1196A) in haloarchaea. In a thermophilic archaeon, both enzymes participated in glycolysis but only deletion of the pyruvate, water dikinase gene completely omitted growth on sugars (Imanaka et al. 2006).

Complete gene sets for the reverse EM pathway (gluconeogenesis) from pyruvate to phosphorylated glucose were identified in all four haloarchaeal genomes. Consistently, labeling experiments (Sonawat et al. 1990; Ghosh and Sonawat 1998) and enzyme activity studies (Rawal et al. 1988) have confirmed gluconeogenesis in *H. salinarum*, which is required to synthesize hexoses for membrane constituents. For example, glucose was found to be incorporated into different sugar moieties (glucose, mannose, galactose) of halobacterial glycolipids (Weik et al. 1998). Furthermore, saccharide units are attached to surface proteins of *H. salinarum* such as the S-layer protein and flagellins (Sumper 1987). Sugar moieties of lipids and proteins are likely synthesized via nucleotide sugars. In accordance with this, several nucleotide sugar enzymes such as UDP-glucose 4-epimerase (EC 5.1.3.2, e.g. NP4662A) and UDP-glucose 6-dehydrogenase (EC 1.1.1.22, e.g. NP2322A, NP4668A) are present in haloarchaeal genomes.

Entner-Douderoff pathway

Variants of the classical Entner-Douderoff pathway are common in the archaeal domain of life (Danson and Hough 1992). Halophilic archaea such as *H. vallismortis* (Altekar and Rangaswamy 1992) operate the semi-phosphorylated pathway, where glucose is converted to 2-dehydro-3-deoxygluconate (KDG). This intermediate is then phosphorylated to KDPG and subsequently split into pyruvate and glyceraldehyde 3-phosphate. In *Sulfolobus* and *Thermoplasma*, a non-phosphorylated ED pathway has been described (in addition to the semi-phophorylated pathway variant), where KDG is cleaved without prior phosphorylation by a novel bifunctional KD(P)G aldolase (same COG0329 as dihydrodipicolinate synthase) (Ahmed et al. 2005). Orthologs for all genes of the characterized *Sulfolobus* ED pathway cluster (Ahmed et al. 2005; Kim and Lee 2005) were found in *H. salinarum*, *H. marismortui* and *H. walsbyi*, namely genes encoding D-gluconate dehydratase (EC 4.2.1.39, *gnaD*, COG4948, e.g. OE1664R), KDG kinase (EC 2.7.1.45, *kdgK*, COG0524, e.g. OE1266R), and KD(P)G aldolase (*kdgA*, COG0329, e.g. OE1665R). The ED pathway genes, except *kdgK*, are located in one gene cluster in the *H. salinarum* genome, which additionally contains a glucose 1-dehydrogenase gene (EC 1.1.1.47, e.g. OE1669F) (Bonete et al. 1996) as well as two conserved genes (e.g. OE1668R, OE1672F), which are candidates for the yet missing gluconolactonase (EC 3.1.1.17). The non-carbohydrate-utilizing strain *N. pharaonis* lacks all enzyme genes for the ED pathway.

In addition to their archaeal KD(P)G aldolase (rrnAC0960, HQ2365A), *H. marismortui* (rrnAC3121), *H. walsbyi* (HQ1495A), and *Haloferax alicantei* (AAB40121, co-located with *kdgK*, AAB40122) also encode the canonical bacterial-type KDPG aldolase (EC 4.1.2.14, *kdgA*, COG0800), which is absent in *Halobacterium*, *Sulfolobus*, and *Thermoplasma* species. This suggests operation of different ED pathway variants in the two (halo)archaeal groups, and, probably, a 'reduced' semi-phosphorylated ED pathway in *H. salinarum*, which needs to be investigated in future. So far, experiments with glucose-grown cells of *H. salinarum* have shown the conversion from glucose to gluconate (Sonawat et al. 1990; Bhaumik and Sonawat 1999), but have not detected subsequent reactions from gluconate to pyruvate and glyceraldehyde 3-phosphate when applying indirect enzyme activity tests (Rawal et al. 1988).

Oxidative pentose phosphate pathway

Through the oxidative pentose phosphate pathway, phosphorylated glucose is oxidized to gluconate 6-phosphate (C_6) and then converted to ribulose 5-phosphate (C_5) by oxidative decarboxylation. In the archaeal domain of life, the oxidative PP pathway seems not to exist, because enzyme genes for this pathway are absent in archaeal genomes. However, haloarchaea show glucose 6-phosphate dehydrogenase activity (EC 1.1.1.49) in spite of lacking the respective enzyme gene (Aitken and Brown 1969) and encode orthologs of 6-phosphogluconate dehydrogenase (EC 1.1.1.44, COG1023, e.g. NP0286A). Thus, an operative, albeit modified oxidative PP pathway is indicated for haloarchaea.

Pentose metabolism

In bacteria, pentoses are synthesized via the non-oxidative part of the PP pathway, where fructose 6-phosphate (C_6)

and glyceraldehyde 3-phosphate (C_3) are converted to ribulose 5-phosphate (C_5) in a complex five-step pathway. The enzyme gene set for this pathway, consisting of transaldolase 1 and 2 (EC 2.1.2.1), transketolase (EC 2.2.1.1), and ribulose-phosphate 3-epimerase (EC 5.1.3.1), is absent in most archaea except *Methanococcus jannaschii* and *Thermoplasma* spp. (Soderberg 2005). Individual genes encoding ribose 5-phosphate isomerase (EC 5.3.1.6, e.g. NP0786A) for the conversion of ribose 5-phosphate to ribulose 5-phosphate are present in archaeal genomes.

Although *Methanocaldococcus jannaschii* encodes all enzyme genes for an operative non-oxidative PP pathway, relevant intermediates (i.e. erythrose 4-phosphate, xylose 5-phosphate, sedoheptulose 7-phosphate) are absent in its cells (Grochowski et al. 2005). Instead, ribulose 5-phosphate is exclusively produced through the ribulose monophosphate (RuMP) pathway in *M. jannaschii*. This pathway is commonly employed for formaldehyde fixation and detoxification in bacteria but operates in reverse mode in archaea and, thus, substitutes for the classical non-oxidative PP pathway (Orita et al. 2006; Grochowski et al. 2005). The RuMP pathway converts fructose 6-phosphate to D-arabino-3-hexulose 6-phosphate and then to formaldehyde and ribulose 5-phosphate involving 6-phospho-3-hexuloisomerase (COG0794) and 3-hexulose-6-phosphate synthase (COG0269). These enzymes are encoded in all archaea except *Thermoplasma* and haloarchaea, which presumably operate the non-oxidative and a modified oxidative PP pathway for pentose formation, respectively (Soderberg 2005).

Absence of the non-oxidative branch of the PP pathway in most archaea also results in the absence of the pathway intermediate erythrose 4-phosphate (C_4), which is the precursor of aromatic amino acids in bacteria. While a group of archaea (e.g. *Pyrococcus abyssi*) encodes transketolase required for erythrose 4-phosphate synthesis, many archaea, amongst them haloarchaea, lack transketolase. These archaea operate an alternative pathway for aromatic amino acid synthesis starting from different precursors, 6-deoxy-5-ketofructose-1-phosphate (DKFP) and L-aspartate semialdehyde (see below) (White 2004).

The key enzyme of the reductive branch of the PP pathway for CO_2 fixation is ribulose-bisphosphate carboxylase (EC 4.1.1.39, RuBisCO). RuBisCO activity was previously detected in haloarchaea such as *H. mediterranei* but not in *H. salinarum* (Rawal et al. 1988; Rajagopalan and Altekar 1994). The CO_2 acceptor and substrate of RuBisCO, ribulose 1,5-bisphosphate is not directly synthesized from ribulose 5-phosphate in archaea, but from the purine precursor 5-phospho-D-ribosyl-1-pyrophosphate (PRPP) (Finn and Tabita 2004). The required gene for this conversion is present in all haloarchaea (e.g. NP5174A), while RuBisCO (NP2770A) is only encoded in the *N. pharaonis* genome. Recently, it has been shown that purine and pentose metabolism are connected in archaea, as archaeal type III RuBisCO is involved in an AMP recycling pathway that is present in *N. pharaonis* (see below) (Sato et al. 2007). This novel pathway might be part of a cyclic CO_2 fixation pathway in archaea consisting of (i) pentose formation (PRPP) from fructose 6-phosphate (e.g. via the RuMP pathway), (ii) conversion of PRPP and adenine to AMP by adenine phosphoribosyltransferase (EC 2.4.2.7, e.g. NP1254A, NP1426A), (iii) AMP recycling releasing adenine and 3-phosphoglycerate (and involving the CO_2 fixation step), and (iv) gluconeogenesis for the conversion of 3-phosphoglycerate back to fructose 6-phosphate (Sato et al. 2007).

Glycerol metabolism

In many hypersaline habitats, glycerol is a highly abundant carbon source that is produced by the halotolerant green algae *Dunaliella* to protect itself from osmotic pressure (Borowitzka et al. 1977; Phadwal and Singh 2003). Haloarchaea are able to catabolize the abundant glycerol through phosphorylation to glycerol 3-phosphate and subsequent formation of dihydroxyacetone phosphate (DHAP) (Rawal et al. 1988; Nishihara et al. 1999). Consistently, haloarchaeal genomes encode glycerol kinase (EC 2.7.1.30, e.g. OE3762R), the multi-subunit glycerol 3-phosphate dehydrogenase (EC 1.1.99.5, e.g. OE3763F-OE3765F) and a potential *sn*-glycerol-3-phosphate ABC transport system (e.g. OE5166F-OE5170F). The haloalkaliphile *N. pharaonis* lacks glycerol degrading enzymes, which might be due to its soda lake habitat, where *Dunaliella* is not a main primary producer. It should be noted that glycerol degradation and lipid formation occur through separate pathways in archaea, meaning that the intermediate glycerol 3-phosphate derived from glycerol catabolism is not used for the synthesis of the glycerophosphate backbone of archaeal lipids (see below) (Nishihara et al. 1999).

In *H. salinarum*, glycerol can also be converted to DHA by glycerol dehydrogenase (Rawal et al. 1988). For this reaction, a plasmid-encoded glycerol dehydrogenase (EC 1.1.1.6, OE5160F) is employed, which has been characterized and structurally elucidated (Offermann 2003; Horn 2006). The produced DHA might be phosphorylated by DHA kinase (EC 2.7.1.29) and fed into the lower EM pathway. However, potential DHA kinases genes (HQ2672A, HQ2673A) are only encoded by *H. walsbyi*, where they probably depend on a cytosolic phosphoenolpyruvate-dependent phosphotransferase system (HQ2709A) (Bolhuis et al. 2006).

Pyruvate metabolism and tricarboxylic acid cycle

Pyruvate metabolism

The central metabolite pyruvate is converted to acetyl-CoA by pyruvate-ferredoxin oxidoreductase (EC 1.2.7.1, *porAB*, OE2623R/OE2622R) (Kerscher and Oesterhelt 1981a, b) and subsequently fed into the tricarboxylic acid (TCA) cycle. NMR spectroscopy experiments for *H. salinarum* have shown that 90% of the flux is channelled into the TCA cycle via pyruvate-ferredoxin oxidoreductase (Ghosh and Sonawat 1998; Bhaumik and Sonawat 1994). The remaining 10% of the pyruvate is converted to the TCA intermediate oxaloacetate by pyruvate carboxylase (EC 6.4.1.1), in order to fill up the oxaloacetate pool of the TCA cycle, when its intermediates are drawn off for biosynthetic purposes (Ghosh and Sonawat 1998). However, haloarchaea do not encode archaeal-type pyruvate carboxylase (Mukhopadhyay et al. 2000), but biotin carboxylases that are more likely to be involved in fatty acid degradation (e.g. NP4250A/NP4252A located within a fatty acid degradation cluster NP4230A–NP4258A). *H. marismortui* and *H. walsbyi* encode a phosphoenolpyruvate carboxylase (EC 4.1.1.31, rrnAC0562, HQ3197A), which has been proposed to be involved with a novel cytosolic phosphotransferase system (pNG7387-pNG7391, HQ1667A, HQ2709A) (Bolhuis et al. 2006). During gluconeogenesis, phosphoenolpyruvate is synthesized from oxaloacetate through malic enzyme (EC 1.1.1.38/39/40, e.g. NP0132A, NP1772A) and pyruvate, water dikinase (EC 2.7.9.1, e.g. NP1196A) in haloarchaea. The former anaplerotic enzyme has been shown to be active in *H. salinarum*, while the anaplerotic reactions catalyzed by phosphoenolpyruvate carboxykinase (EC 4.1.1.32/38/49) and oxaloacetate decarboxylase (EC 4.1.1.3) are missing in haloarchaea (Bhaumik and Sonawat 1994; Ghosh and Sonawat 1998).

Under anaerobic conditions, it has been shown for *H. salinarum* that pyruvate is primarly converted to alanine, presumably by an aspartate transaminase, and to a larger extent to lactate and acetate (Bhaumik and Sonawat 1994; Ghosh and Sonawat 1998). In spite of proven lactate dehydrogenase activity in *H. salinarum* cell extracts (Bhaumik and Sonawat 1994), no clear lactate dehydrogenase homolog has been identified in haloarchaeal genomes. In *M. jannaschii*, it has been shown that lactate is produced from lactaldehyde, which might be derived from methylglyoxal (Grochowski et al. 2006a). The *M. jannaschii* lactaldehyde dehydrogenase is similar to several probable aldehyde dehydrogenases encoded in haloarchaea (e.g. NP1686A, NP3020A).

Tricarboxylic acid cycle

Although TCA cycles are highly variable and often incomplete within the archaeal domain of life (Huynen et al. 1999), haloarchaeal genomes encode the complete set of enzymes. For *H. salinarum*, activity of all these enzymes has been proven (Aitken and Brown 1969; Hubbard and Miller 1972; Kerscher and Oesterhelt 1981a, b; Gradin et al. 1985) and an operative TCA cycle has been shown by NMR spectroscopy (Ghosh and Sonawat 1998).

In haloarchaea, pyruvate and 2-oxoglutarate are not converted by classical 2-oxoacid dehydrogenase complexes but by 2-oxoacid-ferredoxin oxidoreductases encoded by *porAB* and *korAB* genes, respectively (Kerscher and Oesterhelt 1981a, b). However, haloarchaeal and other archaeal genomes further contain gene clusters encoding all components of a 2-oxoacid dehydrogenase complex. In *Thermoplasma acidophilum*, it has recently been shown that the E1 component of the encoded 2-oxoacid dehydrogenase complex accepts branched-chain 2-oxoacids (Heath et al. 2004). Most likely, the haloarchaeal 2-oxoacid dehydrogenase complex is also involved in branched-chain amino acid degradation.

When grown on acetate, *Haloferax volcanii* operates a glyoxylate bypass operon involving isocitrate lyase (EC 4.1.3.1) and a new type of malate synthase (EC 4.1.3.2) (Serrano and Bonete 2001). Homologs of both enzymes are present in the *N. pharaonis* (NP4432A, NP4430A) and *H. walsbyi* (HQ1720A, HQ3094A) genomes, while only a probable malate synthase is encoded in *H. marismortui* (rrnAC1965). *Halobacterium* lacks both glyoxylate cycle genes, although activity of both glyoxylate cycle enzymes has been demonstrated previously in this haloarchaeon (Aitken and Brown 1969).

Nucleotide metabolism

De novo synthesis of nucleotides

The complete gene sets that are required for de novo synthesis of purines from ribose 5-phosphate and for de novo synthesis of pyrimidines from carbamoylphosphate and ribose 5-phosphate are present in haloarchaeal genomes. Haloarchaea reveal an unusual domain fusion pattern of purine synthesis enzymes (Fig. 2), because they contain a unique fusion of GAR and AICAR transformylases (EC 2.1.2.2/EC 2.1.2.3, *purN/purH*, e.g. NP1662A, OE1620R). Haloarchaea do not encode the novel AICAR transformylase (*purP*) (Ownby et al. 2005) present in most archaea, but encode the novel archaeal variant of IMP cyclohydrolase (*purO*, e.g. NP0732A, OE4329F) (Graupner et al. 2002). The four haloarchaeal strains show only few differences in

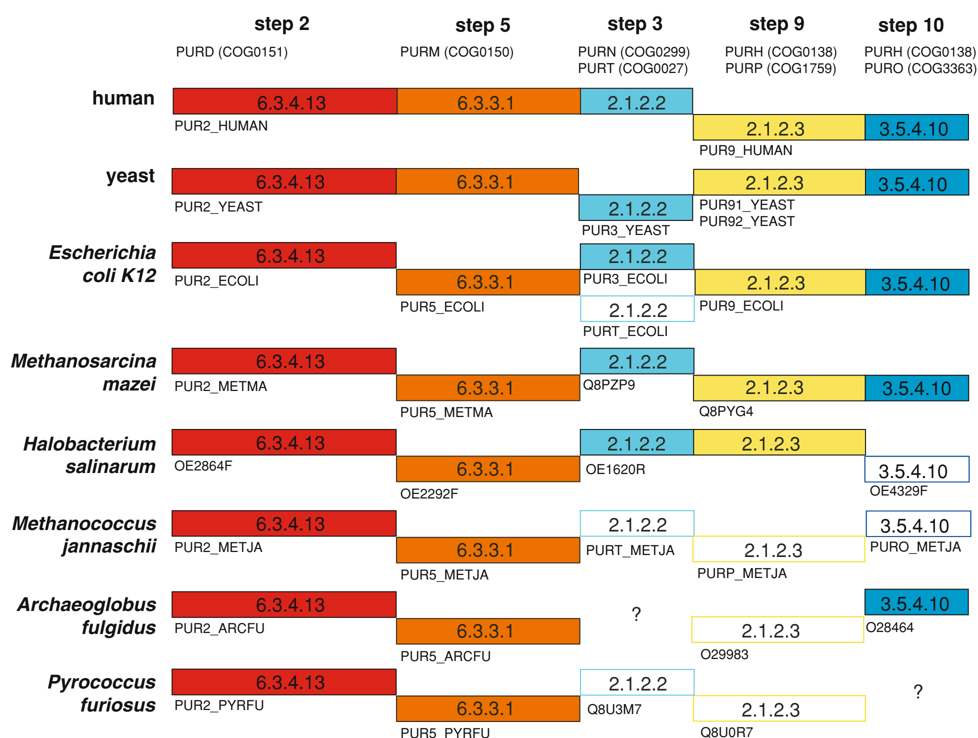

Fig. 2 Domain rearrangement of enzymes involved in the de novo synthesis of purines. The pathway comprises 10 steps from PRPP to IMP. Fusions of enzyme genes are indicated by *linked boxes*. Non-orthologous enzymes are known for steps *3*, *9*, and *10* (*unfilled boxes*) but further archaeal enzymes for purine synthesis are unknown (*question marks*)

their nucleotide metabolism, namely in the occurrence of pyrimidine kinases (EC 2.7.1.21: OE3159R, HQ1795, EC 2.7.1.48: OE2749F) (Supplementary Material S2).

Archaeal type III RuBisCO functions in AMP metabolism

Ribulose-bisphosphate carboxylase (EC 4.1.1.39, RuBis CO) is a key enzyme for CO_2 fixation via the Calvin-Benson-Bassham cycle in plants. However, RuBisCO was found to be involved in bacterial methionine cleavage and in the AMP metabolism of archaea (Sato et al. 2007). The latter pathway recycles the intracellular pool of AMP produced by ADP-dependent (AMP-forming) sugar kinases, which are involved in glycolytic pathways of archaea (Kengen et al. 1994; Selig et al. 1997). *N. pharaonis* is the only haloarchaeal species encoding all enzyme genes for this novel AMP recycling pathway, i.e. AMP phosphorylase (NP3958A), ribose-1,5-biphosphate isomerase (NP3202A), and RuBisCO (NP2770A), while *H. salinarum* exhibits only the isomerase gene (OE3610R) (Sato et al. 2007). *N. pharaonis* and some other archaea do not possess ADP-dependent sugar kinases that would produce AMP though. Instead, AMP recycling might be part of a cyclic pathway for CO_2 fixation as described earlier.

Lipid metabolism

Membrane lipids of archaea consist of glycerol diether lipids with prenyl side chains instead of diacylglycerol esters. Specifically, membranes of *H. salinarum* contain core lipids with two phytanyl side chains (C_{20}), and to lesser extents also other isoprenoid constituents such as squalenes (C_{30}), phytoenes (C_{40}), menaquinones (C_{40}), and dolichol (C_{60}) (Oesterhelt 1976; Lechner et al. 1985; Kushwaha et al. 1976) (Fig. 3). Furthermore, *H. salinarum* synthesizes several carotenoids from prenyl precursors, preferentially bacterioruberins (C_{50}) and photoactive retinal (C_{20}) (Oesterhelt 1976; Oesterhelt and Stoeckenius 1973). Retinal is incorporated into bacteriorhodopsin and other retinal proteins, which are unique to haloarchaea within the archaeal domain of life. Although fatty acids are not part of archaeal membrane lipids, small amounts of fatty acids (C_{14}, C_{16}, C_{18}) have been detected in membrane proteins of *H. salinarum* (Pugh and Kates, 1994). Other haloarchaeal species likely possess similar membrane constituents as *H. salinarum* because they have the same gene set for the de novo synthesis of isoprenoids. However, specific prenyl-based compounds might vary from species to species, as in the case of diether core lipids found in haloalkaliphiles, e.g. *N. pharaonis* (C_{20}–C_{20} and C_{20}–C_{25} prenyl chains) (Tindall et al. 1984).

Prenyl side chains of membrane lipids and other isoprenoids are derived via the mevalonate pathway in haloarchaea (Ekiel et al. 1986), while the glycerophosphate backbone of membrane lipids is formed from glycerol 1-phosphate. This membrane precursor is derived from the glycolytic intermediate DHAP via glycerol-1-phosphate dehydrogenase (EC 1.1.1.261, e.g. NP4492A), which is ubiquitously found in archaea (Nishihara et al. 1999).

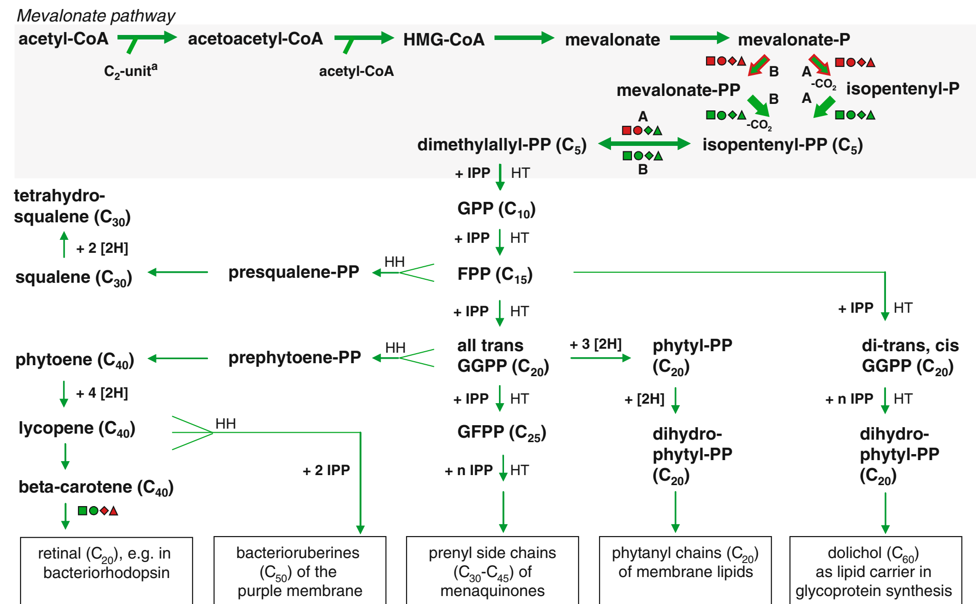

Fig. 3 Biosynthesis of isoprenoids in halophilic archaea. The isoprenoid precursor IPP is synthesized via the mevalonate pathway as shown by labeling studies (*green* reaction exists, *red* reaction absent, *bold* experimental verification). Various isoprenoids detected in membranes of *H. salinarum* (listed in *boxes*) are synthesized by a series of condensation reactions with IPP, which is added in head–tail (HT) or head–head (HH) fashion, and through desaturase reactions ([2H]). Enzyme gene sets for isoprenoid synthesis differ only slightly between halorarchaea (square: *H. marismortui*, circle: *H. walsbyi*, diamond: *N. pharaonis*, triangle: *H. salinarum*, *green* gene exists, *red* gene absent). Bacterial- ([B]) or archaeal-type ([A]) enzyme variants are indicated. *Superscript "a"* indicates C_5-prenyl units are synthesized via the mevalonate pathway starting from two acetyl-CoA molecules and a still unknown C_2-unit arising from amino acid degradation (Ekiel et al. 1986)

De novo synthesis of isoprenoids (mevalonate pathway)

Like other archaea, halophiles synthesize activated C_5-units [dimethylallyl and isopentenyl diphosphate (IPP)] for polycondensation of prenyl chains via the mevalonate pathway (Fig. 3). A previous comparative analysis of the mevalonate pathway (Smit and Mushegian 2000) identified gaps for three pathway steps in archaea, namely the lack of bacterial-type phosphomevalonate kinase (EC 2.7.4.2, COG3890), diphosphomevalonate decarboxylase (EC 4.1.1.33, COG3407), and IPP isomerase (EC 5.3.3.2, COG1443). Recently, an alternative type II IPP isomerase has been identified in archaea, which belongs to the same COG1304 as lactate dehydrogenase (Barkley et al. 2004). Furthermore, it has been suggested that mevalonate phosphate is first decarboxylated and then phosphorylated to synthesize IPP, while phosphorylation of mevalonate phosphate precedes the decarboxylation step in bacteria (Grochowski et al. 2006b). The archaeal-specific reactions involve a predicted phosphomevalonate decarboxylase (COG1355) and a characterized isopentenyl phosphate kinase (COG1608).

Interestingly, haloarchaea may operate a chimeric mevalonate pathway (Fig. 3). Like other archaea, they encode isopentenyl phosphate kinase (e.g. NP2852A) instead of a bacterial phosphomevalonate kinase. However, haloarchaea lack the proposed archaeal phosphomevalonate decarboxylase gene, and, instead, encode a bacterial-type diphospho-mevalonate decarboxylase (e.g. NP1580A). Thus, neither the classical bacterial nor the proposed archaeal mevalonate pathway is complete. For the last mevalonate pathway step, only *N. pharaonis* and *H. salinarum* encode an archaeal-type II IPP isomerase (e.g. NP0360A), while all four haloarchaea possess a bacterial-type IPP isomerase (e.g. NP4826A). Future investigations are needed in order to clarify whether archaeal and bacterial enzymes are employed simultaneously in haloarchaea. The acquired bacterial enzymes might possibly have a higher substrate specificity or turnover for covering increased isoprenoid demands for retinal and bacterioruberin biosynthesis.

A functional mevalonate pathway has been verified for *H. salinarum* by labeling studies (Ekiel et al. 1986), which lead to the proposal of an unusual first step. Lipid labeling patterns indicated that mevalonate is not synthesized from three activated acetate precursors but rather from two acetyl-CoA molecules and an unknown C_2-unit. The latter is not derived from acetate but from degraded amino acids such as lysine. C_5-isoprenoid precursors derived via the mevalonate pathway are elongated to *trans*- and *cis*-polyprenyl chains in head-to-tail fashion by (E)- and (Z)-prenyltransferases, respectively (E: NP3696A, NP4556A,

NP0604A, Z: NP4550A, NP4544A). Exact chain-specificity of prenyltransferase orthologs needs to be determined experimentally, but the biosynthesis of dolichol and menaquinones found in *H. salinarum* requires prenyltransferases with long-chain specifities. Potential enzymes for squalene and phytoene synthesis through head-to-head condensation are also encoded in haloarchaeal genomes.

Synthesis of carotenoids and retinal

For carotene biosynthesis, phytoene is reduced to lycopene by phytoene desaturase that is encoded in haloarchaeal genomes (e.g. NP4764A, NP0204A). Lycopene is the branching point for the synthesis of β-carotene (C_{40}) and bacterioruberins (C_{50}) (Oesterhelt 1976). The reactions that lead to bacterioruberins have not yet been elucidated in detail, but lycopene cyclase activity (e.g. NP0652A) converting lycopene to β-carotene has been shown for *H. salinarum* (Peck et al. 2002). The derived β-carotene is cleaved by β-carotene mono-oxygenase into two retinal molecules (C_{20}), which are incorporated in haloarchaeal rhodopsins. *H. walsbyi* encodes two cyanobacterial-like (HQ2381A, HQ2020A) and one plant-like β-carotene mono-oxygenase homologs (HQ3007A), and *H. marismortui* acquired a cyanobacterial-like β-carotene mono-oxygenase gene (pNG7272) on one of its plasmids (Bolhuis et al. 2006). *H. salinarum* and *N. pharaonis* lack β-carotene mono-oxygenase homologs and must therefore possess a still unknown non-orthologous enzyme for the oxidative cleavage of β-carotene. Previously, *brp* and *blh* have been shown to play a role in regulation or synthesis of retinal (Peck et al. 2001).

Fatty acid metabolism

Even-numbered fatty acids like palmitic and stearic acid are part of membrane proteins but not of membrane lipids in archaea (Pugh and Kates 1994). For example, palmitic acid is associated with halorhodopsin as a free fatty acid in *H. salinarum* (Kolbe et al. 2000). The origin of these fatty acids is unclear because archaea do not encode components of a fatty acid synthase complex. In methanogenic archaea, biosynthesis of fatty-acid-like compounds from 2-oxoglutarate by repeated 2-oxoacid chain elongation has been reported (White 1989).

For the degradation of activated fatty acids via the ß-oxidation pathway, gene candidates are present in haloarchaea and most other archaea. However, since chain-length specifity of these enzymes is currently unknown, fatty acid degradation might still be limited to short chain lengths (e.g. for derivatives of branched-chain amino acids). A series of genes were assigned for *Natronomonas* (37 genes) and *Haloarcula* (29 genes) for the repeated four-step reaction sequence of the ß-oxidation pathway indicating a versatile fatty-acid metabolism in these two species. In contrast, few ß-oxidation genes are present in *H. salinarum* (12 genes) and *H. walsbyi* (6 genes). In accordance to these findings, growth experiments showed that *N. pharaonis* is able to grow on fatty acids of various lengths as carbon source (especially C_{14}), while fatty acids seem not to be utilized by *H. salinarum*, as growth is reduced or diminished by long-chain (C_{14}–C_{18}) and medium-chain fatty acids ($<C_{14}$), respectively (Konigsmaier 2006).

Haloarchaea likely degrade odd-numbered fatty acids, because they possess probable propionyl-CoA carboxylase (EC 6.4.1.3, e.g. NP4250A/NP4252A), methylmalonyl-CoA epimerase (EC 5.1.99.1, e.g. NP1228A) as well as methylmalonyl-CoA mutase (EC 5.4.99.2, e.g. NP1226A, NP2710A). *Natronomonas* further encodes enzymes that are probably involved in propionate catabolism (NP6212A, NP4432A, NP4820A).

Amino acid synthesis

While certain haloarchaea such as *Haloarcula hispanica* (Hochuli et al. 1999) do not require any amino acids for growth, *H. salinarum* is grown in synthetic media with 10 to 15 amino acids (Oesterhelt and Krippahl 1973; Grey and Fitt 1976). Through metabolic pathway reconstruction and comparison, it can be proposed that *H. salinarum* has indeed reduced capabilities to synthesize amino acids. Specifically, *H. salinarum* lacks gene clusters for valine, leucine, isoleucine, lysine, and arginine (Supplementary Material S2). Furthermore, because of reduced folate biosynthesis, methionine biosynthesis via folate-dependent methionine synthase (EC 2.1.1.14) might be affected in *H. salinarum*. The predicted set of essential amino acids fits well to the set of amino acids that can be sensed by *H. salinarum* (Oren 2002) except for lysine, which is not an attractant signal, and for cysteine, which is sensed by BasT (Kokoeva et al. 2002) in spite of the fact that all enzyme genes for cysteine biosynthesis are present in its genome. The synthesis of several amino acids (glutamate, glutamine, proline, aspartate, asparagine, alanine, serine, phenylalanine, tryptophan, histidine) by *H. salinarum* has been verified by NMR labeling studies (Ekiel et al. 1986; Bhaumik and Sonawat 1994; Ghosh and Sonawat 1998; Engelhard et al. 1989) (Fig. 1).

For *H. marismortui*, *N. pharaonis*, and *H. walsbyi*, complete independence from supplemented amino acids can be concluded from comparative analysis (Supplementary Material S2). De novo synthesis of all amino acids has

already been proven for *N. pharaonis* by the development of a synthetic medium without amino acid supplements (Falb et al. 2005; Oberwinkler 2006). The three haloarchaea that do not require amino acids are also able to utilize several nitrogen sources because they posess gene sets required for urea conversion as well as for nitrate and ammonia assimilation (Falb et al. 2005) (Supplementary Material S2). Amino acid biosynthesis pathways of the three haloarchaea show only few differences, i.e. for proline, lysine, serine, and glycine synthesis (see below).

Glutamate family (glutamate, glutamine, proline, arginine)

The biosynthesis of glutamate from the TCA cycle intermediate 2-oxoglutarate is a major metabolic conversion in haloarchaea as shown by NMR labeling studies in *Halobacterium*. Labeled pyruvate, alanine, acetate, and glycerol were mainly found to be incorporated into glutamate, and a considerable part of the flux through the TCA cycle was shown to be channelled to glutamate (Ghosh and Sonawat 1998; Ekiel et al. 1986). Three paralogous glutamate dehydrogenase genes were found in *Natronomonas* (NP1582A, NP1806A, NP6184A), *Halobacterium* (OE1270F, OE1943F, OE2728R), and *Haloarcula* (rrnAC0384, rrnAC0775, pNG7157), but only one gene in *Haloquadratum* (HQ1880A). The activity of two *Halobacterium* glutamate dehydrogenases with $NADP^+$ (OE1943F) and NAD^+ (OE1270F) cofactor specificity has been investigated in detail (Bonete et al. 1987, 1989, 1990; Perez-Pomares et al. 1999; Hayden et al. 2002). In *N. pharaonis*, *H. walsbyi*, and *H. marismortui*, glutamate is also derived by glutamate synthase (e.g. NP1794A) from 2-oxoglutarate and glutamine, a reaction that is part of a proposed ammonia assimilation pathway (e.g. NP4224-NP4228A) (Falb et al. 2005). It should be noted that haloarchaeal cells amass glutamate in high concentrations as a compatible osmolyte (Kokoeva et al. 2002; Desmarais et al. 1997).

Glutamine is derived from glutamate by glutamate-ammonia ligase (EC 6.3.1.2, e.g. NP4376A) which is present in all haloarchaea. Glutamate might further be converted to L-glutamate 5-semialdehyde and subsequently to proline. The required enzymes encoded by the *proCBA* gene cluster are only found in the genomes of *Natronomonas* (NP3974A-NP3978A) and *Haloquadratum* (HQ1844A-HQ1846A), but are absent in *Halobacterium* and *Haloarcula*. Biosynthesis of proline by *H. salinarum* has been shown previously (Ghosh and Sonawat 1998) indicating an alternative pathway in the latter two haloarchaea. Proline might be synthesized via 1-pyrroline-5-carboxylate dehydrogenase (EC 1.5.1.2) and proline dehydrogenase (EC 1.5.99.8, *putA*). This notion is supported by the fact that the phylogenetic profiles of *putA* is complementary to that of the *proABC* cluster, i.e. *putA* is only encoded in *H. salinarum* (OE3955F) and *H. marismortui* (rrnAC2471), but is missing in *N. pharaonis* and *H. walsbyi*. Proline might also be derived by the cyclisation of ornithine as shown for *M. jannaschii* (Graupner and White 2001). Homologs of ornithine cyclodeaminase (EC 4.3.1.12) are encoded in all haloarchaea (e.g. NP0448, NP3802A).

Glutamate is also a precursor of ornithine, which is converted to arginine by urea cycle enzymes. The genomes of *Natronomonas, Haloquadratum*, and *Haloarcula* possess *argXCBDEF* clusters (e.g. NP5258A-NP5268A) containing all required enzymes (except the *argA* gene) for the de novo synthesis of ornithine as well as a probable transcription regulator ArgX. The complete gene set for arginine biosynthesis is absent in *Halobacterium*. Instead, halobacterial arginine requirements are covered by the uptake of external arginine via a verified arginine-ornithine antiporter (OE5204R) (J. Tittor, unpublished results) encoded next to the arginine deiminase pathway cluster on plasmid PHS3.

Aspartate family (aspartate, alanine, asparagine, threonine, methionine, lysine)

Aspartate is derived from the TCA cycle intermediate oxaloacetate by aspartate transaminase (EC 2.6.1.1) of which several paralogs are present in haloarchaeal genomes (e.g. NP0824A, NP1666A, NP4024A, and NP4410A). These seem to be also involved in pyruvate transamination to alanine since experiments showed that the transaminase, which is involved in alanine synthesis in *Halobacterium*, uses aspartate instead of glutamate as amino group donor (Bhaumik and Sonawat 1994). In agreement with this, no clear orthologs of glutamate-pyruvate transaminase (EC 2.6.1.2) have been found in haloarchaeal genomes.

Labeling studies showed that aspartate is converted to asparagine in*H. salinarum* (Engelhard et al. 1989), and, consistently, all haloarchaeal genomes encode asparagine synthase (EC 6.3.5.4, e.g. NP2978A). In a pathway analogous to proline synthesis from glutamate, aspartate is likely to be converted to L-aspartate semialdehyde. This compound is not only a precursor of threonine, methionine, and lysine in archaea, but is also required for aromatic amino acid synthesis via a modified shikimate pathway (see below). Enzyme genes for threonine biosynthesis from L-aspartate semialdehyde via L-homoserine and *O*-phospho-L-homoserine are present in all haloarchaea (e.g. NP0302A, NP4524A, NP5280A). In *M. jannaschii*, *O*-phospho-L-homoserine is not only converted to threonine but also to homocysteine (White 2003). Additionally,

haloarchaea might synthesize homocysteine from homoserine via *O*-acetyl-L-homoserine (but not via *O*-succinyl-L-homoserine). This pathway requires clustered metX and metY genes (e.g. NP0280A-NP0284A) in case of direct sulfhydrylation of *O*-acetyl-L-homoserine. Alternatively, *O*-acetyl-L-homoserine could be converted to cystathionine and subsequently to homocysteine by thiol-lyase/-synthase (e.g. NP4746A). In the final step, methionine is likely derived from homocysteine by cobalamine-independent methionine synthase (EC 2.1.1.14, *metE*), of which two to three paralogs are found in haloarchaea (NP3670A/NP3672A) except Halobacterium (OE2668R). Since methionine synthase depends on the availability of folate, reduced folate synthesis in *H. salinarum* might also affect its synthesis of methionine.

The diaminopimelate (DAP) pathway for the synthesis of lysine has already been proven for *H. hispanica* (Hochuli et al. 1999). Enzymes for the DAP pathway are present in *Natronomonas, Haloquadratum*, and *Haloarcula*, i.e. the *dapABD* gene cluster (e.g. NP1490A-NP1494A, *dapC* is synonym with *argD*) as well as *lysA* (e.g. NP1646A) and *argG* genes (e.g. NP5252A). Gaps within the DAP pathway (*dapE, dapF*) occuring in *Haloarcula* and *Natronomonas* are likely to be bridged by still unknown non-orthologous enzymes. *Halobacterium* is not capable of lysine biosynthesis because it does not encode any of the *dap* genes.

Serine family (serine, glycine, cysteine)

Serine biosynthesis might occur via two different pathways. Within the phosphorylated pathway, glycerate 3-phosphate, a glycolytic intermediate, is oxidized to 3-phospho-hydroxy-pyruvate, which is further converted to phosphoserine and to serine by transaminase and phosphatase activity. Alternatively, non-phosphorylated glycerate is directly oxidized to hydroxypyruvate and then transaminated. All four haloarchaea possess genes for phosphoglycerate dehydrogenase (EC 1.1.1.95, *serA*, e.g. NP0272A) and phosphoserine phosphatase (EC 3.1.3.3, *serB*, e.g. NP0274A) of the phosphorylated synthesis pathway. However, the enzyme gene for the intermediate pathway step, phosphoserine transaminase (EC 2.6.1.52, *serC*), is missing. Nevertheless, studies in *Methanococcales* revealed that broad-specificity class V aspartate transaminases (e.g. NP0884A, NP2578A) catalyze this reaction (Helgadottir et al. 2007). Apart from the phosphorylated serine synthesis pathways, *Natronomonas, Haloquadratum*, and *Haloarcula* might also operate the non-phosphorylated pathway because these species encode a probable hydroxypyruvate reductase (EC 1.1.1.81, e.g. NP1162A) for the initial step from glycerate to hydroxypyruvate. One of the class V aspartate transaminases might then catalyze the transaminase reaction from hydroxypyruvate to serine.

Glycine is potentially derived from serine by glycine hydroxymethyltransferase (EC 2.1.2.1, e.g. NP2050A) in all haloarchaea. *Halobacterium* might additionally synthesize glycine from threonine employing threonine aldolase (EC 4.1.2.5, OE4436R), which is unique to the archaeal domain of life. *H. salinarum* would thus be adapted to its reduced folate synthesis (see below) as the folate-dependent glycine hydroxymethyltransferase is circumvented. All haloarchaea except *H. walsbyi* encode components of the glycine cleavage system (EC 1.4.4.2) which is encoded in a single gene cluster in *Halobacterium* (OE3274R-OE3278R). Future investigations might elucidate whether metabolic fluxes differ for the serine metabolism of *Halobacterium* and the other haloarchaea.

Similar to the conversion of homoserine to homocysteine via *O*-acetyl-L-homoserine, serine is likely to be converted in all halobacteria to *O*-acetyl-serine (*cysE*, e.g. NP4172A) and then to cysteine by the incorporation of sulfide (*cysK*, e.g. NP4748A). An alternative cysteine biosynthesis pathway employed by *M. jannaschii*, which lacks *cysE* and *cysK* genes, starts from homocysteine and phosphoserine and leads via cystathionine to cysteine (White 2003). This pathway requires thiol-lyases and might be also operative in haloarchaea. Recently, also a tRNA-dependent synthesis pathway has been reported for *M. jannaschii* that leads from phosphoserine, an intermediate of the phosphorylated serine pathway, to Cys-tRNA (Sauerwald et al. 2005). Similar tRNA-dependent pathways have been suggested for glutamine and asparagine synthesis from glutamate and aspartate (Di Giulio 2003). For cysteine biosynthesis, sulfide needs to be assimilated from sulfate via adenylylsulfate, 3P-adenylylsulfate, and sulfite. Although archaea encode a gene for the small subunit (*cysD*) of the sulfate adenylyltransferase (EC 2.7.7.4, e.g. NP4570A), they lack the GTPase subunit (*cysN*). Genes for the two subsequent sulfate assimilation steps (*cysC, cysH*) are present in some archaea (e.g. *Aeropyrum pernix*) but are not present in any of the haloarchaeal genomes. Alternatively, thiosulfate might be converted to sulfite by thiosulfate sulfurtransferase [EC 2.8.1.1, e.g. NP3186A, NP4004A (N-terminus)] and subsequently to sulfide by one of the potential sulfite reductases [e.g. NP4004A (C-terminus), not in *H. salinarum*].

Biosynthesis of branched chain amino acids (valine, leucine, isoleucine)

All enzyme genes for the de novo synthesis of valine, leucine, and isoleucine are present in *Natronomonas,*

Haloquadratum, and *Haloarcula* but not in *Halobacterium*. Threonine-ammonia lyase (EC 4.3.1.19, e.g. NP1076A), which is required to synthesize the isoleucine precursor 2-oxobutyrate, is encoded in all haloarchaea. The threonine pathway leading to isoleucine is employed by *H. hispanica* (56% flux) but it is simultaneuosly operated together with the pyruvate pathway leading via citramalate to isoleucine (44% flux) (Hochuli et al. 1999). However, no sequences are yet available in public repositories for enzymes of this pathway, e.g. citramalate lyase (EC 4.1.3.22). A third potential pathway from glutamate to isoleucine via methylaspartate (mesaconate pathway) is not employed by *H. hispanica* (Hochuli et al. 1999). Mesaconate pathway genes (*mamABC*) were found in the genomes of *H. salinarum* and *H. marismortui*, however (see below). In *Halobacterium*, 2-oxobutyrate may further be derived from methionine by methionine gamma-lyase (EC 4.4.1.11), a reaction that releases volatile methanthiol from the cells (Nordmann et al. 1994). The fact that *Halobacterium* encodes enzyme genes for 2-oxobutyrate synthesis but lacks other isoleucine synthesis enzymes implies that 2-oxobutyrate is a precursor for further metabolic pathways in haloarchaea.

Biosynthesis of aromatic amino acids (phenylalanine, tyrosine, tryptophan, histidine)

The complete gene set for the classical shikimate pathway, where 3-dehydroquinate is derived from erythrose 4-phosphate (C_4) and phosphoenolpyruvate (C_3), is only present in *Thermoplasma* spp. and some other archaea (Soderberg 2005). In methanogenic archaea, 3-dehydroquinate is synthesized via a novel pathway starting from L-aspartate semialdehyde (C_4) and 6-deoxy-5-ketofructose-1-phosphate (DKFP, C_6) (White 2004). The DKFP pathway is also employed by haloarchaea as shown by the incorporation of labeled aspartate into the C11-position of tryptophan in *H. salinarum* (Engelhard et al. 1989). Furthermore, the two novel DKFP pathway enzymes for alternative 3-dehydroquinate synthesis, 2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate synthase (COG1830 together with DhnA-type fructose bisphosphate aldolase; e.g. NP3160A) and 3-dehydroquinate synthase type II (COG1465, e.g. NP2238A) (Porat et al 2006) are encoded in haloarchaeal genomes.

The precursor of the modified shikimate pathway, DKFP, presumably derives from methylglyoxal rather than from nucleoside diphosphate sugars in archaea (White and Xu 2006). Methylglyoxal is not created by methylglyoxal synthase (EC 4.2.3.3), which is absent in archaea, but from glyceraldehyde 3-phosphate using triosephosphate isomerase (EC 5.3.1.1, e.g. NP2182A, NP3716A). Another glycolytic enzyme, a multifunctional class I fructose-biphosphate aldolase (e.g. NP1594A) catalyzes the subsequent transaldolase reaction of methylglyoxal with fructose 1,6-diphosphate (or fructose 1-phosphate) to DKFP.

The remaining biosynthesis steps from 3-dehydroquinate to chorismate follow the classical shikimate pathway in haloarchaea. Enzyme genes for biosynthesis of tryptophan, tyrosine, and phenylalanine from chorismate are also present in haloarchaeal genomes. Consistent with this, de novo synthesis of phenylalanine and tryptophan has been proven for *H. salinarum* (Ekiel et al. 1986; Engelhard et al. 1989). A second synthesis pathway for aromatic amino acids in archaea has recently been described for *M. maripaludis* (Porat et al. 2004), where aromatic amino acids are synthesized through the incorporation of exogenous aryl acids catalyzed by a ferredoxin-dependent indolepyruvate oxidoreductase (*ior*). This pathway is presumably not present in halophilic archaea, because their only two ferredoxin-dependent oxidoreductases have been already assigned otherwise (*porAB*, *korAB*) (Kerscher and Osterhelt 1981a, b).

Histidine biosynthesis genes are partly clustered within haloarchaeal genomes. All enzymes for histidine biosynthesis starting from ribose 5-phosphate are encoded except for histidinol-phosphatase (EC 3.1.3.15). An unknown alternative enzyme is indicated for the missing pathway step, especially since histidine biosynthesis has already been proven experimentally in *H. salinarum* (Ekiel et al. 1986).

Amino acid degradation

Glutamate and aspartate degradation

Glutamate and aspartate are major carbon substrates for halophilic archaea that are fed into the TCA cycle and subsequently into the respiratory chain for ATP production. Several transaminases are encoded in haloarchaea which likely catalyze the conversion of aspartate, glutamate, and other amino acids to TCA cycle intermediates. In *H. salinarum* and *H. marismortui*, glutamate might further be degraded to mesaconate by methylaspartate mutase (EC 5.4.99.1) and methylaspartate ammonia-lyase (EC 4.3.1.2). Genes for the mesaconate pathway (*mamABC*, OE4204F-OE4207F, rrn0684-rrn0687) are only encoded in the two haloarchaea and very few bacteria, i.e. two *E. coli* strains as well as a *Clostridium* and *Treponema* species. In thermophilic anaerobic bacteria, conversion of mesaconate to citramalate and subsequently to pyruvate and acetate has been proven (Plugge et al. 2001). However, the respective

enzymes for the established reactions are not yet known. Mesaconate is also a potential precursor of isoleucine in haloarchaea, although not for the haloarchaeon *H. hispanica* (Hochuli et al. 1999).

Arginine metabolism

Urea cycle genes for the conversion of ornithine to arginine were found in all halophilic strains, but arginase (EC 3.5.3.1, rrnAC0383, rrnAC0453) converting arginine back to ornithine is only present in *H. marismortui*. Instead, *H. salinarum* metabolizes arginine to ornithine with concomitant ATP production via the arginine deiminase pathway (Hartmann et al. 1980). This pathway is rare amongst prokaryotes, and *H. salinarum* is the only archaeon that has acquired an arginine deiminase gene cluster (*arcRACB*, OE5205R-OE5209R, plasmid PHS3) (Ruepp and Soppa 1996) encoding all required arginine deiminase pathway enzymes as well as a probable transcription regulator ArcR. The gene for an arginine-ornithine antiporter (OE5204R) (J. Tittor, unpublished results) is also located next to the arginine deiminase gene cluster.

Archaea also utilize arginine for the biosynthesis of polyamines such as putrescine, spermidine, or spermine (Graham et al. 2002b), which seem to be required in archaea for nucleosome maintenance in high-temperature environments (Higashibata et al. 2000). Orthologs of a novel arginine decarboxylase (EC 4.1.1.19, e.g. NP4484A) (Graham et al. 2002b) and agmatinase (EC 3.5.3.11, e.g. NP3022A, NP4754A) for the conversion of arginine to putrescine are present in haloarchaeal genomes. For pathway steps leading from putrescine to spermidine, no enzyme genes are encoded in halophilic archaea.

Branched-chain amino acid degradation

For amino acids with a more complex carbon scaffold, i.e. branched-chain and aromatic amino acids, separate biosynthesis and degradation pathways, regulated independently from each other, have evolved. Catabolism of valine, leucine, and isoleucine first involves a transaminase (e.g. NP5036A) for the conversion of branched-chain amino acids to the respective 2-oxoacids. The following decarboxylation is likely catalyzed by a previously unassigned 2-oxoacid dehydrogenase multienzyme complex (e.g. OE4113F-OE4116F), whose E1 component has recently been shown to accept branched-chain 2-oxoacids in T. acidophilum (Heath et al. 2004). The derived activated fatty acids are likely to be further degraded to acetyl-CoA and propionyl-CoA.

Aromatic amino acid degradation

Histidine is likely to be degraded to glutamate via the urocanate pathway by *H. salinarum* and *H. marismortui* encoding *hutUGIH* gene clusters (e.g. OE2734F–OE2739F). The two haloarchaea (and *A. pernix*) also acquired the only archaeal tryptophanases (EC 4.1.99.1, OE4331R, rrnAC2439) and *H. salinarum* the only archaeal kynureninase (EC 3.7.1.3, OE2332F) for potential indole or anthranilate formation from tryptophan, respectively. Further, haloarchaea (except *H. walsbyi*) encode orthologs (e.g. NP1194A) of the recently described L-tyrosine decarboxylase (EC 4.1.1.25) (Kezmarsky et al. 2005), which is required for synthesis of methanofuran in *M. jannaschii*. Complete aromatic amino acid degradation pathways could not be reconstructed but probable aromatic compound dioxygenases (COG0346, e.g. NP2650A) are encoded in all haloarchaeal genomes.

Cofactor metabolism

Bacteria and archaea are commonly able to synthesize cofactors de novo. However, the relevant biosynthesis pathways are often not completely understood, i.e. early pathway steps leading to biotin and thiamine. In fact, many genes (e.g. *thiI, moaA*) which have been associated with coenzyme biosynthesis have not yet been assigned to specific metabolic reactions. Upon metabolic reconstruction, archaea reveal many gaps where enzyme genes are replaced by still unknown non-orthologous genes. Although some novel enzymes have been elucidated in recent years, e.g. novel classes of GTP cyclohydrolases (e.g. NP4142A, NP2514A) (Graham et al. 2002a; El Yacoubi et al. 2006; Grochowski et al. 2007), several pathway gaps in archaeal coenzyme metabolism remain to be filled. Taking these limitations into account, de novo synthesis pathways for common cofactors are likely in haloarchaea.

The compared haloarchaeal genomes show surprisingly different gene sets for cofactor synthesis pathways, and might have differing synthetic capabilities for coenzymes (Supplementary Material S2). *N. pharaonis* has the most complete set of cofactor synthesis genes and has been shown to grow independently of cofactors (Falb et al. 2005). It is capable of synthesizing menaquinone, coenzyme A, tetrahydrofolate (THF), molybdopterin, hemes, cobamide, flavins, nicotinamide derivates (NAD^+/$NADP^+$), biotin, thiamine, and pyridoxal 5-phosphate. The latter is presumably synthesized by novel pyridoxal 5-phosphate synthesis enzymes (*pdxS/pdxT*, e.g. NP4528A, NP0464A) like in *B. subtilis* (Raschle et al. 2005). While most enzymes for biotin synthesis are encoded in *N. pharaonis* (*bioA* absent) and *H. marismortui* (*bioA* and *bioD* absent),

H. salinarum and *H. walsbyi* lack the complete biotin synthesis gene set. Thiamine biosynthesis pathways might also differ amongst haloarchaea because a couple of probable thiamine biosynthesis genes found in *N. pharaonis* are partly missing in other haloarchaea, i.e. *thiM* (NP4052A), *thiE* (NP4054A), and *tenA* (NP4080A, NP4082A). For the latter two enzymes genes, there are non-orthologous variants in haloarchaea (including *N. pharaonis*), *tenA* is analogous to *thiC* (NP2210A) and *thiN* (NP5168A, NP0546A (fused to *thiD*)) to *thiE* (Morett et al. 2003). Finally, gene sets for folate metabolism differ greatly between various haloarchaeal strains (see below) leading to the reduced folate synthesis capability in *H. salinarum* (Levin et al. 2004). Consistent with these findings, biotin, thiamine, and folate are supplied to the synthetic medium of *Halobacterium* (Oesterhelt and Krippahl 1973).

The utilization of several cofactors has been confirmed in haloarchaea such as the use of nicotinamide derivates by glutamate dehydrogenases (NAD^+: OE1270F, $NADP^+$: OE1943F) (Hayden et al. 2002), the association of coenzyme A and ferredoxin to pyruvate- and 2-oxoglutarate ferredoxin oxidoreductases (OE1710R, OE1711R, OE2622R, OE2623R) (Kerscher and Oesterhelt 1981a, b) and the incorporation of flavin in dodecin (OE3073R) (Bieger et al. 2003). Respiratory chains of haloarchaea further involve menaquinone and certain hemes (Oesterhelt 1976; Sreeramulu et al. 1998; Scharf et al. 1997; Mattar and Engelhard 1997; Falb et al. 2005). Several copies of proteinaceous cofactors, e.g. ferredoxin, thioredoxin, halocyanin (Mattar et al. 1994), and Fe-S proteins, were also found in haloarchaeal genomes. These are likely used in various redox reactions.

Heme and cobamide synthesis

Menaquinone (*men*) and heme (*hem*) biosynthesis gene clusters are present in all haloarchaeal genomes. All heme synthesis genes for steps leading from glutamate to uroporphyrinogen III have been found, but genes for successive modifications of the porphyrin system to protoheme (e.g. by *hemE*) are absent as in most other archaea (Supplementary Material S3). Thus, an alternative heme biosynthesis pathway via precorrin-2 as shown for *M. barkeri* seems likely (Buchenau et al. 2006). A novel archaeal variant of heme A synthase (COG1612) (Lewin and Hederstedt 2006) for the conversion of heme O to heme A is present in haloarchaea (e.g. NP1770A, OE3306R).

Halobacterium assimilates corrinoid precursors and cobamides through a high-affinity transport system, but is also able to synthesize cobamide (coenzyme B_{12}) de novo (Woodson et al. 2003). Cobamide synthesis starting from uroporphyrinogen III has mainly been studied in bacteria, e.g. in *Pseudomonas denitrificans* (*cob* genes) which uses an aerobic pathway and in *Salmonella typhimurium* (*cbi/cob* genes) and *Bacillus megaterium* which employ an anaerobic pathway (Rodionov et al. 2003). The two pathway variants differ in their cobalt integration step occurring early in the anaerobic (*S. typhimurium cbiK, B. megaterium cbiX*) but late within the aerobic pathway (*P. denitrificans cobN*). Haloarchaea encode both cobalt chelatases, the oxygen-dependent (*cobN*, e.g. NP1092A) and the oxygen-independent type (*cbiX*, e.g. NP1108A) (Supplementary Material S3). Experimental data showed, however, that cobamides are synthesized under aerobic conditions in *Halobacterium* (Woodson et al. 2003). Haloarchaeal genomes encode most known *cob* genes but homologs of *P. denitrificans cobG, cobF*, *cobK* (*S. typhimirium cobJ*), and *cobP* (*S. typhimirium cobU)* are absent. This indicates alternative enzymes and reactions in archaeal cobalamin synthesis. For example, the bifunctional bacterial *cobP* enzyme gene (EC 2.7.1.156/2.7.7.62) is replaced by two novel genes *cbiZ* (e.g. NP5300A) and *cobY* (e.g. NP5304A) in archaea (Woodson et al. 2003; Woodson and Escalante-Semerena 2004). The novel archaeal variant of α-ribazole-5′-phosphate phosphatase (EC 3.1.3.71, *cobZ*, COG1267) (Zayas et al. 2006) for the synthesis of the cobamide α-ligand is not present in haloarchaea, however. Their genomes (except *H. salinarum*) contain the bacterial-type phosphatase gene (*cobC* in *Salmonella*, COG0406, e.g. NP1332A) encoded next to the *hemCXD* cluster (NP1326A–NP1330A) in *Natronomonas*. The cobamide α-ligand is derived by cleavage of the ribityl tail from flavin mononucleotide cofactor by BluB (COG0778) (Campbell et al. 2006), of which distant homologs are present in haloarchaea (e.g. NP0176A).

Folate synthesis

Folic acid and its derivates are synthesized from *p*-aminobenzoate (PAB) and a pteridine intermediate (Fig. 4, Supplementary Material S4). The latter is derived from GTP by a series of reactions involving *folE, folB*, and *folK* genes; all of them are missing in the archaeal domain of life except for *H. marismortui.* This species has acquired an extensive folate metabolism gene cluster located on one of its plasmid, which contains canonical GTP cyclohydrolase I (EC 3.5.4.16, *folE*, pNG7382). Recently, a novel archaeal GTP cyclohydrolase (e.g. NP2514A) producing a cyclic phosphate intermediate has been reported in archaea (El Yacoubi et al. 2006; Grochowski et al. 2007), but novel enzyme genes replacing *folB* and *folK* remain to be elucidated. PAB is usually derived from chorismate by aminodeoxychorismate synthase (EC 2.6.1.85, *pabAB*) and aminodeoxychorismate lyase (EC 4.1.3.38, *pabC*) which are clustered in

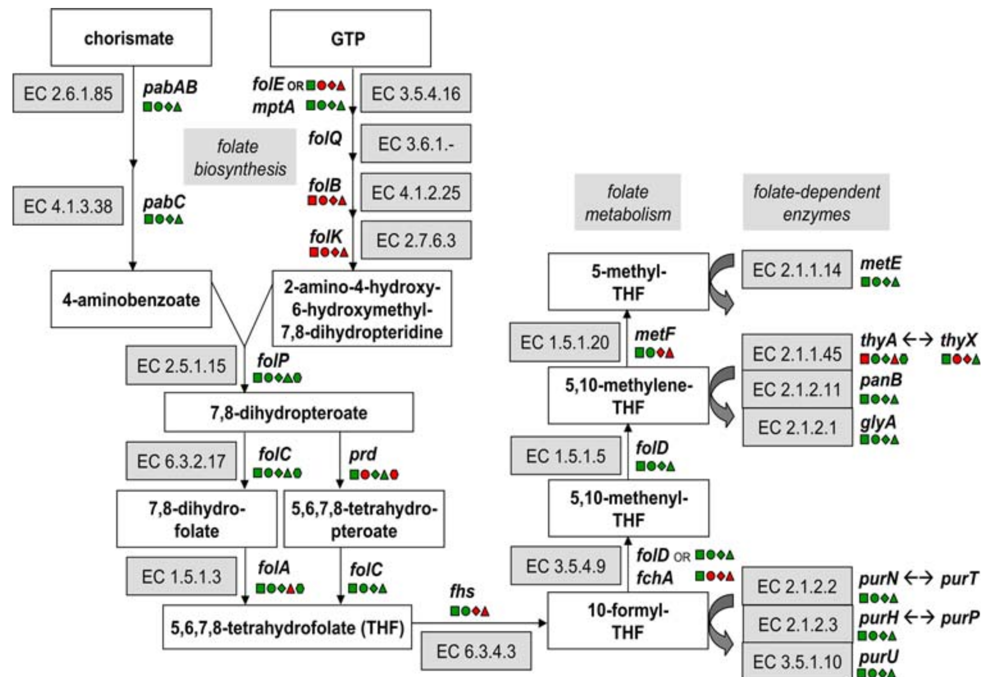

Fig. 4 Folate biosynthesis and metabolism in halophilic archaea. Sets of enzyme genes, which are involved in folate metabolism, differ considerably between haloarchaeal species (square: *H. marismortui*, circle: *H. walsbyi*, diamond: *N. pharaonis*, triangle: *H. salinarum*, hexagon: *H. volcanii*, *green* gene exists, *red* gene absent). For details see Supplementary Material S4

haloarchaeal genomes (e.g. NP0798A-NP0802A). These enzymes have previously been annotated as copies of anthranilate synthase (same COG0147/COG0512) and branched-chain amino acid aminotransferase (same COG0115), respectively. The *pabA* gene (OE1570F) of *H. salinarum* strain R1 (but not of strain NRC-1) is disrupted by an insertion element, though, so that *de novo* synthesis of folate is likely omitted in this strain. Proposed haloarchaeal *pab* genes have not yet been characterized, and it should be noted that labeling studies for *M. maripaludis* suggest the synthesis of PAB from 3-dehydroquinate and not from chorismate in this archaeon (Porat et al. 2006).

For folate synthesis, the pteridine intermediate and PAB are converted to 7,8-dihydropteroate and subsequently to 7,8-dihydrofolate by the gene products of *folP* and *folC* (Fig. 4). Dihydrofolate is then reduced to 5,6,7,8-tetrahydrofolate by dihydrofolate reductase (EC 1.5.1.3, *folA*), which is encoded in all haloarchaea (e.g. NP2922A) except *H. salinarum*. Tetrahydrofolate synthesis in *H. salinarum* occurs via a novel alternative pathway, instead, where 7,8-dihydropteroate is reduced to 5,6,7,8-tetrahydropteroate by a flavin-binding Prd linker domain of the FolC-Prd-FolP fusion protein (e.g. OE1615R, NP1478A) (Levin et al. 2004, 2007). Tetrahydropteroate is subsequently converted to tetrahydrofolate by the FolC domain.

The canonical dihydrofolate reductase FolA is not only involved in de novo synthesis of folate but is also required for the regeneration of dihydrofolate produced by thymidylate synthase (EC 2.1.1.45, *thyA*, NP2924A, HQ2456A). This enzyme is replaced by an alternative folate-independent enzyme encoded by *thyX* (OE2898R, rrnAC1121) in case a chromosomal *folA* gene is absent like in *H. salinarum* and *H. marismortui* (Supplementary Material S4). Tetrahydrofolate is likely metabolized to formyl- and methyl-THF in haloarchaea. However, only *H. walsbyi* and the *H. marismortui* plasmid pNG700 encode all required enzyme genes involved in folate coenzyme metabolism (e.g. HQ1768A, HQ2790A, HQ1756A).

Conclusions

Comparative analysis of enzyme gene sets of four halophilic archaea reveals analogous metabolic routes for the biosynthesis of nucleotides and prenyl-based lipids. Biosynthesis pathways for all proteinogenic amino acids are present for *H. marismortui*, *H. walsbyi*, and *N. pharaonis*, while *H. salinarum* has no biosynthetic capabilities for five amino acids. The loss of amino acid synthesis genes and the acquisition of a catabolic gene clusters for arginine and glutamate fermentation in *Halobacterium* might have been driven by the constant availability of external amino acids under extreme salt conditions, where few other halophiles compete for nutrients. Several pathway variations were observed for folate-dependent enzymes such as the occurrence of different types of thymidylate synthases or the circumvention of glycine hydroxymethyltransferase by threonine aldolase. These differences might be due to varying gene sets for folate metabolism, in particular the replacement of the canonical dihydrofolate reductase by dihydropteroate reductase in *H. salinarum* (Levin et al. 2004, 2007). While some haloarchaea may require

thiamine and biotin supplements, all halophilic archaea possess the required enzyme genes for the de novo synthesis of cobamide, hemes, flavin and nicotinamide nucleotides, folate, coenzyme A, menaquinone, molybdopterin, pyridoxal 5-phosphate, and several proteinaceous cofactors like halocyanin.

The compared haloarchaeal species can best be distinguished by their catabolic and central intermediary pathways. While *H. marismortui* has a versatile sugar and nitrogen metabolism, *H. walsbyi* encodes a unique sugar phosphotransferase system and *H. salinarum* is characterized by a variable energy metabolism. Its plasmids acquired arginine deiminase and cytochrome *d* oxidase gene clusters (OE6185F/OR6186F) as well as the gene for glycerol dehydrogenase. *N. pharaonis* is not able to catabolize glycerol (that is not common in its alkaline environment) and is further incapable of sugar degradation. However, the haloalkaliphilic strain encodes an archaeal-type RuBisCO that is probably involved in a novel carbon dioxide-fixation pathway (Sato et al. 2007). It appears that halophilic archaea have adopted several strategies in order to adapt to the nutritional conditions of hypersaline environments, e.g. by gain/loss of metabolic pathways, acquirement of plasmid-encoded enzymes, and utilization of secretion enzymes (Supplementary Material S2). Interestingly, haloarchaeal genomes often encode bacterial-type enzymes instead of (or alongside) non-orthologous archaeal variants, e.g. bacterial-type α-ribazole-5′-phosphate phosphatase (*cobC*), folate reductase (*folA*), and β-carotene monooxygenases. Within the mevalonate pathway, two bacterial-type enzymes (IPP isomerase, diphosphomevalonate decarboxylase) exist, which might have been acquired to cover high isoprenoid demands of haloarchaea. Future studies might elucidate whether halophilic archaea employ different enzyme sets for the synthesis of their membrane lipids and carotenes, the latter of which are unique to the haloarchaeal branch of the archaeal domain.

The compiled data on haloarchaeal metabolism present a valuable resource for future system biology approaches (Gonzalez et al. 2008), because current knowledge of enzyme variants, domain rearrangements, and enzyme substrate specifities has been included throughout. Further, many of the predicted reactions and enzymes have been linked to available experimental data from enzyme activity tests and labeling studies conducted in the model species *H. salinarum*. Some of these experimental results however disagree with genomic findings, e.g. for enzymes of the glyoxylate cycle (Aitken and Brown 1969) and the Entner-Douderoff pathway (Rawal et al. 1988) (Fig. 1). These inconsistencies might be due to still unknown non-orthologous enzymes or may result from investigating different strains of *Halobacterium*, which have been renamed several times within the past decades. Although many pathway gaps in archaeal metabolic networks have been closed in recent years, novel enzyme and pathway variants still remain to be discovered within the archaeal domain of life. In haloarchaea, pathways for ribose 5-phosphate synthesis and for retinal formation need to be investigated. A future in-depth analysis of abundant non-orthologous gene displacements in halophilic and other archaea might further give interesting insights into the evolution of metabolic pathways.

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