Neisseria gonorrhoeae Pilus and Associated Proteins

By Iryna Boiko and Kathleen Nicholson

Type IV Pili - Introduction

- *Neisseria gonorrhoeae* (Gc) primarily express T4P
- Type IV pili (**T4P**) are filamentous cell surface structures essential for *Neisseria gonorrhoeae* (Gc) colonization and virulence
- T4P play a role in:
  - Initial attachment to host cells and tissues
  - Twitching motility
  - Natural transformation
  - Microcolony formation
  - Biofilm development
  - Protection against neutrophil-mediated killing
- T4P are assembled by a complex machinery, spanning the entire cell envelope (Figure 1)

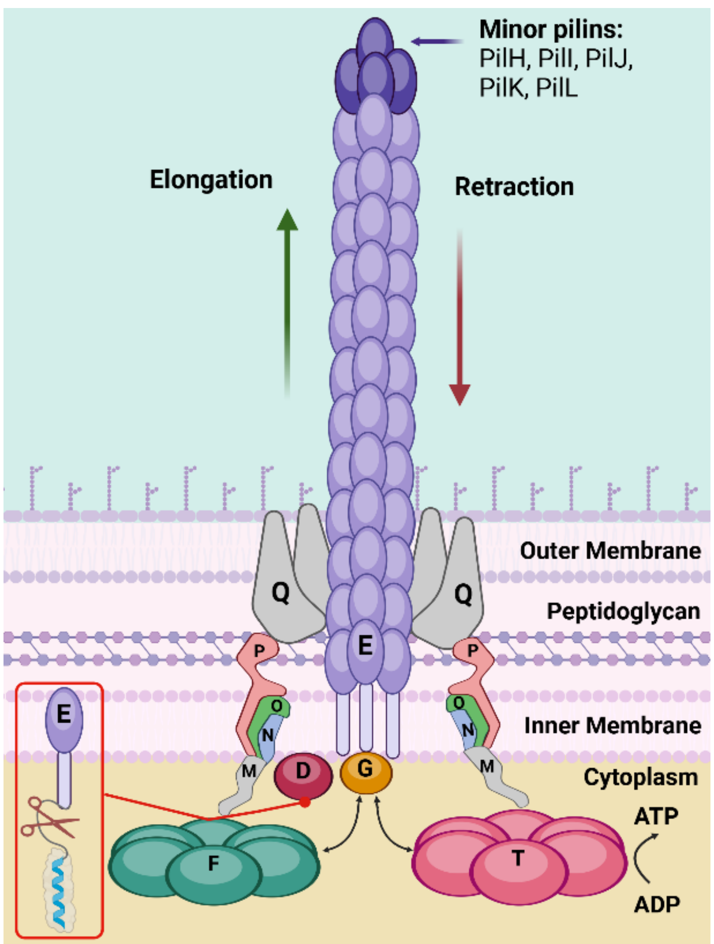

Figure 1. Architecture the Gc T4p apparatus. Model is adapted from Craig *et al*., 2004 and Hospenthal *et al*., 2017.

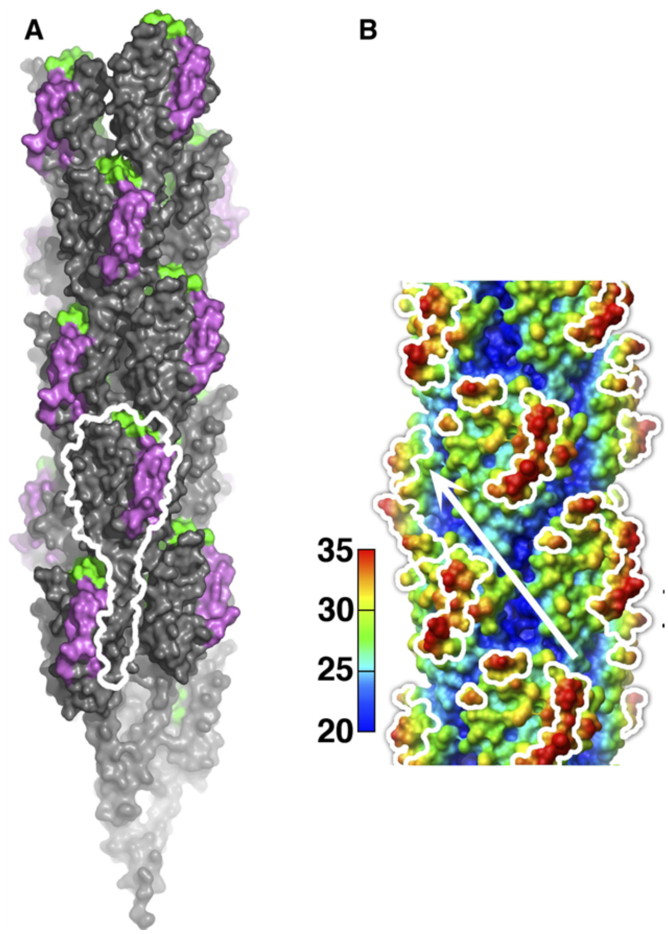

Figure 2. Gc pilus fiber (Craig et al., 2006). Hypervariable epitopes (A, pink) and epitopes exposed to posttranslational modifications (B, white) are predominant at the pilus surface.

Pilus-associated Proteins:

Table 1. Architectural Proteins

<table>
<tr><th>Protein name
NGO, PubMLST, and
UniProt entry</th><th>Protein Designation</th><th>Other Designation(s)</th><th>Function(s) &
Notable Characteristics</th></tr>
<tr><td>Major pilin subunit
No NGO number, NEIS0210, P02974</td><td>PilE</td><td>Protein α, pilin</td><td>Extensive sequence variation due to antigenic variation</td></tr>
<tr><td>Minor pilin
Type II secretion system protein H,
NGO_0452, NEIS0827, Q5F9E6</td><td>PilH</td><td>FimT</td><td rowspan="5">Gc aggregation
Microcolony formation
Pilus-pilus interactions</td></tr>
<tr><td>Minor pilin
NGO_0453, NEIS0828, Q5F9E5</td><td>PilI</td><td>-</td></tr>
<tr><td>Minor pilin
NGO_0454, NEIS0829, Q5F9E4</td><td>PilJ</td><td>PilW</td></tr>
<tr><td>Minor pilin
NGO_0455, NEIS0830, Q5F9E3</td><td>PilK</td><td>-</td></tr>
<tr><td>Minor pilin
NGO_0456, NEIS0831, Q5F9E2</td><td>PilL</td><td>PilX</td></tr>
<tr><td>Minor pilin
NGO_1441, NEIS0487, Q5F6V4</td><td>PilV</td><td>-</td><td>Interacts with PilC
Host-cell adhesion
ComP antagonist</td></tr>
<tr><td>Pilus adhesin
NGO_0055, NEIS0371, Q5FAG7</td><td>PilC1</td><td rowspan="2">Pilus tip adhesin</td><td rowspan="2">Located at the pilus tip
Adhesion
Phase variation via a poly-G tract</td></tr>
<tr><td>Pilus adhesin
NGO_10270, NEIS0033, A0A0H4IWH7</td><td>PilC2*</td></tr>
<tr><td>DNA binding pilin
NGO_1177, NEIS1995, Q5F7J8</td><td>ComP</td><td>-</td><td>DNA uptake sequence (DUS) recognition, DNA binding, natural transformation</td></tr>
</table>

*Not present in all strains

Table 2. T4P Biogenesis Proteins

Protein name NGO, PubMLST, and UniProt entry	Current Designation	Other Designation(s)	Function(s) & Notable Characteristics
Secretin NGO_0094, NEIS0408, Q5FAD2	PilQ	Outer membrane protein macromolecular complex; OMP-MC	Dodecameric secretin; Forms the channel for pilus fiber growth and passage through the outer membrane
Inner membrane lipoprotein NGO_0095, NEIS0409, Q5FAD1	PilP	-	PilM/N/O/P complex; Aligns pilus fiber with secretin channel
Periplasmic alignment protein NGO_0096, NEIS0410, Q5FAD0	PilO	-	
Periplasmic alignment protein NGO_0097, NEIS0411, Q5FAC9	PilN	-	
Inner membrane platform protein NGO_0098, NEIS0412, Q5FAC8	PilM	-	
Platform protein NGO_1669, NEIS1838, Q5F693	PilG	-	Inner membrane protein; Coordinates pilus assembly
T4P assembly protein NGO_0783, NEIS1125, Q5F8J0	TfpC	-	Stabilizes pilus in extended conformation
T4P biogenesis/stability protein NGO_0595, NEIS1246, Q5F912	PilW	-	Not yet studied in Gc; May play a role in assembly and stability of the PilQ secretin
Assembly ATPase NGO_1673, NEIS1844, Q5F689	PilF	PilB	Hexameric ATPase that powers pilus assembly
Pilus assembly protein PilF inhibitor NGO_0348, NEIS0723, Q5F9P5	PilZ	-	Not yet studied in Gc; May control PilF activity
Retraction ATPase NGO_1908, NEIS0036, Q5F5L5	PilT	PilT1	Twitching motility protein; Primary ATPase, powers pilus retraction; Provides energy for depolymerization

Secondary retraction ATPase NGO_0346, NEIS0721, Q5F9P7	PilT2	PilU	Secondary retraction ATPase, works with PilT; Twitching motility-like protein
Prepilin leader peptidase / N- methyltransferase NGO_1670, NEIS1839, Q5F692	PilD	Leader peptidase; Type IV prepilin peptidase	Cleaves leader sequence and N-methylating mature proteins (PilE, PilH, PilI, PilJ, PilK, ComP, PilV, and PilL)
DD-carboxypeptidase/ endopeptidase Mpg NGO_1686, NEIS1856, Q5F676	Mpg	-	Periplasmic protein; Regulatory roles in pilus biogeneisis
T4P pilus secretin-associated protein NGO_1873, NEIS0101, Q5F5P4	TsaP	-	Anchors the secretin complex to peptidoglycan

PilE - Major Pilin Subunit Domains and Structure:

- PilE is the major structural component of the gonococcal pilus fiber.
- PilE is comprised of:
  - **Leader sequence (N-terminal):** 6-7 amino acids, cleaved and methylated by PilD
  - **N-terminal α-helix**: Highly conserved, hydrophobic, forms pilus fiber core, not immunogenic
  - **C-terminal region**: Contains semi-variable regions, a hypervariable loop, and a tail, which are primary sites for antigenic variation; whereas all undergo frequent changes, the semi-variable regions exhibit less sequence diversity.

Antigenic Variation

- PilE undergoes extensive antigenic variation (Figure 2) through:
  - RecA-dependent gene conversion between expressed *pilE* and silent *pilS* loci
  - Multiple silent *pilS* cassettes (18-19 copies within 5-6 loci) provide sequence repertoire for the *pilE* gene variation (see Tables 3 and 4)
- The *sRNA* and guanine quartet (*G4*) structures upstream of the *pilE* gene are essential for antigenic variation of the *pilE* gene
- *pilE* gene variation rate: ~4×10^{-3} variants/CFU/generation

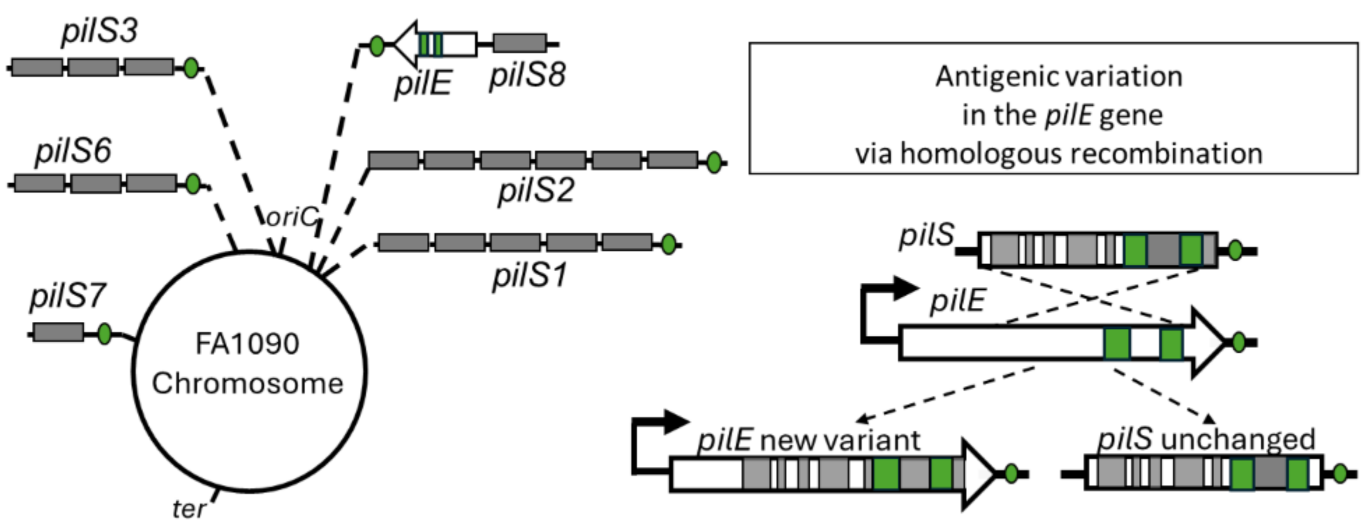

Figure 3. Genomic organization of *pilE* and *pilS* loci in *Gc* FA1090 and the process of antigenic variation in *pilE.* The model is adapted from Rotman & Seifert, 2014 and Rotman *et al*., 2016.

Table 3. ***pilS*** **Silent loci in** ***N.gonorrhoeae*** **F1090**

pilS locus	Partial gene copies	UniProt Number	NCBI Accession number
pilS1, 5 copies	*pilS1c1*	V9GZV8	U58846
	pilS1c2	V9H082	
	pilS1c3	V9H040	
	pilS1c4	V9GZU4	
	pilS1c5	V9GZX1	
pilS2, 6 copies	*pilS2c1*	V9H054	U58848
	pilS2c2	V9GZV6	
	pilS2c3	V9GZY2	
	pilS2c4	V9GZW6	
	pilS2c5	V9H084	
	pilS2c6	V9H048	
pilS3, 3 copies	*pilS3c1*	V9H090	U58850
	pilS3c2	V9H056	
	pilS3c3	V9GZW3	
pilS6, 3 copies	*pilS6c1*	V9GZZ0	U58849
	pilS6c2	None	
	pilS6c3	V9H087	
pilS7, 1 copy	*pilS7c1*	V9GZX4	U58851
pilE8, 1 copy	*pilS8c1*	None[a]	U58847

a – UniProt designation is not available/unassigned

NgoRS

Table 4. *pilS* Silent loci in *N.gonorrhoeae* MS11

<table>
<tr><th>pilS locus</th><th>Partial gene copies</th><th>UniProt Number[a]</th><th>NCBI Accession number</th></tr>
<tr><td rowspan="6">pilS1, 6 copies</td><td>pilS1c1</td><td rowspan="6">None</td><td rowspan="6">M11663</td></tr>
<tr><td>pilS1c2</td></tr>
<tr><td>pilS1c3</td></tr>
<tr><td>pilS1c4</td></tr>
<tr><td>pilS1c5</td></tr>
<tr><td>pilS1c6</td></tr>
<tr><td rowspan="2">pilS2, 2 copies</td><td>pilS2c1</td><td rowspan="2">None</td><td rowspan="2">None[b]</td></tr>
<tr><td>pilS2c2</td></tr>
<tr><td>pilS5, 1 copy</td><td>pilS5c1</td><td>None</td><td>X60748</td></tr>
<tr><td rowspan="3">pilS6, 3 copies</td><td>pilS6c1</td><td rowspan="3">None</td><td rowspan="3">X60749</td></tr>
<tr><td>pilS6c2</td></tr>
<tr><td>pilS6c3</td></tr>
<tr><td>pilS7, 1 copy</td><td>pilS7c1</td><td>None</td><td>X60750</td></tr>
<tr><td rowspan="2">pilE8, 2 copies</td><td>pilS8c1</td><td rowspan="2">None</td><td rowspan="2">None[b]</td></tr>
<tr><td>pilS8c2</td></tr>
</table>

a – At present, UniProt designations are not available for *N. gonorrhoeae* strain MS11
b - NCBI accession numbers are not available for *pilS2* and *pilS8*

Table 5. Summary of the Role of Pilus Variation in Gc pathogenesis

Mechanism	Effect	Clinical Significance
Antigenic variation	Escape from antibody recognition Immune evasion Different adhesin phenotypes	Persistent infection Vaccine challenges
Phase variation	On/off expression of pili Cell tropism changes Modulation of adherence capability Immune evasion	Dissemination Transmission Persistent infection Vaccine challenges
Glycosylation changes	Altered receptor binding Different host targets	Protection from proteolysis Colonization efficiency Immune recognition avoidance Persistent infection
PilC variation	Different adherence properties Tissue specificity	Changes in piliation levels Dissemination potential

References:

1. Aas FE, Egge-Jacobsen W, Winther-Larsen HC, Løvold C, Hitchen PG, Dell A, Koomey M (2006) *Neisseria gonorrhoeae* type IV pili undergo multisite, hierarchical modifications with phosphoethanolamine and phosphocholine requiring an enzyme structurally related to lipopolysaccharide phosphoethanolamine transferases. *J Biol Chem*. 281(38):27712-23.
2. Aas FE, Løvold C, Koomey M (2002) An inhibitor of DNA binding and uptake events dictates the proficiency of genetic transformation in *Neisseria gonorrhoeae*: mechanism of action and links to Type IV pilus expression. *Mol Microbiol*. 46:1441–1450.
3. Brossay L, Paradis G, Fox R, Koomey M, Hébert J (1994) Identification, localization, and distribution of the PilT protein in *Neisseria gonorrhoeae*. *Infect Immun*. 62:2302–2308.
4. Cahoon LA, Seifert HS (2013) Transcription of a cis-acting, noncoding, small RNA is required for pilin antigenic variation in *Neisseria gonorrhoeae*. *PLoS Pathog.* 9:e1003074.
5. Carbonnelle E, Hélaine S, Prouvensier L, Nassif X, Pelicic V (2005) Type IV pilus biogenesis in *Neisseria meningitidis*: PilW is involved in a step occurring after pilus assembly, essential for fibre stability and function. *Mol Microbiol.* 55:54–64.
6. Craig L, Pique ME, Tainer JA (2004) Type IV pilus structure and bacterial pathogenicity. *Nat Rev Microbiol* 2:363–378.
7. Craig L, Volkmann N, Arvai AS, Pique ME, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell*. 23:651-62.
8. Criss AK, Katz BZ, Seifert HS (2009) Resistance of *Neisseria gonorrhoeae* to non-oxidative killing by adherent human polymorphonuclear leucocytes. *Cell Microbiol.* 11:1074–1087.
9. Criss AK, Kline K, Seifert HS (2005) The frequency and rate of pilin antigenic variation in *Neisseria gonorrhoeae*. *Mol Microbiol.* 58:510-519.
10. Davies JK, Harrison PF, Lin YH, Bartley S, Khoo CA, Seemann T, Ryan CS, Kahler CM, Hill SA (2014) The use of high-throughput DNA sequencing in the investigation of antigenic variation: application to *Neisseria* species. *PLoS One* 9(1):e86704.
11. Dietrich M, Bartfeld S, Munke R, Lange C, Ogilvie LA, Friedrich A, Meyer TF (2011) Activation of NF-**κ**B by *Neisseria gonorrhoeae* is associated with microcolony formation and type IV pilus retraction. *Cell Microbiol*. 13(8):1168-82.
12. Drake SL, Koomey M (1995) The product of the pilQ gene is essential for the biogenesis of type IV pili in *Neisseria gonorrhoeae*. *Mol Microbiol*. 18:975–986.
13. Drake SL, Sandstedt SA, Koomey M (1997) PilP, a pilus biogenesis lipoprotein in *Neisseria gonorrhoeae*, affects expression of PilQ as a high-molecular-mass multimer. *Mol Microbiol.* 23(4):657-68.
14. Elkins C, Thomas CE, Seifert HS, Sparling PF (1991) Species-specific uptake of DNA by gonococci is mediated by a 10-base-pair sequence. *J Bacteriol.* 173:3911–3913.
15. Folster JP, Dhulipala V, Nicholas RA, Shafer WM (2007) Differential regulation of ponA and pilMNOPQ expression by the MtrR transcriptional regulatory protein in *Neisseria gonorrhoeae*. *J Bacteriol.* 189(13):4569-77.
16. Freitag NE, Seifert HS, Koomey M (1995) Characterization of the *pilF-pilD* pilus-assembly locus of *Neisseria gonorrhoeae*. *Mol Microbiol.* 16:575–586.
17. Fussenegger M, Rudel T, Barten R, Ryll R, Meyer TF (1997) Transformation competence and type-4 pilus biogenesis in *Neisseria gonorrhoeae*--a review. *Gene* 192:125–134.
18. Guzzo CR, Salinas RK, Andrade MO, Farah CS (2009) PILZ protein structure and interactions with PILB and the FIMX EAL domain: implications for control of type IV pilus biogenesis. *J Mol Biol*. 393:848–866.
19. Hamilton HL, Dillard JP (2006) Natural transformation of *Neisseria gonorrhoeae*: from DNA donation to homologous recombination. *Mol Microbiol.* 59:376–385.
20. Hansen JK, Demick KP, Mansfield JM, Forest KT (2007) Conserved regions from *Neisseria gonorrhoeae* pilin are immunosilent and not immunosuppressive. *Infect Immun*. 75:4138–4147.
21. Helaine S, Dyer DH, Nassif X, Pelicic V, Forest KT (2007) 3D structure/function analysis of PilX reveals how minor pilins can modulate the virulence properties of type IV pili. *Proc Natl Acad Sci USA.* 104:15888–15893.
22. Hospenthal MK, Costa TRD, Waksman G (2017) A comprehensive guide to pilus biogenesis in gram-negative bacteria. *Nat Rev Microbiol.* 15:365–379.

23. Hu LI, Yin S, Ozer EA, Sewell L, Rehman S, Garnett JA, Seifert HS (2020) Discovery of a new *Neisseria gonorrhoeae* type IV pilus assembly factor, TfpC. *mBio*. 11(5):e02528-20.
24. Jennings MP, Jen FE, Roddam LF, Apicella MA, Edwards JL (2011) *Neisseria gonorrhoeae* pilin glycan contributes to CR3 activation during challenge of primary cervical epithelial cells. *Cell Microbiol.* 13(6):885-896.
25. Jolley KA, Bray JE, Maiden MCJ (2018) Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* 3:124.
26. Jonsson AB, Rahman M, Normark S (1995) Pilus biogenesis gene, pilC, of *Neisseria gonorrhoeae*: pilC1 and pilC2 are each part of a larger duplication of the gonococcal genome and share upstream and downstream homologous sequences with opa and pil loci. *Microbiol Read Engl.* 141(Pt 10):2367–2377.
27. Kurre R, Höne A, Clausen M, Meel C, Maier B (2012) PilT2 enhances the speed of gonococcal type IV pilus retraction and of twitching motility. *Mol Microbiol.* 86:857–865.
28. Maier B, Wong GCL (2015) How bacteria use type IV pili machinery on surfaces. *Trends Microbiol.* 23:775–788.
29. Mehr IJ, Seifert HS (1998) Differential roles of homologous recombination pathways in *Neisseria gonorrhoeae* pilin antigenic variation, DNA transformation and DNA repair. *Mol Microbiol.* 30:697–710.
30. Meyer TF, Billyard E, Haas R, Storzbach S, So M (1984) Pilus genes of *Neisseria gonorrheae*: chromosomal organization and DNA sequence. *Proc Natl Acad Sci USA*. 81:6110–6114.
31. Obergfell KP, Seifert HS (2016) The pilin N-terminal domain maintains *Neisseria gonorrhoeae* transformation competence during pilus phase variation. *PLoS Genet.* 12:e1006069.
32. Prister LL, Yin S, Cahoon LA, Seifert HS (2020) Altering the *Neisseria gonorrhoeae pilE* guanine quadruplex loop bases affects pilin antigenic variation. *Biochem*. 59:1104–1112.
33. Punsalang AP, Sawyer WD (1973) Role of pili in the virulence of *Neisseria gonorrhoeae*. *Infect Immun.* 8:255–263.
34. Rohrer MS, Lazio MP, Seifert HS (2005) A real-time semi-quantitative RT-PCR assay demonstrates that the *pilE* sequence dictates the frequency and characteristics of pilin antigenic variation in *Neisseria gonorrhoeae*. *Nucleic Acids Res.* 33:3363–3371.
35. Rotman E, Seifert HS (2014) The genetics of *Neisseria* species. *Annu Rev Genet* 48:405–431.
36. Rotman E, Webber DM, Seifert HS (2016) Analyzing *Neisseria gonorrhoeae* pilin antigenic variation using 454 sequencing technology. *J Bacteriol.* 198:2470–2482.
37. Segal E, Hagblom P, Seifert HS, So M (1986) Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. *Proc Natl Acad Sci USA.* 83:2177–2181.
38. Seifert HS (1997) Insertionally inactivated and inducible *recA* alleles for use in *Neisseria*. *Gene* 188: 215–220.
39. Siewering K, Jain S, Friedrich C, Webber-Birungi MT, Semchonok DA, Binzen I, Wagner A, Huntley S, Kahnt J, Klingl A, Boekema EJ, Søgaard-Andersen L, van der Does C (2014) Peptidoglycan-binding protein TsaP functions in surface assembly of type IV pili. *Proc Natl Acad Sci USA*. 111(10):E953-961.
40. Steichen CT, Cho C, Shao JQ, Apicella MA (2011) The *Neisseria gonorrhoeae* biofilm matrix contains DNA and an endogenous nuclease controls its incorporation. *Infect Immun.* 79:1504–1511.
41. Stohl EA, Dale EM, Criss AK, Seifert HS (2013) *Neisseria gonorrhoeae* metalloprotease NGO1686 is required for full piliation, and piliation is required for resistance to H2O2- and neutrophil-mediated killing. *mBio* 4:e00399-13.
42. Tammam S, Sampaleanu LM, Koo J, Sundaram P, Ayers M, Chong PA, Forman-Kay JD, Burrows LL, Howell PL (2011) Characterization of the PilN, PilO and PilP type IVa pilus subcomplex. *Mol Microbiol*. 82(6):1496-1514.
43. Tønjum T, Freitag NE, Namork E, Koomey M (1995) Identification and characterization of pilG, a highly conserved pilus-assembly gene in pathogenic *Neisseria*. *Mol Microbiol*. 116(3):451-464.
44. UniProt Consortium. (2025) UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res.* 53: D609–D617.
45. Winther-Larsen HC, Hegge FT, Wolfgang M, Hayes SF, van Putten JP, Koomey M (2001) *Neisseria gonorrhoeae* PilV, a type IV pilus-associated protein essential to human epithelial cell adherence. *Proc Natl Acad Sci USA*. 98:15276-15281.
46. Winther-Larsen HC, Wolfgang M, Dunham S, van Putten JP, Dorward D, Løvold C, Aas FE, Koomey M (2005) A conserved set of pilin-like molecules controls type IV pilus dynamics and organelle-associated functions in *Neisseria gonorrhoeae*. *Mol Microbiol.* 56:903-917.

47. Wolfgang M, Park HS, Hayes SF, van Putten JP, Koomey M (1998) Suppression of an absolute defect in type IV pilus biogenesis by loss-of-function mutations in pilT, a twitching motility gene in *Neisseria gonorrhoeae*. *Proc Natl Acad Sci USA*. 95:14973–14978.
48. Wolfgang M, van Putten JP, Hayes SF, Koomey M (1999) The comP locus of *Neisseria gonorrhoeae* encodes a type IV prepilin that is dispensable for pilus biogenesis but essential for natural transformation. *Mol Microbiol.* 31:1345–1357.