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## **Three cheers for the goblet cell: Maintaining homeostasis in mucosal epithelia**

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## **Abstract**

Many organs throughout the body maintain epithelial homeostasis by employing a mucosal barrier which acts as a lubricant and helps to preserve a near-sterile epithelium. Goblet cells are largely responsible for secreting components of this mucosal barrier and represent a major cellular component of the innate defense system. In this review, we summarize what is known about the signaling pathways that control goblet cell differentiation in the intestine, the lung and the ocular surface and discuss a novel functional role for goblet cells in mucosal epithelial immunology. We highlight the cell type specificity of the circuitry regulating goblet cell differentiation and shed light on how alteration of these pathways affects goblet function, which represent prominent features of mucosal-associated diseases.

## **Mucosal epithelia employ specialized cell types to maintain tissue homeostasis**

Throughout the body, internal organs and passages that come into contact with the exterior environment are lined by mucosal epithelium, which is required for lubrication and barrier function against outside pathogens and debris. The gastrointestinal tract, genitourinary system, upper and lower respiratory tract and ocular surface are all vastly distinct tissues functionally, but they share common mucosal features. Mucosal epithelium differentiates (see Glossary) and functions uniquely for the processes required in each tissue type, but there are some important features common to the health and homeostasis of all mucosal epithelia.

Goblet cells are specialized secretory cells found throughout mucosal epithelia, and play an important role in maintaining tissue homeostasis by secreting a variety of factors, including proteins, trefoil factors, and mucins, all of which contribute to the mucus layer protecting mucosal epithelium. Mucins are large, high molecular weight glycoproteins that can be membrane bound or secreted, and over 20 mucin genes have been identified to date [1]. Combinations of membrane-bound and secreted mucins vary between tissues, with goblet cells as the main source of secreted gel-forming mucins in mucosal epithelia. The contents of goblet cells are tightly condensed and stored in secretory granules awaiting release. Goblet cells can secrete a small, baseline amount of mucins via exocytosis under physiologic conditions, but primarily release their entire secretory contents in an apocrine manner or compound exocytosis in response to external stimuli [2-8]. Release of mucins into the extracellular environment is controlled by microbial factors [6], growth factors [4, 7, 9], inflammatory cytokines [6, 7], inflammasomes [8] and autonomic neural pathways, which are mediated in part by

adrenergic and cholinergic receptors present on the surface of goblet cells [3, 4, 10-12]. Mucus is then moved through the gastrointestinal tract by peristalsis, through the respiratory epithelium by mucociliary clearance, and through the ocular surface by blinking [1, 13, 14]. Intestinal and conjunctival epithelia turn over within the span of a few days [15, 16], whereas pulmonary epithelial cells have a much longer lifespan under homeostatic conditions [17]. The mucus layer of all three tissues turns over in a matter of hours [1], but goblet cells are capable of producing large mucin secretions multiple times within their lifespans and may live much longer than their non-secretory epithelial neighbors, at least in the conjunctiva [16].

While the importance of goblet cells in mucosal biology has been appreciated for decades, the genetic regulation of goblet cell differentiation has only begun to be understood in the past ten years. One gene that is of special interest in goblet cell differentiation is sterile  $\alpha$  motif pointed domain epithelial specific transcription factor (*Spdef*). *Spdef* was originally described as a prostate-specific transcription factor [18, 19], but has since been shown to play an integral role in goblet cell differentiation in the intestine [20, 21], the lung [22] and the conjunctiva [23, 24] (Box 1). However, upstream regulators of *Spdef* vary contextually or have not been tested globally, thus hindering a complete understanding of goblet cell differentiation. Alterations in goblet cell number or secretory function is associated with many mucosal diseases (Table 1). In this review, we highlight the current knowledge of genetic pathways regulating goblet cell differentiation, with special emphasis on how regulation of common genes differs contextually. We discuss the role of the goblet cell-secreted mucus layer in innate defense and consider new evidence implicating goblet cells in immune surveillance.

Lastly, we examine alterations of goblet cell gene expression, number, and function in disease states.

### **Mucosal epithelial architecture in the gastrointestinal tract, the airway and the ocular surface**

Mature goblet cells are integral components of the gastrointestinal tract, the airway epithelium and the conjunctival epithelium (Figure 1), but their differentiation from progenitor cells and placement among other specialized cell types varies contextually.

The gastrointestinal tract is organized into functionally and anatomically distinct tissues which are specialized for nutrient uptake, including the mouth, esophagus, stomach, small intestine and colon. Epithelium of the small intestine is organized into villi and crypts and is comprised of a variety of differentiated cell types, including absorptive enterocytes and secretory cells, which encompasses goblet cells, enteroendocrine cells, anti-bacterial Paneth cells, and tuft cells [25-30] (Figure 1). Colonic epithelium differs in that there are no Paneth cells, enterocytes are termed colonocytes, and the differentiated cells of the villi are flush with the luminal surface [25, 31]. Both small and large intestinal epithelia are maintained by well-characterized stem cell pools located in the crypts [25, 27, 30]. Goblet cells are the most abundant secretory cell type within the intestinal epithelium [25] and mainly secrete trefoil factors and Muc2 [1, 6, 32-34]. In the intestine, mucus plays dual roles of a lubricant and a physical barrier between luminal contents and the intestinal epithelium [35] (Table 1). Goblet cells are found in increasing numbers from the proximal small intestine to the distal colon as stool

becomes compacted [27, 29, 36]. Both the small and large intestine are covered by mucus comprised mainly of Muc2, but the mucus covering the small intestine is unattached and in a single layer, whereas the colon maintains a two-layered mucous gel, with the inner layer providing a bacteria-free environment adjacent to the epithelium and the luminal, less viscous layer harboring gut microflora [1, 6, 14, 33].

The airway epithelium consists of the cartilaginous trachea which branches into primary bronchi, bronchioles, and alveolae, which are the sites of gas exchange [37, 38] (Figure 1). The tracheobronchial epithelium contains basal cells, ciliated cells, club (Clara) cells and goblet cells [37, 39-41]. Under physiological conditions, the mouse lung is almost entirely devoid of goblet cells, contrasting with human conducting airways, which do contain some goblet cells in the absence of external stressors [7]. Basal cells are considered the resident stem cells in the tracheobronchial epithelium, and can differentiate into goblet cells [39, 40, 42, 43]. In the smaller conducting airways, club (Clara) cells are considered progenitor cells, and can differentiate into goblet cells in response to allergen, inflammation or exposure to inhaled toxins [41, 44]. The most abundant mucins produced by goblet cells in the tracheobronchial epithelium are MUC5AC and MUC5B [1, 7, 45]. These mucins combine with other protein, lipid and glycosylated factors to form airway surface liquid (ASL), a two-layered, low-viscosity medium with the mucus layer in contact with inhaled air and a sterile, periciliary layer adjacent to the respiratory epithelium [7, 41]. The mucus layer protects the respiratory epithelium from inhaled particles and pathogens by mucociliary clearance, in which cilia moves mucus toward the mouth where it is swallowed [7, 38, 41]. Repression of goblet cell differentiation in the lung is vital to pulmonary health and function. Excessive

secretion of mucins by goblet cells can change the viscosity of mucus, leading to inefficient mucociliary clearance and pulmonary obstruction in respiratory diseases [1, 7, 38, 41] (Table 1).

The conjunctiva lines the inner surface of the eyelid and is continuous with the eyelid epithelium and the corneal epithelium via the limbus [46] (Figure 1). In contrast to gastrointestinal and pulmonary epithelia, conjunctival epithelium is comprised of only two cell types: the keratinocyte and the goblet cell [47]. Goblet cells are concentrated in the fornix and are also present in the bulbar and palpebral conjunctiva, but are absent in the eyelid, the cornea and the limbus (Figure 1). Conjunctival epithelial stratification and goblet cell differentiation begin around eye opening, when the eye is first subjected to photo, oxidative and environmental stresses [5, 48]. There is evidence to suggest that the conjunctival fornix contains a population of cells with stem cell characteristics. While it is known that goblet cells and conjunctival keratinocytes share a common progenitor [49, 50], a definitive goblet cell precursor cell in the conjunctiva has yet to be identified [23]. It has been proposed that goblet cells are terminally differentiated cells arising from the stratified conjunctival keratinocyte [2, 50]. Conjunctival goblet cells secrete components of the tear film, including peroxidase, trefoil factors, defensin and the gel-forming, aqueous mucin Muc5AC, which protects the surface of the eye by maintaining lubrication, clearing molecules, and maintaining mucosal barrier integrity [4, 23, 47, 48, 51, 52] (Table 1). Human conjunctival goblet cells secrete MUC5AC, while murine conjunctival goblet cells secrete both Muc5ac and Muc5b [23]. While goblet cells are solely responsible for secreting the gel-forming Muc5AC, this is only one component of tears; lacrimal glands, Meibomian glands, and stratified squamous

epithelial cells contribute to the aqueous layer, lipid layer, and glycocalyx layer, respectively [47].

Proper specification and differentiation of goblet cells throughout the body is essential for health and normal mucosal epithelial function; however, the mechanisms governing goblet cell differentiation vary in a context-dependent manner.

### **Context-dependent roles of common genes involved in goblet cell differentiation**

While the function of goblet cells in maintaining mucosal epithelial homeostasis is consistent between the intestine, the lung and the conjunctiva, genetic mechanisms regulating their differentiation vary between tissues. The requirement of *Spdef* for proper goblet cell differentiation, maturation, and function remains constant between these tissues (Box 1). Murine models have revealed that many upstream regulators of *Spdef*, including the Notch and Wnt signaling pathways, act in differing ways on goblet cell differentiation in a tissue-dependent manner.

Notch signaling represses goblet cell differentiation in the intestine [53], but promotes goblet cell metaplasia in the lung [54] and is required for normal goblet cell differentiation in the conjunctiva [48]. It is plausible that Notch-mediated lateral inhibition is dependent on tissue-specific promoters and that it regulates cell lineage specification differently in stratified versus simple epithelia. In the gastrointestinal tract, Notch-mediated lateral inhibition is the driving force in promoting differentiation of intestinal cell types and controls selection between absorptive and secretory cell lineages. Progenitor cells with activated Notch signaling, via *Hes1*, become enterocytes, whereas

cells without activated Notch commit to secretory lineages (goblet cells, Paneth cells, enteroendocrine cells or tuft cells) via *Atoh1* [25, 28, 36, 55, 56] (Figure 2A). *Gfi1* acts downstream of the Notch-mediated selection between enterocyte and secretory progenitor cell fates, and is required for normal distribution of enteroendocrine, Paneth and goblet cells within the intestine, and acts upstream of *Spdef* [57, 58]. Conversely, expression of an activated Notch mutant in post-mitotic intestinal epithelium results in an expansion of differentiated goblet cells in a *Hes5*-driven, *Hes1*-independent mechanism [59], supporting the concept that the effects of Notch signaling vary contextually and are dependent on a cell's proliferative state. Additionally, *Gata4/6* negatively regulates SPDEF in the intestine [60] but functions independently of *Gfi1* [61].

Opposite to its role in intestinal progenitor cells, activation of the Notch pathway in airway basal cells [40] or in the entire lung epithelium [54] results in increased numbers goblet cells at the expense of other non-goblet cell types (Figure 3A). Complementary studies with loss of Notch signaling in pulmonary epithelium result in a loss of secretory club (Clara) cells, with a concomitant increase in ciliated cells [62, 63]. In the eye, loss of canonical Notch signaling results in a failure of goblet cell formation during eye development and the loss of pre-existing conjunctival goblet cells in the adult [48] (Figure 4A). Furthermore, Notch signaling induces MUC5AC expression *in vitro* [64].

Notch signaling functions upstream of other factors required for goblet cell differentiation, such as the Krüppel-like transcription factors (KLF) 4 and 5 in the intestine [65, 66] (Figure 2A) and in the ocular surface [48] (Figure 4A). In the intestine, KLF4 is restricted to differentiated, post-mitotic cells, including goblet cells, and induces

cell-cycle arrest [46, 67-70], whereas KLF5 is expressed in basal cells within the crypts and promotes cellular proliferation [67, 71, 72]. Notch signaling has been shown to inhibit KLF4, providing one possible mechanism for restriction of KLF4 expression and goblet cell differentiation in the crypts [65, 66]. Loss of Notch signaling results in goblet cell metaplasia in the intestine; however, KLF4 is not required in this context, as goblet cell metaplasia persists with loss of both Notch signaling and KLF4 [73], indicating that Notch acts on intestinal differentiation in parallel pathways (Figure 2A). Mice null for *Klf4* display a 90% reduction of mature goblet cells in the colon at postnatal day 1; however, goblet cell maturation is minimally affected in the small intestine [31], highlighting differences in tissue-specific regulation of goblet cell differentiation. Loss of *Klf5* in the embryonic intestine revealed severely disrupted crypt-villus architecture and lack of differentiated cell types, including goblet cells [74]. However, conditional loss of *Klf4* at 2 weeks of age [73] or *Klf5* in adult mice [75] results in relatively normal intestinal architecture and cellular differentiation, suggesting that KLF4/5 are required for initial specification events but are dispensable for intestinal maintenance and homeostasis.

KLF4 [5] and KLF5 [71] are both expressed throughout conjunctival epithelium, including in goblet cells, and deletion of *Klf4* [68] or *Klf5* [71] results in complete loss of conjunctival goblet cells. *Klf4* and *Klf5* mRNA levels were unchanged in microarray analysis of the conjunctiva of *Spdef* null mice [23], indicating that *Klf4* and *Klf5* function upstream of *Spdef* (Figure 4A), while microarray analysis of the conjunctiva of *Klf4* deficient mice showed a decrease in *Spdef* expression as well as the winged-helix transcription factors *Foxa1* and *Foxa3* [5]. *Foxa3* and SPDEF mutually induce each other's expression in keratin 14-expressing cells *in vitro* [24].

In the intestine, Foxa1 and Foxa2 promote Muc2 expression and goblet cell maturation and/or differentiation [76], as combined loss of these factors results in decreased numbers of mature goblet cells [77]. Conversely, Foxa2 is a potent inhibitor of goblet cell differentiation in the lung, and Foxa2 expression is mutually exclusive with goblet cell gene expression [78] (Figure 3A). Inhibition of goblet cell differentiation in the lung is critical to pulmonary function and a number of additional factors integral to this process have been identified, such as *Nkx2.1* [79]. Activated  $\beta$ -catenin [80], Foxa3 [81] and SPDEF [22] inhibit expression of Foxa2, and SPDEF and *Nkx2.1* reciprocally inhibit each other [22, 79], placing SPDEF in a central role of cell fate determination within the lung (Figure 3A). SPDEF and Foxa3 reciprocally regulate each other's expression and both promote expression of goblet cell genes such as the protein disulfide isomerase *Agr2* [22, 82], which is required for respiratory goblet cell differentiation [83]. Foxa3 and *Agr2* are both downstream targets of SPDEF [22], and *Spdef*-null mice lack Foxa3 expression in the lung [82]. Foxa3 also regulates *Agr2* and SPDEF expression in primary human airway epithelial cells; however, SPDEF is not required for Foxa3-mediated goblet cell metaplasia, indicating that Foxa3 functions both in conjunction with SPDEF as well as independently of SPDEF in regulating goblet cell differentiation in the lung [81].

Wnt/ $\beta$ -catenin is another important signaling pathway involved in the regulation of goblet cell differentiation. Disruption of canonical Wnt signaling in the intestine results in reduction of goblet, enteroendocrine and Paneth cells [28, 84, 85]. *Spdef* was identified as a downstream target of Wnt signaling in a genetic screen of *Tcf4*-deficient mice [86] (Figure 2A). In the lung, activated  $\beta$ -catenin inhibits expression of Foxa2, promoting

goblet cell metaplasia [80] (Figure 3A). Murine conjunctival goblet cells express the secreted Wnt inhibitor *Frzb*, suggesting that the Wnt signaling pathway may play a role in conjunctival goblet cell differentiation [23].

Transforming growth factor  $\beta$  (TGF $\beta$ ) has an established role in various biological processes, including epithelial cell proliferation and differentiation, inflammation and immune response [87-91]. It was recently reported that TGF $\beta$  signaling is required for normal restriction of goblet cell differentiation in the conjunctiva by negatively regulating *Spdef* in a Smad3-dependent manner [24] (Figure 4A). Elements of the TGF $\beta$  signaling pathway were not identified in gene expression analysis comparing wild-type and *Klf4* deficient conjunctival fornix [5], suggesting that TGF $\beta$  functions independently of KLF4 in regulating goblet cell differentiation. Loss of either Smad3 [92] or Smad4 [93], the common mediator Smad, results in gastric and duodenal mucinous adenocarcinoma, characterized by an abundance of goblet cells, suggesting a global role for TGF $\beta$  in restriction of normal goblet cell differentiation. Furthermore, TGF $\beta$  has an established role in immune response, which is implicated in goblet cell metaplasia in the lung, although a cell-intrinsic role for TGF $\beta$  signaling has not been identified in this context.

We have summarized much of the work examining genetic regulation of goblet cell differentiation in the intestine (Figure 2), the lung (Figure 3) and the conjunctiva (Figure 4). A complete understanding of the factors required in normal goblet cell differentiation will facilitate our understanding of goblet cell dysfunction in a variety of mucosal diseases. However, an additional layer of complexity surrounding goblet cell differentiation and function exists, as we must consider interactions between mucosal epithelia and the immune response.

## **Goblet cells are integral players in innate and adaptive immunity**

Mucosal epithelial cells are active players in both innate and adaptive immunity and serve as the first line of defense against external insults such as pathogens, toxins and allergens. The mucus layer secreted by goblet cells is essential for clearing these external pathogens and allergens from the underlying epithelium and, as such, prevents many potential threats. However, the mucus layer is not simply a static barrier to entry; goblet cells modulate their secretions in response to external stimuli. The most well characterized example is in the intestinal epithelium, where gut microflora and the mucus layer interact dynamically [6, 94]. The intestines of germ-free mice contain fewer goblet cells than normal mice, and maintain a thinner mucosal layer, whereas cholera toxin and listeria toxin, among other infectious agents, rapidly induce mucin production by goblet cells, highlighting the ability of goblet cells to respond to the needs of their mucosal environment [6, 94]. Upregulation of goblet cells and mucus production is a critical response to harmful stimuli in mucosal epithelium, and is accomplished in response to a wide variety of pro-inflammatory cytokines [6]. Furthermore, a novel role for *Muc2* has been discovered as a direct signaling intermediate controlling dendritic cell immunoregulation [94, 95]. Several studies have recently proposed a new role for goblet cells as antigen-presenting cells in addition to their secretory function. Goblet cells in the small intestine can present luminal antigens to underlying dendritic cells via goblet cell-associated antigen passages in response to the luminal environment [96, 97]. In this way, goblet cells perform a unique and integral role in allowing dendritic cells within the underlying epithelium to sense environmental insults without a break in barrier integrity [96], and have emerged as major players in intestinal immunology.

Goblet cells have long been known to play a role in pulmonary immunology. Goblet cells are normally scarce in the respiratory epithelium, secreting a small, baseline amount of mucus crucial for homeostasis, but are robustly increased in response to allergens and inflammation. While transient increases in goblet cell numbers or mucus production are normal physiological responses to infection or allergy, chronic elevation of mucus production and goblet cell metaplasia are classical features of many severe pulmonary diseases [38, 41]. Many experimental models have linked genetic pathways with inflammatory signaling in respiratory goblet cell metaplasia. Pro-inflammatory cytokines, such as IL13, are upregulated in T helper 2 (Th2) and innate immune lymphoid cells in response to inhaled allergens or irritants, which in turn affect respiratory gene expression [98]. SPDEF and Foxa3, which are both critical factors in normal goblet cell differentiation, are induced by viral infections, pro-inflammatory cytokines and STAT6 [22, 81, 82, 99] (Figure 3A), and can, in turn, promote Th2 immune responses. Whether respiratory goblet cells have antigen-presenting functions, as recently discovered in the intestine, remains an open avenue of investigation.

As in the intestine and the lung, conjunctival goblet cells are exquisitely sensitive to external and inflammatory stimuli. Conjunctival goblet cells and intraepithelial lymphocyte populations participate in a dynamic relationship by which inflammatory cytokines, such as TGF $\beta$  [87], leukotrienes [12] and IL13 [100, 101], regulate goblet cell numbers and function in steady-state and pathogenic conditions. Recently, as in the intestine, a new role for goblet cells as antigen-presenting cells was reported in which goblet cells act as an immunomodulator of dendritic cells within the conjunctival stroma

[102]. Goblet cell-derived soluble factors, including TGF $\beta$ 2, maintain dendritic cells in an immature state and modulate local immune response [102].

Inflammation and autoimmunity are major drivers of mucosal disease and, thus, a more complete understanding of the complex interactions between genetic and environmental factors is essential for therapeutic improvements.

### **Goblet cell alteration and dysfunction in mucosal-associated diseases**

Mucosal-associated diseases are a major healthcare concern and affect a significant number of the population. In many cases, incomplete understanding of the mechanisms involved hinder treatment options.

Changes in the mucus index are markers of several intestinal diseases, such as infection, inflammatory bowel disease and cancer [6] (Table 1). Loss or dysfunction of intestinal goblet cells is implicated in inflammatory bowel disease, such as ulcerative colitis and Crohn's disease, and intestinal biopsies from patients indicate similar degrees of inflammation as measured by inflammatory cytokines [103]. However, inflammation stimulates goblet cell differentiation in Crohn's disease via Hath1 (the human homolog of Math1 or Atoh1) and KLF4, whereas there is a defect in induction of these genes resulting in defective goblet cell maturation and an overall decrease in goblet cell numbers in ulcerative colitis [103]. Therefore, the mucus layer is thinned in patients with ulcerative colitis and thickened in patients with Crohn's disease [35]. In ulcerative colitis, defects in the mucous layer enable bacteria to come into contact with the epithelium [14].

Cystic fibrosis is a severe disease that affects all mucous-producing organs (Table 1) and is caused by a mutation in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) channel, rendering it non-functional [14, 104, 105]. The CFTR channel is responsible for secreting bicarbonate, which is thought to be required for the pH-dependent unfolding of both membrane-bound and secreted mucins and proper formation of the mucous gel in the intestine [33, 105]. Experimental models of cystic fibrosis reveal intestinal goblet cell hyperplasia and that the mucus layer in the intestine is attached to the epithelium and thus cannot be moved along the gastrointestinal tract by peristalsis [14, 105]. However, a direct relationship between goblet cell differentiation and cystic fibrosis is still under investigation, as CFTR is normally expressed apically on neighboring epithelial cells in the gastrointestinal and respiratory epithelia, not goblet cells [105], suggesting that the mucus phenotype in cystic fibrosis is an effect on the secreted products of goblet cells rather than in goblet cell production or differentiation. Airway mucus obstruction is the leading cause of morbidity and mortality in patients with cystic fibrosis [105]. Mouse models of cystic fibrosis result in mucus overproduction as a result of non-infectious inflammation independently of CFTR, although in humans, mutation in CFTR is causative of cystic fibrosis [105]. Intriguingly, while patients with cystic fibrosis present with increased mucous secretion in the intestine and the lung, they frequently suffer from dry eye and a lack of conjunctival goblet cells [106]. As in the intestine and the lung, CFTR is expressed apically on neighboring conjunctival epithelial cells, but not goblet cells themselves [107], suggesting that the mucosal symptoms of cystic fibrosis are not intrinsic to goblet cells.

Goblet cells are robustly induced in response to allergens or inflammation, and are greatly increased in human pulmonary diseases, such as chronic obstructive pulmonary disease and asthma [7, 37] (Table 1). Mouse models of allergen-induced goblet cell metaplasia have been essential to our understanding of the mechanisms regulating goblet cell differentiation in the lung, and have uncovered a complex network by which inflammatory stimuli govern goblet cell-associated genes [22, 44, 81, 82, 108]. Furthermore, expression changes in Notch signaling [109, 110] Foxa2 [78], Foxa3 [81, 82], Agr2 [82] and SPDEF [81, 82] have been reported in bronchial samples from patients suffering from chronic obstructive pulmonary disease, asthma or cystic fibrosis (Table 1). While transient increases in mucin production or goblet cell numbers is essential for clearing allergens and infections, chronic goblet cell metaplasia remains a pressing healthcare concern.

Abnormalities in goblet cell function and/or reduction in the number of conjunctival goblet cells negatively impact ocular health and vision by increasing corneal permeability, causing inflammation, changing tear volume and composition, and causing molecular alterations in mucin expression, are associated with atopic keratoconjunctivitis, keratoconjunctivitis sicca ocular cicatricial pemphigoid, Sjögren's syndrome, and other drying diseases [5, 23, 48, 52, 111] (Table 1). Sjögren's syndrome is an autoimmune disorder which presents with ocular and oral dryness, with a concomitant loss of conjunctival goblet cells [23, 52, 112]. Mechanisms regulating goblet cell differentiation in the conjunctiva are not well understood, thereby limiting therapeutic options for ocular surface disorders, such as dry eye syndrome, largely to analgesic measures, such as artificial tears [112]. SPDEF is known to be expressed in

healthy human conjunctival goblet cells, and both *Muc5ac* and *Spdef* mRNA are substantially decreased in samples obtained from patients with Sjögren's syndrome [23, 111]. Members of the Notch signaling pathway, including ligands and receptors, are significantly downregulated in conjunctival samples obtained from patients with dry eye syndrome [48]. Allergic conjunctivitis is characterized by an influx of mast cells, neutrophils and T cells that release histamine, leukotrienes, proteases and cytokines, which stimulate conjunctival goblet cells to produce increased Muc5AC as a protective mechanism to clear allergens from the ocular surface [12, 52]. Treatment of cells or patients with anti-inflammatory or anti-allergy agents is capable of returning Muc5AC levels and the number of conjunctival goblet cells to normal [12, 52].

Taken together, our understanding of a complete picture of goblet cell differentiation on a global scale will improve treatment options for mucosal-associated diseases affecting the gastrointestinal tract, the respiratory epithelium and the ocular surface.

### **Concluding remarks**

Mucosal epithelia form a vital barrier against external assaults which is essential for the maintenance of mucosal homeostasis and health. This protective function is in part accomplished by goblet cells, which are specialized secretory cells that also help lubricate mucosal epithelia. Genetic regulation of goblet cell differentiation differs contextually and is altered in many mucosal-associated diseases. A more complete understanding of the network of genes responsible for goblet cell formation and function, and how these genes interact with the immune response, is essential for

designing new therapeutic targets for these diseases, many of which have limited treatment options. Furthermore, goblet cells are poorly studied outside their secretory role, and their association with a wide variety of diseases suggests that they may have unexplored functions. Recently, a role for goblet cells in immune surveillance [97] has opened the possibility of goblet cells as a new target in the treatment of inflammatory bowel disease and food allergy. Many important and fundamental questions remain (Box 2), and addressing these questions will help in elucidating the full spectrum of functions of this specialized cell type and in designing new therapeutic strategies for mucosal-associated diseases.

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## Figure Legends

**Figure 1. Goblet cells are present in the intestine, lung and eye.** Goblet cells (blue) are localized in the villi of the small intestine and in the colonic surface. In the airway epithelium, a sparse scattering of goblet cells is found throughout the larger conducting airways. In the eye, goblet cells are found in the palpebral conjunctival epithelium, and are concentrated at the fornix. Detailed depictions of the mucosal epithelial architecture of each boxed area in the top panel are shown below each tissue type. Note that not all anatomical structures or stromal cell types are depicted.

**Figure 2. Gene regulatory networks governing goblet cell differentiation in the intestine.** (A) Schematic diagram of key genes identified in intestinal goblet cell regulation, including SPDEF (yellow), which responds downstream of both Notch and Wnt signaling. (B) *Spdef* null mice display a reduction in mature, differentiated goblet cells in the intestine, as shown by PAS staining. Reproduced and modified with the permission of Dr. Hans Clevers and *Gastroenterology* [20]. (C) Overexpression of SPDEF in the intestine using *Fabp-Cre* results in an expansion of Muc2-expressing goblet cells at the expense of other intestinal cell types [21]. Reproduced and modified with the permission of Dr. Noah Shroyer and *Experimental Cell Research*.

**Figure 3. Gene regulatory networks governing goblet cell differentiation in the lung.** (A) Schematic diagram of key genes identified in airway goblet cell regulation, including SPDEF (yellow), which responds downstream of IL-13, Notch and Wnt signaling and is central in cell fate determination within the lung. (B) Expression of SPDEF in club (Clara) cells results in expansion of Alcian blue-positive goblet cells in

the airway epithelium [22]. Reproduced and modified with the permission of Dr. Jeffrey Whitsett and the *Journal of Clinical Investigation*.

**Figure 4. Gene regulatory networks governing goblet cell differentiation in the conjunctiva.** (A) Schematic diagram of key genes identified in conjunctival goblet cell regulation, including SPDEF (yellow), which responds downstream of both Notch and TGF $\beta$  signaling. (B) *Spdef* null mice display a complete loss of goblet cells in the conjunctiva, as shown by PAS staining [23]. Reproduced and modified with the permission of Dr. Ilene Gipson and the *American Journal of Pathology*. (C) Expression of SPDEF in keratin 14-positive cells, including conjunctival epithelial cells, results in increased goblet cell density in the fornix, as shown by Muc5AC staining [24]. Reproduced and modified with the permission of *Development*.

## Glossary

**Apocrine:** mechanism of exocytosis in which secreted products bud off in plasma-membrane bound vesicles and are shed into the lumen.

**Conjunctiva:** the mucosal epithelium lining the inner surface of the eyelid; **forniceal** refers to the folded region near the limbus and **palpebral** refers to the flat region between the fornix and the eyelid epithelium.

**Dendritic cells:** Antigen presenting cells important in innate and adaptive immunity.

**Differentiation:** the process by which a multipotent stem or progenitor cell becomes restricted in function to become a more specialized cell type.

**Exocytosis:** mechanism of releasing secreted contents of a cell into the extracellular environment.

**Homeostasis:** process by which the cellular environment is kept stable and constant in response to fluctuating external conditions.

**Luminal:** the inner space of a cylindrical tube, such as the intestine.

**Metaplasia:** abnormal conversion of one mature differentiated cell type into a new differentiated cell type in response to stimuli.

### **Box 1: SPDEF is a central player in goblet cell differentiation**

SPDEF is required for proper goblet cell differentiation, maturation and function in mucosal epithelia, making this transcription factor a focal point for studies of normal and abnormal goblet cell differentiation. Loss of *Spdef* results in impairment of goblet cell maturation in the intestine [20], failure of goblet cell induction in response to allergen in the lung [82], and complete loss of goblet cell differentiation in the conjunctiva [23]. Conversely, expression of SPDEF results in an expansion of goblet cells at the expense of other cell types in the intestine [21], the lung [22] and the conjunctiva [24]. It is clear that SPDEF plays an integral role in the differentiation and maturation of goblet cells; however, upstream regulators of *Spdef* vary substantially in a tissue-dependent manner. SPDEF functions downstream of many common genetic pathways, including the Notch pathway, Wnt/ $\beta$ -catenin/Tcf4 signaling and TGF $\beta$  signaling (Figures 1A, 2A, 3A), and is tightly regulated to maintain mucosal homeostasis.

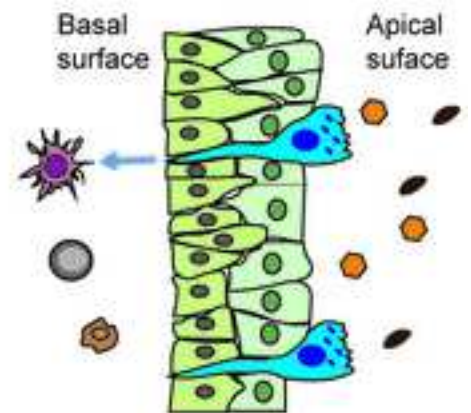
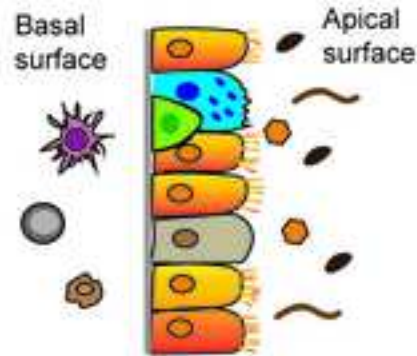
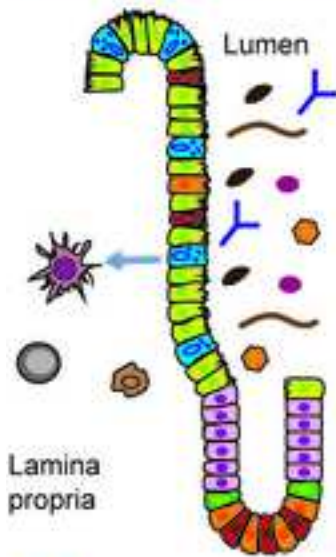
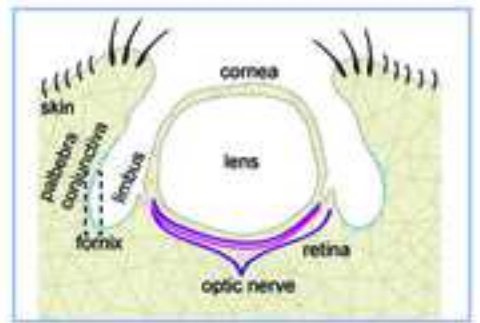
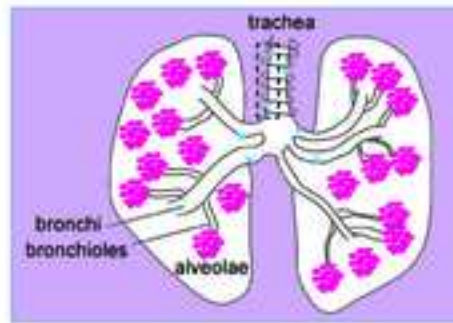
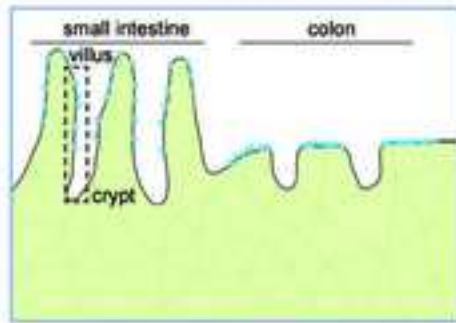
### **Box 2: Outstanding questions**

- What is the full spectrum of functions performed by goblet cells?
- Do goblet cells perform different functions according to their anatomical location?
- Are goblet cells a homogeneous population of cells, or are there subtypes of goblet cells?
- Do signaling pathways regulating goblet cell differentiation vary depending on goblet cell function (secretory versus immune surveillance)?

**Table 1: Goblet cell function in mucosal epithelia homeostasis and disease**

	Intestine	Lung	Eye
<b>Function</b>	Lubrication	Lubrication	Lubrication
	Provide a bacteria-free environment	Contribute to the mucus layer	Component of tears
	Maintain a barrier between the sterile mucosal epithelium and the intestinal microbiota	Protect the respiratory epithelium from inhaled particles and pathogens	Maintain mucosal barrier integrity
<b>Associated diseases</b>	<p>Ulcerative colitis – reduced number of goblet cells and thinner colonic mucus layer</p> <p>Crohn’s disease – enhanced goblet cell differentiation due to inflammation and thicker colonic mucus layer</p> <p>Cystic fibrosis - increased number of goblet cells and thick mucus secretion</p>	<p>Asthma – increased number of goblet cells</p> <p>Chronic obstructive pulmonary disease – increased number of goblet cells</p> <p>Cystic fibrosis – increased number of goblet cells and thick mucus secretion</p>	<p>Sjogren’s syndrome – autoimmune disease resulting in loss of goblet cells</p> <p>Keratoconjunctivitis sicca (dry eye) – loss of goblet cells</p> <p>Allergic conjunctivitis – increased numbers of goblet cells</p> <p>Inverted mucoepidermoid papilloma – increased number of goblet cells</p>

McCauley et al., Figure 1



- Enterocyte absorptive cell
- Enteroendocrine cell
- Tuft cell
- Goblet cell
- Progenitor cell
- +4 stem cell
- Paneth cell
- Crypt base columnar cell

- Ciliated cell
- Goblet cell
- Basal cell
- Clara cell

- Basal cell
- Suprabasal cell
- Goblet cell

- Lymphocyte
- Dendritic cell
- Macrophage
- Pathogens, toxins
- Allergens
- Bacteria
- Inflammatory cytokines
- Immunoglobulin

- Lymphocyte
- Dendritic cell
- Macrophage
- Pathogens, toxins
- Allergens
- Inflammatory cytokines

- Lymphocyte
- Dendritic cell
- Macrophage
- Allergens
- Inflammatory cytokines

McCauley et al., Figure 2

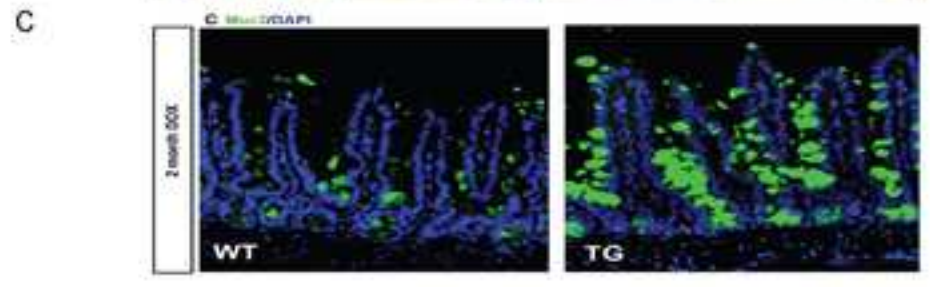
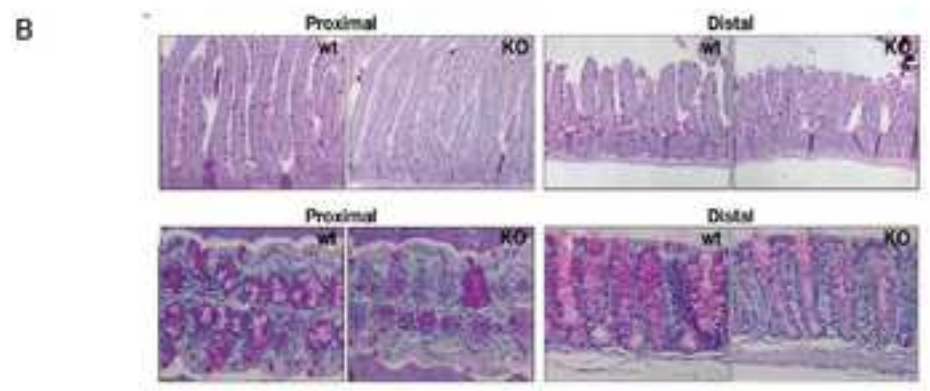
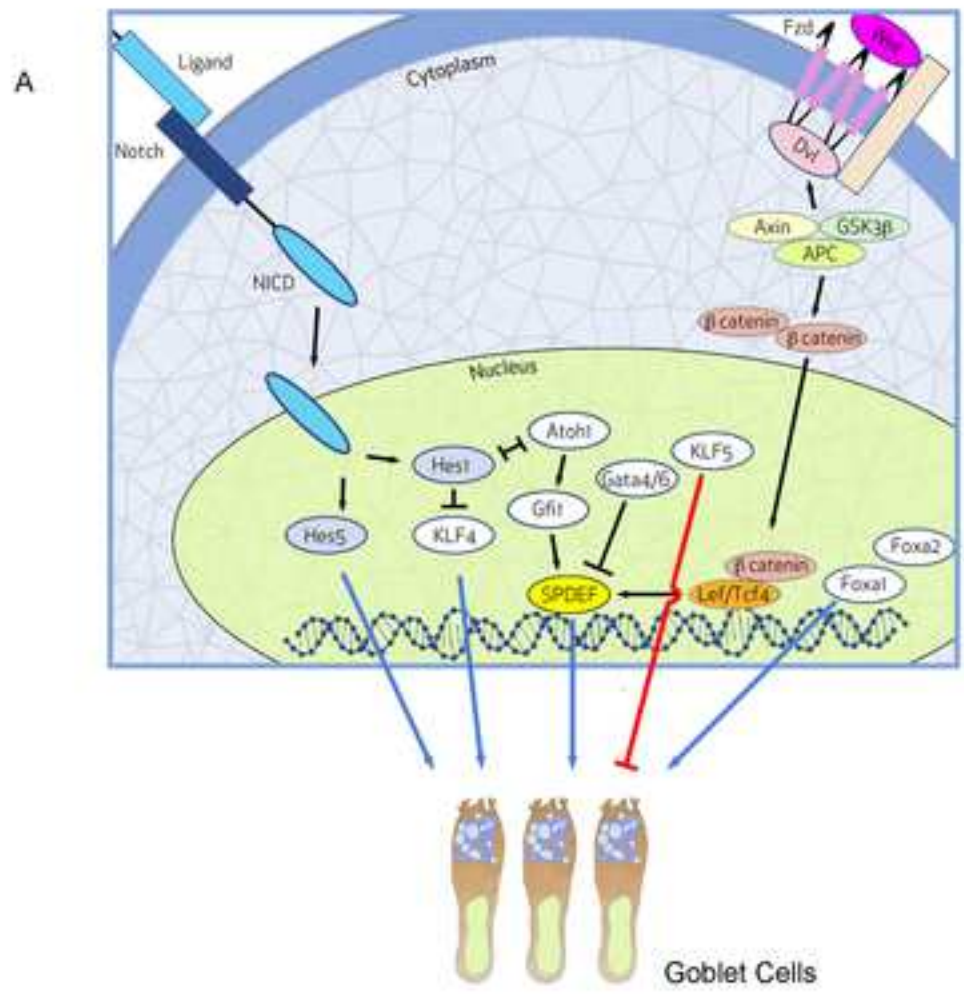
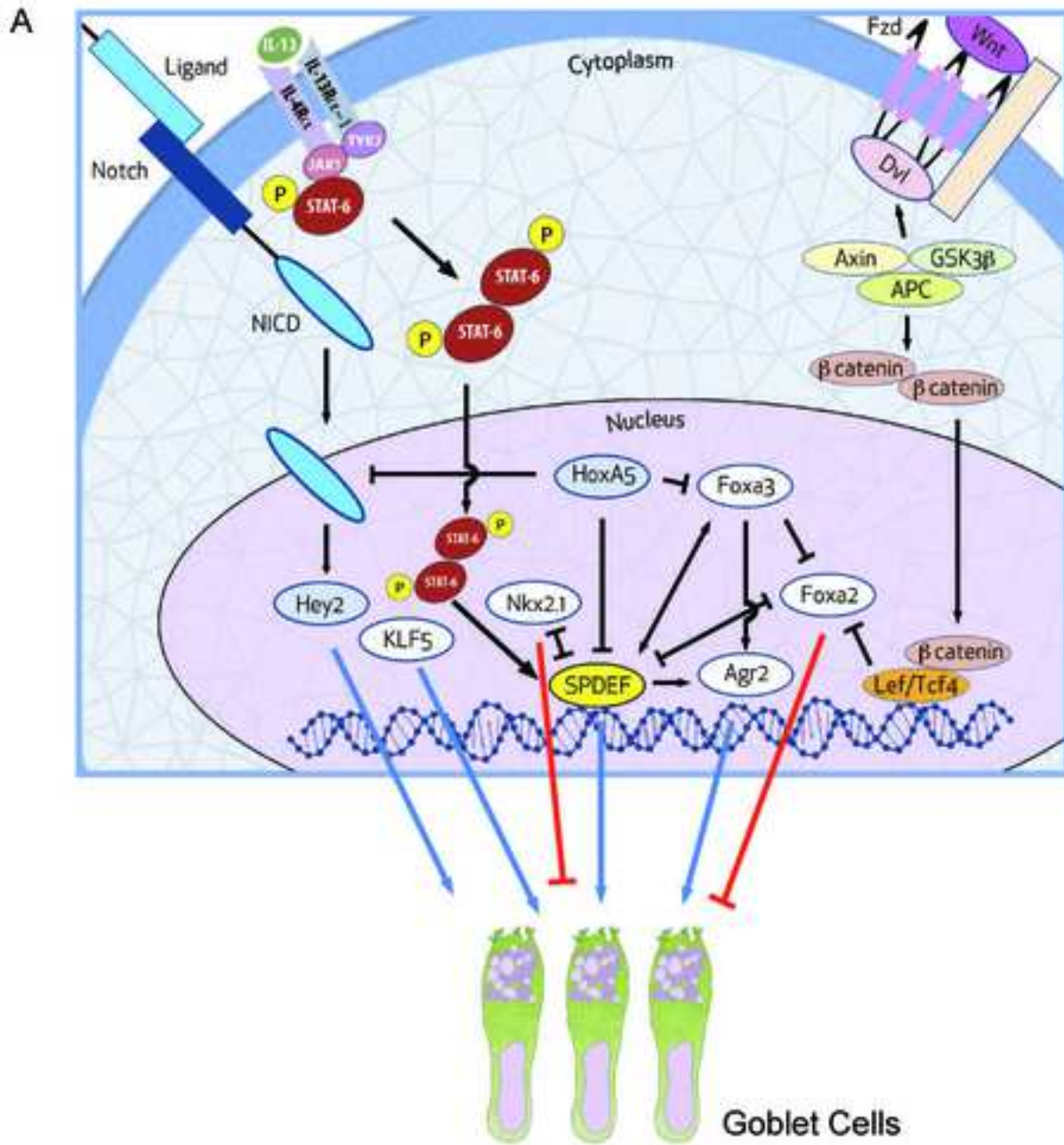


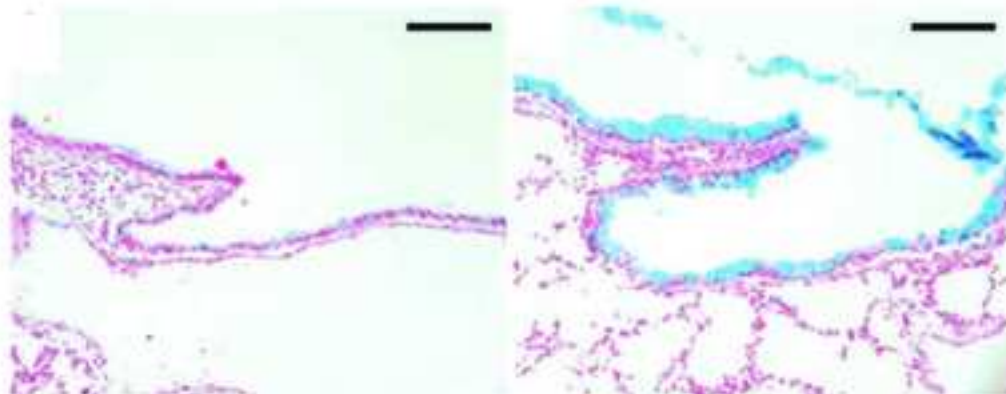
Figure 3

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McCauley et al., Figure 3



**B**



McCauley et al., Figure 4

