

Ocular mucins in dry eye disease

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Short title: Mucins in dry eye disease

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Abstract

Dry eye disease is a common and multifactorial disease with a high prevalence worldwide. Water loss, reduced expression of glycocalyx mucins, and loss of goblet cells secreting gel-forming mucins are hallmarks of dry eye disease. Mucins are large and complex heavily glycosylated proteins. Their organization in the tear film remains unclear, but they play a key role to protect and maintain integrity of the ocular surface. Mice have been extremely valuable mammalian models with which to study ocular physiology and disease, and to evaluate eye therapies. Genetically modified mice and spontaneously occurring mutants with eye defects have proven to be powerful tools for the pharmaceutical industry, clinicians, and basic researchers investigating dry eye disease. However, ocular mucins remain relatively under-studied and inadequately characterized. This review aims to summarize current knowledge about mucin production at the ocular surface in healthy individuals and in dry eye disease, and to compile an overview of mouse models available for the study of mucins in dry eye disease.

Keywords: Dry eye; Mouse model; Mucin; Mucus

Abbreviations: ADDE, aqueous deficient dry eye; BAK, benzalkonium chloride; BTX, botulinic toxin; EDE, evaporative dry eye; MAM, membrane-associated mucin

1. Introduction

Mucus gel is the first defense barrier at the ocular surface. This hydrogel traps bacteria, dust, and pollen, and removes them by eye blinking. Mucus also lubricates the underlying tissue and is crucial to dissipate the energy generated from eye blinking, preventing desiccation of the ocular surface. Dry eye disease (DED) is a common and multifactorial disease. All forms of DED are due to water loss. In aqueous deficient dry eye (ADDE), water loss is due to a deficient lacrimal secretion but a normal rate of evaporation of the tear film, whereas evaporative dry eye (EDE) is due to an excessive evaporation of tears in the presence of normal lacrimal secretion. Regardless, every kind of dry eye enters a self-perpetuating detrimental cycle where the tear film hyperosmolarity induces an inflammatory response that leads to a reduced expression of glycocalyx mucins, apoptotic death of surface epithelial cells, and a loss of goblet cells that secrete gel-forming mucins (Bron et al., 2017). This decreased goblet cell density is proportional to the disease severity (Murube and Rivas, 2003). The aim of this comprehensive review is to summarize current knowledge of mucin production and its role at the ocular surface during health and DED, and to overview the mouse models available for the study of mucins during dry eye.

2. Mucins

Mucus is a hydrogel that covers secretory epithelium. Its primary functions are the protection, lubrication, and hydration of mucosal tissues. It also constitutes a semipermeable protective barrier between the environment and underlying epithelial cells. Mucus gels are composed mainly of water (> 95%) and mucins (MUCs) represent their major organic component. Mucins are high molecular weight proteins characterized by long amino acid sequences enriched in Ser, Thr, and Pro that are *O*-glycosylated. *O*-glycosylation is a posttranslational modification that consists on the addition of lateral sugars on the mucin backbone, also called apomucin. Apomucins are heavily *O*-glycosylated, giving them their specific “bottle brush” structure (Fig. 1). *O*-

glycosylation is essential for the gelation process and the mucus rheological properties, to maintain the mucin tertiary structure (linear instead of globular) and to modulate the interactions between mucins with foreign particles (bacteria, dust...) (Corfield, 2015; Demouveau et al., 2018).

Human *MUC* genes and rodent *Muc* genes encode human MUC and mouse Muc products that can be classified into two families: membrane-associated mucins (MAMs) and secreted mucins (Fig. 1). MUC3A/B, MUC4, MUC11/12, MUC16, and MUC17, are part of large human MAMs found at the apex of epithelial cells of the secretory mucosa (Desseyn et al., 2008) and may to a lower extent contribute to the mucus gel. Several small MAMs are nonspecific to secretory mucosal tissues, such as MUC1, MUC13, MUC15, MUC18, and MUC20-22 (Pallesen et al., 2002; Shih et al., 1998; Uhlén et al., 2015; Woodward and Argüeso, 2014). Because these MAMs are not “mucus molecules”, they should not be classified as mucins but mucin-like molecules. Among the secreted human mucins, MUC7 and MUC8 are small mucins that do not form oligomers while the five others MUC2, MUC5AC, MUC5B, MUC6 and MUC19 are truly gel-forming. These mucins are macromolecules synthesized and secreted as polymers by specialized cells that are mainly goblet cells. They are highly conserved between human and mouse (Culp et al., 2004; Desseyn and Laine, 2003) and impart rheological properties to mucus gels (Demouveau et al., 2018; Thornton et al., 2008).

3. Mucus of the ocular surface

3.1. Tear film

The tear film thickness ranges from 2 to 5.5 μm and covers the ocular surface exposed to the external environment. The most simple and logical representation of tear film is the three-layered model: a mucin layer in contact with the corneal epithelium, an aqueous internal layer, and a lipid external layer that prevents evaporation (Fig. 2a). However, it is increasingly considered that the mucins and aqueous layers are actually a single layer, termed the mucoaqueous layer, with a mucin

gradient toward the ocular surface (Fig. 2b) (Willcox et al., 2017). The exact structure of the tear film is continually debated but there are clinical observations suggesting that “*the fluid drawn into the menisci from the nascent tear film, in the upstroke of the blink, is more watery than the precorneal film itself and it seems likely that an aqueous layer is retained between TFLL [tear film lipid layer] and the subjacent mucoaqueous layer behavior is distinctly gel-like*” (Bron et al., 2017).

The tear film lipid layer, with a mean thickness of 42 nm, is produced by the Meibomian glands and is spread onto the tear film with each blink. The main role of this layer is to stabilize the tear film (Willcox et al., 2017). The aqueous layer is secreted at the conjunctival fornix by the lacrimal gland (Treuting et al., 2012). The mucin layer, formed by MAMs and gel-forming mucins, is produced by corneal and conjunctival epithelia and lacrimal glands.

3.2. Mucin expression at the ocular surface

Mucins contribute to the epithelial barrier, prevent binding of debris and pathogens to the ocular surface, maintain hydration of the ocular surface and stabilize the tear film (Willcox et al., 2017). The production of ocular mucins starts very early during human embryonic development. Indeed, goblet cells containing sialylated mucins are visible in the fornix of the conjunctiva as soon as the 9th week of amenorrhea (Miyashita et al., 1992). In BALB/c mice, conjunctival goblet cells producing Muc5ac are visible 21 days after birth by immunohistochemistry using the 45M1 antibody, which is directed against the human MUC5AC counterpart (Pajooohesh-Ganji et al., 2016).

The three major human ocular MAMs are MUC1, MUC4, and MUC16 (Muc1, Muc4, and Muc16 in the mouse). In humans, MUC1, MUC4, and MUC16 are produced in both the cornea and the conjunctiva (Gipson, 2004; Pflugfelder et al., 2000), whereas only Muc1 and Muc4 are produced in both the mouse cornea and conjunctiva (Kim et al., 2016; Marko et al., 2014; Menon et al., 2015) (Fig. 3). In mice, Muc16 is produced in the conjunctiva but not in the cornea (Gipson et al., 2016). Interestingly, MUC4 is found mainly at the surface of conjunctival epithelial cells and its

expression decreases from the edge of the corneal epithelium to the center of the cornea (Gipson, 2004; Pflugfelder et al., 2000). To our knowledge, the gradient location of Muc4 has not been verified in mouse ocular surface. In mouse, it has been shown that, in addition to conjunctival and corneal epithelial cells, Muc1 is also produced by the conjunctival goblet cells, and is more produced in the conjunctiva than in the cornea (Kardon et al., 1999). In both humans and mice, MUC16/Muc16 is produced by epithelial cells and goblet cells in the conjunctiva (Gipson et al., 2016). MUC16 is a remarkable MAM since it has been shown that it locates in the mucin granules in the goblet cells (Gipson et al., 2016). The authors suggested that one of the roles of MUC16 could be the facilitation of gel-forming mucin release by preventing the gel-forming mucins to attach to the mucin granule membrane. Soluble forms of MUC1, MUC4 and MUC16 have also been detected in human tear film, in addition to the gel-forming mucins MUC5AC and MUC2 (Spurr-Michaud et al., 2007). Human conjunctival and corneal epithelial cells also produce MUC20 (Woodward and Argüeso, 2014). In mouse, *Muc20* gene is expressed in the conjunctiva (Gupta et al., 2011), however its expression in the cornea has not been studied. Low expression of *MUC11* and *MUC15* at the mRNA level has been also reported at the human ocular surface (Gipson, 2004).

Goblet cell distribution within the conjunctiva differs between humans and mice. Gel-forming mucins are produced by goblet cells, which occur as single cells in humans, whereas they are usually associated in clusters forming basket like structures in mice (Gipson and Tisdale, 1997) (Fig. 3). Human conjunctival goblet cells secrete the two gel-forming mucins MUC5AC and MUC19 (Yu et al., 2008). Low expression of *MUC2* and *MUC5B* at the RNA level have been reported at the ocular surface (Gipson, 2004). Unlike in humans, *Muc19* gene seems not to be expressed in the mouse eye (Das et al., 2010). As in humans, mouse conjunctival goblet cells express high levels of *Muc5ac* while low levels of *Muc5b* have been reported (Marko et al., 2014). Nevertheless, it appears that Muc5b production has been greatly underestimated according to our data using a reporter transgenic fluorescently-tagged Muc5b mouse engineered by homologous

recombination (Portal et al., 2017b). This study revealed that almost half of the conjunctival goblet cells produced Muc5b. Furthermore, it demonstrated that Muc5b+ goblet cells represent a good mouse biomarker in an experimental model of dry eye.

Mucins are also produced by ocular glands. Lacrimal gland and Meibomian gland secretions contribute to the mucin content of the tear film. Mucins MUC1, MUC5AC, MUC5B, MUC7, and MUC19 are produced by human lacrimal glands (Paulsen et al., 2004; Yu et al., 2008). MUC1 is also produced by human Meibomian glands (Tektaş et al., 2012). Transcriptome analysis have shown that *Muc1*, *Muc2*, *Muc3*, *Muc4*, *Muc5ac*, *Muc5b*, *Muc6*, *Muc10*, *Muc13*, *endomucin* (*Muc14*), *Muc15*, *Muc16*, *Muc19* and *Muc20* genes are expressed in mouse Meibomian glands (Chen et al., 2019; Parfitt et al., 2016). To our knowledge, mucin production by mouse ocular glands has not yet been analyzed at the protein level.

4. Mucins in dry eye

4.1. Dry eye disease

As stated by the Definition and Classification Subcommittee of the TFOS DEWS II, “*Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles*” (Craig et al., 2017).

The global prevalence of DED ranges from 5% to 50% worldwide, with a higher prevalence of dry eye for women than for men, and a higher prevalence in Asian than in Caucasian populations (Stapleton et al., 2017). For example, in France, the prevalence of dry eye is 21.9% in the elderly (> 73 years), with a higher prevalence in women (27.1% compared with 13.6% for men) (Malet et al., 2014). Thirty-five percent of French patients with dry eye suffer from a mild form, 47% from a moderate form, and 18% from a severe form (Clegg et al., 2006). In China, the prevalence of dry

eye by symptoms is higher than 50% in the elderly (≥ 70 years) and is 38.9% in the general population (from 5 to 89 years) (Song et al., 2018). In Japan, the dry eye prevalence is 66.2% (30-65 years), with a higher prevalence in women (76.5% compared with 60.2% for men) (Uchino et al., 2013).

The etiopathogenic classification of dry eye distinguishes ADDE and EDE, even if they are not exclusive (Craig et al., 2017). However, whatever the etiology of DED, they enter a final common pathway creating a self-perpetuating detrimental cycle: tear hyperosmolarity stimulates ocular surface inflammation, that decreases glycocalyx mucin expression and goblet cell density, and induces surface epithelial cell death that increases ocular surface hyperosmolarity (Bron et al., 2017).

4.2. Alteration of mucins in dry eye

A common feature of all forms of DED is goblet cell loss (Bron et al., 2017). The decrease of goblet cell density, related to the severity of the disease, is accompanied by a decrease of the gel-forming mucin MUC5AC production. It has been shown a decreased concentration of MUC5AC in the tears of patients with DED (Uchino et al., 2014). Depending on the studies, the level of expression of *MUC5AC* gene remains unchanged (Uchino et al., 2014) or is decreased (Corrales et al., 2011b; Machida et al., 2016; Shimazaki-Den et al., 2013) in the conjunctiva of patients with DED compared to healthy subjects. This discrepancy is likely due to different severity of the DED. A decreased expression of *MUC2* gene has also been described (Corrales et al., 2011b). Moreover, it is believed that changes of mucin glycosylation can occur during DED (Mantelli et al., 2013). Changes in MUC2 and MUC5AC glycosylation have been described in patients with symptoms but no sign of dry eye (Berry et al., 2004). The two gel-forming mucins MUC2 and MUC5AC play a pivotal role in the rheological properties of the tear film. Changing in their amount or degree of

glycosylation could affect the amount of water retained in the tear film, so the wettability of the ocular surface and its lubrication.

In patients with DED, it has also been reported a decreased expression of the MAM genes *MUC1*, *MUC4* and *MUC16* (Corrales et al., 2011b; Machida et al., 2016; Shimazaki-Den et al., 2013). Disrupted expression of these mucins that are members of the glycocalyx, can lead to an impaired protection of the ocular surface, as well as a less effective lubrication of the ocular surface. Changes in *MUC1* glycosylation has been described in patients with DED, with an increase of *MUC1* sialylation in mild to moderate cases and a decrease in severe cases (Hayashi et al., 2004). The authors suggested a compensatory mechanism of the MAM in response to the decrease of *MUC5AC* in milder DED to reduce DED symptoms, that is not maintained as the severity of the disease increase.

Moreover, a decreased expression of the *MUC5AC* gene in the conjunctiva and a decreased concentration of *MUC5AC* product in the tears in patients with Sjögren's syndrome, a systemic autoimmune disease characterized in particular by severe dry eye, has been reported (Argüeso et al., 2002). Furthermore, it has been shown a decrease of the expression and production of *MUC19* in patients with Sjögren's syndrome (Yu et al., 2008). Concerning the MAM, the concentration of soluble *MUC16* is increased in the tear film of patients with Sjögren's syndrome, but the level of membrane bound *MUC16* in the conjunctiva remained the same than control subjects (Caffery et al., 2008). It has also been reported an increased concentration of the soluble *MUC1* in the tear film of patients with Sjögren's syndrome, as well as an increased level of membrane bound *MUC1* in their conjunctiva (Caffery et al., 2010). The authors suggested that this increased production of *MUC1* and *MUC16* is a compensatory response of the conjunctival epithelium during Sjögren's syndrome dry eye in order to protect the ocular surface and to maintain a healthy ocular surface.

How does DED affect the mucin pattern expression in specific areas of the ocular surface in patients with Sjögren's syndrome or dry eye patients without Sjögren's syndrome has not been

studied. Mucin levels of expression/production are usually determined from tear and/or impression cytology samples, giving only a global vision on how the mucin expression/production is affected at the ocular surface during DED. In addition to modifications in mucin levels of expression/production, *O*-glycosylation changes appear to be a main feature of DED. This affects directly the rheological properties of the tear film and then its properties to trap and to clear foreign particles, as well as to retain water and so provide hydration and lubrication to the ocular surface (Chaudhury et al., 2016; Ricciuto et al., 2008).

5. Mouse models for the study of mucins in dry eye

Many animal models are used to study DED, and the mouse appears to be a good model. Mice are less expensive than bigger animals, and are widely used to study absorption, distribution, metabolism, and excretion and toxicity tests. Numerous genetic and spontaneous mutant models are now available.

5.1. Dry eye mouse models

Numerous mouse models have been developed over the past 20 years for the study of ADDE or EDE. For ADDE, a wide variety of mouse models have been developed, including surgical models with excision of the extraorbital lacrimal gland (Stevenson et al., 2014) and/or cauterization of the lacrimal duct (Flores et al., 2016), and physical models by placing mice in a desiccating environment with or without scopolamine transdermic application (Barabino et al., 2005; Dursun et al., 2002) or by submitting mice to a therapeutic dose of radiation (Rocha et al., 2013). Chemical models by topical application of benzalkonium chloride (BAK) (Lin et al., 2011), injection of botulinic toxin (BTX)-B (Suwan-Apichon et al., 2006) or BTX-A (Kim et al., 2013), or human recombinant interleukin-1 (Zoukhri et al., 2007) into the lacrimal gland, or injection of concanavalin A intraorbitally or into the lacrimal glands (Lee et al., 2015b, 2015a) are models

allowing the use of one eye as a model of dry eye and the other as an internal control. A plethora of genetic mouse models with dry eye have been also reported including *NRTN*^{-/-} (Song et al., 2003), *Klf4*CN (Swamynathan et al., 2007), Tet-mev-1 (Uchino et al., 2012), *Sod1*^{-/-} (Kojima et al., 2012), *Spdef*^{-/-} (Marko et al., 2013), *Itpr2*^{-/-};*Itpr3*^{-/-} double-KO (Inaba et al., 2014), and *NHE8*^{-/-} mice (Xu et al., 2015). The nonobese NOD strain known to develop diabetes is a polygenic model for autoimmune diabetes, and male NOD mice have been reported to develop lacrimal lesions leading to a dry eye phenotype (Takahashi et al., 1997).

Mouse models for EDE are essentially genetically modified mice that have no, or abnormal, Meibomian glands. These models include the strains *ACAT-1*^{-/-} (Yagyu et al., 2000), *SCD1*^{-/-} (Miyazaki et al., 2001), *TRAF6*^{-/-} (Naito et al., 2002), K14-Noggin (Plikus et al., 2004), *Smad4*CKO (Huang et al., 2009), K14-CreER^{tam};C/EBPα^{ff};C/EBPβ^{ff} (House et al., 2010), *Barx2*^{-/-} (Tsau et al., 2011), *Klf5*CN (Kenchegowda et al., 2011), *Fatp4*^{-/-} (Lin et al., 2013), *Fgfr2*CKO mice (Reneker et al., 2017), and the transgenic lines APOC1 (Jong et al., 1998), and Tabby-EDA (Cui et al., 2005). In addition, numerous mouse models of primary and secondary Sjögren's syndrome, which is a long-term autoimmune disease characterized by an extensive dryness of many organs of the body, were developed and reviewed by Lavoie and colleagues (Lavoie et al., 2011).

5.2 Mucins in dry eye mouse models

Whatever the type of DED, the predominant method of studying mucin as an ocular sensor is periodic acid-Schiff (PAS) staining used to detect all polysaccharides including mucins and which allows assessment of the decrease of goblet cell density (Table 1).

A decrease in the expression of the secreted gel-forming mucins *Muc5ac* and *Muc5b* has been shown at the RNA level in *Spdef*^{-/-} mice, which lack conjunctival goblet cells (Marko et al., 2013). By contrast, in EDE Tabby mice, the absence of Meibomian glands induced an increase of *Muc5ac* and *Muc5b* expression, suggesting a different response of goblet cells according to the dry

eye model (Wang et al., 2016). Nevertheless, the genetically ablation of the gel-forming mucins genes *Muc5b* or *Muc5ac* alone seemed insufficient to induce dry eye (Marko et al., 2014), even if modifications of the ocular surface have been shown in several *Muc5ac*^{-/-} mice (Floyd et al., 2012). The absence of *Muc5ac* was compensated for by an increase of *Muc5b* production, which can explain in part why there are only few symptoms observed in *Muc5ac*^{-/-} mice (Marko et al., 2014). In *MUC5AC-Tox176* mice, for which the toxicity of diphtheria toxin A was driven by the human mucin *MUC5AC* promoter, the conditional loss of conjunctival goblet cells was accompanied by a compensatory upregulation of the MAM *Muc4* (Wang et al., 2009). Using the ADDE model produced by topical applications BAK twice a day for ten days, we showed that the *Muc5b*+ conjunctival goblet cell density decreased and reflected the entire goblet cell density. Importantly, this decrease of conjunctival goblet cell density was compensated for by an increased expression of both *Muc5ac* and *Muc5b* at the RNA levels and *Muc5b* production at the peptide level in the remaining conjunctival goblet cells (Portal et al., 2017a).

A decrease of the MAM *Muc4* in an ADDE mouse model induced by excision of the exorbital lacrimal gland (Kim et al., 2016) and a reduced expression of the two MAMs *Muc1* and *Muc4* in *NRTN*^{-/-} mice, which have a defective parasympathetic innervation of the lacrimal gland, have been reported (Song et al., 2003). However, the genetic ablation of *Muc1* seems to not be enough to induce DED. No ocular phenotype was described in *Muc1* null mice (C57BL/6 background) and the absence of *Muc1* was not compensated by an increase of *Muc4* expression (Danjo et al., 2000). Conjunctivitis and blepharitis, accompanied with a goblet cell hyperplasia, were observed in another *Muc1* null mouse line in the C57BL/6 X SVJ129 genetic background only when mice are housed in a conventional facility (Kardon et al. 1999). The authors suggested that *Muc1* plays an important role in maintaining resistance to bacterial infection. *Muc4*^{-/-} mouse line has also been obtained, and does not have obvious abnormal phenotype (Das et al., 2016). To our knowledge, the ocular phenotype of *Muc4*^{-/-} mice has not been studied in details yet.

Moreover, in *Aire*^{-/-} mice, a mouse model of ADDE associated with Sjögren's syndrome, an increased acidification of goblet cell mucins and an increased sialylation of terminal galactose residues in goblet cell mucins were observed (Stephens and McNamara, 2015).

6. Conclusion

MAMs and gel-forming mucins are a key component of the ocular barrier, as they both contribute to the epithelial barrier, prevent binding of debris and pathogens to the ocular surface, maintain hydration of the ocular surface and stabilize the tear film. Clinical evidence of mucin production disruption has been reported during DED. Despite a wide variety of well characterized mouse models of DED, mucins are still poorly studied and well-characterized molecular tools are missing. A better understanding of the role of ocular mucin in health and disease remains elusive due mainly to the technical limitations of observing *in vivo* or *ex vivo* how mucins are organized and interact in the tear film. Additionally, mucins are difficult to study, primarily because of their high level of glycosylation, which restricts specific antibody development. This has led to underestimated levels of mucin production.

The development of transgenic mouse strains has allowed better characterization of mucin abnormalities during DED, especially in fluorescently-tagged mice. It would be interesting to develop other fluorescently-tagged mice, with different fluorescent reporter, to allow simultaneous visualization of either both gel-forming mucins Muc5ac and Muc5b or a combination of gel-forming and MAMs. The development of knock-out models has revealed that a specific deficiency in one mucin is not sufficient to induce dry eye (no significant ocular phenotype observed in *Muc1*^{-/-}, *Muc5ac*^{-/-} and *Muc5b*^{-/-} mice). The development of mice genetically deficient for both *Muc5b* and *Muc5ac* genes should be considered while interconnection between ocular MAMs and gel-forming mucins should be investigated. Compensatory mechanisms of mucin production and glycosylation were reported in patients, especially in those with mild to moderate dry eye. The loss

of goblet cells seems to appear later as the disease progresses or in more severe cases. More attention should be given to mucin glycosylation changes in the different dry eye mouse models. The pertinence of several mouse models to study the role of the mucins in DED remains to be shown as most of them only evaluated the goblet cell density by PAS staining. Despite the technical limitations to study mucins, mice are a good model to study dry eye since it is possible to mimic ADDE and EDE. Physical models using a desiccating environment are widely used and robust. Chemical models have the advantage to reduce the number of animal used by experiment since one eye can be used as a control while the other one is treated. Moreover, the variety of transgenic mice can bring insights of the molecular mechanisms of the disease.

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Table 1. Evaluation of goblet cell density and mucin expression or production in mouse models of dry eye. Only methods allowing to study goblet cell density and mucins are listed. See references for additional methods to assess dryness used by the authors.

Mouse model	Methods to study mucins and goblet cell density	Ref
DS with scopolamine	PAS (↓ GC density)	(Dursun et al., 2002)
Ovariectomized mice	PAS (ns) / <i>Muc4</i> (expressed in corneal and conjunctival epithelia) and <i>Muc5ac</i> (expressed in conjunctival goblet cells) <i>in situ</i> hybridization / <i>Muc4</i> (ns) and <i>Muc5ac</i> (ns) expression / <i>Muc4</i> (ns) and <i>Muc5ac</i> (ns) staining	(Lange et al., 2003)
<i>NRTN</i> ^{-/-} mice	PAS (↓ GC density) / <i>Muc4</i> (↓) and <i>Muc5ac</i> (↓) staining / <i>Muc1</i> (↓) and <i>Muc4</i> (↓) expression	(Song et al., 2003)
DS	PAS (↓ GC density)	(Barabino et al., 2005)
Klf4CN mice	PAS (absence of GC) / AB (absence of GC)	(Swamynathan et al., 2007)
MUC5AC-DTA mice	AB-PAS (↓ GC density) / mucicarmine staining (↓ GC density) / <i>Muc1</i> (ns), <i>Muc2</i> (↓), <i>Muc4</i> (↑) and <i>Muc5ac</i> (↓) staining / <i>Muc1</i> (ns), <i>Muc2</i> (↓), <i>Muc4</i> (↑) and <i>Muc5ac</i> (↓) expression / <i>Muc4</i> (↑) and <i>Muc5ac</i> (↓) <i>in situ</i> hybridization	(Wang et al., 2009)
Aire-deficient mice into the NOD Lt/J background	AB (↑ AB ⁺ GC = mucin acidification) / PAS (ns)	(Chen et al., 2010)
BAK topical application	<i>Muc5ac</i> staining (↓ GC density)	(Lin et al., 2011)
DS with scopolamine	PAS (↓ GC density) / <i>Muc5ac</i> staining (<i>Muc5ac</i> trapped in GC) / <i>Muc5ac</i> expression (ns) / <i>Muc5ac</i> concentration (↓)	(Corrales et al., 2011a)
DS with scopolamine	<i>Muc5ac</i> staining (↓)	(Zhang et al., 2011)
Klf5CN	PAS (absence of GC)	(Kenchegowda et al., 2011)
Aire-deficient mice into the NOD Lt/J background	AB-PAS (↑ mucin acidification and ↓ GC density)	(Chen et al., 2012)
DS with scopolamine	AB-PAS (ns) / <i>Muc5ac</i> expression (ns)	(Contreras-Ruiz et al., 2013)
<i>Muc5ac</i> ^{-/-} mice	AB-PAS (larger GC) / <i>Muc5ac</i> (absent) and (↑) <i>Muc5b</i> staining / <i>Muc1</i> (ns), <i>Muc2</i> (ns), <i>Muc4</i> (ns), <i>Muc5ac</i> (↓) and <i>Muc5b</i> (↑) expression	(Floyd et al., 2012)
<i>Spdef</i> ^{-/-} mice with DS	PAS (absence of GC)	(Marko et al., 2013)
Aire-deficient mice into the NOD background	Lectin staining: MAL-1 (↑ = ↑ sialylation) and SNA (no SNA ⁺ GC)	(Vijmasi et al., 2013)
<i>Sod1</i> ^{-/-} mice	PAS (↑ GC density after rebamipide treatment) / <i>Muc1</i> (ns after rebamipide treatment), <i>Muc4</i> (ns after rebamipide treatment) and <i>Muc5ac</i> (↑ after rebamipide treatment) expression	(Ohguchi et al., 2013)
DS with scopolamine	PAS (↓ GC density)	(X. Zhang et al., 2014)
<i>Muc5ac</i> ^{-/-} mice	PAS (ns) / <i>Muc5ac</i> (absent) and <i>Muc5b</i> (↑) staining / <i>Muc1</i> (ns), <i>Muc4</i> (ns), <i>Muc5ac</i> (↓) and <i>Muc5b</i> (↑) expression	(Marko et al., 2014)
<i>Muc5b</i> ^{-/-} mice	PAS (ns) / <i>Muc5b</i> staining (absent) / <i>Muc1</i> (ns), <i>Muc4</i> (ns), <i>Muc5ac</i> (ns) and (↓) <i>Muc5b</i> expression	(Marko et al., 2014)
BAK topical application	PAS (↓ GC density)	(Z. Zhang et al., 2014)
NFS/sld mice	<i>Muc5ac</i> expression (↑ after rebamipide treatment)	(Arakaki et al., 2014)
<i>Itp2</i> ^{-/-} ; <i>Itp3</i> ^{-/-} mice	PAS (↓ GC density and presence of abundant mucin complexes)	(Inaba et al., 2014)
<i>Sod1</i> ^{-/-} mice	PAS (↓ GC density) / <i>Muc5ac</i> expression (↓)	(Kojima et al., 2014)
Intraorbital injection of concanavalin A	PAS (↓ GC density)	(Lee et al., 2015b)
<i>NHE8</i> ^{-/-} mice	PAS (↓ GC density) / <i>Muc5ac</i> expression (↓)	(Xu et al., 2015)

Intraorbital and extraorbital gland injection of concavalin A	PAS (↓ GC density)	(Lee et al., 2015a)
NOD.BIO.H2b mice	PAS (↓ GC density)	(Lee et al., 2015a)
EDA-Tabby mice	PAS (ns) / <i>Muc1</i> (↑ only in cornea at 4 week-old), <i>Muc4</i> (ns), <i>Muc5ac</i> (↑), <i>Muc5b</i> (↑), <i>Muc13</i> (↑ in cornea and conjunctiva at 8 week-old) and <i>Muc15</i> (ns) expression / <i>Muc5ac</i> staining (↑)	(Wang et al., 2016)
Lacrimal duct cauterly	PAS (↑ GC density after CFTR _{act} -K089 treatment)	(Flores et al., 2016)
Excision of one exorbital lacrimal gland	<i>Muc4</i> staining (↓)	(Kim et al., 2016)
BAK topical application	AB-PAS (↓ GC density) / <i>Muc5ac</i> (↓ <i>Muc5ac</i> ⁺ GC density) and GFP (↓ <i>Muc5b</i> -GFP ⁺ GC density) / <i>Muc5b</i> staining on <i>Muc5b</i> -GFP reporter mouse / <i>Muc5ac</i> (↑) and <i>Muc5b</i> (ns) expression / Live imaging of <i>Muc5b</i> with <i>Muc5b</i> -GFP reporter mouse (↓ <i>Muc5b</i> -GFP ⁺ GC density)	(Portal et al., 2017a)
<i>Spdef</i> ^{+/−} mice	<i>Muc2</i> (only studied in WT) and <i>Muc5ac</i> staining (only studied in WT)	(Barbosa et al., 2017)
NFS/sld mice	PAS (↓ GC density)	(Ushio et al., 2017)
<i>SCD-1</i> ^{−/−} mice	PAS (↑ GC density) / <i>Muc5ac</i> expression (↑)	(Inaba et al., 2018)
NOD.BIO.H2b mice with DS with scopolamine hydrobromide	PAS (↓ GC density) / AB (↓ GC density) / <i>Muc1</i> (↓), <i>Muc4</i> (↓), <i>Muc5ac</i> (↓) and <i>Muc16</i> (↓) staining	(Kim et al., 2018)
DS with scopolamine	PAS (↓ GC density)	(Žiniauskaitė et al., 2018)
DS with scopolamine hydrobromide	PAS (↑ after treatment with cyclosporine, prednisolone, rebamipide or diquafosol tetrasodium treatment) / <i>Muc1</i> staining (↑ after treatment with cyclosporine, prednisolone, rebamipide or diquafosol tetrasodium treatment) / <i>Muc1</i> (↓), <i>Muc4</i> (↓), <i>Muc16</i> (↓) and <i>Muc5ac</i> (↓) expression / <i>Muc1</i> (↓), <i>Muc5ac</i> (↓) and <i>Muc16</i> (↓) expressing-cells by fluorescence-activated cell sorting	(Moon et al., 2018)
NOD.BIO.H2b mice with DS	PAS (↓ GC density) / AB (↓ GC density) / <i>Muc1</i> (↓), <i>Muc4</i> (↓), <i>Muc5ac</i> (↓) and with scopolamine hydrobromide: <i>Muc16</i> (↓) staining	(Lee et al., 2019)

AB, alcian blue; BAK, benzalkonium chloride; DS, dessication stress; GC, goblet cell; ns, non significant; PAS, periodic acid-Schiff; WT, wild-type

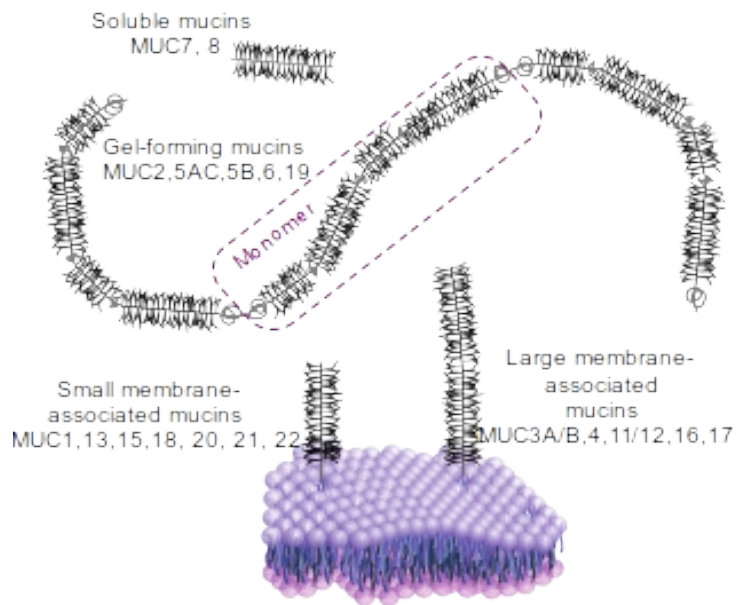


Fig. 1. Schematic representation of the human membrane-associated and secreted mucins. The secreted gel-forming mucins MUC2, MUC5AC, MUC5B, MUC6, and MUC19 form oligomers and are responsible for the rheological properties of the mucus gels. By contrast with the other secreted mucins, MUC7 and MUC8 do not form oligomers. Large MAMs MUC3A/B, MUC4, MUC11/12, MUC16 and MUC17 may also contribute to the mucus gels to a lesser extent. Small MAMs are not specific to secretory mucosal tissues.

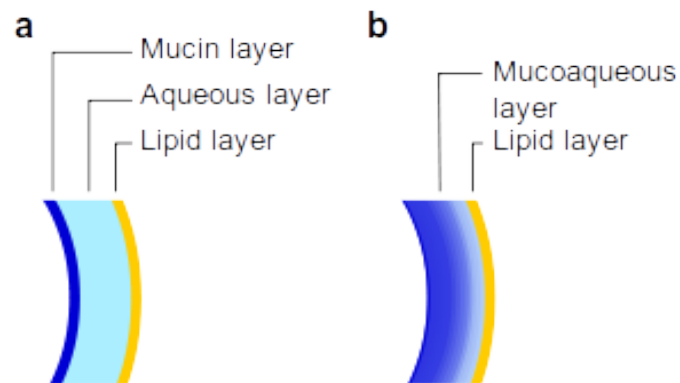


Fig. 2. Schematic representation of the tear film. (a) The classic representation of the tear film is the three-layer model composed of a mucin layer covering the corneal epithelium, an aqueous internal layer, and a lipid layer preventing evaporation. (b) Because the boundary between the mucin and the aqueous layers is not clear, increasingly considered that these two layers actually form a single mucoaqueous layer.

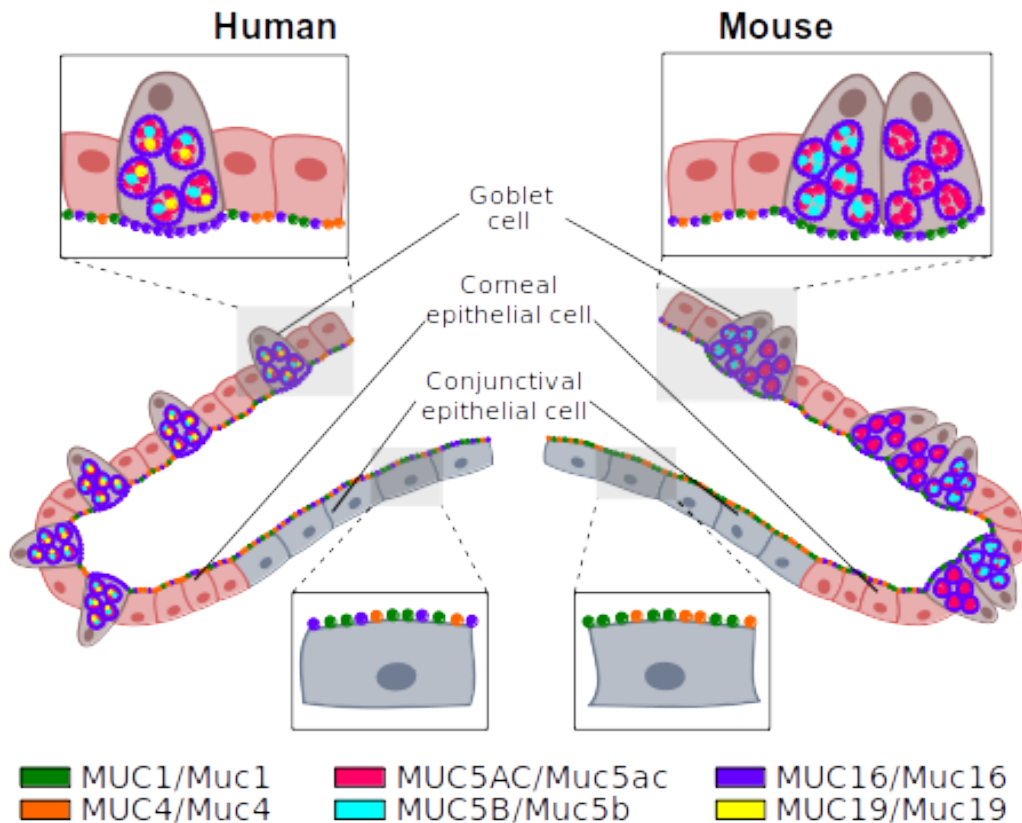


Fig. 3. Mucin production by corneal and conjunctival epithelia in humans and mice. The main MAM produced on the ocular surface of both humans (MUC) and mice (Muc) are MUC1/Muc1, MUC4/Muc4 and MUC16/Muc16. The expression of MUC1 at the surface of human conjunctival goblet cells has never been reported, contrary to mice. Contrary to humans, Muc16 is not produced by the mouse corneal epithelial cells. Regarding the gel-forming mucins, MUC5AC/Muc5ac is the main one in humans and mice. Gel-forming mucins are produced and secreted by specialized cells, the goblet cells. Goblet cells occur as single cells in human conjunctiva, whereas they form basket like clusters in mouse conjunctiva. In humans, conjunctival goblet cells produce MUC19, while *Muc19* gene seems not expressed in the mouse conjunctiva. Very low levels of *MUC5B* expression have been reported in humans, whereas Muc5b seems more expressed in mice for which half of the conjunctival goblet cells produce the mucin.