Review

Microbial adhesins to gastrointestinal mucus

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The gastrointestinal tract (GIT) is lined by a layer of mucus formed by mucin glycoproteins. This layer constitutes a physical and chemical barrier between the intestinal contents and the underlying epithelia. In addition to this protective role, mucins harbor glycan-rich domains that provide preferential binding sites for pathogens and commensal bacteria. Although mucus–microbial interactions in the GIT play a crucial role in determining the outcome of relationships of both commensal and pathogens with the host, the adhesins and ligands involved in the interaction are poorly delineated. This review focuses on the current knowledge of microbial adhesins to gastrointestinal mucus and mucus components.

Mucus and mucin glycans as receptors of gastrointestinal microbes

The mucus layer covering the gastrointestinal tract (GIT) is a biochemically complex medium, rich in glycoproteins, antimicrobial peptides, immunoglobulins and many other intestinal proteins, as well as lipids and electrolytes. The thickness of the mucus layer varies with the region of the GIT, but is thickest in the colon and rectum (Figure 1). The gastrointestinal mucus is divided into an outer loose layer which can be easily removed and an inner layer which is firmly attached to the epithelium. Recent studies showed that the presence of bacteria is restricted to the outer mucus layer, whereas the compact stratified inner layer isolates the epithelium from the enormous bacterial load in the colon lumen [1] (Figure 1). The process underlying the formation of these layers remains elusive.

Mucins constitute the major structural components of the mucus layers. Currently, the human mucin (MUC) family includes 17 members (MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC12, MUC13, MUC14, MUC15, MUC16, MUC17, MUC19, MUC20 and MUC21) with a common structural feature: a tandem repeat domain comprising sequences of amino acids repeated in tandem, which are rich in proline, threonine and serine residues, constituting the PTS (Pro/Thr/Ser) domains (for a review, see [2]). These domains are extensively *O*-glycosylated (Box 1). These high-molecular-mass oligomeric glycoproteins are produced in the intestine as membrane-bound (MUC1, MUC3, MUC4 MUC12, MUC13 and MUC17) and secreted mucins (MUC2, MUC5B, MUC5AC and MUC6). In the small intestine and colon, the mucus layer mainly consists of the secreted mucin MUC2 (in mouse, Muc2) [3], whereas MUC1, MUC5AC and MUC6 are the main mucins in the stomach [2]. Muc2 deficient ($Muc2^{-/-}$) mice suffer from spontaneous colitis intestinal inflammation and/or develop colorectal cancer [1,4], highlighting the importance of a functional mucus layer in maintaining a symbiotic relationship with the microbiota, as recently reviewed [5]. Under the mucus layer, the cell-surface mucins are a dominant feature of the apical surface of all mucosal epithelial cells. Cell-surface mucins are transmembrane glycoproteins; the extensively *O*-glycosylated extracellular domain protrudes at least 800 nm above the cell surface and plays a role in cell adhesion, whereas the cytoplasmic domain has been implicated in signal transduction [6]. The large extracellular domain can be shed from the cell surface and is believed to act as a releasable decoy ligand for bacterial adhesins, thereby limiting attachment of pathogens to other cell-surface molecules and subsequent invasion [7]. These studies have triggered renewed interest in the role of gastrointestinal mucus in preserving gastrointestinal health and the role played by mucins and mucin glycosylation in this process (Box 1).

Structural studies investigating glycosylation of intestinal mucins in humans and rodents showed regio-specific glycosylation along the GIT [8,9]. Human intestinal mucins display decreasing gradients of fucose and ABH blood group and an increasing acidic gradient from ileum to rectum [8]. Recently, the full repertoire of MUC2 *O*-glycans was determined from human sigmoid colons [10]. More than 100 complex oligosaccharides were identified, mostly mono-, di- or trisialylated. This complex MUC2 colon glycan repertoire is relatively conserved between individuals, suggesting a potential role of mucin glycans in the selection of commensal flora by providing preferential binding sites. The capacity to adhere to the mucus layer within the GIT is believed to be a prerequisite for initial colonization and subsequent proliferation of the natural microbiota and could be vital in the realization of certain probiotic properties. Mucin glycans can also act as receptor molecules for pathogenic microorganisms. The interactions between enteric pathogens and mucins, and the mechanisms by which pathogens trigger changes in mucin production and glycosylation have been the focus of much effort in the past decade, as recently reviewed [11]. The development of experimental animal models such as $Muc1^{-/-}$ and $Muc2^{-/-}$ mice has been instrumental in gaining knowledge and awareness of the role of mucins in the maintenance of gut homeostasis, protecting the host from pathogen infection and bacterial invasion (see for example

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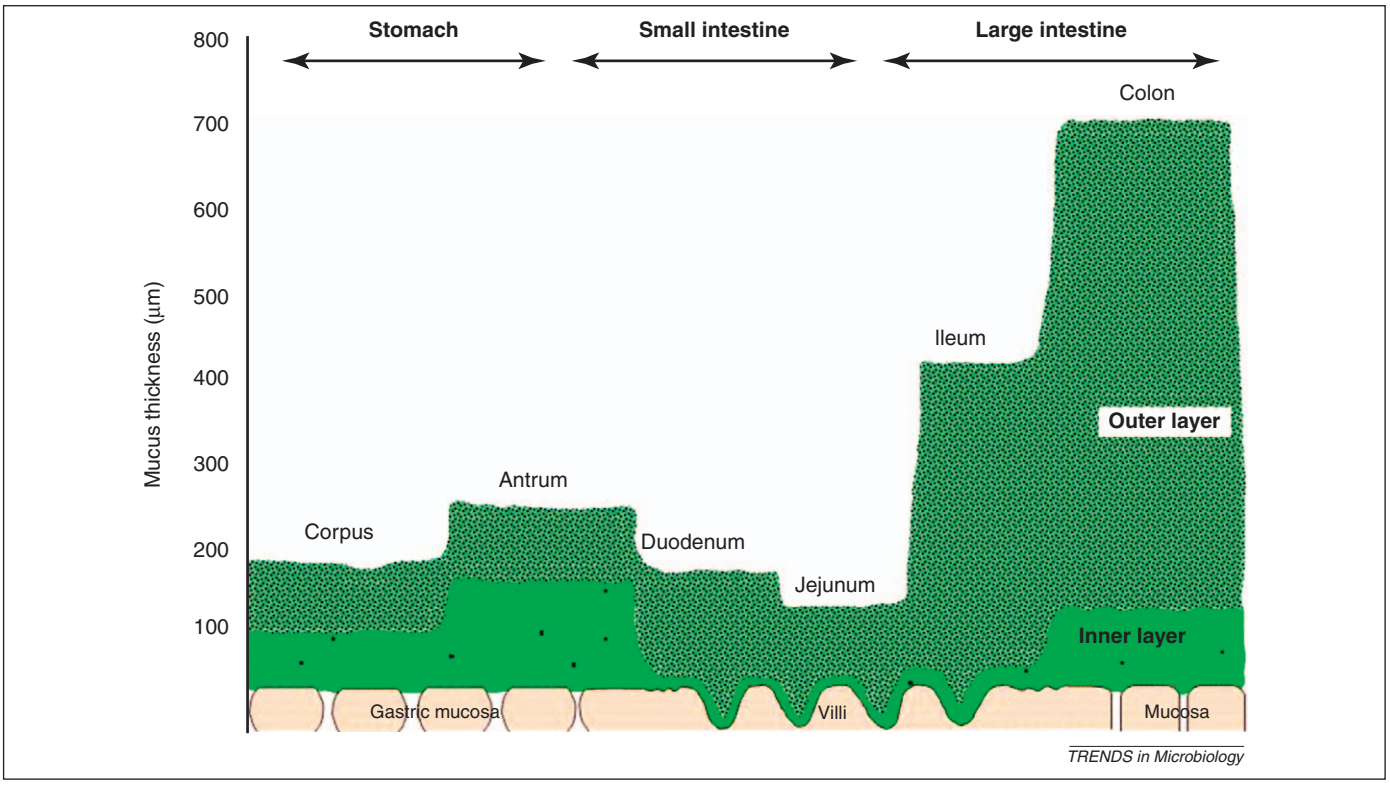

Figure 1. Schematic representation of the mucus layers of the rat gastrointestinal tract. The intestinal epithelial surface is covered by two mucus layers (inner, firmly adherent layer and outer, loosely adherent layer). Microbes are mostly associated with the outer layer (as schematized with black dots). Adapted from [82].

[12–14]). An important factor that correlates with the ability of microbes to adhere to mucus is the presence of specific proteins on the cell surface, referred to as adhesins. With the growing numbers of annotated genomes, new adhesins with mucus-adhering properties have been identified and characterized. In this review, we give a general overview of the biochemical and structural characteristics of the different classes of adhesins from gut microbes which have been implicated in binding to mucus.

Mucus-binding proteins

Mucus-binding proteins (MUBs) have been revealed as one class of effector molecules involved in mechanisms of the adherence of lactobacilli, important commensal bacteria in the GIT, to the host [15]. MUBs are cell-surface proteins containing a typical signal peptide and a LPxTG anchoring motif in the C terminus for covalent attachment to the bacterial cell wall. MUBs are characterized by the presence of multiple Mub repeats, which share homology to the Pfam–MucBP (mucin-binding protein) domains (PF06458). The best studied example of MUBs is from *Lactobacillus reuteri* [16,17], one of the dominant lactobacilli found in the GIT of various animals [18]. The extracellular 353-kDa MUB from *L. reuteri* ATCC 53608 contains two types of related amino acid repeats (Mub1 and Mub2); six copies (RI–RVI) of the type 1 repeat (Mub1) and eight copies (R1–R8) of the type 2 repeat (Mub2). Fusion proteins consisting of different Mub repeats and the maltose-binding protein adhered to pig mucus components, pig gastric mucin (PGM) and hen intestinal mucus [16]. The binding of Mub to mucus components occurred in the pH range 3–7.4, with maximum binding at pH 4–5. Inhibition by asialofetuin or fetuin was of the same order of magnitude, showing that sialic acid was not involved in the interaction. Although these results suggest that Mub interacts with carbohydrates on the mucus components, glucose (Glc), *N*-acetyl-D-galactosamine (GalNAc), *N*-acetylneuraminic acid (Neu5Ac) and *N*-acetyl-D-glucosamine (GlcNAc) did not affect adhesion, whereas only slight inhibition was observed with L-fucose (Fuc) and mannose (Man) and the nature of the mucin glycan ligands remains to be identified [16]. We recently reported the first three-dimensional (3D) structure of a type 2 Mub repeat (Mub-R5) from *L. reuteri* ATCC53608 MUB (PDB 3I57), providing insight into a previously undetected immunoglobulin (Ig)-binding activity for the repeat structural unit of MUBs [17]. MUB folds as two structurally related ubiquitin-like β-grasp-fold (β-GF) domains, an N-terminal domain (B1) and a C-terminal domain (B2) with structural homology to Pfam–MucBP domains. The β-GF domains could potentially provide a scaffold for binding a wide range of ligands (Box 2, Figure 2).

The phylogenetic distribution of the Pfam–MucBP domain is much broader than that of the MUB proteins. For example, MUB is only present in *L. reuteri* ATCC53608, whereas MucBP domains are found in all sequenced *L. reuteri* strains [19]. Across lactobacilli species, 47 proteins with one or more Mub repeats were found in the exoproteomes of six *Lactobacillus* genomes and distributed over six separate Lactobacillales-specific clusters of orthologous protein-coding genes [20]. The Pfam–MucBP domains are also found in the internalin (Inl) family of leucine-rich repeat-containing surface proteins from the foodborne pathogen *Listeria monocytogenes* [21]. However, despite

Box 1. Mucin glycosylation

Mucins carry large numbers of *O*-linked oligosaccharides (*O*-glycans), which account for up to 80% of the total mucin mass and are responsible for many of the rheological and biological properties of mucins. *N*-Glycosylation (mainly high-mannose-, hybrid/complex-type glycans) is relatively minor and mainly associated with the folding and secretion of mucin oligomers [71]. *O*-Linked glycans contain 1–20 residues, which occur both as linear and branched structures, contributing to their structural diversity. In contrast to *N*-glycosylation, which requires a specific amino acid consensus sequence (Asn-Xaa-Ser/Thr), such a relevant motif for *O*-glycosylation has not been identified in glycoproteins. This process is initiated in the Golgi apparatus by the addition of an *N*-acetyl-D-galactosamine (GalNAc) residue to the hydroxyl group of Ser and Thr of the mucin backbone to which is added the core structures. Mucin-type *O*-glycans are built from eight core structures, with core 1, core 2, core 3 and core 4 glycans most commonly found in gastrointestinal mucins (Figure I). After further elongation of the chains to variable lengths based on type 1 or type 2 *N*-acetyl-lactosamine (LacNAc) units, the chains are terminated by L-fucose (Fuc), D-galactose (Gal), GalNAc or sialic acid, also known as neuraminic acid (*N*-acetylneuraminic acid, Neu5Ac) residues, in the peripheral region, forming histo-blood group antigens (HBGAs) such as A, B, H, Lewis a (Le^a), Lewis b (Le^b), Lewis x (Le^x), Lewis y (Le^y), as well as sialyl-Le^a and sialyl-Le^x structures (for a recent review, see [10]). Furthermore, the Sd^a/Cad antigen (GalNAc attached to Gal also substituted with NeuAc) has been shown to be a prominent epitope in the human colon [72,73]. It is generally admitted that the diversity of mucin glycans serves as a bacterial habitat by providing binding sites and energy to sustain the growth of both commensal bacteria and microbial pathogens in the outer mucus layer. Altered *O*-glycosylation profiles of mucins have been associated with increased inflammation in experimental mouse models and humans [74–77], highlighting the importance of mucin glycosylation in maintenance of gut homeostasis.

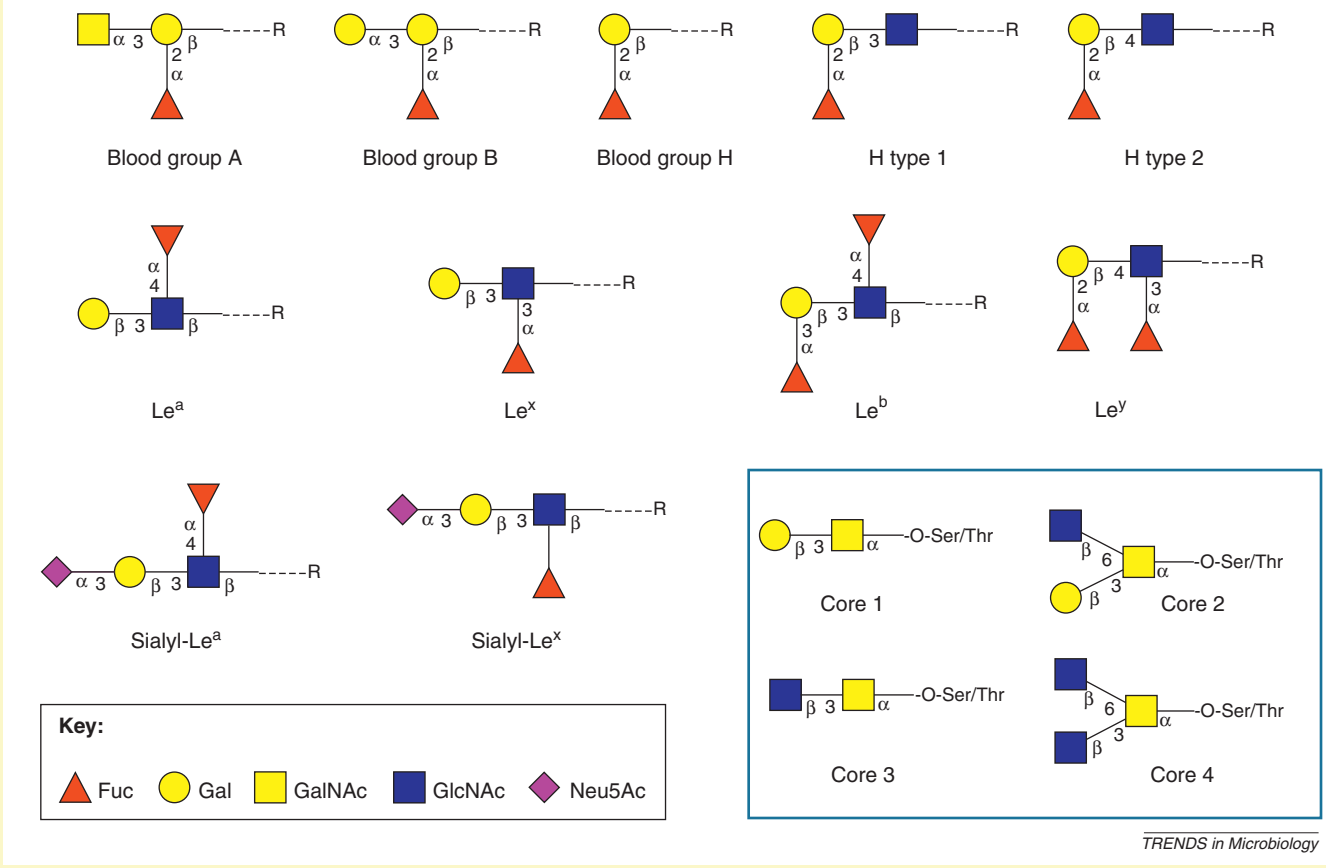

Figure I. Diagrammatic representation of the main core and terminal (ABH structures and Lewis epitopes) structures found in *O*-glycosylated gastrointestinal mucins. Sketched using the nomenclature for monosaccharides (see key).

the multiple copies of MucBP homologs predicted by genome analysis, only a few have been functionally characterized. Recombinant MucBP domains of a predicted cell-surface protein in *Lactobacillus plantarum* WCFS1 containing six MucBP domains [22] were shown to competitively inhibit the binding of *L. plantarum* to Caco-2 cells [23]. The recombinant proteins showed some recognition to human mucus components but more characterization is needed to assess the specificity of the binding. The presence of a putative MucBP was confirmed by PCR in a *L. plantarum* Lp9 isolate [24]. In *L. plantarum* 299 v and WCFS1 strains, the MucBP-containing mannose-specific adhesin (Msa) has been suggested to play a role in adhesion to intestinal epithelial cells [25] and *L. plantarum* Lp6 binding to rat small intestine mucus was reported to be mediated by mannose-specific adhesins [26] but there is no report of purified mannose-adhesin binding to mucin and the specific role of MucBP domains in the adhesion of *L. plantarum* strains to mucin remains to be elucidated. The adherence properties of the only predicted protein (LGG_02337) in *Lactobacillus rhamnosus* GG showing homology with MucBP were recently demonstrated using recombinant purified protein to human intestinal mucus. This adhesin is distributed throughout the cell surface and contributes to the interaction between *L. rhamnosus* GG and mucus, and designated mucus-binding

Box 2. Structural comparisons of MUBs and MucBPs with structurally related domains from other bacterial surface proteins

The first crystal structure of a MUB protein was from *L. reuteri* Mub-R5 repeat (PDB 3I57) [17]. The overall structure of Mub-R5 resembles a distorted cylinder ~110 Å long made up of two discrete domains: an N-terminal domain (B1) domain and a C-terminal (B2) domain. B1 has a canonical β-grasp fold with a four-stranded β-sheet and a single helix and the Ig-binding protein from *Peptostreptococcus magnus* (PpL) is its closest structural homolog. The larger C-terminal B2 domain lacks the helix, and its extra residues form an additional three-stranded β-sheet. The 3D structures of MucBP annotated domains of a predicted adhesion protein PEPE_0118 from *Pediococcus pentosaceus* (PDB 3LYY), of protein LBA1460 from *Lactobacillus acidophilus* (PDB 3Q69) and of a protein annotated as a putative peptidoglycan bound protein (LPXTG motif) lmo0835 (residues 34–128; PDB 2KT7) from *L. monocytogenes* were recently added to the PDB database. Although all annotated as MucBPs, no functional characterization of these proteins has been reported. These structures can be aligned to the functionally characterized *L. reuteri* Mub-R5 repeat (PDB 3I57) and MucBP domain of Spr1345 (PBD 3NZ3) (Figure 3) from the human respiratory pathogen *Streptococcus pneumoniae* R6 [78], an adhesin previously demonstrated to bind mucins and several polysaccharides such as hyaluronan, suggesting specificity for the carbohydrate moiety of mucins [79]. All except the B1 domain of Mub-R5 share the β-grasp fold lacking the helix, suggesting that the conserved C-terminal moiety might participate in the recognition of mucins. Structural similarity was also observed with a domain of adhesion exoprotein from *P. pentosaceus* (PDB 2KYW) and to the B repeat of *L. monocytogenes* internalin B (InlB) (PBD 3NZ3) [80], an invasin mediating entry of the bacteria into eukaryotic cells [21]. The B repeat crystal structure reveals a variation of the β-grasp fold that is most similar to small ubiquitin-like modifiers (SUMOs), providing insights into possible receptor binding sites of mucus-binding proteins with their ligands [80].

factor [27]. Despite these recent advances, further research is warranted to define the precise role of MUB and MucBP adhesins *in vivo* and identify the molecular nature of their receptors.

Multifunctional lactobacillus mucus adhesins

In addition to the structurally characterized MUB and MucBP proteins, multifunctional proteins have been involved in the adhesion of lactobacilli to gastrointestinal mucus [28], although biochemical characterization of the interaction to mucus and/or mucins is often preliminary. A collagen-binding protein (CnBP) of *L. reuteri* NCIB11951 [29], a mucus adhesion-promoting protein (MapA) of *L. reuteri* 104R [30] and mucus/mucin-binding protein (32-Mmubp) of *Lactobacillus fermentum* BCS87 [31] were all identified as components of the ATP-binding cassette

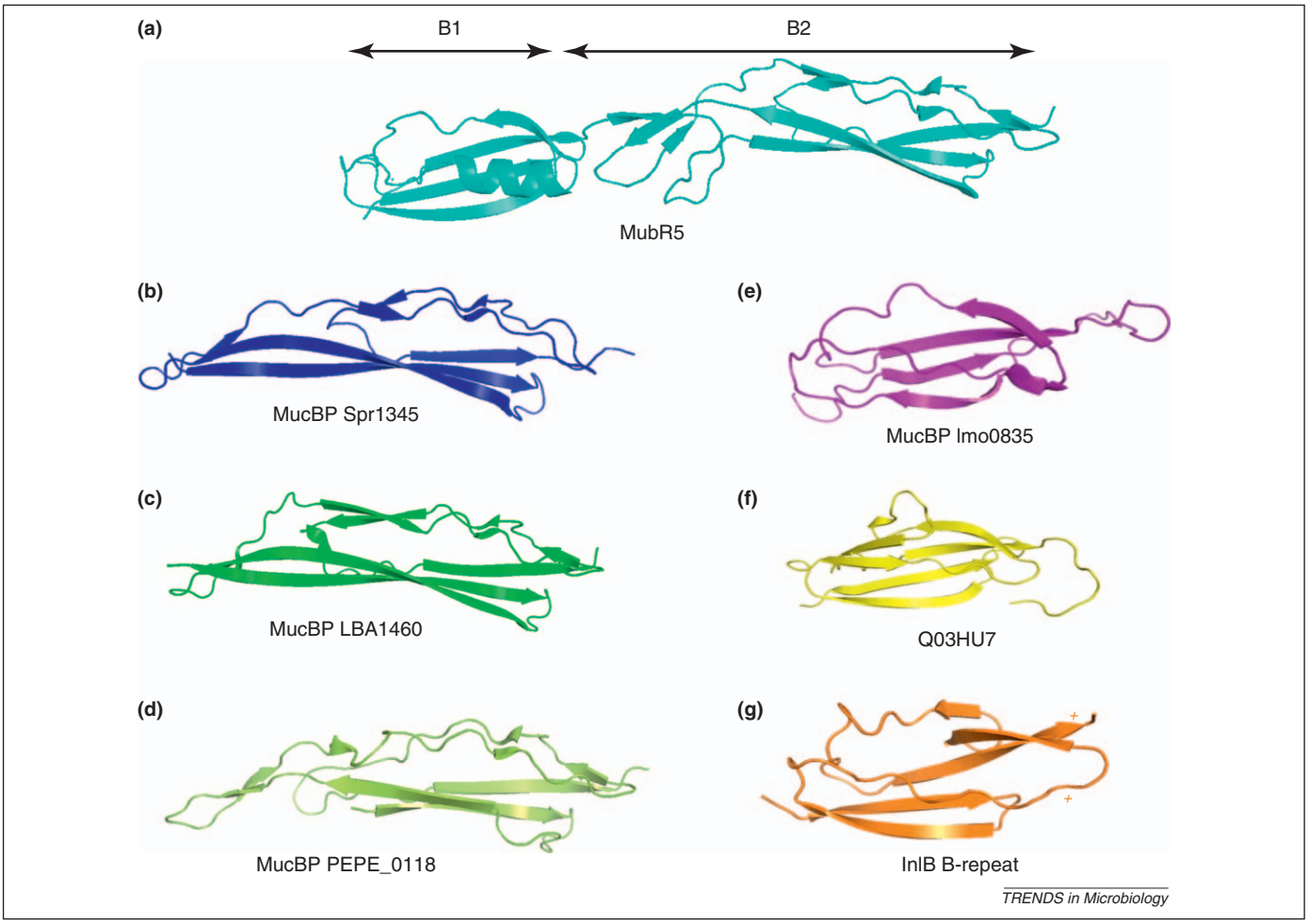

Figure 2. Crystal structures of MUB, MucBPs and related proteins. **(a)** *Lactobacillus reuteri* Mub-R5 repeat (PDB 3I57) [17]; annotated MucBP domains of **(b)** Spr1345 (PBD 3NZ3) from *Streptococcus pneumoniae* [78], **(c)** LBA1460 from *Lactobacillus acidophilus* (PDB 3Q69), **(d)** PEPE_0118 from *Pediococcus pentosaceus* (PDB 3LYY), **(e)** lmo0835 (residues 34–128; PDB 2KT7) from *Listeria monocytogenes*, and structurally related proteins **(f)** adhesion exoprotein from *P. pentosaceus* (PDB 2KYW) and **(g)** InlB B repeat of *L. monocytogenes* (PBD 3NZ3) [80]. For more discussion, see Box 2.

(ABC) transporter and shown to bind gastrointestinal mucus/mucins. Recently, a new 29-kDa adhesin-like protein from *Lactobacillus mucosae* ME340 (Lam29) was identified as a possible mucus binding protein and shown to be 38–77% identical to substrate- and solute-binding proteins of ABC transporters from both Gram-positive and Gram-negative bacteria [32]. Other lactobacilli multifunctional proteins with reported binding ability to human intestinal mucus and/or mucins include the elongation factor Tu (EF-Tu) and heat shock GroEL proteins isolated from *Lactobacillus johnsonii* NCC533 (La1) [33,34] and *L. plantarum* LA 318 glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an important enzyme in glycolysis [35]. EF-Tu, GroEL and GAPDH are referred to as anchorless housekeeping proteins as no established signal sequence or anchoring motif is present in their predicted sequences. Altogether, these findings suggest that proteins involved in bacteria adhesion to mucus could have more than one function or a different function from what is predicted by bioinformatics.

Flagella, fimbriae and pili

Extracellular appendages such as flagella, pili and fimbriae (Box 3) play a major role in the attachment of bacteria to their host and have been implicated in mucus adhesion. Flagella are composed of several thousand copies of flagellin subunits and extensively studied in enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC), both of which cause diarrheal diseases and death worldwide. Recently, the EPEC E2348/69 (O127:H6) and EHEC EDL933 (O157:H7) flagella and their flagellin monomers were shown to possess adhesive properties to a range of host receptors including mucins and bovine mucus [36]. Flagellin mutants of EHEC and EPEC (*fliC*) were significantly less adherent to bovine intestinal tissue than the parental wild-type strains. The adhesive properties of bacterial flagella to mucus were previously reported for *Clostridium difficile*, the main cause of nosocomial infections in elderly and immunocompromised patients. Crude flagella, recombinant flagellar FliC and FliD proteins were shown to bind to murine mucus, whereas no binding to porcine stomach mucus was observed. Flagellated strains seemed to have a better capacity to associate with the cecal wall *in vivo* [37]. These results suggest that the flagellin proteins are involved in attachment of pathogenic bacteria to the mucus layer of the intestine, the first barrier encountered during colonization. To characterize the role of flagellins in the mechanism of action of probiotics, flagellins from the surface of several probiotic *Bacillus* strains were extracted and identified by proteomics [38]. Heterologous expression of *Bacillus cereus* CH flagellin in *Lactococcus lactis* resulted in a strain producing a surface-associated flagellin showing increased adherence to mucin-coated plates, and the ability to competitively inhibit the adhesion of pathogenic *E. coli* LMG2092 and *Salmonella enterica* subsp. *enterica* LMG15860, a Gram-negative pathogen causing enterocolitis in humans and livestock [39]. However, direct binding of this probiotic *B. cereus* flagellin to mucin remains to be demonstrated.

Fimbriae allow bacteria to specifically adhere to a large number of targets, including mammalian cells, host proteins and other microbial cells. The *std* operon encoding a fimbrial adhesin is involved in the ability of *Salmonella enterica* Typhimurium to attach to colonic epithelial cells and for cecal colonization in the mouse [40]. Adherence of Std fimbriated *S.* Typhimurium to Caco-2 cells could be blocked by co-incubation with H2 antigen or by pretreatment of cells with α1–2 fucosidase, whereas pretreatment of Caco-2 cells with neuraminidase or co-incubation with the type 2 disaccharide precursor (Galβ1,4GlcNAc) did not have an effect. Direct binding of purified Std fimbriae to H2 in a solid-phase binding assay was competitively inhibited by *Ulex europaeus* agglutinin-I (UEA-I), a lectin specific for Fucα1–2 moieties. Purified Std fimbriae and UEA both bound to a receptor localized in the mucus layer of the murine cecum [41]. These data suggest that the *std* operon encodes an adhesin that binds an α1–2 fucosylated receptor(s) present in the colonic mucins. Fimbrial adhesins K88 (F4), K99 (F5), F41 and F17 (FY) have been implicated in the adhesion to mucus of the enterotoxigenic *E. coli* strains responsible for diarrhea in swine. Several putative K88 adhesin receptors have been identified on porcine epithelial cells including intestinal mucin-type glycoproteins [42]. Sialic acids and Gal seem to be at least partly responsible for the attachment of K99 [43], whereas F17 and F41 recognize independent receptor sites, the first on sialylated mucus and the second on sialidase-treated mucus [44]. The F17-G adhesin mediates binding to GlcNAc in a terminal nonreducing position or internally in *O*-linked oligosaccharides of intestinal mucins. The F17-G 3D structure (PDB 1O9Z) [45] shares structural similarity to FimH [46,47], a mannose-specific adhesin located on the tip of type 1 fimbriae from enterobacteria such as *E. coli* and *Salmonella* (Figure 3), providing insights into the molecular basis of the diversity in fimbrial lectins and their possible implication in binding to mucin glycans.

Box 3. Flagella, pili and fimbriae

Various cell-surface multisubunit protein polymers, known as flagella, pili or fimbriae, have a pivotal role in the colonization of specific host tissues by Gram-positive and Gram-negative bacteria (for a review, see [81]). Flagella are the most thoroughly studied of all prokaryotic motility structures, and are used for swimming in aqueous environments and in some organisms, for swarming across solid surfaces. Many bacteria have other surface appendages of various sizes and appearances called pili and fimbriae and these terms have often been used interchangeably. Fimbriae are composed of major protein subunits, have diameters ranging from 2 to 8 nm and usually extend 1–2 μm from the bacterial surface. The fimbriae of Gram-negative bacteria have been extensively studied; particularly, those of the Enterobacteriaceae including *E. coli* and *Salmonella* spp. Pili are longer than fimbriae and composed mainly of subunit proteins (pilins) organized into a tube-like structure, which allows the passage of genetic material during conjugation. Although pili are used for adherence by both Gram-negative and Gram-positive bacteria, the structures themselves are substantially different. Gram-negative bacterial pili are composed of pilins which are not covalently linked, and initiation of the structure requires the presence of a protein that ends up on the tip, whereas pili of Gram-positive bacteria, by contrast, are composed of multiple pilin subunits covalently coupled to each other by the transpeptidase activity of the pilin-specific sortase. On average, one to ten conjugative pili and up to more than 400 fimbriae may be present on the surface of a bacterial cell. To maximize their contact with the environment, adhesins are often present on the ends of these long hair-like structures. Flagella, fimbrial and pilin proteins have all been implicated as cell adhesins of microorganisms.

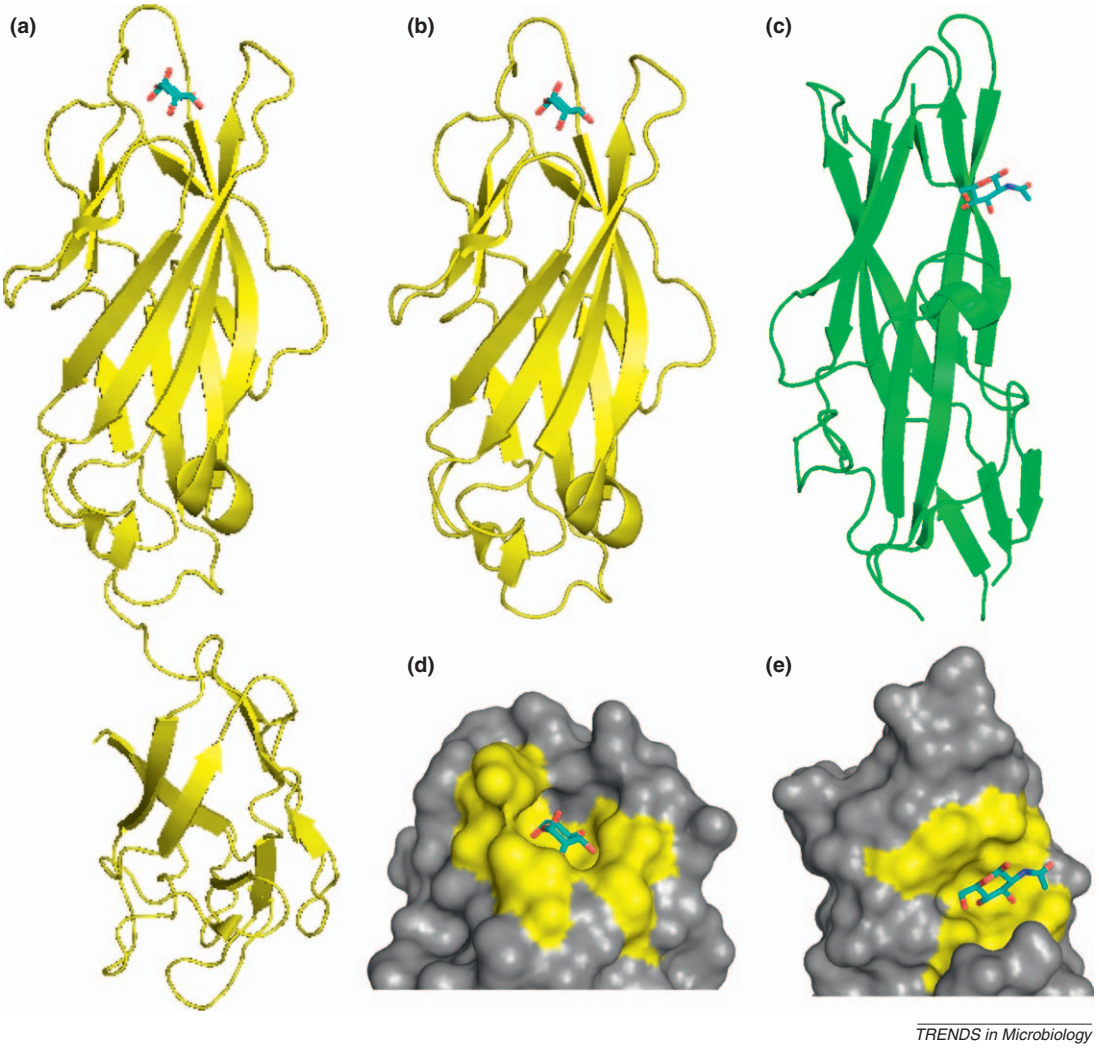

Figure 3. Crystal structures of F17-G and FimH fimbrial adhesins. **(a)** The overall structure of FimH (PDB 1KLF) consists of two Ig-like domains: a C-terminal domain (pilin) that anchors the adhesin into the fimbrial tip and an N-terminal carbohydrate-specific lectin domain [46,47]. Side-by-side comparisons of **(b)** FimH and **(c)** F17-G (PDB 1O9Z) [45] lectin domains with bound D-mannose and GlcNAc ligands, respectively. Both domains share the Ig-like fold of the pilins, despite lack of sequence identity. Close-up view of **(d)** FimH and **(e)** F17-G carbohydrate-binding sites (surface representation). The mannoside-binding pocket of FimH is deep, with a large surface for interactions with the D-mannose monosaccharide, whereas the GlcNAc ligand does not fill the whole F17-G-binding site, providing structural insights into differences of ligand affinity and specificity.

Pili are used for adherence by both Gram-negative and Gram-positive bacteria. Recent studies have established the presence of mucus-binding pili on the surface of a Gram-positive bacterium, *L. rhamnosus* GG, a well-established probiotic strain. Two pilus gene clusters (*spaCBA* and *spaFED*) were identified in the genome of this strain, each of which contained the predicted genes for three pilin subunits and a single sortase [48]. Purified recombinant SpaB, SpaC and SpaF pilin subunits exhibited substantial binding to mucus, which can be inhibited competitively in a dose-related manner [48,49]. In contrast to SpaC and SpaF, the binding between the SpaB pilin subunit and mucus seemed to operate through electrostatic contacts and was not related to a recognized mucus-binding domain. Immunogold labeling confirmed the presence of SpaCBA pili on the cell surface of *L. rhamnosus* GG [49]. Further research is required to assess the functional significance of these results in relation to the health-promoting properties of this probiotic strain, which are dependent in part on prolonged residence in the GIT and thus are probably dictated by adherence to the intestinal mucosa.

Blood group antigen adhesins

Several human enteric pathogens bind to human histo-blood group antigens (HBGAs) expressed on the gut mucosa, including *Campylobacter jejuni*, Norwalk virus and *Helicobacter pylori*.

The best-characterized HBGA adhesin implicated in mucus binding is the blood group binding adhesin (BabA) from *H. pylori*. This bacterial pathogen lives in the mucus niche of the stomach and can cause gastric ulcers and cancer. The binding of *H. pylori* to gastric mucins through BabA and SabA (sialic acid-binding adhesin) protein–carbohydrate interactions has been extensively reviewed (see [50] for a recent example). *H. pylori* binding to mucins differs substantially with the anatomic site, mucin type, pH and gastritis status [51]. BabA binds to the Lewis B (Le^b) HBGA, which is mainly found on MUC5AC, although

MUC1 was also proposed as a Le^b carrier [7]. Some other glycoform structures (e.g. H1 and sialyl-Le^x) are also suggested in binding to *H. pylori* adhesins. In the healthy mucus layer of the stomach, BabA-mediated binding is dominant, and little binding via SabA takes place. During infection, binding through SabA and the charge/low pH-dependent mechanism increases as a result of higher levels of sialylated carbohydrates [51]. The dynamic expression of these adhesins and their ability to be genetically modified might represent mechanisms by which *H. pylori* adapt to changes in mucin expression and/or glycosylation. Although BabA and SabA are the most prominent adhesins studied so far, not every *H. pylori* strain expresses functional BabA or SabA adhesins, suggesting that other bacterial proteins are involved in *H. pylori* adhesion to mucus.

C. jejuni is a highly motile Gram-negative spiral rod bacteria and the most prevalent cause of gastroenteritis in developed countries. A major determinant of pathogenicity in intestinal infection with *C. jejuni* is proposed to be an ability to colonize intestinal mucus and is largely imparted by its glycosylated flagella [52]. *C. jejuni* strains interact with Le^b-like structures present on human milk proteins and to intestinal H2 antigen [53] and binding is inhibited by fucosyl oligosaccharides of human milk [54], suggesting that HBGA lectins could be involved in *C. jejuni* adhesion to mucus. More recently, *C. jejuni* strain 81116 was shown to bind to Muc2 in a pH-dependent manner, with binding highest at intestinal pH 5.5–6.0 [55]. Desialylation of mucin with neuraminidase enhanced binding of *C. jejuni* and eliminated the influence of pH, suggesting at least two different mucin oligosaccharide ligands for *C. jejuni* on murine mucin: a sialylated oligosaccharide bound by a pH-influenced adhesin and a nonsialylated structure bound by a pH-independent adhesin. Unlike *H. pylori*, only negligible binding of *C. jejuni* was observed using synthetic oligosaccharides conjugated to Le^b and sialyl-Le^x, whereas strong binding was obtained with the H2 structure present on gastrointestinal mucins. *C. jejuni* also binds more rapidly to MUC1-expressing cells [55]. Glycan array analyses using *C. jejuni* NCTC11168 isolates grown or maintained under various conditions revealed a range of glycans recognized and bound by *C. jejuni*, including terminal Man, Neu5Ac, Gal and Fuc [56]. Despite these advances in the identification of *C. jejuni* glycan receptors, and the identification of possible candidates upregulated in the presence of MUC2 [57], the nature of the *C. jejuni* lectins involved in binding to HBGAs and mucins is not yet determined.

HBGAs have also been reported as receptors for human noroviruses, a major cause of nonbacterial gastroenteritis in humans. PGM binds to recombinant norovirus particles and competitively inhibits their binding to HBGAs and Caco-2 cells [58]. Different noroviruses recognize different HBGAs, and several receptor binding patterns of noroviruses have been identified [59]. The carboxyl-terminal protruding (P) domain of the norovirus capsids is directly involved in this recognition. The crystal structures of recombinant P protein of a GII-4 strain norovirus, VA387, with synthetic type A or B trisaccharides demonstrated that the receptor binding site lies at the outermost end of the P domain and forms an extensive hydrogen-bonding network with the saccharide ligand. The A and B trisaccharides display similar binding modes, and the common fucose ring plays a key role in this interaction [60]. The surface-exposed carbohydrate-binding domain in the norovirus capsid is under heavy immune selection leading to variations in the capsid ligand-binding pocket and the ability to recognize distinct HBGA carbohydrate receptors on the mucosal surface [61].

The HBGA structures of mucins might also play an essential role in bacterial colonization of nonmotile lactic acid bacteria. Using surface plasmon resonance, Uchida *et al.* [62,63] found that some strains of lactobacilli can recognize and bind to blood group A, B, H antigens on intestinal mucus. The adhesion mechanism of *L. plantarum* LA 318 was shown to be partly due to GAPDH binding to HBGAs expressed on human colonic mucus (HCM). Periodate oxidation of HCM significantly decreased *L. plantarum* LA 318 adhesion. High binding of GAPDH was observed to A and B group antigens and a Galα1,3Fucα1–2 Gal probe, whereas binding to H group antigen was significantly lower and no binding was observed with various monosaccharides, indicating a specificity for the trisaccharide structure of HBGAs [64]. The cell-surface protein Lam29 from *L. mucosae* ME340 was recently implicated in the specific adhesion of *L. mucosae* to A and B blood group antigens, although direct binding to the purified adhesins was not investigated [32]. Altogether, these results indicate that pathogens and commensal adhesins share specificity for HBGAs, providing a platform for the design of strategies to limit infection by pathogens.

Other examples of lectin-like mucus adhesins

Only limited information is available on microbial adhesins or lectins from gut microbes with demonstrated specificity towards mucin glycans. *Entamoeba histolytica*, a human intestinal protozoan parasite causing morbidity and mortality in developing countries, has a Gal/GalNAc cell-surface lectin that has been implicated in its binding to mucin oligosaccharides. This binding can be inhibited *in vitro* using purified rat colonic mucins [65]. The native protein is a 260-kDa heterodimer consisting of a type 1 membrane protein disulfide bonded to a lipid-anchored protein. Each subunit has several isoforms that may form functionally different heterodimers, analogous to the integrin family of proteins. Another 150-kDa Gal/GalNAc lectin was identified in *E. histolytica* that associates with the 260-kDa lectin [66]. The association of these lectins with their host receptors has a crucial impact on the parasite pathogenicity [67]. The functional activity of this lectin has been shown to be involved not only in host cell binding, but also cytotoxicity, complement resistance, induction of encystation and generation of the cyst wall. The role of the lectin in both differentiation and virulence suggests that it may be a pivotal molecule that determines the severity of the infection from a commensal state resulting from increased encystation to an invasive state [68]. Other microbial lectins have recently been reviewed [69], but their implication in mucus or mucin binding is unknown. The chitin-binding protein GbpA (GlcNAc-binding protein A) of *Vibrio cholerae*, the causative agent of the potentially lethal disease cholera, has been described as a common adherence factor for chitin and intestinal surfaces. This

lectin was recently implicated in *V. cholerae* binding to mucin oligosaccharides. Purified recombinant GbpA (rGbpA) specifically bound to GlcNAc residues of intestinal mucin in a dose-dependent and saturable manner. Preincubation of rGbpA with GlcNAc specifically inhibited its binding with mucin. A *gbpA* mutant strain showed a significant decrease in intestinal adherence, leading to less colonization in mice [70]. Altogether, these results suggest that GbpA is a mucin lectin-like adhesin with specificity to GlcNAc residues. The coordinated interaction between GbpA and mucin, where rGbpA can increase MUC2, MUC3 and MUC5AC production and mucin can increase *V. cholerae* GbpA expression, may be key to intestinal colonization and pathogenesis by *V. cholerae* [70]. Understanding of the structure and function of the mucus lectins that control these divergent cell biological processes is essential for prevention and treatment of these infections.

Concluding remarks and future directions

Microorganisms use adhesins to attach to their appropriate environmental niche. Our understanding of the interactions between commensals and pathogens to the mucus layer is increasing but remains incomplete. A widespread paradigm in the field of mucus and mucin biology is that microbes have evolved a multitude of lectins for recognition of the oligosaccharide structures present in mucins. However, little biochemically based evidence of binding to sugars supports this process. Most studies provide indirect or circumstantial evidence by studying the interaction of the whole microbe with mucus and relying on competition experiments to show the involvement of carbohydrates in the interaction. When the adhesins are defined (at the genetic and/or biochemical level), the nature of the glycan receptors is often either lacking or associated with carbohydrate structures known to be present on mucins and it is thus difficult to ascertain the specificity of the interaction for mucin carbohydrate structures. Addressing these questions is hampered by the number of factors involved in the interaction of microbes with mucus, the variation in expression of specific adhesins, and the biochemical heterogeneity of mucus and mucins, and is restricted by technical difficulties in purifying the partners of the interaction, adhesins (often cell-surface multisubunit proteins), mucins from mucus and in characterizing the nature of *O*-glycans in mucin samples to probe carbohydrate–protein interactions.

Because mucus plays a crucial role in bacterial colonization of host cell surfaces, there is an incentive from the scientific community working on probiotics to identify mucus adherent strains. The past decade has witnessed the completion of a large number of genome sequences from probiotic strains and evidence is mounting that mucus adhesins from probiotics are largely diverse, often multifunctional and thus difficult to predict based on sequence homology only. Efforts in elucidating the 3D structure of these adhesins, free or in complex with mucin ligands, will help to define possible common protein folds and provide a molecular basis for the interaction.

Because probiotics and pathogens share the same ecological niche, they might compete for binding sites on gastrointestinal mucins. The increasing antibiotic resistance associated with enteropathogens treated by antimicrobial therapy is raising a need to search for alternative treatment strategies, such as those based on blocking bacterial adhesion to host receptors. To this end, identification of the oligosaccharide structures of the mucins involved in the attachment of pathogens to mucus is essential. Glycan arrays are fast becoming the technique of choice for screening the specificity of lectins and can be used to identify and elucidate the interactions of microbes and microbial adhesins with mucin glycan receptors. However, one limitation of this approach is that the individual oligosaccharide components do not reflect the density, complexity and environment of mucin glycans. The field would be dramatically enhanced by systems that allow assessment of the binding of microbial adhesins to native mucin glycans *in vivo* using specific fluorescent probes. Ascertaining the nature of the interactions between the mucus layer and pathogens or commensals will provide a platform for the rational design of novel strategies to reinforce the intestinal barrier and limit infection by pathogens.

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