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REVIEW

The remarkably multifunctional fibronectin binding proteins of *Staphylococcus aureus*

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Abstract *Staphylococcus aureus* expresses two distinct but closely related multifunctional cell wall-anchored (CWA) proteins that bind to the host glycoprotein fibronectin. The fibronectin binding proteins FnBPA and FnBPB comprise two distinct domains. The C-terminal domain comprises a tandem array of repeats that bind to the N-terminal type I modules of fibronectin by the tandem β-zipper mechanism. This causes allosteric activation of a cryptic integrin binding domain, allowing fibronectin to act as a bridge between bacterial cells and the $\alpha_5\beta_1$ integrin on host cells, triggering bacterial uptake by endocytosis. Variants of FnBPA with polymorphisms in fibronectin binding repeats (FnBRs) that increase affinity for the ligand are associated with strains that infect cardiac devices and cause endocarditis, suggesting that binding affinity is particularly important in intravascular infections. The N-terminal A domains of FnBPA and FnBPB have diverged into seven antigenically distinct isoforms. Each binds fibrinogen by the 'dock, lock and latch' mechanism characteristic of clumping factor A. However, FnBPs can also bind to elastin, which is probably important in adhesion to connective tissue in vivo. In addition, they can capture plasminogen from plasma, which can be activated to plasmin by host and bacterial plasminogen activators. The bacterial cells become armed with a host protease which destroys opsonins, contributing to immune evasion and promotes spreading during skin infection. Finally, some methicillin-resistant *S. aureus* (MRSA) strains form biofilm that depends on the elaboration of FnBPs rather than polysaccharide. The A domains of the FnBPs can interact homophilically, allowing cells to bind together as the biofilm accumulates.

Introduction

The surface of *Staphylococcus aureus* cells is decorated with a diverse array of (up to 24) proteins that are anchored covalently to peptidoglycan by sortases [1, 2]. Many of the proteins have been named after the ligand or function with which they were first associated. Some were given generic names, either *S. aureus* surface (Sas) or serine aspartate repeat (Sdr) proteins, notations that could be changed when a ligand or function was subsequently discovered (e.g. SasH was changed to AdsA when its adenosine synthase activity was reported) [3].

The fibronectin binding proteins (FnBPs) were one of the first proteins of Gram-positive bacteria to be characterised. Initial studies focussed on the mechanism of binding to soluble fibronectin [4, 5]. Later, FnBPs were found to promote adhesion of bacteria to immobilised fibronectin [6]. This explained how bacteria could adhere to implanted medical devices to initiate foreign body infections. *Staphylococcus aureus* was then found to invade a variety of mammalian cells by a mechanism that involved fibronectin, forming a bridge between bacterial surface-located FnBPs and the $\alpha_5\beta_1$ integrin that is widely distributed on mammalian cells [7].

In fact, FnBPs are composite proteins with N-terminal domains that are related to the archetypal fibrinogen binding protein clumping factor A (ClfA), while the C-terminus comprises tandemly repeated fibronectin binding domains (Fig. 1) [8, 9]. Despite the A domains of ClfA and FnBPs each binding to fibrinogen by the same mechanism, they also bind to other ligands. ClfA binds complement factor I and is an important factor in promoting the evasion of neutrophil killing [10], while FnBPs bind to elastin [11] and plasminogen [12] and

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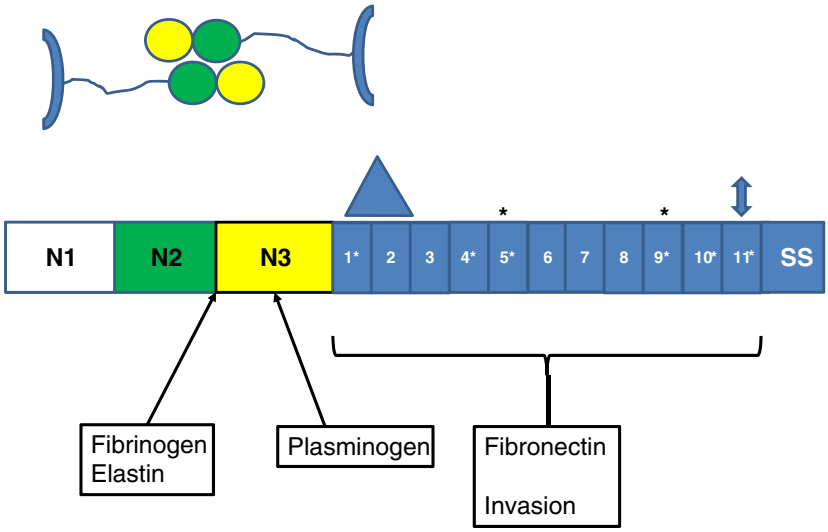

Fig. 1 Structure and organisation of fibronectin binding proteins (FnBPs). The figure summarises the structure and functions of FnBPs. The N-terminal A region of the proteins shown on the left comprises three separately folded subdomains N1, N2 and N3. N2 and N3 form IgG-like folds typical of the microbial surface components recognising adhesive matrix molecules (MSCRAMM) family. The fibronectin binding region usually comprises 10 or 11 tandemly repeated Fn binding domains. Typical FnBPA proteins have 11 fibronectin binding repeats (FnBRs), while FnBPBs have 10, as a result of a recombination event that deleted FnBR2 (indicated by the *triangle*). The numbered FnBRs with an *asterisk* bind Fn with high affinity. The *asterisk above the boxes* indicate FnBRs with amino acid substitutions that increase affinity for Fn. The *double-headed arrow* indicates the location of a substitution that occurs in some strains. The upper diagram indicates that FnBPs can bind each other homophilically, although the precise nature of the binding interaction is not known. *SS*, sorting signal including an LPXTG motif recognised by sortase resulting in anchorage to peptidoglycan

also promote biofilm formation [13]. This paper will review the structure and the diverse functions of FnBPs.

Structure and organisation

The N-terminal A domains of FnBPs comprise separately folded subdomains N1, N2 and N3 (Fig. 1) and are members of the microbial surface components recognising adhesive matrix molecules (MSCRAMM) family. This is defined by having two adjacent IgG-like folded regions with potential to bind ligands by the 'dock, lock and latch' (DLL) mechanism [1]. Located between the A domain and the cell wall is an extended unstructured region comprising tandemly arrayed fibronectin binding repeats (FnBRs) [7, 8]. Most strains express two FnBPs, FnBPA and FnBPB, that differ from each other by (i) the A domains having only about 40 % amino acid sequence identity and (ii) in the archetypal proteins from strain 8325, the FnBR region of FnBPB comprising 10 repeats compared to the 11 repeats in FnBPA. Analysis of the diversity of FnBPA domains in strains from different genetic backgrounds revealed that there are seven distinct isoforms of both FnBPA and FnBPB. They differ in amino acid sequence by between about 60–85 %, with the greatest differences occurring in subdomains N2 and N3 (FnBPA-I to FnBPA-VII and FnBPB-I to FnBPB-VII) [14, 15].Some strains, notably those from CC30 and CC45, do not express FnBPB. Their genomes carry a single *fnbA* gene rather than the closely linked and tandemly arrayed *fnbA* and *fnbB* genes found in the majority of *S. aureus* strains.

Functions of the A domains

Despite the variation in amino acid sequences comprising the N2 and N3 subdomains of FnBPA and FnBPB, both bind to the same site in fibrinogen as the archetypal MSCRAMM clumping factor A, the extreme C-terminus of the γ-chain. A detailed discussion of the DLL mechanism of ligand binding follows in the section below. However, FnBPA and FnBPB differ from ClfA in several respects (Table 1). (i) Both FnBPA and FnBPB bind to elastin, most likely by DLL [16, 17]. (ii) The A domain of FnBPB can bind to fibronectin by a different binding mechanism to the FnBRs [16]. (iii) The FnBPs are important in biofilm formation by some strains of methicillin-resistant *S. aureus* (MRSA). This occurs by homophilic interactions between A domains on neighbouring cells [13, 18, 19]. (iv) Both FnBPA and FnBPB can bind the host plasma protein plasminogen [12].

Binding to fibrinogen

The A domain of FnBPA isoform I (FnBPA-I) has been crystallised in the apo form and in complex with the fibrinogen (Fg) γ-chain peptide [20]. The structures are very similar to ClfA and indicate that ligand binding occurs by DLL. The

Table 1 Properties of the A domains of FnBPA and FnBPB

Ligand	FnBPA	FnBPB
Fibrinogen	SPBA: isoforms I–VII similar half maximum SPR: isoform I K_D 3.7 μM	SPBA: isoforms I–VII similar half maximum SPR: isoform I K_D 2 μM
Elastin	SPBA: isoforms II–VII similar half maximum SPBA: isoform I ~4-fold lower half maximum than II–VII	SPBA: isoforms I–VII similar half maximum SPR: isoform I K_D 3.2 μM
Fibronectin		SPBA: isoforms I–VII similar half maximum SPR: isoform I K_D 2.5 μM
Plasminogen	Only isoform I tested	SPBA: isoforms I–VII similar half maximum SPR: isoform I K_D 0.53 μM
Homophilic interactions	Only isoform I tested AFM: weak binding	Only isoform I tested

SPBA, solid phase binding assay. *SPR*, surface plasmon resonance. *AFM*, atomic force microscopy

FnBPB A domain has not been crystallised but molecular modelling predicted that the protein binds the γ-chain by DLL [16]. This is supported by amino acid substitution mutants of residues predicted to have key roles in ligand binding being defective in Fg binding. The C-terminus of the Fg γ-chain binds in the trench located between the N2 and N3 subdomains (Fig. 2). This triggers a conformational change in the C-terminus of subdomain N3, which locks the γ-chain peptide in place, forming a latch by β-strand complementation with a β-sheet in subdomain N2 [1, 21].

Recently, it was found that the mechanism of ClfA binding to Fg is more complicated than originally proposed. Fibrinogen binds two distinct sites in the ClfA A domain. The γ-chain peptide binds to the trench between N2 and N3

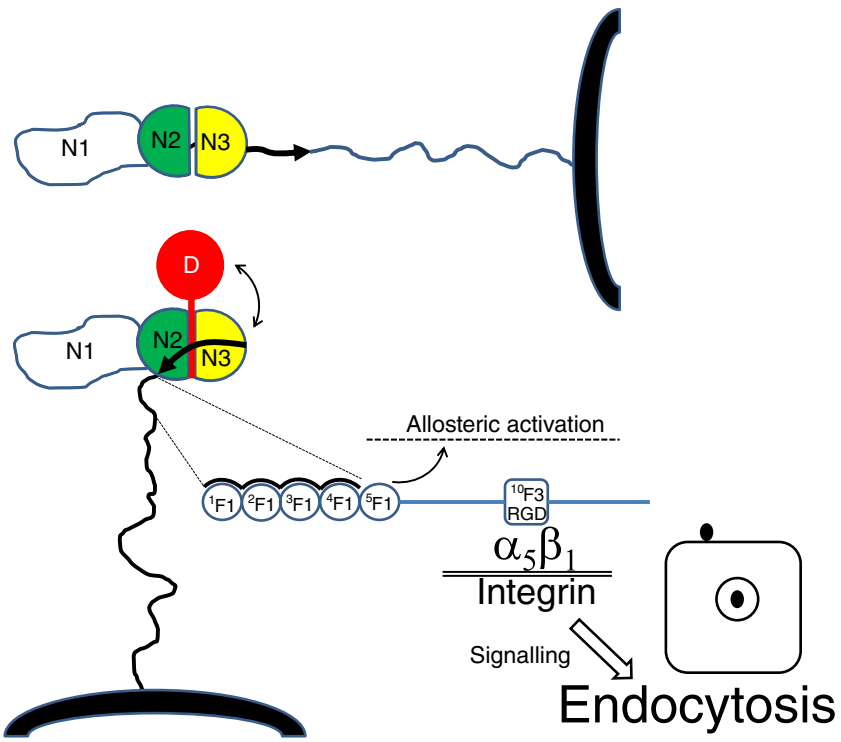

Fig. 2 Conformational changes involved in binding to fibrinogen, fibronectin and host cell invasion. Schematic diagrams illustrating the conformational changes that occur when the A domain of FnBPs bind to the γ-chain peptide that is part of the D domain of fibrinogen (in *red*). The *thick black arrow* emerging from the N3 subdomain in the upper diagram is the latching peptide that is redirected over the bound fibrinogen peptide to lock it in place. It also forms an additional β-strand in a β-sheet in subdomain N2. The *double-headed arrow* indicates the second binding site for Fg in ClfA, which probably does not occur in FnBPs. The lower figure also shows how part of the unstructured fibronectin binding region binds to the N-terminal type I modules by the tandem β-zipper mechanism. Potentially, up to nine such interactions can occur. Intramolecular interactions (*dashed line*) between the N-terminal type I modules and C-terminal type III modules result in allosteric activation of the type III module, exposing an RGD motif which engages an $\alpha_5\beta_1$ integrin to promote invasion by endocytosis

by DLL [22]. In addition, the A domain of ClfA also binds to residues in the Fg D domain at a binding site located on the top of subdomain N3 at a considerable distance from the peptide binding site [23]. The X-ray crystal structure of the ClfA A domain in complex with a function-blocking monoclonal antibody (mAb) bound to a site located on top of subdomain N3. Molecular modelling of ClfA in complex with the Fg D domain indicated that the mAb epitope and the Fg binding site partially overlap, and this was supported by substituting residues in the mAb epitope, which reduced the binding affinity for Fg.

However, it seems unlikely that FnBP binding to Fg also involves a two-site mechanism. The K_D of ClfA for Fg is higher than FnBPA (2.8-fold) or FnBPB (5-fold) [16, 24, 25]. The synthetic γ-chain peptide completely blocked binding of FnBPA to Fg, whereas it only reduced binding to ClfA by 50 %. Also, the affinity of FnBPA for full length Fg and the γ-chain peptide are very similar, whereas the affinity of ClfA for Fg is 10-fold higher than for the peptide. These data are consistent with a single-site DLL mechanism of Fg binding.

A truncated variant of FnBPA-I lacking the latching peptide has only a 2.5-fold lower affinity for Fg [25], which could either be interpreted to favour a second binding site or that the peptide alone is able to bind strongly to the trench. Variants of ClfA and FnBPA-I lacking the latch still bound Fg but the bound protein detached very quickly. The γ-chain peptide seems to bind avidly to the trench but requires the conformational change of locking and latching to be stabilised.

Binding to plasminogen

Staphylococcus aureus cells can capture plasminogen (Plg) from human plasma in a form that can be activated by host or bacterial plasminogen activators (tissue plasminogen activator and staphylokinase, respectively). The presence of the potent serine protease plasmin bound to the bacterial cell surface destroys bound antibody and complement [26] and, thus, promotes immune evasion and probably helps bacterial dissemination during infection. In contrast, a sortase-deficient mutant lacking cell wall-anchored (CWA) proteins bound much less Plg [12]. Both FnBPA and FnBPB bind Plg, but other CWA proteins also contribute. Plg bound to subdomain N3 at a site that did not overlap the Fg binding site. All seven isoforms of FnBPB bound Plg with similar affinities. Indeed, Plg bound to subdomain N3 with the same affinity as it bound to the intact N2N3 region. Several surface-located lysine residues in the N3 subdomain are conserved in all seven FnBPB isoforms. Changing these residues to alanines reduced Plg binding, indicating that they form part of the Plg binding site. The Plg binding site on FnBPA remains to be determined.

Homophilic interactions and biofilm formation

Biofilm formation by some MRSA strains is dependent on the elaboration of surface proteins and not polysaccharide intercellular adhesion [13]. Analysis of FnBP defective mutants of an MRSA strain from CC8 that expresses isoforms I of FnBPA and FnBPB showed that the FnBPs were solely responsible for biofilm accumulation. This did not seem to involve any of the other CWA proteins (e.g. SdrC, SasC, SasG, ClfB) that have been reported to promote biofilm formation in other strains [27–30]. Direct evidence for homophilic binding between A domains on adjacent cells came from the application of single-cell force microscopy and single-molecule force microscopy, which showed that multiple low-affinity interactions between FnBPA molecules are sufficient to anchor cells together [19]. The force needed to separate cells is quite low, which might facilitate cell detachment during the dispersal phase. The mean adhesion force for FnBPA–FnBPA interactions was 192 pN, which is much lower than the remarkably strong binding that occurs by DLL between an MSCRAMM and Fg (2 nN binding force) [31]. Furthermore, the separation of bound FnBPA molecules did not involve any unfolding.

It remains to be seen if MRSA strains from different lineages form protein-dependent biofilm and if other isoforms of FnBPA and FnBPB (II–VII) can also engage in homophilic interactions. Further work must be performed to determine if a single subdomain of the A region is involved and to identify residues that promote binding. The search would be facilitated by knowing if all isoforms bind homophilically. This could help identify conserved residues in binding sites. Identification of the interaction sites will aid the development of inhibitors that prevent biofilm formation.

The function of subdomain N1

Recombinant forms of the full-length A domains of ClfA, FnBPA and FnBPB each bind Fg with the same affinity as their N2 and N3 subdomains, which indicates that subdomain N1 is not involved in ligand binding by DLL. Attempts to construct *S. aureus* strains that constitutively expressed truncates of ClfA and FnBPA isoform I lacking the entire N1 subdomain failed. Using a tightly repressed inducible expression vector, it was established that the N1 subdomain is required for correct expression and elaboration of the protein on the cell surface. Induction of mutants of ClfA expressing truncates lacking N1 resulted in the protein being mislocalised and not being secreted. Since N1 was not required to express a mutant of ClfA lacking SD repeats, it is possible that it is required to support the secretion and anchorage of MSCRAMM with long flexible unstructured domains. However, the mechanistic basis of this is unclear.

Binding to fibronectin

The fibronectin (Fn) binding repeat regions of FnBPs (FnBRs) are intrinsically disordered. They bind with high affinity to the N-terminal region of Fn, which comprises five independently folded type I modules [8, 20, 32, 33]. When bound, the FnBR takes on an ordered structure by forming additional β-strands along triple peptide β-sheets in three or four adjacent F1 modules (Fig. 2). This unusual augmented β-sheet arrangement in a tandem array was termed the tandem β-zipper mechanism [8]. Five of the FnBRs bind to Fn type I modules with a high affinity estimated to be in the low nM range by isothermal titration microcalorimetry and solid-phase binding assays. The stoichiometry of FnBP binding to Fn has been estimated to be between six to nine Fn molecules per FnBP [20]. This provides the potential for receptor clustering, which is important in promoting invasion (see below).

One of the high-affinity Fn binding domains is located closest to the Fg binding A domain. A recombinant protein comprising the A domain and a single FnBR can form a ternary complex in vitro with both Fn and Fg [25]. However, the presence of Fn reduced Fg binding. Even though Fg is present in plasma at a 10-fold higher concentration than Fn, the relative affinities indicate that Fn binding will occur at the expense of Fg binding. It can be argued that Fg binding is more likely to occur where the Fg γ-chain peptide is present at very high local concentrations, such as in blood clots. The same logic applies to FnBP binding to elastin-rich tissue.

Invasion of mammalian cells

Staphylococcus aureus can invade many different types of mammalian cells that do not normally engage in phagocytosis viz. various epithelial cells, endothelial cells, fibroblasts, osteoblasts and keratinocytes [34–42]. This allows bacteria to evade host immune defences and antibiotics. Bacteria can escape into the cytosol by lysing the phagosomal membrane and can proliferate before destroying the integrity of the cell from within. Alternatively, staphylococcal cells can enter a semi-dormant state called small colony variants (SCVs) [43]. These remain quiescent within the host cells and, by not expressing cytolytic toxins, they do not cause much damage. It is noteworthy that SCVs express FnBPs at high levels, which facilitates efficient uptake into nearby cells should they be released [44].

Fibronectin is a dimeric glycoprotein comprising three distinct domains, FI, FII and FIII [45]. In plasma, Fn exists in a compact state held together by specific intramolecular interactions between type I modules in the N-terminal domain and type III modules in the C-terminal cell binding domain (Fig. 2). When the FnBRs of the staphylococcal Fn binding protein bind to the type I modules in the N-terminal domain by the tandem β-zipper mechanism, they compete with the intramolecular bonds, leading to a conformation change in Fn [46]. This results in the cryptic integrin binding module in the tenth type III becoming exposed. The RGD motif is recognised by the $\alpha_5\beta_1$ integrin that occurs widely on the surface of mammalian cells. Thus, Fn acts as a bridge between the bacterium and the host cell. As noted above, a single FnBP protein can bind multiple Fn molecules. This causes clustering of the integrins which, in turn, triggers intracellular signalling by the focal adhesion kinase and Src kinase, and subsequent endocytosis of the bacterial cell [39, 47, 48]. At least one high-affinity Fn binding domain is required for the allosteric activation of Fn and invasion of epithelial cells [49]. In contrast invasion of keratinocytes requires three high-affinity FnBRs [50]. This is perhaps because there are fewer integrin molecules present.

FnBPs and virulence

In order to demonstrate that a particular surface protein is a virulence factor, it is necessary to isolate a knockout mutation in the encoding gene and to show that the mutant lacks virulence in an appropriate infection model. In addition, the mutation should be complemented by reintroducing the wild-type gene on a plasmid. This will confirm that the mutation in the gene of interest and not a secondary mutation acquired during or after mutagenesis is responsible for the altered phenotype [51]. An alternative approach is to introduce the gene into a surrogate non-virulent host, such as *Lactococcus lactis* or *S. carnosus* [52], and to determine that acquisition of the protein promotes virulence.

Several studies over the years investigated the role of FnBPs in virulence, but with mixed results. The outcome likely depended on the bacterial strain, the route of infection and the infection model. Intravenous infection of mice is a widely used model where the outcome can be measured as survival, loss of weight or abscess formation in internal organs such as the kidney or infection of joints (septic arthritis). The Schneewind group performed a systematic analysis of CWA proteins in the pathogenesis of septic infection by *S. aureus* strain Newman [53]. Statistically significant reductions in virulence were observed with a sortase A mutant lacking all CWA proteins and in mutants lacking several individual CWA proteins (e.g. IsdA, IsdB, ClfA, SdrE, ClfB) but not FnBPA or FnBPB. In retrospect, the behaviour of FnBP mutants is perhaps not surprising, since, in strain Newman, both genes have a stop codon at the 5′ end that precludes attachment of the protein to the cell wall by sortase due to lack of the sorting signal [54].

The laboratory strain 8325-4 was used in many early studies of staphylococcal pathogenesis in murine models of infection. This strain is defective in capsule formation and has a

mutation in *rbsU* that prevents expression of the stationary phase sigma factor B and, consequently, the strain expresses FnBPs at a low level. Strain SH1000 is a derivative of 8325-4 which has the *rbsU* gene restored to wild type. This strain has enhanced virulence in the murine model of sepsis and was used for a detailed analysis of the role of FnBPs in pathogenesis [38]. Essentially, both FnBPA and FnBPB were required for full virulence, as measured by weight loss and kidney abscess formation, with FnBPA having the greater effect. FnBP-defective mutants of strain LS1 had reduced virulence in the murine septic arthritis model [55].

Since FnBPs are multifunctional, their precise role in septic infection is difficult to establish. With the bacteria being injected intravenously, it is possible that FnBPs help in evading neutrophils and help promote survival of bacteria during the bacteraemic phase. Invasion of endothelial cells could help in escape from the bloodstream and in the formation of abscesses in internal organs and infection of joints in septic arthritis.

The endocarditis infection model involves bacteria attaching to sterile thrombi of heart valves in rats or rabbits and subsequently invading adjacent healthy tissue to promote expansion of the lesion and further damage to the infected valve. *L. lactis* expressing wild-type and truncated variants of FnBPA showed that the ability of bacteria to adhere to the fibrin clot via the A domain was necessary to establish a foothold on a thrombus, while binding to Fn and invasion of adjacent endothelial cells allowed lesion development [52].

However, it should be emphasised that the rat model of endocarditis involves the creation of a sterile lesion on the heart valve using a catheter. It is possible that the initiation of infection in humans occurs when bacteria attach to and invade undamaged tissue, in which case the event that triggers infection might be attachment to and invasion of undamaged endothelial cells.

Association of FnBP variants with infection

The *fnbA* gene of *S. aureus* strains isolated from cases of infection of cardiovascular devices were compared to those isolated from uncomplicated bacteraemia in patients with implanted cardiac devices [56]. It was found that the former contains one or more non-synonymous single nucleotide polymorphisms (SNPs) compared to the reference gene from 8325-4 (Fig. 1). These result in amino acid changes in FnBR domains that confer a higher affinity for Fn as measured by atomic force microscopy. The same substitutions were found in strains from two continents [57]. In contrast, these substitutions were not associated with strains isolated from prosthetic joint infections [58]. This suggests that strains which have a higher affinity for Fn are more likely to colonise a cardiac device which has been coated with host proteins, including Fn.

Similar polymorphisms were observed in MRSA strains causing endovascular infections [59]. Some strains had an additional FnBR inserted between FnBR-9 and FnBR-10, along with a substitution in FnBR-11 which promoted cell invasion at the expense of Fn binding affinity. This is consistent with the pathogenesis of endovascular infections, which involve invasion of endothelial cells during thrombus expansion on the infected heart valve.

While these associations between SNPs and human infection are interesting, it should be realised that they remain associations that have not been proven experimentally in an appropriate infection model.

Conclusions and future directions

The fibronectin binding proteins of *Staphylococcus aureus* are remarkably diverse and have an impressive array of different functions. In general, cell wall-anchored (CWA) proteins of Gram-positive bacteria are few in number and many have evolved to express multiple functions because of their importance in interaction with the host during colonisation and infection [1].

The A domains of FnBPA and FnBPB have undergone considerable sequence divergence, resulting in distinct isoforms [14, 15]. All of the amino acid sequence changes are predicted to be located on the surface of the proteins and result in antigenic differences. Antibodies raised against one isoform reacted weakly with the others. None of the changes occur in residues involved in Fg binding by ‘dock, lock and latch’ (DLL) and all isoforms bound Fg with similar affinities. The A domains of both FnBPA and FnBPB also bind plasminogen [12]. In the case of FnBPB, Plg binding did not occur by DLL and, indeed, a single FnBPB protein can bind both Plg and Fg simultaneously. All seven isoforms bound Plg with similar affinities, despite the amino acid sequence variation. This information was used to identify conserved lysine residues that are part of the Plg binding site. Further definition of the residues involved in binding will require the X-ray crystal structure of the FnBPB–Plg complex to be solved.

The biological significance of Plg binding is likely to result from the ability of the bound host protein to be activated to plasmin. This could enhance virulence by degrading host immunity proteins [26] and connective tissue to enhance spreading. This can best be tested in murine infection models with mice that express human Plg because the endogenous Plg activator staphylokinase reacts poorly with murine Plg [60]. It remains to be established if FnBPs and, indeed, other Plg binding CWA proteins recognise murine Plg efficiently.

MRSA strains from multilocus sequence type 8 form biofilm that is dependent on FnBP expression during the

accumulation stage. The strains express isoforms I of FnBPA and FnBPB. It is not known if FnBP-dependent biofilm formation is a property of MRSA strains from other lineages or, indeed, if other FnBP isoforms can engage in the homophilic interactions involved. It is also possible that FnBPA and FnBPB interact heterophilically. If this mechanism is widespread, then small-molecule inhibitors might be a useful way to control infection. Definition of the sites of interaction requires the X-ray crystal structure of A domain dimers to be solved, as has been achieved with SraP [61].

Another question is why do most strains of *S. aureus* express two FnBPs when the proteins bind to the same ligands? It is possible that the two genes are regulated differently, allowing expression of at least one of the proteins under different growth conditions. A detailed analysis of the regulatory pathways controlling *fnb* gene expression deserves attention.

Several CWA proteins including FnBPs have been tested as vaccine candidate antigens [62]. The diversity of the A domains and the disordered nature of the fibronectin binding repeats (FnBRs) makes these proteins unsuitable for future vaccine development. Indeed, the FnBRs are poorly immunogenic, with the dominant immune response recognising neoepitopes formed when the FnBR complexes with fibronectin [63].

In conclusion, recent studies with these remarkable proteins have unearthed new and unexpected functions. It is possible that additional functions remain to be discovered and that a more precise understanding of the roles of these proteins in colonisation and pathogenesis will be forthcoming.

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Compliance with ethical standards

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Conflict of interest None.

Ethical approval Not necessary; this is a review.

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