New targets for antibiotic development: biogenesis of surface adherence structures

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Infection of a host by pathogenic bacteria requires that the bacteria are able to bind to the target tissue to colonize and/or begin the process of invasion. This adhesion event involves specific interactions between receptors on the host tissue and external surface structures produced by the bacterial cell. Gram-positive bacteria utilize a special class of surface-anchored proteins for this purpose, while Gram-negative microorganisms use a more complex structure, called a pilus, to achieve the same end. In both cases, a highly conserved pathway is utilized to export, assemble and anchor these surface structures. As such, these pathways represent targets for antibiotic development. Compounds that prevent the assembly of bacterial surface proteins will cripple the ability of bacteria to interact with and colonize host tissue leading to rapid bacterial clearance from the body.

The rise of strains of bacteria resistant to multiple antibiotics is a major threat to human health and one of the most pressing problems facing the medical community today. Two Gram-negative organisms that are responsible for many common infections of the genitourinary tract serve to illustrate this problem. Recent reports indicate that isolates of *Neisseria gonorrhea* resistant to penicillin, tetracycline or both antibiotics are on the rise[1,2]. The numbers vary widely depending on the location; in 1992, over 90% of *N. gonorrhea* isolates in the Philippines and Thailand were resistant to penicillin, whereas, in New York 42% of strains were found to be resistant[3]. These strains carried plasmid-mediated TEM type β-lactamase. Tetracycline resistance in *N. gonorrhea* is mediated by *tetM*, which encodes an efflux pump that efficiently removes tetracycline from the gonococcus[1,3]. Amoxicillin resistance in *Escherichia coli* is common, representing 30% of isolates in the community and 40–50% of isolates in hospital and elder-care settings[3]. *E. coli* has also acquired resistance to trimethoprim-sulphamethoxazole (TMP-SMX); in geriatric-care centers some 40% of strains isolated from urine are resistant to TMP-SMX (Refs 3,4). *E. coli* is responsible for ~6.5 million physician visits per year for urinary tract infections (UTI), which includes 80,000 cases of pyelonephritis. 40% of adult women will suffer symptoms of UTI during their lifetime and many will suffer recurrent infections. UTI is also the most common cause of nosocomial infections (42% of all hospital-acquired infections) and the most common source of bacteremia in community health settings[2].

Gram-positive bacterial pathogens also pose a major threat to human health due to their continued development of resistance to most clinically relevant antibiotics. This is particularly true with *Staphylococcus aureus*: 95% of clinical isolates are penicillin resistant, 30–50% of clinical isolates of *S. aureus* and *S. epidermidis* are resistant to methicillin and its derivatives, >80% are resistant to the fluoroquinolone, ciprofloxacin, and recent isolates have displayed resistance to vancomycin (the antibiotic of last

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resort in the clinic)[1]. Members of the staphylococci are a major source of nosocomial infections and a leading cause of life-threatening maladies such as septicemia, endocarditis, osteomyelitis and toxic-shock syndrome. Staphylococci are also a leading cause of cutaneous infections, opportunistic infections and infections of the urinary tract[1,5]. Furthermore, 20–65% of all infections of prosthetic devices, catheters and shunts are caused by *S. epidermidis*[5]. Antibiotic resistance in the streptococci is a growing problem as well with >30% of *Streptococcus pneumoniae* (pneumococcus) resistant to penicillin[1]. Pneumococcus is a major cause of bacterial pneumonia, causing 500,000 cases in the USA annually, as well as meningitis, otitis media, sinusitis and bacteremia. Nearly 30% of all patients suffering from bacterial pneumonia develop bacteremia[1].

In response to the rapidly rising tide of antibiotic resistance in both Gram-negative and Gram-positive pathogens, many pharmaceutical interests, large and small, are seeking new targets for intervention. The trend, however, is to re-engineer existing agents and to focus on targets that were successful in the past, finding temporary fixes circumventing the resistance mechanism put forth by the microorganism. By way of example, 10,000 β-lactam derivatives have been produced since this important drug class was initially entered into the clinic in the 1940s[6]. Targets of choice in the past include interference with metabolic pathways, protein and nucleic acid synthesis, cell wall synthesis and compromising membrane permeability. The most recent addition to the antibiotic arsenal was the identification of the quinolones in the 1980s, specifically the fluoroquinolones, which attack DNA gyrase[6]. However, resistance to the fluoroquinolone, ciprofloxacin, is currently on the rise with 80% of multiple-resistance strains no longer sensitive to this drug[3]. Another recent advance was the identification of the β-lactamase inhibitors, such as clavulinic acid and sulbactum, which have no inherent antimicrobial activity but bind the active-site serine of the β-lactamase allowing the antibiotic to exert antimicrobial activity[6]. This combination drug therapy has great clinical utility with the broad-spectrum penicillins[6]; however, recently in India, multiple-resistance strains have shown resistance to these compounds[3].

A novel approach has developed from the study of natural products with antimicrobial activity. The magainins are the first group of a growing family of natural peptide antimicrobial agents[6]. One virulence property of *S. typhimurium*, however, is resistance to several antimicrobial peptides, suggesting that spread of this resistance trait could closely follow the use of such compounds in the clinic[7,8]. Two new pharmaceutical companies, Cubist Pharmaceuticals (Cambridge, MA, USA) and Microcide Pharmaceuticals (Mountain View, CA, USA), are focusing on novel pathways: inhibition of efflux pumps[9] and aminoacyl-tRNA synthetases[10], respectively. However, with most new formulations still focused on enhancing the effectiveness of existing antibiotics or working in synergy with existing antibiotics, and also the inherent resilience of microorganisms, it is clear that additional new targets are a necessity.

Pilus biogenesis pathway in Gram-negative bacteria

Pilus biogenesis is essential for bacterial pathogenesis, as in many cases the initial interaction between the pathogen and host occurs via the pilus[11–17]. For example, pyelonephritogenic strains of *E. coli* must gain a 'foothold' in the urinary tract to cause disease. Colonization of the bladder starts by a specific adherence event between an adhesin molecule, localized at the tip of the bacterial type 1 pilus, and a specific receptor – a mannose moiety expressed on the uroepithelium[18]. Likewise, colonization of the kidney requires a specific interaction between the PapG adhesin, a tip component of the Pap pilus, and the receptor Galα1–4Gal, which is presented on the kidney epithelial cell surface[19–21]. Pyelonephritic strains of *E. coli* that express Pap pili lacking the adhesive component PapG are incapable of causing pyelonephritis in cynmolgous monkeys[21]. Similarly, cystitis isolates of *E. coli* that fail to assemble the adhesive component FimH into the type 1 pilus are avirulent in mice[18]. These findings highlight an assembly pathway that has only recently become the focus of a concentrated research program for the development of novel anti-infectives.

As the Pap pilus is universally accepted as essential (i.e. a virulence determinant of pyelonephritogenic *E. coli*), we will focus on the biogenesis of the Pap pilus[14,21]. However, as has been shown in many laboratories, the Pap pilus assembly pathway, and mechanisms therein, is conserved throughout the *Enterobacteriaceae* and virtually all other Gram-negative bacteria[15,22,23].

One virulence factor of a pathogen is the ability to express adherence proteins/factors on the cell surface. These adhesins allow intimate contact with the host, followed by colonization and initiation of the pathogenic program[14,15]. Many adhesins are presented as an integral part of a pilus organelle. Bacterial pili are approximately 1–2 μm

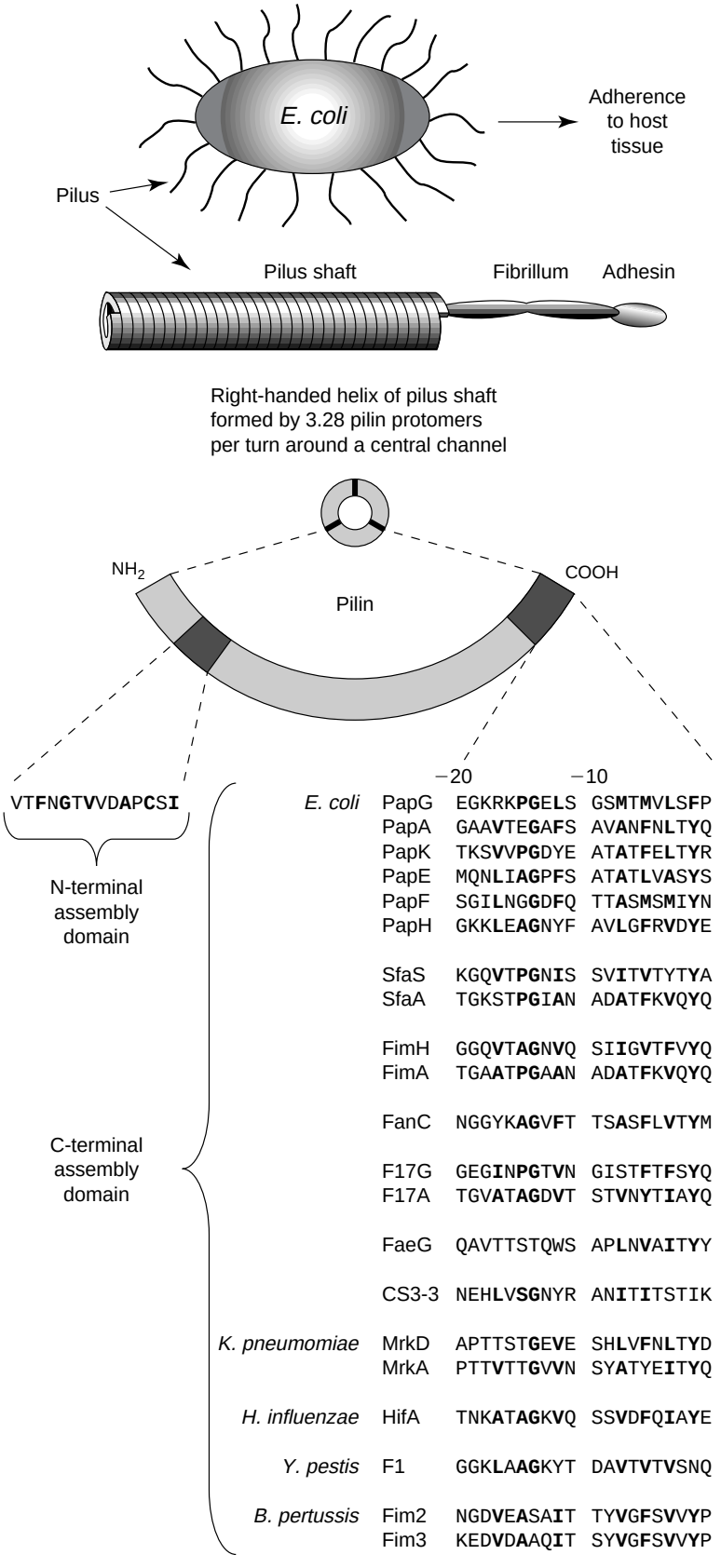

Figure 1. *Structure of* Escherichia coli *Pap pilus and conserved C-terminal assembly domain recognized by the periplasmic chaperone. Pyelonephritogenic strains of* E. coli *produce Pap pili, which are essential for adherence to kidney epithelium*[21]*. Pap pili are bipartite structures having a flexible tip fibrillum attached end-to-end to the right-handed helical shaft. Three PapA molecules occupy a single turn of the pilus shaft and are polymerized such that a pore runs the entire length of the shaft. The adherence protein PapG is localized solely at the tip of the fibrillum. An alignment of several sequenced pilin genes reveals a C-terminal conserved sequence that has since been demonstrated to be a recognition sequence for the chaperone*[40,44,45,75–77]*. Cocrystallization studies revealed the interaction at high resolution between the periplasmic chaperone and the C-terminal recognition sequence*[43]*. Recently, a second conserved region in the N-terminus of the pilin molecule has been shown to be involved in subunit–subunit interactions in the pilus as well as in chaperone–subunit interactions (Soto* et al. *unpublished data).*

long hair-like structures that protrude from the cell surface[12,15,24]. Generally speaking, there are two families of pilus structures: 7–10 nm right-handed helical fibers and 2–5 nm flexible fibers[15,22,25,26]. The 7–10 nm fiber, represented by the Pap and type 1 pilus, presents the adhesin in a flexible 2 nm fiber attached end-to-end to the thick helical rod[27,28]. This tip structure is referred to as the fibrillum. This complex architecture places the adhesin in a more flexible location, presumably to optimize interaction with the receptor[27] (Fig. 1). In the 2–5 nm fibers, represented by the K88 and K99 pili, the adhesin is thought to be the major pilus subunit of the fiber[29–31].

These two types of fibers, as well as the non-pilus adhesins and a collection of atypical surface structures, are assembled by a highly conserved pathway referred to as the chaperone–usher assembly pathway[14,15,23,32] (Table 1). The hallmarks of this pathway are two highly

conserved proteins that are essential for the production of pili: the periplasmic chaperone and the molecular usher. The periplasmic chaperone in the Pap pilus system (i.e. PapD) and the role of the molecular usher PapC are described below, after a brief discription of the biogenesis pathway of the Pap pilus.

Pilus biogenesis

Subunits destined to become an integral part of a pilus must traverse two membranes and the protein-rich periplasm before polymerization into the fiber (Fig. 2a). The highly interactive pilus subunit protomers are 'chaperoned' throughout the pathway from the cytoplasm to the outer-membrane assembly site. When subunits are delivered via the Sec system[33] into the periplasm they are 'met' by the periplasmic chaperone[34]. In fact, efficient release or partitioning from the inner membrane to the periplasm is dependent on the interaction with the chaperone[34]. Further interaction with the chaperone results in a folded subunit that is protected from proteolysis and prevented from premature interaction with other subunits in the periplasm[34]. Chaperone–subunit complexes are subsequently targeted to the outer-membrane-associated usher protein[35,36] where the chaperone is displaced, subunit–subunit interactions are favored and polymerization occurs (Fig. 2a). The usher protein was recently shown to have pore-forming activity *in vitro* and, therefore, may allow pilus subunits to pass through the outer membrane via the pore opening *in vivo*[36].

PapD

PapD is the prototype member of a family of >40 proteins in the *Enterobacteriaceae* that are believed to function as chaperones in the periplasm[22] (Table 1). This protein family shares between 25% and 60% homology and in both mutagenesis studies and genetic complementation a commonality

Table 1. Surface structures assembled by the chaperone/usher pathway[a]

Surface structure	Chaperone/usher	Organism	Disease
7–10 nm fibers			
P pili	PapD/PapC	*E. coli*	UTI
Type 1 pili	FimC/FimD	*E. coli, Salmonella* spp., *K. pneumoniae*	UTI
S pili	SfaE/SfaF	*E. coli*	UTI/NBM
Hif pili	HifB/HifC	*H. influenzae*	Otitis media, meningitis
Pef pili	PefD/PefC	*S. typhimurium*	Gastroenteritis, salmonellosis
MR/P pili	MrpD/MrpC	*P. mirabilis*	Nosocomial UTI
PMF pili	PmfD/PmfC	*P. mirabilis*	Nosocomial UTI
2–5 nm fibers			
K99 pili	FaeE/FaeD	*E. coli*	Neonatal diarrhea in calves, lambs, piglets
K88 pili	FanE/FanD	*E. coli*	Neonatal diarrhea in piglets
F17 pili	F17D/F17C	ETEC	Diarrhea
MR/K	MrkB/MrkC	*K. pneumoniae*	Pneumonia
Nonfimbrial adhesins			
Nfa1-6 family	NfaE/NfaC	*E. coli*	UTI, NBM
Afa-1	AfaB/AfaC	*E. coli*	Pyelonephritis
Dr/AFA-III	DraE/DraD	*E. coli*	UTI, diarrhea
Atypical structures			
CS3	Cs3-1/Cs3-2	ETEC	Traveler's diarrhea
CS6 pili	Css6/CssD	ETEC	Diarrhea
Sef	SefB/SefC	*S. enteritidis*	Gastroenteritis
PH6 antigen	PsaB/PsaC	*Y. pestis, Y. pseudotuberculosis*	Plague
Myf	MyfB/MyfC	*Y. enteritidis*	Enterocolitis

[a]This is a selected list, for a more complete list and relevant references see Refs 15,23 and references therein; UTI, urinary tract infection; NBM, newborn meningitis; ETEC, enteropathogenic *E. coli*.

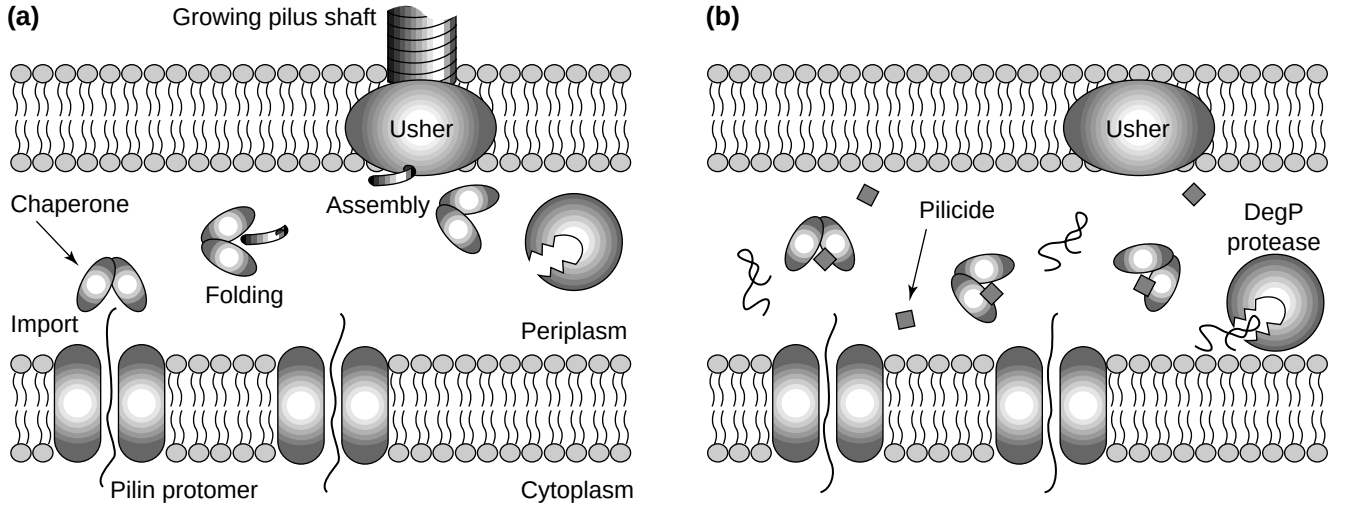

Figure 2. *Pilus biogenesis pathway and a potential multi-site target for antibiotic therapeutic development. (a) As pilin subunits cross the inner membrane they are met by the periplasmic chaperone[34]. Subunits emerge in an unfolded state and reach a folded, protease-resistant state in complex with the chaperone[34]. The chaperone–subunit complex is subsequently targeted to the outer-membrane molecular usher[35,47]. At this site, the chaperone is displaced followed by subunit–subunit interaction and polymerization. The usher forms a pore in the outer membrane[23,36] through which the pilins are believed to pass as the pilus grows. (b) Small-molecule inhibitors of the periplasmic chaperone that block any of the functions along the biogenesis pathway would result in the production of 'bald' bacteria that would be unable to adhere to host tissues.*

of function has been revealed for those members of the family that have been investigated. In the Pap, type 1 and several other pilus systems, it has been demonstrated that chaperone null mutants block pilus biogenesis and result in degradation of pilus subunits in the periplasm[37–39]. Furthermore, specific chaperone–subunit complexes have been purified from the periplasm in all systems tested to date[15,39–42]. The PapD–PapG subunit complex was dissected in detail through X-ray crystallographic determination of a PapD–peptide complex[43]. The peptide that was cocrystallized with the chaperone represents the C-terminal 19 amino acid residues of the PapG adhesin. Among the pilus subunit family of proteins, consisting of proteins from >30 pilus types, the C-terminus is by far the most highly conserved region (Soto *et al.* unpublished data; Fig. 1). The homology region includes a conserved penultimate aromatic residue (−2), a conserved glycine residue (−14) and a pattern of alternating hydrophobic residues between −2 and −14. This conserved region has been demonstrated by both genetic and biochemical means to be a recognition site for the chaperone (Ref. 44 and Soto *et al.* unpublished data). Recently, an N-terminal homology region in pilin subunits was also shown to be essential for piliation and chaperone interaction (G. Soto *et al.* unpublished data). This homology region is well conserved in pilins and was originally described by Lindberg *et al.*[45] (Fig. 1). Point mutations of highly conserved residues in this region affect both pilus biogenesis and chaperone–subunit complex formation (G. Soto *et al.* unpublished data).

PapD is a two-domain V-shaped molecule with a large cleft between the domains. At the base of the cleft lie two invariant residues, arginine 8 (R8) and lysine112 (K112); these residues are invariant in all members of the chaperone family[22]. The cocrystal structure revealed that the C-terminal PapG sequence bound into the conserved cleft of the chaperone forming an extended β-sheet and 'docking' via hydrogen bonding with R8 and K112 (Ref. 43). A wealth of data supports the role of the C-terminal conserved residues in chaperone–subunit interactions[40,44]. Amino acid substitutions at conserved positions in the C-terminus result in pilin subunits that fail to be recognized by the PapD chaperone. Furthermore, point mutations at the invariant R8 and K112 cleft residues of PapD blocked complex formation and pilus assembly[46].

PapC

The second conserved component of the pilus assembly machinery is the molecular usher[14,15,23,36]. This protein's role in assembly is fundamental, as it is in the presence of the usher that the pilus subunit protomers swap interaction with the chaperone for subunit–subunit contacts and polymerization into the highly stable pilus. Specific targeting of chaperone–subunit complexes to the usher has been demonstrated in several *in vitro* systems[35,47]. Recently, it was shown that the usher forms multimeric complexes in the outer membrane and has pore-forming activity *in vitro*[36]. The measured size of the pore formed *in vitro* would allow the polymerizing pilus egress across the outer membrane. The model proposed by Thanassi *et al.* suggests that binding of the chaperone–subunit complex to the usher triggers multimerization and/or pore opening[36,47].

Novel drug targets for Gram-negative pathogens

Small-molecule compounds that inhibit the function of the periplasmic chaperone or molecular usher would result in the inability of bacteria to assemble and/or express adhesin molecules on the surface. Inhibitors of PapD binding to pilin subunits will result in the accumulation of large quantities of unfolded pilin subunits in the periplasm and inner membrane (Fig. 2b). Laboratory studies with *papD* null mutants revealed that the 'off' pathway for subunit expression leads through misfolded intermediates, subunit aggregates and ultimately proteolytic degradation by the DegP protease[34] (C.H. Jones and S.J. Hultgren, unpublished data). A large portion of subunit expressed in the absence of functional PapD remained associated with the inner membrane. Moreover, in the absence of functional DegP protease, these pilin aggregates were found to be toxic and compromised bacterial growth[34]. An inhibitor that blocked only chaperone function would not be expected to be toxic, whereas a dual inhibitor of both the chaperone and the protease may be highly toxic. Such novel drug formulations would result in the invading pathogens being subjected to the mechanical forces that clear particulate matter from mucosal surfaces and the natural cleansing flow of urine through the urinary tract.

Inhibitors of molecular usher function would be expected to block the polymerization of pilin subunits into functional pili. The chaperone–subunit complexes would remain trapped in the periplasm with no way across the outer membrane to the cell surface. Again, this is not expected to be lethal unless paired with an inhibitor of the periplasmic protease DegP. A build-up of chaperone–subunit complexes in the periplasm, as a result of blocked usher function may overwhelm the normal *status quo* in the periplasm, calling into action the protein folding and proteolytic functions that, by a two-component regulatory system, monitor the state of the periplasm[34,48].

Gram-positive surface protein expression pathway

Surface proteins of Gram-positive organisms generally fulfill one of two roles in pathogenesis: either modifying the host immune response or directing adherence to host tissues[49–62]. Staphylococcus Protein A modifies the host response by binding to the complement fixing portion of immunoglobulins which coat the bacterial surface and thus, in effect, the invading pathogen hides from the immune system[56,57]. The two major adhesins of the streptococci are M protein, which binds to keratinocytes, and protein F, which interacts with Langerhans cells in the skin[58–63]. These activities are essential for the invading organism to initiate the infectious process and persist in the host[59]. It has been demonstrated in many laboratories that blocking such activities allows the host to repel the invading organism[49,54,57,64–66]. Jadoun *et al.* demonstrated the essential role of M protein and protein F in invasion of human epithelial monolayers by group A streptococci[59]. Isogenic pairs of strains were used to illustrate that M- and F-mutants were unable to invade. Furthermore, they showed that expression of protein F alone on the surface of a non-invasive strain rendered the strain invasive. Therefore, a clear understanding of the mechanism of surface protein expression provides a unique opportunity to direct inhibitors at a pathway that is important for adherence and survival in the host. The goal is to develop chemotherapeutics that prevent adhesion to host tissue, resulting in clearance of the pathogen.

Cell wall anchoring machinery

Recently, a novel pathway for the secretion and anchoring of cell wall proteins by Gram-positive organisms was described[67–70] (Fig. 3). The mechanism is conserved in all Gram-positive organisms surveyed except *Bacillus* and is responsible for the surface expression of >60 cell surface proteins[70–72] (Table 2). The proper sorting of surface proteins in Gram-positive organisms, such as M protein in *Streptococcus pyogenes*, requires an N-terminal leader peptide to allow secretion across the inner membrane, via a

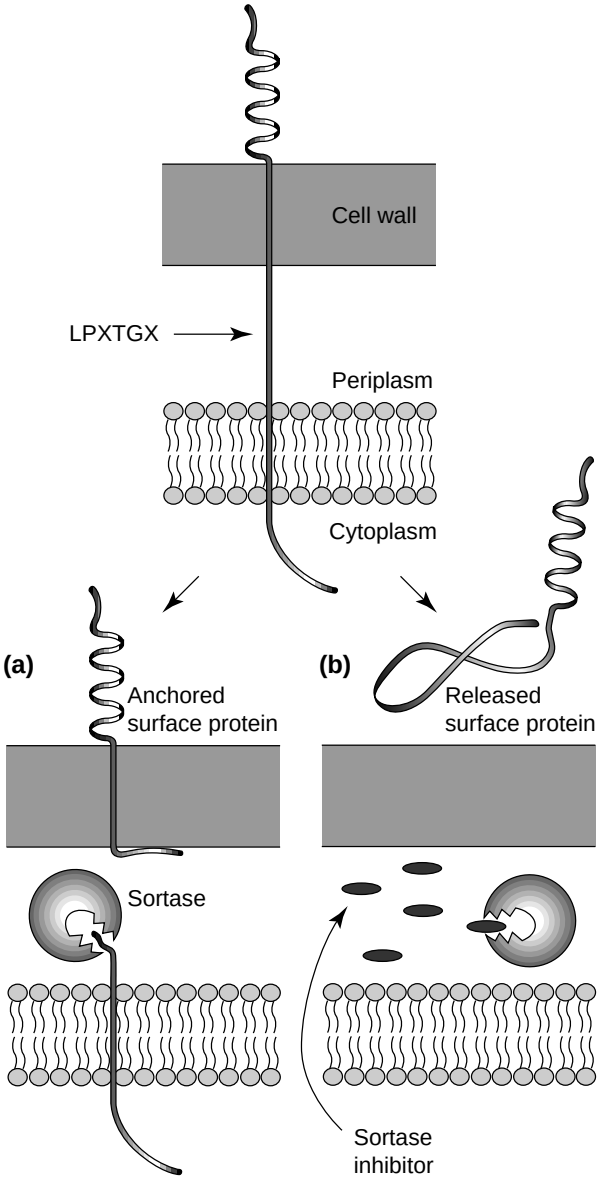

Figure 3. *Mechanism of anchoring surface proteins to the cell wall in Gram-positive bacteria and potential targets for novel antimicrobials. The ability to anchor surface proteins to the Gram-positive cell wall is essential for the success of these pathogens. The 35 C-terminal residues of surface proteins comprise three regions that are required for covalent linkage to the cell wall. (a) The charged tail and hydrophobic region of surface proteins are believed to hold the transiting protein in the inner membrane so that the LPXTGX sequence can be recognized, proteolytically cleaved and covalently linked to the peptidoglycan[68,69,73]. (b) Inhibitors of the pathway, protease or transpeptidase function, will result in release of proteins from the cell and prevent adherence to host tissues.*

Sec-like mechanism, and a C-terminal sorting sequence (~35 residues long) for targeting and anchoring to the cell wall[73] (Fig. 4). The sorting sequence has three elements: the most distal element is a charged tail of 5–12 residues having a mixture of positive and negatively charged amino acids; immediately preceding the charged tail is a 15–22 residue segment of hydrophobic amino acids; and immediately preceding the hydrophobic region is a six-residue highly conserved sequence, LPXTGX (Refs 68–70,72,73).

The current model describing the anchoring of surface proteins to the cell wall suggests that the positively charged tail and the hydrophobic region anchor the transiting protein in the inner membrane. This places the LPXTGX sequence in the proper conformational space for cleavage by a proteolytic enzyme, designated 'sortase' (Fig. 3). Navarre *et al.* demonstrated that cleavage occurs between the threonine and glycine of the LPXTGX in *S. aureus* protein A (Ref. 70). Non-surface-anchored proteins, such as MalE, that have been engineered to contain the sorting signal at the C-terminus are successfully anchored to the cell wall[68]. Moreover, using the MalE fusion protein, the membrane-bound C-terminal fragment was isolated after proteolytic cleavage and the cleavage site was identified. Schneewind *et al.* determined that the anchored proteins are covalently linked to the peptidoglycan via the pentaglycine moiety of the peptidoglycan[69]. They used muramidase, which cleaves between *N*-acetylmuramic acid and *N*-acetylglucosamine in the peptidoglycan, and lysostaphin, which cleaves within the pentaglycine unit of the peptidoglycan, to determine which group was covalently linked to the fusion protein at the threonine residue of LPXTGX. They postulated that the carboxyl group of the threonine residue is covalently linked via an amide bond to the pentaglycine cross-bridge of the peptidoglycan[69]. This mechanism is conserved throughout Gram-positive organisms (Table 2) such that several lactococcus species have been shown to anchor streptococcal M6 protein to their cell wall[74].

The enzyme that actually anchors the protein to the cell wall, via a transpeptidase-like reaction, has not been defined. It is possible that the transpeptidase and sortase are part of a multi-subunit enzyme machine that recognizes, cleaves and links the protein to the peptidoglycan. All of these activities are relevant targets for development of small-molecule inhibitors.

Novel drug targets for Gram-positive pathogens

Point mutations in the LPXTGX anchor recognition signal have been shown to block anchoring of the surface protein

Table 2. Conservation of hexapeptide anchor sequence in Gram-positive surface proteins[a]

Surface protein	Anchor site sequence	Organism	Disease
	LPXTGX		
M protein (M6)	**LP**S**TG**E	*S. pyogenes*	Pharyngitis, scarlet fever, TSS, necrotizing faciitis, bacteremia, rheumatic fever
Alpha antigen (bca)	**LP**A**TG**E	Group B Streptococcus	Neonatal infections[b], UTI, pneumonia, bacteremia, endometriosis
Internalization protein	**LP**T**TG**D	*Listeria monocytogenes*	Neonatal disease, granulomatosis (inlA) infantiseptica, meningitis, bacteremia
Surface protein (PAc)	**LP**N**TG**E	*S. mutans*	Dental caries
Type 1 fimbriae	**LP**L**TG**A	*A. viscosis*	Commensal, secondary actinomycosis
Fibronectin binding protein (FnBP)	**LP**E**TG**G	*S. aureus*	TSS, impetigo, endocarditis, pneumonia, bacteremia
Surface protein (Sec10)	**LP**Q**TG**E	*E. fecalis*	UTI, bacteremia, endocarditis
Wall associated protein (WapA)	**LP**S**TG**E	*S. mutans*	See above
IgA binding protein (IgA-BP)	**LP**S**TG**E	*S. pyogenes* M4	See above
Fc binding protein (Fc-BP)	**LP**S**TG**E	*S. pyogenes*	See above
Protein A	**LP**E**TG**E	*S. aureus*	See above
Protein G	**LP**T**TG**E	Group G *streptococci*	Pharyngitis, scarlet fever, soft tissue, bone and joint infections, pneumonia, endocarditis, TSS neonatal sepsis
Cell wall protease (wg2)	**LP**K**TG**E	*S. cremoris*	N/A[c]
Surface protein (T6)	**LP**S**TG**S	*S. pyogenes*	See above

[a]This is a selected list, as over 60 surface proteins have been identified that contain the conserved hexapeptide; for a more complete list and references see Refs 70–72 and references therein; TSS, toxic shock syndrome; UTI, urinary tract infections.

[b]Neonatal infections include meningitis, pneumonia and bacteremia either *in utero* or shortly after birth.

[c]*S. cremoris* is a group N streptococcus, used in the dairy industry.

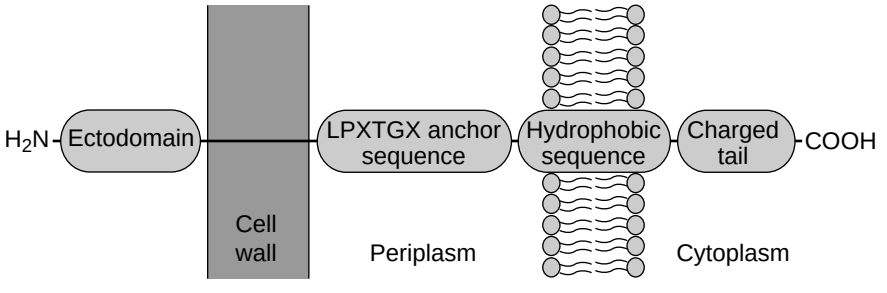

Figure 4. *Structure of cell wall-anchored proteins of Gram-positive bacteria. Over 60 surface molecules from Gram-positive bacteria have been sequenced revealing a striking homology throughout the C-terminal portion of the protein[67,72–74]. The immediate C-terminus is highly charged and is proceeded by a hydrophobic region. Next to the hydrophobic region is the conserved hexapeptide LPXTGX (Refs 67–69, 73). These sequences are essential for anchoring cell wall-associated proteins.*

to the cell wall[73] and the unanchored surface proteins are subsequently shed into the media (Fig. 3b). We propose that an inhibitor of either the protease function or transpeptidase function of the sortase machinery would have a similar result. The inability to anchor essential proteins to the cell wall will leave pathogenic microorganisms 'exposed' to the immune system and subject to mechanical forces that dislodge particulate matter from mucosal surfaces.

Perspectives

Since the discovery and utilization of the first antibiotics, the human race has been striving to keep ahead of the

development of bacterial resistance to these life saving formulations. The development of resistance has now reached a level whereby a new scourge of bacterial disease looms on the horizon. Continued development utilizing novel targets that take into consideration functions that are only essential for the pathogenic process, such as bacterial adherence to host tissue, should allow for the greatest efficacy and also reduce the appearance of resistant strains. Indeed, one goal for this new class of antimicrobial agents is to avoid the development of resistance. Bacteria have developed resistance, to some degree, to all antibiotic formulations that have been developed in the past[3,6]. The strategy behind the development of this new class of antibiotics is to attack mechanisms important for pathogenesis, in this case adherence; thus, it is not intended to kill the pathogen but remove it from the host by allowing physical mechanisms and innate immunity to clear the organisms. It is our belief that treatment of infectious disease by attacking the targets we have discussed herein will have less of a tendency to lead to the rapid development of resistant organisms. Furthermore, because bacterial pili and surface anchored proteins are continuously produced and recycled throughout the infectious process, such antibiotics will, theoretically, exhibit therapeutic as well as prophylactic properties. The definitive answer to this important question awaits the identification of a compound that efficiently blocks adherence.

REFERENCES

1 Murray, P.R. *et al.* (1998) *Medical Microbiology* (3rd edn.) (Brown, M., ed.), Mosby-Year Inc.
2 Warren, J.W. (1996) in *Urinary Tract Infections: Molecular Pathogenesis and Clinical Management* (Mobley, H.L.T. and Warren, J.W., eds), pp. 3–29, ASM Press
3 Neu, H.C. (1992) *Science* 257, 1064–1072
4 Gleckman, R.A. and Czachor, J.S. (1992) in *Infectious Diseases* (Gorbach, S.L., Bartlett, J.G. and Blacklow, N.R., eds), pp. 239–244, W.B. Saunders
5 Sheagren, J.N. and Schaberg, D.R. (1992) in *Infectious Diseases* (Gorbach, S.L., Bartlett, J.G. and Blacklow, N.R., eds), pp. 1395–1400, W.B. Saunders
6 Mann, J. and Crabbe, J.C. (1996) *Bacteria and Antibacterial Agents* (Mann, J., ed.), Spektrum Academic Publishers
7 Groisman, E.A. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 11939–11943
8 Groisman, E.A., Heffron, F. and Solomon, F. (1992) *J. Bacteriol.* 174, 486–491
9 Cubist Pharmaceuticals (1997) Annual company report
10 Barrett, A. *et al.* (1998) *Business Week* 6 April, 104–112
11 Jones, C.H. *et al.* (1992) *Infect. Immun.* 60, 4445–4451
12 Hultgren, S.J., Normark, S. and Abraham, S.N. (1991) *Annu. Rev. Microbiol.* 45, 383–415
13 Hultgren, S.J. *et al.* (1993) in *Adv. Pro. Chem.* (Lorimer, G., ed.), pp. 99–123, Academic Press
14 Hultgren, S.J. *et al.* (1993) *Cell* 73, 887–901
15 Hultgren, S.J., Jones, C.H. and Normark, S.N. (1996) in *Escherichia coli and Salmonella; Cellular and Molecular Biology* (Neidhardt, F.C., ed.), pp. 2730–2756, ASM Press
16 Hultgren, S.J. and Normark, S. (1991) *Curr. Opinion Gen. Dev.* 1, 313–318
17 Baumler, A.J., Tsolis, R.M. and Heffron, F. (1996) *Infect. Immun.* 64, 1862–1865
18 Langermann, S. *et al.* (1997) *Science* 276, 607–611
19 Roberts, J.A. *et al.* (1984) *J. Urol.* 131, 163–168
20 Roberts, J.A. (1987) *South. Med. J.* 80, 347–351
21 Roberts, J.A. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 11889–11893
22 Hung, D.L. *et al.* (1996) *EMBO J.* 15, 3792–3805
23 Thanassi, D.G., Saulino, E.T. and Hultgren, S.J. (1998) *Curr. Opin. Microbiol.* 1, 223–231
24 Brinton, C.C., Jr (1965) *Trans. N.Y. Acad. Sci.* 27, 1003–1165
25 de Graaf, F.K. and Mooi, F.R. (1986) *Adv. Microb. Physiol.* 28, 65–143
26 de Graaf, F.K. and Gaastra, W. (1994) in *Fimbriae, Adhesion, Genetics, Biogenesis and Vaccines* (Klemm, P., eds), pp. 53–70, CRC Press
27 Kuehn, M.J. *et al.* (1992) *Nature* 356, 252–255
28 Jones, C.H. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 2081–2085
29 de Graaf, F.K., Klemm, P. and Gaastra, W. (1981) *Infect. Immun.* 33, 877–883
30 Jacobs, A.A.C., Dimons, C.B.H. and de Graaf, F.K. (1987) *EMBO J.* 6, 1805–1808
31 Jacobs, A.A.C. *et al.* (1987) *J. Bacteriol.* 169, 4907–4911
32 Jacob-Dubuisson, F., Kuehn, M. and Hultgren, S. (1993) *Trends Microbiol.* 1, 50–55
33 Dodd, D.C., Bassford, P.J.J. and Eisenstein, B.I. (1984) *J. Bacteriol.* 159, 1077–1079
34 Jones, C.H. *et al.* (1997) *EMBO J.* 16, 6394–6406
35 Dodson, K.W. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 3670–3674
36 Thanassi, D.G. *et al.* (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 3146–3151
37 Lindberg, F. *et al.* (1989) *J. Bacteriol.* 171, 6052–6058
38 Jones, C.H. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8397–8401
39 Bakker, D. *et al.* (1991) *Mol. Microbiol.* 5, 875–886
40 Striker, R. *et al.* (1994) *J. Biol. Chem.* 269, 12233–12239
41 Jacob-Dubuisson, F. *et al.* (1993) *EMBO J.* 12, 837–847
42 Hultgren, S.J. *et al.* (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 4357–4361
43 Kuehn, M.J. *et al.* (1993) *Science* 262, 1234–1241
44 Bullitt, E. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 12890–12895
45 Lindberg, F.P., Lund, B. and Normark, S. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 1891–1895
46 Slonim, L.N. *et al.* (1992) *EMBO J.* 11, 4747–4756
47 Saulino, E.T. *et al.* (1998) *EMBO J.* 17, 2177–2185
48 Danese, P.N. *et al.* (1995) *Genes Dev.* 9, 387–398
49 Horstmann, R.D. *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 1657–1661
50 Fluckiger, U., Jones, K.F. and Fischetti, V.A. (1998) *Infect. Immun.* 66, 974–979

51 Cheung, A.L. and Fischetti, V.A. (1989) *J. Clin. Invest.* 83, 2041–2049
52 Fischetti, V.A. *et al.* (1988) *Rev. Infect. Dis.* 10, S356–S359
53 Fischetti, V.A. *et al.* (1977) *J. Infect. Dis.* 136, S222–S233
54 Fischetti, V.A. (1989) *Clin. Microbiol. Rev* 2, 285–314
55 Foster, T.G. and McDevitt, D. (1994) *FEMS Microbiol. Lett.* 118, 199–205
56 Moks, T. *et al.* (1986) *Eur. J. Biochem.* 156, 637–643
57 Patel, A.H. *et al.* (1987) *Infect. Immun.* 55, 3103–3110
58 Hanski, E. and Caparon, M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 6172–6176
59 Jadoun, J. *et al.* (1997) *Adv. Exp. Med. Biol.* 418, 511–515
60 Caparon, M.G. *et al.* (1991) *Infect. Immun.* 59, 1811–1817
61 Okada, N. *et al.* (1994) *J. Clin. Invest.* 94, 965–977
62 Ozeri, V. *et al.* (1996) *EMBO J.* 15, 989–998
63 Sela, S. *et al.* (1993) *Mol. Microbiol.* 10, 1049–1055
64 Fischetti, V.A., Hodges, W.M. and Hruby, D.E. (1989) *Science* 244, 1487–1490
65 Fischetti, V.A. *et al.* (1991) *Adv. Exp. Med. Biol.* 303, 159–167
66 Fluckiger, U. and Fischetti, V.A. (1997) *Adv. Exp. Med. Biol.* 418, 909–911
67 Pancholi, V. and Fischetti, V.A. (1989) *J. Exp. Med.* 170, 2119–2133
68 Schneewind, O., Mihaylova-Petkov, D. and Model, P. (1993) *EMBO J.* 12, 4803–4811
69 Schneewind, O., Fowler, A. and Faull, K.F. (1995) *Science* 268, 103–106
70 Navarre, W.W. and Schneewind, O. (1994) *Mol. Microbiol.* 14, 115–121
71 Navarre, W.W., Daefler, S. and Schneewind, O. (1996) *J. Bacteriol.* 178, 441–446
72 Fischetti, V.A., Pancholi, V. and Schneewind, O. (1990) *Mol. Microbiol.* 4, 1603–1605
73 Schneewind, O., Model, P. and Fischetti, V.A. (1992) *Cell* 70, 267–281
74 Piard, J.C. *et al.* (1997) *J. Bacteriol.* 179, 3068–3072
75 Tennent, J.M., Lindberg, F. and Normark, S. (1990) *Mol. Microbiol.* 4, 747–758
76 Normark, S. *et al.* (1988) *Antonie Van Leeuwenhoek* 54, 405–409
77 Normark, S. *et al.* (1986) in *Microbial Lectins and Agglutinins: Properties and Biological Activity* (Mirelman, D., ed.), pp. 113–143, Wiley Interscience