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Review Article

REVIEW ARTICLE

Interactions of microorganisms with host mucins: a focus on *Candida albicans*

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One sentence summary: This review describes the current knowledge on the interactions between microorganisms and host mucins, with a focus on the opportunistic human fungal pathogen and commensal *Candida albicans*.

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ABSTRACT

Mucus is an important host innate defense factor that lines most epithelial cell layers of the body and provides crucial physical and biological protection against pathogenic microorganisms. Mucins are the main glycoproteins of mucus that are responsible for interacting with microorganisms and are critical for the antimicrobial properties of mucus. The mechanisms by which microorganisms interact with mucins are poorly understood, especially in terms of fungi, and these interactions are continually evolving. Work in bacterial pathogens has shown that mucins inhibit bacterial virulence traits, including quorum sensing, toxin secretion and biofilm formation. Among the fungal clade, the common opportunistic human fungal pathogen and commensal *Candida albicans* engages in constant battle with the host innate immune system. This battle creates strong selective pressures for *C. albicans* to evolve in response to the host. Recent work in *C. albicans* found that mucins inhibit specific virulence traits, such as surface adherence, filamentation, biofilm formation and the production of secreted proteases. Here we review the current knowledge of microbial interactions with mucins, with a special emphasis on the interactions between *C. albicans* and mucins.

Keywords: mucus; mucins; mucin monomer; *Candida albicans*; innate immunity; biofilms; host–pathogen interactions; mucosal surface; epithelial cell layer; viscoelasticity

INTRODUCTION

The healthy human microbiota is composed of hundreds of trillions of diverse microorganisms, including bacteria, fungi and archaea, that share and compete for nutrients and environmental niches in the body (Ursell *et al.* 2013; Wang *et al.* 2017). Over the course of millions of years of evolution, these microorganisms have coevolved in a mutually symbiotic relationship with the host, where they contribute to host physiological processes; the host, in turn, provides a hospitable environment for these microorganisms to reside (Relman 2008; Chow *et al.* 2010; Pickard *et al.* 2017). In addition to providing physiological benefits to the host (e.g. through nutrient acquisition and synthesis), these microorganisms also protect the host from invading pathogens that may enter and colonize the host from the outside environment (Chow *et al.* 2010; Buffie and Pamer 2013; Sassone-Corsi and Raffatellu 2015; Chiu *et al.* 2017). Although the majority of members of the microbiota typically behave as mutualists or commensals, some of these microorganisms can have pathogenic potential under certain circumstances (Casadevall and Pirofski 2001; Casadevall 2017; Libertucci and Young 2019). Most research to date has focused on studying bacterial

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members of the microbiota, but fungal members have been found to play increasingly important roles in interacting with the host and in shaping the functions of the microbiota (Underhill and Iliev 2014; Lukeš *et al.* 2015; Kumamoto 2016). Both members of the microbiota and invading pathogens from the environment typically make contact with and often reside on mucosal membranes covering epithelial cell layers found in the body, such as the oral cavity, eyes, nose, ears, respiratory, gastrointestinal and reproductive tracts (Fig. 1; Linden *et al.* 2008; Hansson 2012; Frenkel and Ribbeck 2015). Regardless of where they originated from (the microbiota or the outside environment), those microorganisms that can breach the mucosal barrier have the potential to cause systemic infections in the host (Linden *et al.* 2008; Belkaid and Hand 2014). Consistently, defects in mucus production, mucus physiochemical properties, or in the expression of mucins can lead to several disease states in humans, such as cystic fibrosis, inflammatory bowel disease, ulcerative colitis, Sjogren's syndrome, cancer and preterm birth, which are all associated with microbial dysbiosis and often the presence of an infection (Henke et al. 2004, 2007; Kim and Ho 2010; Williams, Ranjendran and Ramage 2016; Wagner, Wheeler and Ribbeck 2018; Schroeder 2019). In this review, we focus on the interactions between mucins, the major components of mucus responsible for mediating microbial interactions with the host, and microorganisms with pathogenic potential, with an emphasis on *Candida albicans*, a common opportunistic fungal pathogen and commensal of humans.

It is estimated that fungal diseases cost the United States approximately $7.2 billion annually, where *Candida* infections account for approximately 20% of these costs (Benedict *et al.* 2019). *Candida albicans*, the most commonly isolated fungal pathogen from clinical settings, typically resides as a commensal fungus in the microbiota of the skin, vagina, gastrointestinal and urogenital tracts of humans (Kennedy and Volz 1985; Kumamoto 2002, 2011; Achkar and Fries 2010; Ganguly and Mitchell 2011). When alterations to the host microbiota occur, such as by changes in pH or residing microorganisms, *C. albicans* can overgrow, become invasive and cause a wide range of infections (Odds 1987; Kim and Sudbery 2011; Nobile and Johnson 2015). These infections, collectively referred to as candidiasis, can range from superficial skin infections to severe bloodstream infections, the latter of which typically occur in immunocompromised individuals and can be life-threating (Haynes 2001; Kullberg and Oude 2002; Kim and Sudbery 2011). *C. albicans* possesses numerous virulence traits that contribute to its pathogenicity, such as the production of host recognition molecules, the ability to undergo morphological transitions, and the release of secreted aspartyl proteases and phospholipases that can damage host cells (Calderone and Fonzi 2001). In addition, the ability to form biofilms, recalcitrant communities of cells encased in extracellular matrices, is another important virulence trait of *C. albicans* that enhances its survival in the host (Kumamoto 2002; Douglas 2003; Ganguly and Mitchell 2011; Nobile and Johnson 2015; Gulati and Nobile 2016; Lohse *et al.* 2018).

C. albicans biofilm formation in a host setting begins when *C. albicans* cells colonize a mucosal surface covering a layer of epithelial cells or an implanted medical device. The *C. albicans* biofilm life cycle consists of four basic stages (Chandra *et al.* 2001; Douglas 2003; Gulati and Nobile 2016). In the first stage, round yeast form cells adhere to a solid surface (e.g. the intestinal mucosa or an implanted central venous catheter; Kennedy *et al.* 1987; Hawser and Douglas 1994; Baillie and Douglas 1999; Nobile and Johnson 2015). This is followed by proliferation of the adhered cells and early stage filamentation (Baillie and Douglas 1999; Nobile and Johnson 2015). As the biofilm matures, extensive filamentation takes place along with the production of the extracellular matrix, which is comprised of proteins, polysaccharides, nucleic acids and lipids (Baillie and Douglas 1999; Zarnowski *et al.* 2014; Pierce *et al.* 2017). As a result, the mature biofilm architecture is such that it provides structural protection to the cells within the biofilm from both chemical and mechanical insults (Mitchell, Zarnowski and Andes 2016). In addition to this structural protection, cells within the biofilm upregulate drug efflux pumps, further enhancing the resistance and tolerance of biofilms to inhibitory compounds. In the final stage of the *C. albicans* biofilm life cycle, round yeast form cells disperse from the biofilm to colonize new sites (Uppuluri *et al.* 2010; Nobile and Johnson 2015). Taken together, *C. albicans* biofilms can not only be highly resistant and tolerant to chemical and mechanical perturbations but can also act as reservoirs to seed new sites of infection. Interestingly, recent work in *C. albicans* found that specific fungal virulence traits, such as surface adherence, filamentation and biofilm formation, are compromised in the presence of mucins (Kavanaugh *et al.* 2014).

In the following sections of this review, we begin by discussing the properties, functions and structures of mucins. We then review the types of mucins present in the human body and examine their production and biosynthesis. Lastly, we review known interactions between microorganisms and mucins, with a focus on the interactions between *C. albicans* and mucins.

Mucus and mucins—an overview

Mucus is a viscoelastic hydrogel that is comprised of 95% water, 3% mucin glycoproteins and 2% other small molecules, including immunoglobulin A (IgA), lipids and antimicrobial peptides (Celli *et al.* 2005). Mucus provides lubrication and hydration to epithelial linings and is a critical innate defense factor that protects the host against infection; this protection is largely attributed to large glycoproteins called mucins, which can be secreted or membrane-bound (Gendler 1995; Liévin-le Moal and Servin 2017; Petrou and Crouzier 2018). The unique physiochemical properties of mucus are such that mucus limits microbial penetration through the epithelial cell layer, while at the same time permits the passage of water and gases (Cone 2009; Bakshani *et al.* 2018). In addition to these properties, mucosal surfaces continuously regenerate, allowing for the efficient removal of contaminants, and preventing them from reaching the underlying epithelial cell layer (Cone 2009). Mucins within mucus are known to mediate physical interactions with microorganisms, serve as receptor binding sites for the adhesion of molecules, act as nutrient sources for microorganisms, serve as biochemical signals, and support gaseous exchange and nutrient absorption between host cells (Wagner, Wheeler and Ribbeck 2018). Some of these roles depend on where the mucins are produced and localized in the body. For example, a major role of lung mucins is to support gaseous exchange between host cells, while for gut mucins, it is to support nutrient absorption (Corfield 2015). Lastly, it is known that mucins can inhibit virulence traits, such as biofilm formation, motility and cellular morphology changes in opportunistic pathogens, thus maintaining them in a commensal state (Ogasawara *et al.* 2007; Celli *et al.* 2009; Caldara *et al.* 2012; Kavanaugh *et al.* 2014; Co *et al.* 2018).

Mucin monomer molecular weights range from 0.5–20 MDa (Bansil and Turner 2006; Balabushevich *et al.* 2018), where approximately 80% of the molecular weight of a mucin monomer comes from polysaccharides that are attached

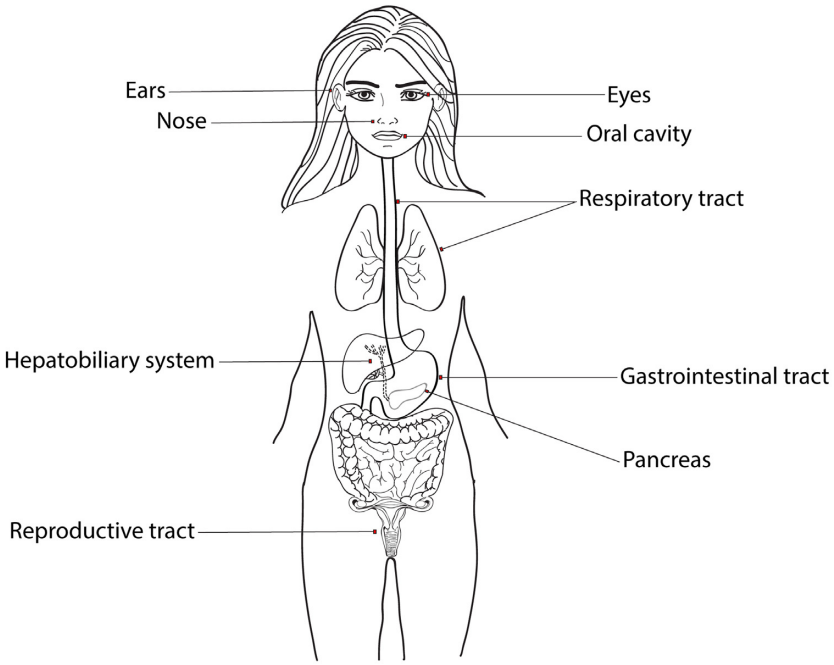

Figure 1. Mucosal membranes covering epithelial cell layers of the human body. Microorganisms are typically associated with mucosal membranes of the eyes, nose, oral cavity, respiratory, gastrointestinal and reproductive tracts. These microorganisms can include both commensals and pathogens. Figure adapted from (Frenkel and Ribbeck 2015)

to the protein core, including N-acetylgalactosamine, N-acetylglucosamine, sialic acid, fucose and galactose (Bansil and Turner 2006; Brockhauser, Schachter and Stanley 2009). The protein core consisting of proline, threonine and serine (called the PTS domain) makes up the remaining 20% of the molecular weight of a mucin monomer, and is the main glycosylated region of the protein (Fig. 2; Bansil and Turner 2006; Brockhauser, Schachter and Stanley 2009).

Human mucin glycoproteins belong to the MUC protein family, which is currently known to consist of 21 secreted and membrane-bound mucins (Corfield 2015, 2018). The five major secreted gel-forming mucins in the human body, which are important contributors to the viscoelasticity of mucus are MUC2, MUC5AC, MUC5B, MUC6 and MUC19 (Thornton, Rousseau and McGuckin 2008). MUC5AC and MUC5B are structurally similar proteins but are found in different niches of the host. For example, MUC5AC is found in mucus of the gastrointestinal and respiratory tracts, and MUC5B is found in salivary and cervical mucus. Currently, there are three known secreted but non-gel forming MUC proteins, MUC7, MUC8 and MUC9 (Corfield 2018). Unlike the gel-forming secreted mucins, MUC7 and MUC8 are not implicated in mediating viscoelasticity, however they have been shown to exhibit microbe-binding and anti-inflammatory properties (Xu *et al.* 2016; Cha and Song 2018). MUC7 specifically has been shown to possess fungicidal activity via a histatin-like domain found at its N-terminal region (Gururaja *et al.* 1999; Puri and Edgerton 2014). The remainder of the MUC protein family is made up of large membrane-bound mucins (also called tethered or cell surface-associated mucins) that form the glycocalyx mucus barrier and are known to mediate adherence to mucosal surfaces and to limit access of microorganisms to epithelial cell layers (Linden *et al.* 2008; Roy *et al.* 2014). These membrane-bound mucins include MUC1, MUC3A/B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, MUC21 and MUC22 (Linden *et al.* 2008; Pelaseyed and Hansson 2020).

Production of mucus and biosynthesis of mucins

Mucus is produced by mucus cells found in the surface epithelium, such as in goblet epithelial cells, in mucus glands, and in mixed glands containing mucus and serous cells (Lillehoj *et al.* 2013; Pelaseyed *et al.* 2014). From there, mucus is secreted onto the epithelial cell layer forming the mucosal surface (Linden *et al.* 2008; Pelaseyed *et al.* 2014; Corfield 2015).

Polymerization and secretion of gel-forming secreted mucins into the mucosa is critical for creating the viscoelastic properties of mucus, while localization of membrane-bound mucins to the cell membrane is critical for forming the glycocalyx mucus barrier. For secreted mucins, oligomerization occurs by rapid dimerization of mucin monomers in the endoplasmic reticulum (ER) (Linden *et al.* 2008; Corfield 2015). This is followed by O-glycosylation in the Golgi apparatus (Linden *et al.* 2008; Corfield 2015). For membrane-bound mucins, which are monomeric, proper synthesis in the ER is dependent on cleavage of an SEA domain into two subunits by autoproteolysis concurrent with N-glycosylation (Macao *et al.* 2006). For membrane-bound mucins, similar to secreted mucins, O-glycosylation also takes place in the Golgi apparatus, but for membrane-bound mucins, the newly synthesized mucin monomer is then tethered to the cell membrane (Linden *et al.* 2008; Corfield 2015).

Microbial interactions with mucins

Mucins in mucus are critical in the host's defense against invading microorganisms. Consistent with this concept, host genes encoding mucins have been found to be upregulated in the presence of invading microorganisms (Wagner, Wheeler and Ribbeck 2018). In one example, exposure of ear epithelial cells to the otitis media causing bacteria *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis* led to an upregulation of host genes encoding MUC2, MUC5AC and MUC5B (Kerschner *et al.*

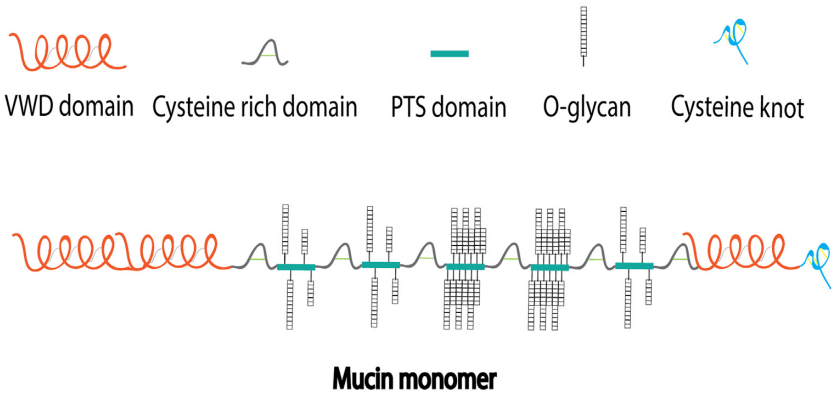

Figure 2. Schematic drawing of a mucin monomer. Each mucin monomer consists of a protein core called the PTS domain that is comprised primarily of proline, threonine and serine. O-glycosylation of polysaccharides occurs at PTS domains between cysteine rich domains. Typically, the C-terminus of the protein backbone contains a cysteine knot and the N-terminus contains several von Willebrand D (VWD) domains.

2014; Wagner, Wheeler and Ribbeck 2018). In another example, exposure of lung epithelial cells to *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyogenes* led to an upregulation of host genes encoding MUC5AC and MUC2 (Dohrman *et al.* 1998; Wagner, Wheeler and Ribbeck 2018). From the pathogen perspective, in response to host mucins, invading microorganisms can defend themselves in a number of different ways. They can, for example, secrete hydrolytic enzymes, such as proteases and glycosidases that degrade mucins and promote microbial invasion into the underlying host epithelial cell layer (Derrien *et al.* 2010). For example, in response to mucins, *E. coli* secretes the metalloprotease SslE, *Vibrio cholerae* secretes the metalloprotease TagA, and *C. albicans* secretes the aspartyl protease Sap2, which can all degrade mucins, potentially allowing for microbial penetration of the mucosal barrier (Colina *et al.* 1996; Szabady *et al.* 2011; Luo *et al.* 2014; Valeri *et al.* 2015).

Interestingly, other than invading microorganisms, some symbiotic members of the microbiota can also degrade mucins and are important in maintaining healthy host metabolic processes (Derrien *et al.* 2010; Tailford *et al.* 2015). For example, *Akkermansia muciniphila*, a bacterial colonizer of the mucosal layer of the intestinal tract that can degrade mucins is depleted in individuals with metabolic disorders, such as in diabetic and obese individuals (Collado *et al.* 2007; Cani and de Vos 2017; Shin *et al.* 2019; Xu *et al.* 2020). *A. muciniphila* is known to degrade and utilize mucins as a nutrient source by producing sialidases, fucosidases, N-acetyl-β-glucosaminidases and GlcNAc-sulfatases, which breakdown mucin monomers (Derrien *et al.* 2004; Ottman *et al.* 2016; Geerlings *et al.* 2018; Xu *et al.* 2020). Using mucins as a nutrient source is particularly useful in the colon, where carbon sources are extremely limited (Derrien *et al.* 2008). Interestingly, *A. muciniphila* colonizes the mucosal layer, but does not invade it, which is an intriguing distinction from most of the mucin degrading microorganisms that have pathogenic potential. In the case of *A. muciniphila*, the host may support its colonization of the mucosal layer in exchange for the benefits it provides to the host in preventing metabolic disorders.

In addition to these mucin degrading enzymes, microorganisms can also directly bind to mucins, an interaction that is continuously evolving between the host and pathogen. Many commensal and pathogenic bacterial species, such as *Helicobacter pylori, Yersinia enterocolitica, P. aeruginosa* and *E. coli*, to name a few, are known to physically bind to mucins (Sajjan and Forstner 1990; Wanke *et al.* 1990; Mantle and Husar 1994; Lindén *et al.* 2009; Dhar and McAuley 2019; Wheeler *et al.* 2019; Hoffman, Lalsiamthara and Aballay 2020). From the host perspective, microbial binding to mucins can be beneficial by allowing for the removal of pathogens through mucus flow and excretion, and even by 'mucin shedding' (Linden *et al.* 2008; Van Putten and Strijbis 2017). In an example of the latter concept, the human opportunistic bacterial pathogen, *H. pylori*, which typically colonizes the digestive tract, has been found to bind via its BabA and SabA adhesins to Lewis^b, sialyl Lewis^a and sialyl Lewis^x extracellular domain antigens of the carbohydrate portion of the membrane-bound mucin MUC1 (Lindén *et al.* 2009; Dhar and McAuley 2019). This binding event induces shedding of the microbe-bound MUC1 from the gastric epithelial cell layer, thereby preventing *H. pylori* from adhering to host epithelial cells (Lindén *et al.* 2009; Dhar and McAuley 2019). This is followed by excretion of the microbe bound MUC1 into the stomach, where it is digested by stomach acids. Mucin shedding can significantly limit disease progression by pathogens, which in the case of *H. pylori*, is the development of chronic peptic ulcers in the host.

In another example of microbe-mucin binding, the opportunistic bacterial pathogen, *P. aeruginosa*, commonly found in the respiratory tract of humans is known to physically bind via multiple strain dependent surface adhesins, including flagellins, to the sialyl Lewis^x extracellular domain antigens of the carbohydrate portion of airway mucins (Carnoy *et al.* 1994; Scharfman *et al.* 1999; Lillehoj, Kim and Kim 2002). From the host perspective, this binding interaction can lead to attenuation of *P. aeruginosa* virulence by inducing a downregulation of numerous bacterial virulence genes, including genes involved in quorum sensing (e.g. *lasR*), toxin secretion (e.g. *pcrV*) and siderophore biosynthesis (e.g. *pvdA*), as well as by inducing active biofilm dispersion (Caldara *et al.* 2012; Wheeler *et al.* 2019). From the pathogen perspective, on the other hand, *P. aeruginosa* can use mucin binding to its advantage to cause disease by enhancing its ability to adhere to and colonize the mucosal surface, and by aiding its penetration to the underlying epithelial cell layer (Derrien *et al.* 2010; Hoffman, Lalsiamthara and Aballay 2020). When this occurs, *P. aeruginosa* binds mucins primarily at the N-acetylgalactosamine and N-acetylglucosamine polysaccharide portions of mucin monomers, leading to their degradation, thus allowing *P. aeruginosa* to access the underlying epithelial cell layer (Hoffman, Lalsiamthara and Aballay 2020).

In terms of pathogenic fungi, there are many unanswered questions on how fungi interact with mucins, but the most mechanistic information is known for *C. albicans* (Fig. 3). One

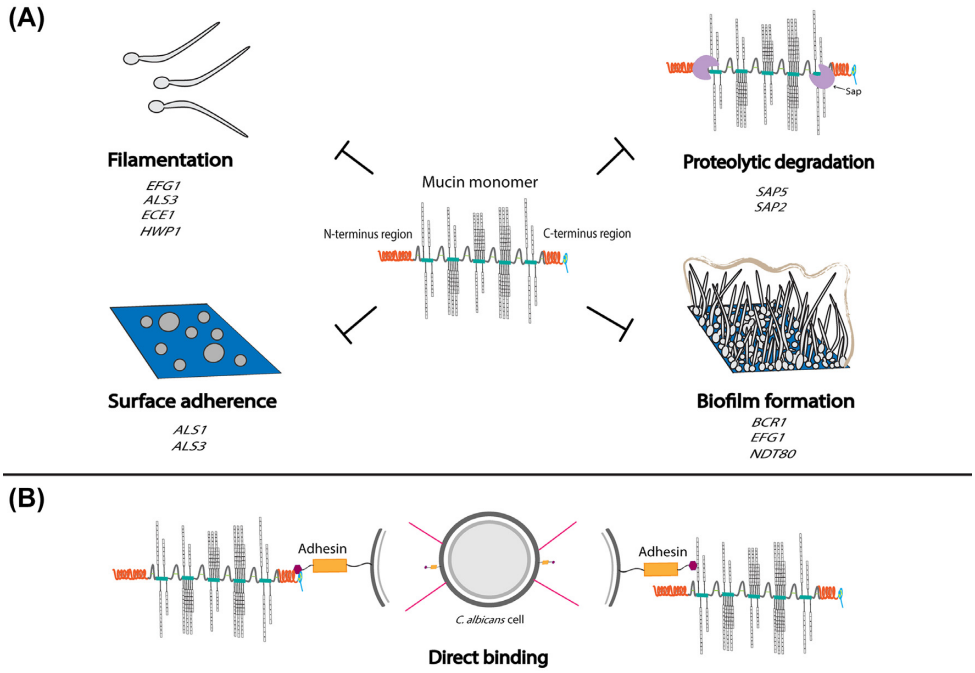

Figure 3. Summary of known and hypothesized interactions between *C. albicans* and mucins. **(A)** Known interactions. These include the suppression of *C. albicans* adherence, filamentation, biofilm formation and secreted protease production in the presence of mucins. Several *C. albicans* genes encoding important virulence processes are known to be downregulated in the presence of mucins, including *ALS1* and *ALS3* (adherence); *EFG1*, *ALS3*, *ECE1* and *HWP1* (filamentation); *BCR1*, *EFG1* and *NDT80* (biofilm formation); and *SAP5* and *SAP2* (proteolytic degradation). **(B)** Hypothesized interactions. *C. albicans* adhesins may directly bind to a mucin monomer at the C-terminus of the PTS domain and/or at a glycan monosaccharide.

study determined that the gel-forming mucins MUC5AC, MUC5B and MUC2 prevent *C. albicans* from transitioning from the round yeast cell state to the elongated hyphal cell state that is critical for this fungus to invade the host epithelial cell layer and is an important structural feature of its biofilms (Kavanaugh *et al.* 2014; Basmaciyan *et al.* 2019). In addition, when hyphal *C. albicans* cells were exposed to mucins under hyphal inducing conditions, newly budded cells from these hyphae were in the round yeast form rather than the elongated hyphal form. In contrast, in the absence of mucins, newly budded cells under these conditions were always in the hyphal form. In addition, methylcellulose, a viscosity control used in this study to mimic the viscosity of mucins did not affect filamentation, suggesting that the biological properties of mucins, rather than their physical properties, are important in their ability to suppress *C. albicans* filamentation. Taken together, these results indicate that mucins suppress the development of hyphae from yeast cells and also suppress the development of new hyphae from existing hyphal cells. Not surprisingly in this study, several *C. albicans* genes involved in filamentation were downregulated in the presence of mucins, including *EFG1*, which encodes a major transcriptional regulator of filamentation, as well as *ALS3*, *ECE1* and *HWP1* (Kavanaugh *et al.* 2014). Interestingly, mucins appear to induce *C. albicans* cells to transition into a novel yeast morphology that phenotypically resembles the oval mating competent opaque cell type of this fungus, but that is functionally distinct since the morphology induced in the presence of mucins is not mating competent (Kavanaugh *et al.* 2014). This morphology also bears some resemblance to the *C. albicans* gastrointestinally induced transition (GUT) cell type identified as a commensal cell state that occurs when *C. albicans* cells are passaged through the gastrointestinal tract of a mouse (Pande, Chen and Noble 2013; Noble, Gianetti and Witchley 2017). It is possible that this novel mucin induced morphology may occur uniquely and specifically in response to mucins.

Another study found that *C. albicans* filamentation was inhibited by salivary mucins in a dose dependent manner (Ogasawara *et al.* 2007). In this study, *C. albicans* cells were grown for 24 hours under hyphal inducing conditions in the absence and presence of different concentrations of salivary mucins ranging from 125 to 1000 μg/mL (Ogasawara *et al.* 2007). In the absence of mucins, *C. albicans* cells formed long and numerous hyphae, while in the presence of mucins, there was a clear dose dependent reduction in hyphal formation as higher concentrations of mucins were used, and at 1000 μg/mL of mucins, no hyphae were observed whatsoever in the culture (Ogasawara *et al.* 2007). No differences in growth rates were observed in the absence versus the presence of mucins at concentrations up to 1000 μg/mL of mucins (Ogasawara *et al.* 2007). The expression of *RAS1*, which encodes the Ras1 GTPase that regulates the cAMP and MAP kinase pathways involved in the induction of hyphal formation, was also measured in this study (Feng *et al.* 1999; Leberer *et al.* 2001; Ogasawara *et al.* 2007). In the absence of mucins under hyphal inducing conditions, *RAS1* expression levels were increased throughout the course of the experiment, while in the presence of mucins under the same conditions, *RAS1* expression levels were significantly repressed (Ogasawara *et al.* 2007). This repression of *RAS1* also correlated with a decrease in the expression of *EFG1*. Taken together, these results indicate that salivary mucins suppress the development of hyphae from yeast cells.

Other than filamentation, it has been shown that mucins, specifically MUC5AC, inhibit adherence of *C. albicans* cells to abiotic (polystyrene) and biotic (human epithelial cell) surfaces (Kavanaugh *et al.* 2014). The adherence inhibitory effects of mucins on these surfaces was observable after 30 minutes and increased significantly over the course of a 1-hour adhesion

assay. Interestingly, the methylcellulose viscosity control also inhibited surface adhesion, suggesting that the physical properties of mucins contribute to their anti-adherence properties. Consistent with the finding that mucins inhibit adherence, a number of *C. albicans* genes involved in adherence were downregulated in the presence of mucins, such as the adhesins *ALS1* and *ALS3* (Kavanaugh *et al.* 2014). Therefore, by preventing *C. albicans* cells from adhering to surfaces, mucins impede the ability of *C. albicans* to achieve the first step necessary in the process of breaching the epithelial cell layer.

Since filamentation and adherence are important processes during *C. albicans* biofilm formation, it follows that biofilm formation would also be inhibited in the presence of mucins. Indeed, over the course of a 48-hour biofilm experiment in the presence of mucins, biofilm formation was severely constrained, where few hyphae were observed throughout the rudimentary (∼60 μm thick) biofilm formed (Kavanaugh *et al.* 2014). This is in contrast to the robust (∼500 μm thick) biofilm formed in the absence of mucins, containing long and extensive hyphae. The methylcellulose viscosity control in this experiment also inhibited biofilm formation, suggesting that the physical properties of mucins contribute to their antibiofilm properties. Consistent with the finding that mucins inhibit biofilm development, the genes *BCR1*, *EFG1* and *NDT80*, encoding three of the six core *C. albicans* biofilm master regulators, were downregulated in the presence of mucins (Nobile *et al.* 2012; Kavanaugh *et al.* 2014).

Other than inhibiting filamentation, adherence and biofilm formation in *C. albicans*, mucins also suppress the expression of *C. albicans* secreted aspartyl protease encoding genes, such as *SAP5* and *SAP2* (Kavanaugh *et al.* 2014). By suppressing the expression of these hydrolytic enzymes, which are known *C. albicans* virulence factors that are similar to those produced by bacterial pathogens, mucins protect themselves from degradation and limit the ability of *C. albicans* to colonize the mucosal surface and invade the underlying epithelial cell layer (Colina *et al.* 1996; Naglik, Challacombe and Hube 2003; Nikou *et al.* 2019).

Although the molecular mechanisms for *C. albicans* direct binding to mucins are unknown, the adhesin Als1 is known to bind via its N-terminal region to fucose-containing glycans (Donohue *et al.* 2011). Based on these findings and the fact that mucins are heavily comprised of fucose glycans, it seems feasible that mucins could directly bind to Als1 as well as to the structurally similar protein Als3. Additionally, the hyphal specific cell surface protein Hwp1 is another candidate for mucin binding that is already known to interact with the host. Hwp1, which has a domain that resembles mammalian transglutaminase substrates, can bind to and form stable bonds of attachment to mammalian transglutaminases on the surfaces of host buccal epithelial cells (Staab *et al.* 1999). Hwp1 is also known to have complementary surface adhesion functions with Als1 and Als3 (Nobile *et al.* 2008). Taken together, it is plausible that Hwp1 could also directly bind to mucins. From the pathogen perspective, binding of *C. albicans* surface proteins and/or adhesins to mucins could increase adherence to the mucosal surface, allowing for *C. albicans* to penetrate to the epithelial cell layer. From the host perspective, binding of *C. albicans* surface adhesins to mucins could allow for mucin shedding to occur, where the *C. albicans* cells bound to mucins could be excreted in mucus flow, thereby reducing the number of *C. albicans* cells available to invade the host epithelial cell layer.

Another study assessing the abilities of several *Candida* species to bind to small intestinal mucins observed a hierarchy of mucin binding capabilities that appears to correlate with the abilities of the different species to cause disease in mammals, suggesting that direct mucin binding is an important virulence factor in the *Candida* clade (De Repentigny *et al.* 2000; Hirayama *et al.* 2020). The authors found that *C. albicans*, *Candida dubliniensis* and *Candida tropicalis* strongly adhered to mucins; *Candida parapsilosis* and *Candida lusitaniae* moderately adhered to mucins; and *Candida krusei* and *Candida glabrata* weakly adhered to mucins (De Repentigny *et al.* 2000). *S. cerevisiae*, which was used as a non-pathogenic outlier species, adhered to mucins the weakest relative to the *Candida* species tested. The binding of *Candida* species to mucins in this study appeared to be, in part, dependent on the *C. albicans* secreted aspartyl protease Sap2 (De Repentigny *et al.* 2000). The authors suggest that the C-terminal glycosylated region of small intestinal mucins may be involved in the direct binding of *Candida* adhesins to mucins, and that this region of the mucin monomer is a substrate specifically for Sap2 (De Repentigny *et al.* 2000). Taken together, although the mucosal surface acts as a barrier to *Candida* species from accessing the epithelial cell layer, mucins are likely substrates for several *Candida* secreted proteases that can degrade mucins, allowing *Candida* cells to access the underlying epithelial cell layer (De Repentigny *et al.* 2000).

Finally, it has been postulated that *C. albicans* cell type heterogeneity in the gastrointestinal tract, which is lined with mucus, can be modulated by the immune status of the host (Kumamoto and Pierce 2011; Pierce and Kumamoto 2012). This model predicts that *C. albicans* produces phenotypic variants with two distinct functions: one optimized for persistence as a commensal in the host and one optimized for pathogenic interactions with the host (Kumamoto and Pierce 2011; Koh 2013). When alterations in the host's immune status occur, the levels of these phenotypic variants are postulated to shift, changing the pathogenic potential of the population (Kumamoto and Pierce 2011). This model is supported by studies showing that variability in the levels of the transcriptional regulators Efg1 and Efh1 in mouse infection models can shift the *C. albicans* population between the commensal and pathogenic states (Pierce and Kumamoto 2012). Specifically, if a change in the host status selects for *C. albicans* cells with low Efg1 activity, then the *C. albicans* cell population shifts to become pathogenic, while if this change selects for cells with low Efh1 activity, then the population shifts to become commensal, and vice versa (White *et al.* 2007; Pierce and Kumamoto 2012). In terms of heterogeneity in cell morphology, another study showed that the *C. albicans* yeast form over other morphological forms is the commensal morphological form in the gastrointestinal tract in a monocolonized gnotobiotic mouse model (Böhm *et al.* 2017). This finding is logical given that the filamentous form is the morphological form that can breach the mucosal barrier (Basmaciyan *et al.* 2019). Interestingly, when the mice were treated with antibiotics, a morphologically heterogeneous population of cell types containing yeast and filamentous forms was formed, thereby increasing the pathogenic potential of the population (Böhm *et al.* 2017). This study also identified three transcriptional regulators, Zcf8, Zfu2 and Try4, that are required for maintaining this yeast form morphology and thus the commensal state of *C. albicans* in the mouse gastrointestinal tract (Böhm *et al.* 2017). Interestingly, these regulators also promote the adherence of *C. albicans* to mucin coated surfaces as well as to mucus producing intestinal epithelial cells (Böhm *et al.* 2017).

Much less is known about the interactions of the non-*Candida* fungal pathogens with mucins. *Aspergillus fumigatus*, an opportunistic human fungal pathogen that can colonize the respira-

tory tract and cause aspergillosis, is known to degrade mucins using hydrolytic enzymes, including proteases and glycosidases (St. Leger and Screen 2000; Oguma *et al.* 2011; Cowley *et al.* 2017). One study found that approximately 75% of the protein portions and 40% of the polysaccharide portions of mucins were degraded by *A. fumigatus* secreted proteases and glycosidases, respectively, that were produced under *in vitro* growth conditions in the presence of mucins (St. Leger and Screen 2000). Consistent with this finding, another study found that the *A. fumigatus* secreted serine protease Alp1 was highly upregulated at both the protein and transcript level in the presence of mucins (Farnell *et al.* 2012). From the pathogen perspective, degrading mucins using secreted proteases and glycosidases can allow *A. fumigatus* cells to access the underlying epithelial cell layer. In addition, studies have suggested that *A. fumigatus* likely uses mucins as a nutrient source (St. Leger and Screen 2000; Oguma *et al.* 2011; Cowley *et al.* 2017). From the host perspective, in response to *A. fumigatus* secreted proteases, the host compensates by upregulating the expression of MUC5AC in airway epithelial cells, which can be a double-edged sword (Cowley *et al.* 2017). The upregulation of MUC5AC could be protective against infection by inhibiting fungal colonization of the mucosal layer, but if MUC5AC becomes highly upregulated or upregulated for too long, this can lead to diseases related to mucus hypersecretion, such as allergic bronchopulmonary aspergillosis, which typically occurs in individuals with asthma or cystic fibrosis (Oguma *et al.* 2011; Gao *et al.* 2012).

CONCLUDING REMARKS

Mucus is an important host innate defense factor that lines most epithelial cell layers of the body and provides crucial physical and biological protection against pathogenic microorganisms. Mucins are the main glycoproteins of mucus that are responsible for interacting with microorganisms and are critical for the antimicrobial properties of mucus. The physiochemical properties of mucins can suppress key virulence traits in microorganisms, maintaining them in a commensal state. In the opportunistic human fungal pathogen *C. albicans*, adherence, filamentation, biofilm formation and the production of secreted proteases are suppressed by mucins, although the molecular mechanisms behind this suppression are unknown. In general, further work is needed to elucidate the molecular mechanisms involved in microbe–mucin interactions, which will be essential for our understanding of how a healthy mucosal barrier is maintained. In order to carry out such studies, there is a need for tractable mucus model systems that can be used to study microbe–mucin interactions. Mucosal model systems would also be helpful in the development of novel therapeutics to treat mucosal diseases, such as cystic fibrosis, inflammatory bowel disease and ulcerative colitis. Finally, given that mucins are such critical players against infection, mechanistically understanding their biological and physical properties will be useful in the development of novel therapeutic strategies against pathogenic microorganisms.

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