Chapter 8
The Distribution and Evolution of C1 Transfer Enzymes and Evolution of the Planctomycetes

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Contents

Abbreviations

H_4MPT	Tetrahydromethanopterin
C1	Single carbon atom as in C1 compound, organic compound containing a single carbon atom
MtdB	NAD(P)-linked methylene-H_4MPT dehydrogenase
MFR	Methanofuran
H_4MPT	Tetrahydromethanopterin
F_{420}	Coenzyme F_{420}
CoM	Coenzyme M
CoB	Coenzyme B. Fae formaldehyde-activating enzyme
MtdB MtdC	Methylene-H_4MPT dehydrogenases

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Mch	Methenyl-H_4MPT cyclohydrolase
FhcABCD	Formyltransferase/hydrolase complex
FdwD	Is homologous to the D subunit of formyl-MFR dehydrogenase
LUCA	Last universal common ancestor

8.1 Introduction: History and Significance of the Question

Since their discovery, Planctomycetes have continued to be a fascinating group of organisms as they possess properties not typical of other bacteria, such as a cell wall lacking peptidoglycan reminiscent of Archaea, intricate cell compartmentalization reminiscent of Eukaryotes, division by budding reminiscent of yeasts, and unique metabolites such as sterols produced by the *Gemmata* species and ladderane lipids produced by autotrophic ammonia-oxidizing planctomycetes (Fuerst and Sagulenko 2011). When the first genomic sequence of a Planctomycete, of *Pirellula* sp. strain 1 (since renamed as *Rhodopirellula baltica*; Schlesner et al. 2004) was sequenced in 2003, it revealed another unusual feature, the presence of genes for C1 metabolism and more specifically genes encoding tetrahydromethanopterin (H_4MPT)-linked reactions for C1 transfers (Glöckner et al. 2003). Why was this discovery so significant? It was significant because it identified Planctomycetes as the third major phylum to possess genes for reactions requiring H_4MPT as a cofactor and a second phylum within the bacterial domain. Only a few years before, such genes were serendipitously discovered in a methylotrophic bacterium, *Methylobacterium extorquens*, and demonstrated to be indispensable for growth on C1 substrates such as methanol (Chistoserdova et al. 1998), which was followed by the identification of these genes in a number of other methylotrophic Proteobacteria (Vorholt et al. 1999), for the first time suggesting that functions thought to be unique to a limited group of Archaea, specifically methanogenic and sulfate-reducing Archaea, all classed within the kingdom Euryarchaeotes, may be in fact more widespread. However, gene/protein homologs from the Archaea and the Proteobacteria were only distantly related. In addition, the pathways they encoded were parts of distinctly different biochemical processes, i.e., methanogenesis (reducing CO_2 to methyl) and methylotrophy (oxidizing methyl to CO_2; Fig. 8.1). As the involvement of the H_4MPT-linked functions in methanogenesis and methylotrophy established the common root of the two bioconversions, the question arose of their evolutionary history. Which pathway evolved first? Did the methanogenesis precede the methylotrophy or vice versa? The possibility of lateral gene transfer between Euryarchaea and Proteobacteria has been discussed in this context, and the most probable direction of the transfer was assumed to be from Archaea into Bacteria (Chistoserdova et al. 1998; Vorholt et al. 1999; DeLong 2000; Gogarten et al. 2002; Boucher et al. 2003; Martin and Russell 2003). A scenario of lateral transfer of these genes from a (aerobic) proteobacterial methylotroph into a euryarchaeon was also suggested (Cavalier-Smith 2002), but this scenario would necessitate aerobic methylotrophy preceding anaerobic methanogenesis, which contradicts the current understanding of the history of Earth's atmosphere (Kasting and Siefert 2002). The discovery of the H_4MPT-linked C1 transfer function in the Planctomycetes, a deeply

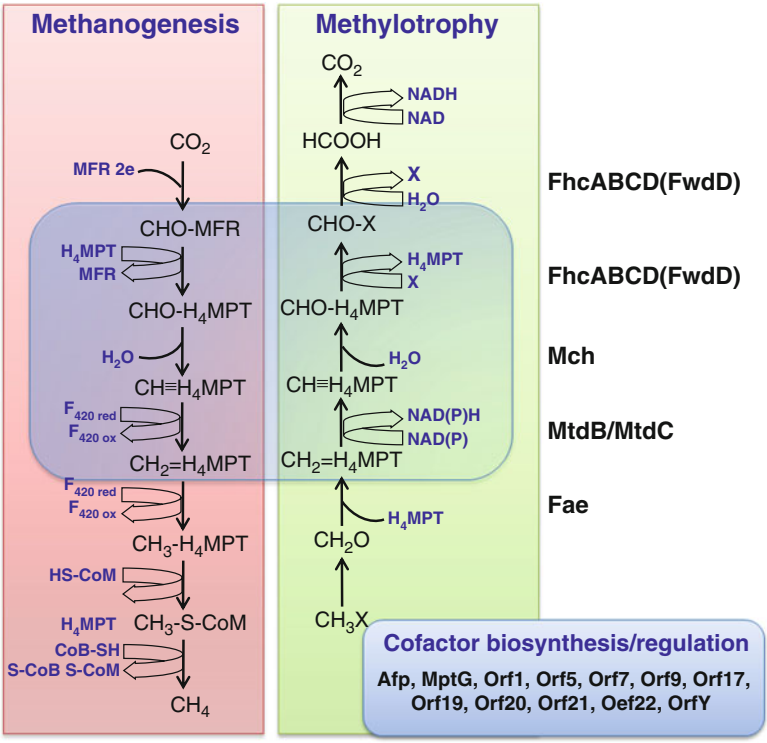

Fig. 8.1 The commonality of methanogenesis and methylotrophy. Analogous reactions are highlighted by a *blue box*. *MFR* methanofuran, *H_4MPT* tetrahydromethanopterin, *F_{420}* coenzyme F_{420}, *CoM coenzyme M*, *CoB* coenzyme B, *Fae* formaldehyde-activating enzyme, *MtdB*, *MtdC* methylene-H_4MPT dehydrogenases, *Mch* methenyl-H_4MPT cyclohydrolase, *FhcABCD* formyltransferase/hydrolase complex, FwdD is homologous to the D subunit of formyl-MFR dehydrogenase

branching division of Bacteria (Brochier and Philippe 2002; Di Giulio 2003), provided a missing link in understanding the history of metabolism and offered an opportunity for refining the picture of the evolution of methanogenesis and methylotrophy, as well as for a better understanding of the evolution of C1 metabolism on Earth.

8.2 Phylogenetic Analysis of C1 Transfer Enzymes in Planctomycetes

One of the major outcomes of discovering the third deeply branching microbial group possessing H_4MPT-linked functions was the potential to obtain new insights into the possible scenarios for the evolution of these functions and to test the then currently prevalent hypothesis of lateral transfer of these genes from Euryarchaeota to Proteobacteria (Gogarten et al. 2002; Boucher et al. 2003; Martin and Russell

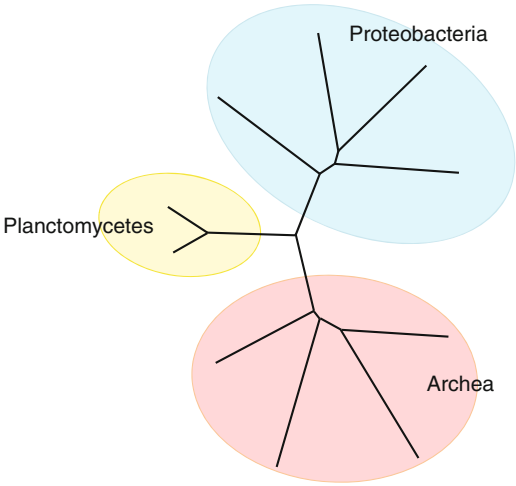

Fig. 8.2 A cartoon depicting a typical topology of phylogenetic trees resolving positions of Planctomycete C1 transfer pathway peptides with respect to the counterparts from Euryarchaea and Proteobacteria

2003). Two groups independently carried out phylogenetic analysis of the polypeptides translated from the C1 genes shared between Planctomycetes (at the time represented by two genera, *Rhodopirellula* and *Gemmata*), Proteobacteria (both methylotrophs and non-methylotroph species), and Archaea (both methanogens and sulfate-reducing species). Chistoserdova and colleagues analyzed a total 16 polypeptides (Chistoserdova et al. 2004), and Bauer and colleagues analyzed a total of seven polypeptides (Bauer et al. 2004). In both cases, phylogenetic analyses showed that, in general, the polypeptide counterparts from Planctomycetes appeared to be distant from both their archaeal homologs and from their proteobacterial homologs, in most cases forming a distinct third group on phylogenetic trees, with significant bootstrap confidence for the node defining the group's monophyly (Fig. 8.2). Notably, this pattern was revealed by the methenyl-H_4MPT cyclohydrolase (Mch) polypeptides that were previously assumed to be some of the most reliable enzymes for following the evolutionary history of methanogenesis (and likely of C1 transfers in bacteria) based on the criteria of its essential function, the lack of duplication in any known organism (at least at that time), and the absence of substitution by functionally equivalent enzymes (Reeve et al. 1997). Mch phylogeny also seemed to agree with the 16S rRNA phylogeny in both Euryarchaeota (Reeve et al. 1997) and Proteobacteria (Vorholt et al. 1999). The data from the phylogenetic analyses suggested that a single event was responsible for the emergence of the functions in question for each major phylum possessing them. However, some of the trees built in these studies showed deviations from this common pattern. In some cases, tree topologies were complicated by the presence of multiple gene homologs, potentially reflecting a more complex evolution of these genes, which suggested early duplications, as well as early and recent gene transfers for some of the genes (Chistoserdova et al. 2004). Another deviation from the common pattern was noted for some of the genes/enzymes in *Rhodopirellula baltica*, specifically for the polypeptides involved in the formyltransferase/hydrolase (Fhc) complex, which tended to cluster within the proteobacterial branch instead of clustering with the *Gemmata* sequences

(Chistoserdova et al. 2004; Bauer et al. 2004), such clustering potentially reflecting either specific selective pressures or results of lateral transfers from Proteobacteria.

As many as seven alternative scenarios were invoked to explain the presence of the H_4MPT-linked C1 transfer genes and their evolution in the three phylogenetically separated prokaryotic divisions: Euryarchaeota, Planctomycetes, and Proteobacteria. These included (a) their presence in the last universal common ancestor (LUCA) followed by gene loss in most of the divisions, as well as most of the Proteobacteria (Chistoserdova et al. 2004; Bauer et al. 2004); (b) lateral transfer from Euryarchaeota into the bacterial domain before the separation of Planctomycetes and Proteobacteria (Chistoserdova et al. 2004; Bauer et al. 2004); (c) independent transfers from Euryarchaeota into Planctomycetes and into Proteobacteria after the latter groups separated (Chistoserdova et al. 2004; Bauer et al. 2004); (d) transfer from Euryarchaeota into Proteobacteria and later from Proteobacteria into Planctomycetes; (e) transfer into Planctomycetes and later from Planctomycetes into Proteobacteria (Bauer et al. 2004); (f) emergence of the genes in Proteobacteria with subsequent independent lateral transfers into Euryarchaeota and Planctomycetes; and (g) emergence in Planctomycetes followed by independent transfers into Proteobacteria and Euryarchaeota (Chistoserdova et al. 2004). While Bauer et al. (2004) have postulated that in all scenarios Euryarchaeotes had to be the ancestral carrier of the H_4MPT-linked C1 transfer genes, Chistoserdova et al. (2004) favored scenarios in which the genes in question were either present in the LUCA or have emerged in Planctomycetes, the conclusion mainly based on the topology of the phylogenetic trees and based on the presumed antiquity of the Planctomycetes (Brochier and Philippe 2002; Di Giulio 2003). It was concluded that, in both scenarios, a selective pressure would be required to prevent the loss of the entire complement of the genes. Thus, for early life on Earth, a fitness advantage corresponded by this pathway could be predicted. Formaldehyde is thought to have been abundant on early Earth (Arrhenius et al. 1994). Therefore, it was argued that early cells could benefit from a system to reduce the toxic effect of formaldehyde, the role that could have been carried out by the H_4MPT-linked C1 transfer pathway in the early Planctomycetes. At later stages, an additional fitness could be derived from the ability to draw energy from these reactions. Whichever scenario was true, it appeared that the H_4MPT-linked C1 transfer pathway between the oxidation levels of formaldehyde and formate was likely an early, important function for life, which provided the essential building block in the formation of both methanogenesis and methylotrophy pathways (Chistoserdova et al. 2004).

8.3 MtdC: A Novel Methylene-H_4MPT Dehydrogenase Found in Planctomycetes

While most of the enzymes involved in the H_4MPT-linked C1 transfer pathway are shared between Bacteria and Archaea, some are Bacteria specific. One of these enzymes, the NAD(P)-linked methylene-H_4MPT dehydrogenase (MtdB), that is unique to Bacteria operates in the pathway in place of its functional counterparts,

that are linked to H_2 or cofactor F_{420} (Fig. 8.1). Based on the lack of sequence similarity, MtdB must have evolved independently of the archaeal functional counterparts. Enzyme properties and mutant analyses demonstrated that MtdB fulfills a dual physiological role in methylotrophic metabolism, in energy generation (in the form of NADH), and in formaldehyde detoxification (Hagemeier et al. 2000). In some methylotrophs, a paralog of MtdB is present, named MtdA, and this has been characterized as a bifunctional methylene-H_4MPT/methylene-tetrahydrofolate (H_4F) dehydrogenase (Vorholt et al. 1998). While their specificities overlap and both can oxidize methylene-H_4MPT, the main function of MtdA is believed to be in reducing methenyl-H_4F to methylene-H_4F (Chistoserdova 2011). In addition, MtdA has a function in general metabolism (e.g., purine biosynthesis) in organisms that do not possess the traditional enzyme, FolD, which is an enzyme that fulfills this function in most bacteria and eukaryotes (Chistoserdova 2011). The origin and evolutionary history of MtdA and MtdB remained poorly understood. While MtdA reveals low levels of sequence similarity to FolD enzymes (15 % identity at the amino acid level), MtdB shares no similarity with FolD (Hagemeier et al. 2000). However, the two paralogs reveal a significant level of similarity to each other (about 30 % at the amino acid level), pointing to their common origin (Vorholt et al. 1998). In the Planctomycete genomes, a single ortholog was identified through BLASTP analysis using either MtdA or MtdB sequences as queries, and these revealed higher similarity to the former (43–53 %) than to the latter (28–32 % at the amino acid level; Vorholt et al. 2005). This finding was unexpected considering the established functions for MtdA and MtdB (i.e., reduction of methenyl-H_4F and oxidation of methylene-H_4MPT, respectively), especially given the fact that Planctomycetes encode FolD (Vorholt et al. 2005). These considerations suggested that the function of Mtd protein orthologs in Planctomycetes could be more similar to the function of MtdB than to the function of MtdA. This hypothesis was tested by expressing the *mtd* gene homolog from *Gemmata* sp. in the mutants of *M. extorquens* containing lesions in either *mtdA* or *mtdB*, which are both negative for growth on methanol (Chistoserdova 2011). Neither of the mutants could be complemented by the *mtd* gene from *Gemmata* sp., suggesting that its product may possess substrate specificities differing from the ones of MtdA or MtdB. Indeed, the purified enzyme, while highly active in catalyzing the methylene-H_4MPT dehydrogenase reaction using NADP as a cofactor, revealed low efficiency in catalyzing the dehydrogenation of either methylene-H_4MPT using NAD as a cofactor, in contrast to the characterized MtdB (Hagemeier et al. 2000), or methylene-H_4F with NADP as a cofactor, in contrast to the characterized MtdA (Vorholt et al. 1998). However, compared to MtdA and MtdB, the new enzyme, named MtdC, was shown to possess a broader substrate range, revealing affinities for NAD, NADP, H_4F, and H_4MPT, with the highest affinity for the H_4MPT/NADP couple. This substrate combination likely represents the physiological activity of this enzyme. Thus, while phylogenetically more related to MtdA, MtdC must be a functional homolog of MtdB, which acts as a part of the oxidative pathway linked to H_4MPT (Fig. 8.1). This conclusion is also supported by the chromosomal location of *mtdC* genes in physical proximity of other genes involved in the pathway (Vorholt et al. 2005), which is also the case with the *mtdB*

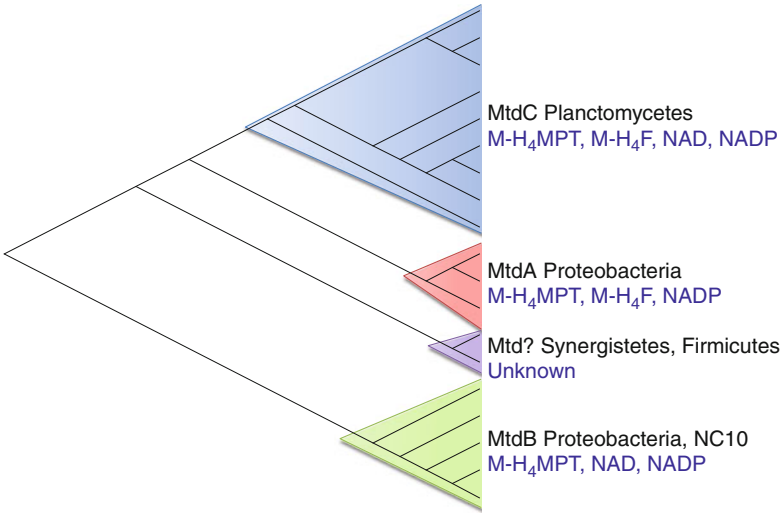

Fig. 8.3 Phylogeny of relevant Mtd proteins, Mtd protein affiliations with specific phyla, and substrate/cofactor specificities

gene (Kalyuzhnaya et al. 2005). Both the substrate "promiscuity" of MtdC and its phylogenetic separation from its homologs (Fig. 8.3) were used as arguments for its ancestral role with respect to MtdA and MtdB (Vorholt et al. 2005).

8.4 Recent Genomic Insights into the Distribution of the H_4MPT-Linked C1 Transfer Functions

Over the past few years, microbial genomic databases expanded substantially, including novel and phylogenetically divergent organisms (Wu et al. 2009; Pagani et al. 2012). The Planctomycete genomic database remains very limited, with a total count of 11 publically available genomes, providing genetic blueprints for *Blastopirellula marina* (5.7 Mb; http://genome.jgi.doe.gov/), *Candidatus* Kuenenia stuttgartiensis (4.2 Mb; Strous et al. 2006), *Gemmata obscuriglobus* (9.1 Mb; http://genome.jgi.doe.gov/), *Isosphaera pallida* (5.5 Mb; Göker et al. 2011), *Pirellula staleyi* (6.2 Mb; Clum et al. 2009), *Planctomyces brasiliensis* (6.0 Mb; http://genome.jgi.doe.gov/), *Planctomyces limnophilus* (5.5 Mb; LaButti et al. 2010), *Planctomyces maris* (7.8 Mb; http://genome.jgi.doe.gov/), *Rhodopirellula baltica* (7.1 Mb; Glöckner et al. 2003), *Singulisphaera acidiphila* (9.7 Mb; http://genome.jgi.doe.gov/), and an unclassified strain, an endophyte of *Porphyra umbilicalis* that is most closely related to *R. baltica* (7.3 Mb; http://genome.jgi.doe.gov/). Analysis of these genomes reconfirms that genes for the H_4MPT-linked C1 transfer reactions are some of the most conserved genes among the diverse

Planctomycetes, one exception being the metabolic specialist *Candidatus* Kuenenia stuttgartiensis, an anaerobic ammonia oxidizer (anammox) that lacks these genes (Strous et al. 2006). As this organism possesses a genome of significantly smaller size compared to other Planctomycetes, it is not unlikely that these genes were lost through genome reduction, which is typical of organisms evolved to specialize in a specific mode of metabolism, and the anammox Planctomycetes are an example of such specialization (Strous et al. 2006). The remaining ten genomes encode complete or nearly complete complements of genes implicated in the C1 transfer pathway. Single exceptions, such as no recognizable homolog for *fhcB* gene in *R. baltica* or *I. pallida* (Glöckner et al. 2003; Göker et al. 2011) or no recognizable *orf21* in *P. limnophilus* (LaButti et al. 2010) in BLAST analyses, are likely results of gene divergence beyond recognition by the BLAST tool or of functional gene replacement. Indeed, extreme divergence of C1 transfer genes in Planctomycetes is one insight resulting from the availability of new genomes, as well as from the growing databases of genes belonging to yet uncultivated Planctomycete species (Kalyuzhnaya and Chistoserdova 2005; Elshahed et al. 2007; Woebken et al. 2007). Despite such sequence divergence (e.g., some proteins involved in C1 transfer functions are less than 30 % identical among different Planctomycetes), phylogenetic analyses typically result in the outcomes similar to the ones presented in Fig. 8.2. The Planctomycete sequences tend to cluster together on phylogenetic trees, forming branches separated from the branches representing other phyla, which reinforces the notion of a monophyletic origin for most of the C1 genes in Planctomycetes. The degree of divergence for these genes, obvious even from the analysis of a very limited set of data, must further support the notion of a long history of the Planctomycetes after their separation from other lineages. In terms of gene clustering, a trend previously noted for the early genomes (Chistoserdova et al. 2004; Kalyuzhnaya et al. 2005; Woebken et al. 2007) maintains: the C1 genes are less clustered in the Planctomycetes than in other Bacteria but more clustered than in Archaea. However, many Planctomycete gene clustering signatures are shared with the signatures found in other phyla. For example, cluster *orf19-mptG* that is conserved in the Planctomycete genomes is not typical of Proteobacteria but is found in the genomes of Synergistetes, Firmicutes, and Division NC10 (Fig. 8.4), cluster *fae-mtdC* is shared between the Planctomycetes and Division NC10, cluster *orf17-orf1-orf9-orf21* is shared with Beta- and Gamma- but not Alphaproteobacteria (Kalyuzhnaya et al. 2005), and cluster *mch-orf5-orf7* is typical (so far) of all Bacteria. The fact that the H_4MPT-linked C1 transfer pathway genes were maintained in the majority of the extant Planctomycetes, likely through vertical inheritance, must further suggest that, despite its enigmatic role, this pathway must be of great physiological and environmental importance to the Planctomycetes. The role proposed originally was the detoxification of formaldehyde (Chistoserdova et al. 2004; Fig. 8.1). The argument for this role is the persistent presence of the (true) *fae* gene (i.e., encoding formaldehyde-activating enzyme that condenses formaldehyde with H_4MPT; Vorholt et al. 2000) for all the Planctomycetes possessing the pathway. Interestingly, *fae*

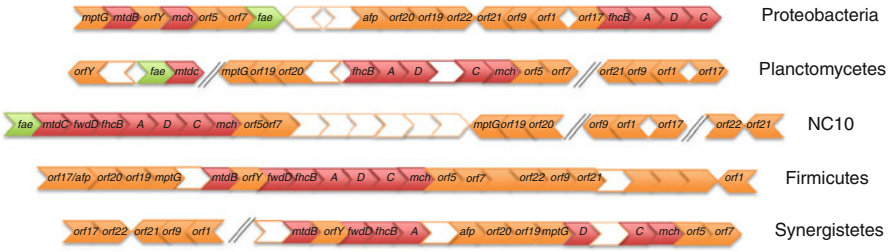

Fig. 8.4 Clustering of the C1 genes on the chromosomes of major phyla. Proteobacteria are represented by *Methylibium petroleiphilum* (Kane et al. 2007); Planctomycetes are represented by *Singulisphaera acidiphila* (note that this organism reveals more clustering than other Planctomycetes; http://genome.jgi.doe.gov/); Division NC10 is represented by *Candidatus* Methylomirabilis oxyfera (Ettwig et al. 2010); Firmicutes are represented by *Halanaerobium hydrogeniformans* (Brown et al. 2011); and Synergistetes are represented by *Anaerobaculum hydrogeniformans* (http://genome.jgi.doe.gov/). Genes for H_4MPT-linked C1 transfer enzymes are in *red*; genes for cofactor biosynthesis/regulation are in *orange* (genes without designation are not conserved among clusters); *fae* genes are in *green* (note their absence on the chromosomes of Firmicutes and Synergistetes); genes not relevant to discussion are *colorless*. *orfY* is not part of a cluster and is not shown for NC10. *Parallel lines* indicate that clusters are not contiguous on the chromosomes

genes are missing (likely through gene loss; Fig. 8.4) from the genomes of Firmicutes and Synergistetes. The lack of Fae potentially suggests that these organisms may employ the pathway as part of a metabolic scheme not involving free formaldehyde. Instead, they would employ (unknown) reactions that would transfer a methyl group directly onto H_4MPT. However, the nature and the metabolic purpose of such variants remain unknown. In contrast to the *true fae* genes, for the ones that are phylogenetically Planctomycete specific (Chistoserdova et al. 2004), some Planctomycetes (6 out of 9) encode distant homologs named Fae3 (Kalyuzhnaya et al. 2005; Chistoserdova 2011). These do not follow the typical Planctomycete phylogenetic pattern (shown in Fig. 8.2). Instead, they cluster together with the proteobacterial sequences, all the known sequences revealing over 80 % identity at the amino acid level, suggesting that recent evolution for these genes involved both intra- and inter-domain transfers. The function of Fae3 remains unknown (Chistoserdova 2011).

Overall, comparisons of genes/enzymes involved in H_4MPT-linked C1 transfers in Planctomycetes with their counterparts in other phyla, in terms of both sequence conservation/divergence and gene clustering, suggest a long evolutionary history for each lineage. While the genes from the newly identified lineages such as Synergistetes, Firmicutes, and Division NC10 show a high degree of divergence with any previously described C1 transfer genes/proteins (Ettwig et al. 2010 and unpublished observations by the author), conservation in gene clustering between different lineages (Fig. 8.4) suggests a common origin.

8.5 New Insights into the Evolution of Microbial C1 Metabolism

Alternative scenarios for the evolution of C1 transfer pathways in the microbial world that remained unresolved due to the limited distribution of the pathway (Chistoserdova et al. 2004; Bauer et al. 2004) can now be revisited. Based on the analysis of recently sequenced genomes, the pathway appears to be much more widespread in both bacterial and archaeal phyla than previously thought. Complete or partial sets of genes have now been identified, besides methanogenic and sulfate-reducing Archaea, Proteobacteria, and Planctomycetes, in the genomes of anaerobic methane oxidizers (which are related to the methanogens; Knittel and Boetius 2009), genomes representing Crenarchaeota (e.g., *Ignisphaera*; incomplete pathway, no confirmed metabolic function); the yet unclassified Division NC10 (complete pathway and confirmed methylotrophy metabolism); phylum Synergistetes (complete pathway with no confirmed metabolic function); Firmicutes (complete pathway, no confirmed metabolic function); and in Actinobacteria (incomplete pathway, no confirmed metabolic function). This broad distribution of the pathway and further expansion of the phylogenetic diversity of the respective genes/enzymes clearly point to the likelihood of the presence of this pathway in the last universal common ancestor (LUCA). Remarkably, some of the new members of the bacterial domain possessing this pathway are obligate anaerobes (e.g., members of the NC10 division, Synergitetes, Firmicutes), supporting the hypothesis of the early emergence of the pathway (Chistoserdova et al. 2004), possibly prior to the emergence of oxygenic photosynthesis. Likely, this pathway, potentially in its formaldehyde-oxidizing capacity, has evolved before any of the primary C1 oxidation (such as methane monooxygenase and methanol dehydrogenase) or C1 reduction modules (such as methyl-CoM reductase) have emerged.

While the existence of the deeply diverging genes in the major microbial lineages reflect the long history of this pathway, it is certain that more recent lateral transfers played a role, which is demonstrated by the *fae3* genes that are shared among Planctomycetes and Alpha-, Beta-, and Gammaproteobacteria (Chistoserdova et al. 2004; Chistoserdova 2011). At least in Betaproteobacteria, genes encoding the H_4MPT-linked pathway appear to be of polyphyletic origin with the sequences of *Burkholderiaceae* separating from the sequences of *Methylophilaceae* (Kalyuzhnaya et al. 2005; Chistoserdova et al. 2007), with both types emerging from a common ancestor of Proteobacteria.

The recent genomic data also argue against the previous assumption that the pathway could not have been lost in many lineages of the Prokaryotes. To the contrary, the pathway in question appears to be a currency easily gained and lost. For example, recent deletion events could be noted by comparing genomes of two closely related *Nitrosococcus* species: while gene synteny and high gene identity are maintained between the C1 transfer gene clusters in *Nitrosococcus halophilus* and *Nitrosococcus oceani,* in the latter, key genes are missing from the cluster

(and from the genome), suggesting that the pathway is no longer operational in this species (Chistoserdova 2011). Another example is the (extensively sampled) Burkholderiales species, many of which encode the entire pathway, but many lack the entire pathway, and some possess multiple (phylogenetically distinct) gene clusters, suggesting that both gene losses and lateral transfers must be taking place (Chistoserdova 2011). More ancient, lineage-specific gene loss is also apparent based on comparisons of the gene complements present. One example is the *fwdD* gene homolog (that would likely be a subunit of the formyltransferase/hydrolase complex) that is maintained in Archaea, Synergistetes, Firmicutes, and Division NC10 (Fig. 8.4) but is missing from most of the known genomes of Planctomycetes or Proteobacteria. Another example is the Afp protein that is encoded in the genomes of Archaea, Synergistetes, Firmicutes, and most Proteobacteria. However, the respective gene is not recognized in the Planctomycete genomes and was shown to be substituted by a nonhomologous gene in the *Methylobacterium* species of Alphaproteobacteria (Marx et al. 2003; Vuilleumier et al. 2009).

8.6 Conclusions: Changing Trees

While the Planctomycetes are ubiquitous in the environment, their lifestyles and their environmental functions remain enigmatic, except for the established function of the anammox Planctomycetes (Strous et al. 2006). Excluding those species, from genomics of the cultivated species and based on culture-independent detection in a variety of environments (Kalyuzhnaya and Chistoserdova 2005; Elshahed et al. 2007; Woebken et al. 2007), the C1 transfer genes appear to be persistently present in diverse Planctomycetes, both aerobes and anaerobes, suggesting their importance for species survival/fitness. Currently, no methylotrophy capability has been documented for a Planctomycete, and no obvious source of formaldehyde in their habitats has been established. Thus, the selective pressure for maintaining C1 metabolism functions, as well as the exact nature of the metabolism involving these functions, remains a mystery. However, Planctomycetes are often detected in environments with high rates of C1 metabolism (Lösekann et al. 2007; Kalyuzhnaya et al. 2008; Webster et al. 2011; Sauter et al. 2012), suggesting that they may be somehow involved, potentially through synergistic relationships in which C1 transfer capabilities provide an advantage. At the same time, as the tree of life is becoming better sampled through genomic approaches, the tree of the organisms encoding H_4MPT-linked C1 transfer functions is also expanding. Based on the genomic information available in 2004, we favored two alternative hypotheses for the evolution of these genes: their emergence in the LUCA with subsequent losses from most microbes or their emergence in the Planctomycetes with subsequent transfers into Euryarchaeota and Proteobacteria (Fig. 8.5a, b). At this time, based on the newly established presence of these genes in a number of deeply branching phyla, such as Firmicutes,

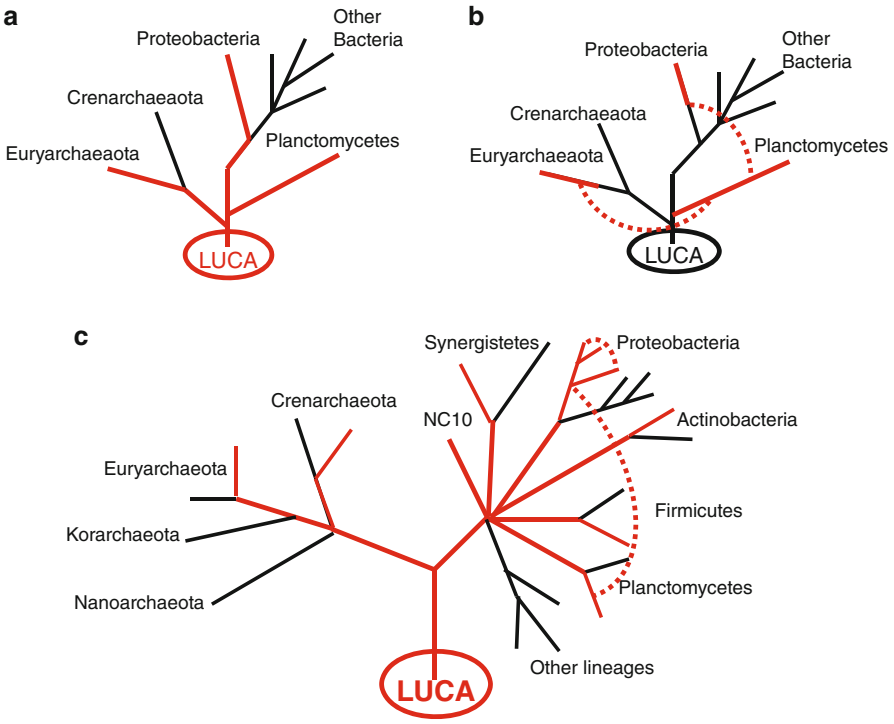

Fig. 8.5 A cartoon depicting the probable scenarios of the evolution of H_4MPT-linked C1 transfer genes in the microbial world. *Red branches* indicate (partial) presence, *black branches* indicate absence, and *dotted lines* indicate lateral transfers. (**a**, **b**) Scenarios proposed in 2004 (Chistoserdova et al.), based on limited sets of genomic data. (**c**) A scenario proposed here based on the newly available genomic data

Synergistetes, Division NC10, and based on the remarkable divergence of the genes within both Bacteria and Archaea, the former scenario appears to be more plausible (Fig. 8.5c), which further suggests that the pathway indeed must be very ancient. Currently, the involvement of the pathway has been established in four physiological processes: methanogenesis and anaerobic methane oxidation, both in Euryarchaeota, aerobic methylotrophy/formaldehyde detoxification in Proteobacteria, and anaerobic methane oxidation in Division NC10. It is entirely possible that the C1 transfer reactions encoded by Planctomycetes and by other phyla may be involved in or linked to metabolic processes that are neither methanogenesis nor methylotrophy. Further sampling of the diversity of the Planctomycetes and delineation of their relationships with other members of microbial communities will be instrumental in exploring such an intriguing possibility.

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