been predicted from comparisons of open binary and closed ternary crystal structures (Johnson et al., 2003; Yin and Steitz, 2004), the simulations reported in Golosov et al. (2010) give the first indications that glycines 711 and 715 are conserved in the A-family DNA polymerases to allow for a specific flexibility in the O- and O1-helices. The importance to translocation of a flexible O-helix is unclear, since bending of the helix during the dynamics simulation occurs before DNA movement, but such flexibility could be the key for binding of the next incoming nucleotide and fingers closure, a phase that may also be studied informatively using the same computational methods.

This work highlights how computational approaches can assist in the design of site-directed mutagenesis, as well as kinetic, crystallographic, and single-molecule experimental approaches (Joyce, 2009) that are necessary to acquire a deep understanding of complex processes. Molecular dynamics is one of the few general methods available to model transient structural states in large molecular machines at the atomic level. As more structures that define a single reaction pathway become available, targeted or steered computational methods are likely to become increasingly important tools in the analysis and understanding of dynamic molecular machines.

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Catching Pneumonia

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Many Gram-positive bacteria have pili attached to their cell walls, but they are much simpler and shorter than their more familiar Gram-negative analogs. The structure of an "adhesin" from the tip of the pneumococcal pilus (Izoré et al., 2010) reveals intradomain insertions of eukaryotic origin that may hold the key to systemic invasion.

Many pathogenic bacteria have evolved to establish themselves in one organ or locale, to move on when conditions are appropriate, and to become systemic should the host be considered dispensable. One such pathogen is *Streptococcus pneumoniae* (sometimes called "pneumococcus"), a major causative agent of pneumonia, bacterial meningitis, and bacteremia/sepsis. It is the primary killer of children in the developing world, and despite the availability of antibiotics, remains a serious threat to the elderly (Finn and Jenkinson, 2006). It is also one of the opportunistic organisms that hastens death, applying the coup-de-grâce as the immune system and major organs begin to fail, giving rise in the nineteenth century to its rather macabre label as "the old man's friend."

As the first step, bacteria must recognize a specific surface of the host target tissue. This often occurs on the mucosal surfaces of the nasal passages and upper respiratory tract, and is mediated by proteins called "adhesins." Adhesins often contain several adhesive domains that recognize distinct host targets either with broad or fine specificity. Many bacteria augment this process by attaching adhesins to long appendages called either pili (singular = pilus = hair) or fimbriae (singular = fimbria = thread or fiber).

The highly versatile helical pili of Gram-negative bacteria were first described nearly a century ago; they are long and (relatively) thick, inserted into the outer membrane, and easily observable by the optical microscope. It is less well known that many Gram-positive bacteria have pili too, attached by covalent bonds to their peptidoglycan cell walls. But their organization is quite different; they are much thinner and shorter than their Gram-negative counterparts, and were first observed in *Corynebacteria* forty years ago using electron microscopy. Even earlier, in the 1930s and 1940s, the microbiologist Rebecca Lancefield isolated the protein components and showed that they were extraordinarily stable, strain-specific antigens (Lancefield, 1933). Although known well enough in the field of oral hygeine, Gram-positive

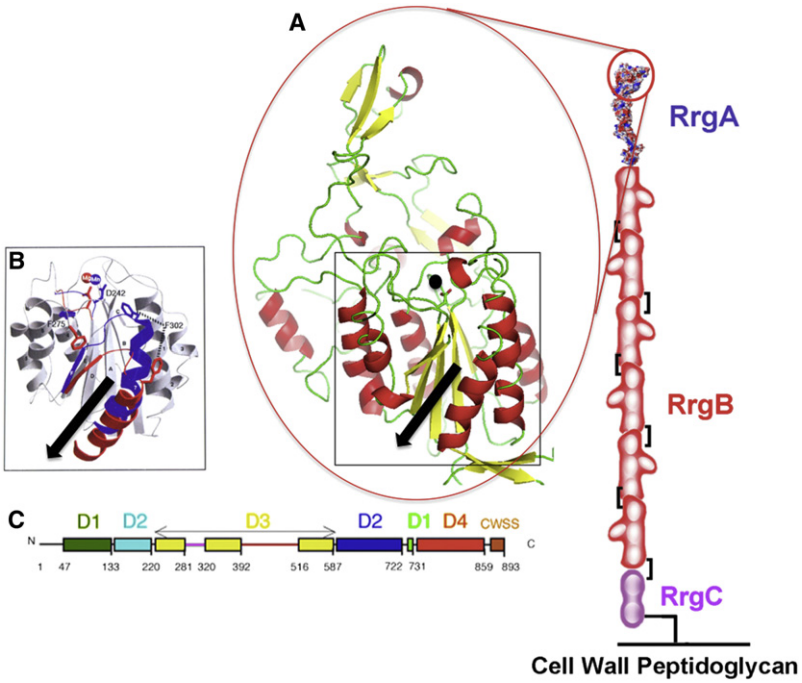

Figure 1. The Pilus of *S. pneumonaie* May Mediate Cell Binding in the Bloodstream
(A) Schematic assembly of the pilus, and structure of the distal domain (D3) of RrgA. Black dot indicates Mg^{2+} ion at the MIDAS motif.
(B) Overlay of two conformations of the I domain of integrin αMβ2. Equivalent region of RrgA is boxed. Black arrow points to conformational switch to a more extended high affinity, state promoted by hydrodynamic shear forces.
(C) Domain organization of RrgA showing "serial insertion" of domains.

pili were otherwise largely ignored by the majority of microbiologists until very recently.

Olaf Schneewind and colleagues may be credited for leading the renaissance in functional and structural analysis of Gram-positive pili (Ton-That et al., 2004). The components of pili are encoded by a gene cluster that typically includes sortases (the enzymes that catalyze the formation of intersubunit isopeptide bonds), and three structural proteins: the major "pilin" that forms the shaft, and two minor pilins that resemble adhesins and were originally thought to decorate the pilus shaft. Very recently, however, a definitive electron microscopy (EM) analysis of the pilus from *S. pneumoniae* (Hilleringmann et al., 2009) has demonstrated what is a much simpler organization at first glance (Figure 1). Although the major pilin proteins (RrgB) stack end to end to form the shaft as expected, the big surprise was that the pilus contains just two minor pilins, one at either end of the tip: the "proximal pilin" (RrgC) links the major pilin to the cell wall, and the "distal pilin" (RrgA) is presented at the end of the tip of the pilus furthest from the cell wall.

The first structure of a major pilin, from *Streptococcus pyogenes,* was published in 2007 (Kang et al., 2007). It showed how the pilin subunits, which form the shaft of pilus, are glued together by covalent isopeptide linkages, a process catalyzed by the sortase enzymes. The greater surprise was the presence of intramolecular isopeptides that form spontaneously within each subunit, between the side chains of lysine and either glutamate or asparagine. This chemistry requires harsh conditions in the test tube, but is achieved in the pilin simply through the close juxtaposition of the reactants in a hydrophobic environment and a general base (another glutamate) that presumably promotes deprotonation of the lysine side chain to create a nucleophile that can attack the amide or carboxylic carbon. The fact that this process seems to be both easy and spontaneous begs a larger question of how the other 99.9% of the proteome have evolved to avoid forming such bonds. It might be easier to address the "why," since proteins that are too stable are a liability inside the cell, as they cannot be readily proteolyzed when they have outlived their usefulness.

The structure of the distal pilin (RrgA) from *S. pneumoniae* (Izoré et al., 2010) reveals several new surprises, as well as intriguing insights into the evolution and the functions of its pilus (Figure 1). RrgA is a large protein comprising four major domains. Three of these may now be considered to cap the pilus shaft. The fourth domain, at the very tip, has a core that is a dead ringer for a eukaryotic integrin I domain. Although the general fold is found in some bacterial chelatases, it is much more likely (see below) that it has occurred through horizontal gene transfer, perhaps via a phage. In fact, *S. pneumoniae* is "naturally competent" for genetic transformation, which means that its genome takes up foreign DNA with great ease, and its genome shows extensive evidence for this (Hakenbeck et al., 2001).

As judged by the structures present in Protein Data Bank (PDB), successful domain insertion nearly always occurs at domain boundaries, where they are least likely to disturb folding of the mother protein (Selvam and Sasidharan, 2004). Insertions into loops of the mother protein are much less common, and serial insertions are very rare. The *rrga* gene seems to be a "serial inserter" (see Figure 1), suggesting that it offers a functional advantage. One prediction is that domain insertion stabilizes the daughter protein. Since the termini of the insert are fixed, this should reduce the entropy of the unfolded state and hence reduce the unfavorable entropy of folding (as in the case of disulfide bonds) if the mother domain is more stable, which it is; the mother domain is stabilized by isopeptide bonds as well as a Ca^{2+} coordination site.

To become systemic and cause meningitis, the bacteria must first enter the bloodstream, where they may replicate rapidly, causing bacteremia and septic shock. But in order to cross the blood-brain barrier, they must first attach to the blood vessel wall. This is much more difficult, since once attached, they are immediately subjected to the force created by the flow of blood. This force is called hydrodynamic "shear," because the blood flows faster in the center of the vessel. One way that attachment might

be achieved is through the use of "catch bonds," bonds that counterintuitively get stronger as the force that would break them increases. This attractive but controversial theory has found much support recently, from the study of both integrins (Kong et al., 2009) and an adhesin from a Gram-negative pilus, FimH (Tchesnokova et al., 2008).

And so, returning to the integrin I domain within RrgA, it is either of eukaryotic origin or a remarkable example of convergent evolution, and I suspect the former. In integrins, engagement by ligands on endothelial cells that line the vasculature triggers a conformational change involving co-engagement of a Mg^{2+} ion at the MIDAS motif (Emsley et al., 2000), which transduces a signal across the plasma membrane. Judged by its sequence, the RrgA I domain preserves both the structural and mechanistic parts of this machine. It will be of great interest to see if *S. pneumoniae* has indeed stolen a piece of its host to perform the same feat as platelets and leukocytes, which arrest on the blood vessel wall in an integrin-dependent fashion.

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