Published in final edited form as:

Environ Microbiol Rep. 2015 June ; 7(3): 414–419. doi:10.1111/1758-2229.12265.

Structural conservation of chemotaxis machinery across Archaea and Bacteria

Ariane Briegel[1], **Davi R. Ortega**[1], **Audrey Huang**[1], **Catherine M. Oikonomou**[1], **Robert P. Gunsalus**[2], and **Grant J. Jensen**[1,3,*]

[1] California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125

[2]University of California Los Angeles, 609 Charles E. Young Dr. S., Los Angeles, CA 90095

[3] Howard Hughes Medical Institute, 1200 E. California Blvd., Pasadena, CA 91125

Summary

Chemotaxis allows cells to sense and respond to their environment. In bacteria, stimuli are detected by arrays of chemoreceptors that relay the signal to a two-component regulatory system. These arrays take the form of highly stereotyped super-lattices comprising hexagonally packed trimers-of-receptor-dimers networked by rings of histidine kinase and coupling proteins. This structure is conserved across chemotactic bacteria, and between membrane-bound and cytoplasmic arrays, and gives rise to the highly cooperative, dynamic nature of the signaling system. The chemotaxis system, absent in eukaryotes, is also found in archaea, where its structural details remain uncharacterized. Here we provide evidence that the chemotaxis machinery was not present in the last archaeal common ancestor, but rather was introduced in one of the waves of lateral gene transfer that occurred after the branching of Eukaryota but before the diversification of Euryarchaeota. Unlike in Bacteria, the chemotaxis system then evolved largely vertically in Archaea, with very few subsequent successful lateral gene transfer events. By electron cryotomography (ECT), we find that the structure of both membrane-bound and cytoplasmic chemoreceptor arrays is conserved between Bacteria and Archaea, suggesting the fundamental importance of this signaling architecture across diverse prokaryotic lifestyles.

Introduction

Single-celled organisms rely on signal transduction pathways to sense and respond to their environments. In Bacteria, one such pathway, the chemotaxis system, relays information on the chemical environment to the flagellar motor to bias swimming direction. Roughly half of all bacteria are chemotactic (Wuichet and Zhulin, 2010). The chemotaxis system, typified by *Escherichia coli*, consists of chemoreceptors (methyl-accepting chemotaxis proteins, or MCPs) networked into cooperative arrays by coupling protein (CheW) and a two-component signaling kinase (CheA). Adaptation to stimulus is mediated by methylation and de-methylation of the receptors, performed by a methyltransferase (CheR) and methylesterase (CheB). CheB is one of the two response regulators controlled by CheA; the other is CheY, which, when phosphorylated, can bind to the flagellar motor, inducing a shift in its direction

*Correspondence: Tel (626) 395-8827, Fax (626) 395-5730, jensen@caltech.edu.

of rotation, and therefore the swimming behavior of the cell, inducing a “tumble” rather than a “run” (Hazelbauer et al., 2008).

In Bacteria, the structure of the chemosensory array is universal across all species imaged to date (Briegel et al., 2009). It is even conserved between membrane-bound and cytoplasmic arrays (Briegel et al., 2014).

Many archaea also contain chemotaxis genes. Decades of work have shown that the halophilic archaeon *Halobacterium salinarum* senses chemical attractants such as oxygen, as well as light, through a chemosensory system that, as in bacteria, translates into a change in direction of flagellar rotation. Interestingly, the archaeal flagellum, or archaellum, is not homologous to that of bacteria (Jarrell and Albers, 2012). Archaeal chemotaxis systems use an additional protein, CheF, to translate signal from CheY to the motor (Schlesner et al., 2009).

Evolutionary genomics has revealed that the majority of archaeal chemotaxis genes are found in Euryarchaeota and exhibit high sequence similarity to those found in Firmicutes and Thermotogales, suggesting that the system was acquired via lateral gene transfer (LGT) (Wuichet and Zhulin, 2010). However, recently sequenced genomes (Blainey et al., 2011; Spang et al., 2012) from the deeply branching Thaumarchaeota (Brochier-Armanet et al., 2008) also contain chemotaxis systems, raising the possibility of a chemotactic Last Archaeal Common Ancestor (LACA).

Here, we apply evolutionary genomics to distinguish between these hypotheses, and electron cryotomography (ECT) to investigate the structure of archaeal chemotaxis systems.

Results and Discussion

To identify chemotactic archaea, we selected 240 genomes spanning all major archaeal branches: Euryarchaeota (178), Crenarchaeota (51), Thaumarchaeota (9), Korarchaeota (1), and Nanoarchaeota (1). Using the MiST2 database (Ulrich and Zhulin, 2010), we determined that half of these genomes contained chemotaxis systems, defined as the presence of at least one CheA and one MCP. Chemotactic species all belonged to one of two archaeal phyla – Euryarchaeota (118) or Thaumarchaeota (2).

Chemotaxis systems are commonly classified on the basis of their signaling kinase, CheA (Wuichet and Zhulin, 2010). In Bacteria, there are more than a dozen such classes: multiple systems that signal to the flagellum (denoted F), another that signals to the Type IV pilus (Tfp), and one involved in alternate cellular functions (ACF). To classify the archaeal chemotaxis systems identified above, we examined the protein sequences of their associated CheA. There are 139 such CheA genes in the archaeal pangenome, representing only two classes: F1 (134) and ACF (5), consistent with previous work (Wuichet and Zhulin, 2010). ACF systems are found only in the Methanomicrobiales order of Archaea, and, based on sequence similarity (Supplemental Table 1), likely arose from LGT from Deltaproteobacteria.

In contrast to ACF systems, F1 systems are widespread across Euryarchaeota, supporting the idea that this system was present in their common ancestor. F1 systems are also found in Thaumarchaeota, a deeply branched phylum, raising the question of whether the LACA was chemotactic. To investigate this question, we used a concatenated alignment of CheA, CheB, and CheR to construct a phylogenetic tree of F1 systems in Bacteria and Archaea. In many genomes, there are multiple copies of these genes, making it difficult to unambiguously assign them to shared classes. Therefore, we chose only species that either contained all three genes in the same cluster or contained only a single copy of each gene of the F1 system. The final dataset contains 203 CheA:CheB:CheR concatenated sequences from 193 organisms: Euryarchaeota (82), Thaumarcheota (2), Firmicutes (93), Thermotogae (3), Synergistetes (3), Cyanobacteria (3), Chloroflexi (1), Planctomycetes (1), Actinobacteria (1), Chlorobi (2) and Nitrospirae (2). Multiple copies of these sequences in the same organism reflect gene duplication, revealed by alignment of CheA sequences by BLAST and comparison of domain architecture. The resulting tree is shown in Supplemental Figure 1. We find that the F1 chemotaxis system in Archaea is monophyletic. In general, the topology of our F1 chemotaxis tree recapitulates that of the organismal tree, with two notable exceptions, highlighted in Figure 1.

First, our results suggest that the F1 chemotaxis system was laterally transferred from the Euryarchaeota to the Thaumarchaeota sometime between the branching of the Methanococcales and the Archaeoglobales (Supplemental Figure 2), which rejects the idea of a chemotactic LACA. Rather, our data suggest that the ancestral archaeal chemotaxis system was laterally transferred from an ancestor of Bacteria before the divergence of the earliest branching bacterial lineages of Firmicutes, Thermotogales and Synergistetes

Our data also suggest more recent LGT events. We find that the chemotaxis system of Methanosarcinales appears to be misplaced in the CheA:CheB:CheR tree (Supplemental Figure 2). Further inspection shows that most Methanosarcinales have a version of the F1 chemotaxis system more similar to that of Clostridia than to any other archaeal clade, consistent with a relatively recent LGT, as has been suggested (Deppenmeier et al., 2002). Some Methanosarcinales (e.g. *Methanosarcina mazei*, *Methanosarcina acetivorans*) appear to have the vertically inherited system, while others (e.g. *Methanococcoides burtonii*) have the laterally transferred system.

Given the bacterial source of archaeal chemotaxis systems, we wanted to see whether their structure was conserved. Electron microscopy of Archaea has lagged behind that of Bacteria. Many species require complex media, extremes of temperature, or anaerobic conditions for growth. Halobacteria are relatively easy to culture but require high salt concentrations. High salt causes catastrophic bubbling even at low electron doses, preventing data collection by ECT, and cells often lose structural integrity upon removal of salt (Trachtenberg et al., 2000). Additionally, many archaeal cells are too large to allow adequate transmission of electrons. With these constraints in mind, we selected four archaeal species, representing three diverse orders of Euryarchaeota: Thermococcales (*Thermococcus kodakarensis*), Methanomicrobiales (*Methanoregula formicica*, *Methanosprillum hungatei*), and Halobacteriales (*Halobacterium salinarum*).

By ECT, we observed membrane-bound chemoreceptor arrays in *T. kodakarensis*, *M. hungatei*, and *H. salinarum* (Figure 2). The order and packing of the chemoreceptors was identical to that of bacteria, with 12 nm between the centers of adjacent hexamers of trimers-of-MCP-dimers. Interestingly, chemoreceptor arrays may have been observed previously in EM images of Archaea, but not identified as such. For instance, in *M. hungatei*, what are identified as cytoskeletal structures are likely chemoreceptor arrays (Toso et al., 2011). Similarly, work in *M. hungatei* and *H. salinarum* identified a “polar organelle,” which is likely the chemoreceptor array (Cruden et al., 1989; Metlina, 2004). The “polar organelle” was first described in *(Aqua)Spirillum serpens* in 1962 (Murray and Birch-Andersen, 1963). That study preceded Julius Adler's seminal 1966 paper on bacterial chemotaxis, which stimulated much work in the field (Adler, 1966). However, the “polar organelle” is described as a structure distinct from the chemoreceptor array as recently as 2001 (Lybarger and Maddock, 2001). Our results, however, lead us to believe that they are, in fact, the same structure (Supplemental Figure 3).

We also observe cytoplasmic chemoreceptor arrays in *M. formicicum* (Figure 3). Again, their structure is strikingly similar to that of bacterial cytoplasmic arrays: two hexagonally-packed lattices of trimers-of-MCP-dimers, presumably interacting at their ligand-binding tips, sandwiched between two CheA/CheW baseplates. As observed in the bacterium *Rhodobacter sphaeroides*, these arrays frequently curve, exhibiting both positive and negative curvature in opposing halves of the array (Briegel et al., 2014). The data from all four species are summarized in Supplemental Table 2.

Both cytoplasmic and membrane-bound arrays exhibited more variation in subcellular localization than their bacterial counterparts. Rather than clustering tightly at the cell pole, as in most chemotactic bacteria, archaeal arrays were frequently observed nearer to mid-cell.

The F1 chemotaxis system was likely transferred from the last bacterial common ancestor in one of the waves of LGT that gave rise to the diversification of Euryarchaeota more than 3.5 billion years ago (Battistuzzi et al., 2004; Nelson-Sathi et al., 2014). It has been suggested that these influxes of genes allowed metabolic divergence, and it may be that chemotaxis helped these diversifying organisms find and colonize new niches. That the transfer occurred after the branching of Eukaryotes from Archaea may explain why chemotaxis systems are not found in Eukaryota (Koretke et al., 2000). It is currently believed that there has been much less LGT to eukaryotes than between prokaryotes, and most of these events happened relatively recently (Keeling and Palmer, 2008). In fact, of the dozen or so subfamilies of two-component signaling systems, only one is found in Eukaryota (Wolanin et al., 2002). Bacteria evolved several classes of chemotaxis systems that were widely exchanged via LGT. In Archaea, by contrast, evolution was largely vertical, with successful LGT of the F1 chemotaxis system occurring maybe as few as three times. It is striking that this signaling architecture, developed in Bacteria, should remain unchanged over billions of years in Archaea as members of this lineage adapted to new lifestyles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Dr. Christine Moissl-Eichinger for sharing *Halobacterium salinarum* (DSM 3754). We thank Drs. Matthias Koch and Kristin Wuichet for discussions. This work was funded by the Department of Energy Biosciences Division award DE-FG02-O8ER64689 and the UCLA-DOE Institute of Genomics and Proteomics award DE-FC03-02ER6342 to R.P.G. and NIGMS award GM101425 to G.J.J.

References

Adler J. Chemotaxis in Bacteria. Science. 1966; 153:708–716. [PubMed: 4957395]

Agulleiro JI, Fernandez JJ. Fast tomographic reconstruction on multicore computers. Bioinformatics. 2011; 27:582–583. [PubMed: 21172911]

Atomi H, Fukui T, Kanai T, Morikawa M, Imanaka T. Description of Thermococcus kodakaraensis sp. nov., a well studied hyperthermophilic archaeon previously reported as Pyrococcus sp. KOD1. Archaea. 2004; 1:263–267. [PubMed: 15810436]

Battistuzzi FU, Feijao A, Hedges SB. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. BMC Evol Biol. 2004; 4:44. [PubMed: 15535883]

Blainey PC, Mosier AC, Potanina A, Francis CA, Quake SR. Genome of a low-salinity ammonia-oxidizing archaeon determined by single-cell and metagenomic analysis. PLoS One. 2011; 6:e16626. [PubMed: 21364937]

Briegel A, Ladinsky MS, Oikonomou C, Jones CW, Harris MJ, Fowler DJ, et al. Structure of bacterial cytoplasmic chemoreceptor arrays and implications for chemotactic signaling. Elife. 2014; 3:e02151. [PubMed: 24668172]

Briegel A, Ortega DR, Tocheva EI, Wuichet K, Li Z, Chen S, et al. Universal architecture of bacterial chemoreceptor arrays. Proc Natl Acad Sci U S A. 2009; 106:17181–17186. [PubMed: 19805102]

Brochier-Armanet C, Forterre P, Gribaldo S. Phylogeny and evolution of the Archaea: one hundred genomes later. Curr Opin Microbiol. 2011; 14:274–281. [PubMed: 21632276]

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P. Mesophilic Crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat Rev Microbiol. 2008; 6:245–252. [PubMed: 18274537]

Cruden D, Sparling R, Markovetz AJ. Isolation and Ultrastructure of the Flagella of Methanococcus thermolithotrophicus and Methanospirillum hungatei. Appl Environ Microb. 1989; 55:1414–1419.

Deppenmeier U, Johann A, Hartsch T, Merkl R, Schmitz RA, Martinez-Arias R, et al. The genome of Methanosarcina mazei: evidence for lateral gene transfer between bacteria and archaea. J Mol Microbiol Biotechnol. 2002; 4:453–461. [PubMed: 12125824]

Hazelbauer GL, Falke JJ, Parkinson JS. Bacterial chemoreceptors: high-performance signaling in networked arrays. Trends Biochem Sci. 2008; 33:9–19. [PubMed: 18165013]

Jarrell KF, Albers SV. The archaellum: an old motility structure with a new name. Trends Microbiol. 2012; 20:307–312. [PubMed: 22613456]

Keeling PJ, Palmer JD. Horizontal gene transfer in eukaryotic evolution. Nat Rev Genet. 2008; 9:605–618. [PubMed: 18591983]

Koretke KK, Lupas AN, Warren PV, Rosenberg M, Brown JR. Evolution of two-component signal transduction. Mol Biol Evol. 2000; 17:1956–1970. [PubMed: 11110912]

Kremer JR, Mastronarde DN, McIntosh JR. Computer visualization of three-dimensional data using Imod. J Struct Biol. 1996; 116:71–76. [PubMed: 8742726]

Lybarger SR, Maddock J. Polarity in Action: Asymmetric Protein Localization in Bacteria. J Bacteriol. 2001; 183:3261–3267. [PubMed: 11344132]

Metlina AL. Bacterial and archaeal flagella as prokaryotic motility organelles. Biochemistry (Mosc). 2004; 69:1203–1212. [PubMed: 15627373]

Murray RGE, Birch-Andersen A. Specialized Structure in the region of the flagella tuft in Spirillum serpens. Can J Microbiol. 1963; 9

Nelson-Sathi S, Sousa FL, Roettger M, Lozada-Chavez N, Thiergart T, Janssen A, et al. Origins of major archaeal clades correspond to gene acquisitions from bacteria. Nature. 2014

Nicastro D, Schwartz CL, Pierson J, Gaudette R, Porter ME, McIntosh JR. The molecular architecture of axonemes revealed by cryoelectron tomography. Science. 2006; 313:944–948. [PubMed: 16917055]

Oesterhelt D, Krippahl G. Phototrophic growth of halobacteria and its use for isolation of photosynthetically-deficient mutants. Ann Microbiol (Paris). 1983; 134B:137–150. [PubMed: 6638758]

Schlesner M, Miller A, Streif S, Staudinger WF, Muller J, Scheffer B, et al. Identification of Archaea-specific chemotaxis proteins which interact with the flagellar apparatus. BMC Microbiol. 2009; 9:56. [PubMed: 19291314]

Spang A, Poehlein A, Offre P, Zumbragel S, Haider S, Rychlik N, et al. The genome of the ammonia-oxidizing Candidatus Nitrososphaera gargensis: insights into metabolic versatility and environmental adaptations. Environ Microbiol. 2012; 14:3122–3145. [PubMed: 23057602]

Toso DB, Henstra AM, Gunsalus RP, Zhou ZH. Structural, mass and elemental analyses of storage granules in methanogenic archaeal cells. Environ Microbiol. 2011; 13:2587–2599. [PubMed: 21854518]

Trachtenberg S, Pinnick B, Kessel M. The cell surface glycoprotein layer of the extreme halophile Halobacterium salinarum and its relation to Haloferax volcanii: cryo-electron tomography of freeze-substituted cells and projection studies of negatively stained envelopes. J Struct Biol. 2000; 130:10–26. [PubMed: 10806087]

Ulrich LE, Zhulin IB. The MiST2 database: a comprehensive genomics resource on microbial signal transduction. Nucleic Acids Res. 2010; 38:D401–407. [PubMed: 19900966]

Wolanin PM, Thomason PA, Stock JB. Histidine protein kinases: key signal transducers outside the animal kingdom. Genome Biol. 2002; 3 REVIEWS3013.

Wuichet K, Zhulin IB. Origins and diversification of a complex signal transduction system in prokaryotes. Sci Signal. 2010; 3:ra. 50.

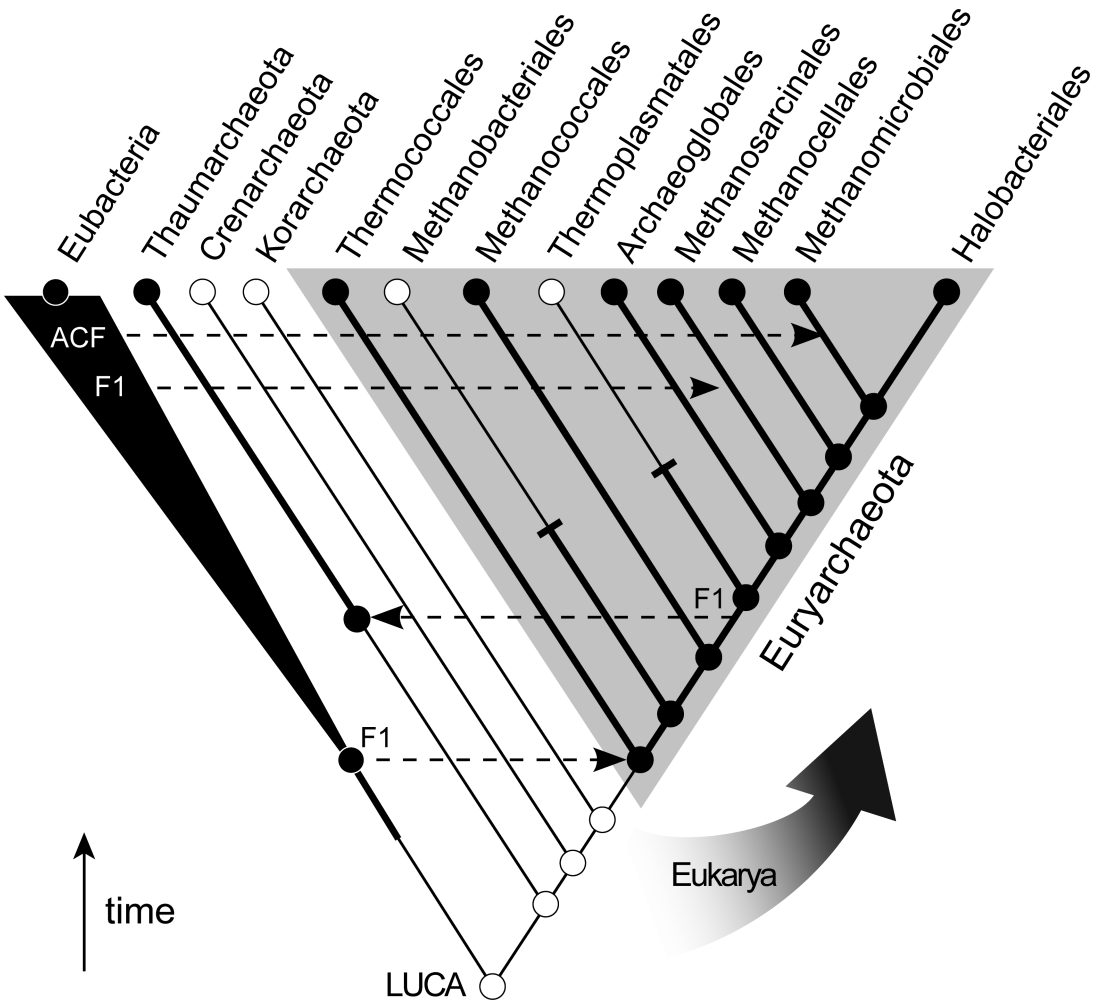

Figure 1. Mapping evolutionary events of the archaeal chemotaxis system onto the tree of life

Tree of life scheme is as in (Brochier-Armanet et al., 2011). Presence (thick lines) and absence (thin lines) of the F1 chemotaxis system are marked by black and white nodes, respectively. Loss events are marked by a bar across the branch. Dashed arrows denote LGT events of the indicated systems. The Euryarchaeota are shaded in grey. LUCA: Last Universal Common Ancestor.

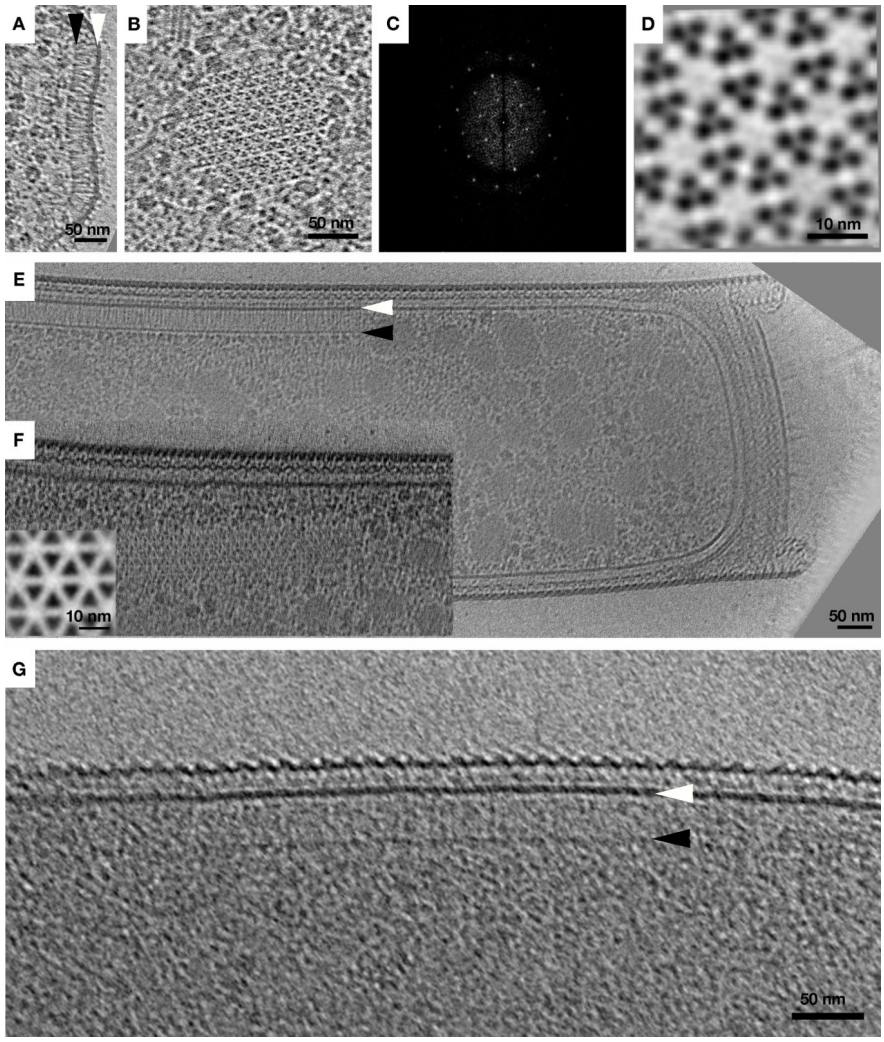

Figure 2. Membrane-bound chemoreceptor arrays in Archaea

T. kodakarensis (**A-D**) was grown anaerobically on elemental sulfur as previously described (Atomi et al., 2004). *M. hungatei* (**E-F**) was grown on hydrogen and carbon dioxide (Toso et al., 2011). *H. salinarum* (**G**) was grown aerobically in complex medium (Oesterhelt and Krippahl, 1983) at 37°C. *H. salinarum* cells were fixed with 2.5% glutaraldehyde then washed into low-salt buffer with 7% PEG-6000. Cells of all strains were mixed with fiducial markers, plunge-frozen on EM grids, and imaged by ECT. Images were aligned, CTF corrected, and reconstructed with IMOD (Kremer et al., 1996). SIRT reconstructions were calculated using TOMO3D (Agulleiro and Fernandez, 2011), and subvolume averages generated using PEET (Nicastro et al., 2006). (**A**, **E**, and **G**) show representative side views of membrane-bound arrays in *T. kodakarensis*, *M. hungatei*, and *H. salinarum*, respectively. (**B** and **F**) show top views, demonstrating the hexagonal packing of the trimers-of-MCP-dimers (power spectrum shown in **C** and subtomogram averages in **D** and inset). Scale bars 50 nm (A, B, E, F, G) or 10 nm (D, F inset); power spectrum (C) not to scale.

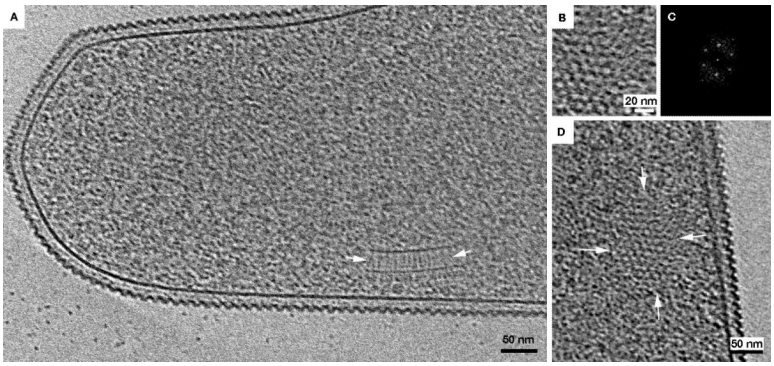

Figure 3. Cytoplasmic chemoreceptor arrays in *M. formicicum*

Live cultures of *M. formicicum* (DSM 22288) were purchased from the DSMZ and frozen on EM grids upon arrival. ECT data collection and image processing was performed as described in Figure 2. (**A**) shows a side view of a cytoplasmic array (white arrows). (**B**, associated power spectrum in **C**) and (**D**) show cross-sections through a top view of an array revealing hexagonal packing of two layers of trimers-of-MCP-dimers, sandwiched between CheA/CheW baseplates. Scale bars 50 nm (A, D) or 20 nm (B); power spectrum (C) not to scale.