Microbiology Spectrum
American Society for Microbiology Press

The Dynamic Structures of the Type IV Pilus

MATTHEW MCCALLUM,[1,2] LORI L. BURROWS,[3] AND P. LYNNE HOWELL[1,2]

[1]Department of Biochemistry, University of Toronto, Toronto, ON M5S 1A8, Canada; [2]Program in Molecular Medicine, Peter Gilgan Centre for Research and Learning, The Hospital for Sick Children, Toronto, ON M5G 0A4, Canada; [3]Department of Biochemistry and Biomedical Sciences and the Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, Hamilton, ON L8N 3Z5, Canada

ABSTRACT Type IV pilus (T4P)-like systems have been identified in almost every major phylum of prokaryotic life. They include the type IVa pilus (T4aP), type II secretion system (T2SS), type IVb pilus (T4bP), Tad/Flp pilus, Com pilus, and archaeal flagellum (archaellum). These systems are used for adhesion, natural competence, phage adsorption, folded-protein secretion, surface sensing, swimming motility, and twitching motility. The T4aP allows for all of these functions except swimming and is therefore a good model system for understanding T4P-like systems. Recent structural analyses have revolutionized our understanding of how the T4aP machinery assembles and functions. Here we review the structure and function of the T4aP.

INTRODUCTION

The fundamental type IVa pilus (T4aP)-like architecture includes a retractable pilus fiber, a motor, an alignment subcomplex, and—in Gram-negative bacteria—an outer membrane secretin pore (Fig. 1). The pilus is an extracellular polymer of pilins. Pilins subunits are stored in the inner membrane and the motor powers their polymerization (extension) and depolymerization (retraction) at the pilus base. The alignment subcomplex connects the secretin with the motor and controls pilus dynamics. Finally, the secretin pore allows the pilus to extend through the outer membrane. Since publication of previous T4aP reviews (1–4), discoveries made using cryo-electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET), X-ray crystallography, and nuclear magnetic resonance (NMR) have dramatically reshaped our understanding of T4P-like systems. Here we put these discoveries in context with the structure and function of the T4aP, using the *Pseudomonas aeruginosa* T4aP system nomenclature.

THE FIBER

The Major Subunit, PilA

The major pilin PilA is the most abundant subunit in the pilus fiber. X-ray crystallography (5–18) and NMR (19–21) structures revealed four typical features: an elongated S-shaped N-terminal α-helix (α1N), a ~4-stranded antiparallel β-sheet, the αβ-loop, and the D-loop (Fig. 2) (Table 1). The αβ-loop and D-loop vary in sequence, structure, and posttranslational modifications between strains and species (7, 13, 18, 22–27). Together, the antiparallel β-sheet, αβ-loop, and D-loop create a globular domain from which the α1N helix protrudes. The hydrophobic α1N helix retains pilins in the inner membrane

Received: 23 August 2018, **Accepted:** 2 January 2019, **Published:** 1 March 2019

Editors: Maria Sandkvist, Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan; Eric Cascales, CNRS Aix-Marseille Université, Mediterranean Institute of Microbiology, Marseille, France; Peter J. Christie, Department of Microbiology and Molecular Genetics, McGovern Medical School, Houston, Texas

Citation: McCallum M, Burrows LL, Howell PL. 2019. The dynamic structures of the type IV pilus. *Microbiol Spectrum* 7(2):PSIB-0006-2018. doi:10.1128/microbiolspec.PSIB-0006-2018.

Correspondence: Lori L. Burrows, burrowl@mcmaster.ca; P. Lynne Howell, howell@sickkids.ca

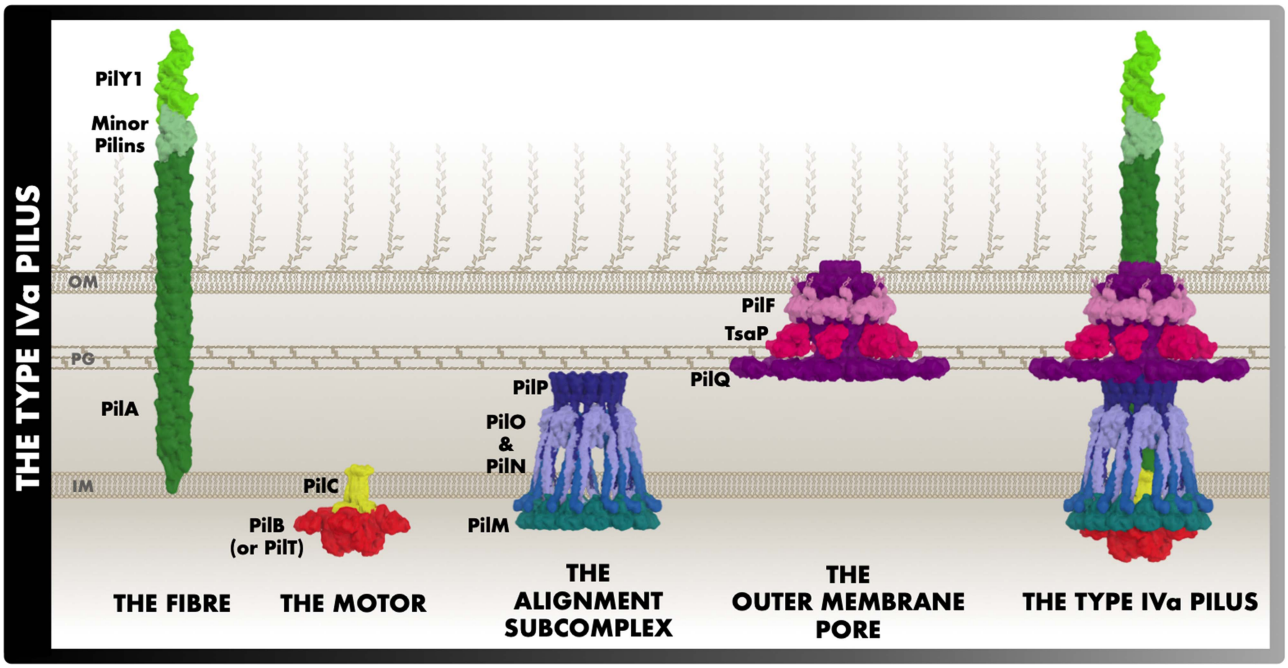


FIGURE 1 Subcomplexes of the T4aP. The protein structures portrayed reflect the full-length structure predictions and their predicted location in the T4aP. This figure is largely consistent with the previously published working model of the *M. xanthus* T4aP (43). Due to limited information, there is uncertainty regarding the locations of PilF, TsaP, PilY1, and the minor pilins.

prior to pilin polymerization and after pilin depolymerization. The peptidase PilD hydrolyzes the cytoplasmic leader sequence of nascent pilins and then methylates the new N terminus (28).

Recent 5- to 8-Å resolution cryo-EM maps of pilus fibers from *Neisseria* and *P. aeruginosa* (29, 30) largely validated older, lower-resolution models (6, 7, 13, 21, 31). With a helical rise of ~10 Å and 80 to 100° twist, the α1N helices bundle to form a hydrophobic core, while Glu-5 forms a salt bridge with the positively charged N-terminal amine of distal PilA molecules (6, 7, 13, 21, 29, 30). The αβ-loop and D-loop are surface exposed in these models, consistent with their sequence variability and posttranslational modifications that facilitate bacteriophage and immune evasion (6, 7, 24, 25). The 5- to 8-Å cryo-EM maps revealed that the segment between conserved Gly-14 and Pro-22 in the α1N helix was unexpectedly disordered in polymerized filaments (29, 30). This disordered region might allow the pilus to reversibly stretch to three times its original length (29, 32). Based on circular-dichroism analyses (33), the α1 helix is structured in pilin monomers (29, 30). Thus, relative to PilA monomers, the α1N in pilus fibers is stretched and may be under tension, or prestress—an architectural concept reviewed here (34), potentially explaining why the pilus appears to act more like a rod than a rope in micrograph animations (35). Rod-like behavior could facilitate preferential adhesion of the pilus tip to substrates and twitching motility (36).

Minor Pilins

The low-abundance minor pilins are similar in architecture to PilA (37, 38). PilV, PilW, and PilX are functionally equivalent to GspI, GspJ, and GspK of the type II secretion system (T2SS), respectively (16, 39, 40). Crystallographic analysis of the globular domains of GspIJK heterotrimers revealed a helical arrangement of the three components with GspK at the tip (Fig. 2) (41). The bulky globular domain of GspK may hinder its insertion anywhere but at the pilus tip (41). This suggests that the GspIJK trimer, and by extension the PilVWX trimer, is located at the pilus tip, though PilX is less bulky than GspK (41). By self-assembling a short stem, minor pilins are thought to prime pilus assembly by reducing the energy barrier to extraction of pilins from the membrane (39, 41–43). Minor pilins FimU and PilE connect PilVWX to the PilA fiber (40, 44), and because they have the α1N helix-destabilizing Pro-22 residue missing in PilVXW (39, 40), they may initiate α1N helix melting during pilus assembly. Some T4aP include additional minor pilins with specialized binding capabili-

ties, like ComP in *Neisseria*, which binds DNA to promote uptake (38, 45–48).

PilY1

PilY1 is an adhesin that likely localizes to the pilus tip with PilVWX (39, 49, 50). The N-terminal region of PilY1 is variable and important for PilY1 adhesive capacities (49, 51). Crystallographic analyses revealed that the conserved C-terminal domain of PilY1 is a 7-bladed β-propeller domain with a calcium binding motif (51). Manipulation of this motif reduces PilY1-based adhesion in *Neisseria gonorrhoeae* (52) and causes retraction defects in *P. aeruginosa* (51).

THE MOTOR

PilC

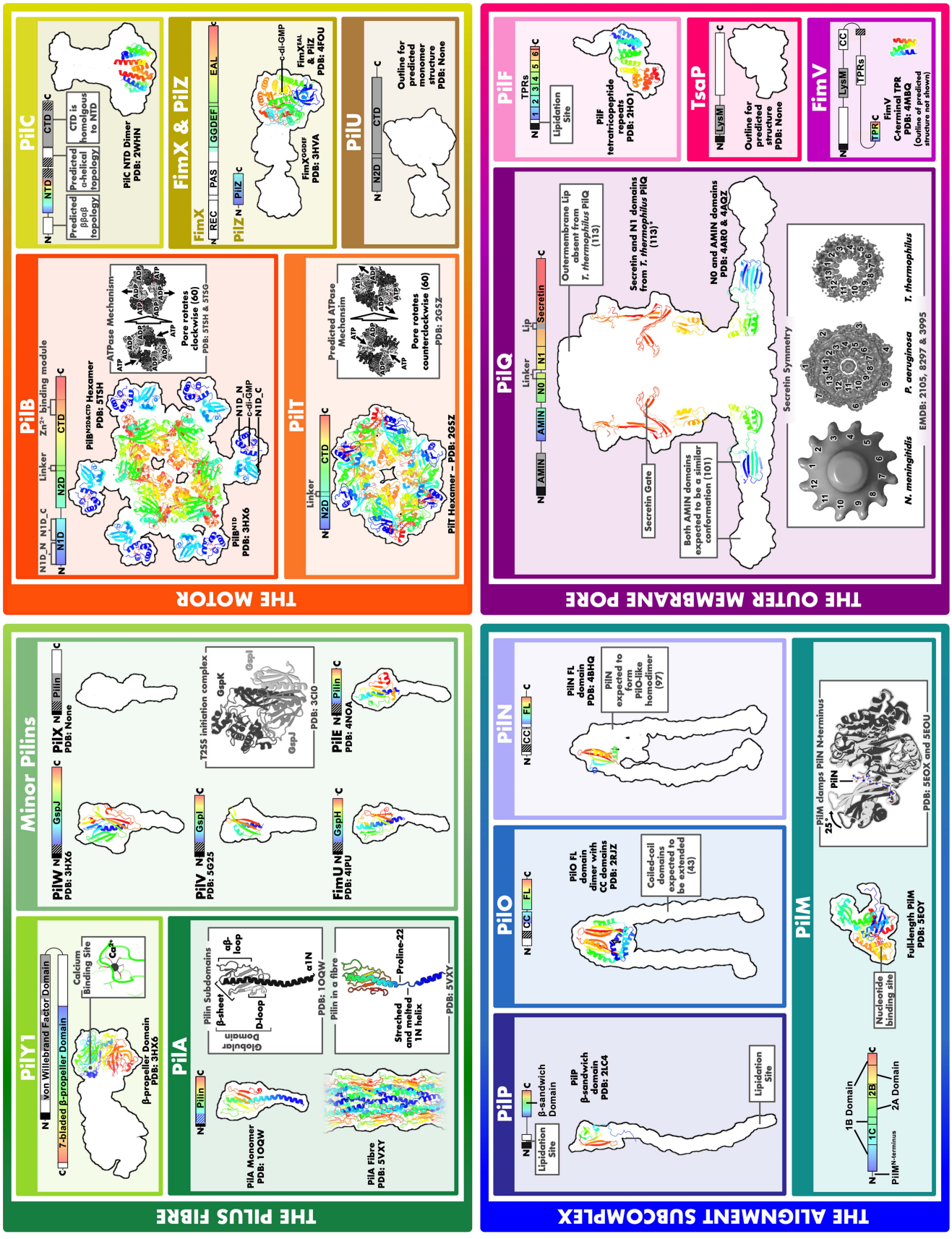
PilC is a 3-pass inner membrane protein with two homologous globular cytoplasmic domains (53, 54) and, in many proteobacteria, a small cytoplasmic N-terminal domain with predicted ββαβ topology (Fig. 2). Cryo-electron tomography (cryo-ET) of the *Myxococcus xanthus* T4aP machinery suggests that PilC is a dimer and localized in the pore of cytoplasmic hexameric ATPases PilB and PilT, while its transmembrane segments may interact with PilA (43). Given this organization, PilC might be rotated by PilB and PilT to catalyze PilA polymerization and depolymerization, respectively (43, 53). *In vitro* analyses support direct interactions between PilB and PilC, PilT and PilC, and PilA and PilC (53, 55–57). Purified PilC is dimeric and tetrameric, consistent with a 22-Å-resolution cryo-EM analysis of PilC from *Neisseria meningitidis* (54, 57, 58). The N-terminal domain of PilC from *Thermus thermophilus* was crystallized as an asymmetric homodimer, and mutating this dimer interface ablated *in vitro* tetramers but not dimers (54).

PilB and PilT

C_2-symmetric structures of PilB from *T. thermophilus* ($PilB^{Tt}$) bound to the slowly hydrolyzable ATP analog ATPγS (59), PilB from *Geobacter metallireducens* ($PilB^{Gm}$) bound to ADP plus the nonhydrolyzable ATP analog AMP-PNP (60), and apo-PilB from *Geobacter sulfurreducens* ($PilB^{Gs}$) (61) were recently determined. The symmetry of $PilB^{Tt}$ initially suggested a C_2 rotary mechanism with a predicted counterclockwise pore rotation (59). In contrast, the two structures of $PilB^{Gm}$ representing pre- and posthydrolysis states indicated a clockwise pore rotation (60) (Fig. 2) and that four of the six ATPγS molecules in the lower-resolution $PilB^{Tt}$ hexamer should have been modeled as ADP and magnesium. We proposed that clockwise rotation of the PilB pore may move PilC clockwise in 60° increments to facilitate the polymerization of PilA into the right-handed helix observed by cryo-EM (29, 30, 60). Note that hydrolysis of ATP by PilB gives the impression of pore rotation, though PilB does not rotate during this process. Recent 8-Å resolution cryo-EM analysis of $PilB^{Tt}$ showed a domain N terminal to the motor domains, the N1D, in a position that may block PilC from directly contacting the pore (62). Thus, the N1D might participate directly in the motor function of PilB (62). Alternatively, the N1Ds could regulate whether the $PilB^{Tt}$ pore is available for binding PilC. Consistent with a rotary model, the PilB homolog from the archaellum FlaI is proposed to facilitate archaellum spinning; consistent with a C_2-symmetric mechanism, purified FlaI, PilB, and PilT have two free ATP binding sites (61, 63–68).

The N1D of PilB is composed of two subdomains, N1D_N and N1D_C (69). The N1D_N subdomain of PilB binds the biofilm-related second messenger, c-di-GMP (69–71); the N1D_C subdomain also contacts c-di-GMP (69) (Fig. 2). A complex consisting of N1D_C of the T2SS PilB homolog, GspE, bound to the cytoplasmic domain of GspL (PilM) was crystallized (72, 73), suggesting a similar interaction in the T4aP (55). Therefore, c-di-GMP binding to PilB might regulate the PilB interaction with PilM and thus PilB engagement with the T4aP. PilB homologs from *P. aeruginosa*, *Xanthomonas campestris*, and *Xanthomonas axonopodis* lack an obvious c-di-GMP-binding motif in their N1D_N subdomains, though they interact with c-di-GMP-binding FimX, which also interacts with PilB-binding PilZ (74–76).

PilT is homologous to the motor domains of PilB and powers pilus depolymerization/retraction with extraordinary forces (35, 77–81). In addition to PilT, its paralogs in some T4aP systems, such as PilU, are also functionally significant (82–84). Pilus retraction is essential for several T4aP functions, including twitching motility, competence, and phage infection (77, 85, 86). In contrast to PilB, structures of PilT exhibit dissimilar rotational symmetries (87, 88). We applied the direction of ATP binding and hydrolysis of PilB to the structure of C_2-symmetric PilT from *Aquifex aeolicus* (89) (Fig. 2). This analysis suggested that the pore and thus PilC would rotate counterclockwise to facilitate PilA depolymermization and pilus retraction (60). Intriguingly, the T4aP of *Vibrio cholerae* was recently reported to retract with low speed and force in a PilT knockout mutant (35), and the type IVb pilus (T4bP) systems of *V. cholerae* and the Tad pilus of *Caulobacter crescentus*, which lack retraction ATPases, were also reported to retract with low speed and force


THE MOTOR
PilB
PilT
PilC
FimX & PilZ
PilU
THE OUTER MEMBRANE PORE
PilQ
PilF
TsaP
FimV
THE PILUS FIBRE
Minor Pilins
PilY1
PilA
THE ALIGNMENT SUBCOMPLEX
PilP
PilO
PilN
PilM

(90, 91). Thus, PilT might simply enhance the speed and force of retraction (35). The energy for retraction in the absence of PilT was proposed to be potential energy stored in the pilus (90), possibly elastic tension in the melted α1N helices of PilA (see above).

THE ALIGNMENT SUBCOMPLEX

PilM

Cryo-ET of *M. xanthus* indicated that PilM forms a ring on the inner leaflet of the inner membrane surrounding PilB or PilT (43), consistent with evidence that PilM binds to both PilB and PilT (53, 55, 57). The X-ray crystallographic structures of PilM from *T. thermophilus* and *P. aeruginosa* bound to the first eight residues of PilN have been solved, revealing that PilM is structurally similar to FtsA (55, 92). Interestingly, the T2SS homolog of PilM, GspL, is equivalent to a fusion of PilM and PilN (73, 92, 93). In the T4aP there is functional relevance for discrete PilM and PilN proteins, since PilN binding favors PilM-PilB interactions while reducing PilM-PilT interactions in bacterial two-hybrid experiments (55). Likewise, PilM subdomain 1C turns on a hinge to bind PilN (55) (Fig. 2), and this hinge is the predicted site for PilM-PilB interactions based on the homologous interactions in the T2SS (55, 72, 73). Thus, PilN binding to PilM might influence which ATPase is associated with the T4aP (55). Consistent with proposed conformational changes, the diameter of the PilM ring of *M. xanthus* is wider in the piliated than in the nonpiliated state (43).

PilN and PilO

PilN and PilO are structurally similar inner membrane proteins with a cytoplasmic N-terminal peptide of ~20 residues, a transmembrane domain followed by a coiled-coil domain, and a C-terminal globular domain with a ferredoxin-like fold (94–96). They form an oligomeric cage-like ring in the inner membrane and periplasm (43). The ferredoxin-like domains from *T. thermophilus* PilN and *P. aeruginosa* PilO crystallized as homodimers (94–96). One PilO structure from *P. aeruginosa* revealed a distinct PilO ferredoxin-like domain homodimer interface (95), and *in vivo* cysteine disulfide cross-linking studies of PilN and PilO homodimers and heterodimers are consistent with this interface (95–97). Cross-linked heterodimers, but not homodimers, interfere with T4aP function, suggesting that the homodimeric interface is stable while the heterodimeric interface may be dynamic (97). Mutagenesis studies suggest that heterodimerization occurs mainly through the coiled-coil domains, and coiled-coil mutations lead to pilus extension and retraction defects (98). Thus, PilN and PilO heterodimers are proposed to interact with PilM to influence which ATPase is bound (43, 55, 98).

PilP

PilP is an inner membrane lipoprotein with a partially disordered N-terminal region followed by a globular β-sandwich domain (99, 100). The partially disordered region binds to PilN and PilO heterodimers but not homodimers (100). Since heterodimeric PilN and PilO interactions are dynamic, the PilP interaction with PilN and PilO may also be dynamic (98). NMR analysis and pulldown experiments demonstrated that the β-domain of PilP also interacts with the N0 domain of PilQ, as do the homologous domains in the T2SS and T4bP (101–104). Thus, the PilMNOP subcomplex links the cytoplasmic ATPases to PilQ (43, 102). The dynamic interactions predicted for PilMNOP may also transduce signals. In *P. aeruginosa*, surface sensing is thought to

FIGURE 2 The structures of the type IV pilus. The four subcomplexes are split into four quadrants, which are further subdivided into individual proteins in boxes colored to correspond to Fig. 1. In the linear domain architecture, domains are displayed to scale as blocks colored to indicate known structures (rainbow colors), segments with high-confidence structure predictions (gray), unknown structures (white), transmembrane segments (diagonal bars), hydrolyzed signal peptides (black), or predicted/known disorder (black line). The known or predicted domain name is written; if a domain has no name, it could not be predicted. In the black outlined cartoon structures, a black outline of the predicted (127–129) full-length homology model is shown to scale for reference. Known structures are displayed as cartoons in rainbow colors corresponding to the colors shown in the linear domain architecture. A short description of the rainbow-colored cartoon structure and the PDB accession code are written in black font. Since the black outline is a *P. aeruginosa* structure prediction while the cartoons correspond to structures sometimes determined in other species, the black outline and cartoons may not fully match. Note that the PDB coordinate file for PilQ from *T. thermophilus* (marked with an asterisk) was obtained from the authors of reference 113 and used here with their permission; only the secretin and adjacent N1 domain (N5 in *T. thermophilus*) are shown here, as the other *T. thermophilus* PilQ domains are divergent or atypical compared to those in *P. aeruginosa*. (The N1 domain is also named N2, N3, N4, or N5 in systems or species where the N1 domain is duplicated.) No black outline is shown for FimV, as high-confidence structure prediction was not possible for most of this component. Unexpectedly, most of TsaP was predicted (127) with high confidence to be structurally similar to the protein with PDB code 3SLU. Gray boxes note interesting features of the protein or other relevant structures; structures in gray boxes are not to scale.

TABLE 1 List of available T4aP structures[a]

Pseudomonas equivalent	Name	Species	PDB (EMDB) code(s)	Reference(s)
The pilus fiber				
PilY1	PilY1	*Pseudomonas aeruginosa* PAO1	3HX6	51
PilV	Tt1218	*Thermus thermophilus*	5G25	16
PilW	Tt1219	*Thermus thermophilus*	5G23, 5G24	16
FimU	FimU	*Pseudomonas aeruginosa* PAO1	4IPU, 4IPV	39
PilE	PilE	*Pseudomonas aeruginosa* PAO1	4NOA	40
	PilX	*Neisseria meningitidis*	2OPD, 2OPE	45
	PilV	*Neisseria meningitidis*	5V23,[b] 5V0M[b]	None
PilA	PilA	*Acinetobacter baumannii*	4XA2, 5VAW,[b] 5IHJ, 5CFV	8
	PilA	*Geobacter sulfurreducens*	2M7G	19
	PilA	*Pseudomonas aeruginosa* PAK	1DZO, 1OQW, 1X6P/Q/R/X/Y/Z	6, 12, 17
	PilA	*Pseudomonas aeruginosa* K122-4	1HPW, 1QVE, 1RG0	14, 21
	PilA	*Pseudomonas aeruginosa* Pa110594	JYZ, 3JZZ	5
	PilA	*Pseudomonas aeruginosa* consensus	2PY0	11
	PilA1	*Clostridium difficile*	4TSM, 4OGM, 4PE2	9
	FimA	*Dichelobacter nodosus*	3SOK	10
	PilBac1	*Shewanella oneidensis*	4D40, 4US7	15
	PilE	*Francisella tularensis*	3SOJ	10
	PilE	*Neisseria gonorrhoeae*	1AY2, 2HI2, 2PIL	7, 13, 18
	PilE	*Neisseria meningitidis*	5JW8, 4V1J[b]	29
	Tt1221	*Thermus thermophilus* HB8	4BHR	94
PilA fiber	PilA	*Pseudomonas aeruginosa* PAK	5VXY (8740)	30
	PilA4	*Thermus thermophilus*	None (3024)	119
	PilE	*Neisseria gonorrhoeae*	5VXX (8739)	30
	PilE	*Neisseria gonorrhoeae*	2HIL (1236)	7
	PilE	*Neisseria meningitidis*	5KUA (8287)	29
None known	ComP	*Neisseria meningitidis*	2M3K, 5HZ7	38, 48
	ComP	*Neisseri subflava*	2NBA	38
	PilJ	*Clostridium difficile*	4IXJ	37
	Tt1222	*Thermus thermophilus HB8*	5G2F	16
The motor				
PilB	PilB	*Geobacter metallireducens*	5TSG, 5TSH	60
	PilF	*Thermus thermophilus*	5IT5, 6EJF (3882), 6F8L (4194), (2222), (2223)	59, 62, 130
	PilB	*Geobacter sulfurreducens*	5ZFR	61
PilB (N1D)	MshE	*Vibrio cholerae* serotype O1	5HTL	69
PilT	PilT	*Pseudomonas aeruginosa* PAO1	3JVU, 3JVV	88
	PilT	*Aquifex aeolicus* strain VF5	2EWV, 2EWW, 2EYU, 2GSZ	89
	PilT4	*Geobacter sulfurreducens*	5ZFQ	61
	PilT2	*Thermus thermophilus*	5FL3[b]	None
PilC	PilC	*Thermus thermophilus*	2WHN	54
FimX	FimX	*Pseudomonas aeruginosa* PAO1	4AG0, 3HV9, 3HVA, 3HV8, 4AFY, 4J40	135, 136
	FimX	*Xanthomonas* sp.	4FOK, 4F3H	76, 137
PilZ	PilZ	*Xanthomonas* sp.	3CNR, 3DSG	75, 138
FimX and PilZ	FimX and PilZ	*Xanthomonas* sp.	4FOU, 4F48	76, 137
The alignment complex				
PilM	PilM	*Pseudomonas aeruginosa* PAO1	5EOX, 5EOY, 5EQ6	55
PilM and $PilN^{cyto}$	PilM and $PilN^{cyto}$	*Pseudomonas aeruginosa* PAO1	5EOU	55
	PilM and $PilN^{cyto}$	*Thermus thermophilus*	2YCH	92
PilN	PilN	*Thermus thermophilus*	4BHQ	94
PilO	PilO	*Pseudomonas aeruginosa* PAO1	2RJZ, 5UVR	95, 96
PilMN, PilMNO, and PilMNOA	PilMN, PilMNO, and PilMNOA	*Thermus thermophilus*	None[c] (4157, 4157, 4159)	94
PilP	PilP	*Pseudomonas aeruginosa* PAO1	2LC4, 2Y4X,[b] 2Y4Y[b]	100
	PilP	*Neisseria meningitidis*	2IVW	99

(continued)

TABLE 1 List of available T4aP structures[a] *(continued)*

Pseudomonas equivalent	Name	Species	PDB (EMDB) code(s)	Reference(s)
The outer membrane pore				
PilQ	PilQ	*Pseudomonas aeruginosa* PAO1	None (8297)	114
	PilQ	*Thermus thermophilus*	None[c] (3985, 3995, 3996, 3997, 3998)	113
	PilQ	*Candidatus pelagibacter*	None (8330)	117
PilQ and PilP	PilQ and PilP	*Neisseria meningitidis*	4AV2 (2105)	101
PilQ (N0 and linker from N0 to N1)	PilQ (N0 and linker from N0 to N1)	*Neisseria meningitidis*	4AR0	101
PilQ (AMIN)	PilQ (β2)	*Neisseria meningitidis*	4AQZ	101
PilF	PilF	*Pseudomonas aeruginosa* PAO1	2HO1, 2FI7	134, 139
PilF	PilW	*Neisseria meningitidis*	2VQ2	126
FimV	FimV	*Pseudomonas aeruginosa* PAO1	4MBQ, 4MAL	140
In situ structures of the T4aP		*Myxococcus xanthus*	3JC8, 3JC9 (3247–3264)	43
		Thermus thermophilus	None (8224, 3021–3024)	118, 119

[a]These are the published T4aP structures, not those from related T2SS, T4bP, Com pilus, Tad/Flp pilus, or archaellum.
[b]Peer-reviewed article describing the structure has not been published yet.
[c]A model could be built into the density, but the model was not published to the Protein Data Bank.

initiate with PilY1, proceed through PilMNOP, require PilT, and ultimately activate the diguanylate cyclase SadC (105–108).

THE OUTER MEMBRANE PORE

PilQ

The secretin domain of PilQ forms the outer membrane pore, and until recently this domain resisted structure determination. The structures of the other PilQ subdomains were determined by NMR: one of two peptidoglycan-binding AMIN (β) domains located near the N terminus, the N0 domain, and the N1 domain (101). In *P. aeruginosa*, the AMIN domains localize PilQ to sites of cell division for preinstallation of the T4aP complex into the nascent septa of the daughter cells (109). Since the N0 domain of PilQ is bound by PilP (see above), PilQ recruits and localizes PilMNOP (110, 111). In the absence of an outer membrane, Gram-positive bacteria with T4aP mostly lack PilP and PilQ homologs (112).

Using single-particle cryo-EM, ~19-Å-, 7.4-Å-, and 7.0-Å-resolution maps of full-length PilQ from *N. meningitidis*, *P. aeruginosa*, and *T. thermophilus*, respectively, have been determined (101, 113, 114). Two-dimensional (2D) cryo-EM top views are also available for outer membrane-embedded *N. gonorrhoeae* and *N. meningitidis* PilQ (115, 116). These maps are 12-, 13-, or 14-fold symmetric, consistent with dodecamers, tridecamers, or tetradecamers (101, 113, 114, 117). Given that PilQ ultimately connects to hexameric ATPases in the cytoplasm via the alignment subcomplex, there may be stoichiometry mismatches between subcomplexes in some T4aP systems.

Two internal gates have been identified in PilQ: the secretin gate and the periplasmic gate (43, 101, 113, 114, 118, 119), although some cryo-EM maps are missing one or the other. Model building in the 7.0-Å-resolution *T. thermophilus* PilQ cryo-EM map was made possible by using homology models of new 3- to 4-Å-resolution GspD cryo-EM models (113, 120–123). Based on these models, it is clear how the secretin gate prevents leakage of molecules in the absence of the pilus (113, 124). The function of the periplasmic gate is less clear. The linker between the N0 domain and N1 domain forms the periplasmic gate in *T. thermophilus* PilQ, though this portion of PilQ shows limited homology to more typical T4aP systems (113). Since the gates must reorient during pilus extension and after retraction, it is conceivable that PilP binding to the N0 domain could sense these movements and transmit a signal to the cytoplasm via PilMNO.

PilF, TsaP, and FimV

The PG-binding protein FimV is widely distributed in T4aP systems (125) and was originally thought to be a PilQ-stabilizing protein, as mutants had reduced levels of PilQ (126). Recent data suggest that FimV plays a role in localizing PilQ and contributes to the expression of multiple T4aP proteins via a cAMP-dependent surface sensing mechanism (109, 127–130).

The PilF pilotin protein is required for stability, outer membrane localization, and multimerization of PilQ (131–134). PilF is a six-tetratricopeptide repeat lipoprotein localized to the outer membrane (126, 134). In the cryo-EM 2D class averages of *Neisseria* PilQ and the 3D reconstruction of *P. aeruginosa* PilQ, 7-fold symmetric spokes were detected around 14-fold symmetric PilQ (114–116).

In the *P. aeruginosa* 3D reconstruction, the spokes are localized to the inner leaflet of the outer membrane (114). Density consistent with these spokes was also present in cryo-ET images of *M. xanthus* T4aP and the 3D reconstruction of *N. meningitidis* PilQ (43, 101). Similar spokes in the T2SS correspond to the unrelated T2SS pilotin (120), suggesting the T4aP spokes could be PilF. Indeed, the spoke symmetry suggests that the spokes may simultaneously bind at least two PilQ protomers in the assembled secretin, potentially stabilizing nascent PilQ oligomers to assist PilQ multimerization. Alternatively, it has been proposed that the peptidoglycan-binding protein TsaP may form the spokes, since deleting TsaP from *M. xanthus* or *N. gonorrhoeae* causes the spokes to disappear (43, 116). It is possible that these spokes comprise multiple proteins.

CONCLUSION

Recent advances in cryo-EM and cryo-ET allow us to put crystallographic and NMR structures of individual T4aP components into biological context (Fig. 1). With these advances, new mysteries have emerged. For example, how does PilC interface with PilA and the pilus fiber in a way that facilitates extension and retraction yet opposes pilus shedding? Do the proposed dynamics in PilMNOPQ form a mechanical signal cascade? Structural insights from the T4aP will help rationalize new findings and expedite our understanding of other T4P-like systems.

ACKNOWLEDGMENTS

M.M. has been funded by graduate scholarships from the Canadian Institutes for Health Research (CIHR) and the Province of Ontario. P.L.H. is the recipient of a Canada Research Chair. This work was supported by grant MOP 93585 from CIHR to L.L.B. and P.L.H.

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