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Current Opinion in Microbiology

Transcriptional regulation of methanogenic metabolism in archaea

Katie E Shalvarjian[1] and Dipti D Nayak

Methanogenesis is a widespread metabolism of evolutionary and environmental importance that is likely to have originated on early Earth. Microorganisms that perform methanogenesis, termed methanogens, belong exclusively to the domain Archaea. Despite maintaining eukaryotic transcription machinery and homologs of bacterial regulators, archaeal transcription and gene regulation appear to be distinct from either domain. While genes involved in methanogenic metabolism have been identified and characterized, their regulation in response to both extracellular and intracellular signals is less understood. Here, we review recent reports on transcriptional regulation of methanogenesis using two model methanogens, *Methanococcus maripaludis* and *Methanosarcina acetivorans,* and highlight directions for future research in this nascent field.

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Introduction

Methanogens are a polyphyletic group of archaea that couple growth and energy conservation to the generation of methane gas through a process called methanogenesis [1–3]. These microbes are typically found in anoxic environments such as wetlands and sewage treatment plants, as well as the digestive tract of diverse hosts like termites, cows, and humans [4]. As the predominant source of methane, a potent greenhouse gas, methanogenic archaea rank as a highly influential group of organisms in the context of global climate regulation [1,5–7]. By now, many of the enzymes and respiratory complexes involved in methanogenesis have been identified and characterized in intricate biochemical detail [1,3]. Yet, very little is known about how these genes are regulated in response to biotic or abiotic signals. As the climate crisis exacerbates, the need to understand how methanogens regulate their metabolism in response to environmental cues is crucial to forecast how they will adapt to and impact the warming planet.

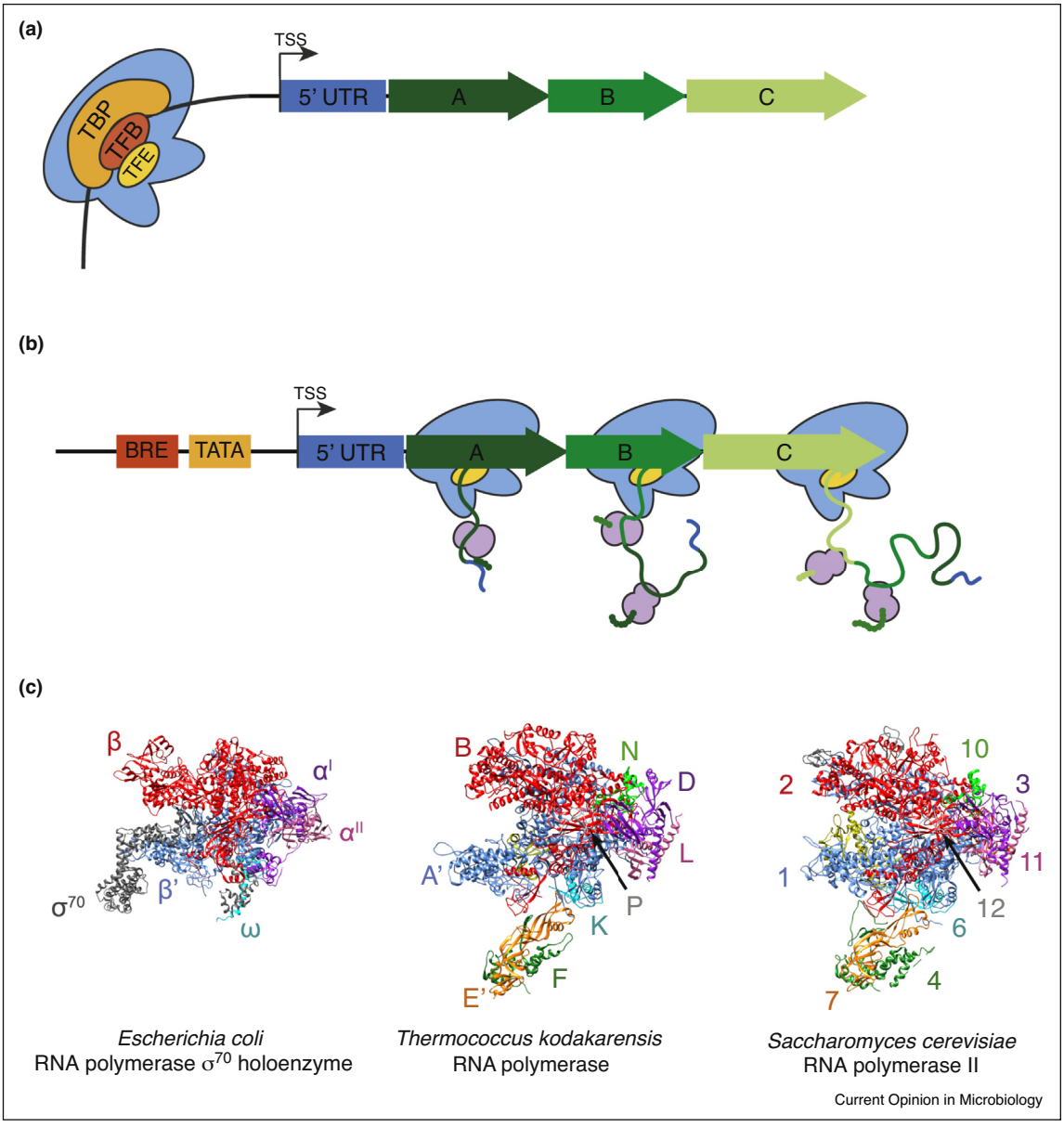
Since all known methanogens are members of the Archaea, the third domain of Life, their transcriptional machinery contains elements related to that of Bacteria and Eukarya, while remaining distinct from either (Figure 1) [8,9]. For instance, the archaeal RNA polymerase (RNAP) is structurally and evolutionarily related to PolII in *Saccharomyces cerevisiae* (Figure 1c) [10]. Similarly, transcription initiation relies on homologs of eukaryotic TATA-binding proteins (TBPs) and transcription factor B (TFB) binding to the TATA box and B-recognition element in the core promoter sequence, respectively (Figure 1a) [11,12]. However, following coordination of RNAP with the promoter, elongation proceeds akin to Bacteria, with transcription of the nascent mRNA coupled directly to its translation (Figure 1b) [13]. This is particularly relevant within the context of polarity as archaeal genomes are generally organized in operons [8]. Although homologs of the canonical, bacterial two component system (TCS) and some eukaryotic transcription factors have been identified in methanogens, the fundamental mechanisms of signal transduction and gene regulation in archaea are distinct and largely uncharacterized *in vivo* [14,15,16•]. However, with the advent of new genetic techniques, these questions are becoming more feasible to address within the context of the cell.

This review summarizes current knowledge and identifies future directions for research related to the regulation of methanogenesis in *Methanococcus maripaludis* and *Methanosarcina acetivorans*. These well-studied species have emerged as genetically tractable model organisms that represent two distinct energy conservation strategies in methanogens. *M. maripaludis* lacks cytochromes and is restricted to growth on H_2 + CO_2 or formate [17]; whereas *M. acetivorans* contains cytochromes and has a broader substrate range, which can include acetate and methylated compounds like methanol or methylamines [18]. Together, these two species provide an ideal genetic framework to identify signaling cascades and transcription factors that regulate methanogenesis *in vivo*.

Methanogens without cytochromes

Methanogens belonging to the Orders *Methanococcales*, *Methanobacteriales*, *Methanomicrobiales*, and *Methanopyrales*

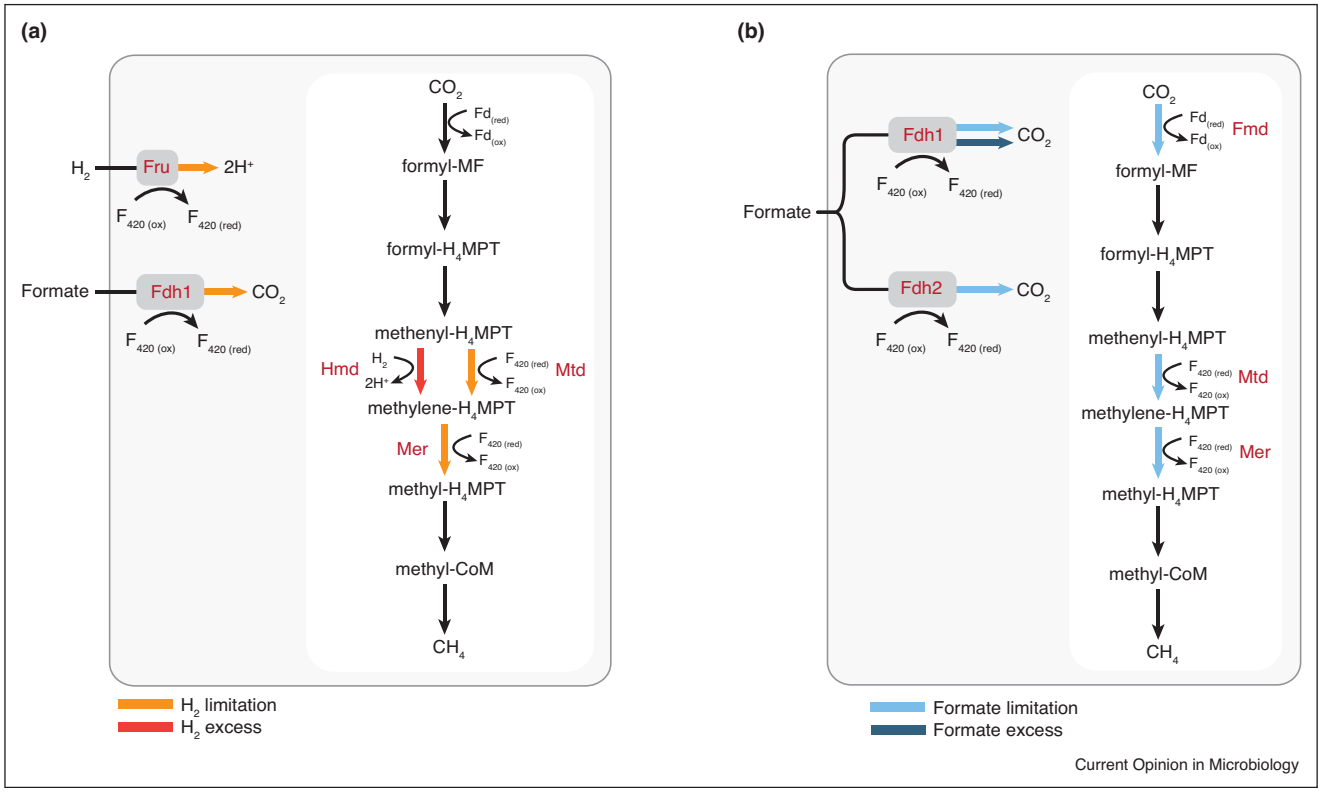
Figure 1



Transcription in Methanogenic Archaea. **(a)** Two transcription factors, TBP (TATA binding protein; in **orange**) and TFB (transcription factor B; in orange-red), that bind to the TATA-box and the B-recognition element (BRE) in the promoter region respectively are required to recruit the RNA polymerase (in light blue) for transcription initiation in methanogenic archaea. A third transcription factor, Transcription factor E (TFE; in yellow) is not essential but enhances the recruitment of RNA polymerase *in vitro.* The 5′ untranslated region (UTR) is highlighted in blue. **(b)** Genes in methanogenic archaea are arranged in operons and transcription of a multi-gene operon (in green) produces polycistronic RNA. Assembly of the corresponding polypeptide is facilitated by a translating ribosome (light purple). **(c)** Structural overview of RNA polymerase (RNAP) derived from a bacterium (*Escherichia coli*; PDB ID: 4YG2), an archaeon (*Thermococcus kodakarensis* PDB ID: 4QIW) and a eukaryote (Polymerase II from *Saccharomyces cerevisiae* PDB ID: 1WCM). Orthologous subunits of RNAP are shown using the same color.

within the *Euryarchaeota*, lack cytochromes and differ in their energy metabolism from members of *Methanosarcinales*. The vast majority of these cytochrome-lacking methanogens are restricted to growth on CO_2 with H_2 or formate as the electron donor [1,4]. However, a few notable exceptions can use methylated compounds as a carbon source and short-chain alcohols, like ethanol, as an electron donor as discussed in [2,19,20]. Although methanogens with and without cytochromes share a conserved seven-step pathway for the stepwise reduction of CO_2 to methane, the two differ significantly in their energy conservation strategies [1]. Methanogens without cytochromes, like *M. maripaludis*, also lack the

Figure 2



Condition Specific Regulation of Methanogenesis in *Methanococcus maripaludis*. Steps highlighted with colored arrows denote upregulation of the corresponding enzyme based on substrate availability. **(a)** Transcriptomic analyses of methanogenesis in H_2-replete and H_2-limited conditions has shown that genes catalyzing the fourth and the fifth step in the sequential reduction of CO_2 to methane are transcriptionally regulated as a function of H_2 availability. Additionally, the F_{420}-reducing hydrogenase complex (Fru) and formate dehydrogenase (Fdh1) are upregulated upon H_2 limitation. **(b)** During growth on formate, three steps of the seven-step pathway for CO_2 reduction are regulated as a function of substrate concentration. In addition, the two formate dehydrogenase orthologs are differently expressed: Fdh1 is expressed in formate replete media whereas Fdh2 is only expressed when formate concentrations are growth limiting.

quinone-like compound methanophenazine and rely on flavin-based electron bifurcation (FBEB) for the regeneration of coenzyme M (CoM) and coenzyme B (CoB) from the oxidized heterodisulfide (CoM-CoB) produced during the final step of methanogenesis [1,21–23]. As a result, the Na^+-dependent N^5-methyl-tetrahydromethanopterin:CoM methyltransferase (Mtr) singularly generates the ion gradient for ATP synthesis by the membrane-bound ATPase [1]. Until recently, *M. maripaludis* was the only genetically tractable member of this group of methanogens [24,25]. While its nitrogen regulon has been characterized and reviewed in detail in [26,27], regulation of methanogenesis in *M. maripaludis* has only been inferred from genome-wide transcriptomic data [28]. Therefore, despite extensive evidence for the regulation of methanogenic enzymes (as discussed below) transcriptional regulators involved in this process are yet to be characterized.

H_2 dependent gene regulation

Whole cell transcriptomic analyses of *M. maripaludis* in chemostat cultures where growth was limited by H_2 revealed extensive regulation in core methanogenesis genes [28–30]. Significantly, expression of metabolic genes that use F_{420}, an NAD(P) analog used by methanogens as a redox carrier, is linked to extracellular H_2 levels [29]. Two enzymes can carry out the fourth step of the methanogenesis pathway: an F_{420}-dependent tetrahydromethanopterin dehydrogenase (Mtd) or a H_2-dependent tetrahydromethanopterin dehydrogenase (Hmd) (Figure 2a). As H_2 levels drop, transcripts for Mtd become more abundant relative to Hmd, favoring the use of F_{420} as an intermediate under low H_2 conditions (Figure 2a) [30,31••]. Similarly, transcripts for the F_{420}-dependent methylene tetrahydromethanopterin reductase (Mer) and F_{420}-dependent hydrogenase (Fru) are more abundant under H_2-limiting conditions (Figure 2a) [29]. This upregulation of genes encoding F_{420}-dependent enzymes under H_2-limiting conditions might be beneficial to sustain metabolic flux for methanogenesis fueled by reduced F_{420} rather than H_2 [17,23,31••]. Similar studies with other methanogens like *Methanocaldococcus janaschii* and *Methanothermobacter thermoautotrophicum ΔH*, have also revealed substantial transcriptional regulation of Mtd

versus Hmd—along with other methanogenic enzymes—as a function of H_2 availability [32,33]. Altogether, while it is clear that there is an extensive H_2 regulon in *M. maripaludis*, a H_2 sensor and its downstream signaling cascade remains elusive.

Formate dependent gene regulation

In methanogens without cytochromes, formate dehydrogenase (encoded by *fdhAB*) catalyzes the oxidation of formate to CO_2 with the concomitant reduction of F_{420}. *M. maripaludis* encodes two copies of *fdhAB* where only *fdh1* is essential for growth on formate and the Δ*fdh2* mutant is indistinguishable from wildtype [31••,34]. Whole cell transcriptomics and gene specific lacZ fusions have shown that *fdh1* is upregulated during H_2 limitation and in the presence of formate, whereas *fdh2* is only upregulated under formate limiting conditions (Figure 2b) [34]. Likewise, many core methanogenesis genes appear to be regulated in response to formate (Figure 2b) [31••]. Whether the regulatory signals for formate and H_2 are the same, somewhat overlapping, or completely distinct is yet to be determined.

Methanogens with cytochromes

Members of the Order *Methanosarcinale*s encode cytochromes and a membrane bound electron transport chain for energy conservation during methanogenesis [1,2,4]. These methanogens are metabolically diverse [4,35•], providing a unique opportunity for the characterization of genes involved in the substrate specific regulation of methanogenesis. While many species within the Genus *Methanosarcina* are genetically tractable [36,37], *M. acetivorans* is emerging as a preeminent model system for understanding gene regulation in methanogens and perhaps, even more broadly, in the Archaea. Consequently, *M. acetivorans* encodes the largest number of transcription factors (TFs) in an archaeal genome [14] and substantial work has been done to show that several members of the ArsR family TFs act as both activators and repressors of genes involved in methanogenic metabolism [38,39••].

Methylotrophic methanogenesis

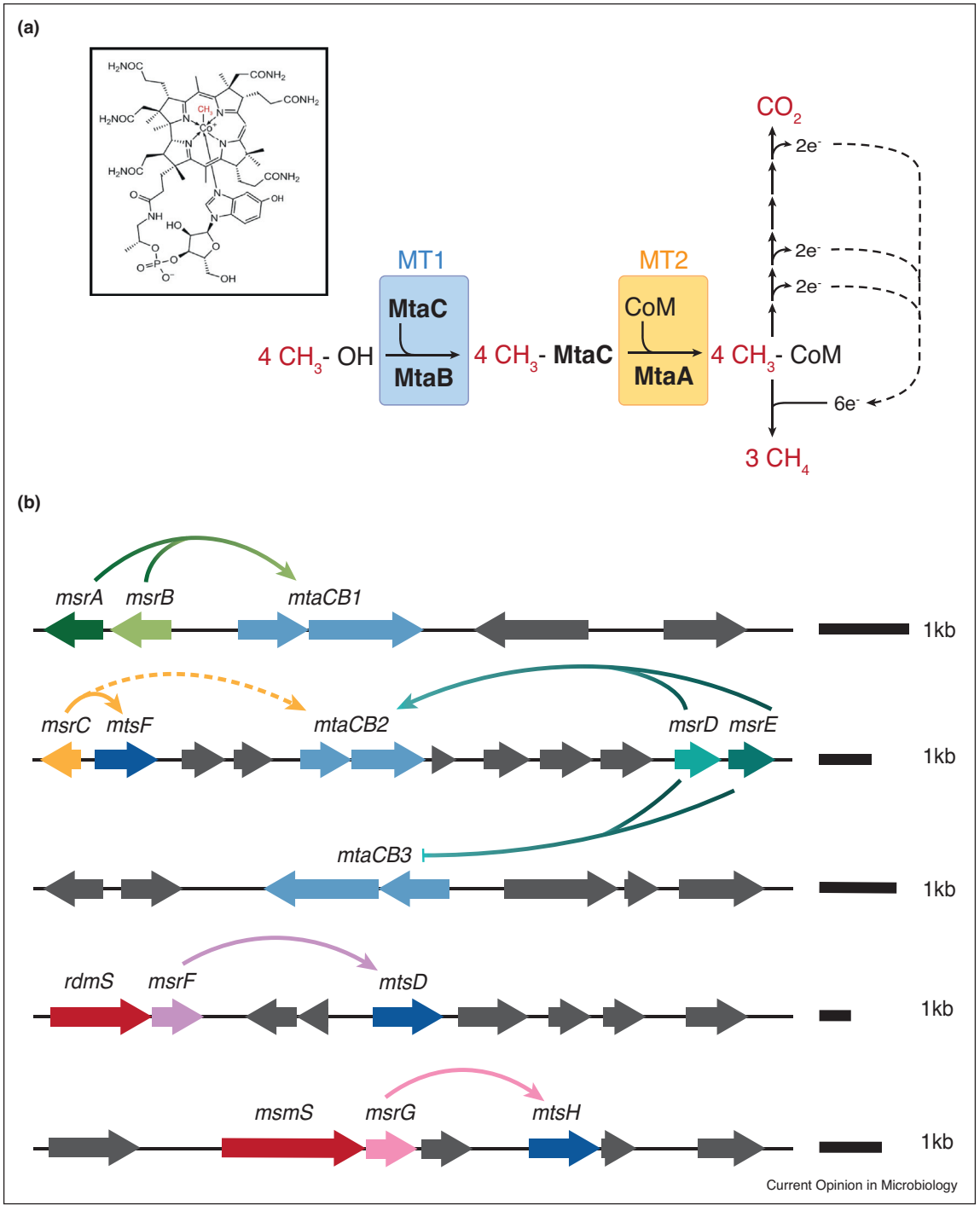
During growth on methylated compounds, also referred to as methylotrophic methanogenesis, 1/4th of the C_1 substrate is oxidized to CO_2 via a reversal of six steps of the core methanogenesis pathway and 3/4th is reduced to methane using the enzyme methyl coenzyme reductase (Figure 3a) [3,40]. During this process, the methyl group from the substrate enters core methanogenesis at the level of methyl-CoM via two stepwise methyl-transfer reactions mediated by two different methyltransferases, referred to as MT1 and MT2 (Figure 3a) [41]. The MT1 complex is substrate-specific and consists of a methyltransferase that transfers the methyl group to its associated corrinoid containing protein, whereas MT2 catalyzes the transfer of the methyl group from the corrinoid protein to CoM to form methyl-CoM (Figure 3a) [42–45]. For the purpose of this review, we will focus *only* on the regulation of MT1 and MT2 during methylotrophic methanogenesis.

Methanosarcina spp. often encode multiple copies of the substrate-specific MT1 that are dynamically regulated (Figure 3b) [18,46]. In *M. acetivorans*, the three copies of methanol-specific MT1 operons, *mtaCB1*, *mtaCB2*, and *mtaCB3* are differentially regulated with respect to changes in growth phase and substrate availability [38,39••]. Several members of the ArsR family of TFs that are in the genomic proximity of these operons have been designated as members of the Msr (**m**ethanol **s**pecific **r**egulator) family upon functional characterization (Figure 3b) [39••]. Msr proteins can both activate and repress the expression of *mtaCB* in *M. acetivorans*. Mutational analyses coupled to transcriptional fusions have revealed that MsrA and MsrB act as positive regulators of *mtaCB1* while MsrD and MsrE function in the dual activation and repression of *mtaCB2* and *mtaCB3*, respectively (Figure 3b) [39••]. This model of Msr-mediated regulation can be extended to a class of fused MT1/MT2 proteins (MtsD, MtsF, MtsH) that are required for the production of methylated sulfur compounds during growth on carbon monoxide, or for growth on methylated sulfur compounds [47,48]. Genetic studies have shown that MsrF, MsrC, and MsrG are required for the transcriptional activation of MtsD, MtsF, and MtsH respectively (Figure 3b) [49••]. While each of the Msr proteins contains a DNA-binding domain, whether these proteins directly bind to the promoter region of the corresponding MTs or interact with other TFs to regulate transcription is unknown and warrants further investigation [39••].

Recent work has identified two redox-active cytoplasmic sensor kinases that are likely involved in the regulation of MTs [50,51]. A methylsulfide-specific sensor, named MsmS, is encoded directly upstream of the regulator MsrG and undergoes autophosphorylation when the iron in its covalently bound heme group is oxidized from Fe (II) to Fe(III) (Figure 3b) [50]. Interestingly, a *ΔmsmS* knockout constitutively produces MtsF, indicating that MsmS may act as a negative effector of *mtsF* expression [50]. Similarly, another sensor kinase, RdmS, is autophosphorylated at a conserved tyrosine residue when intramolecular disulfide bonds between two cysteine residues are formed under oxidizing conditions [51]. These sensor kinases are promising models for understanding archaeal two-component systems, but whether they interact with Msr family regulators to fine-tune the expression of MTs remains unclear.

Beyond transcription initiation, regulation of methylotrophic methanogenesis also occurs at the post-transcriptional level. Curiously, when the *mtaCBA* genes are expressed from a tetracycline-inducible $P_{mcrB(tetO1)}$ promoter, RNA and protein levels can vary substantially (>10 fold) depending on the growth conditions [52]. This observation is supported by recent work identifying that

Figure 3



Methylotrophic Methanogenesis in *Methanosarcina acetivorans*. **(a)** A substrate-specific methyltransferase (MtaB) catalyzes a methyl-transfer reaction from methanol to a corrinoid-containing protein (MtaC). The heterodimer of MtaB and MtaC (in blue) is called the MTI. A second methyltransferase (MtaA2; in yellow) catalyzes a methyl-transfer reaction from methyl-MtaC to form methyl-coenzyme M (CoM), which is disproportionated in a 3:1 ratio to methane and CO_2 using the conserved seven-step methanogenesis pathway. The MtaC corrinoid cofactor, methylated 5-hydroxybenzimidazolylcobamide, is depicted in the inset above MT1. **(b)** Msr proteins regulate the expression of three different MtaCB operons in *M. acetivorans.* The dashed arrow indicates that MsrC is a weak activator of MtaCB2 compared to MsrDE.

global changes in mRNA half-life in *M. acetivorans* depend on its growth conditions [53], in addition to work characterizing small RNAs that regulate expression of MTs in *Methanosarcina mazei* [54]. However, a model describing the extent to which post-transcriptional regulation fine tunes gene expression and cellular protein levels has yet to be uncovered.

Acetoclastic methanogenesis

In *Methanosarcina* spp. growth on acetate proceeds through the dismutation of acetate to CO_2 and methane using two dedicated enzymes: acetate kinase (Ack) and phosphotransacetylase (Pta) [3,55]. First, acetate is activated to acetyl-CoA by Ack and Pta, after which it is split into enzyme-bound methyl and CO groups by acetyl-CoA decarbonylase/synthase (ACDS). The methyl group then enters the central pathway as methyl-tetrahydrosarcinopterin (methyl-H_4SPT), and the CO group is oxidized to CO_2 [3,55].

Mutational analyses coupled with RNA-seq have revealed that a TrmB family regulator, named MreA, plays a role in the dual activation and repression of acetoclastic and methylotrophic methanogenesis, respectively [56••]. RNA-seq analyses of the *ΔmreA* mutant revealed wholesale downregulation of genes required for acetoclastic methanogenesis with concurrent upregulation of MTs and the Msr family regulatory proteins involved in methylotrophic methanogenesis [56••]. Additionally, purified MreA has been shown to bind the promoter region of *pta* and *fpo* (F_{420} dehydrogenase); the former is essential to acetoclastic methanogenesis, while the latter is a membrane bound energy conservation complex upregulated during methylotrophic growth. Taken with the observation of a substantial growth defect in a *ΔmreA* mutant, these results indicate that MreA may play a role in the global regulation of methanogenesis [56••]. However, the underlying mechanism of MreA mediated regulation is yet to be characterized.

Conclusions and future directions

Methanogenesis is a widespread, environmentally relevant, and evolutionarily important metabolism. While there is considerable phenomenological evidence of regulation in methanogens, a clear picture of transcriptional and post-transcriptional regulatory processes in these pivotal organisms has yet to emerge. The work outlined in this review represents a foundation for future research threads that will shed light on how methanogens sense their environment and tune their metabolism in response. Recent methodological advances, such as genome-wide functional screens coupled with high-throughput global gene expression profiling, are likely to facilitate future efforts to map the complex regulatory network in methanogens. Furthermore, besides its myriad practical applications, studying the regulation of methanogenesis also presents an opportunity to deepen our fundamental understanding of gene regulation in the domain Archaea.

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Conflict of interest statement

Nothing declared.

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