Review

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Twitch or swim: towards the understanding of prokaryotic motion based on the type IV pilus blueprint

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Abstract: Bacteria and archaea are evolutionarily distinct prokaryotes that diverged from a common ancestor billions of years ago. However, both bacteria and archaea assemble long, helical protein filaments on their surface through a machinery that is conserved at its core. In both domains of life, the filaments are required for a diverse array of important cellular processes including cell motility, adhesion, communication and biofilm formation. In this review, we highlight the recent structures of both the type IV pilus machinery and the archaellum determined *in situ*. We describe the current level of functional understanding and discuss how this relates to the pressures facing bacteria and archaea throughout evolution.

Keywords: archaellum; cryoEM; flagellum; structure; twitching motility; type IV pili.

Introduction

For more than 2 billion years, early prokaryotes were the only life form on earth. These tiny single cells most likely lived in the water and were immotile, using only currents to transport them throughout primordial ponds and oceans. Development of cell motility can be thought of as the keystone of evolution, enabling cells to actively migrate to new environments and establish ecological niches in which they could thrive and proliferate, leading to the cellular diversity that we know today.

Most prokaryotes produce a type of cell surface appendage, or filament, that acts to propel them in a specific direction. The power for movement can be thought of as being driven by a type of molecular engine, using a fuel source such as an ion gradient or ATP, which is converted into mechanical force. Such molecular engines are highly complex, being comprised of many different protein components (membrane bound and soluble forms) present in varying stoichiometries (Maier and Wong, 2015; Albers and Jarrell, 2018).

There are two domains of prokaryotes, the bacteria and the archaea, with both unique and unifying properties. The cells are similar in basic morphology and function, yet at the level of gene transcription and translation, archaea possess features which are more typical of eukaryotes (Eme et al., 2017). Both bacteria and archaea appear to have developed motility devices based on an ancestrally conserved filamentous prototype. This machinery, in bacteria named the type IV pilus (T4P) (Maier and Wong, 2015) and in archaea called the archaellum (Albers and Jarrell, 2018) has diversified in a way that can provide clues about the different constraints and opportunities facing the two domains of life throughout their evolution.

One key difference in the way that bacteria and archaea move is in the type of motion generated. The most well-studied form of prokaryotic motility stems from the bacterial flagellum (Imada, 2017; Terashima et al., 2017; Khan and Scholey, 2018), which is used primarily for swimming in aqueous environments. It is a massive structure, powered by an ion gradient, that acts as a rotary propeller to drive directional movement (Imada, 2017; Terashima et al., 2017; Khan and Scholey, 2018). In terms of mechanics, this type of motility would not be particularly well suited for movement along surfaces, which a large proportion of bacteria colonise. In this case, bacteria use the T4P to 'walk' in a type of jerky motion referred to as twitching (Maier and Wong, 2015). This involves the rapid extension of the filament from the cell body, adherence of the tip to a

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surface, and then rapid retraction from the base, ensuring that the cell is pulled forwards in the direction of filament assembly. Archaea have been less well studied to date, and the only appendage used for motility that has been identified so far is the archaellum, which amalgamates features from both types of bacterial motile filaments (Albers and Jarrell, 2018). Superficially, the archaellum functions as a propeller-type driven machine like the flagellum, but on a structural level is much more similar to the T4P (Albers and Jarrell, 2018). In this review, we explore the building blocks of T4P and archaella, and relate this to their function and evolution from a common ancestor.

The filaments and their protomers

Both the T4P and the archaellum are filaments comprised of thousands of copies of individual protomers (pilins or archaellins), arranged in a helical formation (Figure 1). These extend from the cell up to several micrometres in length and are predominantly found in polar regions (Bardy et al., 2002; Nudleman et al., 2006; Gold et al., 2015; Daum et al., 2017) (Figure 1), presumably ensuring co-ordinated and directional movement. Pilins and archaellins are synthesised typically as membrane-integral preproteins that contain a conserved class III signal sequence. The sequences are usually positively charged oligopeptides that terminate in lysine-glycine residues, and are followed by a hydrophobic stretch of ~20 amino acids (Albers et al., 2003). Signal peptides are cleaved by a dedicated type IV prepilin peptidase, PilD in bacteria (Nunn and Lory, 1991) and FlaK/PibD in archaea (Albers et al., 2003; Bardy and Jarrell, 2003) (Figure 1). Bacterial PilD is bifunctional, both cleaving and N-methylating type IV pilin precursors (Strom et al., 1993) whereas the archaeal homologue PibD lacks the methylation domain (Szabó et al., 2006).

Pilins and archaellins share a characteristic 'lollipop' shape consisting of a hydrophobic N-terminal kinked α-helix followed by a C-terminal β-strand rich globular domain (Figure 2) (Braun et al., 2016; Kolappan et al., 2016; Poweleit et al., 2016; Daum et al., 2017; Wang et al., 2017). Differences in the sizes of individual monomers lead to variations in the filament packing and diameters of the assembled filaments (Braun et al., 2016; Kolappan et al., 2016; Poweleit et al., 2016; Daum et al., 2017; Wang et al., 2017). The central core of the filament is formed from bundles of the N-terminal α-helices of individual monomers (Figure 2). This α-helix is conserved between bacterial and archaeal species, highlighting the importance of the region in biosynthesis, assembly and stability. Diversification of function appears to arise predominantly from differences in the C-terminal domain, which is exposed on the filament surface. For example, bacterial pilins possess a small number of known sites for post-translational modification, and archaellins have several (Craig et al., 2006; Ng et al., 2006). In the bacterium *Neisseria gonorrhoeae*, the major pilin subunit PilE undergoes phospho-modifications with phosphoethanolamine and phosphocholine at a specific residue, as well as glycosylation at a second site (Hegge et al., 2004). Studies have revealed a high degree of conservation, with similar post-translational modifications also observed in other *Neisseria* strains (Stimson et al., 1995; Craig et al., 2006). Archaella from *Pyrococcus furiosus*, possess five N-linked glycosylation sites in each $FlaB_0$ archaellin (Daum et al., 2017), while the *Methanospirillum hungatei* filament possesses six O-linked and two N-linked glycans per subunit (Poweleit et al., 2016).

The physiological role of post-translational modification is not entirely clear, but it is likely that it provides a flexible means of expanding small genomes by generating diversity. For example, the presence of saccharides is thought to increase the adhesive properties of proteins (Lis and Sharon, 1993). In archaea, glycosylated archaella mediate cell-surface adhesion as well as contacts between neighbouring cells (Näther et al., 2006), a key process in biofilm formation for both types of prokaryote. Surface glycans that possess species-specific sequences also act as recognition tags for cell-cell communication in archaea (Näther et al., 2006). Glycosylation of bacterial pilins has been shown to increase virulence by increasing resistance of *Pseudomonas aeruginosa* to phagocytosis (Tan et al., 2015). Glycosylation-defective mutants of both bacteria and archaea are impaired in filament assembly and possess reduced motility (Smedley et al., 2005; Chaban et al., 2006), and bacteria become more sensitive to pilus-specific bacteriophages (Harvey et al., 2017). In extremophiles, surface glycans also increase protein robustness under harsh environmental conditions, likely due to the formation of additional hydrogen bonds (Lis and Sharon, 1993). Diversity within the C-terminal domain is therefore widespread between different species of both bacteria and archaea, likely as a result of different evolutionary pressures owing to their individual habitats and functional requirements.

Interestingly, T4P and archaella do not appear to be exclusively comprised of one type of protomer. Bacteria and archaea can possess multiple pilin/archaellin-like proteins within their genome, often encoded in the same operon. However, the filaments seem to be comprised of one major form: PilA/PilE in bacteria (Gold and

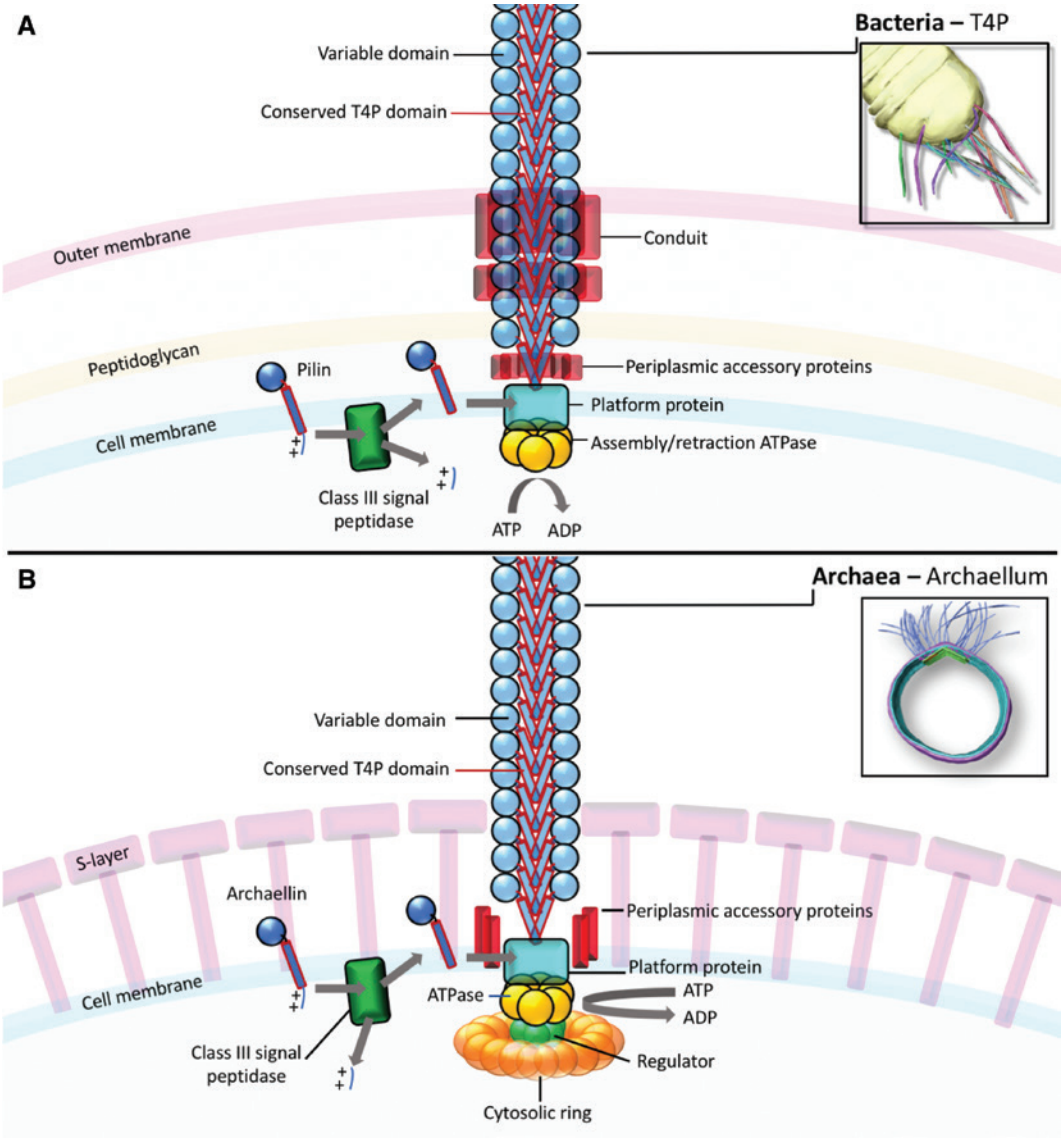

Figure 1: Schematic comparison of the T4P and archaellum machineries.
Schematic views of (A) the T4P and (B) the archaellum machinery highlights similarities (outlined in black and red) and differences (not outlined) in protein composition. Both filaments (blue) consist of lollipop-shaped subunits. Each subunit harbours a conserved hydrophobic N-terminal α-helical segment (red outline) and a hydrophilic globular β-strand-rich C-terminal domain (black outline). Pilins and archaellins are expressed as transmembrane proteins. Removal of the conserved positively charged signal peptide by a class III signal peptidase (green) primes the protomers for filament assembly. Assembly is supported by a cell membrane integral platform protein (cyan) and powered by ATP hydrolysis through a soluble cytosolic ATPase (yellow). The two machineries differ with respect to accessory proteins. Periplasmic passage of the bacterial T4P is regulated by a massive outer-membrane bound conduit called PilQ (transparent red) in Gram-negative bacteria. Such a conduit is missing in single-membrane bound archaea. Passage of the archaellum may be aided by the small membrane-bound periplasmic proteins FlaF and FlaG (red) and requires local disassembly of the S-layer (transparent pink). While T4P use distinct ATPases for filament assembly and retraction, archaea employ one bifunctional enzyme. Switch between archaellum assembly and rotation is thought to be performed by the regulator FlaH (light green). A cytosolic ring surrounding the archaellum motor complex (orange) (FlaX in crenarchaeota and likely FlaC, FlaD, FlaE in euryarchaeota) may act as a stator or transmit signals from chemoreceptors. Such a cytosolic ring is not found in the T4P machinery. Boxed inserts; tomographic surface representations of (A) the bacterium *T. thermophilus* (Gold et al., 2015) and (B) the archaeon *P. furiosus* (Daum et al., 2017).

Kudryashev, 2016), and members of the FlaB family in archaea, such as $FlaB_0$ in *P. furiosus* (Daum et al., 2017) and $FlaB_3$ in *M. hungatei* (Poweleit et al., 2016). T4P can also be classified into T4aP and T4bP subgroups, based on their major pilin protein (in particular the length of leader peptide) and the organisation of their pilus genes (Pelicic,

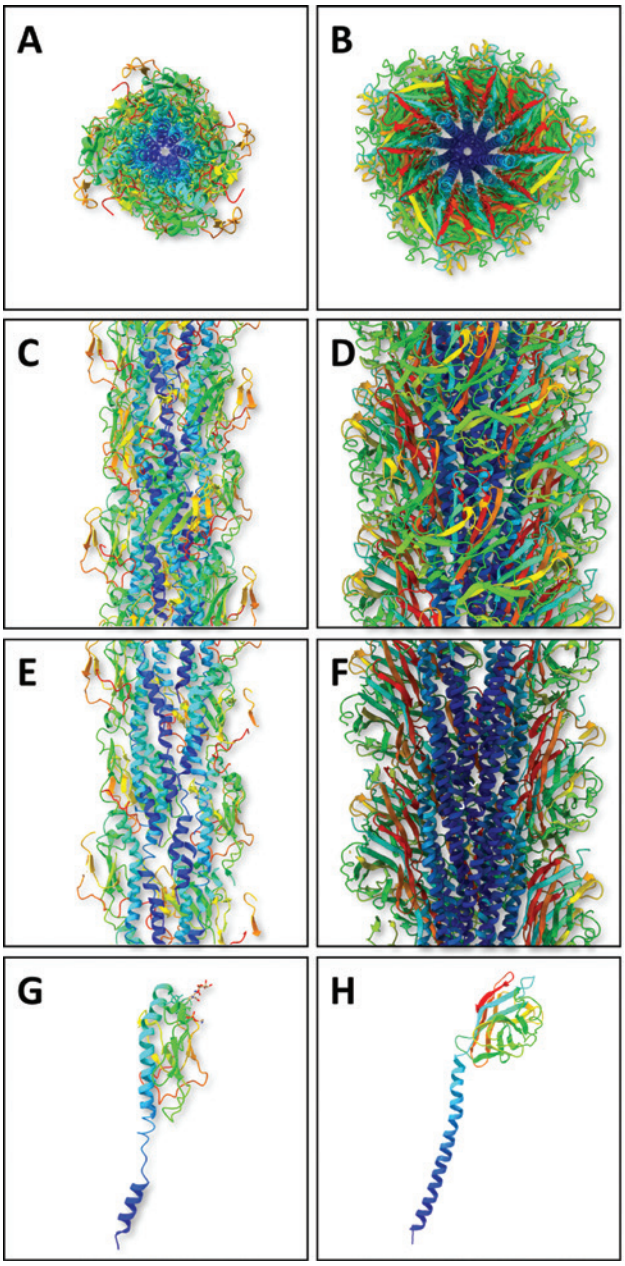

Figure 2: Atomic models of the T4P and archaellum filaments. Atomic model of the *N. gonorrhoeae* T4P (PDB-5VXX; Wang et al., 2017) in (A) top view, (C) side view and (E) cross section. (G) The PilE protomer. Post-translational modifications are shown in ball and stick representation. Atomic model of the *P. furiosus* archaellum (PDB-5O4U; Daum et al., 2017) in (B) top view, (D) side view and (F) cross section. (H) The $FlaB_0$ protomer. Each protomer is depicted in rainbow colours, from the N-terminus (blue) to the C-terminus (red).

2008). In haloarchaea, different archaellins are expressed under different environmental conditions (Syutkin et al., 2014), suggesting that distinct filaments may exist. Questions remain as to whether the minor pilins/archaellins are found at specific or sporadic locations along the filament. Perhaps they can form cap structures at the filament termini, are important in providing flexibility or strength, or can form different filaments entirely.

Filament assembly and force generation

Both T4P and archaella must cross cell envelopes to span the distance from the site of assembly at the cytoplasmic membrane to the cell exterior. Most archaea are surrounded by only one membrane and a proteinaceous cell wall called an S-layer, whereas bacteria can be either single- (Gram-positive) or double- (Gram-negative) membrane bound, and in some cases may also assemble an S-layer (Figure 1). The conserved N-terminal parts of the pilin/archaellin protomers act initially as transmembrane segments prior to assembly, and are subsequently integrated into the hydrophobic core of the growing filament. It has been hypothesised that assembly is facilitated by the polytopic membrane integral assembly platform protein PilC in bacteria and the homologous protein FlaJ in archaea (Friedrich et al., 2002; Karuppiah et al., 2010; Albers and Jarrell, 2018). PilC/FlaJ are thought to assemble individual pilin/archaellin monomers into the filament, which are concomitantly pushed out of the membrane (Figure 1).

The mechanical energy for the assembly process is delivered by cytosolic ATPases with RecA-fold domain homology. Bacteria use distinct ATPases for filament assembly (PilF/PilB) and disassembly (PilT/PilU) (Salzer et al., 2014), whereas archaea possess a single ATPase, FlaI (Reindl et al., 2013). The hexameric ATPases are predicted to interact with PilC (bacteria) or FlaJ (archaea) (Reindl et al., 2013; Albers and Jarrell, 2018), and has been demonstrated in the bacterium *Myxococcus xanthus* (Bischof et al., 2016). ATP hydrolysis likely results in conformational changes in the motor proteins, which are transduced into PilC/FlaJ to generate the growing filament (Figure 1) (Reindl et al., 2013; Albers and Jarrell, 2018).

The precise mechanism in which individual protomers assemble into filaments is not known, but different models have been proposed for bacterial T4P. Based on a known helical structure of the filament, the original rotary model suggests either a rotation of the point of subunit insertion or a rotation of the filament itself (Mattick, 2002). In a subsequent 3-start model, pilin subunits in the inner membrane are added to three active sites around the filament circumference, which does not require rotation of the growing filament upon addition of each new subunit (Craig et al., 2006). More recent spooling-rotational models now combine rotation of the ATPase with

a progressive twisting and tightening up of the filament as subunits are added (Nivaskumar et al., 2014; McCallum et al., 2017); the filament itself would not rotate in this case. The latter hypothesis appears more energetically favourable than a 3-start model, and also is consistent with the observation that bacteria tethered by their T4P do not rotate (Sun et al., 2000), unlike archaea tethered by archaella (Kinosita et al., 2016).

The function of bacterial T4P and archaella is dependent on a switching mechanism that alters the machinery from an 'assembly mode' to a 'force-generating mode'. In twitching bacteria, the T4P machinery toggles between filament assembly and retraction by exchanging the assembly ATPase (PilF/PilB) for the retraction ATPase (PilT/PilU) (Salzer et al., 2014). Although the precise mechanism is unknown, it is plausible that sensing surface attachment can trigger a conformational change in the filament, which ripples back to the cell to activate retraction (Ghosh et al., 2014). As the archaellum machinery uses only one ATPase (FlaI), a molecular 'gear stick' needs to be in place that stops filament assembly at a defined length and modulates the ATPase to power only filament gyration. The switch between archaellum assembly and rotation is thought to be regulated by the soluble protein FlaH, another RecA-fold protein that binds FlaI in an ATP-dependent manner (Chaudhury et al., 2016; Meshcheryakov and Wolf, 2016). Whilst the exact nature of this switch is so far unclear, homology between FlaH and the circadian clock protein KaiC (Meshcheryakov and Wolf, 2016) may suggest a time-dependent autophosphorylation mechanism.

Conduits and accessory proteins

Operons that encode T4P and archaella contain additional genes for proteins that have regulatory roles, for

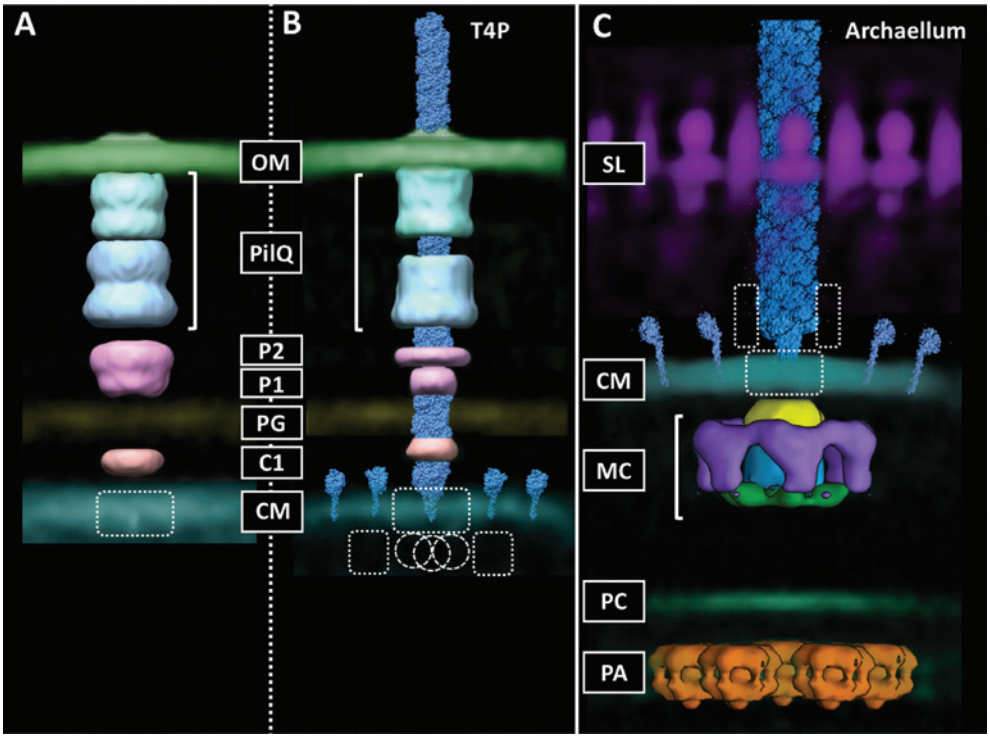

Figure 3: Composite models of the T4P and archaellum machineries.
Composite models of the T4P machinery from *T. thermophilus* in the (A) closed state (EMD-3021; Gold et al., 2015) without assembled pilus and the (B) open state with pilus assembled (EMD-3023; Gold et al., 2015). The helical reconstruction of the *N. gonorrhoeae* T4P filament is docked into the open state of the PilQ channel (PDB-5VXX; Wang et al., 2017). Unknown protein densities (C1, P1 and P2) are probably comprised of the proteins PilN, PilO and PilW, central components of the T4P machinery in *Thermus*. The dashed rectangle indicates the position of the inner membrane protein PilC, which may link to the ATPases PilF/PilT1/PilT2 (dashed circles) via PilM in the cytoplasm (dashed squares). (C) Composite model of the archaellum machinery (motor complex, EMD-3759; polar cap protein array, EMD-3760 and helical reconstruction of the filament, PDB-5O4U) (Daum et al., 2017). Dashed lines indicate proposed positions of accessory proteins FlaJ (horizontal rectangle), FlaF and FlaG (vertical rectangles) that were not resolved. Abbreviations are: OM, outer membrane; P1, central periplasmic ring 1; P2, central periplasmic ring 2; PG, peptidoglycan; C1, proximal to the cytoplasmic membrane; CM, cell membrane; SL, S-layer; MC, motor complex; PC, polar cap; PA, protein array of unknown identity.

example in guiding the filament across the periplasm. A number of essential proteins have been identified in the periplasm of both Gram-negative and archaeal machineries. In Gram-negative bacteria, by far the largest component of the T4P machinery consists of a multimeric protein complex called PilQ (Figures 1A, 3A and B), which is embedded in the outer membrane and projects into the periplasm. The *Thermus thermophilus* major pilin PilA4 was no longer found in the outer membrane in a PilQ mutant, supporting the suggestion that PilQ guides the pilus across the periplasm (Rumszauer et al., 2006). Structures of two T4aP (Gold et al., 2015; Chang et al., 2016) machineries obtained *in situ* show PilQ to be gated and highly dynamic, with the length of PilQ correlating to the width of the bacterial periplasm. An additional protein TsaP is associated with PilQ in some species, suggested to anchor PilQ to the peptidoglycan layer (Siewering et al., 2014). PilW (named PilF in *P. aeruginosa,* Tgl in *M. xanthus*) is a predicted lipoprotein, essential for the outer membrane localisation of PilQ (Rumszauer et al., 2006; Koo et al., 2008).

The proteins PilM, PilN, PilO, and in some species additionally PilP, form an alignment subcomplex, bridging PilQ and the motor ATPases in the cytoplasm (Hospenthal et al., 2017). PilN and PilO are embedded in the cytoplasmic membrane, connecting PilM to the membrane-associated lipoprotein PilP, which in turn interacts with PilQ. Proposed functions of the alignment subcomplex include providing a connection between PilQ and the cytoplasmic ATPases, concentrating pilins at the appropriate site, and also transducing signals from the motor proteins to open or close the channel (Tammam et al., 2011, 2013).

PilQ-like conduits do not exist in archaea studied to date, including the species *P. furiosus* (Daum et al., 2017) and *Thermococcus kodakarensis* (Briegel et al., 2017), which are both surrounded by a single membrane and an S-layer (Figures 1B and 3C). Instead of a conduit through the periplasm, the archaella traverse a gap in the S-layer (Daum et al., 2017), which may be the result of mechanical replacement or proteolytic degradation during filament assembly.

Guidance of the filament towards the S-layer in archaea is thought to be provided by the proteins FlaF and FlaG (Banerjee et al., 2015) (Figure 1B). These proteins are homologous to bacterial pilins, possessing an N-terminal T4P-like signal peptide followed by a hydrophobic α-helical domain, as well as a β strand-rich C-terminus. However, instead of forming filaments, the proteins may form a ring-shaped complex on the extracellular surface of the cell membrane, which could integrate the archaellum into the S-layer (Banerjee et al., 2015).

Archaellum operons also encode accessory proteins with putative regulatory roles. Euryarchaeota express the proteins FlaC, FlaD and FlaE, which have been suggested to form a cytosolic ring around a central complex formed of FlaJ, FlaI and the cytosolic regulator FlaH (Figures 1B and 3C) (Daum et al., 2017). FlaC, FlaD and FlaE have been shown to interact with chemoreceptors via adaptor proteins (Schlesner et al., 2009), suggesting that they convey chemotropic signals to the motor complex. Interestingly, these cytosolic rings co-localise with a structure called the polar cap, which has been proposed to act as an organising centre for archaellar bundles (Briegel et al., 2017; Daum et al., 2017) (Figure 3C). In crenarchaeota such as *Sulfolobus acidocaldarius*, the FlaJ/I/H core is surrounded by a membrane-anchored oligomeric ring of FlaX proteins, which has been suggested to form the static part for archaellum force generation and recruitment of FlaB into the filament (Banerjee et al., 2012).

We have only recently begun to understand some of the functions of these accessory proteins, and hence many questions remain open. For example, T4P have only been identified in Gram-positive bacteria in the last few years (Piepenbrink and Sundberg, 2016) and thus their function and components are less well understood. Presumably mechanisms must exist to guide filaments through the thick peptidoglycan layer, perhaps in a similar manner to the archaellum traversing the S-layer. Similarly, whilst double membrane bound archaea such as *Ignicoccus hospitalis* also possess T4P-like filaments (Braun et al., 2016), the structure or molecular composition of their assembly machinery is unknown. It is conceivable that complexes analogous to bacterial PilQ may exist, which allow the filaments to cross the outer cell membrane. Although bacterial twitching motion is clearly directional (Burrows, 2012), we are only just beginning to reveal how targeted twitching motion is controlled.

To twitch versus swim

The mechanical response of a single bacterial pilus, in terms of tensile strength, elongation and retractive force has been well studied (Merz et al., 2000; Maier et al., 2002, 2004; Biais et al., 2010). Less is understood about the coordination of multiple filaments, which appears to be species-dependent. In rod-shaped bacteria such as *Thermus* and *Myxococcus*, filaments have been shown to assemble at one cell pole predominantly (Bulyha et al., 2009; Gold et al., 2015), clearly allowing for directed twitching motility. In *Myxococcus*, the core assembly machinery was

shown to form at both cell poles, but the motor ATPases would oscillate with PilB at the leading and PilT at the lagging end (Bulyha et al., 2009). In coccoid bacteria such as *Neisseria*, cells are piliated uniformly and appear to move in a random walk with directional memory (Marathe et al., 2014). In the coccoid cyanobacterium *Synechocystis* however, the PilB ATPase was shown to concentrate in crescents adjacent to the plasma membrane, correlating strongly with the direction of twitching motility (Schuergers et al., 2015). In line with this, the archaeal cytosolic ring (putatively comprised of FlaC, FlaD and FlaE) is juxtaposed to the crescent-shaped polar cap organising centre (Briegel et al., 2017; Daum et al., 2017).

It is not yet clear which factors control the assembly of filaments at a particular region of the cell to allow for directional changes in motility. Clues come from a recent study of *Synechocystis* bacteria, where local differences in light intensity were shown to induce asymmetric activation of the T4P machinery (Nakane and Nishizaka, 2017). In archaea, FlaC, FlaD and FlaE have been shown to interact with chemoreceptors via adaptor proteins (Schlesner et al., 2009) in a potentially similar manner, suggesting that they convey chemotropic signals to the motor complex. It seems plausible that directional movement can also be controlled by taxis signals in bacteria (Black et al., 2006), and indeed T4P function in *P. aeruginosa* is controlled in part by the Chp chemosensory signal transduction pathway, activated in this case by mechanical cues (Persat et al., 2015).

In both swimming and twitching motility, directionality is thus provided by the localisation of protein components, which likely rearrange in response to specific environmental signals. In swimming, movement is controlled by the speed of rotation of the ATP-driven propeller-like filament. The gyration of the *Halobacterium salinarium* archaellum was measured at 23 ± 5 Hz with a motor torque at 50 pN nm (Kinosita et al., 2016). Rotating archaella were observed to pause intermittently at ~36° or 60°, which likely represents individual ATP hydrolysis steps and suggests that 10 or six ATP molecules are consumed per revolution (Kinosita et al., 2016). In bacterial twitching however, three phases appear to be involved: T4P assembly, surface adhesion and finally retraction, coordinated by the two different motor ATPases. A 100 pN force is generated from a single T4P retraction event, which is sufficiently large to drive the cell forwards (Maier et al., 2002). It is thought that individual T4P can retract independently (Skerker and Berg, 2001), but a co-ordinated effort generates forces 10 times stronger (Biais et al., 2008). In *N. gonorrhoeae*, the speed of retraction has been shown to correlate to the removal of ~1500 subunits per second (Merz et al., 2000), and is dependent on oxygen concentration and proton motive force (Kurre and Maier, 2012; Kurre et al., 2013). Similarly, multiple archaella per cell will likely enhance swimming speed and efficiency of movement. Whilst the polar cap has been suggested to establish this coordination (Daum et al., 2017), details on the dynamic orchestrated action of multiple archaella and their consequence on cell propulsion remain to be explored.

The summation of many homologous and some unique proteins into different structures thus results in two related systems that function in very different ways. T4P and archaella show extraordinary conservation on the level of sequence and structure, including the overall 'lollipop'-like shape of the pilin/archaellin protomers, their amphipathic surfaces and the way in which they are processed and organised within the assembled filaments (Giltner et al., 2012; Poweleit et al., 2016; Daum et al., 2017). The highest degree of conservation is found in the N-terminal α-helix, which is essential for filament assembly. On the other hand, the surface-exposed β-strand-rich domain is highly variable and gives each filament its specificity with regards to environmental adaption and functional needs.

The ubiquity and conservation of pilins and archaellins highlights their evolutionary success in terms of forming stable, yet flexible and versatile filaments. The presence of this blueprint in both domains of life also raises the intriguing hypothesis that a common progenitor protein and filament existed in the last universal common ancestor (LUCA), before the two domains of life diverged more than 3 billion years ago (Makarova et al., 2016). Whilst the function of this ancient filament may remain elusive to us, we now witness that archaea have developed a powerful propeller, whereas bacteria have evolved an intricate twitching ratchet. Both machines are driven by the action of central ATPases and assembled on homologous platform proteins, whilst the accessory proteins differ greatly. Bacterial and archaeal membrane lipids are distinct, thus it is plausible that assembly of filaments in membranes could require different capabilities and thus evolution of different facilitator proteins. The difference in regulatory proteins is clearly owed to functional diversification, but the lack of the large periplasmic conduit in archaea studied so far (Briegel et al., 2017; Daum et al., 2017) may reflect a bacterial evolutionary pressure owing to the emergence of the outer membrane.

Recent progress in investigating the T4P and archaellum machineries on both a structural and molecular level have shed new light on the function and evolutionary relationship of bacterial and archaeal motility. Future

research promises to expand this knowledge across scales to obtain a holistic understanding of prokaryotic motion, from the behaviour of cellular communities all the way down to dynamic structural models of individual motor complexes.

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