Phylogenomics of the archaeal flagellum: rare horizontal gene transfer in a unique motility structure
Elie Desmond, Céline Brochier-Armanet, Simonetta Gribaldo

To cite this version:
Elie Desmond, Céline Brochier-Armanet, Simonetta Gribaldo. Phylogenomics of the archaeal flagellum: rare horizontal gene transfer in a unique motility structure. BMC Evolutionary Biology, BioMed Central, 2007, 7, pp.53-70. 10.1186/1471-2148-7-106 . hal-00698404

HAL Id: hal-00698404
https://hal.archives-ouvertes.fr/hal-00698404
Submitted on 7 Apr 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Phylogenomics of the archaeal flagellum: rare horizontal gene transfer in a unique motility structure

Elie Desmond¹, Celine Brochier-Armanet²,³ and Simonetta Gribaldo*¹

Address: ¹Unite Biologie Moléculaire du Gène chez les Extremophiles, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France, ²Université de Provence Aix-Marseille I, Marseille, France and ³Laboratoire de chimie bactérienne, Institut de Biologie Structurale et de Microbiologie (CNRS), Marseille, France

Email: Elie Desmond - edesmond@pasteur.fr; Celine Brochier-Armanet - celine.brochier@ibsm.cnrs-mrs.fr; Simonetta Gribaldo* - simo@pasteur.fr

* Corresponding author

Abstract

Background: As bacteria, motile archaeal species swim by means of rotating flagellum structures driven by a proton gradient force. Interestingly, experimental data have shown that the archaeal flagellum is non-homologous to the bacterial flagellum either in terms of overall structure, components and assembly. The growing number of complete archaeal genomes now permits to investigate the evolution of this unique motility system.

Results: We report here an exhaustive phylogenomic analysis of the components of the archaeal flagellum. In all complete archaeal genomes, the genes coding for flagellum components are co-localized in one or two well-conserved genomic clusters showing two different types of organizations. Despite their small size, these genes harbor a good phylogenetic signal that allows reconstruction of their evolutionary histories. These support a history of mainly vertical inheritance for the components of this unique motility system, and an interesting possible ancient horizontal gene transfer event (HGT) of a whole flagellum-coding gene cluster between Euryarchaeota and Crenarchaeota.

Conclusion: Our study is one of the few exhaustive phylogenomics analyses of a non-informational cell machinery from the third domain of life. We propose an evolutionary scenario for the evolution of the components of the archaeal flagellum. Moreover, we show that the components of the archaeal flagellar system have not been frequently transferred among archaeal species, indicating that gene fixation following HGT can also be rare for genes encoding components of large macromolecular complexes with a structural role.

Background

Motile archaeal species swim by means of rotating flagella structures driven by a proton gradient force [1,2], as in bacteria [3]. Interestingly, although they are both responsible for swimming, archaeal and bacterial flagella are not homologous, either in terms of overall structure, components and assembly (for a recent review see [4,5]). The bacterial flagellum is a complex rotary structure made up of as much as 20 proteins and composed of three major parts -the basal body, the hook, and the filament. Rotation is provided by an ATPase exploiting a proton gradient force, and can be switched by specific proteins in...
response to attractants or repellents in the environment through the chemotaxis system. The filament is a hollow structure about 20 nm in diameter and is composed of a single type of protein called flagellin. Bacterial flagellins are assembled by a complex type III secretion system located in the basal body and are added to the distal tip of the flagellum after passing through the hollow cavity [4].

Much less is known about the archaeal flagellum. It has been extensively studied in terms of components, assembly, and mutation experiments, in Halobacteria and Methanococcales (for recent reviews [4-6]). The archaeal flagellum is a structure thinner than its bacterial counterpart, where at least a filament and a hook are evident [7-9]. The archaeal flagellum has been shown to have a unique symmetry in *Halobacterium salinarum*. In fact, it has 3.3 subunits/turn of a 1.9 nm pitch left-handed helix compared to 5.5 subunits/turn of a 2.6 nm pitch right-handed helix for plain bacterial flagellum filaments [10,11]. The archaeal filament can be made up of different types of homologous flagellin proteins (called FlaA or FlaB). The filament is ~10 nm in diameter and is not hollow, resembling more to bacterial type IV pili in this respect [10]. A few other characteristics of archaeal flagella make them more alike bacterial pili than flagella: as bacterial pilins, archaeal flagellins (i) are made as preproteins with short signal peptides that are processed by a recently identified archaeal-specific signal peptidase (called FlaK) [12-14] that shows weak sequence similarity with the bacterial pili leader peptidase PilD, (ii) are likely added at the base of the filament as in bacterial pili, and (iii) undergo glycosylation as post-translational modification [15] (see [5] for a recent review). Moreover, one component of archaeal flagella (FlaI) is homologous to bacterial PilT, an ATPase involved in bacterial pilin export (a type II/IV secretion system) and pilus retraction during twitching motility [16]. However, none of the remaining archaeal flagellum components are homologous to those of bacterial pili [5]. Moreover, bacterial pili are not rotating structures, and no specific anchoring structures have ever been observed, indicating substantial differences between these two cellular structures.

A number of putative flagellum accessory genes lie close to flagellin genes in archaeal genomes (called flaC, flaD/ flaE, flaF, flaG, flaH, flaI, and flaJ) [5]. Their putative role in flagellum structure and assembly was tentatively deduced based on their sequences, cellular location, and mutation experiments [4-6].

FlaC, FlaD, FlaH and FlaI are associated with the membrane fraction in *Methanococcus voltae* and may thus be peripheral components of the archaeal flagellum [5,6]. FlaH, FlaI and FlaJ may be important for the assembly of archaeal flagella and possibly form a secretion complex [5,6]. FlaH harbors a domain similar to that found in bacterial RecA-like ATPases, and FlaH mutants are nonmotile and nonflagellated [17]. FlaI contains many transmembrane domains, while FlaI probably encodes an ATPase that may be important for flagellins export, similarly to the role of its bacterial homologue, the pilin export ATPase PilT, and/or for providing the force for rotation. No experimental data are presently available for FlaG and FlaJ, although FlaG may be a component of the anchoring system between the hook and the filament [6]. It may be possible that some of the multiple flagellin proteins have different roles in flagellum substructures other than the filament [6]. Finally, additional components of the archaeal flagellum may be encoded by genes that have not yet been identified.

The uniqueness of the archaeal flagellum in terms of components, structure, and assembly indicates that archaeal and bacterial flagella have distinct origins (i.e. they are analogous systems). Interestingly, homologues of most bacterial chemotaxis genes are found in archaeal genomes [5,18], suggesting that archaeal and bacterial chemotaxis systems are evolutionary related. However, their interaction with the flagellum system in Archaea remains largely unknown (for a recent review see [19]). In this work, we sought to contribute to the research on archaeal flagella and archaeal motility in general performing an accurate phylogenomic study (*sensu* Eisen [20]) of the archaeal motility apparatus in terms of taxonomic distribution of the genes coding for its components, their genomic context, and their phylogeny. This allowed us to sketch a fairly detailed image on the origin and evolution of this macromolecular structure.

**Results**

**Taxonomic distribution and genomic context**

The taxonomic distribution of the genes coding for archaeal flagellum components is congruent with that presented in a recent review [5] and is generally consistent with species descriptions [21]. Gene homologues for all components of the archaeal flagellum are found in the complete genomes of Archaea that are described as motile [5] (indicated by M and ● signs in Figure 1). Conversely, no homologues of genes coding archaeal flagellum proteins are found in the complete genomes of Archaea that are described as non motile [5] (indicated by NM and ○ signs in Figure 1). Although the representative of the *Methanosarcina* genus (i.e. *Methanosarcina mazei*, *Methanosarcina acetivorans* and *Methanosarcina barkeri*) are described as non-motile [21], at least a full complement of homologues of the genes coding for archaeal flagellum components is present in the complete genomes of these species [5,22] (Red rectangles in Figure 1). Conversely, no homologues of the genes coding for archaeal flagellum components are found in the complete genome of *Pyrob-
aculum aerophilum [5] (Yellow rectangle in Figure 1). This is surprising since the genus *Pyrobaculum* is described as “motile due to flagellation” in the Bergey’s manual and a picture is included of a “platinum shadowed cell showing flagella of *P. aerophilum*” [21]. Similarly, no homologues of the genes coding for archaean flagellum components are present in the complete genome of *Methanopyrus kandleri* [5] (Yellow rectangle in Figure 1), which is described as motile in the Bergey’s manual [21].

In all archaean genomes harboring flagellum components, the corresponding genes are always organized into one or two very well conserved clusters [5] (*fla* clusters, Figure 2). The only exception is the gene coding for the preflagellin peptidase FlaK, which is located close to the *fla* cluster in *Methanococcus jannaschii* only [5]. *flaK* homologues are nevertheless always present, at least in single copy at different locations in the other archaean genomes, to the notable exception of *Aeropyrum pernix*, *Thermoplasma acidophilum* and the Archaeoglobales. We verified that, Nevertheless, psi-Blast searches revealed that the genes lie in another region of the chromosome (two are clustered together and an additional small one is isolated (Figure 2B)), suggesting a disruption of the original cluster. This is also the case of the *fla1* cluster of *M. burtonii*, which presents no nearby flagellin genes (a single isolated *flaB* gene was possibly part of this cluster before disruption, Figure 2A and see below). In *S. solfataricus* a transposase disrupts the gene coding for FlaG (Figure 2B). However, both the N- and C-ter sequences of FlaG are still present in *S. solfataricus* (CheY, CheA, and CheD) and in *N. pharaonis* (CheY, CheC, and CheD). In this archaean, the operon is disrupted, the second half lying at ~50 ORFs downstream (Figure 2A). Interestingly, *M. maez* and *M. acetivorans* each harbor two copies of the *fla2* cluster (hereafter called *fla2A* and *fla2B*), that differ in the number of flagellin gene copies (two in *fla2A* and one in *fla2B*), and in the presence of two different hypothetical genes (possibly very distant homologues of flaD, see above) lying in between the genes coding for FlaB and FlaG (Figure 2B). According to these characteristics, the *A. fulgidus*, the *M. hungatei* and one of the *M. burtonii* gene clusters resemble more to the *fla2A* cluster, while that from *M. barkeri* resembles more to the *fla2B* cluster (Figure 2B).

A careful observation of gene order within each cluster revealed two types of organizations, that we will hereafter call *fla1* and *fla2* (Figure 2). *fla1* clusters are characterized by the presence of flaC, plus one or few copies of flaD (also annotated as flaE) or by a fusion of flaC and flaD (Figure 2A), whereas *fla2* clusters lack these genes (Figure 2B). Nevertheless, psi-Blast searches revealed that the genes (hyp1 and hyp2, Figure 2B) lying between flaB and flaG in *fla2* clusters from Methanomicrobia (Methanosarcinales and the Methanomicrobiaceae *Methanospirillum hungatei*) and Archaeoglobales may be a very distant homologue of PilD -the bacterial prepilin peptidase- that they may have recruited by horizontal gene transfer, and how they cope with the absence of this enzymatic activity (or which non-homologous enzyme performs the function) remains puzzling.

Desulfurococcales (Aeropyrum pernix) and Sulfolobales (Sulfolobus solfataricus Sulfolobus tokodaii Sulfolobus acidocaldarius) and in some Euryarchaeota: Methanomicrobia (*Methanospirillum hungatei*, Methanococoides burtonii, Methanosarcina acetivorans, Methanosarcina mazei and Methanosarcinaarkeri) and the Archaeoglobales *Archaeoglobus fulgidus* (Figure 2B). In Halobacteriales and Sulfolobales the clusters also include non-flagellum genes (Figure 2A). In particular, a gene coding for a homologue of a bacterial chemotaxis component (MCP domain signal transducer) is present in *Halobacterium* sp., and three genes coding for components of the chemotaxis system are present in *H. marismortui* (CheY, CheA, and CheD) and in *N. pharaonis* (CheY, CheC, and CheD). In this archaean, the operon is disrupted, the second half lying at ~50 ORFs downstream (Figure 2A). Interestingly, *M. maez* and *M. acetivorans* each harbor two copies of the *fla2* cluster (hereafter called *fla2A* and *fla2B*), that differ in the number of flagellin gene copies (two in *fla2A* and one in *fla2B*), and in the presence of two different hypothetical genes (possibly very distant homologues of flaD, see above) lying in between the genes coding for FlaB and FlaG (Figure 2B). According to these characteristics, the *A. fulgidus*, the *M. hungatei* and one of the *M. burtonii* gene clusters resemble more to the *fla2A* cluster, while that from *M. barkeri* resembles more to the *fla2B* cluster (Figure 2B). Multiple copies of flagellin genes (*flaB/flaA*) are found in most archaean genomes, especially in Thermococcales, whereas Thermoplasmatales and Sulfolobales harbor single gene copies (Figure 2), confirming earlier studies on the composition of the flagella from *T. volcanium* and *S. shibatae* [23]. In *M. hungatei*, the flagellin genes lie in another region of the chromosome (two are clustered together and an additional small one is isolated (Figure 2B)), suggesting a disruption of the original cluster. This is also the case of the *fla1* cluster of *M. burtonii*, which presents no nearby flagellin genes (a single isolated *flaB* gene was possibly part of this cluster before disruption, Figure 2A and see below). In *S. solfataricus* a transposase disrupts the gene coding for FlaG (Figure 2B). However, both the N- and C-ter sequences of FlaG are still very similar to FlaG homologues found in *S. solfataricus* close relatives (e.g. *S. acidocaldarius* and *S. tokodaii*), suggesting that the disruption of flaG is recent. Indeed, the sequenced genome of *S. solfataricus* presents indeed a high number of insertion elements that may have recently invaded this strain [24]. Interestingly, cells of this strain appear non-flagellated under the electron microscope (P. Redder, personal communication) even if the disruption of the flaG gene does not affect the transcription of the downstream operon genes [25]. This suggests that FlaG (possibly involved in the flagellum anchoring system [6]) is an essential flagellum component.
Finally, additional copies of flagellum components lie in a few instances outside of the clusters (examples are additional flaB genes in M. burtonii, the two Thermoplasmatales, H. marismortui and N. pharaonis; an additional flaG in Halobacterium sp.; and an additional flaD in H. marismortui, N. pharaonis, and M. burtonii; an additional flaF in M. maripaludis, and an additional flaK in Methanococcales) (Figure 2).

**Phylogenetic analysis**

Phylogenetic analyses were performed on six amino acid sequence datasets corresponding to FlaA/B, FlaD/E, FlaG, FlaH, FlaI, and FlaJ. Phylogenetic analysis of FlaC, FlaF and FlaK could not be performed due to a too restricted phylogenetic distribution of FlaC, and the poor sequence conservation of FlaF and FlaK.

**FlaG, FlaH, FlaI, FlaJ**

Among all archaeal flagellum components, FlaH, FlaI and FlaJ are the most conserved at the sequence level and always lie close to each other in all the analyzed genomes (Figure 2) strengthening their likely fundamental role in flagellum assembly and function (see above). FlaI has a number of bacterial homologues belonging to type IV and type II secretion systems, including the type IV pili component PilT [26]. Moreover, FlaI has additional archaeal homologues that are also probably part of yet to describe secretion machineries [27,28]. In a phylogeny including all these homologues FlaI sequences form a monophyletic group and are most closely related to their archaeal counterparts (not shown). FlaH shares a RecA-like ATPase domain with distant archaeal and bacterial homologues that are not involved in motility structures. Psi-blast
searches revealed (i) that FlAJ harbours a few distant archaeal homologues annotated as involved in type II secretion and (ii) weak similarities with the bacterial pilus assembly protein TadC and TadB.

FlAJ shares the domain GSPII F with TadC and TadB. However, this may not be significant, given that the domain was defined on the basis of an alignment that included both archaeal and bacterial sequences. The similarity between the FlAJ sequences and TadB and TadC sequences is very weak (16% and 35% of identity and similarity with TadB sequences, respectively and 14% and 32% of identity and similarity with TadC sequences, respectively) and is mainly the result of the sharing of small hydrophobic amino acids. To our point of view this sequence similarity is too weak to definitively conclude that these sequences are homologues although this has been claimed [27].

After removal of ambiguously aligned positions, 104, 193, 392 and 353 amino acids could be kept for phylogenetic analysis of FlA, FlH, FlA, and FlAJ, respectively. The resulting trees are strikingly congruent (Figure 3). Notably, all major archaeal groups except Methanomicrobia (Methanomicrobiales plus Methanosarcinales) are well defined and strongly supported statistically (Bootstrap Values -BV- > 990%) and/or Posterior Probabilities -PP- = 1), suggesting that no recent horizontal transfer of flAH, flAG, flAJ and flAL genes occurred across these groups.

Figure 2
Genomic organization of the genes coding for flagellum components in complete archaeal genomes (fla clusters). Numbers within brackets correspond to the locus tags of each gene. A // sign indicates that the following components are elsewhere in the hypothetical. A Genomic organization of type I clusters (flaI). These are found only in Euryarchaea and are characterized by the presence of flAC and flaDE and by a conserved gene order flaAB, flaC, flaD, flaF, flaG, flaH, flaI, flaJ. B Genomic organization of the type II clusters (fla2). These are found in Crenarchaea and in some Euryarchaea and are characterized by the absence of flAC and flaDE and by a conserved gene order flaAH, flaC, flaD, flaF, flaG, flaH, flaI, flaJ.
The sequences from Methanomicrobia form a well supported cluster (BV > 980‰ and/or Posterior Probabilities -PP- = 1) that also includes sequences from A. fulgidus. It is not possible for the time being to decide whether this is due to a HGT or a hidden paralogy. Importantly, the corresponding trees are also strongly consistent with gene cluster organization. In fact, homologues from fla1 and fla2 clusters (characterized by a flaF-flaG and by a flaG-flaF gene order, respectively) form two distinct strongly groups (BV > 975‰ and PP = 1, Figure 3). In particular, in all four trees, the homologues from the fla2 clusters of M. hungatei, the four Methanosarcinales and A. fulgidus appear close to Crenarchaeota (Figure 3). This is in contrast to their expected position as sister-group of Halobacteriales within Euryarchaeota (Figure 1, [29,30]). Interestingly, such expected position is shown by the M. burtonii sequences belonging to its fla1 cluster (Figure 2A).

This suggests that the fla1 and fla2 clusters from M. burtonii have different origins (see below). Independent species-specific duplications of flaG appear to have occurred in Halobacterium sp., N. pharaonis, and M. hungatei (and gene duplications in these last two species). Moreover, in the FlaH, Fla1 and Fla1 phylogenies, the sequences from M. burtonii, M. hungatei, M. barberi and A. fulgidus fla2 clusters with the sequences belonging to the fla2B cluster from M. mazei and M. aceticivorans (Figures 3B, 3C and 3D, respectively), supporting a close relationship of these clusters, as suggested by their gene organization (Figure 2B).

FlaD/E

As discussed above, homologues of FlaD/E genes are missing in all fla2 clusters from Crenarchaeota. In fla2 clusters from Methanomicrobia and A. fulgidus the two hypothetical proteins hyp1 and hyp2 could be distantly related to FlaD/E (Figure 2B). However they are too distant to be included in any phylogenetic analysis. The small number of unambiguously aligned positions (77 amino acids positions) that could be kept for phylogenetic analysis gives a poor resolution of the relationships between major euryarchaeal groups (Figure 4A). However, sequences from phyla form monophyletic groups generally well supported (BV = 996‰, PP = 0.93, BV = 1000‰ and BV = 958‰ for Halobacteriales, Methanosarcinales, Thermoplasmatales and Thermococcales, respectively, Figure 4A). This indicates that, similarly to FlaG, Fla1, Fla1, and FlaH, no recent HGT involving the flaD/E gene occurred among these groups. Interestingly, a tandem duplication event appears to have occurred in an ancestor of Methanococcales, with the two copies having been conserved within the cluster. Halobacteria also harbor two copies of flaD. One of the two copies is fused with flaC and always resides within the fla cluster, whereas the second copy resides within the fla cluster only in Halobacterium sp. This suggests that the fusion of flaC and flaD genes occurred before the divergence of the three Halobacterales, but after the duplication event and that one of the two copies was displaced after the duplication event in the ancestor of H. marismortui and N. pharaonis. A similar duplication of FlaD followed by a fusion of one of the two copies of FlaD and FlaC also appears to have occurred in M. burtonii. As in most Halobacterales one of the two copies resides outside the fla cluster (Figure 2A) suggesting its displacement after the duplication event. The poor resolution of the relationships between groups (due to the small number of positions kept for the phylogenetic analysis) does not permit to determine if a single fusion event of FlaC and FlaD occurred in ancestor of Halobacterales and Methanomicrobia or if this event occurred two times independently in both lineages.

FlaB

Given the use of only 72 unambiguously aligned positions for analysis, the FlaB tree presents a number of poorly resolved nodes (Figure 4B). Nevertheless, the monophyly of a number of groups is recovered and supported by BV > 850‰, except for Thermococcales, Methanococcales and Methanomicrobia. As for FlaG, Fla1, Fla1, and FlaH, the FlaB tree is again strongly consistent with gene cluster organization (Figure 4B). In particular, the FlaB from the fla2 clusters of M. hungatei, the four Methanosarcinales and A. fulgidus appear close to Crenarchaeota, while the isolated FlaB from M. burtonii appear close to Halobacteria, and thus likely functions with the flagellum components of fla1 cluster (Figure 4B). Interestingly, the multiple copies of flagellins group in a group-specific manner (Figure 4B), suggesting that flagellin gene family expansion occurred mainly by gene duplication and multiple times independently in different species, and not by HGT.

Discussion and conclusion

The archael flagellum is a complex cellular structure composed of multiple subunits and anchored to the membrane. The striking conservation of the genomic context of the genes coding for these subunits indicates a likely highly coordinated expression and assembly mechanisms. Coupled to the high sequence conservation of the different subunits across archael species inhabiting very different habitats, this underlines the importance for structural maintenance of the archael flagellum. However, we highlighted an important dimorphism of the genetic context organization, with two types of gene clusters harboring differences in both gene content and gene order (Figure 2). In fact, most Euryarchaeaa exhibit the fla1 cluster, whereas all the Crenarchaeae and some Euryarchaeae have the fla2 cluster (e.g. Methanomicrobia and A. fulgidus). The difference between the two clusters is strongly supported by phylogenetic analysis, and indicates that Methanomicrobia and A. fulgidus flagellum
components encoded by fla2 gene clusters are more closely related to their crenarchaeal homologues than to the homologues encoded by the fla1 gene clusters of their close relatives (i.e. M. burtonii, Thermoplasmatales and Halobacteriales, Figures 1, 3 and 4). Two different evolutionary scenarios can be proposed to explain our results. In the first scenario (Figure 5), the last ancestor of Archaea was flagellated and had the two types of clusters (i.e. both fla1 and fla2, blue and red clusters, respectively, Figure 5), resulting from the duplication of an ancestral cluster (purple cluster, Figure 5), and these were secondarily and differently lost during archael lineages evolution (seven losses of the fla1 cluster and nine losses of the fla2 cluster). Importantly, some of these losses would have also occurred recently in the Euryarchaeota (for example the loss of the fla1 cluster in the Methanosarcinaceae lineage would have occurred after the divergence of M. burtonii, that would have kept the two types of clusters). Finally, a duplication event of the whole fla2 cluster occurred in an ancestor of Methanosarcina (red circle, Figure 5) leading to the fla2B cluster (orange cluster, Figure 5). This first scenario involves 16 losses, and implies that the ancestor of Archaea and the ancestor of each euryarchaeal group had two types of flagella. Moreover, it does not explain the position of A. fulgidus sequences within the Methanomicrobia group and not as sister of this group, as generally indicated by molecular phylogeny of multiple markers (Figure 1 and 30). A second scenario involves less losses (three losses of the fla2 cluster and seven losses of the fla1 cluster) (Figure 6 and Figure 7 for a more detailed scenario). Here, the ancestor of Archaea was also flagellated, but had only a single type of fla cluster (either fla1, fla2, or else, purple cluster in Figure 6 and Figure 7). After the divergence of Crenarchaeota and Euryarchaeota (black circle,

Figure 3
Unrooted maximum likelihood phylogenetic trees of FlaG (A), FlaH (B), FlaI (C) and FlaJ (D). Numbers at nodes indicate bootstrap values for 1000 replicates of the original dataset and posterior probabilities, computed by PHYML and MrBayes, respectively. The scale bar represents the average number of substitutions per site. The phylogenies show a clear separation between the type I clusters (characterized by a FlaF FlaG order of genes) and type II clusters (characterized by a FlaG FlaF order of genes).
Figure 4

A. Unrooted maximum likelihood phylogenetic trees of FlaD/E. Numbers at nodes indicate bootstrap values for 1000 replicates of the original dataset and posterior probabilities, computed by PHYML and MrBayes, respectively. The scale bar represents the average number of substitutions per site. The light blue circles indicate duplication events. B. Unrooted maximum likelihood phylogenetic trees of FlaA/B. Numbers at nodes indicate bootstrap values for 1000 replicates of the original dataset and posterior probabilities, computed by PHYML and MrBayes, respectively. The scale bar represents the average number of substitutions per site. Detailed cluster organizations are not shown. White arrows are used to schematize the clusters. The phylogenies show a clear separation between the type I clusters (characterized by a FlaF FlaG order of genes) and type II clusters (characterized by a FlaG FlaF order of genes).

Figure 6 and Figure 7), evolution led to the fla1 and fla2 clusters. A single HGT of the fla2 cluster would have then occurred from some ancestors of Sulfolobales and Desulforococcales to an ancestor of Methanomicrobia (Figure 6 Figure 7, HGT 1), and was followed by a HGT from some ancestors of Methanosarcinales to A. fulgidus (Figure 6 Figure 7, HGT 2). Methanosarcina, M. hungatei and A. fulgidus would thus have lost their original fla1 gene cluster before or after their replacement by a fla2 gene cluster of crenarchaeal origin. We favor a HGT from Crenarchaeota to Methanomicrobia rather than the opposite, since in this case we would expect to find the M. burtonii fla1 genes more closely related to their paralogues belonging to the fla2 cluster than to their fla1 orthologues from Halobacteriales.

Both scenarios imply that the archaean flagellum would have appeared prior to the last archaean ancestor.

Apart these two likely cases of HGT of the entire fla2 gene cluster, we found no clear evidence for recent transfers of the genes coding for flagellum components among archaean species. Indeed, the poor resolution of interphyla relationships in some trees is more likely due to lack of phylogenetic signal rather than horizontal gene transfer. One explanation for the rarity of HGT is that it is pos-
Figure 5
An evolutionary scenario for the origin and evolution of the archaeal flagellum. Blue, red, orange and purple clusters represent fla1 cluster, fla2A cluster, fla2B cluster and their ancestor, respectively. The black circle represents the last common ancestor of Archaea. The green circle represents the duplication event that led to the fla1 and fla2 clusters. This duplication event occurred before the last common ancestor of Archaea, which thus harbored the two types of clusters. The red circle represents the recent duplication event of the fla2 cluster in ancestor of Methanosarcina. The blue and red crosses represent the loss of the fla1 and fla2A clusters, respectively.

Possible the result of the high level of integration of flagellum components within the macromolecular structure. Importantly, this contradicts the generally assumed notion that HGT of "informational" genes (i.e. those coding for proteins involved in the expression and the transmission of genetic information) are less frequent than those involving the remaining ones ("operational") genes. Nevertheless, the acquisition of a whole flagellum component coding gene cluster from distant donors seems possible. Interestingly, even if two clusters coexist within a genome (i.e. in *M. butronii*) neither gene recombination is observed between homologous genes belonging to the two clusters, nor losses, suggesting that the components of a cluster may interact preferentially due to coevolution, although they form similar cellular structures. The presence in *M. butronii* of two types of fla clusters (possibly one native and one acquired by HGT from crenarchaeota) represents an interesting experimental model to study. It would be in fact particular interesting to know the difference between the components encoded by the two fla clusters in terms of expression and assembly, and how two different flagellum systems coexist in this archaean.

Finally, it has been suggested that archaea are modified Actinobacteria and that archaeal flagella are derived from bacterial flagella following an adaptation to acidic environments by the recruitment of an already acid-stable glycoprotein from the pilus machinery that would have replaced the original flagellin while leaving intact the basal rotary motor [31]. We find this hypothesis unlikely for the fact that archaeal flagella do not resemble to bacterial flagella in major structure, assembly, and components, and not only concerning flagellin. Indeed, no even distant homologues to any component, including the basal rotary motor, of bacterial flagella can be recovered in archaeal genomes, including the related type III secretion system components [28]. Moreover, flagella of acidophilic bacteria (such as *Thiobacillus*) show a typical bacterial structure (e.g. a diameter of approximately 20 nm), so they adapted to acidic conditions without radically modi-
An evolutionary scenario for the origin an
d the evolution of the archaeal flagellum.
Blue, red, orange and purple clusters repre-
sent fla1 cluster, fla2A cluster, fla2B cluster and their ancestor, respectively. The black circle represents the last common
ancestor of Archaea. The red circle represents the recent duplication event of the fla2 cluster in ancestor of Methanosarcina.
The blue and red crosses represent the loss of the fla1 and fla2A clusters, respectively. The green arrows represent horizontal
gen transfers.

Figure 6

An evolutionary scenario for the origin and the evolution of the archaeal flagellum. Blue, red, orange and purple clusters rep-
resent fla1 cluster, fla2A cluster, fla2B cluster and their ancestor, respectively. The black circle represents the last common
ancestor of Archaea. The red circle represents the recent duplication event of the fla2 cluster in ancestor of Methanosarcina.
The blue and red crosses represent the loss of the fla1 and fla2A clusters, respectively. The green arrows represent horizontal
gen transfers.

fying their motility structures and components [28].
Indeed, the uncomplete genome of the extreme acidophilic bacterium (optimal pH<3) Acidobacterium capsulatum contains a clear homologue of bacterial flagellin, indicating that adaptation to acidic environments was possible without its replacement.

The lack of congruence between the description of archaeal species as motile or non-motile and the taxono-
mic distribution of homologues of flagellum compo-
ment coding genes[5] is particularly striking and underlines the need to explore more in depth the motility
systems in Archaea. The presence of two gene clusters in non motile Methanosarcinales is particularly puzzling.
Either these species can be flagellated under particular
conditions that have not yet been tested, or the flagellum
component homologues are involved in other functions
than motility (for example, they could be required for cell-
cell adhesion to form cell aggregates, a peculiarity of this
archaeal family). It will be extremely interesting to study
the expression and localization of the flagellum compo-
nents in Methanosarcinales, in the light of the fact that
flagellum genes of Methanosarcinales may have been
recruited from the distantly related Crenarchaeota, since
this event may have been an important step in the evolu-
tion of this archaeal lineage. Moreover, virtually nothing
is known about other types of motility than swimming in
archaea [28], while in bacteria these are starting being
investigated [4]. The fact that no homologues of flagellum
components are encoded in the genomes of at least two
archaeal species that are described as motile (M. kandleri
and P. aerophilum) is also puzzling. Although it is possible
that the strains used for genome sequencing have lost the
flagellum operon following lab cultivation (see for exam-
ple [32], it would surely be interesting to test motility in
these archaeal species. Alternatively, this observation
could also suggest that other types of motility may occur
in archaea and are made possible by still unknown mole-
cular structures. It would also be very useful to investigate
the relationship between the flagellum and the secretion
systems in Archaea. Indeed, archaeal genomes harbor only a few homologues of bacterial TypeII/IV secretion systems [28], and it is not known whether they form pili, despite rare observations [33-35] and evidence for conjugation [36-38].

To sum up, two radically different options for motility were adopted at the divergence of the two prokaryotic domains, and much still remain to be uncovered on archaeal motility systems.

**Methods**

**Data set construction**

The list of archaeal flagellum components was retrieved from the Kyoto Encyclopedia of Genes and Genomes [39]. Homologous sequences of each archaeal flagellum component were retrieved from the nr database at the National Center for Biotechnology Information [40] using theblastp program with different seeds [41] and the ALIBABA program (P. Lopez personal communication). For each dataset, additional searches using psi-Blast were performed to search for divergent homologues (especially from bacteria) [41]. tBlastN searches on the unfinished genomes of the Halobacteriaie Haloferax volcanii were performed at TIGR [42]. Multiple alignments were done with ClustalW [43] and MUSCLE [44]. For each dataset, the quality of the alignments obtained with CLUSTALW and MUSCLE, was evaluated with T-COFFEE (CLUSTALW and MUSCLE provided alignments of comparable quality, not shown) [45]. All the alignments were edited and manually improved using the ED program from the MUST package [46]. Regions where the homology between sites was

---

**Figure 7**

A detailed evolutionary scenario for the origin and the evolution of the archaeal flagellum based on figure 6. The purple cluster represents the ancestor of fla1 and fla2 clusters. The black circle represents the last common ancestor of Archaea. Blue arrows represent the recent duplication events. Black arrows indicate gene movements to different locations from their original positions in the cluster. Dark green { symbols indicate gene insertions within the clusters. The blue, red and black crosses represent the loss of the fla1 cluster, fla2A cluster, or of single genes, respectively. A red arrow indicates gene inversion. F indicates the fusion of FlaC and FlaD. The green arrows represent horizontal gene transfers.
doubtfully were manually removed from the datasets for phylogenetic analyses.

**Phylogenetic analysis**

Maximum Likelihood (ML) phylogenetic trees were computed with Phyml [47,48] and the JTT model of amino acid substitution (Jones, Taylor and Thornton [49]). A gamma correction with 4 discrete classes of sites was used to take into account the heterogeneity of evolutionary rates across sites. The alpha parameter and the proportion of invariant sites were estimated for each dataset. The robustness of each branch was estimated by non-parametric bootstrap analysis (1000 replicates) using PHYML. In addition, bayesian analyses were performed using MrBayes [50] with a mixed model of amino acid substitution. As for ML tree reconstruction, a gamma correction with 4 discrete classes of sites was used and the alpha parameter and the proportion of invariant sites were estimated. MrBayes was run with four chains for 1 million generations and trees were sampled every 100 generations. To construct the consensus tree, the first 1500 trees were discarded as "burnin".

**Genomic context analysis**

The genomic localization of each archaeal flagellum component was manually investigated in all archaeal complete genomes available at the NCBI.

**Authors' contributions**

SG conceived the study and supervised the analyses. ED carried out the analyses. CB and SG refined and completed the analyses and wrote the manuscript. All authors read and approved the final manuscript.

**References**

1. Alam M, Oesterhelt D: Morphology, function and isolation of halobacterial flagella. J Mol Biol 1984, 176(4):459-475.
2. Marwan W, Alam M, Oesterhelt D: Rotation and switching of the flagellar motor assembly in Halobacterium halobium. J Bacteriol 1991, 173(6):1971-1977.
3. Macab RM: The bacterial flagellum: reversible rotary propel- lor and type III export apparatus. J Bacteriol 1999, 181(23):7149-7153.
4. Bardy SL, Ng SY, Jarrell KF: Prokaryotic motility structures. Microbiology 2003, 149(Pt 2):295-304.
5. Ng SY, Chaban B, Jarrell KF: Archaeal flagella, bacterial flagella and type IV pilis: a comparison of genes and posttranslational modifications. J Mol Microbiol Biotechnol 2006, 11(3-5):167-191.
6. Thomas NA, Bardy SL, Jarrell KF: The archaeal flagellum: a different kind of prokaryotic motility structure. FEMS Microbiol Rev 2001, 25(2):147-174.
7. Faguy DM, Koval SF, Jarrell KF: Physical characterization of the flagella and flagellins from Methanospirillum hungatei. J Bacteriol 1994, 176(24):7491-7498.
8. Metlina AL: Bacterial and archaeal flagella as prokaryotic motility organelles. Biochemistry (Moscow) 2004, 69(11):1203-1212.
9. Cruden D, Sparling R, Markovetz AJ: Isolation and Ultrastructure of the Flagella of Methanococcus thermolithotrophicus and Methanospirillum hungatei. Appl Environ Microbiol 1989, 55(6):1414-1419.
10. Cohen-Krausz S, Trachtenberg S: The structure of the archaea- phage flagellar filament of the extreme halophile Halo- bacterium salinarum R1M1 and its relation to eubacterial flagellar filaments and type IV pilis. J Mol Biol 2002, 321(3):383-395.
11. Trachtenberg S, Cohen-Krausz S: The archaeabacterial flagellar filament: a bacterial propeller with a pilus-like structure. J Mol Microbiol Biotechnol 2006, 11(3-5):208-220.
12. Bardy SL, Jarrell KF: FlaK of the archaeon Methanococcus maripaludis possesses prefagellin peptide activity. FEMS Micro- Biol Lett 2002, 208(1):53-59.
13. Bardy SL, Jarrell KF: Cleavage of prefagellins by an aspartic acid signal peptide is essential for flagellation in the archaeon Methanococcus voltae. Mol Microbiol 2003, 50(4):1339-1347.
14. Albers SV, Szabo Z, Driessen AJ: Archaeal homolog of bacterial type IV prepilin signal peptides with broad substrate spe- cificity. J Bacteriol 2003, 185(13):3918-3925.
15. Wieland F, Paul G, Sumpner M: Halobacterial flagellins are sul- fated glycoproteins. J Biol Chem 1985, 260(28):15180-15185.
16. Mattick JS: Type IV pil and twitching motility. Annu Rev Microbiol 2002, 56:289-314.
17. Thomas NA, Pawson CT, Jarrell KF: Insertional inactivation of the flaH gene in the archaeon Methanococcus voltae results in non-flagellated cells. Mol Genet Genomics 2001, 265(4):596-603.
18. Redder P, Garrett RA: Mutations and rearrangements in the genome of Sulfolobus solfataricus. Mol Biol Evol 1996, 13(8):902-905.
19. Szurmant H, Ordal GW: Diversity in chemotaxis mechanisms among the bacteria and archaebacteria. Microbiol Mol Biol Rev 2004, 68(2):301-319.
20. Eisen JA: A phylogenomic study of the MutS family of pro- teins. Nucleic Acids Res 1998, 26(18):4291-4300.
21. Garrity GM: Bergey’s Manual of Systematic Bacteriology. 2nd edition. Edited by: Garrity GM, New York , Springer-Verlag; 2001.
22. Glaugan JE, Nusbaum CG, Roy A, Endrizzi MG, Rondonal P, FitzHugh W, Calvo S, Engels R, Smirnov S, Atoono D, Brown A, Allen N, Naylor J, Stange-Thomann N, DeArellano K, Johnson R, Linton L, McEwan P, McKernan K, Talanaz J, Tilurre A, Ye W, Zimmer A, Barber RD, Cannon I, Graham DE, Grameade AM, Hedderich R, Ingram-Smith C, Kuetterer HC, Krazycki JA, Leigh JA, Li W, Liu J, Multielphadphy A, Reeeje JN, Smith K, Springer TA, Unayam LA, White O, White RH, Conway de Macario E, Ferry JR, Jarrell KF, Jing H, Macario AJ, Paulsen I, Pritchett M, Sowers KR, Swanson RV, Zinder SH, Lander E, Metchall WW, Birren B: The genome of M. acetivorans reveals extensive metabolic and physiological diversity. Genome Res 2002, 12(4):532-542.
23. Faguy DM, Bayley DP, Kostykova AS, Thomas NA, Jarrell KF: Isolation and characterization of flagella and flagelin proteins from the Thermocloidophilic archaea Thermoplasma volca- nium and Sulfofobulob shaibatae. J Bacteriol 1996, 178(3):902-905.
24. Redder P, Garrett RA: Mutations and rearrangements in the genome of Sulfolobus solfataricus P2. J Bacteriol 2006, 188(12):4198-4206.
25. Albers SV, Driessen AJ: Analysis of ATPases of putative secre- tion operons in the thermocloidophilic archaeon Thermoplasma volca- nium and Sulfofobulob shaibatae. J Bacteriol 1996, 178(3):902-905.
26. Redder P, Garrett RA: Mutations and rearrangements in the genome of Sulfolobus solfataricus P2. J Bacteriol 2006, 188(12):4198-4206.
27. Albers SV, Driessen AJ: Analysis of ATPases of putative secre- tion operons in the thermocloidophilic archaeon Thermoplasma volca- nium and Sulfofobulob shaibatae. J Bacteriol 1996, 178(3):902-905.
32. Labes A, Schonheit P: Sugar utilization in the hyperthermophilic, sulfate-reducing archaean Archaeoglobus fulgidus strain 7324: starch degradation to acetate and CO2 via a modified Embden-Meyerhof pathway and acetyl-CoA synthetase (ADP-forming). Arch Microbiol 2001, 176(5):329-338.

33. Leadbetter JR, Breznak JA: Physiological ecology of Methanobrevibacter cutularis sp. nov. and Methanobrevibacter curvatus sp. nov., isolated from the hindgut of the termite Reticulitermes flavipes. Appl Environ Microbiol 1996, 62(10):3620-3631.

34. Miroshnichenko ML, Gongadze GM, Rainey FA, Kostyukova AS, Lysenko AM, Chernykh NA, Bonch-Osmolovskaya EA: Thermococcus gorgonarius sp. nov. and Thermococcus pacificus sp. nov.: Heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. Int J Syst Bacteriol 1998, 48 Pt 1:23-29.

35. Weiss RL: Attachment of bacteria to sulfur in extreme environments. J Gen Microbiol 1973, 77:501-507.

36. Prangishvili D, Albers SV, Holz I, Arnold HP, Stedman K, Klein T, Singh H, Hiort J, Schweier A, Kristjansson JK, Zillig W: Conjugation in archaea: frequent occurrence of conjugal plasmids in Sulfolobus. Plasmid 1998, 40(3):190-202.

37. Miroshnichenko ML, Gongadze GM, Rainey FA, Kostyukova AS, Lysenko AM, Chernykh NA, Bonch-Osmolovskaya EA: Thermococcus gorgonarius sp. nov. and Thermococcus pacificus sp. nov.: Heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. Int J Syst Bacteriol 1998, 48 Pt 1:23-29.

38. KEGG: [http://www.genome.jp/kegg/](http://www.genome.jp/kegg/).

40. NCBI: [http://www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/).

41. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997, 25(17):3389-3402.

46. Philippe H: MUST, a computer package of Management Utilities for Sequences and Trees. Nucleic Acids Res 1993, 21(22):5264-5272.

48. Guindon S, Gascuel O: PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res 2005, 33(Web Server issue):W557-9.

49. Jones DT, Taylor WR, Thornton JM: The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 1992, 8(3):275-282.

50. Huelsenbeck JP, Ronquist F: MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 2001, 17(8):754-755.