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Sticky and sweet: the role of post-translational modifications on neisserial pili

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A commentary on

***Neisseria gonorrhoeae* pilin glycan contributes to CR3 activation during challenge to primary cervical epithelial cells**
by Jennings, M. P., Jen, F. E.-C., Roddam, L. F., Apicella, M. A., and Edwards, J. L. (2011). Cell. Microbiol. doi: 10.1111/j.1462-5822.2011.01586.x

Neisseria gonorrhoeae and *N. meningitidis* are obligate human pathogens of medical importance which are highly related at the genomic level and have a conserved array of pathogenicity determinants (Virji, 2009). *N. meningitidis* resides in the human nasopharynx and is the causative agent of transmissible meningitis and septic shock (Stephens et al., 2007). *N. gonorrhoeae* is a resident of the urogenital tract, is sexually transmitted and is the causative agent of gonorrhoeae and pelvic inflammatory disease in women (Edwards and Apicella, 2004). Both organisms colonize the mucosal epithelial cell surface via Type IV pili (Tfp), which engages the I-domain containing integrins (Edwards and Apicella, 2004) triggering the recruitment of factors ultimately responsible for the formation and extension of pseudopodia from the epithelial cell (Virji, 2009). During this period, the bacteria proliferate on the cell surface forming microcolonies which eventually disperse on the surface of the epithelial cells. Intimate association between the bacterium and host cell is mediated by the bacterial opacity proteins (Opa) with the epithelial host cell receptor *c*arcino*e*mbryonic *a*ntigen-related *c*ell *a*dhesion *m*olecule (CEACAM, CD66) which triggers a cascade of signals resulting in internalization of the bacteria into the host cell (Virji, 2009).

Not only is Tfp required for the initial attachment events to the host cell surface, it is also involved in twitching motility (Merz et al., 2000). Tfp is extruded through an outer membrane secretin, PilQ, and is retracted by the motor protein PilT into the periplasm where the fiber is depolymerized. Cycles of Tfp extension, surface binding, and subsequent retraction result in the ability of the bacterium to crawl across a solid surface. Retraction of a single Tfp fiber generates forces of 50–100 pN, however, this is greatly enhanced when the fibers form bundles, typically containing 8–10 strands, which are co-operatively retracted resulting in the generation of forces in the nanonewton range (Biais et al., 2008). Thus the Tfp motor of *Neisseria sp.* is one of the most powerful biological motors currently known. Recent work has now shown that post-translational modifications to the pilin subunit, PilE, are the major contributors to the formation and disaggregation of bundled pili.

PilE is expressed as a prepilin which undergoes processing by a prepilin peptidase, PilD, during export into the periplasm where subunits are arranged in a helix for extrusion through PilQ to form the shaft of the pilus. During this process of transport, cleavage and polymerization, PilE also undergoes multisite hierarchical post-translational modification (Aas et al., 2006). These O-linked additions are zwitterionic polar residues such as phosphocholine (PC), phosphoethanolamine (PE), and phosphoglycerol (PG), in addition to a separate glycan subunit of variable composition (Aas et al., 2006). The addition of PC and PE to PilE is catalyzed by the *p*ilin *p*hosphoethanolamine *t*ransferase (PptA; Aas et al., 2006) which can add up to five residues (four PE and one PC) to serine 68 and serine 156 of PilE from *N. gonorrhoeae* strain MS11 (Aas et al., 2006). The appearance of PC is due to the conversion of PE to PC by an unknown mechanism after attachment to PilE (Naessan et al., 2008). Variable substitution patterns can arise through the phase variable expression of PptA and the presence of the pilin-like protein, PilV, in the shaft (Aas et al., 2006). The addition of phosphoglycerol (PG) is catalyzed by PptB which adds a single residue to either serine 69 (100% occupancy) or serine 93 (15% occupancy) of PilE from *N. meningitidis* strain 8013 (Chamot-Rooke et al., 2011). The equivalent positions in the gonococcal PilE from gonococcal strain MS11 are occupied with phosphoserine (serine 68) and PG (serine 94; Forest et al., 1999). Why there is a difference in the occupancy of serine 68/69 with PG has so far not been explored. However, both PilE structures are glycosylated with different glycans at serine 63 and therefore these attachments may influence the availability of serine 68/69 to PptB. Chamot-Rooke et al. (2011) have shown that the occupancy of PG on PilE controls the ability to form bundled pili. To demonstrate this, they used *N. meningitidis* strain 8013 which has pilin decorated with GATDH and two PG residues. They showed that PptB is upregulated during attachment to host cells, leading to increased substitution of PilE with PG, ultimately resulting in the separation of the bundled pili into single strands. This observation was used to suggest that the bacterial cells without bundled pili were being released from the microcolony for further dissemination across the host cell surface.

Depending on the host strain, glycosylation at serine 63 can consist of the addition of an O-linked trisaccharide (Oac) Gal(β1-4)Gal(α1-3)2,4-diacetamido-2,4,6-trideoxyhexose (DATDH) or truncated versions thereof (Power et al., 2003). The genes encoding the enzymes required for the synthesis and transfer of the glycan to PilE have been named *p*ilin *g*lycosylation (*pgl*) genes. Truncated glycan variants, in which the terminal O-acetyl group (OAc) is missing on the trisaccharide or in which either one or both galactosyl groups are absent, are generated by the phase variable expression of the O-acetyl transferase, PglI (Warren et al., 2004), and the galactosyl transferases, PglA ,and PglE (Power et al., 2003). DATDH is synthesized by the concerted action of three enzymes PglBCD. In a subset of meningococcal isolates, a variant glycosyl group, glyceroamido acetamido trideoxyhexose

(GATDH), replaces DATDH at the glycosylation site (Chamot-Rooke et al., 2007). The synthesis of this residue is due to the presence of a variant of PglB, PglB2, and the acquisition of a new ORF (Orf8) with homology to L-2-haloalkanoic acid dehalogenase (Kahler et al., 2001) which is believed to result in the modification of DATDH to GATDH (Chamot-Rooke et al., 2007). The glycan trisaccharide is built as a lipid linked oligosaccharide on the inner surface of the cytoplasmic membrane and transferred to the periplasmic face by the flippase, PglF (Kahler et al., 2001, Power et al., 2003). The glycan group is then transferred en-block onto PilE by pilin glycosylation ligase, PglL (Power et al., 2006), which is promiscuous for many substrates (Faridmoayer et al., 2008).

Original work using *N. meningitidis* strain 8013 indicated that Tfp biogenesis structure and function was not affected by the presence of the GATDH on PilE (Marceau et al., 1998). However, Jennings et al. (2011) have now provided direct evidence for the involvement the pilin glycan in events leading to invasion of *N. gonorrhoeae* into host cells. To demonstrate this, they used *N. gonorrhoeae* strain strain MS11 which has the trisaccharide at position serine 63 and a low level of PG at serine 94 and obtained similar results with strain 1291. They have shown that non-glycosylated mutants displayed an initial slight increase in association (adherence plus invasion) with primary human cervical epithelial (pex) cells within the first 15 min, but this gradually decreased with time. Competitive inhibition assays demonstrated that purified pili decorated with glycan was more effective than non-glycosylated pili in blocking this initial phase of bacterial association with the host cell. However, this effect was not the result of a loss in adhesiveness of the non-glycosylated pilin for the I-domain of CR3. However, the I-domain of CR3 is dynamically equilibrated between two forms, an open (high-affinity) and closed (low-affinity) conformation, which modulates the binding of ligands to the receptor. Using recombinant I-domains in the high and low affinity states, they demonstrated that both the mono- or disaccharide forms of pilin interact with the low-affinity, closed I-domain of CR3. They hypothesized that the interaction between the glycan of pili and the closed I-domain may lead to the "opening" of the I-domain of CR3 for the engagement of multiple binding sites within the integrin receptor, thus resulting in high affinity binding. They observed that only 30% of CR3 on non-infected pex cells were present in the high affinity conformation. However, upon exposure to gonococcal strains expressing glycosylated pilin the concentration of active high affinity CR3 rose on the pex cell surface. Conversely, gonococcal strains expressing pili without the glycan, PC or PE had no effect on CR3 activation.

In conclusion, a "sticky and sweet" model has emerged that explains the roles of post-translational modifications to pilin during attachment of *Neisseria sp.* to host cells. The dynamic expression of the zwitterionic polar residues on PilE determines the formation and disaggregation of bundled pili thus governing the release of bacteria from microcolonies. Conversely, the dynamic expression of the glycan on PilE leads to the engagement of the I-domain of CR3 to promote attachment to the host cell surface. There are many outstanding questions regarding this work. What is the distribution of CR3 on nasopharyngeal mucosal surfaces and does it play a role in the ability of meningococci expressing the trisaccharide to colonize this niche? Although no role for GATDH on PilE from meningococci could be found, was this the result of using cell lines that may not express the appropriate receptor? And lastly, is this "sticky and sweet" mechanism of engagement with host cells shared with the non-pathogenic *Neisseria sp.*? *N. lactamica,* a commensal of the human nasopharynx, also expresses glycosylated Tfp which appear to be indistinguishable from that of gonococci and meningococci (Bourd et al., 2010). Therefore, is this mechanism of glycosylated Tfp mediated engagement with host cells related to commensalism or virulence? It will be very interesting to see the resolution of these aspects of neisserial biology.

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