**Enzymes methanogens have in common:**

* Mcr- methyl-coenzyme M reductase catalyzes final step of methanogenesis: CoM-CH3 to CH4
* Hdr- heterodisulfide reductase catalyzes the reduction of CoM-S-S-CoB to CoM-SH and CoB-SH. While all methanogens have Hdr, subunits vary. HdrABC are soluble and often bound to MvhAGD. HdrDE are membrane bound and are the final enzyme in the electron transport chain where the acceptor is heterodisulfide.

**Hydrogenotrophic**

*Redox of substrate(s):* donor- hydrogen, acceptor- CO2

*Energy conservation step(s):* (1) methyl-transfer reaction carried out by membrane bound mtr that translocates sodium across membrane leading to sodium motive force to generate ATP via ATP synthase

*Key genes (necessary for hydrogenotrophic- and some methylotrophic with hydrogen, see section below)*:

* F420-reducing hydrogenase- oxidizes dihydrogen to reduce F420 to F420H2 which is subsequently re-oxidized during reduction of carbon dioxide to methane (Frh/Fpo)
* Mvh hydrogenase (complexed with Hdr)- couples the oxidation of dihydrogen to the reduction of ferredoxin and the heterodisulfide CoM-S-S-CoB in a process called flavin-based electron bifurcation, reduced ferredoxin is required for first step of methanogenesis

*Other important processes that are either not unique or not required:*

* Formate dehydrogenase (Fdh, not required)- produces reduced coenzyme F420- many hydrogenotrophic methanogens can use formate in place of H2 to make methane from CO2; this enzyme can also be coupled to electron bifurcating enzyme complex to reduce heterodisulfide
* Mtr membrane bound methyltransferase (not unique to hydrogenotrophic but is required)- translocates sodium ions across the membrane leading to the buildup of a sodium motive force that is subsequently used by an ATP synthase
* For methanogens without cytochromes, Eha hydrogenase maybe vital for energy conservation

**Acetoclastic**

*Redox of substrate:* donor- carbonyl group of acetate, acceptor- methyl group of acetate

*Energy conservation step(s):* (1) Mtr membrane bound methyltransferase- translocates sodium ions across the membrane leading to the buildup of a sodium motive force that is subsequently used by an ATP synthase (2) membrane-bound electron transport chain utilization of reduced ferredoxin and heterodisulfide which both are produced during methanogenesis

*Key genes (unique to acetoclastic)*:

* acetyl-CoA decarbonylase/synthase (cdh CODH included)- dismutates acetyl-coA; carbonyl group is oxidized to carbon dioxide whereas the methyl group is funneled into the central methanogenic pathway to be reduced to methane

*Other important processes that are either not unique or not required:*

* Acetate transporter (not unique to acetoclastic but is required)- transports acetate into cell
* Activation of acetate to acetyl-CoA is carried out one of two ways and requires ATP (not unique to acetoclastic but is required):
  + Acetate kinase and (phospho)transacetylase
  + Acetyl-CoA synthetase
* Mtr membrane bound methyltransferase (not unique to acetoclastic but is required)- translocates sodium ions across the membrane leading to the buildup of a sodium motive force that is subsequently used by an ATP synthase
* Electron transport systems (not unique to acetoclastic but is required): Acetoclastic methanogens are operating at the lowest thermodynamic limits because acetate to methane is only -36kJ/mol and activation of acetate requires ATP investment, thus needs some energy-conserving systems. They have electron transport systems: Ech hydrogenase acts as primary proton pump when Fdred is formed from acetyl-CoA, forming H2 and translocating one proton across the cytoplasmic membrane. The hydrogen molecule diffuses out of the cell and is oxidized by the Mph-reducing hydrogenase (Vho) that transfers electrons to Mph and releases two protons to the extracellular side of the membrane. Then the heterodisulfide reductase as terminal enzyme of the anaerobic respiratory chain that also contributes to the electrochemical proton gradient by the transfer of 2H+/2e−. Rnf can operate in the same capacity as Ech.
  + Ech or Rnf
  + Mph-reducing hydrogenase (Vho/Fpo)

**Methylotrophic (does not require hydrogen)**

*Redox of substrate(s):* donor- 1 quarter of methyl groups are oxidized to CO2, acceptor- three quarters of the methyl groups are reduced to methane

*Energy conservation step(s):*

* Energy conservation only happens during membrane-bound electron transport

*Key genes (unique to methyltrophic- or methylotrophic with hydrogen, see section below)*:

* a substrate-specific methyltransferase (MT1) that transfers the methyl group to a corrinoid protein (see *Key genes for specific substrates* below for specifics*)*
* substrate specific corrinoid protein
* a second methyltransferase (MT2) funnels the methyl group into the methanogenic pathway at the stage of methyl-CoM
* *NOTE:* the membrane-bound methyltransferase operates in the reverse reaction, thereby dissipating the proton/sodium motive force- thus even though they may possess Mtr, it is not an energy conservation step as running in reverse direction (to CO2)

*Other important processes that are either not unique or not required:*

* Electron transport systems (not unique to methylotrophic but is required): Methyltorophic methanogens are operating at the lowest thermodynamic limits because acetate to methane is only -36kJ/mol and activation of acetate requires ATP investment, thus needs some energy-conserving systems. They have electron transport systems: Ech hydrogenase acts as primary proton pump when Fdred is formed from acetyl-CoA, forming H2 and translocating one proton across the cytoplasmic membrane. The hydrogen molecule diffuses out of the cell and is oxidized by the Mph-reducing hydrogenase (Vho) that transfers electrons to Mph and releases two protons to the extracellular side of the membrane. Then the heterodisulfide reductase as terminal enzyme of the anaerobic respiratory chain that also contributes to the electrochemical proton gradient by the transfer of 2H+/2e−. Rnf can operate in the same capacity as Ech.
  + Ech or Rnf
  + Mph-reducing hydrogenase (Vho/Fpo)
* Mtr membrane bound methyltransferase (not unique to methylotrophic but is required)-
* Multi-heme c-type cytochrome thought to enhance acetoclastic methanogenesis during redox cycling of nanoFe3O4

*Key genes for specific substrates:*

* Methyl groups bound to an N- For these genes, A is MT2, B is MT1, C is the corrinoid protein
  + Trimethylamine- MttBC, B contains pyrrolysine
  + Dimethylamine- MtbBC, B contains pyrrolysine
  + Monomethylamine- MtmBC, B contains pyrrolysine
  + Betaine- MtgBC
  + Proline-betaine- MtpBC
  + Carnitine- MtcBC
  + Choline- MthBC
  + Butyrobetaine- MtyBC
* Methyl groups bound to an S- these genes were largely not named to the nomenclature set by Krzycki and Thauer- and they are only two subunits instead of three like all the others
  + MtsD- MT1 with preferable substrate dimethylsufide
  + MtsF- MT1 with preferable substrate methanethiol
  + MtsH- MT1 for dimethylsulfide or methanethiol
  + MtpC- corrinoid protein for MMPA
  + MtpA- MT1 and MT2 for MMPA
  + THIS IS NOT THE SAME AS ALL THE OTHERS DEPSITE HAVING A AND B SUBUNITS: MtsB- corrinoid protein and MtsA corresponding methyltransferase – together these proteins catalyze a DMS:CoM methyltransferase reaction analogous to that catalyzed by the MtsA/B system of *M. barkeri*
* Methyl groups bound to an O
  + Methanol
    - MtaB- methanol specific MT1
    - MtaC- methanol specific corrinoid protein
    - MtaA- methanol specific MT2
  + Methoxylated compounds- this does not function like methyl-N. While the mechanism is not totally clear, it appears that methyls are transferred to H4MPT instead of CoM.
    - MtvB- MT1
    - MtrH- MTII
    - MtoC- methylated by MtoB
    - MtoB- transfers methyl group of methoxycompound to Co(1)-MtoC resulting in methylated Co(III)-mtoC (o-demethylase)
    - MtoA- transfers methyl group from methylated Co(III)-MtoC to H4MPT (hypothesized)

**Hydrogen-dependent methylotrophic**

*Redox of substrate(s):* donor- 1 quarter of methyl groups are oxidized to CO2, acceptor- three quarters of the methyl groups are reduced to methane

*Energy conservation step(s):*

* Energy conservation is dependent on the genes in the genome and is not as cut and dry as other types of methanogenesis- some have methanophenazine based electron transport systems while others encode an Fpo complex that translocates 4H+ across the membrane and electrons are channeled to HdrD to reduce heterodisulfide, while others remain unknown
* *NOTE:* the membrane-bound methyltransferase operates in the reverse reaction, thereby dissipating the proton/sodium motive force- thus even though they may possess Mtr, it is not an energy conservation step as running in reverse direction (to CO2)

*Key genes*:

* a substrate-specific methyltransferase that transfers the methyl group to a corrinoid protein (see *Key genes for specific substrates* above for specifics*)*
* substrate specific corrinoid protein
* a second methyltransferase funnels the methyl group into the methanogenic pathway at the stage of methyl-CoM/H4MPT
* Methanogens performing hydrogen dependent methylotrophic methanogenesis will lack or not express the upper part of methanogenesis (methyl-CoM oxidation to CO2)
* *NOTE:* methanogens that lack the upper part of methanogenesis (methyl-CoM oxidation to CO2) are obligately hydrogen dependent methylotrophs, meaning they do not oxidize methyl groups to CO2- this also means that they cannot live on only CO2/H2 or only methyl substrates

*Other important processes that are either not unique or not required:*

* These organisms require a way for hydrogen to be oxidized- some are membrane bound and reduce methanophenazine while others do not- below are examples including how the hydrogenases contribute to energy conservation:
  + Case one: Methanophenazine reducing hydrogenase (Vht)- oxidizes H2 with electrons being shuttled to Hdr via methanophenazine shuttle- both enzymes drive proton gradient for ATP synthase
  + Case two: a soluble hydrogenase/heterodisulfide reductase complex (MvhADG/HdrABC) is used to transfer the electrons resulting from H2 oxidation by MvhADG to the reduction of heterodisulfide and ferredoxin by HdrABC. The reduced ferredoxin then could be reoxidized by the membrane-bound Ehb complex resulting in a sodium motive force.
  + Case three: a Fpo-like complex interacts directly with subunit HdrD, forming an energy-converting ferredoxin:heterodisulfide oxidoreductase. The HdrABC/MvhADG complex catalyzes the H2-dependent reduction of heterodisulfide and the formation of reduced ferredoxin. The reduced ferredoxin is then oxidized by the ‘headless’ Fpo complex thereby translocating up to 4 H+ across the membrane and electrons are channeled to HdrD for reduction of the second heterodisulfide. F420:methanophenazine oxidoreductase (Fpo) lacks the F420-oxidizing subunit FpoF and lack the subunit HdrE of the membrane-bound heterodisulfide reductase HdrDE
  + Case four: membrane-bound hydrogenase (or formate dehydrogenase) acts as electron input module, and electrons are transferred to methanophenazine. The membrane-bound heterodisulfide reductase HdrDE acts as a terminal reductase and reduces the CoM-S-S-CoB heterodisulfide. Interestingly, this respiratory chain contains cytochromes

**Carboxydotrophic acetogenesis and methanogenesis on CO**

* Produces acetate and formate from carbon monoxide along with methane albeit at lower quantities
* Genes required are acetate kinase and phosphoacetyltransferase. CODH is contributes to the process but is not required and CmtA (MA4384) is a soluble CH3-tetrahydrosarcinapterin:HS-CoM methyltransferase postulated to supplement the membrane-bound CH3-tetrahydrosarcinapterin:HS-CoM methyltransferase during CO-dependent growth
* Process quantified and demonstrated in *M. acetivorans*

**Utilization of ethanol for methanogenesis**

* Grows with CO2 and ethanol as eletron donor
* Shown in *Methanomicrobiales*, *Methanofollis ethanolicus*
* encodes three sets of alcohol and aldehyde dehydrogenases (iron-dependent alcohol dehydrogenases: MEFOE\_RS00535, MEFOE\_RS00570, MEFOE\_RS02725; aldehyde dehydrogenases: MEFOE\_RS06760, MEFOE\_RS07165, MEFOE\_RS03840
* *Methanosarcina* and *Methanoculleus* encode homologs but have not been tested in lab

**Utilization of propanol/2-butanol**

* Secondary alcohols are oxidized to their ketones
* Iron-containing alcohol dehydrogenases are encoded in many of these strains including Methanomicrobiales, but whether this enzyme is responsible needs to be experimentally determined.

**Gibb’s free energy for different methanogenesis substrates from Kurth, et al.**

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