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A reconstruction of the metabolism of *Methanococcus jannaschii* from sequence data

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1. Introduction

The complete genome of *Methanococcus jannaschii* was placed in the public databases in late August 1996, just as the event was announced in *Science* (Bult et al., 1996). The actual sequence, along with an emerging estimate of the genes and their functionality, is maintained by The Institute for Genome Research, which did the sequencing. Recognition of the significance of this event was almost immediate (Gray, 1996; Fox, 1996). The availability of the first complete archaeal genome is certainly a major event in the history of microbiology. More archaeal genomes will follow in quick succession, along with many more bacterial and eukaryotic genomes. We are rapidly reaching the point where a goal as ambitious as "characterizing unicellular life" can be openly discussed without inviting scorn.

The work presented in this article is a direct outgrowth of our efforts to accurately identify the coding regions in *Methanococcus jannaschii*. A number of the authors participated in the initial attempt to determine the coding sequences and establish estimates of the function associated with the corresponding protein. It was decided that the development of a metabolic reconstruction for the organism was needed. Evgeni Selkov, working with a team at Argonne National Laboratory, had developed such reconstructions for *Haemophilus influenzae* and *Mycoplasma genitalium*, the first two prokaryotic genomes that were completely sequenced (Fleischmann et al., 1995; Fraser et al., 1995). We decided to formulate an initial metabolic reconstruction that would integrate the sequence data with the known biochemical and phenotypic data.

What emerges is a reconstruction in which much of the metabolism revealed by sequence analysis is in close agreement with the known biochemistry. In these areas of agreement, we believe that the careful depiction of the pathways, labeled with EC numbers and connected to the actual coding sequences corresponding to these functional roles, will be of value to others exploring this genome. However, there is more to be said:

(1) The metabolic reconstruction represents an attempt to formulate a model reconciling the sequence data with known biochemistry. This model goes beyond asserting what can be reliably deduced from the sequence data. It includes assertions that must be viewed as hypotheses to be tested. It also includes numerous assertions of pathways for which some enzymes have not yet been identified in the sequence data. Each such assertion is a judgment that must continually be reconsidered as more data become available.
(2) An accurate understanding of this organism will ultimately arise from many sources, and we believe that this effort is advanced by making the initial reconstruction publicly available, rather than waiting for the experimental evidence required to confirm or reject some of these conjectures. Indeed, one of the central roles of a metabolic reconstruction of the sort we present is to focus experimentation on specific questions of central importance.
(3) Many aspects of the metabolism cannot, at this time, be resolved. Questions relating to the roles of specific transport proteins, whether the Calvin cycle is actually present, and a number of other issues must remain open at this point.

2. The environment of *Methanococcus jannaschii*

M. jannaschii strain JAL-1 was isolated from surface material collected at a "white smoker" chimney at a

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depth of 2600 m in the East Pacific Rise near the western coast of Mexico (Jones et al., 1983). Two similar strains were isolated from hydrothermally active sediments in the Guaymas Basin at a depth of 2000 m (Zhao et al., 1988; Jones et al., 1989).

Cells of *M. jannaschii* are irregular cocci (Jones et al., 1983). The cell envelope is composed of a cytoplasmic membrane and a protein surface layer (Nusser and Konig, 1987). Polar bundles of flagella are also present. This morphology is common among the methanococci.

The characteristics of the source material for these isolates suggest that *M. jannaschii* possesses adaptations for growth at high temperature and pressure, as well as moderate salinity. The water chemistry of the sites suggests an environment rich in sulfide, H_2, CO_2, Fe^{+2}, and Mn^{+2} (Jannasch and Mottl, 1986). This anaerobic environment would be well suited for a H_2-utilizing methanogen that reduces CO_2 to methane. Fixed nitrogen, either as NH_3 or NO_2-, is not abundant. In addition, small amounts of CO are present. Thus, it is possible that CO could be used as an electron donor in place of H_2.

3. Methanogenesis

From its growth characteristics and what little is known about its biochemistry, *M. jannaschii* appears to be typical of H_2-utilizing, autotrophic methanogens. These archaea perform anaerobic respiration with CO_2 as the terminal electron acceptor according to the general equation:

$$4H_2 + CO_2 \text{--->} CH_4 + 2H_2O.$$

So far, all methanogens isolated appear to be obligate methanogens and do not possess additional sources of energy capable of supporting growth. As expected, *M. jannaschii* does not grow in a rich heterotrophic medium in the absence of H_2 (Jones et al., 1983) and related methanococci do not metabolize glucose or most amino acids. However, the current evidence does not exclude alternative but minor pathways of energy metabolism. For instance, *M. jannaschii* produces glycogen as an intracellalar storage material (Konig et al., 1985). Presumably, it also possesses the pathways to utilize this carbohydrate (Yu et al., 1994).

The pathway of methanogenesis from CO2 is complex and requires five unique coenzymes: methanofuran, tetrahydromethanopterin (H_4MPT), coenzyme M (HS-CoM), 7-mercaptoheptanoylthreonine phosphate (HS-HTP), and coenzyme F420 (for reviews, see Thauer et al., 1993; Muller et al., 1993). Simply, the pathway involves the stepwise reduction of CO_2 with H_2 as the ultimate electron donor. It contains three coupling sites to the proton motive force (PMF). In the first, the PMF is utilized to drive the endergonic reduction of CO_2 to the formyl level. The second and third coupling sites generate the PMF by coupling exergonic steps in CO_2 reduction to proton or sodium pumps. Each of the three coupled reactions is catalysed by a membrane protein complex. In addition, the methylreductosome is a large complex attached to the interior of the cytoplasmic membrane, which contains at least one "soluble" enzyme of the pathway.

methanogenesis (plasma membrane)		
1.2.99.5	TUNGSTEN FORMYLMETHANOFURANDEHYDROGENASE SUBUNIT A	MJ1169
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT B	MJ1194
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT C	MJ1171
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT D	MJ1168
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT E	MJ1165
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT F	MJ1166
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT G	MJ1167
	TUNGSTEN FORMYLMETHANOFURAN DEHYDROGENASE SUBUNIT C RELATED PROTEIN	MJ0658
2.3.1.101	FORMYLMETHANOFURAN–TETRAHYDRO-METHANOPTERIN N-FORMYLTRANSFERASE	MJ0318
3.5.4.27	METHENYLTETRAHYDROMETHAN-OPTERIN CYCLOHYDROLASE	MJ1636
1.5.99.9	COENZYME F420-DEPENDENT METHYLENETETRAHYDROMETHANOPTERIN DEHYDROGENASE	MJ1035
1.12.99.-	COENZYME F420-INDEPENDENT METHYLENETETRAHYDROMETHANOPTERIN DEHYDROGENASE	MJ0784
1.-.-.-	METHYLENETETRAHYDROMETHANOPTERIN OXIDOREDUCTASE	MJ1534
2.1.1.86	METHYLENETETRAHYDROMETHANOPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT A	MJ0851
	METHYLENETETRAHYDROMETHAN-OPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT B	MJ0850
	METHYLENETETRAHYDROMETHANOPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT C	MJ0849
	METHYLENETETRAHYDROMETHAN-OPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT D	MJ0848
	METHYLENETETRAHYDROMETHANOPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT E	MJ0847
	METHYLENETETRAHYDROMETHANOPTERIN:	

	COENZYME M METHYLTRANSFERASE SUBUNIT F	MJ0852
	METHYLENETETRAHYDROMETHANOPTERIN: COENZYME M METHYLTRANSFERASE SUBUNIT G	MJ0853
	METHYLENETETRAHYDROMETHAN-OPTERIN: COENZYME MMETHYLTRANSFERASE SUBUNIT H	MJ0854
1.8.-.-	METHYL-COENZYME M REDUCTASE ALPHA SUBUNIT	MJ0846
	METHYL-COENZYME M REDUCTASE BETA SUBUNIT	MJ0842
	METHYL-COENZYME M REDUCTASE GAMMA SUBUNIT	MJ0845
	METHYL-COENZYME M REDUCTASE OPERON PROTEIN C	MJ0844
	METHYL-COENZYME M REDUCTASE OPERON PROTEIN D	MJ0843
	METHYL COENZYME M REDUCTASE II ALPHA SUBUNIT	MJ0083
	METHYL COENZYME M REDUCTASE II BETA SUBUNIT	MJ0081
	METHYL COENZYME M REDUCTASE II GAMMA SUBUNIT	MJ0082

There is substantial sequence evidence for the existence of formate dehydrogenase, which suggests that this organism is capable of utilizing formate in place of H_2. This property is widespread among H_2-utilizing methanogens. Although *M. jannaschii* does not grow on formate, cell extracts appear to have the ability to oxidize formate, and a closely related isolate grows with formate (Jones et al., 1983, 1989).

formate oxidation (plasma membrane)		
1.2.1.2	FORMATE DEHYDROGENASE ALPHA CHAIN	MJ1353
		M_jannaschii_chromosome_1304115_1303648 MJ0006
	FORMATE DEHYDROGENASE BETA CHAIN	MJ0005
	FORMATE DEHYDROGENASE IRON–SULFUR SUBUNIT	MJ0155
	FDHD PROTEIN	MJ0295

Electron carriers for many of the reactions in methanogenesis are not known with certainty. It is likely that Fe/S proteins are utilized for many steps. For some reactions, coenzyme F_{420}, a deazaflavin that was discovered in methanogens but subsequently found in the bacteria, is utilized. Methanococci also contain NAD(P)H and flavins, although cytochromes and ubiquinone or menaquinone are believed to be absent. The proton motive force generated during methanogenesis is utilized for ATP synthesis, transport, motility, and other cellular functions. In the related archaeon, *Methanococcus voltae*, the sodium motive force is probably the major component of the membrane potential (Jarrell and Sprott, 1985). It is coupled to ATP synthesis by a Na^+-translocating ATPase and to the proton gradient by a Na^+/H^+ antiporter (Dybas and Konisky, 1992; Carper and Lancaster, 1986; Chen and Konisky, 1993). Similarly, transport is dependent on sodium (Dybas and Konisky, 1992; Ekiel et al., 1985; Jarrell et al., 1984). Presumably, other bioenergetic processes in methanococci such as motility will prove to be coupled to the sodium motive force.

4. Carbohydrate metabolism

M. jannaschii grows autotrophically and there is little evidence that it assimilates organic compounds. Thus, it must biosynthesize all its cellular components from CO_2. In the related methanogen *Methanococcus maripaludis*, CO_2 is assimilated via a modified Ljungdahl–Wood pathway of acetyl-CoA biosynthesis (Shieh and Whitman, 1988; Ladapo and Whitman, 1990). In this pathway, the methyl carbon of acetyl-CoA is derived from methyl-H_4MPT, an intermediate in the pathway of methanogenesis. The carboxy carbon is derived from CO_2 via reduction to CO. These reactions are catalyzed by an enzyme complex named acetyl-CoA decarbonylase/synthase. Because the complex also oxidizes CO, it is sometimes called carbon monoxide dehydrogenase (EC 1.2.99.2). Both of its subunits were identified in *M. jannaschii*.

Acetyl-CoA synthase pathway (plasma membrane)		
1.2.99.2	CARBON MONOXIDE DEHYDROGENASE	
	ALPHA SUBUNIT (EC 1.2.99.2) CARBON MONOXIDE DEHYDROGENASE	MJ0153
	BETA SUBUNIT	MJ0152
		MJ0156
	CARBON MONOXIDE DEHYDROGENASE	
	EPSILON SUBUNIT	MJ0154
	CORRINOID/IRON–SULFUR PROTEIN,	
	LARGE SUBUNIT	MJ0112
	CORRINOID/IRON–SULFUR PROTEIN,	
	SMALL SUBUNIT	MJ0113

4.1. Glycogen metabolism

The following enzymes participating in metabolism of glycogen were found in the sequence data: glycogen synthetase (EC 2.4.1.11), glycogen phosphorylase (EC 2.4.1.1), UDPglucose pyrophosphorylase (EC 2.7.7.9), and phosphoglucomutase (EC 5.4.2.2).

Although we could not locate the glycogen branching (EC 2.4.1.18) and debranching (EC 3.2.1.33/2.4.1.25) enzymes, which are required to support glycogen metabolism, we believe that further analysis will locate these enzymes in the genome.

glycogen degradation		
2.4.1.1	PHOSPHORYLASE	MJ1631
2.4.1.25	4-ALPHA-GLUCANOTRANSFERASE	missing
3.2.1.33	AMYLO-1,6-GLUCOSIDASE	no sequences
5.4.2.2	PHOSPHOGLUCOMUTASE	MJ0399

glycogen synthesis		
5.4.2.2	PHOSPHOGLUCOMUTASE	MJ0399
2.7.7.9	UTP–GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE	MJ1334
2.4.1.11	GLYCOGEN (STARCH) SYNTHASE	MJ1606
2.4.1.18	1,4-ALPHA-GLUCAN BRANCHING ENZYME	missing

Entries in the tables of assignments that have no sequence represent enzymes for which no sequence is available for any organism. Since our assignments of function are based on similarity to known, characterized sequences, no attempt could be made to locate sequences within *M. jannaschii* corresponding to these functions. On the other hand, enzymes characterized as missing (which occur in the tables below) represent functions for which representative sequences do exist in the databases.

4.2. Embden–Meyerhof pathway

Six of nine enzymes of the Embden–Meyerhof pathway (EMP) catabolizing glucose-6-phosphate to pyruvate and lactate were found in the sequence data, although three important enzymes of glycolysis (6-phosphofructokinase (EC 2.7.1.11 or EC 2.7.1.90), fructose bisphosphate aldolase (EC 4.1.2.13), and phosphoglycerate mutase (EC 5.4.2.1)) have not been located. A glucokinase (EC 2.7.1.2 or EC 2.7.1.63), which phosphorylates glucose at the expense of ATP or polyphosphate, has not been identified. However, this enzyme would not be required if glycogen was the major carbohydrate metabolized. Recent results (Kengen et al., 1994, 1995) show that *P. furiosus* uses novel ADP-dependent (AMP-forming) forms of glucokinase and 6-phosphofructokinase. The ADP-dependent versions appear more appropriate to high-temperature environments. This is a most remarkable development and strongly suggests that a similar situation may exist in *M. jannaschii*. We suspect that the divergence of these two enzymes from the more common forms is substantial enough to make detection difficult.

Glycolytic pathway (ADP, ATP)		
5.3.1.9	GLUCOSE-6-PHOSPHATE ISOMERASE	MJ1605
2.7.1.-	6-PHOSPHOFRUCTOKINASE (ADP)	missing
4.1.2.13	FRUCTOSE BISPHOSPHATE ALDOLASE	missing
5.3.1.1	TRIOSEPHOSPHATE ISOMERASE	MJ1528
1.2.1.12	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (PHOSPHORYLATING)	MJ1146
2.7.2.3	PHOSPHOGLYCERATE KINASE	MJ0641
5.4.2.1	PHOSPHOGLYCERATE MUTASE	missing
4.2.1.11	PHOSPHOPYRUVATE HYDRATASE	MJ0198 MJ0232
2.7.1.40	PYRUVATE KINASE	MJ0108

Although we cannot yet verify the existence of ADP-dependent versions of these key enzymes, we believe that the possible implications are worth considering, should their presence be confirmed. In the more common versions of glycolysis, the ADP generated by the early stages is immediately phosphorylated in thelater steps. If, instead, AMP is produced from ADP, recycling AMP becomes an issue. The most probable means of recycling AMP uses adenylate kinase (EC 2.7.4.3):

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AMP + ATP <-> 2 ADP
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The adenylate kinase reaction here is far from equilibrium: to maintain stationary turnover of AMP, it must have a velocity twice as high as the glucose consumption rate. Therefore, we expect the adenylate kinase found in this organism to have a high affinity for AMP and ATP and a very high specific activity with respect to glucokinase and 6-phosphofructokinase.

We have found solid sequence evidence in favor of NAD-dependent GAP dehydrogenase (EC 1.2.1.12). It must be noted that NADP-dependent GAP dehydrogenase, as well as an ATP-dependent version of 6-phosphofructokinase (EC 2.7.1.11) have been reported in M. maripaludis (Yu et al., 1994). These differences may reflect the considerable evolutionary distance that separates the mesophilic and hyperthermophilic methanococci. The presence or absence of the NADP-dependent GAP dehydrogenase is an issue that directly relates to the presence or absence of the oxidative portion of the pentose-phosphate shunt (see below).

Phosphonopyruvate decarboxylase (EC 4.1.1.-) potentially links glycolysis with a largely unknown metabolism of phosphonates.

No enzymes involved in the nonphosphorylated Entner–Doudoroff pathway were detected in the sequence data. This result agrees with the known biochemical evidence (Yu et al., 1994; Kengen et al., 1995).

4.3. Gluconeogenesis

Hexoses are made by gluconeogenesis. Phosphoenolpyruvate biosynthesis for gluconeogenesis is catalyzed by pyruvate, water dikinase. Seven of nine enzymes of this pathway have been reliably identified. The three that have not are the phosphoglycerate mutase

and aldolase, mentioned above, and fructose bisphosphatase (EC 3.1.3.11). All the enzyme activities of the pathway have also been detected in M. maripalidus (Shieh et al., 1987; Yu et al., 1994).

Gluconeogenesis (via EC 2.7.9.2)		
2.7.9.2	PYRUVATE, WATER DIKINASE	MJ0542
4.2.1.11	PHOSPHOPYRUVATE HYDRATASE	MJ0198 MJ0232
5.4.2.1	PHOSPHOGLYCERATE MUTASE	missing
2.7.2.3	PHOSPHOGLYCERATE KINASE	MJ0641
1.2.1.12	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (PHOSPHORYLATING)	MJ1146
5.3.1.1	TRIOSEPHOSPHATE ISOMERASE	MJ1528
4.1.2.13	FRUCTOSE BISPHOSPHATE ALDOLASE	missing
3.1.3.11	FRUCTOSE BISPHOSPHATASE	missing
5.3.1.9	GLUCOSE-6-PHOSPHATE ISOMERASE	MJ1605

4.4. Reductive tca

Biochemical evidence strongly supports the hypothesis that the reductive branch of the tricarboxylic acid cycle is utilized to make 2-oxoglutarate and glutamate from oxaloacetate (Shieh and Whitman, 1987; Sprott et al., 1993). We were able to locate four of the five required enzymes (EC 1.1.1.37/1.1.1.82, EC 4.2.1.2, EC 1.3.99.1, and EC 6.2.1.5); the sequence of the fifth, 2-oxoglutarate synthase (EC 1.2.7.3), has not yet been identified in any organism. The alternative would require the existence of a portion of the oxidative TCA cycle. We doubt the presence of the three enzymes from the oxidative portion of the cycle leading to 2-oxoglutarate (citrate synthase, aconitase, and isocitrate dehydrogenase), although both the aconitase and isocitrate dehydrogenase were listed in Bult et al. (1996). The similarities between MJ1596 and MJ0720 and known versions of both isocitrate dehydrogenase and isopropylmalate dehydrogenase (EC 1.1.1.85, which is used in leucine biosynthesis) are very strong. MJ0499 is very similar to 3-isopropylmalate dehydratase (EC 4.2.1.33, which also is utilized in leucine biosynthesis) and less so to aconitase.

truncated reductive tricarboxylic acid cycle (cytosol, plasma-membrane) (via EC 1.2.7.3)		
4.1.1.3	OXALOACETATE DECARBOXYLASE	MJ1231
1.1.1.37/1.1.1.82	MALATE DEHYDROGENASE	MJ1425
4.2.1.2	FUMARATE DEHYDRATASE	MJ1294 MJ0617
1.3.99.1	FUMARATE REDUCTASE FLAVOPROTEIN SUBUNIT	MJ0033
6.2.1.5	SUCCINATE–COA LIGASE (ADP-FORMING)	MJ0210 MJ1246
1.2.7.3	2-OXOGLUTARATE SYNTHASE	no sequences

We have found membrane-bound, Na-dependent oxaloacetate decarboxylase (EC 4.1.1.3), which converts pyruvate into oxaloacetate. Pyruvate is formed by reductive carboxylation of acetyl-CoA catalyzed by pyruvate oxidoreductase (EC 1.2.7.1) (Shieh and Whitman, 1987) or by the glycolytic system. Based upon N-terminal sequence information for the pyruvate oxidoreductase from M. maripalidus (Yang and Whitman, unpublished data), four genes encoding subunits of the pyruvate oxidoreductase (EC 1.2.7.1) have been identified.

pyruvate synthase reaction		
1.2.7.1	PYRUVATE SYNTHASE	MJ0266 MJ0267 MJ0268 MJ0269

4.5. Pentose biosynthesis

Two pathways have been proposed for pentose biosynthesis in the methanococci. In one proposal, a nonoxidative pathway is composed of transketolase, transaldolase, ribose-5-phosphate epimerase, and ribulose-5-phosphate isomerase (Yu et al., 1994). In the second proposal, erythrose-4-phosphate is formed via carboxylation of dihydroxyacetone phosphate instead of transketolase (Choquet et al., 1994b).

Sequence analysis has identified genes that encode enzymes of the nonoxidative pentose-phosphate shunt; they are used to produce ribose phosphate for nucleotide biosynthesis. The two dehydrogenases (EC 1.1.1.49 and EC 1.1.1.44) required for the oxidative part of the shunt have not yet been found and thir activities are not detectable in *M. maripalidus* (Yu et al., 1994). Isotope labeling of *M. jannaschii* provides additional evidence that the oxidative pentose phosphate pathway is absent (Sprott et al., 1993).

Non-oxidative hexose monophosphate pathway		
5.3.1.6	RIBOSE 5-PHOSPHATE EPIMERASE	MJ1603
5.1.3.1	RIBULOSE-PHOSPHATE 3-EPIMERASE	MJ0680
2.2.1.1	TRANSKETOLASE	MJ0679 MJ0681
2.2.1.2	TRANSALDOLASE	MJ0960

4.6. CO_2 fixation

The large subunit of RuBisCo (EC 4.1.1.39) has been identified, which raises the question "Is the entire Calvin Cycle actually present?" The answer to this question will hinge on whether phosphoribulokinase (EC 2.7.1.19) is present; it has not yet been identified.

One conjecture is that the phosphoribulokinase, which is normally a two-subunit enzyme (neither subunit of which has been located), might be ADP-dependent. Such coenzyme substitutions have been proposed in the glycolytic pathway, and they often make recognition of the enzyme from sequence data difficult or impossible. Another possibility is that protein MJ1235 is only paralogous to RuBisCo and has a different metabolic function which has yet to be identified.

Calvin cycle (via EC 1.2.1.12)		
4.1.1.39	RIBULOSE BISPHOSPHATE CARBOXYLASE	MJ1235
2.7.2.3	PHOSPHOGLYCERATE KINASE	MJ0641
1.2.1.12	GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (PHOSPHORYLATING)	MJ1146
5.3.1.1	TRIOSEPHOSPHATE ISOMERASE	MJ1528
4.1.2.13	FRUCTOSE BISPHOSPHATE ALDOLASE	missing
3.1.3.11	FRUCTOSE BISPHOSPHATASE	missing
2.2.1.1	TRANSKETOLASE	MJ0679 MJ0681
5.1.3.1	RIBULOSE PHOSPHATE 3-EPIMERASE	MJ0680
5.3.1.6	RIBOSE 5-PHOSPHATE EPIMERASE	MJ1603
2.7.1.19	PHOSPHORIBULOKINASE	missing

4.7. Inositol metabolism

Di-myo-inositol-1,1-phosphate (DIP) is an abundant osmolyte in *M. igneus*, a hyperthermophile related to *M. jannaschii* (Ciulla et al., 1994). A gene encoding one of the enzymes necessary for inositol biosynthesis from glucose-6-phosphate has been found.

"Myo"-inositol biosynthesis		
5.5.1.4	MYO-INOSITOL-1-PHOSPHATE SYNTHASE	missing
3.1.3.25	MYO-INOSITOL-1 (OR 4)-MONOPHOSPHATASE	MJ0109

4.8. Other pathways of carbohydrate metabolism

("S")-lactate–pyruvate catabolism (NAD(′+))		
1.1.1.27	L-LACTATE DEHYDROGENASE	MJ0490

5-phosphoribose 1-diphosphate biosynthesis		
2.2.1.1	TRANSKETOLASE	MJ0490 MJ0681
2.2.1.2	TRANSALDOLASE	MJ0960
5.1.3.1	RIBULOSE PHOSPHATE 3-EPIMERASE	MJ0680
5.3.1.6	RIBOSE 5-PHOSPHATE EPIMERASE	MJ1603
2.7.6.1	RIBOSE PHOSPHATE PYROPHOSPHOKINASE	MJ1366
GDPrhamnose biosynthesis		
5.3.1.8	MANNOSE-6-PHOSPHATE ISOMERASE	MJ1618
5.4.2.8	PHOSPHOMANNOMUTASE	MJ1100 MJ0399
2.7.7.22	MANNOSE-1-PHOSPHATE GUANYLYLTRANSFERASE (GDP)	MJ1618
4.2.1.47	GDP-MANNOSE 4,6-DEHYDRATASE	no sequences
1.1.1.187	GDP-4-DEHYDRO-D-RHAMNOSE REDUCTASE	no sequences
UDP-"N"-acetyl-D-galactosamine biosynthesis		
2.6.1.16	GLUCOSAMINE–FRUCTOSE-6-PHOSPHATE AMINOTRANSFERASE (ISOMERIZING)	MJ1420
2.3.1.4	GLUCOSAMINE PHOSPHATE N-ACETYLTRANSFERASE	no sequences
5.4.2.3	PHOSPHOACETYLGLUCOSAMINE MUTASE	missing
2.7.7.23	UDP-N-ACETYLGLUCOSAMINE PYROPHOSPHORYLASE	missing
5.1.3.7	UDP-N-ACETYLGLUCOSAMINE 4-EPIMERASE	no sequences
UDPglucose metabolism		
5.1.3.2	UDP-GLUCOSE 4-EPIMERASE	MJ0211
UDPglucuronate anabolism		
2.7.7.9	UTP–GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE	MJ1334
1.1.1.22	UDP-GLUCOSE 6-DEHYDROGENASE	missing
"Alpha"-glucose 1,6-bisphosphate anabolism (via EC 5.4.2.2)		
5.4.2.2	PHOSPHOGLUCOMUTASE	MJ0399
Cyclic 2,3-bisphosphoglycerate biosynthesis		
2.7.2.-	2-PHOSPHOGLYCERATE KINASE	MJ1482
5.4.2.-	CYCLIC 2,3-DIPHOSPHOGLYCERATE SYNTHETASE	no sequences
dTDP-L-rhamnose biosynthesis		
2.7.7.24	GLUCOSE-1-PHOSPHATE THYMIDYLYLTRANSFERASE	MJ1101
4.2.1.46	DTDP-GLUCOSE 4,6-DEHYDRATASE	missing
5.1.3.13	DTDP-4-DEHYDRORHAMNOSE 3,5-EPIMERASE	missing
1.1.1.133	DTDP-4-DEHYDRORHAMNOSE	missing

	REDUCTASE	
Deoxyribose 1,5-bisphosphate anabolism (via EC 5.4.2.2)		
5.4.2.2	PHOSPHOGLUCOMUTASE	MJ0339
Oxaloacetate decarboxylation		
4.1.1.3	OXALOACETATE DECARBOXYLASE	MJ1231
Phosphoglycerylglycosyl teichoic acid–diphosphoundecaprenol biosynthesis		
2.7.8.15	UDP-N-ACETYLGLUCOSAMINE–DOLICHYL PHOSPHATE N-ACETYLGLUCOSAMINE PHOSPHOTRANSFERASE	MJ1113
2.4.1.187	N-ACETYLGLUCOSAMINYL DIPHOSPHOUNDECAPRENOL N-ACETYL-BETA-D-MANNOSAMINYLTRANSFERASE	no sequences
2.7.8.12	CDP-GLYCEROL GLYCEROPHOSPHOTRANSFERASE	no sequences
Pyruvate–("S")-lactate anabolism (NADH)		
1.1.1.27	L-LACTATE DEHYDROGENASE	MJ0490
Trehalose synthesis		
5.4.2.2	PHOSPHOGLUCOMUTASE	MJ0399
2.7.7.9	UTP–GLUCOSE-1-PHOSPHATE URIDYLYLTRANSFERASE.	MJ1334
2.4.1.15	ALPHA,ALPHA-TREHALOSE PHOSPHATE SYNTHASE (UDP-FORMING)	missing
3.1.3.12	TREHALOSE PHOSPHATASE	missing

The presence of glycerol dehydrogenase (EC 1.1.1.6) appears clear. This would imply the presence of glycerone kinase (EC 2.7.1.29), since the only apparent way to consume glycerone is by conversion to glycerone phosphate (a glycolytic intermediate).

It was believed until now that methanogenic archaea known to accumulate glycogen do not synthesize cyclic 2,3-biphosphoglycerate (Konig et al., 1985). Nevertheless, in this organism both storage mechanisms seem to exist, since 2-phosphoglycerate kinase (EC 2.7.2.-) has been clearly identified.

5. Amino acids and polyamine metabolism

On the basis of labeling and enzymatic data, the biosynthesis of most amino acids, nucleosides, and hexoses in methanogens appears to occur by pathways common in bacteria (for a review see Simpson and Whitman, 1993). Some noteworthy features are described below. Nearly all of the biosynthetic pathways for amino acids (including selenocysteine) have been detected, although a few of the required enzymes have not yet been found. The one main exception is the biosysthesis of cysteine; we have been unable to locate the enzymes of cysteine biosynthesis.

All of the enzymes involved in the common biosynthetic pathway leading from aspartate to diaminopimelate and then to lysine and methionine (including the multifunctional enzyme aspartokinase I (EC 2.7.2.4)/homoserine dehydrogenase I (EC 1.1.1.3)) were found. The identified methionine synthase (EC 2.1.1.14) has a high similarity score to a known cobalamine-independent counterpart in *M. thermoautotrophicum* (Vaupel et al., 1996). This enzyme catalyses the synthesis of methionine from homocysteine using (we believe) 5-methyl-tetrahydromethanopterin, rather than 5-methylmethyltetrahydrofolate, as the donor of the required methyl group. Lysine is made by the diaminopimelic pathway. There are biochemical data that in Methanobacteria isoleucine is synthesized from pyruvate and acetyl-CoA via the citramalate pathway (Eikmanns et al., 1983). Enzymes participating in the citramalate pathway have not been sequenced in any organism yet, so it is impossible to confirm its existence in *M. jannascii* from the sequence data. All enzymes of arginine biosynthesis via ornithine carbamoyltransferase were found, which agrees with Meile and Leisinger (1984). Other amino acids appear to be derived using well-known common pathways (Ekiel et al., 1983). It is likely that polyamine biosynthesis begins with spermidine synthase (EC 2.5.1.16), which has been located.

4-Aminobutanoate catabolism		
2.6.1.19	4-AMINOBUTYRATE AMINOTRANSFERASE	missing
1.2.1.16	SUCCINATE-SEMIALDEHYDE DEHYDROGENASE ($NAD(P)^+$)	MJ1411
"N"-acetylglutamate cycle		
2.3.1.1	AMINO ACID N-ACETYLTRANSFERASE	MJ0186
2.7.2.8	ACETYLGLUTAMATE KINASE	MJ0069
1.2.1.38 -	N-ACETYL-GAMMA GLUTAMYL PHOSPHATE REDUCTASE	MJ1096
2.6.1.11	ACETYLORNITHINE AMINOTRANSFERASE	MJ0721
2.3.1.35	GLUTAMATE N-ACETYLTRANSFERASE	MJ0186

"S"-adenosylhomocysteine catabolism		
3.3.1.1	ADENOSYLHOMOCYSTEINASE	MJ1388
Acetamide degradation		
3.5.1.4	AMIDASE	MJ1160
Alanine biosynthesis		
2.6.1.2	ALANINE AMINOTRANSFERASE	MJ1479
Alanyl-tRNA biosynthesis		
6.1.1.7	ALANINE–TRNA LIGASE	MJ0564
Allothreonine degradation		
2.1.2.1	GLYCINE HYDROXY-METHYLTRANSFERASE	MJ1597

Arginine biosynthesis

6.3.5.5	CARBAMOYL PHOSPHATE SYNTHASE (GLUTAMINE-HYDROLYSING)	MJ1378
		MJ1381
		MJ1019
2.1.3.3	ORNITHINE CARBAMOYLTRANSFERASE	MJ0881
6.3.4.5	ARGININOSUCCINATE SYNTHASE	MJ0429
4.3.2.1	ARGININOSUCCINATE LYASE	MJ0791

Arginyl-tRNA biosynthesis

6.1.1.19	ARGININE–TRNA LIGASE	MJ0237

Asparagine biosynthesis (glutamine-hydrolysing)

6.3.5.4	ASPARAGINE SYNTHASE (GLUTAMINE-HYDROLYSING)	M_jannaschii_ chromosome_ 994624_995571
		MJ1116
		MJ1056

Asparagine degradation

3.5.1.1	ASPARAGINASE	MJ0020

Aspartate aminotransferase reaction

2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391
		MJ0001
		MJ0684
		MJ0959

Aspartate biosynthesis

2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391
		MJ0001
		MJ0684
		MJ0959

Aspartyl-tRNA biosynthesis

6.1.1.12	ASPARTATE–TRNA LIGASE	MJ1555

Chorismate biosynthesis

4.1.2.15	2-DEHYDRO-3-DEOXY-PHOSPHOHEPTONATE ALDOLASE	missing
4.6.1.3	3-DEHYDROQUINATE SYNTHASE	missing
4.2.1.10	3-DEHYDROQUINATE DEHYDRATASE	MJ1454
1.1.1.25	SHIKIMATE 5-DEHYDROGENASE	MJ1084
2.7.1.71	SHIKIMATE KINASE	missing
2.5.1.19	3-PHOSPHOSHIKIMATE 1-CARBOXYVINYLTRANSFERASE	MJ0502
4.6.1.4	CHORISMATE SYNTHASE	MJ1175

Chorismate metabolism

4.1.3.27	ANTHRANILATE SYNTHASE	MJ0238
		MJ1075

Citramalate pathway

4.2.1.34	(S)-2-METHYLMALATE DEHYDRATASE	no sequences
4.1.3.25	CITRAMALYL-COA LYASE	no sequences
2.8.3.11	CITRAMALATE COA-TRANSFERASE	no sequences

Dipicolinate anabolism

4.2.1.52	DIHYDRODIPICOLINATE SYNTHASE	MJ0244
	DIPICOLINATE SYNTHASE	missing

Glutamate biosynthesis (alanine)

2.6.1.2	ALANINE AMINOTRANSFERASE	MJ1479

Glutamate deamination

2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391
		MJ0001
		MJ0684
		MJ0959

Glutamate synthase (NADPH) reaction

1.4.1.13	GLUTAMATE SYNTHASE (NADPH)	MJ1351

Glutamine biosynthesis

6.3.1.2	GLUTAMATE–AMMONIA LIGASE	MJ1346

Glutamyl-tRNA biosynthesis

6.1.1.17	GLUTAMATE–TRNA LIGASE	MJ1377

Glycyl-tRNA biosynthesis

6.1.1.14	GLYCINE–TRNA LIGASE	MJ0228

Histidine biosynthesis

2.4.2.17	ATP PHOSPHORIBOSYLTRANSFERASE	MJ1204
3.6.1.31	PHOSPHORIBOSYL-ATP PYROPHOSPHATASE	MJ0302
3.5.4.19	PHOSPHORIBOSYL-AMP CYCLOHYDROLASE	MJ1430
5.3.1.16	N-(5′-PHOSPHO-D-RIBOSYLFORMIMINO)-5-AMINO-1-(5"-PHOSPHORIBOSYL)-4-IMIDAZOLE CARBOXAMIDE ISOMERASE	MJ1532
	HISF PROTEIN	MJ0411
2.4.2.-	AMIDOTRANSFERASE HISH	MJ0506
4.2.1.19	IMIDAZOLEGLYCEROL PHOSPHATE DEHYDRATASE	MJ0698
3.1.3.15	HISTIDINOL PHOSPHATASE	missing
2.6.1.9	HISTIDINOL PHOSPHATE AMINOTRANSFERASE	MJ0955
1.1.1.23	HISTIDINOL DEHYDROGENASE	MJ1456

Histidine biosynthesis (Archaeal)

2.4.2.17	ATP PHOSPHORIBOSYLTRANSFERASE	MJ1204
3.6.1.31	PHOSPHORIBOSYL-ATP PYROPHOSPHATASE	MJ0302
3.5.4.19	PHOSPHORIBOSYL-AMP CYCLOHYDROLASE	MJ1430
5.3.1.16	N-(5′-PHOSPHO-D-	

	RIBOSYLFORMIMINO)-5-AMINO-1-(5′-PHOSPHORIBOSYL)-4-IMIDAZOLE CARBOXAMIDE ISOMERASE	MJ1532
4.2.1.19	IMIDAZOLEGLYCEROL PHOSPHATE DEHYDRATASE	MJ0698
2.6.1.9	HISTIDINOL PHOSPHATE AMINOTRANSFERASE	MJ0955
3.1.3.15	HISTIDINOL PHOSPHATASE	missing
1.1.1.23	HISTIDINOL DEHYDROGENASE	MJ1456

Histidyl-tRNA biosynthesis

6.1.1.21	HISTIDINE–TRNA LIGASE	MJ1000

Homoserine biosynthesis

2.7.2.4	ASPARTATE KINASE	MJ0571
1.2.1.11	ASPARTATE-SEMIALDEHYDE DEHYDROGENASE	MJ0205
1.1.1.3	HOMOSERINE DEHYDROGENASE	MJ1602 MJ0571

Isoleucine biosynthesis (NADPH, NADH)

4.1.3.18	ACETOLACTATE SYNTHASE	MJ0663 MJ0277 MJ0161
1.1.1.86	KETOL-ACID REDUCTOISOMERASE	MJ1543
4.2.1.9	DIHYDROXY-ACID DEHYDRATASE	MJ1276
2.6.1.42	BRANCHED-CHAIN AMINO ACIDAMINOTRANSFERASE	MJ1008

Isoleucyl-tRNA biosynthesis

6.1.1.5	ISOLEUCINE–TRNA LIGASE	MJ0947

Leucine biosynthesis (via EC 2.6.1.42)

2.6.1.42	BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE	MJ1008
4.1.3.12	2-ISOPROPYLMALATE SYNTHASE	MJ1195 MJ0503
4.2.1.33	3-ISOPROPYLMALATE DEHYDRATASE	MJ1271 MJ1277 MJ1003 MJ0499
1.1.1.85	3-ISOPROPYLMALATE DEHYDROGENASE	MJ1596 MJ0720

Leucyl-tRNA biosynthesis

6.1.1.4	LEUCINE–TRNA LIGASE	MJ0633

Lysine anabolism

4.2.1.52	DIHYDRODIPICOLINATE SYNTHASE	MJ0244
1.3.1.26	DIHYDRODIPICOLINATE REDUCTASE	MJ0422
2.3.1.117	2,3,4,5-TETRAHYDROPYRIDINE-2-CARBOXYLATE N-SUCCINYLTRANSFERASE	missing
2.6.1.17	SUCCINYLDIAMINOPIMELATE AMINOTRANSFERASE	no sequences
3.5.1.18	SUCCINYL-DIAMINOPIMELATE DESUCCINYLASE	MJ0457
5.1.1.7	DIAMINOPIMELATE EPIMERASE	MJ1119
4.1.1.20	DIAMINOPIMELATE DECARBOXYLASE	MJ1097

Lysine anabolism

4.1.3.21	HOMOCITRATE SYNTHASE	MJ1392
4.2.1.79	2-METHYLCITRATE DEHYDRATASE	no sequences
4.2.1.36	HOMOACONITATE HYDRATASE	missing
1.1.1.155	HOMOISOCITRATE DEHYDROGENASE	no sequences
2.6.1.39	2-AMINOADIPATE AMINOTRANSFERASE	no sequences
1.2.1.31	AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE	missing
1.5.1.10	SACCHAROPINE DEHYDROGENASE ($NADP^+$, L-GLUTAMATE FORMING)	missing
1.5.1.8	SACCHAROPINE DEHYDROGENASE ($NADP^+$, L-LYSINE FORMING)	no sequences

Lysine anabolism (ATP, NADPH, acetyl-CoA)

2.7.2.4	ASPARTATE KINASE	MJ0571
1.2.1.11	ASPARTATE-SEMIALDEHYDE DEHYDROGENASE	MJ0205
4.2.1.52	DIHYDRODIPICOLINATE SYNTHASE	MJ0244
1.3.1.26	DIHYDRODIPICOLINATE REDUCTASE	MJ0422
	ACETYL-L,L-DIAMINOPIMELATE AMINOTRANSFERASE	no sequences
	TETRAHYDRODIPICOLINATE ACETYLTRANSFERASE	no sequences
3.5.1.47	N-ACETYLDIAMINOPIMELATE DEACETYLASE	no sequences
5.1.1.7	DIAMINOPIMELATE EPIMERASE	MJ1119
4.1.1.20	DIAMINOPIMELATE DECARBOXYLASE	MJ1097

Lysine anabolism (via EC 1.4.1.16)

4.2.1.52	DIHYDRODIPICOLINATE SYNTHASE	MJ0244
1.3.1.26	DIHYDRODIPICOLINATE REDUCTASE	MJ0422
1.4.1.16	DIAMINOPIMELATE DEHYDROGENASE	missing
4.1.1.20	DIAMINOPIMELATE DECARBOXYLASE	MJ1097

Methionyl-tRNA biosynthesis

6.1.1.10	METHIONINE–TRNA LIGASE	MJ1263

Phenylalanine biosynthesis (via EC 2.6.1.9/2.6.1.1)

2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391 MJ0001 MJ0684 MJ0959
2.6.1.9	HISTIDINOL PHOSPHATE AMINOTRANSFERASE	MJ0955
5.4.99.5	CHORISMATE MUTASE	MJ0246

		MJ0612
4.2.1.51	PREPHENATE DEHYDRATASE	MJ0637
Phenylalanyl-tRNA biosynthesis		
6.1.1.20	PHENYLALANINE–TRNA LIGASE	MJ0487
		MJ1108
		MJ1660
Prephenate biosynthesis		
5.4.99.5	CHORISMATE MUTASE	MJ0246
		MJ0612
Prolyl-tRNA biosynthesis		
6.1.1.15	PROLINE–TRNA LIGASE	MJ1238
Selenocysteinyl-tRNA biosynthesis		
2.7.9.3	SELENIDE, WATER DIKINASE	MJ1591
2.9.1.1	CYSTEINYL-TRNA(SER) SELENIUM TRANSFERASE	missing

Serine biosynthesis		
1.1.1.95	PHOSPHOGLYCERATE DEHYDROGENASE	MJ1018
2.6.1.52	PHOSPHOSERINE AMINOTRANSFERASE	missing
3.1.3.3	PHOSPHOSERINE PHOSPHATASE	MJ1594
Serine biosynthesis		
2.1.2.1	GLYCINE HYDROXYMETHYLTRANSFERASE	MJ1597
Serine degradation		
2.1.2.1	GLYCINE HYDROXYMETHYLTRANSFERASE	MJ1597
Spermidine biosynthesis		
4.1.1.17	ORNITHINE DECARBOXYLASE	missing
2.5.1.16	SPERMIDINE SYNTHASE	MJ0313
Spermine biosynthesis		
4.1.1.19	ARGININE DECARBOXYLASE	missing
3.5.3.11	AGMATINASE	MJ0309
2.5.1.16	SPERMIDINE SYNTHASE	MJ0313
2.5.1.22	SPERMINE SYNTHASE	no sequences

Threonine biosynthesis		
2.7.1.39	HOMOSERINE KINASE	MJ1104
4.2.99.2	THREONINE SYNTHASE	MJ1465
Threonine biosynthesis		
2.7.2.4	ASPARTATE KINASE	MJ0571
1.2.1.11	ASPARTATE-SEMIALDEHYDE DEHYDROGENASE	MJ0205
1.1.1.3	HOMOSERINE DEHYDROGENASE	MJ1602
		MJ0571
2.7.1.39	HOMOSERINE KINASE	MJ1104
4.2.99.2	THREONINE SYNTHASE	MJ1465
Threonine catabolism (NADPH, NADH)		
4.2.1.16	THREONINE DEHYDRATASE	missing
4.1.3.18	ACETOLACTATE SYNTHASE	MJ0663
		MJ0277
		MJ0161
1.1.1.86	KETOL-ACID REDUCTOISOMERASE	MJ1543
4.2.1.9	DIHYDROXY-ACID DEHYDRATASE	MJ1276
2.6.1.42	BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE	MJ1008
Threonyl-tRNA biosynthesis		
6.1.1.3	THREONINE–TRNA LIGASE	MJ1197

Tryptophan biosynthesis		
4.1.3.27	ANTHRANILATE SYNTHASE	MJ0238
		MJ1075
2.4.2.18	ANTHRANILATE PHOSPHORIBOSYLTRANSFERASE	MJ0234
5.3.1.24	PHOSPHORIBOSYLANTHRANILATE ISOMERASE	MJ0451
4.1.1.48	INDOLE-3-GLYCEROL-PHOSPHATE SYNTHASE	MJ0918
4.2.1.20	TRYPTOPHAN SYNTHASE	MJ1038
		MJ1037
Tryptophanyl-tRNA biosynthesis		
6.1.1.2	TRYPTOPHAN–TRNA LIGASE	MJ1415

Tyrosine biosynthesis (NAD($^{+}$)) (via EC 2.6.1.1)		
2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391
		MJ0001
		MJ0684
		MJ0959
5.4.99.5	CHORISMATE MUTASE	MJ0246
		MJ0612
1.3.1.12	PREPHENATE DEHYDROGENASE	MJ0612
Tyrosine biosynthesis (NAD($^{+}$)) (via EC 2.6.1.9/2.6.1.1)		
2.6.1.1	ASPARTATE AMINOTRANSFERASE	MJ1391
		MJ0001
		MJ0684
		MJ0959
2.6.1.9	HISTIDINOL PHOSPHATE AMINOTRANSFERASE	MJ0955
5.4.99.5	CHORISMATE MUTASE	MJ0246
		MJ0612
1.3.1.12	PREPHENATE DEHYDROGENASE	MJ0612
2.6.1.5	TYROSINE AMINOTRANSFERASE	missing
Tyrosyl-tRNA biosynthesis		
6.1.1.1	TYROSINE–TRNA LIGASE	MJ0389
Valine anabolism (NADPH, NADH)		
4.1.3.18	ACETOLACTATE SYNTHASE	MJ0663
		MJ0277
		MJ0161
1.1.1.86	KETOL-ACID REDUCTOISOMERASE	MJ1543
4.2.1.9	DIHYDROXY-ACID DEHYDRATASE	MJ1276
2.6.1.42	BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE	MJ1008
Valine catabolism		
2.6.1.42	BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE	MJ1008
Valyl-tRNA biosynthesis		

6.1.1.9	VALINE–TRNA LIGASE	MJ1007

6. Nucleotide metabolism

Although pyrimidines and purines appear to be derived from common pathways, C1 groups may be also contributed from methanogenesis (Ekiel et al., 1983). The entire set of reactions for interconversions between nucleotides and their reduced forms listed below is present in *M. jannaschii*. This organism uses anaerobic nucleoside triphosphate reductase (probably B12-dependent) to generate the precursors of DNA. Both thioredoxin and glutaredoxin are present and could be used by the reductase.

Purine metabolism		
"de novo" purine biosynthesis		
2.4.2.14	AMIDOPHOSPHORIBOSYLTRANSFERASE	MJ0204
6.3.4.13	PHOSPHORIBOSYLAMINE–GLYCINE LIGASE	MJ0937
2.1.2.2	PHOSPHORIBOSYLGLYCINAMIDE FORMYLTRANSFERASE	missing
6.3.5.3	PHOSPHORIBOSYLFORMYL-GLYCINAMIDINE SYNTHASE	MJ1264 MJ1648
6.3.3.1	PHOSPHORIBOSYLFORMYLGLY-CINAMIDINE CYCLO-LIGASE	MJ0203
4.1.1.21	PHOSPHORIBOSYLAMINOIMIDAZOLE CARBOXYLASE	MJ0616
6.3.2.6	PHOSPHORIBOSYLAMINOIMIDAZOLE-SUCCINOCARBOXAMIDE SYNTHASE	MJ1592
4.3.2.2	ADENYLOSUCCINATE LYASE	MJ0929
2.1.2.3	PHOSPHORIBOSYLAMINO-IMIDAZOLECARBOXAMIDE FORMYLTRANSFERASE	missing
3.5.4.10	IMP CYCLOHYDROLASE	missing

ADP biosynthesis		
2.7.4.3	ADENYLATE KINASE	MJ0479
ADP phosphorylation		
2.7.4.6	NUCLEOSIDE-DIPHOSPHATE KINASE	MJ1265
AMP biosynthesis		
6.3.4.4	ADENYLOSUCCINATE SYNTHASE	MJ0561
4.3.2.2	ADENYLOSUCCINATE LYASE	MJ0929
ATP biosynthesis		
2.7.4.3	ADENYLATE KINASE	MJ0479
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
GTP anabolism		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
IMP–GMP,_pyrophosphate_anabolism		
1.1.1.205	IMP DEHYDROGENASE	MJ1616
6.3.5.2	GMP SYNTHASE (GLUTAMINE-HYDROLYSING)	MJ1131 MJ1575
ITP anabolism		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265

Adenine catabolism		
3.5.4.2	ADENINE DEAMINASE	MJ1459
Adenine salvage pathway		
2.4.2.7	ADENINE PHOSPHORIBOSYLTRANS-FERASE	MJ1655
5-amino-4-imidazolecarboxamide salvage pathway		
2.4.2.7	ADENINE PHOSPHORIBOSYLTRANS-FERASE	MJ1655
Adenosine catabolism		
2.4.2.1	PURINE NUCLEOSIDE PHOSPHORYLASE	MJ0060
dATP biosynthesis		
2.7.4.3	ADENYLATE KINASE	MJ0479
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dGTP anabolism		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dITP anabolism		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265

Deoxyadenosine catabolism		
2.4.2.1	PURINE NUCLEOSIDE PHOSPHORYLASE	MJ0060
Deoxyguanosine catabolism		
2.4.2.1	PURINE NUCLEOSIDE PHOSPHORYLASE	MJ0060
Deoxyribose 1-phosphate biosynthesis		
2.4.2.1	PURINE NUCLEOSIDE PHOSPHORYLASE	MJ0060
2.4.2.4	THYMIDINE PHOSPHORYLASE	missing
Guanosine catabolism		
2.4.2.1	PURINE NUCLEOSIDE PHOSPHORYLASE	MJ0060

Pyrimidine Metabolism		
"de novo" pyrimidine biosynthesis		
6.3.5.5	CARBAMOYL PHOSPHATE SYNTHASE (GLUTAMINE-HYDROLYSING)	MJ1378 MJ1381 MJ1019
2.1.3.2	ASPARTATE CARBAMOYLTRANSFERASE	MJ1406 MJ1581
3.5.2.3	DIHYDROOROTASE	MJ1490
1.3.3.1	DIHYDROOROTATE OXIDASE	MJ0654
2.4.2.10	OROTATE PHOSPHORIBOSYLTRANS-	MJ1109

	FERASE	MJ1646
4.1.1.23	OROTIDINE-5′-PHOSPHATE DECARBOXYLASE	MJ1109
dCDP biosynthesis		
2.7.4.14	CYTIDYLATE KINASE	missing
dCTP biosynthesis		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dCTP biosynthesis		
2.7.4.14	CYTIDYLATE KINASE	missing
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dCTP degradation		
3.5.4.13	DCTP DEAMINASE	MJ0430
CDP biosynthesis		
2.7.4.14	CYTIDYLATE KINASE	missing
CTP biosynthesis		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
CTP biosynthesis		
6.3.4.2	CTP SYNTHASE	MJ1174
TDP biosynthesis		
2.7.4.9	THYMIDYLATE KINASE	MJ0293
TTP biosynthesis		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dTMP anabolism (via EC 2.4.2.2)		
2.4.2.2	PYRIMIDINE NUCLEOSIDE PHOSPHORYLASE	MJ0667
2.7.1.21	THYMIDINE KINASE	missing
dTMP biosynthesis		
2.1.1.45	THYMIDYLATE SYNTHASE	MJ0511
dTTP biosynthesis		
2.7.4.9	THYMIDYLATE KINASE	MJ0293
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dTTP biosynthesis		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dTTP biosynthesis (dATP)		
2.7.4.9	THYMIDYLATE KINASE	MJ0293
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265
dUDP biosynthesis		
2.7.4.9	THYMIDYLATE KINASE	MJ0293
dUMP biosynthesis (via EC 2.4.2.2)		
2.4.2.2	PYRIMIDINE NUCLEOSIDE PHOSPHORYLASE	MJ0667
2.7.1.21	THYMIDINE KINASE	missing
dUTP biosynthesis		
2.7.4.6	NUCLEOSIDE DIPHOSPHATE KINASE	MJ1265

7. Lipid metabolism

Like other archaea, *M. jannaschii* contains isoprenoid-based ether lipids (for a review see Koga et al., 1993). In addition to the common archaeol (2,3-di-O-phytanyl-sn-glycerol diether) and caldarchaeol (2,2′,3,3′-diphytanyl-sn-diglycerol tetraether), *M. jannaschii* contains a unique macrocyclic diether (2,3-di-o- cyclic-biphytanyl-sn-glycerol). The polarlipids contain phosphoethanolamino, 6-(aminoethylphospho)glucosyl, glucosyl and gentiobiosyl residues. Mevalonate is a precursor for the isoprenoid groups, as expected from common pathways (Sprott et al., 1993).

M. jannaschii must have the whole set of enzymes required to generate membrane lipids from glycolytic intermediates. However, since few sequences exist for this metabolism, few similarities were detected, and very little can be inferred directly from the sequence data. Even so, the key enzymes from the mevalonate pathway (EC 1.1.1.34 and 2.7.1.36) can be clearly recognized; this is the central pathway of archaeal lipid de novo biosynthesis. The end-product of this pathway is isopentenyl pyrophosphate, which must be polymerized to forms of prenyl-pyrophosphates. We have located the trifunctional protein that polymerizes the isopentenyl-pyrophosphate to geranylgeranyl pyrophosphate and farnesyl pyrophosphate (EC 2.5.1.10, EC 2.5.1.29 and EC 2.5.1.1). The fatty-acid synthase complex, which occurs in both bacteria and eukaryotes, is absent.

Lipid metabolism		
Dolichyl phosphate degradation		
3.1.3.51	DOLICHYL PHOSPHATASE	no sequences
Farnesyl diphosphate biosynthesis		
2.3.1.16	ACETYL-COA C-ACYLTRANSFERASE	missing
4.1.3.5	HYDROXYMETHYLGLUTARYL-COA SYNTHASE	missing
1.1.1.34	HYDROXYMETHYLGLUTARYL-COA REDUCTASE (NADPH)	MJ0705
2.7.1.36	MEVALONATE KINASE	MJ1087
2.7.4.2	PHOSPHOMEVALONATE KINASE	missing
4.1.1.33	DIPHOSPHOMEVALONTE DECARBOXYLASE	no sequences
5.3.3.2	ISOPENTENYL-DIPHOSPHATE DELTA-ISOMERASE	missing
2.5.1.1	DIMETHYLALLYLTRANSFERASE	MJ0860
2.5.1.29	FARNESYLTRANSTRANSFERASE	MJ0860
2.5.1.10	GERANYLTRANSTRANSFERASE	MJ0860
phosphatidylserine biosynthesis		
2.7.8.8	CDP-DIACYLGLYCEROL–SERINE O-PHOSPHATIDYLTRANSFERASE	MJ1212

The reliable identification of UDP-N-acetylglucosamine–dolichyl-phosphate-N-acetylglucosaminephosphotransferase indicates that dolichol biosynthesis from

farnesyl diphosphate is also present. The presence of acetyl-CoA carboxylase indicates that malonyl-CoA is probably used as a building block for complex lipids. We were able to reliably identify only a few enzymes dealing with metabolism of phospholipids. In particular, CDP-diacylglycerol–serine O-phosphatidyltransferase, ω-3 fatty acid desaturase, and phospholipase C were identified.

8. Metabolism of coenzymes and prosthetic groups

As was mentioned above, methanogens have a unique set of the coenzymes, including methanofuran, tetrahydromethanopterin (H4MPT), coenzyme M (HS-CoM), 7-mercaptoheptanoylthreonine phosphate (HS-HTP), and coenzyme F430 (for reviews, see (DiMarco et al., 1990)). Methanogenes also use a number of familiar coenzymes and cofactors participating in various metabolic processes (for a review, see (Jones et al., 1989)), such as thiamine, riboflavin, pyridoxine, cobamides, biotin, niacin, and panthotenate.

The autotrophic nature of *M. jannaschii* implies its capability to synthesize all coenzymes and prosthetic groups required for its metabolism. In many cases, however, the enzymes involved in these biosyntheses have not been thorough characterized in any organism. We found at least partial evidence for genes encoding the biosynthesis of the following enzymes: methanopterin, NAD, cobalamine, riboflavin, FMN, FAD, thiamine pyrophosphate and biotin.

Coenzymes and vitamins		
NAD(′+) biosynthesis		
2.4.2.19	NICOTINATE-NUCLEOTIDE PYROPHOSPHORYLASE (CARBOXYLATING)	MJ0493
2.7.7.18	NICOTINATE-NUCLEOTIDE ADENYLYLTRANSFERASE	no sequences
6.3.5.1 (GLUTAMINE-HYDROLYSING)	NAD(+) SYNTHASE	MJ1352
Biotin biosynthesis		
6.2.1.14	6-CARBOXYHEXANOATE–COA LIGASE	MJ1297
2.3.1.47	8-AMINO-7-OXONONANOATE SYNTHASE	MJ1298
2.6.1.62	ADENOSYLMETHIONINE–8-AMINO-7-OXONONANOATE AMINOTRANSFERASE	MJ1300
6.3.3.3	DETHIOBIOTIN SYNTHASE	MJ1299
2.8.1.-	BIOTIN SYNTHETASE	no sequences
Porphyrin biosynthesis		
6.1.1.17	GLUTAMYL–TRNA SYNTHETASE	MJ1377
1.2.1.-	GLUTAMYL-TRNA REDUCTASE	MJ0143
5.4.3.8	GLUTAMATE-1-SEMIALDEHYDE 2,1-AMINOMUTASE	MJ0603
4.2.1.24	PORPHOBILINOGEN SYNTHASE	MJ0643
4.3.1.8	HYDROXYMETHYLBILANE SYNTHASE	MJ0569
4.2.1.75	UROPORPHYRINOGEN-III SYNTHASE	MJ0994
4.1.1.37	UROPORPHYRINOGEN DECARBOXYLASE	missing
1.3.3.3	COPROPORPHYRINOGEN OXIDASE	MJ1487
1.3.3.4	PROTOPORPHYRINOGEN OXIDASE	MJ0928
4.99.1.1	FERROCHELATASE	missing
Siroheme biosynthesis		
6.1.1.17	GLUTAMYL–TRNA SYNTHETASE	MJ1377
1.2.1.-	GLUTAMYL-TRNA REDUCTASE	MJ0143
5.4.3.8	GLUTAMATE-1-SEMIALDEHYDE 2,1-AMINOMUTASE	MJ0603
4.2.1.24	PORPHOBILINOGEN SYNTHASE	MJ0643
4.3.1.8	HYDROXYMETHYLBILANE SYNTHASE	MJ0569
	SIROHEME SYNTHASE (CONTAINS:	
	2.1.1.107/1.-.-.-/4.99.1.- UROPORPHYRIN-III C-METHYLTRANSFERASE/ PRECORRIN-2 OXIDASE/ FERROCHELATASE)	missing
Vitamin B12 biosynthesis		
2.1.1.107	UROPORPHYRIN-III C-METHYLTRANSFERASE	MJ0965
1.3.3.-	ANAEROBIC PROTOPORPHYRINOGEN OXIDASE	MJ0928
	COBYRIC ACID SYNTHASE	MJ0484
	COBYRINIC ACID A,C-DIAMIDE SYNTHASE	MJ1421
5.-.-.-	PRECORRIN ISOMERASE	MJ0930
2.1.1.-	S-ADENOSYL-L-METHIONINE–PRECORRIN-2 METHYLTRANSFERASE	MJ0771
2.1.1.-	PRECORRIN-3 METHYLASE	MJ0813 MJ1578
2.1.1.-	PRECORRIN-6Y METHYLASE	MJ1522
1-.-.-	PRECORRIN-8W DECARBOXYLASE	MJ0391
	CBIB PROTEIN	MJ1314
	CBID PROTEIN	MJ0022
	CBIJ PROTEIN	MJ0552
	CBIM PROTEIN	MJ1091
	CBIM PROTEIN	MJ1569
	CBIN PROTEIN	MJ1090
	CBIO PROTEIN	MJ1088

	CBIQ PROTEIN	MJ1089
	COBN PROTEIN	MJ0908
	COBALAMIN (5′-PHOSPHATE) SYNTHASE	MJ1438

Riboflavin biosynthesis

3.5.4.25	GTP CYCLOHYDROLASE II	MJ0055
3.5.4.26	DIAMINOHYDROXYPHOSPHO RIBOSYLAMINOPYRIMIDINE DEAMINASE	no sequences
1.1.1.193	5-AMINO-6-(5-PHOSPHO RIBOSYLAMINO)URACIL REDUCTASE	no sequences
2.5.1.9	RIBOFLAVIN SYNTHASE	MJ0303

Like those for thiamine, niacin, and panthotenate, we believe that the *M. jannaschii* counterparts of some biosynthetic enzymes either have diverged too far from the bacterial or eukaryotic versions to be recognizable or are analogs, but not homologs, of them.

Biochemical evidence indicates that folic acid levels are extremely low in methanogens (Leigh, 1983) and that tetrahydrofolate coenzymes are probably not present (Purwantini and Daniels, 1996). Our analysis of the sequence data also indicates an absence of enzymes using these coenzymes.

Some evidence exists that lipoic acid occurs in archaea (Noll et al., 1988). Its main function is as a prosthetic group within the pyruvate dehydrogenase complex and the 2-oxoglutarate dehydrogenase complex. Of the five enzymes normally involved in these complexes, only the lipoate dehydrogenase (EC 1.8.1.4) has been located. This result leads to a puzzling situation in which there seems no apparent physiological function for lipoic acid, but the mechanism for reoxidizing it appears to exist. However, it has been recently shown (Bunik and Follman, 1993) that lipoate dehydrogenase can also use thioredoxin as a substrate, and thioredoxin may play a significant role in *M. jannaschii*.

9. Enzymatic activities coupled to oxidation or reduction of F_{420}

F_{420} can act as a replacement for ferredoxin in some methanogens, including *Methanococcus jannaschii*. It functions as a low-potential two-electron acceptor. The following table summarizes the instances in which enzymatic activities using F_{420} were detected:

F_{420}-dependent enzymes		
1.12.99.1	COENZYME F420 HYDROGENASE ALPHA SUBUNIT	MJ0727
1.12.99.1	COENZYME F420 HYDROGENASE ALPHA SUBUNIT	MJ0029
1.12.99.1	COENZYME F420 HYDROGENASE ALPHA SUBUNIT	M_jannaschii_ chromosome_ 29808_31007
	COENZYME F420 HYDROGENASE BETA SUBUNIT	MJ0725 MJ0032 MJ0870
	COENZYME F420 HYDROGENASE GAMMA SUBUNIT	MJ0726 MJ0031
	COENZYME F420 HYDROGENASE DELTA SUBUNIT	MJ0030
1.2.1.2	FORMATE DEHYDROGENASE ALPHA CHAIN	MJ1353 M_jannaschii_ chromosome_ 1304115_1303648 MJ0006
	FORMATE DEHYDROGENASE BETA CHAIN	MJ0005
	FORMATE DEHYDROGENASE IRON–SULFUR SUBUNIT	MJ0155
	FDHD PROTEIN	MJ0295
1.5.99.9	METHYLENETETRAHYDRO-METHANOPTERIN OXIDOREDUCTASE	MJ1534

10. Membrane transport

Like many autotrophic methanogens, *M. jannaschii* has a limited capacity to assimilate organic molecules (Sprott et al., 1993). Compounds assimilated well include leucine, isoleucine, phenylalanine, formate, pyruvate and malate. Compounds assimilated poorly include mevalonate, glycerol, and lysine. Compounds assimilated in very low amounts or not at all include serine, aspartate, citrate, glucose, and acetate. The inability to assimilate acetate is unusual for methanogens, and acetate kinase, phosphotransacetylase, and acetyl coenzyme A synthetase activities are not detectable.

Sequence data reveal a wide spectrum of membrane transport proteins, the substrates for which have not yet been identified.

Membrane transport is driven by both ATP-dependent and osmotic-potential-based mechanisms. The proton motive force is generated during methanogenesis and drives a classical H-ATPase (EC 3.6.1.34) for ATP biosynthesis; the key subunits have been reliably identified. A second H-ATPase (EC 3.6.1.35), more typical of plants and fungi is also present.

11. Summary

The interpretation of the *Methanococcus jannaschii* genome will inevitably require many years of effort. This initial attempt to connect the sequence data to aspects of known biochemistry and to provide an overview of what is already apparent from the sequence data will be refined.

Numerous issues remain that can be resolved only by direct biochemical analysis. Let us draw the reader's attention to just a few that might be considered central:

(1) We are still missing key enzymes from the glycolytic pathway, and the conjecture is that this is due to ADP-dependency. The existence of glycolytic activity in the cell-free extract should be tested.
(2) The issue of whether the Calvin cycle is present needs to be examined.
(3) We need to determine whether the 2-oxoglutarate synthase (ferredoxin-dependent) (EC 1.2.7.3) activity is present.
(4) The issue of whether cyclic 2,3-bisphosphate is detectable in the cell-free extracts needs to be checked. If it is, this result would confirm our assertion of the two pathways controlling synthesis and degradation of cyclic 2,3-bisphosphate.

We will provide the current metabolic reconstruction, which will be updated as new interpretations and data emerge, via the WIT system, which is a Web application that can be accessed via the URLhttp://www.cme.msu.edu/WIT/

Our sincere hope is that others will find this initial model useful and will forward criticisms, corrections, and updates to Evgeni Selkov at the e-mail address: Evgeni@mcs.anl.gov

12. Unlinked References

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