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AMYLOIDS — A FUNCTIONAL COAT FOR MICROORGANISMS

Martijn F. B. G. Gebbink^{*}, Dennis Claessen[‡], Barend Bouma^{*}, Lubbert Dijkhuizen[‡] and Han A. B. Wösten[§]

Abstract | Amyloids are filamentous protein structures ~10 nm wide and 0.1–10 µm long that share a structural motif, the cross-β structure. These fibrils are usually associated with degenerative diseases in mammals. However, recent research has shown that these proteins are also expressed on bacterial and fungal cell surfaces. Microbial amyloids are important in mediating mechanical invasion of abiotic and biotic substrates. In animal hosts, evidence indicates that these protein structures also contribute to colonization by activating host proteases that are involved in haemostasis, inflammation and remodelling of the extracellular matrix. Activation of proteases by amyloids is also implicated in modulating blood coagulation, resulting in potentially life-threatening complications.

Amyloid fibrils (BOX 1) have historically been associated with pathology in a class of degenerative diseases including Alzheimer's disease and Creutzfeldt–Jakob disease. However, recent data have shown that amyloid fibril formation not only results in toxic aggregates but also provides biologically functional molecules¹. Such functional amyloids have been identified on the surfaces of fungi and bacteria. The proteins forming these fibrils are not related; HYDROPHOBINS² and CHAPLINS^{3–6} form amyloid fibrils on the surfaces of fungi and Gram-positive streptomycetes, respectively, whereas CURLI and thin aggregative fimbriae (TAFI, formerly known as SEF17) form on the cell surfaces of Gram-negative *Escherichia* and *Salmonella* species⁷ (FIG. 1). In this review, we discuss how amyloids are assembled at microbial surfaces and discuss their roles in bacterial and fungal invasion of biotic and abiotic substrates. In particular, we consider their potential role in human and animal infections. By activating host proteases involved in the haemostatic system, microbial amyloids have been implicated in colonization of the host and might contribute to complications during sepsis. Evidence also indicates that they might form a protective coat enabling certain pathogens to evade the immune system. It is also possible that the host immune system could recognize microbial amyloids as a signal that indicates potential danger.

Amyloids and fungi

Hydrophobins and their encoding genes have been identified in both the ascomycetes and the basidiomycetes — phyla that represent most species in the fungal kingdom⁸. These proteins have not been found in yeasts but they are expressed in dimorphic fungi. The presence of hydrophobins is not associated with a specific microbial lifestyle — they are produced by SAPROPHYTIC fungi, pathogenic fungi and fungi that establish a mutually beneficial symbiosis.

Functions of amyloid fibrils of hydrophobins. At hydrophilic–hydrophobic interfaces class I hydrophobins self-assemble into an amphipathic membrane that consists of a mosaic of amyloid fibrils known as rodlets (BOX 2). This membrane has multiple functions. It allows fungi to escape an aqueous environment⁹, confers hydrophobicity to air-exposed fungal surfaces^{10–18}, and mediates the attachment of fungi to hydrophobic solid substrates, such as the surface of a host^{17,19,20}. Hydrophobins thereby facilitate dispersion of spores and contribute to invasion of abiotic and biotic substrates.

Fungal spores are often produced by aerial structures, which develop from hyphae that have escaped their aqueous environment. Aerial growth is mediated by

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The diagram illustrates the structure of a protein fibre. It features a central core of parallel blue arrows representing β -sheets, oriented along a horizontal 'Fibre axis' indicated by a blue arrow at the top. These sheets are stacked vertically, with a label ' β -Sheet stacking' and a downward-pointing blue arrow on the right. The sheets are connected by red and orange loops representing α -helices. A specific inter-strand hydrogen bond is highlighted with a double-headed arrow and labeled '4.7 Å Inter β -strand hydrogen bond'.

HYDROPHOBIN

CHAPLINS

CURLI

TAFI

SAPROPHYTIC

APPRESSORIUM

The role(s) of hydrophobins, once spores have entered an animal or human, has been most extensively studied in *Aspergillus fumigatus*. This fungus causes several respiratory diseases in immunocompromised patients. The hydrophobins RodA (HYP1)^{18,23} and RodB²⁴ are present on the conidial surface of this pathogen. RodA is involved in rodlet formation^{18,23} but the function of RodB is not known²⁴. The conidia of an *A. fumigatus* strain in which the *rodA* gene was disrupted adhered equally well to pneumocytes, fibrinogen and laminin when compared with wild-type conidia, whereas adhesion to collagen and albumin was reduced¹⁸. These data indicate that RodA is not the main component that determines adherence of conidia to host tissue, a conclusion that might have been expected as experiments indicate that rodlets primarily mediate attachment to hydrophobic surfaces. However, RodA does seem to have an important role in allowing conidia to avoid clearance by neutrophils and macrophages in the early stages of infection. In an *in vitro* assay, more than 40% of conidia lacking rodlets ($\Delta rodA$ and $\Delta rodA \Delta rodB$) were killed by alveolar macrophages, compared with ~20% of the wild-type and $\Delta rodB$ spores²⁴. These data

Box 2 | Assembly of hydrophobins into amyloid fibrils

The amino-acid sequences of hydrophobins are diverse, but all contain eight conserved cysteine residues¹²² that form four disulphide bridges^{123–125}. SC3 from *Schizophyllum commune* is the best characterized hydrophobin². Mature SC3 — after the secretion signal sequence has been cleaved — consists of 112 amino acids and has a molecular mass of 14 kDa¹²⁶. At hydrophilic–hydrophobic interfaces it self-assembles into an amphipathic protein film consisting of a mosaic of 10-nm-wide parallel amyloid fibrils, called rodlets^{11,20,21,127}. These rodlets are composed of two tracks of 2–3 protofilaments and bind thioflavin-T and Congo red². Rodlets are not formed in the aqueous environment due to the presence of the disulphide bridges in SC3 (REF. 128). Self-assembly of SC3 is accompanied by several conformational changes. The water-soluble form^{126,129} proceeds via an intermediate form with a greater amount of α -helical structure (known as the α -helical state) to a stable end form with a greater amount of β -sheet structure (known as the β -sheet state)^{126,130}. SC3 in the β -sheet state initially has no clear ultrastructure (β -sheet I state), but after prolonged incubation the protein organizes itself into rodlets (β -sheet II state)^{2,130}. These rodlets form a stable semi-permeable membrane through which molecules >200 Da cannot pass (X. Wang and G. T. Robillard, personal communication). This membrane lowers the water surface tension from 72 mJ m⁻² to 24 mJ m⁻² and is therefore highly surface active⁹.

SC3 is not a paradigm for all hydrophobins. It is a typical example of a class I hydrophobin¹³¹. Class II hydrophobins also assemble at hydrophilic–hydrophobic interfaces, but they do not form amyloid fibrils^{2,132}. Their membranes are much less stable, possibly owing to their inability to form rodlets. It has been suggested that the difference in amyloid formation between class I and class II hydrophobins is due to the amino-acid sequence between the third and fourth cysteine residue^{126,133}. Class II hydrophobins have an intervening sequence of 11 residues, whereas class I hydrophobins have 17–39 residues.

agree with the finding that *in vivo* growth of the Δ rodA strain of *A. fumigatus* is reduced in rat lungs during the first 5 days of infection²⁵. Assuming that RodA forms a semi-permeable layer around spores (like SC3; BOX 2), this protein film would not be expected to hamper the diffusion of oxidative species from the macrophage into the fungal cell. However, it is possible that these reactive molecules are neutralized by reacting with the hydrophobins in the rodlet layer²⁴. Although RodA protects the spore during the initial stages of infection, no differences were observed in the mean length of survival of mice infected with Δ rodA spores compared with wild-type spores^{18,25}. This observation may be explained by ongoing swelling of spores caused by water uptake — the resulting increase in spore volume disrupts the continuity of the hydrophobin membrane, which no longer protects against oxidative species or shields antigens located in the cell wall.

MYCELIUM
A network of fungal or streptomyces hyphae.

Amyloids and bacteria

Chaplin amyloids are present on the surfaces of the Gram-positive actinomycetes *Streptomyces* spp.³ Genetic evidence indicates that chaplin genes are present in other actinomycetes (including *Thermobifida fusca*⁵) but are absent from *Mycobacterium* spp. and the closely related *Corynebacterium* spp. Curli and Tafi amyloids have been found on the surfaces of the Gram-negative γ -proteobacteria *Escherichia coli* and *Salmonella enterica*⁷, as well as *Citrobacter* spp.²⁶ Genetic evidence has indicated that the genomes of other Enterobacteriaceae (for example, *Enterobacter* spp. and, possibly, *Shigella* spp.) also encode curli genes^{26–28}. Whether amyloid fibrils are more widespread within the bacterial domain is not yet known.

Streptomycetes, such as *Streptomyces coelicolor*, are soil-dwelling bacteria, whereas other related streptomycete species are pathogens of animals or plants. The life cycle of streptomycetes is remarkably similar to that of filamentous fungi. After a colonizing MYCELIUM has been formed in an aqueous substrate, hyphae grow into the air to form chains of exospores. These spores are dispersed and can give rise to a new colonizing mycelium. *E. coli* and *Salmonella* spp. inhabit the intestinal tract of humans and other warm-blooded animals and can cause enteric disease, septicaemia or non-enteric localized diseases such as urinary tract infections, meningitis or pneumonia.

Chaplin amyloids. Like hydrophobins, chaplins self-assemble into an amphipathic membrane that consists of amyloid fibrils of these proteins (BOX 3). The functions of the chaplin membrane are similar to those of hydrophobin membranes — they mediate attachment to hydrophobic surfaces²⁹ and allow hyphae to escape the aqueous environment³. The membrane is also responsible for making the surface of hyphae and spores hydrophobic^{3,4}, which prevents aerial hyphae aggregating and probably facilitates the dispersal of spores by the wind (FIG. 2). Streptomycete spores can be introduced into humans or animals by a process similar to that of fungal spores. These spores are abundant in damp environments, including buildings, and can result in occupants suffering inflammatory responses^{30,31} and even immunotoxic effects³². In tropical and subtropical regions, *Streptomyces somaliensis* causes actinomycetoma, which is associated with progressive swelling and severe skin deformations³³. It is not yet clear whether chaplins contribute to the development of this disease by, for example, mediating attachment to, or colonization of, host tissue.

Curli and Tafi amyloids. Curli and Tafi amyloid fibrils of *E. coli* and *Salmonella* spp. are mainly composed of the highly homologous subunit proteins CsgA and AgfA, respectively (BOX 4). These structures are involved in the attachment of the bacteria to inert solid surfaces and also function in biofilm formation^{34,35}. In the case of *Salmonella enterica* serovar Enteritidis, Tafi fibrils have been shown to promote bacterial cell–cell interactions — a process that contributes to the thick biofilms

Figure 2 | Formation of amyloid structures allows streptomycetes and filamentous fungi to invade the air. The mechanism by which aerial structures are formed by streptomycetes and filamentous fungi is remarkably similar. In both cases the water surface tension at the substrate–air interface is reduced by proteins that self-assemble into an amphipathic rigid membrane. This membrane is composed of amyloid fibrils of the hydrophobins and chaplins in filamentous fungi and streptomycetes, respectively. Aerial hyphae continue to secrete hydrophobins or chaplins and these molecules assemble at the hyphal surface making them hydrophobic. Modified, with permission, from REF. 3 © (2003) Cold Spring Harbor Laboratory Press, and REF. 9 © (1999) Elsevier Science.

formed by strains expressing these extracellular structures³⁴. Curli and Tafi fibrils also function in bacterial virulence; Tafi fibrils, like other fimbriae, probably fulfill this role by mediating colonization of the small intestine^{36–38}. Moreover, curli fibrils have been shown to have a role in the intracellular invasion of host cells³⁹.

Interaction of microbial amyloid with host proteins

As mentioned above, amyloid fibrils share a structural motif — the cross-β structure^{40,41} — which indicates that these fibrils have common properties⁴². Indeed, evidence is now accumulating that several mammalian proteins bind amyloids irrespective of their amino-acid sequence. Proteins that bind amyloids include fibronectin, tissue-type plasminogen activator (tPA) and factor XII. These proteins all contain a specific module, the fibronectin type I domain, and several have

been shown to interact with amyloid (Bouma, B. *et al.*, unpublished; FIG. 3). Interestingly, curli and Tafi fibrils also bind fibronectin, tPA and factor XII^{43–45}.

Fibronectin is a multi-domain, adhesive glycoprotein that circulates in blood and is a component of the extracellular matrix (ECM). This glycoprotein supports cell adhesion and has an important role in cell migration during wound healing, cancer and bacterial infection. Invasion of host cells by curli-coated *E. coli* could be blocked by competitors (for example, RGD peptides) of fibronectin⁴⁶. It is proposed that invasion is a two-step process — in the first step, the curli fibril interacts with fibronectin and in the second step the fibronectin-coated bacteria are internalized through interactions with integrins⁴⁶. This proposed mechanism is similar to the process by which Group A streptococci are internalized by eukaryotic cells⁴⁷.

Both tPA and factor XII are serine proteases that are part of the haemostatic system. This system consists of fibrinolytic and coagulation cascades that control clot dissolution and blood clotting, respectively (BOX 5). tPA is the initiator of the fibrinolytic system, whereas factor XII initiates the contact system of coagulation. Both enzymes require an activator that functions as a scaffold, bringing enzyme and substrate into close contact. Fibrin, present either in blood clots or as a component of the ECM, is the classical cofactor for tPA. On binding tPA and plasminogen to fibrin, the rate of plasminogen activation is markedly increased⁴⁸. It was recently shown that fibrin and fibrin-derived peptides adopt a cross-β structure and form amyloid fibrils⁴⁹. The formation of these fibrils correlates with tPA binding and stimulation of tPA-mediated plasminogen activation. Interestingly, prototype amyloid peptides, including amyloid-β (Aβ) and islet amyloid polypeptide (IAPP; which is associated with pancreatic β-cell toxicity in type II diabetes), also bind tPA and can substitute for fibrin in plasminogen activation. These results suggest that any amyloid can activate tPA, so microbial curli, chaplin and hydrophobin amyloids might also have plasminogen activation activity. Furthermore, these microbial amyloids could also activate factor XII, which is structurally related to tPA. Activation of the contact and fibrinolytic systems by microbial surface-located amyloid could be a method by which certain microorganisms exploit the host haemostatic system.

Pathogens exploit the haemostatic system

The host fibrinolytic and contact systems are activated during infection with bacteria, viruses, protozoa and fungi (REFS 50–58 and refs therein). The resulting host-derived extracellular proteolytic activity contributes to inflammation and the pathogenesis of sepsis^{43,51–53,59–72}. Activation of plasminogen by bacterial pathogens results in proteolysis of the ECM^{73–77}, allowing bacteria to penetrate the basement membrane^{67,78–81} and leading to increased invasion of human endothelial⁸², pharyngeal⁸³ or HeLa cells⁸⁴. Pathogens can synthesize their own plasminogen activators or rely on host tPA to generate plasmin on the cell surface (REFS 50,56 and

M PROTEINS

A family of proteins expressed by *Streptococcus pyogenes* that mediate interaction with human plasma proteins and promote bacterial adherence to epithelial cells.

Box 3 | **Assembly of chaplins into amyloid fibrils**

Streptomyces coelicolor contains eight chaplins⁵. The mature forms of five of these chaplins (ChpD–H) are ~55 amino acids long, whereas ChpA–C consist of ~225 amino acids. ChpA–C contain two so-called chaplin domains (that is, sequences similar to those of mature ChpD–H), a hydrophilic region and a cell-wall-anchoring domain, which is recognized by sortase enzymes that covalently attaches it to the growing peptidoglycan layer^{134,135}. Linkage to the cell wall would explain why ChpA–C cannot be extracted from cell walls of aerial hyphae³. By contrast, ChpD–H dissociate on treatment with trifluoroacetic acid (TFA), and the resulting monomers are water soluble. These unstructured monomers assemble at the water–air interface into amyloid fibrils with a diameter of 4–6 nm. The fibrils form a rigid, light-reflecting film that reduces the water surface tension from 72 to 26 mJ m⁻² (REF. 3). Water-soluble chaplins do not spontaneously assemble at a hydrophobic solid substrate²⁹. Instead, they adopt an α -helical conformation. Heating in diluted detergent induces the protein to proceed to the β -sheet-rich amyloid form. The α -helical conformation could therefore be an intermediate in the assembly process as observed for hydrophobins (BOX 2) and other amyloid proteins^{2,130,136}. Chaplins assemble in solution when a seed of the assembled form of the protein is added. So, in contrast to hydrophobins, once a nucleus of amyloid has been formed, self-assembly of chaplins becomes independent of a hydrophilic–hydrophobic interface. It is not yet known whether streptomycetes form nucleation proteins as has been observed for curli fibres (BOX 4).

Chaplin fibrils are organized in rodlets at the surface of *S. coelicolor* aerial hyphae and spores (FIG. 1b). These rodlets consist of two tracks, each track containing two chaplin fibrils⁴. It has been proposed that the rodlin RdlA and RdlB align the chaplin fibrils into rodlets (see figure). This hypothesis is based on the observation that mutants in which either or both *rdl* genes have been deleted lack the rodlet structures but instead have fibrils at their surfaces similar in appearance to those formed by chaplins *in vitro*. Figure modified, with permission, from REF. 4 © (2004) Blackwell Publishing.

refs therein). Various microbial tPA receptors have been described^{83,85,86–88}, including curli amyloids of *E. coli*^{45,85,89}. Interestingly, a curli-deficient mutant strain of *E. coli*, which is no longer virulent, also fails

to bind tPA and activate plasminogen^{45,85,89}, which indicates that amyloid may be a virulence factor.

Several bacteria, including *E. coli*^{43,63}, *Salmonella enterica* serovar Typhimurium⁶³, *Staphylococcus aureus*⁹⁰ and *Streptococcus pyogenes*^{59,91,92}, assemble proteins from the contact system of clot dissolution at their cell surface. Adsorption of these proteins to the cell surface results in activation of factor XII and subsequent release of bradykinin and other pro-inflammatory peptides that induce vasodilation, vasopermeability and cytokine release^{43,63,90,92}. The mechanisms of assembly and activation of proteins of the contact system are remarkably similar to those of recruitment and activation of components of the fibrinolytic system. In fact, the same cell-surface receptors have been implicated — curli of *E. coli*^{43,63}, Tafi of *Salmonella* spp.⁶³ and the M PROTEINS of *S. pyogenes*^{59,91}. Mutant strains of *E. coli* or *S. pyogenes* lacking curli or M protein, respectively, fail to bind and activate contact proteins^{43,63,91,92}. So far, only curli and Tafi are known to form amyloid. It remains to be established whether M proteins and other receptors for tPA and factor XII also form amyloid or whether they activate these proteases by another mechanism. The potential consequences of the interaction between amyloid and factors of the haemostatic system are illustrated schematically in FIG. 3.

Box 4 | **Formation of curli and Tafi amyloid**

CsgA is the main structural component of curli of *Escherichia coli*^{7,137}. The mature form of this protein is 131 amino acids in length (15 kDa) and is characterized by several glycine repeats and the absence of cysteines¹³⁷. A purified His-tagged version of CsgA is initially soluble, but after incubation at 4°C for 4–12 hours the protein forms amyloid fibrils that are similar to those observed at the surfaces of *E. coli*⁷. These fibrils are 4–7 nm and 6–12-nm wide, as shown by negative staining⁴⁴ and shadowing⁷ (FIG. 1c), respectively. For formation of curli fibrils *in vivo*, nucleation and precipitation machinery is required that probably prevents self-assembly in the cell and accelerates assembly at the cell surface. This machinery is encoded by *csgB* and *csgEFG*¹³⁸. Experimental evidence indicates that CsgB and CsgF nucleate CsgA fibres at surfaces of cells^{7,139}. As CsgB is homologous to CsgA it is possible that these proteins co-assemble¹³⁸. The lipoprotein CsgG, which localizes to the inner leaflet of the outer membrane, was shown to be necessary for CsgA stabilization and secretion^{7,140}.

Tafi fibrils of *Salmonella* spp. are mainly composed of AgfA¹⁴¹, the amino-acid sequence of which shares 74% identity and 86% similarity with that of *E. coli* CsgA¹⁴². The mechanism of nucleation and aggregation of AgfA is identical to that of CsgA¹⁴¹⁻¹⁴³. But the physical appearance of Tafi can be different to curli, caused by the fact that Tafi interacts avidly with cellulose produced by *Salmonella* spp.¹⁴⁴ In the absence of this polysaccharide, Tafi and curli fibres are indistinguishable from each other.

Box 5 | **The haemostatic system**

The haemostatic system controls blood clotting (coagulation) and clot dissolution (fibrinolysis). The coagulation system comprises the extrinsic and intrinsic pathways.

The extrinsic pathway is triggered by tissue factor that is exposed on cells on injury. The intrinsic pathway is initiated when blood contacts a negatively charged surface, and is therefore also referred to as the contact system of coagulation. Activation of both coagulation pathways activates a cascade of proteases

that ultimately leads to the conversion of prothrombin into thrombin. Thrombin cleaves fibrinogen, which in turn triggers fibrin polymerization and clot formation. The extrinsic pathway is considered the main route of coagulation to prevent blood loss, whereas the intrinsic (contact) pathway has been postulated to function during inflammation.

Contact system

The contact system is activated by the conversion of factor XII into the active serine protease factor XIIa, induced by binding of factor XII to an activator. *In vitro* activators often have negatively charged groups and include glass, kaolin, dextran sulphate and phospholipids. The *in vivo* activator is unknown, but recent evidence indicates that amyloid may be able to act in this regard (Bouma, B. *et al*, unpublished). Formation of factor XIIa ultimately yields the nona-peptide bradykinin, which is released from high-molecular-weight kininogen (HK)¹⁴⁵ by kallikrein (K). Bradykinin induces vasodilation, hypotension, increased vasopermeability and broncho-constriction¹⁴⁶, as well as the release of intracellular vesicles containing tissue-type plasminogen activator (tPA)^{147,148}. The latter is considered the main activator of fibrinolysis. Factor XIIa and kallikrein also activate the complement system and stimulate neutrophils^{64,149}. Taken together, the contact system contributes to inflammatory reactions^{68,150}.

Fibrinolytic system

Plasminogen (Plg) and its active form plasmin (Pls) are central components in fibrinolysis. Plasminogen is activated by the serine proteases tPA or urokinase, both of which are produced by endothelial cells, among other cell types. Plasmin has a broad substrate specificity and degrades not only fibrin but also other extracellular matrix components such as laminin and fibronectin. In addition, plasmin activates pro-collagenases, allowing degradation of collagen matrices^{151,152}, and cleaves certain pro-hormones (for example, latent transforming growth factor- α)¹⁵³. Given its broad specificity, plasmin is believed not only to have a role in fibrinolysis but also in several physiological and pathophysiological processes¹⁵⁴. Indeed, it is associated with cell-mediated degradation of the extracellular matrix during inflammation, wound healing, tissue remodelling, metastasis and invasion of tumour cells, neuronal cell migration and long-term potentiation¹⁵⁴, PK, prekallikrein; PL, phospholipids; Va, factor Va.

Microbial amyloid and the innate immune system

In addition to proteins that contain the fibronectin type I module, other proteins also interact with amyloid, including components of the innate immune system⁹³ such as the serum amyloid P component (SAP)^{94–96}, the scavenger receptors A, B1 (REFS 97,98), CD36 (REFS 99–102) and RAGE (receptor for advanced glycation end products)^{103–106}. The physiological function of SAP is not completely understood, but it is believed to control clearance of chromatin and apoptotic cells and prevents autoimmunity to chromatin and apoptotic cells^{107,108}. SAP stabilizes amyloid fibrils⁹⁵, thereby contributing to the pathogenesis of amyloidosis¹⁰⁹. SAP also interacts with bacteria including *S. pyogenes*¹¹⁰ and *E. coli*¹¹¹, and it has been suggested that the binding of SAP to these bacteria is mediated by surface-located amyloid. Interestingly,

by binding SAP, pathogenic bacteria evade neutrophil phagocytosis and have enhanced virulence¹¹¹.

So far, experiments indicate that the presence of surface-located amyloid only benefits the microorganism. However, scavenger receptors of the innate immune system might recognize amyloid as a pathogen-associated molecular pattern. In support of this hypothesis is the finding that scavenger receptor type A is involved in phagocytosis of bacteria^{112,113} and is known to bind amyloid^{97,114,115}. Interestingly, *S. typhimurium* with cell-surface-located Tafi is cleared more rapidly from the host than strains that do not express these amyloid fibrils¹¹⁶. However, Tafi is also a virulence factor and provides the pathogen with a selective advantage by increasing the efficiency of colonization of the host intestine¹¹⁷. It could be that in the case of *Shigella*, clearance of the microorganism

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16. Stringer, M. A., Dean, R. A., Sewall, T. C. & Timberlake, W. E. Rodletless, a new *Aspergillus* developmental mutant induced by directed gene inactivation. *Genes Dev.* **5**, 1161–1171 (1991).
17. Talbot, N. J. *et al.* MPG1 encodes a fungal hydrophobin involved in surface interactions during infection-related development of *Magnaporthe grisea*. *Plant Cell* **8**, 985–999 (1996).
18. Thau, N. *et al.* rodletless mutants of *Aspergillus fumigatus*. *Infect. Immun.* **62**, 4380–4388 (1994).
19. Talbot, N. J., Ebbole, D. J. & Hamer, J. E. Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell* **5**, 1575–1590 (1993).
20. Wösten, H. A. B., Schuren, F. H. J. & Wessels, J. G. H. Interfacial self-assembly of a hydrophobin into an amphipathic protein membrane mediates fungal attachment to hydrophobic surfaces. *EMBO J.* **13**, 5848–5854 (1994).
21. Wösten, H. A. B., Ruudt, T. G., van der Mei, H. C., Busscher, H. J. & Wessels, J. G. H. Interfacial self-assembly of a *Schizophyllum commune* hydrophobin into an insoluble amphipathic membrane depends on surface hydrophobicity. *Colloids Surf. B: Biointerf.* **5**, 189–195 (1995).
22. St Leger, R. J., Staples, R. C. & Roberts, D. W. Cloning and regulatory analysis of starvation-stress gene, *ssgA*, encoding a hydrophobin-like protein from the entomopathogenic fungus, *Metarhizium anisopliae*. *Gene* **120**, 119–124 (1992).
23. Parta, M., Chang, Y., Rulung, S., Pinto-DaSilva, P. & Kwon-Chung, K. J. *HYP1*, a hydrophobin gene from *Aspergillus fumigatus*, complements the rodletless phenotype in *Aspergillus nidulans*. *Infect. Immun.* **62**, 4389–4395 (1994).
24. Paris, S. *et al.* Conidial hydrophobins of *Aspergillus fumigatus*. *Appl. Environ. Microbiol.* **69**, 1581–1588 (2003).
25. Shibuya, K. *et al.* Histopathology of experimental invasive pulmonary aspergillosis in rats: pathological comparison of pulmonary lesions induced by specific virulent factor deficient mutants. *Microb. Pathog.* **27**, 123–131 (1999).
26. Zogaj, X., Bokranz, W., Nimtz, M. & Romling, U. Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. *Infect. Immun.* **71**, 4151–4158 (2003).
27. Doran, J. L. *et al.* DNA-based diagnostic tests for *Salmonella* species targeting *agfA*, the structural gene for thin, aggregative fimbriae. *J. Clin. Microbiol.* **31**, 2263–2273 (1993).
28. Sakellaris, H. *et al.* Curli loci of *Shigella* spp. *Infect. Immun.* **68**, 3780–3783 (2000).
29. Claessen, D. Structural proteins involved in morphological differentiation of streptomycetes. PhD Thesis (Univ. of Groningen, The Netherlands, 2004).
30. Hirvonen, M. R., Nevalainen, A., Makkonen, N., Mönkkönen, J. & Savolainen, K. *Streptomyces* spores from mouldy houses induce nitric oxide, TNF- α and IL-6 secretion from RAW264.7 macrophage cell line without causing subsequent cell death. *Environ. Toxicol. Pharmacol.* **3**, 57–63 (1997).
31. Hirvonen, M. R., Ruotsalainen, M., Savolainen, K. & Nevalainen, A. Effect of viability of actinomycete spores on their ability to stimulate production of nitric oxide and reactive oxygen species in RAW264.7 macrophages. *Toxicology* **124**, 105–114 (1997).
32. Jussila, J. *et al.* Systemic immunoresponses in mice after repeated exposure of lungs to spores of *Streptomyces californicus*. *Clin. Diagn. Lab. Immunol.* **10**, 30–37 (2003).
33. Fahal, A. H. Mycetoma: a thorn in the flesh. *Trans. R. Soc. Trop. Med. Hyg.* **98**, 3–11 (2004).
34. Austin, J. W., Sanders, G., Kay, W. W. & Collinson, S. K. Thin aggregative fimbriae enhance *Salmonella enteritidis* biofilm formation. *FEMS Microbiol. Lett.* **162**, 295–301 (1998).
35. Vidal, O. *et al.* Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new *ompR* allele that increases curli expression. *J. Bacteriol.* **180**, 2442–2449 (1998).
36. van der Velden, A. W. M., Baumlér, A. J., Tsois, R. M. & Heffron, F. Multiple fimbrial adhesins are required for full virulence of *Salmonella typhimurium* in mice. *Infect. Immun.* **66**, 2803–2808 (1998).
37. Sukupolvi, S. *et al.* Expression of thin aggregative fimbriae promotes interaction of *Salmonella typhimurium* SR-11 with mouse small intestinal epithelial cells. *Infect. Immun.* **65**, 5320–5325 (1997).
38. Dibb-Fuller, M. P., Allen-Vercoe, E., Thorns, C. J. & Woodward, M. J. Fimbriae- and flagella-mediated association with and invasion of cultured epithelial cells by *Salmonella enteritidis*. *Microbiology* **145**, 1023–1031 (1999).
39. Gophna, U. *et al.* Curli fibers mediate internalization of *Escherichia coli* by eukaryotic cells. *Infect. Immun.* **69**, 2659–2665 (2001).
40. Serpell, L. C. *et al.* The protofilament substructure of amyloid fibrils. *J. Mol. Biol.* **300**, 1033–1039 (2000).
41. Sunde, M. *et al.* Common core structure of amyloid fibrils by synchrotron X-ray diffraction. *J. Mol. Biol.* **273**, 729–739 (1997).
42. Rochet, J. C. & Lansbury, P. T. Jr. Amyloid fibrillogenesis: themes and variations. *Curr. Opin. Struct. Biol.* **10**, 60–68 (2000).
43. Ben Nasr, A., Olsén, A., Sjöbring, U., Müller-Esterl, W. & Björck, L. Assembly of human contact phase proteins and release of bradykinin at the surface of curli-expressing *Escherichia coli*. *Mol. Microbiol.* **20**, 927–935 (1996).
- The first example of activation of components of the intrinsic pathway of coagulation by curli and Tafi fibrils.**
44. Olsén, A., Jonsson, A. & Normark, S. Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*. *Nature* **338**, 652–655 (1989).
45. Sjöbring, U., Pohl, G. & Olsén, A. Plasminogen, absorbed by *Escherichia coli* expressing curli or by *Salmonella enteritidis* expressing thin aggregative fimbriae, can be activated by simultaneously captured tissue-type plasminogen activator (t-PA). *Mol. Microbiol.* **14**, 443–452 (1994).
- The first example of activation of components of the fibrinolytic system by curli and Tafi fibrils.**
46. Gophna, U., Oelschlaeger, T. A., Hacker, J. & Ron, E. Z. Role of fibronectin in curli-mediated internalization. *FEMS Microbiol. Lett.* **212**, 55–58 (2002).
47. Ozeri, V., Rosenshine, I., Mosher, D. F., Fassler, R. & Hanski, E. Roles of integrins and fibronectin in the entry of *Streptococcus pyogenes* into cells via protein F1. *Mol. Microbiol.* **30**, 625–637 (1998).
48. Suenson, E., Lutzen, O. & Thorsen, S. Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. *Eur. J. Biochem.* **140**, 513–522 (1984).
49. Kraneburg, O. *et al.* Tissue-type plasminogen activator is a multiligand cross-beta structure receptor. *Curr. Biol.* **12**, 1833–1839 (2002).
- This article shows that the common feature of tPA-activating ligands is the amyloid cross- β structure.**
50. Heller, M. V. *et al.* Early markers of blood coagulation and fibrinolysis activation in Argentine hemorrhagic fever. *Thromb. Haemost.* **73**, 368–373 (1995).
51. Lottenberg, R., Minning-Wenz, D. & Boyle, M. D. Capturing host plasmin(ogen): a common mechanism for invasive pathogens? *Trends Microbiol.* **2**, 20–24 (1994).
52. Coleman, J. L. & Benach, J. L. Use of the plasminogen activation system by microorganisms. *J. Lab. Clin. Med.* **134**, 567–576 (1999).
53. Läähteenmäki, K., Kuusela, P. & Korhonen, T. K. Bacterial plasminogen activators and receptors. *FEMS Microbiol. Rev.* **25**, 531–552 (2001).
54. Goto, H., Wells, K., Takada, A. & Kawaoka, Y. Plasminogen-binding activity of neuraminidase determines the pathogenicity of influenza A virus. *J. Virol.* **75**, 9297–9301 (2001).
55. Handley, M. A., Steigbigel, R. T. & Morrison, S. A. A role for urokinase-type plasminogen activator in human immunodeficiency virus type 1 infection of macrophages. *J. Virol.* **70**, 4451–4456 (1996).
56. Huang, Y. H. *et al.* Antibodies against dengue virus E protein peptide bind to human plasminogen and inhibit plasmin activity. *Clin. Exp. Immunol.* **110**, 35–40 (1997).
57. Huang, Y. H. *et al.* Tissue plasminogen activator induced by dengue virus infection of human endothelial cells. *J. Med. Virol.* **70**, 610–616 (2003).
58. Monroy, V. & Ruiz, B. H. Participation of the Dengue virus in the fibrinolytic process. *Virus Genes* **21**, 197–208 (2000).
59. Ben Nasr, A., Herwald, H., Müller-Esterl, W. & Björck, L. Human kininogens interact with M protein, a bacterial surface protein and virulence determinant. *Biochem. J.* **305**, 173–180 (1995).
60. Boyle, M. D. & Lottenberg, R. The interaction of pathogens with humans. *Methods* **21**, 99–102 (2000).
61. Hack, C. E. & Colman, R. W. The role of the contact system in the pathogenesis of septic shock. *Sepsis* **3**, 111–118 (1999).
- A recommended review on the role of the haemostatic system during infection.**
62. Hack, C. E. Derangements of coagulation and fibrinolysis in infectious diseases. *Contrib. Microbiol.* **10**, 18–37 (2003).
- A recommended review of haemostasis during infection.**
63. Herwald, H. *et al.* Activation of the contact-phase system on bacterial surfaces — a clue to serious complications in infectious diseases. *Nature Med.* **4**, 298–302 (1998).
64. Jansen, P. M. *et al.* Inhibition of factor XII in septic baboons attenuates the activation of complement and fibrinolytic systems and reduces the release of interleukin-6 and neutrophil elastase. *Blood* **87**, 2337–2344 (1996).
65. Kalter, E. S., van Dijk, W. C., Timmerman, A., Verhoef, J. & Bouma, B. N. Activation of purified human plasma prekallikrein triggered by cell wall fractions of *Escherichia coli* and *Staphylococcus aureus*. *J. Infect. Dis.* **148**, 682–691 (1983).
66. Korhonen, T. K. *et al.* Plasminogen receptors. Turning *Salmonella* and *Escherichia coli* into proteolytic organisms. *Adv. Exp. Med. Biol.* **412**, 185–192 (1997).
67. Läähteenmäki, K., Kuusela, P. & Korhonen, T. K. Plasminogen activation in degradation and penetration of extracellular matrices and basement membranes by invasive bacteria. *Methods* **21**, 125–132 (2000).
68. Levi, M., Keller, T. T., van Gorp, E. & ten Cate, H. Infection and inflammation and the coagulation system. *Cardiovasc. Res.* **60**, 26–39 (2003).
69. Persson, K., Russell, W., Morgelin, M. & Herwald, H. The conversion of fibrinogen to fibrin at the surface of curliated *Escherichia coli* bacteria leads to the generation of proinflammatory fibrinopeptides. *J. Biol. Chem.* **278**, 31884–31890 (2003).
70. Psuja, P. *et al.* Plasma markers of hypercoagulability in patients with serious infections and risk of septic shock. *Clin. Appl. Thromb. Hemost.* **8**, 225–230 (2002).
71. Saetre, T., Lindgaard, A. K. & Lyberg, T. Systemic activation of coagulation and fibrinolysis in a porcine model of serogroup A streptococcal shock. *Blood Coagul. Fibrinolysis* **11**, 433–438 (2000).
72. Holder, I. A. & Neely, A. N. Hageman factor dependent activation and its relationship to lethal *Pseudomonas aeruginosa* burn wound infections. *Agents Actions Suppl.* **38**, 329–342 (1992).
73. Darenfed, H., Grenier, D. & Mayrand, D. Acquisition of plasmin activity by *Fusobacterium nucleatum* subsp. *nucleatum* and potential contribution to tissue destruction during periodontitis. *Infect. Immun.* **67**, 6439–6444 (1999).
74. Fuchs, H., Wallich, R., Simon, M. M. & Kramer, M. D. The outer surface protein A of the spirochete *Borrelia burgdorferi* is a plasmin(ogen) receptor. *Proc. Natl Acad. Sci. USA* **91**, 12594–12598 (1994).
75. Coleman, J. L. & Benach, J. L. The generation of enzymatically active plasmin on the surface of spirochetes. *Methods* **21**, 133–141 (2000).
76. Coleman, J. L., Roemer, E. J. & Benach, J. L. Plasmin-coated *Borrelia burgdorferi* degrades soluble and insoluble components of the mammalian extracellular matrix. *Infect. Immun.* **67**, 3929–3936 (1999).
77. Virkola, R. *et al.* Interaction of *Haemophilus influenzae* with the mammalian extracellular matrix. *J. Infect. Dis.* **173**, 1137–1147 (1996).
78. da Silva, C. M., de Abreu Viripo, L., Nishi, R. & Cristina, P. M. Binding of plasminogen to *Pseudomonas aeruginosa* results in formation of surface-associated plasmin and enhanced bacterial invasiveness. *Microb. Pathog.* **36**, 59–66 (2004).
79. Korhonen, T. K. *et al.* Penetration of fimbriated enteric bacteria through reconstituted basement membranes: a hypothesis. *FEMS Microbiol. Lett.* **79**, 307–312 (1992).
80. Läähteenmäki, K. *et al.* Bacterial plasminogen receptors: *in vitro* evidence for a role in degradation of the mammalian extracellular matrix. *Infect. Immun.* **63**, 3659–3664 (1995).
81. Eberhard, T. H., Kronvall, G. & Ullberg, M. Surface bound plasmin promotes migration of *Streptococcus pneumoniae* through reconstituted basement membranes. *Microb. Pathog.* **26**, 175–181 (1999).
82. Jong, A. Y. *et al.* Binding of *Candida albicans* enolase to plasmin(ogen) results in enhanced invasion of human brain microvascular endothelial cells. *J. Med. Microbiol.* **52**, 615–622 (2003).
83. Pancholi, V., Fontan, P. & Jin, H. Plasminogen-mediated group A streptococcal adherence to and pericellular invasion of human pharyngeal cells. *Microb. Pathog.* **35**, 293–303 (2003).
84. Yavlovich, A., Higazi, A. A. & Rottem, S. Plasminogen binding and activation by *Mycoplasma fermentans*. *Infect. Immun.* **69**, 1977–1982 (2001).
85. Kukkonen, M. *et al.* Identification of two laminin-binding fimbriae, the type 1 fimbria of *Salmonella enterica* serovar *Typhimurium* and the G fimbria of *Escherichia coli*, as plasminogen receptors. *Infect. Immun.* **66**, 4965–4970 (1998).
86. Urdaneta, L., Vanegas, G., Premoli, G. & Avilan, L. Plasminogen interaction and activation on *Streptococcus mutans* surface. *Oral Microbiol. Immunol.* **19**, 257–261 (2004).
87. Bergmann, S. *et al.* Identification of a novel plasmin(ogen)-binding motif in surface displayed α -enolase of *Streptococcus pneumoniae*. *Mol. Microbiol.* **49**, 411–423 (2003).
88. Kuusela, P. *et al.* Surface-associated activation of plasminogen on gram-positive bacteria. Effect of plasmin on the adherence of *Staphylococcus aureus*. *Acta Ophthalmol. Suppl.* 42–46 (1992).