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were not templated, with a strong association of these untemplated changes with the region just 5′ of a templated change on the *vlsE* coding strand. This junction thus may represent a hot spot for repair errors during the recombination process. The mechanism for this increased error rate has not as yet been determined; the only gene products that have been shown to facilitate *vlsE* recombination are RuvAB that constitute a branch migrase [7,8], which would not be involved directly in strand synthesis or ligation. One limitation of the analysis is that PacBio sequencing has an inherently high error rate, even with the SMRT-Bell modification; although filtration is used, it is likely that at least some of the untemplated changes identified are actually sequence errors.

Other findings of the study include an estimate of the *vlsE* switch rate (approximately 0.033 per cell generation) [3]; it is of interest that *vlsE* recombination has not been reported during *in vitro* culture or tick infection, indicating that the *in vivo* environment may change gene expression or the activity of protein or nucleic acid cofactors in this process. As observed previously, there was some preference of certain silent cassettes (e.g., 4, 5, and 6) as sources for DNA sequences. There was also a decreased switch rate associated with cassettes that lack one or both 17 bp direct repeats that typically interconnect the silent cassettes, although it is unclear whether this finding may be related to whether these cassettes also have truncations or internal deletions. The average length of the recombination events was longer in *B. burgdorferi* recovered from immunocompetent mice as opposed to immunodeficient mice, which, as indicated by the authors, is likely due to immunologic selection of organisms in which VlsE has more sequence differences and thus fewer shared epitopes with preceding clones. Finally, switching events tended to be clustered, and their ends also were preferentially localized in regions of high homology between *vlsE* and the donating silent cassette. It is of interest that multimeric G stretches, which are common on the coding strand of the *vlsE* cassette region, were not associated with switch region boundaries. These poly-G stretches would likely affect DNA replication and transcription rates, although not as much as the more complex quadruplex G structures.

The article by Verhey *et al.* represents a significant advance, both quantitatively and qualitatively, in our knowledge of the nature of *vlsE* recombination events. However, the Lyme disease spirochetes are elusive beasts, and we still have quite a way to go to ‘catch up’ in our understanding how this immune evasion mechanism works. Perhaps future studies will overcome the current barrier regarding genetic manipulation of the *vls* system, fueling a sprint toward identifying the *cis*- and *trans*-acting elements important in this process.

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Forum

Mechanomicrobiology: How Mechanical Forces Activate *Staphylococcus aureus* Adhesion

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During colonization of biomaterials and host tissues, surface-attached bacteria are subjected to mechanical stresses, including hydrodynamic flow and cell-surface contacts. Two publications show that mechanical force activates the adhesive function of *Staphylococcus aureus* surface proteins, thereby providing the pathogen with a means to withstand high shear stress during colonization.

Bacteria that colonize surfaces must cope with physical stresses, such as fluid flow and cell-surface interactions [1]. There is compelling evidence that mechanics can have a profound influence on bacterial behaviors, such as cell adhesion, but how exactly cell-surface components respond to mechanical cues is poorly understood. A well known example of stress-induced adhesion mechanism is found in the prototypical adhesin FimH of *Escherichia coli*. FimH binds mannose residues on epithelial cells via a catch bond, that is, a receptor–ligand bond that is strengthened by mechanical force [2,3]. The FimH–mannose bond plays an

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important role in urinary tract infections by promoting strong adhesion at a high flow rate. The underlying mechanism involves a force-induced allosteric switch to a high-affinity, strong binding conformation of the adhesin. In a review published in 2008 [3], the authors suggested that catch-bond mechanisms are likely to be widespread among bacterial adhesins. Today, we must recognize that while shear-enhanced adhesion has been described for a variety of microbial species, there is still very little direct evidence for the existence of catch bonds. Perhaps a major reason for this is the paucity of single-molecule tools for studying the mechanical response of adhesins.

During the colonization of human tissues, *Staphylococcus aureus* is subjected to mechanical forces associated with fluid flow, cell-surface contacts or epithelial turnover [4]. To resist mechanical stress, the pathogen uses a family of surface proteins that promote cell adhesion to host extracellular proteins such as fibronectin and fibrinogen, as well as cell–cell adhesion and biofilm formation. Flow experiments have revealed that *S. aureus* adhesion can be enhanced under high

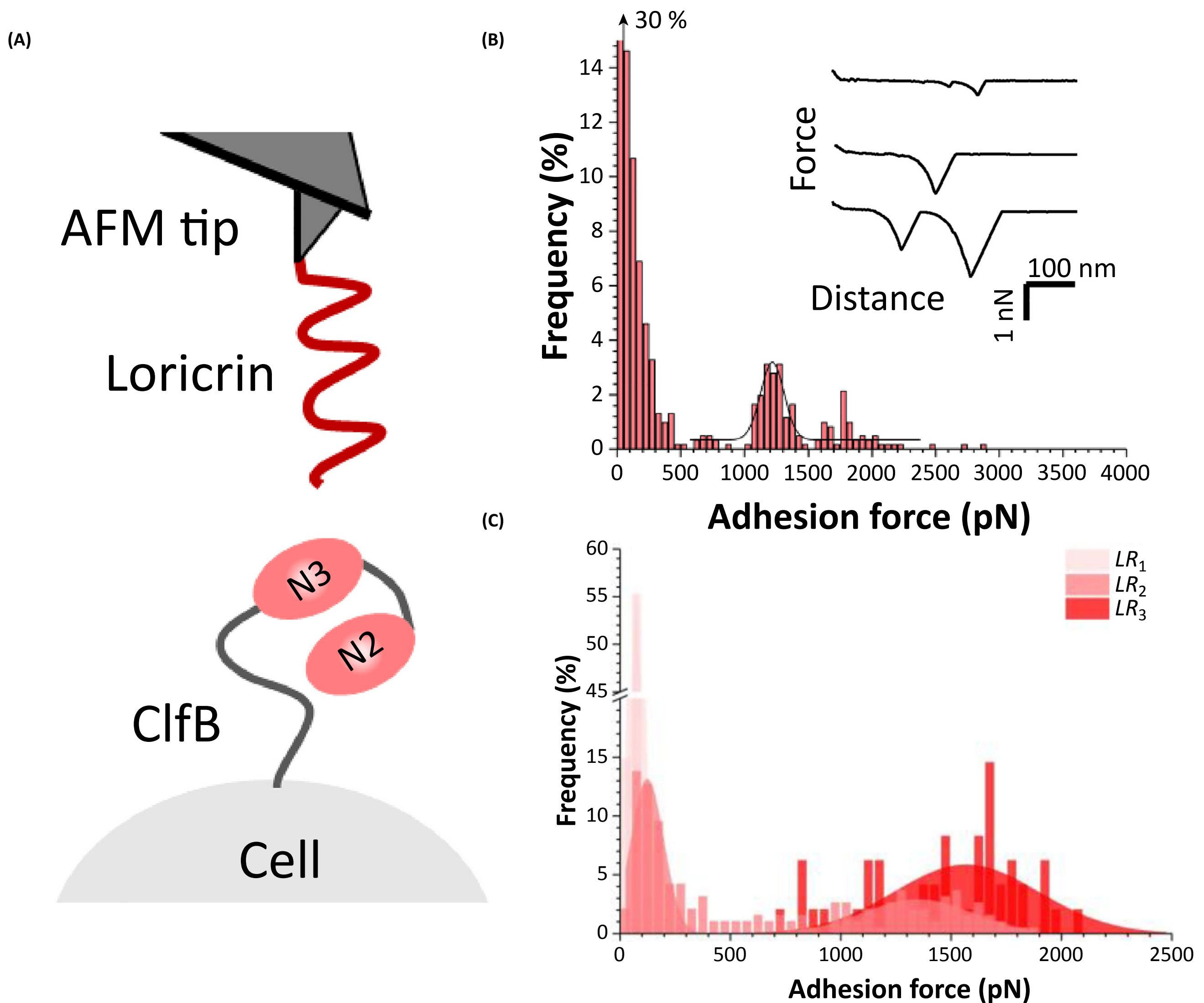

Figure 1. The ClfB–Loricrin Bond Is Dramatically Strengthened by Mechanical Tension. (A) Single-molecule analysis of the ClfB–loricrin interaction. For the sake of clarity the N1 subdomain of ClfB is not shown. (B) ClfB binds to loricrin through weak and strong interactions. (C) The probability of forming strong bonds increases when mechanical force is applied rapidly. AFM, atomic-force microscopy. Adapted with permission from [7] and *mBio*.

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shear. Binding of fibrinogen by the cell-surface protein clumping factor A (ClfA) promotes platelet capture and thrombus formation under high-shear conditions [5]. ClfA is also involved in the shear-dependent adhesion of *S. aureus* to von Willebrand factor (VWF) [6]. While these reports show that *S. aureus* adhesion can be strengthened under high shear, how this is achieved at the molecular level is not known.

Two recent studies have demonstrated that the *S. aureus* adhesins ClfA and ClfB behave as force-sensitive molecular switches that potentiate staphylococcal adhesion under mechanical stress [7,8]. The results highlight the importance of mechanobiology in regulating staphylococcal adhesion. Central to these discoveries is atomic-force microscopy (AFM), which offers unprecedented opportunities to study bacterial cell walls at the single-molecule level [9,10]. AFM enables researchers to measure the localization, adhesion, elasticity, and interactions of individual surface components, thereby providing novel insights into cellular structure and function. Importantly, unlike classical assays such as surface plasmon resonance (SPR), AFM probes functional adhesins directly in living cells, thus in their biologically relevant conformation and environment. Using this technology, Vitry *et al*. [7] studied the influence of mechanical force on the interaction between ClfB and the squamous epithelial cell envelope protein loricrin (Figure 1). To quantify the strength of single ClfB bonds, they measured the forces between AFM probes functionalized with ligands and bacterial cells expressing the adhesin (Figure 1A). They found that ClfB mediates *S. aureus* adhesion to loricrin through weak and strong molecular bonds (Figure 1B). Strong bonds are among the strongest receptor–ligand bonds measured so far (~1500 pN) and originate from a multistep 'dock, lock, and latch' (DLL) interaction. AFM and steered molecular dynamics simulations recently revealed that the extreme mechanical stability of DLL bonds originates from an intricate hydrogen bond network between the ligand peptide backbone and the adhesin. So the high binding strength results from the simultaneous rupture of numerous hydrogen bonds [11]. Such ultrastrong binding forces are in contrast to the classical affinity values measured by SPR, indicating that single-molecule forces (at nonequilibrium) and binding constants (at equilibrium) are not correlated. This observation suggests that the strength of molecular bonds measured under force is more relevant than the affinity constant to describe bacterial adhesion under shear. The team next demonstrated that the ClfB–loricrin bond is dramatically strengthened by mechanical tension, that is, weak bonds dominate at low stress, while strong bonds are favored at high stress (Figure 1C). These findings favor a two-state model whereby bacterial adhesion to loricrin is enhanced through force-induced conformational changes in the ClfB molecule, from a weakly binding folded state to a strongly binding extended state.

Single-molecule AFM has also been used to unravel the force-sensitive binding mechanism of ClfA. The interaction between ClfA and the plasma protein fibrinogen (Fg) is weak at low tensile force, but is dramatically enhanced (~1500 pN) by mechanical tension (Figure 2). Strong bonds, but not weak ones, are inhibited by a peptide mimicking the C terminal segment of the Fg γ-chain. These results point to a mechanism whereby ClfA interacts with Fg via two distinct binding sites, whose adhesive function is regulated by mechanical tension (Figure 2). In the absence of tensile force, Fg binds to the top of the ClfA N3 domain via weak bonds. Under mechanical tension, extension and conformational changes in the ClfA molecule triggers a DLL interaction by the ClfA N2N3 subdomains.

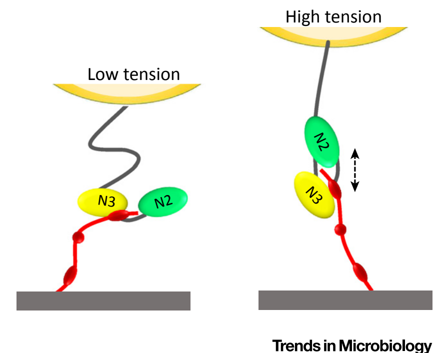

Figure 2. ClfA Interacts with Fg via Two Distinct Binding Sites, Whose Adhesive Function Is Tightly Regulated by Mechanical Tension. Under low force, Fg weakly binds to the top of the ClfA N3 domain. Under high force, extension and conformational changes of the ClfA N2N3 subdomains enable the γ-chain of Fg to dock in the ligand-binding trench and form strong DLL-like bonds. Adapted with permission from [8].

The single-molecule studies discussed here demonstrate that *S. aureus* adhesins feature unanticipated mechanical responses when subjected to mechanical stress. The force-enhanced adhesion of ClfA and ClfB is reminiscent of a catch-bond mechanism, which until now has never been reported for *S. aureus*. A unique feature of the ClfA/B-ligand complexes is their very high mechanical stability, that is, they can withstand forces equivalent to that of covalent bonds without breaking the polypeptide backbones. This intriguing phenomenon suggests that the complex may direct force along pathways nonparallel to the pulling direction, as reported for the mechanically stable multidomain cellulosome protein complex [12]. The force-dependent adhesion of ClfA and ClfB is of biological relevance as it enables *S. aureus* to finely modulate their adhesion to host tissues and implanted biomaterials, helping cells to attach firmly under high shear stress and to detach and spread under low stress. Perhaps *S. aureus* has evolved this sophisticated mechanism to provide the bacteria with a competitive advantage during colonization. We anticipate that single-molecule AFM experiments will

contribute to the identification of novel binding mechanisms in *S. aureus* adhesins, which may in turn help to develop novel antistaphylococcal strategies.

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