Cell. Mol. Life Sci. (2011) 68:3635–3641
DOI 10.1007/s00018-011-0822-3

Cellular and Molecular Life Sciences

MULTI-AUTHOR REVIEW

Composition and functional role of the mucus layers in the intestine

Malin E. V. Johansson · Daniel Ambort · Thaher Pelaseyed · André Schütte · Jenny K. Gustafsson · Anna Ermund · Durai B. Subramani · Jessica M. Holmén-Larsson · Kristina A. Thomsson · Joakim H. Bergström · Sjoerd van der Post · Ana M. Rodriguez-Piñeiro · Henrik Sjövall · Malin Bäckström · Gunnar C. Hansson

Received: 17 June 2011 / Revised: 7 September 2011 / Accepted: 7 September 2011 / Published online: 25 September 2011

Abstract In discussions on intestinal protection, the protective capacity of mucus has not been very much considered. The progress in the last years in understanding the molecular nature of mucins, the main building blocks of mucus, has, however, changed this. The intestinal enterocytes have their apical surfaces covered by transmembrane mucins and the whole intestinal surface is further covered by mucus, built around the gel-forming mucin MUC2. The mucus of the small intestine has only one layer, whereas the large intestine has a two-layered mucus where the inner, attached layer has a protective function for the intestine, as it is impermeable to the luminal bacteria.

Keywords Mucus · Mucin · MUC2 · Colon · Commensal bacteria · Small intestine

Introduction

The intestine is a remarkable organ. It is filled with 10^{13}–10^{14} bacteria and also with digestive enzymes that are capable of degrading the molecules we are composed of. Despite this, we manage to survive and are neither invaded by bacteria nor digested. It is still not well understood how this sophisticated system works, although several of the important ingredients have been studied individually and are well described. An important process is the continuous distal propulsion of the intestinal content, as controlled by the enteric nervous system. Without this, the bacteria would expand quickly, especially in the small intestine with all its non-absorbed nutrients. The epithelial cells with their tight junctions line the intestinal tube and form the major defense line between the host and the intestinal content. However, in between the two there is also a mucus layer, an often forgotten and ignored part of the defense system. The ignorance of the mucus has several reasons, including the fact that it is normally transparent and only observed when charcoal is added on top of it. It is also due to the difficulties working with the major component of mucus, the mucins, as they are very large, complex, and often insoluble. Our recent discovery of a two-layered mucus in the colon that physically separates the luminal bacteria from the epithelial cells has renewed the interest in mucus and mucin research [1]. In fact, it has been outlined as one of the most important areas for a better understanding of mucosal immunology [2].

Mucins

All mucins are characterized by having at least one PTS domain, consisting of abundant Ser, Thr, and Pro amino acid residues. The Ser and Thr are decorated with the for mucins typical *O*-glycans, which gives the mucin domains an extended and stiff conformation (‘bottle brush’-like). The protein core of these mucin domains is often characterized by multiply repeated sequences called tandem repeats. However, this is not always the case, as the typical repeated sequence is degenerated during evolution and several mucins do not show any repetitive characteristics.

M. E. V. Johansson · D. Ambort · T. Pelaseyed · A. Schütte · J. K. Gustafsson · A. Ermund · D. B. Subramani · J. M. Holmén-Larsson · K. A. Thomsson · J. H. Bergström · S. van der Post · A. M. Rodriguez-Piñeiro · H. Sjövall · M. Bäckström · G. C. Hansson (✉)
Department Medical Biochemistry, University of Gothenburg, PO Box 440, 405 30 Gothenburg, Sweden
e-mail: gunnar.hansson@medkem.gu.se

Because of this, we prefer to call the DNA and protein sequences PTS domains, although not all such domains contain both Ser and Thr amino acids [3]. In fact, one can mine DNA sequences for PTS domains only by searching for long DNA stretches that encode a high frequency of Thr and/or Ser amino acid residues together with a lower frequency of Pro residues, an approach that detects more mucins than searching for repetitive sequences only [3, 4]. The PTS domains show, in contrast to what is normally characterizing protein domains, little sequence conservation also between closely related species [3]. Typical for the PTS domains is also that they are found within one long exon. The variation in length that is observed for some mucins is caused by allelic variation and is thus inherited. The length of these domains is probably very important, as the second largest PTS domain of MUC2 has a similar length in humans and mice/rats, even if there is no sequence similarity. Interestingly, the human sequence shows a very high sequence similarity between the repeats whereas the mouse and rat sequences have lost most of their repetitive nature. This suggests that the human PTS was replaced more recently than the evolutionary separation. The polymorphism in the length of the PTS domains has been linked to disease, as in the case of MUC1 and *Helicobacter pylori* infection [5].

The most prominent feature of mucins is the *O*-glycosylation of their PTS domains to generate the mucin domain glycopeptide (Fig. 1). This is initiated by any of the peptidyl-GalNAc-transferases (20 different in humans), which have a more or less restricted specificity for the peptide core sequences. The added GalNAc is then the substrate for further glycosylation by Gal- and GlcNAc-transferases. The glycosyltransferases expressed in a certain cell determine which oligosaccharides are formed, but their Golgi localization is also important. The transferases are localized according to the pH gradient over the Golgi stack, as we have shown [6]. Mucin *O*-glycosylation was for long considered to be static. However, we have shown glycosylation to be a dynamic process, as exemplified by transient glycosylation alterations during intestinal infections [7, 8].

There are two different types of molecules called mucins (Fig. 1). The classical gel-forming mucins (**MUC2**, MUC5AC, MUC5B, and MUC6) form extremely large polymers (MUC2 > 100 MDa) (intestinal mucins in bold). The second group, the transmembrane (TM) mucins (MUC1, **MUC3**, MUC4, **MUC12**, **MUC13**, MUC16, and **MUC17**), cover the apical surface of the enterocytes (or other epithelial cells) (glycocalyx). The transmembrane mucins all have a cytoplasmic tail and an enormous extracellular mucin domain with masses from one million up to more than ten million Da (except MUC13, which is smaller).

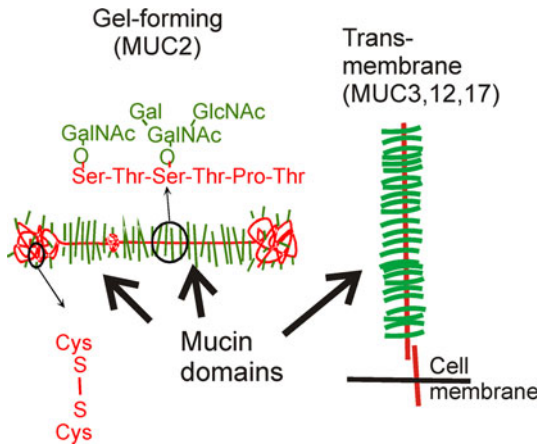

Fig. 1 Schematic presentation of intestinal gel-forming and transmembrane mucins with their highly *O*-glycosylated mucin domains. *Red* protein core, *Green* oligosaccharides

Epithelial cells and their glycocalyx

Before we look at the enterocytes and their glycocalyx, we should consider its dimensions in relation to the normal mucus of colon. As illustrated in Fig. 2a, the distal colon is protected by a two-layered mucus that reaches up to a millimeter from the epithelial cells [1, 9]. The inner of these layers is impermeable to bacteria and is 50–100 μm thick in mice and rat and probably even thicker in humans. The two mucus layers are formed by the gel-forming MUC2 mucin, with MUC2 being their main component and forming their skeleton. Below the two layers is the glycocalyx of the enterocytes, which has been estimated to reach at least 1 μm from the apical cell membrane. Although formally not proven, the transmembrane mucins MUC3, MUC12, and MUC17 are the major components of the intestinal glycocalyx. In addition, the very short MUC13 mucin is also found in the intestinal epithelium and is contributing to its protection [10]. The PTS domains of MUC3, MUC12 and MUC17 are 4,000–5,000 amino acids long (see http://www.medkem.gu.se/mucinbiology/databases) and when the abundant Ser and Thr have been *O*-glycosylated they form long and extended rods that are best described as bottle brushes. Assuming the same length per amino acid as determined for the mucin domains of MUC2 [11], the mucin domains of MUC3, MUC12, and MUC17 are about 0.8 μm long. These mucins are densely packed and will in this way provide a barrier with similar properties as those of the mucus layers on the luminal side. The glycocalyx has not been studied in great detail, but it is assumed that they are quite similar on the enterocytes in the small and large intestine.

MUC3, MUC12, and MUC17 belong to the family of SEA-(sea urchin sperm protein/enterokinase/agrin) domain-containing TM mucins and are located at the 7q22 locus [3].

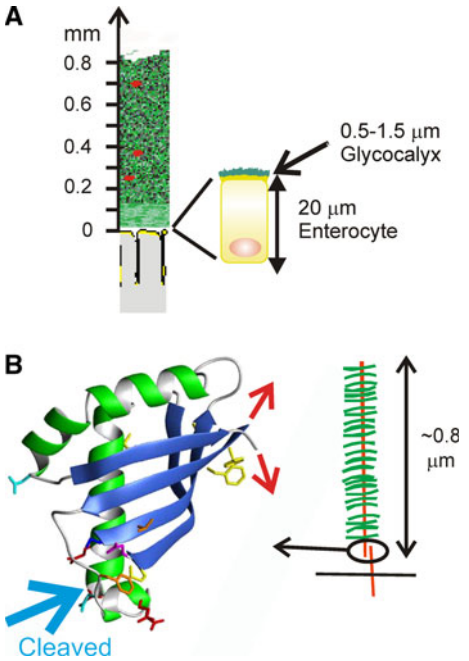

Fig. 2 The enterocyte glycocalyx. **a** Schematic presentation of the mucus layers of rat distal colon, illustrating their dimensions in comparison with the glycocalyx of the apical membrane of the enterocyte. **b** The transmembrane mucins of the SEA-type have an autoproteolytically cleaved SEA domain just outside of the cell membrane. The structure of the MUC1 SEA domain is shown to the *left* with the cleaved site indicated by a *blue arrow* [12]

The single SEA domain in these mucins is located extracellularly, close to the cell membrane (Fig. 2b), and have a sequence similar to that of the SEA domain in the MUC1 mucin [12]. The structure of this domain has been determined by us and shows a tightly packed domain with four anti-parallel β-pleated sheets, two in the outer domain and two in the membrane-anchored domain, with an intervening cleavage site (Fig. 2b). Interestingly, this cleavage is necessary for proper folding of the domain in the endoplasmic reticulum (ER) and this autoproteolysis, which occurs between the amino acids Gly and Ser, is in fact triggered by the folding energy [12]. To have such a break-point in the polypeptide chain, close to the membrane, might be a way to protect the cell from physical stress by allowing the two parts of these mucins to separate, while maintaining an intact epithelial cell layer.

The function of the TM mucins is most likely primarily cell protection, but they can probably also act as sensors for the luminal milieu, as suggested by the structure of the SEA domain. There are a few hydrophobic patches in the SEA domain, where other proteins might bind [12]. Moreover, the MUC3, MUC12, and MUC17 mucins all have a cytoplasmic C-terminal PDZ-binding motif that we have shown to bind several of the cytoplasmic PDZ proteins [13, 14]. These proteins are scaffolds for organizing and sorting transmembrane proteins, especially in polarized cells [15]. Many of the membrane proteins with C-terminal PDZ-binding motifs are ion channels, suggesting a connection between the TM mucins and ion transport. In fact, MUC17 binds PDZK1 and MUC3 binds GOPC, both being PDZ proteins known to interact with the CFTR ion channel [13, 14].

The gel-forming mucin MUC2 forms the skeleton of the intestinal mucus

As already pointed out, the gel-forming mucin MUC2 is the major component of the mucus layers of the intestine, which are produced by the goblet cells. The mucus is built around a primary MUC2 translation product of about 5,200 amino acids (this is not absolute, as the gene has not yet been fully sequenced and also because of genetic variation in the number of tandem repeats). The protein is translocated into the ER lumen where it folds and forms disulfide-bonded dimers due to the cysteine-knot in its far C-terminal end. The folding of MUC2 is a challenge for the cell due to the abundant Cys residues and there is a need for special chaperone proteins like AGR2 for this process [16]. The ER stress response system probably needs to be at least partly activated to increase the number of chaperones, as lack of the master regulator of the ER stress response, XBP1, causes accumulation of misfolded mucins and also inflammation [17].

In the Golgi apparatus, the MUC2 dimers are decorated with *O*-glycans to reach a molecular mass in the range of 5 MDa. The dimers are then sorted to the regulated secretory pathway where the mucins are densely packed in secretory vesicles. At this stage, the N-terminal ends of the dimers are coupled together by disulfide bonds into trimeric structures, Fig. 3a [18]. Once released from the cells, the MUC2 molecules are expanded and will form large net-like structures due to the C-terminal dimers and the N-terminal trimers (Fig. 3b). Such a model should have a built-in property to form flat sheets.

The disulfide-bonded di- and trimers are not the only interaction points for the MUC2 mucin. Several years ago Carlstedt et al. [19] discovered that MUC2 is also interconnected via non-reducible bonds. The chemical nature of this bond is still unknown, but it is formed during biosynthesis in the goblet cells and it also gives the MUC2 mucin its typical property of insolubility in chaotropic salts [20]. Moreover, all the human gel-forming mucins, except MUC6, have two or more CysD domains interrupting the PTS domains [3]. These domains are 110 amino acid long, with ten conserved Cys amino acids that form five intramolecular disulfide bonds [21]. The MUC2 mucin has two

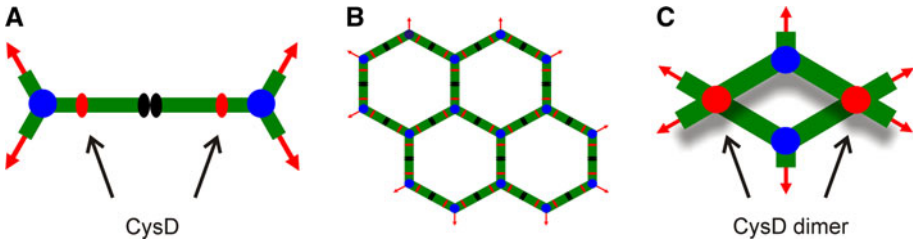

Fig. 3 The MUC2 mucin. **a** The MUC2 mucin forms dimers in its C-terminus and trimers in its N-terminus. The localization of the second CysD domain are marked with *arrows*. **b** A model of the net-like polymeric network, as organized by the MUC2 mucin, forming large sheets. **c** The CysD domains form non-covalent dimers between two sheets of MUC2, that further organizes the mucin net-work

such domains and we could recently show that its second CysD domain forms very stable, non-covalent dimers [21]. It is very likely that other CysD domains have similar properties and that they are there to form non-covalent cross-links in the mature mucus gel for further stability (Fig. 3c). However, this would imply that the CysD domains do not interact during vesicular storage, before secretion of the mucin. The exact mechanisms for this remain to be elucidated, but may involve differences in Ca^{2+}-concentrations and pH. Finally, we have discovered that there is a large protein called FCGBP (immunoglobulin Fc γ binding protein) that is covalently attached to the MUC2 mucin in the colonic mucus [22]. FCGBP has 13 von Willebrand D domains (vWD), 11 of which have a GDPH (Gly–Asp–Pro–His) sequence suggesting an autocatalytic cleavage, as found in the fourth vWD of the MUC2 mucin. This was shown to be cleaved at low pH between the Asp and Pro and by this generating a new C-terminus that forms an internal anhydride with the side-chain of the Asp [23]. The vWD domain is still held together via disulfide bonds across the cleavage site, bridging the cleaved parts of the protein. The newly formed anhydride is very unstable and will react with primary amines or hydroxyl groups (but only if not hydrolyzed in water). As FCGBP has up to 11 of these attachment sites, it can be predicted to cross-link components of the mucus to the MUC2 mucin. However, it is not known how and when the GDPH cleavage is triggered, nor if the attachment sites are random or if there is a specificity. A similar system connecting bikunin with heavy chain 3 of the pre-α-inhibitor protein has been shown to be specific for the C6 hydroxyl group of GalNAc in the bikunin chondroitin sulfate [24]. Taken together, this suggests that the MUC2 mucin can be further covalently cross-linked by endogenous properties of the FCGBP protein once the mucus gel has been secreted and formed.

The basic structure of the MUC2 mucin thus has a net-like appearance that can be further cross-linked by both non-covalent and covalent mechanisms. This suggests that the mucus gel, as organized by MUC2, is well structured and well stabilized.

The mucus of the small intestine

The small intestine is protected by mucus that has the MUC2 mucin as its main and structural component, just as in the large intestine. Despite this, the properties of the mucus in these two locations are different. As described by Atuma et al. [9], the small intestine has a mucus that is easy to aspirate and remove. We recently extended our studies of the small intestine, using explant systems. In the ileum, the mucus covers the villi (Fig. 4) and this mucus is easy to aspirate, removing the mucus all the way down to the crypt openings. Atuma et al. have, in contrast, in their experimental set-up, seen a thin layer of non-removable mucus in the small intestine. These differences may be caused by the technical difficulties in removing mucus in the small intestine due to the presence of villi. Our preliminary studies also suggest that this small intestinal mucus is permeable to bacteria. The observations that the ileal mucus has only one layer, is not attached to the epithelium,

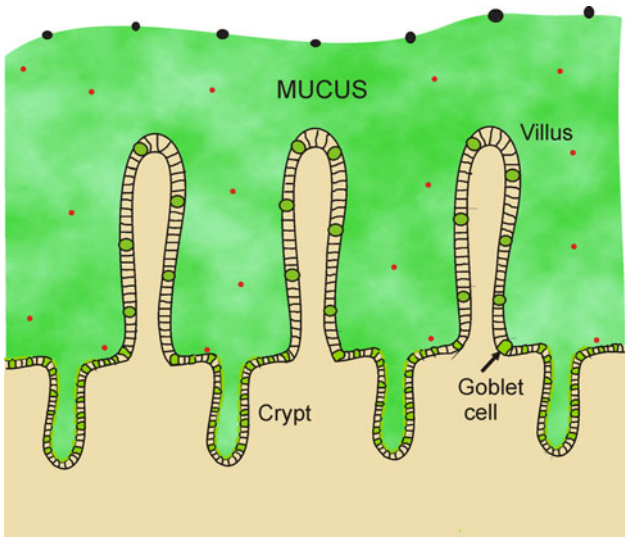

Fig. 4 The mucus organization of the small intestine. The organization of the ileal mucus is shown, as revealed by the addition of charcoal to the mucus surface (*black dots on top*). This mucus is permeable, as represented by *red beads* (in the size of bacteria) in the mucus

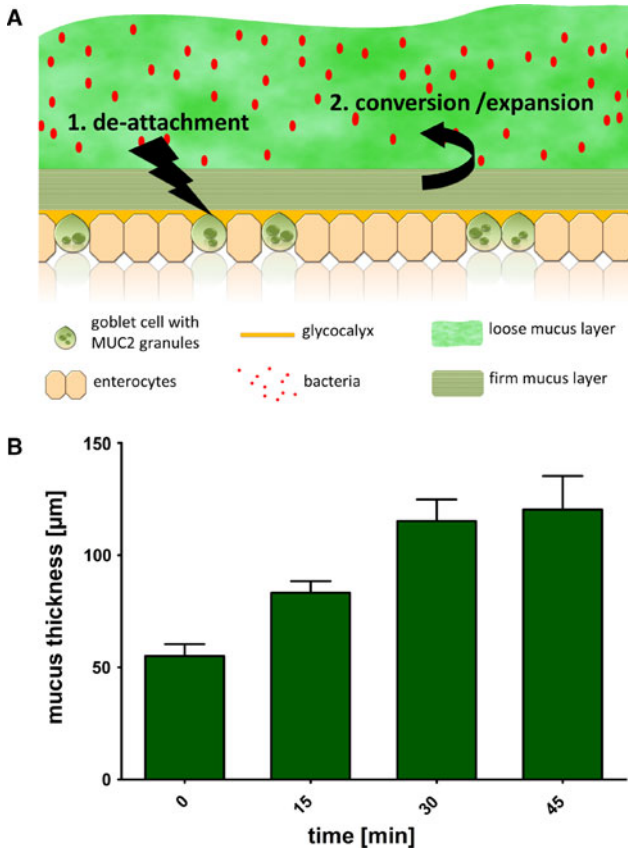

Fig. 5 The mucus organization of the distal large intestine. **a** The two mucus layers as organized by the MUC2 mucin. **b** Measurements of the total mucus (inner and outer layers) in distal colon in an explant culture system. The loose mucus was first removed by gentle aspiration and the growth of the mucus was monitored by measuring the increase in the total mucus thickness over time

and is permeable to bacteria are all in contrast to the large intestine. The molecular reasons for why MUC2 is forming another type of mucus in the small as compared to the large intestine are not understood today.

The mucus of the distal large intestine

As already pointed out, the colon has a two-layered mucus (Fig. 5a). The inner mucus layer is formed from the goblet cell secretion, remains attached to the epithelium and is not possible to remove experimentally by gentle aspiration. This inner layer has a stratified appearance as could be predicted from the previously discussed model of the MUC2 polymers, with flat sheets of MUC2 (Fig. 3). This inner layer has special physical properties as it is not soluble in for example guanidinium chloride [1]. This layer is then converted to the outer mucus layer by processes that are not yet understood, followed by a protease-dependent fourfold volume expansion [25]. The outer layer can be aspirated and this mucus layer is thus also called the loose mucus. Immunostained tissue sections, made with techniques that preserve the mucus, show a sharp border between the inner and outer layer, suggesting a very well controlled system. As germ-free animals also show both an inner firm layer and an outer loose layer, this conversion and expansion must happen through endogenous mechanisms and proteases [1]. Using the experimental explant tissue set-up, the inner layer is determined to be about 50 µm in mice, the same thickness as in live animals [1]. As also observed in live animals, the mucus layer grows over time, as shown in Fig. 5b for distal colon. After 45 min, the mucus has grown almost 100 µm (Fig. 5b). Thus, the colon has a sophisticated mucus protection system that is under control of the host, even though it is located at a large distance from the epithelial cell surface.

Localization of bacteria in the distal large intestine

With this elaborate mucus system in the colon, it was obvious to ask where the commensal bacteria were to be found. Surprisingly, the bacteria were found only in the outer loose mucus layer and were totally absent in the inner attached layer [1]. In fact, the commensal bacteria have the outer mucus layer as their habitat. This is illustrated in Fig. 6, where the commensal *Lactobacilli* are revealed by specific in situ hybridization in the outer mucus layer, with the inner mucus layer being totally devoid of bacteria [26]. Thus, the inner mucus layer is impermeable to bacteria. This organization is probably critical for a normal homeostasis and also effectively separates bacteria from cells of the immune system. This means that the immune

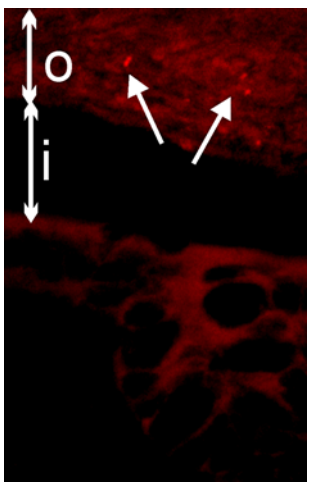

Fig. 6 Commensal bacteria is only found in the outer mucus layer of distal colon. Lactobacilli were stained by in situ hybridization and found in the outer (o) mucus layer. The lack of bacteria in the inner stratified mucus layer (i) makes it appear as an empty space. The epithelium is revealed by epifluorescence

system does not need to monitor bacterial friends from bacterial foes in the colon. This model has led to the questioning of several of the more popular models of how the immune system handles the commensal flora of the colon. Interestingly, lack of the MUC2 mucin allow the bacteria to come in direct contact with the epithelial cells, penetrate down into the normally sterile crypts, and even into the epithelial cells [1]. These mice develop a relatively severe inflammation with diarrhea, rectal prolapse, weight loss and, after some time, also colon cancer [1, 27, 28], a situation that is similar to the human disease ulcerative colitis.

Why is the outer mucus layer such a good habitat for the commensal microbiota? A first prerequisite is that the bacteria are able to penetrate this mucus. Secondly, the bacteria can use the high number of glycans on the mucins as attachment sites. Many bacteria are known to carry adhesins of lectin-type that can be used to anchor the bacteria. As the glycan repertoire varies considerably between species, it can be suggested that there are only certain bacteria that has the appropriate adhesins to adapt to each host. In fact, it is now known that there is a core microbiota in each species, supporting this model [29] and that the glycans on mucosal surfaces also varies dramatically [30, 31]. Further proof for such a model is our recent observation that the MUC2 *O*-glycans in the sigmoid colon are uniform in all humans, except in individuals with an active colon inflammation [32, 33]. This is in contrast to the mucin *O*-glycans on other mucosal surfaces of the body. Together, these observations suggest a co-evolution between host and bacteria for an optimal symbiotic relation. Last but not least, all the oligosaccharides present on the mucins provide an enormous food supply for the bacteria. It is well known that the commensal bacteria have an especially rich source of glycan-degrading enzymes [34]. These enzymes are typically of the exo-glycosidase type that removes one sugar residue at a time, starting from the non-reducing end. This is probably important, as this will slow down the degradation that will finally reach the protein core of the mucin, and when this is degraded the whole mucin polymer network will be dissolved. Such a scenario is suggested by our recent studies, in collaboration with Dr. Lijun Xia, of mice lacking the Core 1 glycosyltransferase. This glycosylation deficiency will give rise to a MUC2 mucin with shorter *O*-glycans and this will allow a faster degradation. These animals develop a relatively severe colitis, just as the animals that are totally devoid of MUC2 [35]. The released sugars are then utilized as food for the bacteria, but they are also converted to short fatty acids that can diffuse through the mucus and reach the epithelium, which will in this way regain some of the energy used for the biosynthesis of the quickly turned-over mucus.

Conclusions

The two-layered mucus system of the large intestine, as organized by the MUC2 mucin, is sophisticated, and more complex than understood before. The outer mucus provides an optimal symbiotic milieu for our bacteria. The inner mucus is well structured in such a way that it is impermeable to bacteria and by this it will keep our bacteria at a long distance from the epithelium. Finally, the dense glycocalyx, as organized by the transmembrane mucins, will protect the enterocyte apical membrane and might at the same time act as an important sensor of the luminal milieu. Although we have made considerable advances in our conceptual understanding of the mucus as a protective system of the intestine, there are still many molecular mechanisms controlling this complex system that remain to be elucidated. We expect many exciting discoveries about both mucins and mucus in the years to come.

Acknowledgments This work was supported by the Swedish Research Council (no. 7461, 21027, and 342-2004-4434), The Swedish Cancer Foundation, The Knut and Alice Wallenberg Foundation (KAW2007.0118), IngaBritt and Arne Lundberg Foundation, Sahlgren's University Hospital (LUA-ALF), EU-FP7 IBDase (no. 200931), Wilhelm and Martina Lundgren's Foundation, Torsten och Ragnar Söderbergs Stiftelser, The Sahlgrenska Academy Foundations, and The Swedish Foundation for Strategic Research—The Mucosal Immunobiology and Vaccine Center (MIVAC) and the Mucus-Bacteria-Colitis Center (MBC) of the Innate Immunity Program (2010-2014). We also acknowledge the Mammalian Protein Expression Core Facility at the University of Gothenburg.

References

1. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci USA 105:15064–15069
2. Gill N, Wlodarska M, Finlay BB (2010) The future of mucosal immunology: studying an integrated system-wide organ. Nat Immunol 11:558–560
3. Lang T, Hansson GC, Samuelsson T (2007) Gel-forming mucins appeared early in metazoan evolution. Proc Natl Acad Sci USA 104:16209–16214
4. Lang T, Alexandersson M, Hansson GC, Samuelsson T (2004) Bioinformatic identification of polymerizing and transmembrane mucins in the puffer fish *Fugu rubripes*. Glycobiology 14:521–527
5. Vinall LE, King M, Novelli M, Green CA, Daniels G, Hilkens J, Sarner M, Swallow DM (2002) Altered expression and allelic association of the hypervariable membrane mucin MUC1 in *Helicobacter pylori* gastritis. Gastroenterology 123:41–49
6. Axelsson MA, Karlsson NG, Steel DM, Ouwendijk J, Nilsson T, Hansson GC (2001) Neutralization of pH in the Golgi apparatus causes redistribution of glycosyltransferases and changes in the *O*-glycosylation of mucins. Glycobiology 11:633–644
7. Olson FJ, Johansson ME, Klinga-Levan K, Bouhours D, Enerbäck L, Hansson GC, Karlsson NG (2002) Blood group A

glycosyltransferase occurring as alleles with high sequence difference is transiently induced during a *Nippostrongylus brasiliensis* parasite infection. J Biol Chem 277:15044–15052
8. Holmén JM, Olson FJ, Karlsson H, Hansson GC (2002) Two glycosylation alterations of mouse intestinal mucins due to infection caused by the parasite *Nippostrongylus brasiliensis*. Glycoconj J 19:67–75
9. Atuma C, Strugala V, Allen A, Holm L (2001) The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo. Am J Physiol Gastrointest Liver Physiol 280:G922–G929
10. Sheng YH, Lourie R, Linden SK, Jeffery PL, Roche D, Tran TV, Png CW, Waterhouse N, Sutton P, Florin TH, McGuckin MA (2011) The MUC13 cell-surface mucin protects against intestinal inflammation by inhibiting epithelial cell apoptosis. Gut. on-line
11. Carlstedt I, Herrmann A, Karlsson H, Sheehan J, Fransson LA, Hansson GC (1993) Characterization of two different glycosylated domains from the insoluble mucin complex of rat small intestine. J Biol Chem 268:18771–18781
12. Macao B, Johansson DG, Hansson GC, Härd T (2006) Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin. Nat Struct Mol Biol 13:71–76
13. Malmberg EK, Pelaseyed T, Petersson AC, Seidler UE, De Jonge H, Riordan JR, Hansson GC (2008) The C-terminus of the transmembrane mucin MUC17 binds to the scaffold protein PDZK1 that stably localizes it to the enterocyte apical membrane in the small intestine. Biochem J 410:283–289
14. Pelaseyed T, Hansson GC (2011) CFTR anion channel modulates expression of human transmembrane mucin MUC3 via the PDZ protein GOPC. J Cell Sci 124:3074–3083
15. Lamprecht G, Seidler U (2006) The emerging role of PDZ adapter proteins for regulation of intestinal ion transport. Am J Physiol Gastrointest Liver Physiol 291:G766–G777
16. Park SW, Zhen G, Verhaeghe C, Nakagami Y, Nguyenvu LT, Barczak AJ, Killeen N, Erle DJ (2009) The protein disulfide isomerase AGR2 is essential for production of intestinal mucus. Proc Natl Acad Sci USA 106:6950–6955
17. Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H, Nieuwenhuis EE, Higgins DE, Schreiber S, Glimcher LH, Blumberg RS (2008) XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 134:743–756
18. Godl K, Johansson ME, Lidell ME, Mörgelin M, Karlsson H, Olson FJ, Gum JR Jr, Kim YS, Hansson GC (2002) The N terminus of the MUC2 mucin forms trimers that are held together within a trypsin-resistant core fragment. J Biol Chem 277:47248–47256
19. Herrmann A, Davies JR, Lindell G, Martensson S, Packer NH, Swallow DM, Carlstedt I (1999) Studies on the "insoluble" glycoprotein complex from human colon. Identification of reduction-insensitive MUC2 oligomers and C-terminal cleavage. J Biol Chem 274:15828–15836
20. Axelsson MA, Asker N, Hansson GC (1998) *O*-glycosylated MUC2 monomer and dimer from LS 174T cells are water-soluble, whereas larger MUC2 species formed early during biosynthesis are insoluble and contain nonreducible intermolecular bonds. J Biol Chem 273:18864–18870
21. Ambort D, van der Post S, Johansson ME, Mackenzie J, Thomsson E, Krengel U, Hansson G (2011) Function of the CysD domain of the gel-forming MUC2 mucin. Biochem J 436:61–70
22. Johansson ME, Thomsson KA, Hansson GC (2009) Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. J Proteome Res 8:3549–3557
23. Lidell ME, Johansson ME, Hansson GC (2003) An autocatalytic cleavage in the C terminus of the human MUC2 mucin occurs at the low pH of the late secretory pathway. J Biol Chem 278: 13944–13951
24. Zhuo L, Hascall VC, Kimata K (2004) Inter-alpha-trypsin inhibitor, a covalent protein–glycosaminoglycan–protein complex. J Biol Chem 279:38079–38082
25. Johansson ME, Larsson JM, Hansson GC (2011) The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci USA 108:4659–4665
26. Subramani DB, Johansson MEV, Dahlén G, Hansson GC (2010) *Lactobacillus* and *Bifidobacterium* species do not secrete proteases that can cleave the MUC2 mucin protein core which organizes the colon mucus layers. Beneficial Microbes 1:343–350
27. Velcich A, Yang W, Heyer J, Fragale A, Nicholas C, Viani S, Kucherlapati R, Lipkin M, Yang K, Augenlicht L (2002) Colorectal cancer in mice genetically deficient in the mucin Muc2. Science 295:1726–1729
28. Van der Sluis M, De Koning BA, De Bruijn AC, Velcich A, Meijerink JP, Van Goudoever JB, Buller HA, Dekker J, Van Seuningen I, Renes IB, Einerhand AW (2006) Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. Gastroenterology 131:117–129
29. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102:11070–11075
30. Gagneux P, Varki A (1999) Evolutionary considerations in relating oligosaccharide diversity to biological function. Glycobiology 9:747–755
31. Hansson GC (1988) Structural aspects of blood group glycosphingolipids in the gastrointestinal tract. Adv Exp Med Biol 228:465–494
32. Larsson JM, Karlsson H, Sjovall H, Hansson GC (2009) A complex, but uniform *O*-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn. Glycobiology 19:756–766
33. Larsson JM, Karlsson H, Crespo JG, Johansson ME, Eklund L, Sjövall H, Hansson GC (2011) Altered O-glycosylation profile of MUC2 mucin occurs in active ulcerative colitis and is associated with increased inflammation. Inflamm Bowel Dis. on-line
34. Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI (2003) A genomic view of the human–*Bacteroides thetaiotaomicron* symbiosis. Science 299:2074–2076
35. Fu J, Wei B, Wen T, Johansson ME, Liu X, Bradford E, Thomsson KA, McGee S, Mansour L, Tong M, McDaniel JM, Sferra TJ, Turner JR, Chen H, Hansson GC, Braun J, Xia L (2011) Loss of intestinal core 1-derived *O*-glycans causes spontaneous colitis in mice. J Clin Invest 121:1657–1666