REVIEW ARTICLE

The unique features of glycolytic pathways in Archaea

Corné H. VERHEES*[1], Servé W. M. KENGEN*, Judith E. TUININGA*, Gerrit J. SCHUT†, Michael W. W. ADAMS†, Willem M. DE VOS* and John VAN DER OOST*[2]

*Laboratory of Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands, and †Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA 03602, U.S.A.

An early divergence in evolution has resulted in two prokaryotic domains, the Bacteria and the Archaea. Whereas the central metabolic routes of bacteria and eukaryotes are generally well-conserved, variant pathways have developed in Archaea involving several novel enzymes with a distinct control. A spectacular example of convergent evolution concerns the glucose-degrading pathways of saccharolytic archaea. The identification, characterization and comparison of the glycolytic enzymes of a variety of phylogenetic lineages have revealed a mosaic of canonical and novel enzymes in the archaeal variants of the Embden–Meyerhof and the Entner–Doudoroff pathways. By means of integrating results from biochemical and genetic studies with recently obtained comparative and functional genomics data, the structure and function of the archaeal glycolytic routes, the participating enzymes and their regulation are re-evaluated.

Key words: Archaea, Embden–Meyerhof pathway, Entner–Doudoroff pathway, glycolysis, glycolytic enzyme, polysaccharide.

INTRODUCTION

Polysaccharides are a major source of carbon to support heterotrophic growth in the three domains of life: Bacteria, Archaea and Eukaryota. Their utilization generally involves extracellular hydrolysis of polysaccharides, uptake of oligosaccharides by specific transporters and their intracellular hydrolysis to generate hexoses (e.g. glucose, galactose, mannose and fructose) and pentoses (e.g. xylose and arabinose). Subsequently, these monosaccharides are being oxidized via a well-conserved set of central metabolic pathways.

A variety of pathways is involved in the degradation of glucose to pyruvate (Schemes 1A and 1B). The Embden–Meyerhof (EM) pathway, or glycolysis, is the general route for glucose degradation in all domains of life. Some micro-organisms use an alternative pathway for glucose degradation, i.e. the Entner–Doudoroff (ED) pathway. In addition, some organisms are capable of alternative routes and bypasses in sugar degradation, include the oxidative pentose phosphate (PP) pathway (also referred to as the hexose monophosphate pathway) [1]. Established pathways for biosynthetic purposes include gluconeogenesis and the non-oxidative PP pathway (Scheme 1).

Extensive research over several decades has resulted in detailed information on the composition of sugar metabolic pathways and the regulation thereof in bacteria and eukaryotes [2,3]. Because most archaea have been discovered only 1–2 decades ago, studies addressing archaeal glycolytic pathways were first initiated in the early 1990s. The most progress has been made with hyperthermophilic archaea that grow optimally above 80 °C on a variety of sugars. The approaches taken to unravel these metabolic routes include (i) identification of fermentation end products by HPLC and ^{13}C-NMR [4], (ii) identification of intermediates of sugar metabolism following the conversion of ^{14}C-labelled glucose [5,6], (iii) enzyme-activity measurements in cell extracts [7], (iv) characterization of purified enzymes [8], and (v) molecular analysis of genes encoding glycolytic enzymes, and comparative genome analysis [9,10]. In the present review, the sugar metabolism of archaea is re-evaluated by integrating physiological, biochemical and genetic data with recent insights provided by comparative and functional genomics.

SACCHAROLYTIC ARCHAEA

Owing to the efforts of several research groups over the past decade, insight is emerging into the sugar metabolism of archaea in general and that of hyperthermophiles in particular. Several modified sugar-degrading pathways that are operational at extremely high temperatures have been identified in some of these hyperthermophiles (reviewed in [10–14]).

The Archaea comprise both autotrophic (e.g. methanogens, sulphur reducers and sulphate reducers) and heterotrophic representatives [12]. Most hyperthermophilic archaea are heterotrophs that use polypeptides as carbon and energy sources. However, a growing number of archaeal species has been shown to be saccharolytic as well, and efficient growth has been observed on various poly-, oligo- and mono-saccharides (Table 1). Among the archaea, major differences occur in their energy-transducing capacity. Several archaea have been reported to perform respiration either aerobically with oxygen as a terminal electron acceptor (e.g.

Abbreviations used: AOR, aldehyde oxidoreductase; ED, Entner–Doudoroff; EM, Embden–Meyerhof; FBA, fructose-1,6-bisphosphate aldolase; FBP, fructose-1,6-bisphosphatase; FOR, formaldehyde ferredoxin oxidoreductase; GAP, glyceraldehyde 3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAPN, NAD$^+$-dependent GAPDH; GAPOR, glyceraldehyde-3-phosphate ferredoxin oxidoreductase; GLK, glucokinase; KDG, 2-keto-3-deoxygluconate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; LDH, lactate dehydrogenase; ORF, open reading frame; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PFKB, minor PFK; PGI, phosphoglucose isomerase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; PP, pentose phosphate; PPS, PEP synthase; PTS, phosphotransferase system; PYK, pyruvate kinase; SIS, sugar isomerase; T_{opt}, optimum growth temperature; TIM, triosephosphate isomerase.

[1] Present address: DSM Research, A. Fleminglaan 1, 2613 AX Delft, The Netherlands
[2] To whom correspondence should be addressed (e-mail john.vanderoost@wur.nl).

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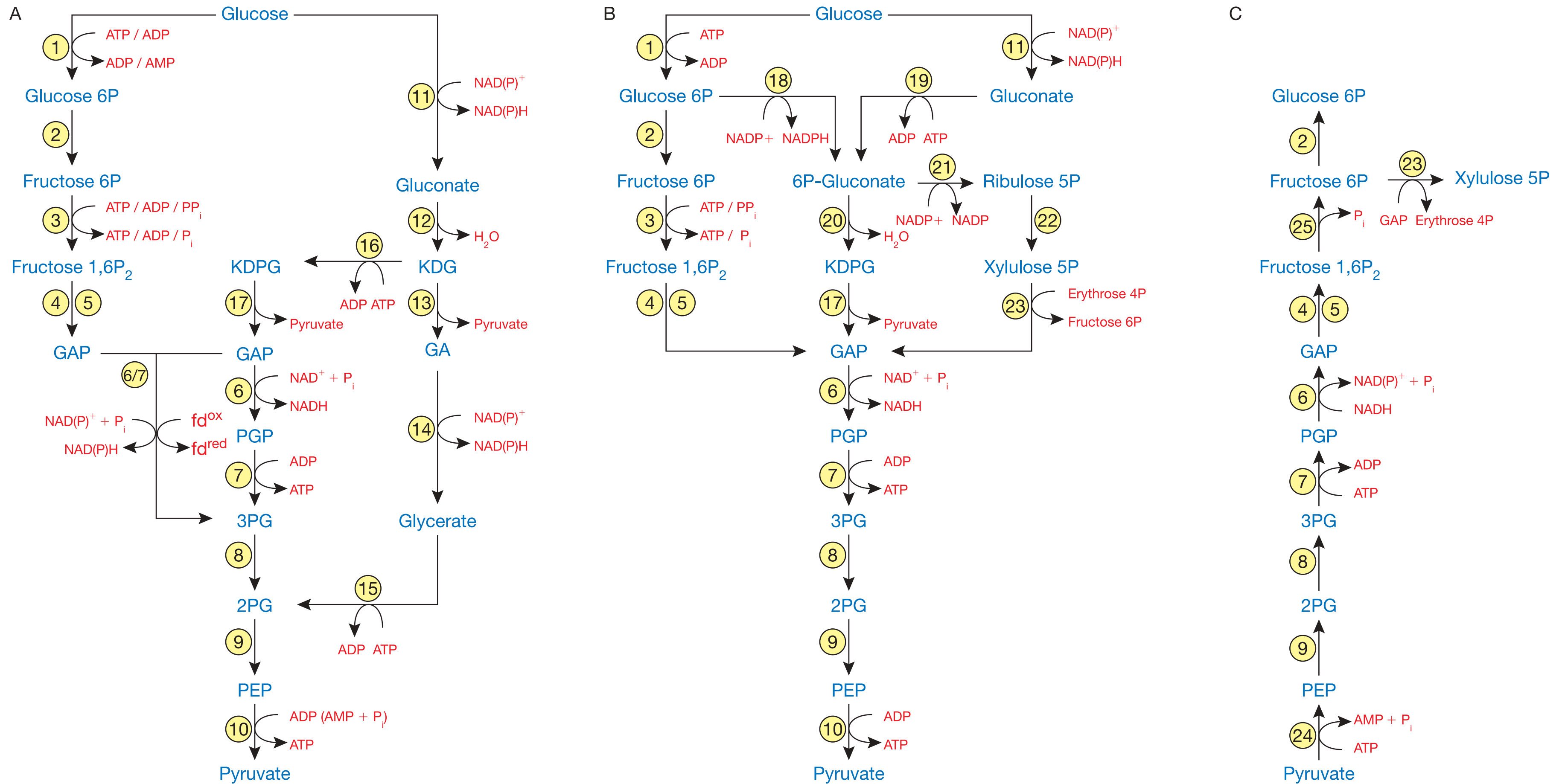

Scheme 1 Glycolysis and gluconeogenesis

(**A**) Glucose degradation in Archaea via variants of the EM and ED pathways. (**B**) Bacterial/eukaryal routes of glucose catabolism: EM, ED and oxidative PP pathway. (**C**) Gluconeogenesis in archaea and bacteria/eukarya results in the reduction of pyruvate to fructose 6-phosphate (Fructose 6P), which may be further converted into glucose 6-phosphate (Glucose 6P) or xylulose 5-phosphate (Xylulose 5P) via the non-oxidative PP pathway. Abbreviated metabolites in (**A**)–(**C**) are: GA, glyceraldehyde; Fructose 1,6P$_2$, fructose 1,6-bisphosphate; PGP, 2,3-bisphosphoglycerate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; fd^{ox}, oxidized ferredoxin; fd^{red}, reduced ferredoxin. Key to enzymes: (1) GLK; (2) PGI; (3) PFK; (4) FBA; (5) TIM; (6) GAPDH; (7) PGK; (6/7) GAPOR and GAPN; (8) PGM; (9) enolase; (10) PYK; (11) Glc DH/Glc-lac; glucose dehydrogenase/gluconolactonase; (12) G-hydr, gluconate dehydratase; (13) KDG ald, KDG aldolase; (14) Ald DH, glyceraldehyde dehydrogenase; (15) Gly kin, glycerate kinase; (16) KDG kin, KDG kinase; (17) KDPG ald, KDPG aldolase; (18) G6P DH/6PG-lac; glucose-6-phosphate dehydrogenase/6-phosphogluconolactonase; (19) GlcA-kin, gluconate kinase; (20) PG-hydr, 6-phosphogluconate kinase; (21) PG DHDC, 6-phosphogluconate dehydrogenase (decarboxylase); (22) RP epi, ribulose-5-phosphate 3-epimerase; (23) Tr.ketolase, transketolase; (24) PPS; (25) FBP.

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Table 1 Carbohydrate-metabolizing archaea

Organism	T_{opt} (°C)	Metabolism	Habitat	Carbohydrate substrates	Pathway	Reference
Sulfolobales						
Sulfolobus solfataricus	80	Aerobic	Terrestrial	Starch, dextrin, xyloglucan, maltose, sucrose, lactose, glucose, xylose	ED*	[13,29]
Sulfolobus shibatae	81	Aerobic	Terrestrial	Starch, glucose, galactose, arabinose		[13]
Sulfolobus acidocaldaricus	75	Aerobic	Terrestrial	Sucrose, lactose, glucose, galactose		[13]
Thermoproteales						
Thermoproteus tenax	88	Anaerobic	Terrestrial	Starch, glycogen, glucose	EM*/ED*	[13]
Desulfurococcales						
Desulfurococcus amylolyticus	90	Anaerobic	Terrestrial	Starch, glycogen	EM*	[5,13]
Desulfurococcus saccharovorans	85	Anaerobic	Terrestrial	Glucose		[13]
Desulfurococcus mucosus	88	Anaerobic	Terrestrial	Starch		[13]
Pyrodictium abyssi	97	Anaerobic	Marine	Starch, glycogen, raffinose, lactose		[13]
Thermococcales						
Pyrococcus furiosus	100	Anaerobic	Marine	Starch, pullulanan, glycogen, maltose, cellobiose, glucose, lactose†, melibiose†	EM*	[13,21]; C. Verhees, unpublished work
Pyrococcus woesei	100	Anaerobic	Marine	Starch, glycogen, maltose, cellobiose	EM*	[13]
Pyrococcus glycovorans	95	Anaerobic	Marine	Starch, maltose, cellobiose, glucose		[118]
Thermococcus celer	88	Anaerobic	Marine	Sucrose		[119]
Thermococcus stetteri	75	Anaerobic	Marine	Starch	EM*	[13]
Thermococcus zilligii	75	Anaerobic	Terrestrial	Maltose	EM*	[56,120]
Thermococcus litoralis	88	Anaerobic	Marine	Starch, maltose	EM*	[13,121]
Thermococcus profundus	80	Anaerobic	Marine	Starch, maltose		[13]
Thermococcus hydrothermalis	90	Anaerobic	Marine	Cellobiose, maltose		[122]
Thermococcus aggregans	88	Anaerobic	Marine	Starch, maltose		[123]
Thermococcus guaymasensis	88	Anaerobic	Marine	Starch, maltose		[123]
Thermococcus pacificus	85	Anaerobic	Marine	Starch		[124]
Thermococcus fumicolans	85	Anaerobic	Marine	Maltose		[125]
Thermococcus profundus	80	Anaerobic	Marine	Starch, maltose		[126]
Archaeoglobales						
Archaeoglobus fulgidus strain 7324	83	Anaerobic	Marine	Starch	EM*	[48]
Thermoplasmales						
Thermoplasma acidophilum	59	Aerobic	Terrestrial	Glucose	ED*	[79]
Halobacteriales‡						
Halococcus saccharolyticus	37	Aerobic	High salt§	Arabinose, lactose, fructose, glucose	EM/ED*	[84,127]
Haloferax mediteranei	35	Aerobic	High salt§	Starch, lactose, sucrose, fructose, glucose	EM/ED*	[87,128,129]
Haloarcula vallismortis	37	Aerobic	High salt§	Fructose, glucose	EM/ED*	[129]
Halobacterium saccharovorum	37	Aerobic	High salt§	Glucose	ED*	[130]

* Modification in EM/ED pathway.
† *Pyrococcus furiosus* degrades the glucose moiety of lactose and melibiose. Galactose is mainly secreted in the medium.
‡ Halophiles use an EM-like pathway for the degradation of fructose and a modified ED pathway for the degradation of glucose.
§ $>$ 12 % (2 M) NaCl.

Sulfolobus, *Pyrobaculum*, *Thermoplasma* and halophilic archaea), or anaerobically with alternative terminal electron acceptors, such as nitrate (e.g. *Pyrobaculum*), sulphur (e.g. *Thermoproteus*), sulphate (e.g. *Archaeoglobus*) or carbon dioxide (e.g. methanogens). In some organisms, the generation of membrane potential has never been demonstrated experimentally, and hence they may depend on substrate-level phosphorylation only (members of the orders Thermococcales and Desulphurococcales) [12]. However, comparative genomic analyses of Thermococcales (e.g. *Pyrococcus* spp.) have revealed the presence of putative membrane-potential-generating protein complexes [15,16] that require experimental verification.

The saccharolytic archaea *Pyrococcus furiosus* and *Sulfolobus solfataricus* have been studied in considerable detail, and, as such, form the basis of the present review. *Pyrococcus furiosus* is an anaerobic micro-organism with an optimum temperature for growth (T_{opt}) of 100 °C that was isolated from marine hot springs, and belongs to the Euryarchaeota [17]. *S. solfataricus* is an aerobic micro-organism ($T_{opt} = 80$ °C) that was isolated from acidic solfatara fields, and belongs to the Crenarchaeota [18]. Both archaea are able to grow on a variety of α- and β-linked glucose saccharides and glucose [17,19–24]. Polysaccharides are degraded by specific extracellular glycosyl hydrolases to oligosaccharides [20,21,25–29], which are subsequently transported into the cell by either ABC (ATP-binding cassette)-type or secondary transporters [23,30–35]. Sugar transport via the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) is very common in bacteria, but is apparently absent from archaea and eukaryotes. Interestingly, genomic analyses reveal that PTS is also lacking from the thermophilic bacteria *Thermotoga maritima* and *Aquifex aeolicus*. Transported oligosaccharides are hydrolysed further to glucose by specific intracellular glycoside hydrolases [19,24,29,36–39]. Glucose is metabolized to pyruvate via variants of two main sugar catabolic routes: the EM pathway, that is operating in *Pyrococcus furiosus*, or the ED pathway, which is found in *S. solfataricus* [5,13]. Subsequently, pyruvate is converted into acetyl-CoA and CO_2 by pyruvate ferredoxin oxidoreductase [40]. *S. solfataricus* and other respiring archaea have the capacity to completely oxidize acetyl-CoA to CO_2 via the citric acid cycle, when in combination with respiratory-coupled oxidation of NAD(P)H and ferredoxin [41]. Based on analysis of the *S. solfataricus* genome, it has been proposed that ferredoxin, rather than NADH, may be the main physiological electron carrier in *Sulfolobus*, and maybe in archaea in general [42]. Obligatory

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Table 2 Molecular reconstruction of archaeal and thermophilic sugar metabolism

Numbering of the genes is according to http://www-archbac.u-psud.fr/projects/sulfolobus/. The 16 archaea for which genome sequences are available have been clustered (based on similar genomic profiles) for the sake of clarity; the genes of the species between parentheses have not been included. PF, *Pyrococcus furiosus* (PH, *Pyrococcus horikoshii*; PAB, *Pyrococcus abyssi*); MA, *Methanosarcina acetivorans* (MM, *Methanosarcina mazei*); MJ, *Methanocaldococcus jannaschii*; MT, *Methanobacterium thermoautotrophicum* (MK, *Methanopyrus kandleri*); AFU, *Archaeoglobus fulgidus*; VNG, *Halobacterium* NRC-1; TA, *Thermoplasma acidophilum* (TV, *Thermoplasma volcanium*); SSO, *Sulfolobus solfataricus* (STO, *Sulfolobus tokadaii*); APE, *Aeropyrum pernix*; PAE, *Pyrobaculum aerophilum*. The hyperthermophilic bacterium *Thermotoga maritima* (TM) is included to allow comparison of the archaeal genomes with a bacterial genome. Experimentally confirmed gene products are in bold and are underlined [46,51,52,57,58,66,67,72–74,76,81,85,86,97,98,131,132]. It should be stressed that the proposed gene functionality requires experimental verification; parentheses indicate uncertain substrate specificity. The numbers and enzyme abbreviations are as in Scheme 1; EC, Enzyme Commission (http://www.genome.ad.jp/kegg); COG, Clusters of Orthologous Groups (http://www.ncbi.nlm.nih.gov/COG). Asterisks (*) in the COG column indicate identity; in the species columns, asterisks indicate clustering of genes (per column, genes marked with one, two or three asterisks link with other genes marked similarly), and # indicates a fusion of two enzyme-encoding genes. For the Domain column: A, Archaea; B, Bacteria; E, Eukaryota.

						Euyarchaea							Crenarchaea			Bacteria
Number	Enzyme	Remark	EC	COG	Domain	PF (PH/PAB)	MJ	MA (MM)	MT (MK)	AFU	VNG	TA (TV)	SSO (STO)	APE	PAE	TM
EM pathway																
1	GLK	ADP/glucose	2.7.1.147	4809*	A	0312	(1604)									
		ATP/glucose	2.7.1.2	0837	BE						2629	0825		2091	3437	1469
		ATP/hexose	2.7.1.1		E											
2	PGI		5.3.1.9	2140	A	0196		0821		(1494)						
			5.3.1.9	0166	ABE		1605*				1992	(1419)	(2281)	(0768)	1610	1385
3	PFK	ADP	2.7.1.146	4809*	A	1784	1604*	3562								
		ATP	2.7.1.11	0205	ABE									0012*	0835	0209
		PP_i	2.7.1.90		ABE						0289					0289
4	FBA		4.1.2.13	1830	A	1956	0400	0439	0579	0108	0683		3226	0011*		
			4.1.2.13	0191	BE											0273
5	TIM		5.3.1.1	0149	ABE	1920[4]	1528	4607	1041*	1304	1027	0313	2592	1538	1501	0689*#
6/7	GAPOR	Ferredoxin	1.2.1.-	2414	A	0464	1185								1029	
	GAPN	NAD	1.2.1.-	1012	A	(0755)	(1411)	(1355)	(0978)		0937	0809	3194*[3]	1786		
6	GAPDH	NAD(P)	1.2.1.12	0057	ABE	1874[4]	1146	1018	1009	1732	0095	1103	0528**	0171**	1740*	0688*
7	PGK	ADP	2.7.2.3	0126	ABE	1057	0641	2669	1042*	1146	1216	1075	0527**	0173**	1742*	0689*#
8	PGM	2,3-PG	5.4.2.1	0406	ABE							1347	2236			1374
		Independent	5.4.2.1	0696	ABE	1959	1612	0132	1591	1751	1887	0413	0417	1616	2326	1774
9	Enolase		4.2.1.11	0148	ABE	0215	0232	1672	0043	1132	1142	0882	0913[5]	2458	0812	0877
10	PYK	ADP	2.7.1.40	0469	ABE	1188[2]	0108	3890			0324	0896	0981[5]	0489	0819	0208
Gluconeogenesis																
21	PPS	ATP	2.7.9.2	0574	ABE	0043	0542	2667	1119	0710	0330	0886	0883	0650	2423	0272
9	Enolase		4.2.1.11	0148	ABE	0215	0232	1672	0043	1132	1142	0882	0913	2458	0812	0877
8	PGM	2,3-PG	5.4.2.1	0406	ABE							1347	2236			1374
		Independent	5.4.2.1	0696	ABE	1959	1612	0132	1591	1751	1887	0413	0417	1616	2326	1774
7	PGK	ATP	2.7.2.3	0126	ABE	1057	0641	2669	1042	1146	1216	1075	0527	0173	1742	0689
6	GAPDH	NAD(P)H	1.2.1.12	0057	ABE	1874[4]	1146	1018	1009	1732	0095	1103	0528	0171	1740	0688
5	TIM		5.3.1.1	0149	ABE	1920[4]	1528	4607	1041*	1304	1027	0313	2592	1538	1501	0689**
4	FBA		4.1.2.13	1830	A	1956	0400	0439	0579	0108	0683		3226	0011		
			4.1.2.13	0191	BE											0273
22	FBP	Type V	3.1.3.11	1980	A	0613[1]	0299		1686	1442		1428	0286	1109	0944	
		Type IV	3.1.3.11	0483	ABE	2014	0109	3344	0871	2372			2418	1798		1415
		Type I	3.1.3.11	0158	ABE						0684					
2	PGI		5.3.1.9	2140	A	0196		0821		(1494)						
			5.3.1.9	0166	ABE		1605				1992	(1419)	(2281)	(0768)	1610	1385
ED pathway																
11	Glc DH	NAD(P)	1.1.1.47	1063	ABE						0446*	0897	3003 3042** (3204)			0298
	Glc-lac		3.1.1.17	3386	ABE					0648	3041**					
12	G-hydr		4.2.1.39	4948**	A						0442*	0085	3198*[3]			(0006)
13	KDG ald		4.1.2.-	0329***	A							0619	3197*[3]			
14	Ald DH	NAD(P)	1.2.1.-	1012	A							(0809)	(1629)			
15	Gly kin	ATP	2.7.1.-	2379	A							0453	0666			
16	KDG kin		2.7.1.45	0524	AB						0158		3195*			0067**
18	G6P DH	NAD(P)	1.1.1.49	0364	BE											1155***
	6PG-lac		3.1.1.31	0363	BE											1154***
19	GlcA-kin	ATP	2.7.1.31		BE						0443					
20	PG-hydr		4.2.1.12	4948**	BE											(0006)
17	KDPG ald			0329***	A						0444*					
	KDPG ald		4.1.2.14	0800	BE											0066**
6	GAPDH	NAD(P)	1.2.1.12	0057	ABE	1874	1146	1018	1009	1732	0095	1103	0528**	0171*	1740*	0688*
7	PGK	ADP	2.7.3.2	0126	ABE	1057	0641	2669	1042	1146	1216	1075	0527**	0173*	1742*	0689*
6/7	GAPOR	Ferredoxin	1.2.1.-	2414	A	0464	1185								1029	
	GAPN	NAD	1.2.1.-	1012	A	(0755)	(1411)	(1355)	(0978)		0937	0809	3194*[3]	1786		
8	PGM	2,3-PG	5.4.2.1	0406	ABE							1347	2236			1374
	Independent		5.4.2.1	0696	ABE	1959	1612	0132	1591	1751	1887	0413	0417	1616	2326	1774
9	Enolase		4.2.1.11	0148	ABE	0215	0232	1672	0043	1132	1142	0882	0913	2458	0812	0877
10	PYK	ADP	2.7.1.40	0469	ABE	1188	0108	3890			0324	0896	0981	0489	0819	0208

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Table 2 (Contd.)

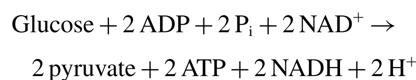
						Euyarchaea							Crenarchaea			Bacteria
Number	Enzyme	Remark	EC	COG	Domain	PF (PH/PAB)	MJ	MA (MM)	MT (MK)	AFU	VNG	TA (TV)	SSO (STO)	APE	PAE	TM
PP pathway																
21	PG DHDC		1.1.1.44	0362	BE	(0716)					(2553)		(1560)		(1145)	0438
	R5P iso		5.3.1.6	0120	A	1258	1603	1683	0608	0943	2272	0878	0978	0665	1027	
			5.3.1.6	0698	BE											1080
22	RP epi		5.1.3.1	0036			0680*									1718
23	Tr.ketolase	N-terminal subunit	2.2.1.1	3959*		1688*	0679*					0617*	0299*	0583*	1929*	0954*
		C-terminal subunit		3958*		1689*	0681*					0618*	0297*	0586*	1927*	0953*
	Tr.aldolase		2.2.1.2	0176			0960					0616*				0295

[1] Characterized in *Thermococcus kodakaraensis* [99].
[2] Characterized in *Pyrococcus furiosus* (J. E. Tuininga and S. W. M. Kengen, unpublished results).
[3] Characterized in *Sulfolobus solfataricus* (B. Siebers, T. J. G. Ettema and J. van der Oost, unpublished results).
[4] Characterized in *Pyrococcus woesei* [64,133].
[5] Characterized in *Thermoproteus tenax* [114].

anaerobic fermenting archaea such as *Pyrococcus furiosus* produce mainly acetate and CO_2 as end products via an archaeal-specific acetyl-CoA synthetase [43,44].

ARCHAEAL VARIANTS OF THE EM PATHWAY

The classical EM pathway is well-conserved in bacteria and eukaryotes, where ten enzymes catalyse the step-wise oxidation of glucose to pyruvate (Table 2). Glycolysis starts with the phosphorylation of glucose, followed by the isomerization to fructose 6-phosphate and a second phosphorylation, the aldol cleavage of fructose 1,6-bisphosphate and a P_i-dependent activation of glyceraldehyde 3-phosphate (GAP), which is further converted into pyruvate [1] (Scheme 1B). The net reaction of the total EM pathway is:



$$\text{Glucose} + 2\,\text{ADP} + 2\,\text{P}_i + 2\,\text{NAD}^+ \rightarrow 2\,\text{pyruvate} + 2\,\text{ATP} + 2\,\text{NADH} + 2\,\text{H}^+$$

The best-studied archaeal EM pathway is the one of *Pyrococcus furiosus*. Biochemical studies and comparative genomics have revealed that only four of the ten glycolysis enzymes of bacteria/eukaryotes have orthologues in *Pyrococcus*: triosephosphate isomerase (TIM), phosphoglycerate mutase (PGM), enolase and pyruvate kinase (PYK) [9] (Table 2). Novel enzymes, unprecedented conversions and different control points have been elucidated in the pyrococcal glycolysis. Each glycolytic enzyme is described separately below, and the case of *Pyrococcus* is compared with that of bacteria/eukaryotes and, when data are available, with that of other archaea. Obviously, several glycolytic enzymes are absent in organisms (e.g. *S. solfataricus*) that degrade glucose via an ED-like pathway (see below; Table 2). Although an alternative has been proposed (see discussion below; [45]), the general view of the net reaction of the EM-like pathway of *Pyrococcus furiosus* is:

$$\text{Glucose} + 4\,\text{fd}^{\text{ox}} \rightarrow 2\,\text{pyruvate} + 4\,\text{fd}^{\text{red}} + 4\,\text{H}^+$$

where fd is the single-electron carrier ferredoxin, which is either oxidized (fd^{ox}) or reduced (fd^{red}).

GLK (Glucokinase)

The enzyme that is responsible for the activation of glucose in *Pyrococcus furiosus* is an ADP-dependent kinase (ADP-GLK) that is not related to the classical ATP-dependent enzymes of bacteria (GLK) or eukaryotes (hexokinase) [8,46]. The *Pyrococcus furiosus* GLK is member of a unique family that also includes ADP-dependent phosphofructokinases (ADP-PFKs), as discussed below [8,46,47]. ADP-GLK is also found in other *Pyrococcus* and *Thermococcus* species [47]. The activity of ADP-GLK has been measured in extracts of *Archaeoglobus fulgidus* strain 7324 [48], but the genome sequence of a closely related strain (VC16) does not contain a homologue of either *Pyrococcus furiosus* GLK or classical bacterial/eukaryal kinase (Table 2). Rather than ADP-GLK, homologues of ATP-GLK are found among other archaea (Table 2), including *Thermoproteus tenax*, *Thermoplasma volcanium*, *Thermoplasma acidophilum*, *Aeropyrum pernix* and *Pyrobaculum aerophilum* (www.genome.ad.jp/kegg/kegg2.html). So far, only the archaeal ATP-GLKs from *Ae. pernix* [49] and *Thermoproteus tenax* [50] have been characterized biochemically. They actually appear to have broad substrate specificity, and, as such, they were referred to as hexokinases.

PGI (Phosphoglucose isomerase)

The genes encoding the PGI of *Pyrococcus furiosus* and *Thermococcus litoralis* have been cloned and characterized [51–53]. This type of PGI is unrelated to its bacterial and eukaryal counterparts. Instead it belongs to the so-called cupin superfamily [52]. Members of this superfamily have a wide variety of functions that often appear to be related to the binding or conversion of saccharides. The closest functional analogue of the archaeal PGI is phosphomannose isomerase [52,53a]. This type of PGI seems to be restricted to the *Pyrococcus*, *Thermococcus* and *Methanosarcina* species; a gene in *Ar. fulgidus* appears to encode an enzyme that is more closely related to cupin proteins with distinct substrate specificity (Table 2) [52]. The *pgi* gene of *Pyrococcus furiosus* overlaps 25 nt with an adjacent ORF (open reading frame) (PF0196), suggesting that the two ORFs are co-transcribed. However, it was demonstrated by Northern blotting that PGI is monocistronic [52], a conclusion supported by microarray analysis [54].

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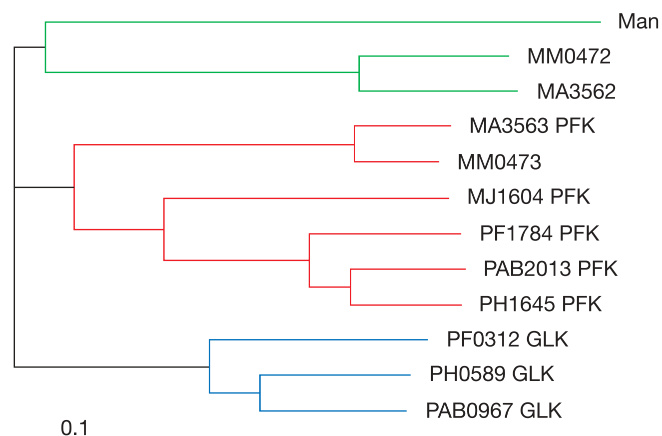

Figure 1 ADP-dependent kinases in archaea and eukaryotes

Phylogenetic analysis of ADP-dependent kinases in archaea (PF, *Pyrococcus furiosus*; PH, *Pyrococcus horikoshii*; PAB, *Pyrococcus abyssi*; MJ, *Methanocaldococcus jannaschii*; MM, *Methanosarcina mazei*; MA, *Methanosarcina acetivorans*). Red branches, PFKs; blue branches, GLKs; also included are related kinases (green branch) with unknown specificity from *Methanosarcina* spp. (MM0472, MA3562) and *Homo sapiens* (Man; Gene Identifier 13543940). The dendrogram was based on a ClustalX alignment.

Genome analysis reveals that orthologues of classical PGI are present not only in the bacterium *Thermotoga maritima*, but also in several archaea, such as *Methanocaldococcus* (*Methanococcus*) *jannaschii* and *Halobacterium* NRC-1 (Table 2). According to the COG (clusters of orthologous groups of proteins) database (http://www.ncbi.nlm.nih.gov/COG), additional orthologues are present in the genomes of *Thermoplasma acidophilum* (TA1419), *S. solfataricus* (SSO2281) and *Ae. pernix* (APE0768). However, only a sugar isomerase (SIS) domain is identified in the latter protein sequences, whereas both a SIS domain and a phosphoglucose (PGI) domain are present in the other orthologues. Apart from PGIs, the SIS domain has also been demonstrated to play a role in the isomerization of other sugars [55]. Thus TA1419 and APE0768 cannot unambiguously be classified as PGIs (Table 2).

PFK

Analysis of PFK from *Pyrococcus furiosus* again revealed that this kinase is ADP-dependent, just like its counterpart from the related *Thermococcus zilligii* [4,46,56]. Sequence analysis indicated that the ADP-PFKs are paralogues of the ADP-GLKs, sharing the same COG 4809 (Table 2 and Figure 1). An orthologue of the *Pyrococcus furiosus* ADP-PFK was also identified in the non-saccharolytic *Methanocaldococcus jannaschii* [57]. The characterized enzyme (MJ1604) has recently been reported to have dual activity towards both glucose and fructose 6-phosphate [58]. ADP-PFK homologues appeared to be present in the sulphate-reducing *Ar. fulgidus* strain 7324 [48], and in both thermophilic and mesophilic glycogen-degrading methanogenic species belonging to the Methanococcales and the Methanosarcinales [57]. The *pfk* gene of *Methanocaldococcus jannaschii* is located adjacent to predicted homologues of glucose isomerase (MJ1605) as well as glycogen synthase (MJ1606) (Table 2), strongly suggesting a concerted role in glycogen synthesis. Interestingly, the genes encoding the predicted ADP-PFKs in *Methanosarcina* spp. are clustered with paralogous genes, probably encoding ADP-dependent kinases with an unknown specificity (Figure 1). No homologues have been found in bacterial genomes. Interestingly, however, uncharacterized genes with significant identity with the ADP-dependent archaeal kinases were identified in several eukaryal genomes, including that of man (Figure 1) [57]. The ADP-dependent sugar kinases do not share overall sequence similarity with classical sugar kinase sequences in bacteria and eukaryotes [57]. The recently solved structure of ADP-GLK from the archaeon *Thermococcus litoralis*, closely related to *Pyrococcus furiosus*, shows a similar fold as ribokinase and adenosine kinase [59]. The 'minor PFK' from *Escherichia coli* (PFKB) belongs to this ribokinase family as well. In bacteria/eukaryotes, the ATP-dependent hexokinases/GLKs on one hand, and the ATP-dependent PFKs on the other, belong to different monophyletic families (COG 0837 and 0205 respectively). Hence, based on the established primary and the tertiary structures, it is concluded that the archaeal ADP-dependent kinases are not related to their counterparts in bacteria and eukaryotes.

The genome sequences of some hyperthermophilic archaea such as *Pyrobaculum aerophilum* and *Ae. pernix* contain homologues of bacterial/eukaryal ATP-dependent sugar kinases. The PFK of *Ae. pernix* has been characterized and represents the first ATP-dependent PFK to be characterized from a hyperthermophile, an enzyme that is apparently not allosterically regulated [60]. A third type of PFK exists in *Thermoproteus tenax*. This enzyme is PP_i-dependent and is also found among some bacteria and eukaryotes, and is distantly related to ATP-PFK [61] (both belonging to COG 0205) (Table 2). The genome of *Ar. fulgidus* strain VC16 appears not to encode any homologue of PFK (Table 2), although in cell extracts of strain 7423, ADP-PFK activity has been detected [48].

FBA (Fructose-1,6-bisphosphate aldolase)

A distantly related archaeal type of FBA has recently been identified in *Thermoproteus tenax* and *Pyrococcus furiosus* [62], confirming earlier function prediction [63]. Orthologues of this 'archaeal type class I aldolase' are present in all sequenced archaeal genomes, except for *Thermoplasma* and *Pyrobaculum*. Paralogues of the aldolase are present in *Methanocaldococcus jannaschii* (MJ1585), *Ar. fulgidus* (AF0230), and *Halobacterium* NRC-1 (VNG0309), and the encoded enzymes were predicted to function either as deoxyribose phosphate aldolase or as transaldolase [63].

TIM

All sequenced archaea have an obvious TIM homologue (Table 2), indicating that TIM does not display extensive variation, unlike some of the other glycolytic enzymes. Although this enzyme does not take part in the ED pathway (regular or modified), it seems to be required for those organisms harbouring an ED-type pathway apparently in order to perform gluconeogenesis. It has previously been noticed that TIM of hyperthermophiles is smaller (220–230 amino acids) than TIM found in mesophiles such as *E. coli* (250–260 amino acids) [64]. The structure of the enzyme from the hyperthermophile *Pyrococcus woesei* has been determined and compared with that of the mesophilic homologue. It is a more compact protein ('tiny TIM') [65], with several peptides 'missing' throughout the protein sequence. Interestingly, all archaea possess this smaller version of TIM, whereas eukaryotes and bacteria, as well as the hyperthermophilic bacterium *Aq. aeolicus*, contain the larger version.

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GAPOR (Glyceraldehyde-3-phosphate ferredoxin oxidoreductase)

In bacteria and eukaryotes, the (reversible) conversion of GAP into 3-phosphoglycerate is catalysed by the enzyme couple of the NAD^+-dependent GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (GAPN) and the ATP-generating phosphoglycerate kinase (PGK). In *Pyrococcus furiosus*, a distinct enzyme is responsible for the single-step conversion of GAP into 3-phosphoglycerate in a phosphate-independent manner: GAPOR [66]. Unlike the bacterial system, the physiological role of GAPOR appears to be solely the oxidation of GAP (glycolysis) and not the reduction of 3-phosphoglycerate [67] (see the Regulation of archaeal glycolysis section, below). GAPOR is a member of the aldehyde oxidoreductase (AOR) family of tungsten-containing enzymes. Three of the five AOR-family paralogues of *Pyrococcus furiosus* [AOR, FOR (formaldehyde ferredoxin oxidoreductase) and GAPOR] have been characterized biochemically, and each catalyses the ferredoxin-dependent oxidation of aldehydes [68]. The crystal structure of AOR revealed that the tungsten is co-ordinated by two pterin molecules, and that the protein contains a [4Fe–4S] cluster [68]. The pterin ligands and the iron–sulphur cluster are co-ordinated by conserved amino acid residues. Based on sequence alignments, a typical GAPOR signature in the [4Fe–4S] cluster-co-ordinating residues was observed (Cys-Gly-Glu-Pro-Cys-Pro-Xaa-Xaa-Cys), while the other AOR family members contain a less conserved signature (Cys-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Cys) [67]. Using this conserved signature, potential homologues of GAPOR can be found in *Pyrococcus horikoshii*, *Pyrococcus abyssi*, *Pyrobaculum aerophilum* and *Methanocaldococcus jannaschii* (Table 2). GAPOR activity was measured in cell extracts of *Methanocaldococcus jannaschii* (G. J. Schut and J. van der Oost, unpublished work), the starch-degrading *Ar. fulgidus* strain 7324 [48], *Desulfurococcus amylolyticus* and *Thermococcus litoralis* [5]. The presence of GAPOR in *Methanocaldococcus jannaschii* probably reflects the capacity of this methanogen to degrade glycogen [57].

An interesting variation occurs in the crenarchaeon *Thermoproteus tenax*, which contains a distinct allosterically controlled GAPN that catalyses the phosphate-independent, single-step oxidation of GAP to 3-phosphoglycerate in glycolysis [69]. Although both GAPOR and GAPN are novel glycolytic enzymes only identified in archaea, they both have distantly related enzymes, at least in bacteria. In enterobacteria, such as *E. coli*, a molybdenum-containing oxidoreductase has been identified that is structurally related to the AOR family, but is certainly not involved in glycolysis [67]. GAPN is member of a large family of aldehyde dehydrogenases that are also present in bacteria and eukaryotes [69]. The physiological role of the numerous GAPOR/AOR and GAPN-like enzymes that appear to be encoded by many archaeal genomes (Table 2) remains to be established.

PGM

The last 'missing-link' of the *Pyrococcus furiosus* glycolytic pathway was PGM, responsible for interconverting 3-phosphoglycerate and 2-phosphoglycerate. Archaeal PGM has been predicted by comparative analysis of metabolic pathways in different genomes [9,70,71]. The prediction of the archaeal PGMs (11 % amino acid identity with its *E. coli* counterpart) has been confirmed experimentally for *Pyrococcus furiosus* and *Methanocaldococcus jannaschii* (MJ1612) [72]. Apparently, a gene-duplication event has led to a second copy of this gene in *Methanocaldococcus jannaschii* (MJ0010), *Methanobacterium thermoautotrophicus* (MT0418) and *Ar. fulgidus* (AF1425) (Table 2). It has been demonstrated that both MJ0010 and MJ1612 encode enzymes with PGM activity [73]. The physiological role of these paralogues remains to be addressed. This new subfamily of PGM is present in all archaea whose genome has been sequenced (Table 2). As in several bacteria, two PGM types coexist in *Sulfolobus* and *Thermoplasma* [72]; this apparent redundancy would make sense when both systems evolved distinct catalytic features.

Enolase

The enolase of *Pyrococcus furiosus* has been characterized and shows high similarity to enolases from various sources [74]. Enolases can be identified in all archaeal genomes and represent the bacterial/eukaryal type. They appear to be highly conserved throughout the three domains and have been helpful in unravelling phylogenetic relationships [75].

PYK

As in bacteria and eukaryotes, the final step in the archaeal glycolytic pathways appears to be catalysed by PYK. Homologues of PYK are present in the genomes of saccharolytic archaea, and appear to be absent in the autotrophs *Methanopyrus kandleri* and *Methanobacterium thermoautotrophicus*, as well as in the non-saccharolytic *Ar. fulgidus* strain VC16 (Table 2). It had been suggested that the *Pyrococcus furiosus* PEP synthase (PPS) might play a novel glycolytic role, synthesizing ATP from PEP and AMP [45], but kinetic data do not support such a conclusion [76].

Enzyme-activity measurements of key glycolytic enzymes in various archaea on the one hand, and comparative genomics on the other (Table 2), indicate that archaeal EM pathways constitute different combinations of classical (bacterial/eukaryal) and novel (archaeal) enzymes. For example, *D. amylolyticus* was found to contain a partially modified EM pathway, including GAPOR, but with classical ATP-GLK and ATP-PFK (Scheme 2) [5,6,60]. The *Thermoproteus tenax* EM pathway includes an ATP-GLK, a PP_i-PFK and GAPN (Scheme 2) [5,61]. It is concluded that all archaeal EM pathways that have been characterized to date are relatively inefficient: during the oxidation of glucose to pyruvate by *Pyrococcus furiosus*, no net ATP appears to be produced (Scheme 1A). This is a direct consequence of the single-step, non-phosphorylating conversion of GAP by GAPOR or GAPN, in contrast with substrate-level phosphorylation by the enzyme couple GAPDH/PGK in classical glycolysis (Scheme 1B). The ADP-dependent sugar kinases (*Pyrococcus furiosus*) do not appear to affect the overall glycolytic efficiency. The PP_i-PFK (*Thermoproteus tenax*) appears to partially restore the loss of efficiency, since a waste product (PP_i) is used as phosphoryl donor; however, the yield of the *Thermoproteus tenax* pathway (1 ATP/glucose) would still be less than in classical glycolysis (2 ATP/glucose). Another difference is that GAPOR activity in *Thermococcus*, *Pyrococcus*, *Desulfurococcus* spp. and *Ar. fulgidus* strain 7324 results in the production of reduced ferredoxin, instead of NADH in classical glycolysis. Although the above discussion suggests that the *Pyrococcus furiosus* glycolysis does not result in the net generation of ATP, it has been deduced from yield studies that *Pyrococcus furiosus* has a net production of at least 1 mol of ATP during the glycolytic oxidation of 1 mol of glucose to pyruvate [4]. This could be achieved by the function of PPS in glycolysis ($PEP + AMP + P_i \rightarrow pyruvate + ATP$) [45], or by an alternative membrane-potential-generating system involving ferredoxin and hydrogenase [15].

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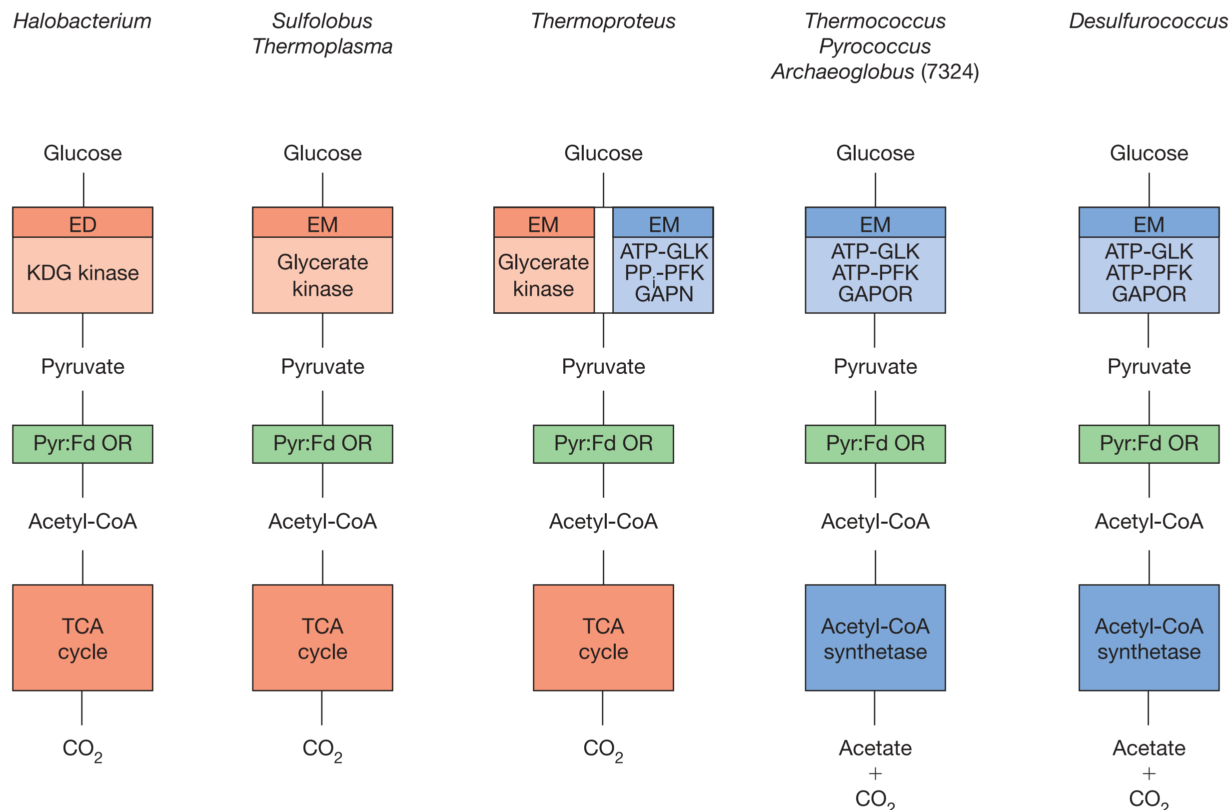
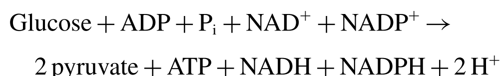


Scheme 2 Overview of glucose metabolism in archaea

Summary of relevant variations in the glucose-degrading pathways of archaea, as compared with the classical bacterial/eukaryal pathways. Pyr:Fd OR, pyruvate ferredoxin oxidoreductase; TCA, trichloroacetic acid. Adapted from Archives of Microbiology, Comparative analysis of the Embden–Meyerhof and Entner–Doudoroff glycolytic pathways in hyperthermophilic archaea and the bacterium *Thermologa*, Selig, M., Xavier, K. B., Santos, H. and Schönheit, P., **167**, p. 230, Figure 11, 1997 © Springer-Verlag GmbH & Co. [5].

ED-LIKE PATHWAYS IN ARCHAEA

The classical ED pathway of bacteria (and a limited number of eukaryal micro-organisms) consists of nine enzymes, and starts with the phosphorylation of either glucose or its oxidized derivative gluconate (Scheme 1B). The generated 2-keto-3-deoxy-6-phosphogluconate (KDPG) is converted by an aldol cleavage, resulting in the formation of GAP and pyruvate. The triose phosphate is activated by P_i, and is further degraded to pyruvate by enzymes that are identical with those of the EM pathway [1]. The net reaction of the bacterial ED pathway is:



$$\text{Glucose} + \text{ADP} + \text{P}_i + \text{NAD}^+ + \text{NADP}^+ \rightarrow$$
$$2\,\text{pyruvate} + \text{ATP} + \text{NADH} + \text{NADPH} + 2\,\text{H}^+$$

It should be noted that the main difference between the ED and EM pathways is that, in the former, both NADPH and NADH are formed, instead of 2 NADH, and that substrate-level phosphorylation only yields a single ATP per glucose molecule [1].

ED-like pathways found in archaea are modified in two ways. Halophilic archaea (and some bacteria) use a variant ED pathway in which 2-keto-3-deoxygluconate (KDG) is phosphorylated to KDPG by KDG kinase, rather than the general conversion of glucose into glucose 6-phosphate in bacteria and some eukaryotes (Scheme 1A). Phosphorylation at either level of the C_6-stage in the pathway (glucose, gluconate or KDG) allows the gain of ATP by substrate-level phosphorylation during conversion of GAP via 1,3-diphosphoglycerate into 3-phosphoglycerate in the canonical EM pathway (Scheme 1B) [11]. *Thermoproteus*, *Thermoplasma* and *Sulfolobus* spp. use an ED-like pathway in which none of the hexose intermediates are phosphorylated. Instead, the activation via phosphorylation does occur at a later stage in the pathway: at the level of KDG (e.g. halophilic archaea) or glycerate (e.g. *Sulfolobus* and *Thermoplasma*) (Scheme 1). Whereas the ED pathway in halophiles (via KDPG) is expected to have a similar net result as the bacterial route, there appears to be no net generation of ATP by the latter 'non-phosphorylating' ED pathway (via KDG) (Scheme 1A):

$$\text{Glucose} + 2\,\text{NAD(P)}^+ \rightarrow 2\,\text{pyruvate} + 2\,\text{NAD(P)H} + 2\,\text{H}^+$$

Both archaeal ED variants merge at some stage with the enzymes of the C_3-stage of the EM pathway [5,11,77–79]. Specific features of the archaeal ED pathway are discussed below.

Glucose dehydrogenase

The first enzyme of the variant ED pathways of archaea is the NAD(P)^+-dependent glucose dehydrogenase [80]. The first gene encoding an archaeal glucose dehydrogenase was isolated from *Thermoplasma acidophilum* [81]. Subsequently homologues were purified from cell extracts of *Thermoproteus tenax* and *Haloferax mediterranei* [82,83]. Three paralogues of this protein sequence

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were identified in the genome of *S. solfataricus*, and a single copy was identified in the genome of *Halobacterium* NRC-1 (Table 2). The protein sequences of these archaeal glucose dehydrogenases resemble zinc-containing alcohol dehydrogenases, and are distantly related to bacterial/eukaryal glucose dehydrogenases (COG 1063; Table 2).

Gluconate dehydratase

A potential gluconate dehydratase (SSO3198) was identified in the genome of *S. solfataricus* based on the presence of a dehydratase-like domain, and on its clustering with KDG aldolase (SSO3197; see below), a kinase (SSO3195) and a GAPN homologue (SSO3194) (Table 2) (T. J. G. Ettema and J. van der Oost, unpublished work). Clustering of these genes could not be observed in any of the other sequenced archaeal genomes. An orthologue of SSO3198 was identified in the genome of the bacterium *Thermotoga maritima* (TM0006), where it may encode the missing phosphogluconate dehydratase (Table 2).

KDG kinase

Strains of the halophile genera *Halobacterium*, *Haloferax* and *Halococcus* appear to be the only archaea that phosphorylate C_6 metabolites of the ED-like pathway via the phosphorylation of KDG by a specific kinase (Scheme 1). The generated KDPG appears to be converted as in the bacterial ED pathway (reviewed in [84]), and, as such, is the only example to date of an archaeal ED pathway with net ATP production (however, see discussion below).

KDG aldolase

A novel aldolase that catalyses the conversion of the non-phosphorylated KDG has been purified and characterized from *S. solfataricus* [85]. In the genome of *Halobacterium*, a distantly related gene (VNG0444) has been identified by similarity search and gene context, and was predicted to encode the KDPG aldolase (Table 2). Recently, biochemical evidence has been gained that TM0066 in the *Thermotoga maritima* genome (orthologous to VNG0444) indeed encodes an active KDPG aldolase [86].

Glyceraldehyde dehydrogenase

An archaeal glyceraldehyde dehydrogenase sequence has not yet been identified unambiguously. Several GAPN homologues are present in *Sulfolobus*, which are predicted to encode aldehyde dehydrogenases [42]; the specificity of these paralogues remains to be established experimentally. It has been confirmed biochemically that glyceraldehyde is specifically converted into glycerate by a glyceraldehyde dehydrogenase in *Thermoplasma acidophilum* and *S. solfataricus* [79,87]. In *Halobacterium* NRC-1, GAP is generated by aldol cleavage of KDPG, as in bacteria (Schemes 1A and 1B). Although a homologue of a non-phosphorylating GAPOR (GAPN; VNG 0937) appears to be encoded by the genome of *Halobacterium* NRC-1, the oxidation of GAP may also be catalysed by the classical GAPDH/PGK couple (Table 2). Among the glycolytic pathways in archaea, the ED pathway of *Halobacterium* is most similar to its bacterial (eukaryal) counterpart.

Glycerate kinase

Phosphorylation activity on glycerate has been demonstrated in *Thermoplasma acidophilum* cell extracts. The absence of PGM activity suggests that 2-phosphoglycerate (instead of 3-phosphoglycerate) is produced from the phosphorylation reaction of glycerate [79]. Based on these data, this conversion has been proposed to occur in *Sulfolobus* as well [42,87]. Candidate genes that might encode glycerate kinase have been identified in the genome of *S. solfataricus* [42], and in that of other archaea (Table 2). PGM protein sequences (dPGM-type; Table 2) that have been identified in *S. solfataricus* and *Thermoplasma acidophilum* would probably be produced only under gluconeogenic conditions. The specificity of the proposed glycerate kinases and the PGMs remain to be resolved.

Overall, the ED pathways of *Sulfolobus* and *Thermoproteus* yield: glucose $\rightarrow$ 2 pyuvate + 2 NADPH, due to the apparent lack of a GAP oxidation/phosphorylation system. Therefore, it does not matter at what level phosphorylation occurs in these archaea. Since the archaea that contain ED pathways appear to be capable of a relatively efficient cofactor reoxidation, either via oxidative phosphorylation (e.g. *Sulfolobus*) or via sulphur respiration (e.g. *Thermoproteus*), the low efficiency of their ED pathway might be of less importance. Alternatively, it has been suggested that the archaeal non-phosphorylating ED pathway may be reversible, i.e. an alternative gluconeogenesis pathway (reviewed in [1]).

PENTOSE CONVERSION IN ARCHAEA

Some archaea (e.g. *Sulfolobus*) have the capacity to use pentoses as a carbon source. Moreover, all autotrophic and heterotrophic archaea must be able to generate pentoses as building blocks for the biosynthesis of nucleotides and certain amino acids. Hence all archaea should possess enzymes that are responsible for the pentose metabolism. At present, however, little information is available on the identity of the archaeal enzymes or on the actual composition of the pathway(s) involved.

A general pathway for pentose degradation and biosynthesis in bacteria and eukaryotes is the PP pathway, consisting of an oxidative and a non-oxidative branch [1]. Obviously, the non-oxidative branch is essential for growth on pentoses. In addition, there may be a link with the gluconate metabolism (Scheme 1B). The oxidative branch is used to produce C_5 sugars (ribulose 5-phosphate, xylulose 5-phosphate and ribose 5-phosphate) from C_6 sugars (6-phosphogluconate) by means of oxidation and decarboxylation reactions, releasing CO_2 (Scheme 1B). Together with the non-oxidative branch, it forms a bypass between glucose 6-phosphate and fructose 6-phosphate, i.e. the hexose monophosphate pathway. Biosynthetic functions of the PP pathway are the synthesis of NADPH and ribose. At present, there is no evidence that catabolism of glucose in archaea proceeds via a PP-like pathway (Scheme 1A). Genes encoding canonical enzymes of the oxidative branch of the PP pathway, such as glucose-6-phosphate dehydrogenase and gluconate-6-phosphate dehydrogenases, have not been identified in archaea (Table 2) [42,88,89]. However, the possibility cannot yet be excluded that the archaeal enzymes that constitute a PP-like pathway are unrelated or only distantly related to their classical counterparts, and are therefore not easily detectable by comparative genomics.

As pointed out above, most, if not all, archaea are expected to have the capacity to generate pentoses, most likely via a non-oxidative branch of the EM/gluconeogenesis pathway. Genome analysis indicates that some of the key enzymes may indeed be present in several archaea: transketolase, ribulose-5-phosphate 3-epimerase, ribulose-5-phosphate isomerase (Table 2 and

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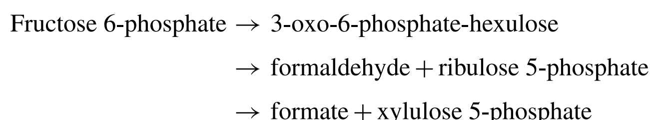
Scheme 1C). Interestingly, the recent DNA microarray analysis on the complete *Pyrococcus furiosus* genome revealed significant up-regulation on maltose (versus peptides) of the amino-acid-biosynthesis pathways, including the biosynthesis routes of histidine and the aromatic amino acids [90]. In the latter biosynthesis pathways, ribose 5-phosphate is an essential precursor. The predicted transketolase genes (PF1688 and PF1689), whose products catalyse the conversion of fructose 6-phosphate and GAP into erythrose 4-phosphate and the pentose xylulose 5-phosphate (Scheme 1C), reside in a gene cluster that is involved in the biosynthesis of aromatic amino acids. Indeed, this strongly suggests that the transketolase (together with specific isomerases) is involved in the synthesis of pentoses from glycolytic intermediates in archaea. It is obvious that several missing links in this pathway still remain to be discovered. Alternative synthesis of pentoses from GAP and acetaldehyde has recently been suggested to be catalysed by paralogues of the FBA sequence from *Methanocaldococcus jannaschii* (MJ1585) and *Ar. fulgidus* (AF0230) [63].

A novel glycolytic pathway that potentially involves pentoses has recently been proposed in *Thermococcus zilligii*. Based on [^{13}C]glucose-labelling experiments, it has been suggested that a C_6 intermediate (gluconate 6-phosphate) is cleaved into a C_5 intermediate (xylulose 5-phosphate) and formate, involving a novel type lyase [91]. The PP is presumably degraded via the pentose phosphoketolase pathway, commonly found in lactic acid bacteria. [92]. A relative contribution of 2:1 (novel pathway versus the EM pathway) was detected for cells grown on tryptone. The presence of glucose in the growth medium appears to repress the enzymes in this novel pathway, and results in the inversion of the relative contributions of the two pathways [91]. An alternative interpretation of the labelling pattern would be the conversion:



$$\begin{aligned}\text{Fructose 6-phosphate} &\rightarrow \text{3-oxo-6-phosphate-hexulose}\\ &\rightarrow \text{formaldehyde} + \text{ribulose 5-phosphate}\\ &\rightarrow \text{formate} + \text{xylulose 5-phosphate}\end{aligned}$$

involving two isomerases, a lyase/synthase and the well-characterized FOR. Obviously, a more detailed analysis of enzyme activities is required to address this matter.

GLUCONEOGENESIS IN ARCHAEA

Gluconeogenesis is a ubiquitous biosynthetic pathway via which pyruvate is converted into fructose 6-phosphate or glucose 6-phosphate, and sometimes all the way into glucose (as in multicellular eukaryotes) (Scheme 1C). Several of the pathway's intermediates serve as precursors for a large number of anabolic pathways, including the aforementioned ribose synthesis (PP pathway). In addition, several archaea (e.g. methanogens and *Sulfolobus*) [93] have been demonstrated to have the capacity to generate glucose polymers (glycogen) as storage material. The gluconeogenic pathway shares many enzymes with the EM pathway, and is therefore partly reversible to the EM pathway. However, three enzymes are rather specific for the bacterial/eukaryal gluconeogenesis (irreversible) and are thus not present in glycolysis: (i) the phosphorylation of pyruvate to PEP is catalysed by PPS instead of by PYK, (ii) the dephosphorylation of fructose 1,6-bisphosphate is catalysed by fructose-1,6-bisphosphatase (FBP) instead of by ATP-PFK, and (iii) in some organisms, glucose 6-phosphate can be dephosphorylated to glucose by glucose-6-phosphatase [94]. Specific features of the archaeal gluconeogenesis pathway will be discussed below.

PPS

Homologues of classical PPS sequences are present in all sequenced archaeal genomes, despite their saccharolytic capacity, suggesting that the function of the enzyme in gluconeogenesis. The PPS from *Pyrococcus furiosus* was purified and characterized by two groups [45,76]. Strikingly, transcription of the gene encoding the PPSs has been reported to be enhanced by growth on maltose [95], possibly suggesting a role in glycolysis (see also below). However, the latter finding is not in agreement with recent biochemical and DNA microarray analyses, which would suggest the enzyme to function in gluconeogenesis [76,90,96]. In that case, classical PYK present in this archaeon (Table 2) might catalyse the reaction in the glycolytic direction. Despite the bioenergetic data, the true function of PPS in *Pyrococcus furiosus* is still a matter of debate, and is under current investigation (J. E. Tuininga and S. W. M. Kengen, unpublished work).

GAPDH/PGK

All archaea contain the enzyme couple GAPDH and PGK (Table 2). It has been shown for *Pyrococcus furiosus* and *Thermoproteus tenax* that this enzyme couple acts solely in gluconeogenesis, which also explains its presence in non-sugar-degrading *M. thermoautotrophicum* and *Ar. fulgidus* strain VC-16. The novel GAP-oxidizing enzymes, GAPOR and GAPN, have been shown to function solely in glycolysis in *Pyrococcus furiosus* and *Thermoproteus tenax* respectively [66,67,69].

FBP

A homologue of a canonical FBP (type I) could be identified in the archaeal genome sequence of *Halobacterium* NRC-1. No obvious orthologues of this gene are present in the other archaeal genomes (Table 2). However, characterization of a bifunctional FBP/*myo*-inositol-1-phosphatase from *Methanocaldococcus jannaschii* (MJ0109) [97] resulted in the identification of the corresponding gene in the genomes of several archaea as well as *Thermotoga maritima* (FBP type IV) (Table 2) [98]. Recent studies revealed the presence of yet another FBP (type V) in *Thermococcus kodakaraensis* [99]. Homologues of this enzyme were identified in all (except *Halobacterium* spp.) euryarchaeal and crenarchaeal genomes (Table 2), indicating that the archaea apparently contain two distinct types of FBPs, probably with functions in gluconeogenesis and the PP pathway (Table 2). An orthologue of the latter gene appears to be up-regulated during operation of gluconeogenesis in *Pyrococcus furiosus* (see below), and, as such, is the most likely candidate for the archaeal FBP (G. J. Schut and M. W. W. Adams, unpublished work). It should be noted that the PP_i-dependent PFK of *Thermoproteus tenax* has been shown to have phosphatase activity as well [61]; it is not yet known, however, whether or not this enzyme also substitutes FBP *in vivo*.

Archaea that do not possess PGI protein sequences (e.g. *Methanobacterium thermoautotrophicus* and *Ar. fulgidus*) (Table 2) might possess a gluconeogenesis pathway up to the level of fructose 6-phosphate, which probably suffices for a link to the non-oxidative PP pathway, but certainly not to glycogen synthesis (Scheme 1C). As pointed out above, it has been suggested that the archaeal non-phosphorylating ED pathway may be reversible [1]; future research should reveal whether alternative pathways for gluconeogenesis exist.

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REGULATION OF ARCHAEAL GLYCOLYSIS

Regulation of the classical glycolysis is a very complex process. Modulation of the glycolytic flux relies on the co-ordinated triggering of multiple events, both at the DNA level (modulation of gene expression) and at the enzyme level (regulation of enzymic activities either by allosteric effectors or by covalent modifications) [3,100].

Classical control sites of glycolysis are the unidirectional conversions, catalysed by the kinases/phosphatases: during glycolysis, PFK and PYK, and during gluconeogenesis, PPS and FBP. Regulation of the main metabolic pathways of the model bacterium *E. coli* has been studied thoroughly. A thorough proteome analysis study has revealed that a shift from glucose to acetate resulted in down-regulation of the glycolysis-specific genes encoding PFK (*pfkA*) and PYK(*pykF*), and an up-regulation of FBP (*fbp*) and PPS (*ppsA*). Recent DNA microarray analysis in which similar conditions (*E. coli* growth on acetate compared with glucose) were compared did confirm most relevant conclusions, i.e. induced transcription of gluconeogenesis (*ppsA* 13-fold and *fbp* 3-fold up-regulated), and repression of glycolysis (*pykF* 5-fold and *pfkA* 2-fold down-regulated) [101]. In *E. coli*, FruR (fructose repressor protein, also referred to as Cra, for catabolite-repressor activator) is the transcriptional regulator that senses the intracellular concentration of fructose phosphates, and, depending on the cell's metabolic state, represses transcription of the glycolytic enzyme *pfkA* and *pykF* genes, and induces transcription of the *ppsA* and *fbp* genes encoding gluconeogenic enzymes [102]. A second regulator (Mlc) monitors the glucose supply, and controls the expression of genes that encode PTS-type transport systems for glucose, maltose and mannose [103]. In addition, CRP (cAMP-receptor protein; also referred to as CAP, catabolite-activating protein) is involved in the global regulation of sugar uptake and hydrolysis systems (e.g. the *lac* operon) [104]. In Gram-positive bacteria (e.g. *Lactococcus lactis*), the catabolite-control protein (CcpA) is a global regulator that, amongst others, has been demonstrated to be a transcriptional activator of the *las* operon, consisting of genes encoding the key glycolytic enzymes PFK and PYK, as well as lactate dehydrogenase (LDH) [105,106]. The latter enzyme is crucial for NADH re-oxidation, and, as such, plays a key role in enhancing the glycolytic flux.

A key control point of yeast glycolysis is the second irreversible step of the pathway, which is catalysed by PFK (Scheme 1B). Upon sugar availability, PFK activity increases as a result of rising concentrations of its substrate, fructose 6-phosphate, and its main allosteric activator, fructose 2,6-bisphosphate. Activation of the PFK reaction increases the concentration of its product, fructose 1,6-bisphosphate, which is, in turn, an allosteric activator of PYK, the second specific glycolytic enzyme. Glycolysis is therefore progressively activated by increasing concentrations of enzyme substrates and allosteric activators [3]. In addition, the transcription of the genes encoding PFK (*pfk*) and PYK (*pyk1*) is mainly induced upon an increased glucose concentration in the medium [107]. In the absence of glucose, a yeast DNA-binding protein (GRC1) was found to strongly decrease the transcription levels of genes encoding the key glycolytic enzymes [108,109].

In contrast with the situation for some model bacteria and eukaryotes, the glycolytic control in archaea is still poorly understood. At present, nothing is known about post-translational control of the archaeal central metabolic pathways. Some insight has been gained into the control of the modified EM pathways of *Pyrococcus furiosus* and *Thermoproteus tenax*, including modulation of gene expression and allosteric regulation of enzymic activities. The activity levels of *Pyrococcus furiosus* glycolytic enzymes are generally elevated in cells grown on sugars compared with cells grown on peptides or pyruvate [4,52,96,110]. This is in good agreement with transcript analyses of *Pyrococcus furiosus* glycolytic genes, that reveal up-regulation in sugar-grown cells, and down-regulation in peptide- or pyruvate-grown cells [52,54,62,67].

Clustering of glycolytic genes is frequently observed in bacteria. As mentioned above, the *las* operon of *L. lactis* encodes the irreversible key enzymes PFK, PYK and LDH. Context analysis (http://www.bork.embl-heidelberg.de/STRING/) reveals that, in many bacterial genomes, some reversible glycolysis/gluconeogenesis enzymes cluster as well: TIM, GAPDH, PGK, and sometimes enolase and FBA (results not shown) [111,112]. In *Thermotoga maritima*, the genes encoding the former three enzymes cluster as well; moreover, TIM and PGK are fused (Table 2). As indicated by asterisks in Table 2, several gene clusters encode archaeal glycolytic enzymes. The operon that encodes the PP_i-PFK and FBA of *Thermoproteus tenax* appears to be up-regulated 6-fold during growth on glucose compared with autotrophic growth [62]. Higher transcript levels of genes encoding reversible enzymes under catabolic conditions probably reflect the necessity of a higher carbon flux through the glycolytic pathway.

No archaeal transcriptional regulators have yet been identified that are involved in glycolysis regulation. A potential *cis*-regulatory element has recently been identified in promoter sequences of the genes encoding glycolytic enzymes in *Pyrococcus furiosus* [113]. This inverted repeat was not present in promoter sequences of genes encoding FBP, GAPDH and PGK, enzymes that act solely in gluconeogenesis in this organism. Interestingly, the motif was apparent in the promoter region of the PPS-encoding gene as well, but not in that of PYK. Although this observation would agree with a proposed role of PPS during glycolysis [45], it conflicts with a biochemical characterization of the *Pyrococcus furiosus* enzyme [76]. Since the inverted repeat is present in promoter sequences of genes encoding glycolytic enzymes, it may represent a specific site for regulating the *Pyrococcus furiosus* glycolytic pathway by an as-yet-unidentified transcriptional regulator [113].

In classical glycolysis, the reactions catalysed by hexokinase, PFK and PYKs are virtually irreversible. Hence, it makes sense that these enzymes, at least in eukaryotes, are main allosterically regulated control sites [3]. ADP-GLK and ADP-PFK from *Pyrococcus furiosus*, and PP_i-PFK and PYK from *Thermoproteus tenax*, have been investigated with regard to their regulatory roles (C. H. Verhees, unpublished work; [46,61,114]). Interestingly, none of these enzymes was allosterically regulated by any of the known allosteric effectors. Therefore, they presumably do not act as major allosteric control points of the glycolytic pathway.

Regulation of GAPN in the glycolytic pathway of *Thermoproteus tenax* has been demonstrated to occur not transcriptionally, but rather allosterically. Activity of GAPN is inhibited by NADPH, NADP, NADH and ATP, and is positively regulated by AMP, glucose 1-phosphate, fructose 6-phosphate, ADP, fructose 1-phosphate and ribose 5-phosphate [69,115]. Apparently, allosteric regulation does exist for some of the glycolytic enzymes in the hyperthermophilic archaea. On the other hand, the GAPDH enzyme of *Thermoproteus tenax* is regulated at the transcription level, rather than allosterically, and in this sense it resembles the *Pyrococcus furiosus* enzyme (see below). PYK is another example of a regulatory difference between the two organisms. In *Thermoproteus tenax*, PYK is transcriptionally regulated, but this is not the case in *Pyrococcus furiosus* [114].

The non-allosteric GAPOR enzyme has been proposed to be an important enzyme in control of glycolysis in *Pyrococcus furiosus* [67]. Unlike GAPDH in bacteria/eukaryotes, the reaction

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Table 3 Modulation of expression of genes encoding enzymes that are involved in glycolysis (EM pathway) and gluconeogenesis in *Pyrococcus furiosus*

RNA was isolated from cells that were grown in batch cultures on either maltose or peptides [54], and labelled cDNA was hybridized with a complete genomic DNA microarray (G. J. Schut and M. W. W. Adams, unpublished work). Modulation is indicated as fold-change: (+), up-regulation on maltose; (−), down-regulation on maltose.

Number	Glycolysis enzyme	Gene (PF)	Maltose-induced up-regulation (+) or down-regulation (−)	Number	Gluconeogenesis enzyme	Gene (PF)	Maltose-induced up-regulation (+) or down-regulation (−)
1	GLK	0312	+ 2.1				
2	PGI	0196	+ 4.9				
3	PFK	1784	+ 5.7	22	FBP	0613	− 14.9
4	FBA	1956	+ 1.2				
5	TIM	1920	+ 4.7				
6/7	GAPOR	0464	+ 5.7	6	GAPDH	1874	− 9.2
				7	PGK	1057	− 7.5
8	PGM	1959	+ 3.2				
9	Enolase	0215	+ 2.5				
10	PYK	1188	− 1.5	21	PPS	0043	+ 1.1

catalysed by GAPOR is also irreversible (like the eukaryal/bacterial control sites). GAPOR acts solely in glycolysis and the expression of its gene is induced by growth on sugars. In contrast, the expression of the gene encoding GAPDH appears to be enhanced under gluconeogenic conditions (cells grown on tryptone; see below) [90]. This confirms the involvement of GAPOR in pyrococcal glycolysis. This archaeal-specific bypass has been proposed to be a novel site for glycolytic control [67].

Complete genome microarrays of *Pyrococcus furiosus* have recently been performed [54,90]. Recent microarray analysis (maltose compared with peptides) show that the mechanism of regulation for *Pyrococcus furiosus* is mainly at the transcriptional level. As shown in Table 3, four enzymes involved in glycolysis are strongly up-regulated during growth on maltose (PGI, PFK, GAPOR and TPI), whereas three gluconeogenic enzymes are strongly up-regulated in the peptide-grown cultures (GAPDH, PGK and FBP). It seems that the latter three enzymes are the major regulation points for the switch between gluconeogenesis and glycolysis. It is concluded that regulation of the glycolytic flux in archaea, such as *Pyrococcus furiosus*, involves modulation of gene expression, rather than allosteric regulation of enzyme activities.

EVOLUTIONARY ASPECTS OF ARCHAEAL GLYCOLYTIC PATHWAYS

In most organisms, glucose catabolism is accomplished by an EM, an ED or sometimes a PP pathway, or variations thereof. The routes generally converge at the level of GAP (Scheme 1), which is subsequently converted by a common core pathway into pyruvate [1,116]. The variant ED pathways in *Sulfolobus*, *Thermoproteus* and *Thermoplasma* form an exception in that they converge with the EM pathway at the level of 2-phosphoglycerate (Scheme 1). The common branch that consists of the enzymes GAPDH, PGK, PGM, enolase and PPS appears to be ubiquitously present in archaea (Table 2), as well as in bacteria and eukaryotes. The complete conservation of this C_3 stage of glycolysis/gluconeogenesis suggests that it represents an ancient pathway (reviewed in [10]).

Gluconeogenesis is a pathway through which pyruvate is converted into fructose 6-phosphate, glucose 6-phosphate or glucose. This pathway is crucial for biosynthetic purposes and appears to be present in all organisms. Little variation is observed in the reductive enzymes of this pathway between the three domains of life [10]. In archaea, some gluconeogenic enzymes appear to have evolved by early gene duplication and subsequent differential loss, resulting in extremely low sequence similarity with their bacterial and eukaryal counterparts (Table 2). In all archaea, gluconeogenic pathways appear to be present. In contrast, sugar catabolic pathways are absent in non-heterotrophic archaea, except for glycogen-degrading methanogens. Subsequently, the convergent evolution of sugar kinases, PGI and GAPOR, in combination with the gain of glycosyl hydrolases and appropriate transport systems enabled archaea, such as *Pyrococcus*, to adopt a heterotrophic lifestyle [117].

It has been pointed out that many unusual glycolytic enzymes have evolved in the archaea, as compared with the counterparts in bacteria and eukaryotes. This probably reflects the independent evolution of the catabolic branches of the different pathways in bacteria and archaea. Orthologues of the bacterial enzymes were conserved in the subsequently evolving eukaryotes. Originally, the archaeal glycolytic enzymes were considered as novel inventions. More thorough analysis of primary and tertiary structures, however, revealed that structural homologues are often found in bacteria and/or eukaryotes, whereas the functional conservation (substrate specificity) is more distant. The ADP-dependent kinases (ADP-GLK and ADP-PFK) have been demonstrated to be related to the ribokinase family; another member of this family is a redundant glycolytic enzyme of *E. coli* (PFKB). The archaeal isomerase (PGI) is not at all related to the classical counterpart, but has a similar fold as phosphomannose isomerase in bacteria and fungi. The archaeal FBA resembles an *E. coli* aldolase, which is present next to the classical Class II enzyme [62]. Also, the most severe modification of the archaeal pathway in terms of bioenergetics, the oxidation of GAP, is catalysed by the archaea-specific glycolytic enzymes GAPOR or GAPN; these enzymes also have counterparts that have different physiological roles in bacteria and eukarya. Hence, it is concluded that the conserved enzymes in archaeal glycolysis are the result of lateral gene transfer from bacteria, and that the unique enzymes are the result of an independent, convergent evolution. The fact that the bacterial and archaeal pathways are chemically very similar probably reflects the fact that the selective pressure for thermodynamically efficient glucose conversion does not allow too many alternative pathways [1].

CONCLUDING REMARKS

It is evident that glucose catabolism in archaea proceeds via novel pathways that are significantly different from established EM or ED pathways. The purification and characterization of many

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novel archaeal glycolytic enzymes, the emerging data of genome sequences, as well as the recent functional genomics studies, have revealed the complexity of sugar metabolism. Comparisons made with canonical sugar catabolic pathways have revealed major variations mainly in the upper part of the archaeal glycolysis, concerning the steps from glucose to 3-phosphoglycerate. Over the past decade, the composition of several EM- and ED-like glucose-degradation pathways in archaea have been elucidated in considerable detail. Major breakthroughs are still ahead of us concerning the details of its regulation and its bioenergetics. In addition, much is still to be learned about archaeal pentose metabolism. In the post-genome era, however, a significant gain of insight is anticipated in the near future.

Part of this work was supported by the Earth Life Sciences Foundation (ALW), which is subsidized by the Netherlands Organization for Scientific Research (NWO), and by the US Department of Energy (FG05-95ER201750) and the National Science Foundation (MCB0129841).

REFERENCES

1 Meléndez-Hevia, E., Waddell, T. G., Heinrich, R. and Montero, F. (1997) Theoretical approaches to the evolutionary optimization of glycolysis – chemical analysis. Eur. J. Biochem. **244**, 527–543

2 Peekhaus, N. and Conway, T. (1998) What's for dinner?: Entner–Doudoroff metabolism in *Escherichia coli*. J. Bacteriol. **180**, 3495–3502

3 Goncalves, P. and Planta, R. J. (1998) Starting up yeast glycolysis. Trends Microbiol. **6**, 314–319

4 Kengen, S. W., de Bok, F. A., van Loo, N. D., Dijkema, C., Stams, A. J. and de Vos, W. M. (1994) Evidence for the operation of a novel Embden–Meyerhof pathway that involves ADP-dependent kinases during sugar fermentation by *Pyrococcus furiosus*. J. Biol. Chem. **269**, 17537–17541

5 Selig, M., Xavier, K. B., Santos, H. and Schönheit, P. (1997) Comparative analysis of Embden–Meyerhof and Entner–Doudoroff glycolytic pathways in hyperthermophilic archaea and the bacterium *Thermotoga*. Arch. Microbiol. **167**, 217–232

6 Siebers, B. and Hensel, R. (1993) Glucose catabolism of the hyperthermophilic archaeum *Thermoproteus tenax*. FEMS Microbiol. Lett. **111**, 1–8

7 Schäfer, T. and Schönheit, P. (1992) Maltose fermentation to acetate, CO_2 and H_2 in the anaerobic hyperthermophilic archaeaon *Pyrococcus furiosus*: evidence for the operation of a novel sugar fermentation pathway. Arch. Microbiol. **158**, 188–202

8 Kengen, S. W., Tuininga, J. E., de Bok, F. A., Stams, A. J. and de Vos, W. M. (1995) Purification and characterization of a novel ADP-dependent glucokinase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Biol. Chem. **270**, 30453–30457

9 Dandekar, T., Schuster, S., Snel, B., Huynen, M. and Bork, P. (1999) Pathway alignment: application to the comparative analysis of glycolytic enzymes. Biochem. J. **343**, 115–124

10 Ronimus, R. S. and Morgan, H. W. (2003) Distribution and phylogenies of enzymes of the Embden–Meyerhof–Parnas pathway from archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. Archaea, in the press

11 Danson, M. J. (1993) Central Metabolism of the Archaea, Elsevier, Amsterdam

12 Schönheit, P. and Schäfer, T. (1995) Metabolism of hyperthermophiles. World J. Microbiol. Biotechnol. **11**, 26–57

13 Kengen, S. W. M., Stams, A. J. M. and de Vos, W. M. (1996) Sugar metabolism of hyperthermophiles. FEMS Microbiol. Rev. **18**, 119–137

14 de Vos, W. M., Kengen, S. W., Voorhorst, W. G. and van der Oost, J. (1998) Sugar utilization and its control in hyperthermophiles. Extremophiles **2**, 201–205

15 Silva, P. J., van den Ban, E. C., Wassink, H., Haaker, H., de Castro, B., Robb, F. T. and Hagen, W. R. (2000) Enzymes of hydrogen metabolism in *Pyrococcus furiosus*. Eur. J. Biochem. **267**, 6541–6551

16 Cohen, G. N., Barbe, V., Flament, D., Galperin, M., Heilig, R., Lecompte, O., Poch, O., Prieur, D., Querellou, J., Ripp, R. et al. (2003) An integrated analysis of the genome of the hyperthermophilic archaeon *Pyrococcus abyssi*. Mol. Microbiol. **47**, 1495–1512

17 Fiala, G. and Stetter, K. O. (1986) *Pyrococcus furiosus* sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100 °C. Arch. Microbiol. **161**, 168–175

18 Zillig, W., Stetter, K. O., Wunderl, S., Schulz, W., Priess, H. and Scholz, I. (1980) The *Sulfolobus*-"*Caldariella*" group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. Arch. Microbiol. **125**, 259–269

19 Kengen, S. W., Luesink, E. J., Stams, A. J. and Zehnder, A. J. (1993) Purification and characterization of an extremely thermostable β-glucosidase from the hyperthermophilic archaeon *Pyrococcus furiosus*. Eur. J. Biochem. **213**, 305–312

20 Gueguen, Y., Voorhorst, W. G., van der Oost, J. and de Vos, W. M. (1997) Molecular and biochemical characterization of an endo-β-1,3-glucanase of the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Biol. Chem. **272**, 31258–31264

21 Driskill, L. E., Kusy, K., Bauer, M. W. and Kelly, R. M. (1999) Relationship between glycosyl hydrolase inventory and growth physiology of the hyperthermophile *Pyrococcus furiosus* on carbohydrate-based media. Appl. Environ. Microbiol. **65**, 893–897

22 Grogan, D. W. (1989) Phenotypic characterization of the archaebacterial genus *Sulfolobus*: comparison of five wild-type strains. J. Bacteriol. **171**, 6710–6719

23 Elferink, M. G., Albers, S. V., Konings, W. N. and Driessen, A. J. (2001) Sugar transport in *Sulfolobus solfataricus* is mediated by two families of binding protein-dependent ABC transporters. Mol. Microbiol. **39**, 1494–1503

24 Kaper, T., Verhees, C. H., Lebbink, J. H., van Lieshout, J. F., Kluskens, L. D., Ward, D. E., Kengen, S. W., Beerthuyzen, M. M., de Vos, W. M. and van der Oost, J. (2001) Characterization of β-glycosylhydrolases from *Pyrococcus furiosus*. Methods Enzymol. **330**, 329–346

25 Koch, R., Zablowski, P., Spreinat, A. and Antranikian, G. (1990) Extremely thermostable amylolytic enzyme from the archaebacterium *Pyrococcus furiosus*. FEMS Microbiol. Lett. **71**, 21–26

26 Jorgensen, S., Vorgias, C. E. and Antranikian, G. (1997) Cloning, sequencing, characterization, and expression of an extracellular α-amylase from the hyperthermophilic archaeon *Pyrococcus furiosus* in *Escherichia coli* and *Bacillus subtilis*. J. Biol. Chem. **272**, 16335–16342

27 Brown, S. H. and Kelly, R. M. (1993) Characterization of amylolytic enzymes, having both α-1,4 and α-1,6 hydrolytic activity, from the thermophilic archaea *Pyrococcus furiosus* and *Thermococcus litoralis*. Appl. Environ. Microbiol. **59**, 2614–2621

28 Dong, G., Vieille, C., Savchenko, A. and Zeikus, J. G. (1997) Cloning, sequencing, and expression of the gene encoding extracellular α-amylase from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme. Appl. Environ. Microbiol. **63**, 3569–3576

29 Moracci, M., Cobucci Ponzano, B., Trincone, A., Fusco, S., De Rosa, M., van der Oost, J., Sensen, C. W., Charlebois, R. L. and Rossi, M. (2000) Identification and molecular characterization of the first α-xylosidase from an archaeon. J. Biol. Chem. **275**, 22082–22089

30 Koning, S. M., Elferink, M. G., Konings, W. N. and Driessen, A. J. (2001) Cellobiose uptake in the hyperthermophilic archaeon *Pyrococcus furiosus* is mediated by an inducible, high-affinity ABC transporter. J. Bacteriol. **183**, 4979–4984

31 Horlacher, R., Xavier, K. B., Santos, H., DiRuggiero, J., Kossmann, M. and Boos, W. (1998) Archaeal binding protein-dependent ABC transporter: molecular and biochemical analysis of the trehalose/maltose transport system of the hyperthermophilic archaeon *Thermococcus litoralis*. J. Bacteriol. **180**, 680–689

32 Xavier, K. B., Martins, L. O., Peist, R., Kossmann, M., Boos, W. and Santos, H. (1996) High-affinity maltose/trehalose transport system in the hyperthermophilic archaeon *Thermococcus litoralis*. J. Bacteriol. **178**, 4773–4777

33 Wanner, C. and Soppa, J. (1999) Genetic identification of three ABC transporters as essential elements for nitrate respiration in *Haloferax volcanii*. Genetics **152**, 1417–1428

34 Tawara, E. and Kamo, N. (1991) Glucose transport of Haloferax volcanii requires the Na^+-electrochemical potential gradient and inhibitors for the mammalian glucose transporter inhibit the transport. Biochim. Biophys. Acta **1070**, 293–299

35 Albers, S. V., Elferink, M. G., Charlebois, R. L., Sensen, C. W., Driessen, A. J. and Konings, W. N. (1999) Glucose transport in the extremely thermoacidophilic *Sulfolobus solfataricus* involves a high-affinity membrane-integrated binding protein. J. Bacteriol. **181**, 4285–4291

36 Constantino, H. R., Bron, S. H. and Kelly, R. M. (1990) Purification and characterization of an α-glucosidase from a hyperthermophilic archaebacterium, *Pyrococcus furiosus*, exhibiting a temperature optimum of 105 to 115 °C. J. Bacteriol. **173**, 3654–3660

37 Pisani, F. M., Rella, R., Raia, C. A., Rozzo, C., Nucci, R., Gambacorta, A., De Rosa, M. and Rossi, M. (1990) Thermostable β-galactosidase from the archaebacterium *Sulfolobus solfataricus*: purification and properties. Eur. J. Biochem. **187**, 321–328

38 Nucci, R., Moracci, M., Vaccaro, C., Vespa, N. and Rossi, M. (1993) Exo-glucosidase activity and substrate specificity of the β-glycosidase isolated from the extreme thermophile *Sulfolobus solfataricus*. Biotechnol. Appl. Biochem. **17**, 239–250

39 Rolfsmeier, M. and Blum, P. (1995) Purification and characterization of a maltase from the extremely thermophilic crenarchaeote *Sulfolobus solfataricus*. J. Bacteriol. **177**, 482–485

40 Blamey, J. M. and Adams, M. W. (1993) Purification and characterization of pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon *Pyrococcus furiosus*. Biochim. Biophys. Acta **1161**, 19–27

© 2003 Biochemical Society

41 Huynen, M. A., Dandekar, T. and Bork, P. (1999) Variation and evolution of the citric-acid cycle: a genomic perspective. Trends Microbiol. **7**, 281–291

42 She, Q., Singh, R. K., Confalonieri, F., Zivanovic, Y., Allard, G., Awayez, M. J., Chan-Weiher, C. C., Clausen, I. G., Curtis, B. A., De Moors, A. et al. (2001) The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. Proc. Natl. Acad. Sci. U.S.A. **98**, 7835–7840

43 Musfeldt, M., Selig, M. and Schönheit, P. (1999) Acetyl coenzyme A synthetase (ADP forming) from the hyperthermophilic Archaeon *Pyrococcus furiosus*: identification, cloning, separate expression of the encoding genes, acdAI and acdBI, in *Escherichia coli*, and *in vitro* reconstitution of the active heterotetrameric enzyme from its recombinant subunits. J. Bacteriol. **181**, 5885–5888

44 Musfeldt, M. and Schönheit, P. (2002) Novel type of ADP-forming acetyl coenzyme A synthetase in hyperthermophilic archaea: heterologous expression and characterization of isoenzymes from the sulfate reducer *Archaeoglobus fulgidus* and the methanogen *Methanococcus jannaschii*. J. Bacteriol. **184**, 636–644

45 Sakuraba, H., Utsumi, E., Kujo, C. and Ohshima, T. (1999) An AMP-dependent (ATP-forming) kinase in the hyperthermophilic archaeon *Pyrococcus furiosus*: characterization and novel physiological role. Arch. Biochem. Biophys. **364**, 125–128

46 Tuininga, J. E., Verhees, C. H., van der Oost, J., Kengen, S. W., Stams, A. J. and de Vos, W. M. (1999) Molecular and biochemical characterization of the ADP-dependent phosphofructokinase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Biol. Chem. **274**, 21023–21028

47 Koga, S., Yoshioka, I., Sakuraba, H., Takahashi, M., Sakasegawa, S., Shimizu, S. and Ohshima, T. (2000) Biochemical characterization, cloning, and sequencing of ADP-dependent (AMP-forming) glucokinase from two hyperthermophilic archaea, *Pyrococcus furiosus* and *Thermococcus litoralis*. J. Biochem. (Tokyo) **128**, 1079–1085

48 Labes, A. and Schönheit, P. (2001) Sugar utilization in the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324: starch degradation to acetate and CO2 via a modified Embden–Meyerhof pathway and acetyl-CoA synthetase (ADP-forming). Arch. Microbiol. **176**, 329–338

49 Hansen, T., Reichstein, B., Schmid, R. and Schönheit, P. (2002) The first archaeal ATP-dependent glucokinase, from the hyperthermophilic crenarchaeon *Aeropyrum pernix*, represents a monomeric, extremely thermophilic ROK glucokinase with broad hexose specificity. J. Bacteriol. **184**, 5955–5965

50 Dorr, C., Zaparty, M., Tjaden, B., Brinkmann, H. and Siebers, B. (2003) The hexokinase of the hyperthermophile *Thermoproteus tenax*: ATP-dependent hexokinases and ADP-dependent glucokinases, two alternatives for glucose phosphorylation in Archaea. J. Biol. Chem. **278**, 18744–18753

51 Hansen, T., Oehlmann, M. and Schönheit, P. (2001) Novel type of glucose-6-phosphate isomerase in the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Bacteriol. **183**, 3428–3435

52 Verhees, C. H., Huynen, M. A., Ward, D. E., Schiltz, E., de Vos, W. M. and van der Oost, J. (2001) The phosphoglucose isomerase from the hyperthermophilic archaeon *Pyrococcus furiosus* is a unique glycolytic enzyme that belongs to the cupin superfamily. J. Biol. Chem. **276**, 40926–40932

53 Jeong, J. J., Fushinobu, S., Ito, S., Jeon, B. S., Shoun, H. and Wakagi, T. (2003) Characterization of the cupin-type phosphoglucose isomerase from the hyperthermophilic archaeon *Thermococcus litoralis*. FEBS Lett. **535**, 200–204

53a Berrisford, J. M., Akerboom, J., Turnbull, A. P., De Geus, D., Sedelnikova, S. E., Staton, I., McLeod, C. W., Verhees, C. H., van der Oost, J., Rice, D. W. and Baker, P. J. (2003) Crystal structure of *Pyrococcus furiosus* phosphoglucose isomerase: implications for substrate binding and catalysis. J. Biol. Chem. **278**, 33290–33297

54 Schut, G. J., Zhou, J. and Adams, M. W. (2001) DNA microarray analysis of the hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for a new type of sulfur-reducing enzyme complex. J. Bacteriol. **183**, 7027–7036

55 Bateman, A. (1999) The SIS domain: a phosphosugar-binding domain. Trends Biochem. Sci. **24**, 94–95

56 Ronimus, R. S., Koning, J. and Morgan, H. W. (1999) Purification and characterization of an ADP-dependent phosphofructokinase from *Thermococcus zilligii*. Extremophiles **3**, 121–129

57 Verhees, C. H., Tuininga, J. E., Kengen, S. W., Stams, A. J., van der Oost, J. and de Vos, W. M. (2001) ADP-dependent phosphofructokinases in mesophilic and thermophilic methanogenic archaea. J. Bacteriol. **183**, 7145–7153

58 Sakuraba, H., Yoshioka, I., Koga, S., Takahashi, M., Kitahama, Y., Satomura, T., Kawakami, R. and Ohshima, T. (2002) ADP-dependent glucokinase/phosphofructokinase, a novel bifunctional enzyme from the hyperthermophilic archaeon *Methanococcus jannaschii*. J. Biol. Chem. **277**, 12495–12498

59 Ito, S., Fushinobu, S., Yoshioka, I., Koga, S., Matsuzawa, H. and Wakagi, T. (2001) Structural basis for the ADP-specificity of a novel glucokinase from a hyperthermophilic archaeon. Struct. Fold Des. **9**, 205–214

60 Hansen, T. and Schönheit, P. (2000) Purification and properties of the first-identified, archaeal, ATP-dependent 6-phosphofructokinase, an extremely thermophilic non-allosteric enzyme, from the hyperthermophile *Desulfurococcus amylolyticus*. Arch. Microbiol. **173**, 103–109

61 Siebers, B., Klenk, H. P. and Hensel, R. (1998) PP_i-dependent phosphofructokinase from *Thermoproteus tenax*, an archaeal descendant of an ancient line in phosphofructokinase evolution. J. Bacteriol. **180**, 2137–2143

62 Siebers, B., Brinkmann, H., Dorr, C., Tjaden, B., Lilie, H., van der Oost, J. and Verhees, C. H. (2001) Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase. J. Biol. Chem. **276**, 28710–28718

63 Galperin, M. Y., Aravind, L. and Koonin, E. V. (2000) Aldolases of the DhnA family: a possible solution to the problem of pentose and hexose biosynthesis in archaea. FEMS Microbiol. Lett. **183**, 259–264

64 Kohlhoff, M., Dahm, A. and Hensel, R. (1996) Tetrameric triosephosphate isomerase from hyperthermophilic Archaea. FEBS Lett. **383**, 245–250

65 Walden, H., Bell, G. S., Russell, R. J., Siebers, B., Hensel, R. and Taylor, G. L. (2001) Tiny TIM: a small, tetrameric, hyperthermostable triosephosphate isomerase. J. Mol. Biol. **306**, 745–757

66 Mukund, S. and Adams, M. W. (1995) Glyceraldehyde-3-phosphate ferredoxin oxidoreductase, a novel tungsten-containing enzyme with a potential glycolytic role in the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Biol. Chem. **270**, 8389–8392

67 van der Oost, J., Schut, G., Kengen, S. W., Hagen, W. R., Thomm, M. and de Vos, W. M. (1998) The ferredoxin-dependent conversion of glyceraldehyde 3-phosphate in the hyperthermophilic archaeon *Pyrococcus furiosus* represents a novel site of glycolytic regulation. J. Biol. Chem. **273**, 28149–28154

68 Roy, R., Menon, A. L. and Adams, M. W. (2001) Aldehyde oxidoreductases from *Pyrococcus furiosus*. Methods Enzymol. **331**, 132–144

69 Brunner, N. A., Brinkmann, H., Siebers, B. and Hensel, R. (1998) NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase from *Thermoproteus tenax*: the first identified archaeal member of the aldehyde dehydrogenase superfamily is a glycolytic enzyme with unusual regulatory properties. J. Biol. Chem. **273**, 6149–6156

70 Koonin, E. V., Mushegian, A. R., Galperin, M. Y. and Walker, D. R. (1997) Comparison of archaeal and bacterial genomes: computer analysis of protein sequences predicts novel functions and suggests a chimeric origin for the archaea. Mol. Microbiol. **25**, 619–637

71 Galperin, M. Y., Bairoch, A. and Koonin, E. V. (1998) A superfamily of metalloenzymes unifies phosphopentomutase and cofactor-independent phosphoglycerate mutase with alkaline phosphatases and sulfatases. Protein Sci. **7**, 1829–1835

72 van der Oost, J., Huynen, M. and Verhees, C. H. (2002) Molecular characterization of phosphoglycerate mutase in archaea. FEMS Microbiol. Lett. **212**, 111–120

73 Graham, D. E., Xu, H. and White, R. H. (2002) A divergent archaeal member of the alkaline phosphatase binuclear metalloenzyme superfamily has phosphoglycerate mutase activity. FEBS Lett. **517**, 190–194

74 Peak, M. J., Peak, J. G., Stevens, F. J., Blamey, J., Mai, X., Zhou, Z. H. and Adams, M. W. (1994) The hyperthermophilic glycolytic enzyme enolase in the archaeon, *Pyrococcus furiosus*: comparison with mesophilic enolases. Arch. Biochem. Biophys. **313**, 280–286

75 Tracy, M. R. and Hedges, S. B. (2000) Evolutionary history of the enolase gene family. Gene **259**, 129–138

76 Hutchins, A. M., Holden, J. F. and Adams, M. W. (2001) Phosphoenolpyruvate synthetase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Bacteriol. **183**, 709–715

77 De Rosa, M., Gambacorta, A., Nicolaus, B., Giardina, P., Poerio, E. and Buonocore, V. (1984) Glucose metabolism in the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus*. Biochem. J. **224**, 407–414

78 Hensel, R., Laumann, S., Lang, J., Heumann, H. and Lottspeich, F. (1987) Characterization of two D-glyceraldehyde-3-phosphate dehydrogenases from the extremely thermophilic archaebacterium *Thermoproteus tenax*. Eur. J. Biochem. **170**, 325–333

79 Budgen, N. and Danson, M. J. (1986) Metabolism of glucose via a modified Entner–Doudoroff pathway in the thermoacidophilic archaeabacterium *Thermoplasma acidophilum*. FEBS Lett. **196**, 207–210

80 Giardina, P., de Biasi, M. G., de Rosa, M., Gambacorta, A. and Buonocore, V. (1986) Glucose dehydrogenase from the thermoacidophilic archaebacterium *Sulfolobus solfataricus*. Biochem. J. **239**, 517–522

81 Bright, J. R., Bryom, D., Danson, M. J., Hough, D. W. and Towner, P. (1993) Cloning, sequencing and expression of the gene encoding glucose dehydrogenase from the thermophilic archaeon *Thermoplasma acidophilum*. Eur. J. Biochem. **211**, 549–554

82 Siebers, B., Wendisch, V. F. and Hensel, R. (1997) Carbohydrate metabolism in *Thermoproteus tenax*: *in vivo* utilization of the non-phosphorylative Entner–Doudoroff pathway and characterization of its first enzyme, glucose dehydrogenase. Arch. Microbiol. **168**, 120–127

83 Bonete, M.-J., Pire, C., Llorca, F. I. and Camacho, M. L. (1996) Glucose dehydrogenase from the halophilic Archaeon *Haloferax mediterranei*: enzyme purification, characterisation and N-terminal sequence. FEBS Lett. **383**, 227–229

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84 Johnsen, U., Selig, M., Xavier, K. B., Santos, H. and Schonheit, P. (2001) Different glycolytic pathways for glucose and fructose in the halophilic archaeon *Halococcus saccharolyticus*. Arch. Microbiol. **175**, 52–61
85 Buchanan, C. L., Connaris, H., Danson, M. J., Reeve, C. D. and Hough, D. W. (1999) An extremely thermostable aldolase from *Sulfolobus solfataricus* with specificity for non-phosphorylated substrates. Biochem. J. **343**, 563–570
86 Griffiths, J. S., Wymer, N. J., Njolito, E., Niranjanakumari, S., Fierke, C. A. and Toone, E. J. (2002) Cloning, isolation and characterization of the *Thermotoga maritima* KDPG aldolase. Bioorg. Med. Chem. **10**, 545–550
87 Danson, M. J. (1989) Central metabolism of the archaeabacteria: an overview. Can. J. Microbiol. **35**, 58–63
88 Selkov, E., Maltsev, N., Olsen, G. J., Overbeek, R. and Whitman, W. B. (1997) A reconstruction of the metabolism of *Methanococcus jannaschii* from sequence data. Gene **197**, 11–26
89 Makarova, K. S., Aravind, L., Galperin, M. Y., Grishin, N. V., Tatusov, R. L., Wolf, Y. I. and Koonin, E. V. (1999) Comparative genomics of the Archaea (Euryarchaeota): evolution of conserved protein families, the stable core, and the variable shell. Genome Res. **9**, 608–628
90 Schut, G. J., Brehm, S. D., Datta, S. and Adams, M. W. (2003) Whole-genome DNA microarray analysis of a hyperthermophile and an archaeon: *Pyrococcus furiosus* grown on carbohydrates or peptides. J. Bacteriol. **185**, 3935–3947
91 Xavier, K. B., da Costa, M. S. and Santos, H. (2000) Demonstration of a novel glycolytic pathway in the hyperthermophilic archaeon *Thermococcus zilligii* by ^{13}C-labeling experiments and nuclear magnetic resonance analysis. J. Bacteriol. **182**, 4632–4636
92 Kandler, O. (1983) Carbohydrate metabolism in lactic acid bacteria. Antonie van Leeuwenhoek **49**, 209–224
93 Konig, H., Skorko, R., Zillig, W. and Reiter, W. D. (1982) Glycogen in thermoacidophilic archaebacteria of the genera *Sulfolobus*, *Thermoproteus*, *Desulfurococcus* and *Thermococcus*. Arch. Microbiol. **132**, 297–303
94 Foster, J. D. and Nordlie, R. C. (2002) The biochemistry and molecular biology of the glucose-6-phosphatase system. Exp. Biol. Med. (Maywood) **227**, 601–608
95 Robinson, K. A. and Schreier, H. J. (1994) Isolation, sequence and characterization of the maltose-regulated mlrA gene from the hyperthermophilic archaeum *Pyrococcus furiosus*. Gene **151**, 173–176
96 Adams, M. W., Holden, J. F., Menon, A. L., Schut, G. J., Grunden, A. M., Hou, C., Hutchins, A. M., Jenney, Jr, F. E., Kim, C., Ma, K. et al. (2001) Key role for sulfur in peptide metabolism and in regulation of three hydrogenases in the hyperthermophilic archaeon *Pyrococcus furiosus*. J. Bacteriol. **183**, 716–724
97 Stec, B., Yang, H., Johnson, K. A., Chen, L. and Roberts, M. F. (2000) MJ0109 is an enzyme that is both an inositol monophosphatase and the 'missing' archaeal fructose-1,6-bisphosphatase. Nat. Struct. Biol. **7**, 1046–1050
98 Verhees, C. H., Akerboom, J., Schiltz, E., de Vos, W. M. and van der Oost, J. (2002) Molecular and biochemical characterization of a distinct type of fructose-1,6-bisphosphatase from *Pyrococcus furiosus*. J. Bacteriol. **184**, 3401–3405
99 Rashid, N., Imanaka, H., Kanai, T., Fukui, T., Atomi, H. and Imanaka, T. (2002) A novel candidate for the true fructose 1,6-bisphosphatase in archaea. J. Biol. Chem. **277**, 30649–30655
100 Peng, L. and Shimizu, K. (2003) Global metabolic regulation analysis for *Escherichia coli* K12 based on protein expression by 2-dimensional electrophoresis and enzyme activity measurement. Appl. Microbiol. Biotechnol. **61**, 163–178
101 Oh, M. K., Rohlin, L., Kao, K. C. and Liao, J. C. (2002) Global expression profiling of acetate-grown *Escherichia coli*. J. Biol. Chem. **277**, 13175–13183
102 Ramseier, T. M., Bledig, S., Michotey, V., Feghali, R. and Saier, Jr, M. H. (1995) The global regulatory protein FruR modulates the direction of carbon flow in *Escherichia coli*. Mol. Microbiol. **16**, 1157–1169
103 Plumbridge, J. (2002) Regulation of gene expression in the PTS in *Escherichia coli*: the role and interactions of Mlc. Curr. Opin. Microbiol. **5**, 187–193
104 Hengge-Aronis, R. (1999) Interplay of global regulators and cell physiology in the general stress response of *Escherichia coli*. Curr. Opin. Microbiol. **2**, 148–152
105 Llanos, R. M., Harris, C. J., Hillier, A. J. and Davidson, B. E. (1993) Identification of a novel operon in *Lactococcus lactis* encoding three enzymes for lactic acid synthesis: phosphofructokinase, pyruvate kinase, and lactate dehydrogenase. J. Bacteriol. **175**, 2541–2551
106 Luesink, E. J., van Herpen, R. E., Grossiord, B. P., Kuipers, O. P. and de Vos, W. M. (1998) Transcriptional activation of the glycolytic *las* operon and catabolite repression of the *gal* operon in *Lactococcus lactis* are mediated by the catabolite control protein CcpA. Mol. Microbiol. **30**, 789–798
107 Moore, P. A., Sagliocco, F. A., Wood, R. M. and Brown, A. J. (1991) Yeast glycolytic mRNAs are differentially regulated. Mol. Cell. Biol. **11**, 5330–5337
108 Baker, H. V. (1986) Glycolytic gene expression in *Saccharomyces cerevisiae*: nucleotide sequence of GRC1, null mutations, and evidence for expression. Mol. Cell. Biol. **6**, 3774–3784
109 Baker, H. V. (1991) GCR1 of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTTCC sequence motif. Proc. Natl. Acad. Sci. U.S.A. **88**, 9443–9447
110 Schäfer, T. and Schönheit, P. (1993) Gluconeogenesis from pyruvate in the hyperthermophilic archaeon *Pyrococcus furiosus*: involvement of reactions of the Embden–Meyerhof pathway. Arch. Microbiol. **159**, 359–363
111 Plaumann, M., Pelzer-Reith, B., Martin, W. F. and Schnarrenberger, C. (1997) Multiple recruitment of class-I aldolase to chloroplasts and eubacterial origin of eukaryotic class-II aldolases revealed by cDNAs from *Euglena gracilis*. Curr. Genet. **31**, 430–438
112 van den Bergh, E. R., Baker, S. C., Raggers, R. J., Terpstra, P., Woudstra, E. C., Dijkhuizen, L. and Meijer, W. G. (1996) Primary structure and phylogeny of the Calvin cycle enzymes transketolase and fructosebisphosphate aldolase of *Xanthobacter flavus*. J. Bacteriol. **178**, 888–893
113 Verhees, C. H. (2002) Molecular characterization of glycolysis in *Pyrococcus furiosus*. PhD Thesis, Wageningen University, Wageningen, The Netherlands
114 Schramm, A., Siebers, B., Tjaden, B., Brinkmann, H. and Hensel, R. (2000) Pyruvate kinase of the hyperthermophilic crenarchaeote *Thermoproteus tenax*: physiological role and phylogenetic aspects. J. Bacteriol. **182**, 2001–2009
115 Brunner, N. A., Siebers, B. and Hensel, R. (2001) Role of two different glyceraldehyde-3-phosphate dehydrogenases in controlling the reversible Embden–Meyerhof–Parnas pathway in *Thermoproteus tenax*: regulation on protein and transcript level. Extremophiles **5**, 101–109
116 Fothergill-Gilmore, L. A. and Michels, P. A. M. (1993) Evolution of glycolysis. Prog. Biophys. Mol. Biol. **59**, 105–235
117 Ettema, T., van der Oost, J. and Huynen, M. (2001) Modularity in the gain and loss of genes: applications for function prediction. Trends Genet. **17**, 485–487
118 Barbier, G., Godfroy, A., Meunier, J. R., Querellou, J., Cambon, M. A., Lesongeur, F., Grimont, P. A. and Raguenes, G. (1999) *Pyrococcus glycovorans* sp. nov., a hyperthermophilic archaeon isolated from the East Pacific Rise. Int. J. Syst. Bacteriol. **49**, 1829–1837
119 Zillig, W., Holz, I., Janekovic, D., Schäfer, T. and Reiter, W. D. (1983) The archaeabacterium *Thermococcus celer* represents a novel genus within the thermophilic branch of the archaeabacteria. Syst. Appl. Microbiol. **4**, 88–94
120 Ronimus, R. S., Reysenbach, A., Musgrave, D. R. and Morgan, H. W. (1997) The phylogenetic position of the *Thermococcus* isolate AN1 based on 16 S rRNA gene sequence analysis: a proposal that AN1 represents a new species, *Thermococcus zilligii* sp. nov. Arch. Microbiol. **168**, 245–248
121 Xavier, K. B., Peist, R., Kossmann, M., Boos, W. and Santos, H. (1999) Maltose metabolism in the hyperthermophilic archaeon *Thermococcus litoralis*: purification and characterization of key enzymes. J. Bacteriol. **181**, 3358–3367
122 Godfroy, A., Lesongeur, F., Raguenes, G., Querellou, J., Antoine, E., Meunier, J. R., Guezennec, J. and Barbier, G. (1997) *Thermococcus hydrothermalis* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. **47**, 622–626
123 Canganella, F., Jones, W. J., Gambacorta, A. and Antranikian, G. (1998) *Thermococcus guaymasensis* sp. nov. and *Thermococcus aggregans* sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site. Int. J. Syst. Bacteriol. **48**, 1181–1185
124 Miroshnichenko, M. L., Gongadze, G. M., Rainey, F. A., Kostyukova, A. S., Lysenko, A. M., Chernyh, N. A. and Bonch-Osmolovskaya, E. A. (1998) *Thermococcus gorgonarius* sp. nov. and *Thermococcus pacificus* sp. nov.: heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. Int. J. Syst. Bacteriol. **48**, 23–29
125 Godfroy, A., Meunier, J. R., Guezennec, J., Lesongeur, F., Raguenes, G., Rimbault, A. and Barbier, G. (1996) *Thermococcus fumicolans* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent in the north Fiji Basin. Int. J. Syst. Bacteriol. **46**, 1113–1119
126 Kobayashi, T., Higuchi, S., Kimura, K., Kudo, T. and Horikoshi, K. (1995) Properties of glutamate dehydrogenase and its involvement in alanine production in a hyperthermophilic archaeon, *Thermococcus profundus*. J. Biochem. (Tokyo) **118**, 587–592
127 Montera, C. G., Ventosa, A., Rodriguez-Valera, F., Kates, M., Moldoveanu, N. and Ruiz-Berraquero, F. (1989) *Halococcus saccharolyticus* sp. nov., a new species of extremely halophilic non-alkaliphilic cocci. Syst. Appl. Microbiol. **12**, 167–171
128 Rodriguez-Valera, F., Juez, G. and Kushner, D. J. (1983) *Halobacterium mediterranei* spec. nov., a new carbohydrate-utilizing extreme halophile. Syst. Appl. Microbiol. **4**, 369–381

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129 Altekar, W. and Rangaswamy, V. (1990) Indication of a modifies EMP pathway for fructose breakdown in a halophilic archaebacterium. FEBS Microbiol. Lett. **69**, 139–143
130 Rawal, N., Kelkar, S. M. and Altekar, W. (1988) Alternative routes of carbohydrate metabolism in halophilic archaebacteria. Indian J. Biochem. Biophys. **25**, 674–686
131 Verhees, C. H., Koot, D. G. M., Ettema, T. J. G., Dijkema, C., de Vos, W. M. and van der Oost, J. (2002) Biochemical adaptations of two sugar kinases from the hyperthermophilic archaeon *Pyrococcus furiosus*. Biochem. J. **366**, 121–127
132 Jones, C. E., Fleming, T. M., Cowan, D. A., Littlechild, J. A. and Piper, P. W. (1995) The PGK and glyceraldehyde-3-phosphate dehydrogenase genes from the thermophilic archaeon *Sulfolobus solfataricus* overlap by 8-bp: isolation, sequencing of the genes and expression in *Escherichia coli*. Eur. J. Biochem. **233**, 800–808
133 Zwickl, P., Fabry, S., Bogedain, C., Haas, A. and Hensel, R. (1990) Glyceraldehyde-3-phosphate dehydrogenase from the hyperthermophilic archaebacterium *Pyrococcus woesei*: characterization of the enzyme, cloning and sequencing of the gene, and expression in *Escherichia coli*. J. Bacteriol. **172**, 4329–4338

Received 20 September 2002/16 May 2003; accepted 18 August 2003
Published as BJ Immediate Publication 18 August 2003, DOI 10.1042/BJ20021472

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