A 'tad' of hope in the fight against airway disease

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Abstract

Xenopus tadpoles have emerged as a powerful *in vivo* model system to study mucociliary epithelia such as those found in the human airways. The tadpole skin has mucin-secreting cells, motile multi-ciliated cells, ionocytes (control local ionic homeostasis) and basal stem cells. This cellular architecture is very similar to the large airways of the human lungs and represents an easily accessible and experimentally tractable model system to explore the molecular details of mucociliary epithelia. Each of the cell types in the tadpole skin has a human equivalent and a conserved network of genes and signalling pathways for their differentiation has been discovered. Great insight into the function of each of the cell types has been achieved using the *Xenopus* model and this has enhanced our understanding of airway disease. This simple model has already had a profound impact on the field but, as molecular technologies (e.g. gene editing and live imaging) continue to develop apace, its use for understanding individual cell types and their interactions will likely increase. For example, its small size and genetic tractability make it an ideal model for live imaging of a mucociliary surface especially during environmental challenges such as infection. Further potential exists for the mimicking of human genetic mutations that directly cause airway disease and for the pre-screening of drugs against novel therapeutic targets.

Introduction

As we breathe, we inhale particles, toxins and pathogens with the potential to compromise health. The mucociliary epithelium in the airways of the lung provides a robust first line of defence. Mucus provides a physical barrier to entry, whilst beating cilia transport the mucus (and trapped entities) away from the lungs in a process called mucociliary clearance. Breakdown of mucociliary clearance is a feature of airway diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) and asthma[1]. A comprehensive understanding of mucociliary biology and clearance mechanisms is needed to develop more effective therapeutics for these diseases. Mammalian model organisms are commonly used to study the lung mucociliary epithelium because of their anatomical similarity to humans. However, *in vivo* studies are challenging because the airways are not readily accessible and require invasive procedures that remove them from their native environment. Here, I introduce a complementary, easily accessible, non-mammalian model system to study mucociliary biology – the skin of the *Xenopus* tadpole[2]. Rather than an exhaustive account

of the literature, this minireview aims to equip the reader with an overall understanding of the similarities between the tadpole skin and the human airways. The common cell types are discussed, with emphasis placed on the key findings in the tadpole that have proven to be directly relevant to human airways and disease. The review concludes with a look to the future, highlighting where the tadpole model can be of most use in the quest to understand mucociliary biology and for the treatment of airway disease.

Xenopus embryos have been used for many years to research developmental processes because they are produced externally in large numbers, rapidly develop and can be genetically manipulated using simple tools[3]. Two species of *Xenopus*, *X. laevis* and *X. tropicalis*, are used in biomedical research, often interchangeably, since their developmental features and pathways are conserved. By the time the embryo has become a free-swimming tadpole, its skin is a mucociliary epithelium with motile multi-ciliated cells (MCCs), mucin secreting cells and ionocytes, the latter regulating the local ionic environment[4]. These cell types all reside above a population of basal stem cells [5]. A basal lamina containing fibronectin and laminin supports the epithelium [6,7]. This architecture is remarkably similar to the epithelium in the large airways of the human lung, where mucin secreting goblet cells, MCCs and ionocytes form a pseudostratified epithelium with basally residing stem cells[8,9]. Moreover, these cell types are highly similar to their human airway counterparts in development, function and gene expression. A comparison of the large airways and the tadpole skin epithelia is given in Figure 1.

The *Xenopus* model has some unique advantages over other animal and human cell culture models to study mucociliary epithelia. As it is a developing system, the embryonic skin can be used to investigate the dynamic interaction and movement of the different cell types and also how they are initially patterned. The tadpole develops fast – several hours - compared to weeks for human tracheobronchial epithelial cultures. Furthermore, gaining access to the skin mucociliary epithelium is very simple compared to the cumbersome air-liquid interface that needs to be generated for human cultures. In addition, using the embryonic form of *Xenopus* instead of mature animal models (e.g. mice) has an animal welfare benefit. *Xenopus* produce thousands of embryos from a single fertilization, whereas many adult mammals must be sacrificed for similar experiments. Easy access to the skin of *Xenopus* tadpoles provides unique imaging opportunities, whilst rearing the embryos is straightforward and inexpensive, requiring only simple salt solutions.

Motile, multi-ciliated cells (MCCs)

The ciliated cells are the most striking feature of the tadpole skin (Fig. 2A), as noted over half a century ago in seminal electron microscopy studies[10,11]. Many cilia project out from each MCC and beat co-ordinately to generate directional fluid flow, just like in the human airways. Although a large number of studies have been conducted into the structure and function of MCCs, here the focus is on a few selected studies that best demonstrate the similarities between the tadpole and human airway MCCs, but also show how the tadpole model has provided new insight into cilia biology and human disease.

MCC differentiation: An evolutionarily conserved gene regulatory network

As ciliated cells differentiate, cilia are generated in a process called multi-ciliogenesis. The transcription factor, FOXJ1, was one of the earliest identified markers of MCCs in the human airways[12] and a knockout mouse model showed that this transcription factor is essential for ciliogenesis[13]. FoxJ1 was subsequently identified in MCCs in the *Xenopus* tadpole skin and knockdown of FOXJ1 function mimicked the defects observed in mammalian ciliogenesis[14]. This study also identified the genetic targets of FoxJ1 for the first time, showing that the model can be used, not just to confirm, but to extend, our understanding of this cell type. The targets of FoxJ1 were mostly genes encoding the protein machinery required for ciliogenesis and cilia motility, but the study also found that FoxJ1 was insufficient to generate multiple cilia[14]. Further research led to the identification, in *Xenopus*, of Multicilin, which is required for centriole amplification to generate the multiple basal bodies that each act as a template for formation of a single cilium[15]. The transcription factor, Myb, was found to be downstream of Multicilin in both *Xenopus* and mice and is integral to the process of centriole amplification[16]. A protein related to Multicilin, GemC1, was later identified as the key factor at the top of the gene regulatory network simultaneously controlling the pathways of centriole biogenesis, via Multicilin, and ciliogenesis, via FoxJ1[17]. Many of these factors, originally identified in *Xenopus*, were subsequently found to be defective in human disease. For example, mutations in *MCIDAS* (the gene encoding Multicilin) and cyclin O (CCNO – needed for centriole amplification), a downstream target of Myb, led to a reduced number of cilia and defective mucociliary clearance[18,19]. Indeed, in the case of CCNO, knockdown of the gene in the tadpole skin showed a reduction of cilia in the MCCs, supporting the clinical phenotype as well as demonstrating the translational potential of the model.

The polarization of cilia

Polarity is needed both within an individual MCC (rotational polarity), so that the cilia all move in the same direction, and across the plane of an epithelium (tissue-level polarity), to ensure fluid flow. Research using the tadpole model has led the field in understanding how planar cell polarity (PCP) proteins direct this process. For example, the core PCP protein, Dishevelled (Dvl), localizes to one side of the basal body, which has downstream effects on the organisation of actin and microtubules [20,21]. This aligns all the basal bodies in the cell in one direction. When Dvl was inhibited, the basal bodies became randomly orientated[20]. Tissue-level polarity was also shown to involve core PCP proteins, first in *Xenopus*, and then in mouse airways[22,23]. The core PCP proteins, Vangl2 and Frizzled, appear to direct this tissue-level polarity, given that depletion of these proteins in cells adjacent to MCCs affects cilia orientation within MCCs [22]. In the study of MCCs in mouse trachea, asymmetric distribution (i.e. to opposite membranes) of membrane-bound PCP proteins directs the polarity of cilia across the tissue through specific microtubule interactions extending from one pole of the cell and connecting with basal bodies[23]. The importance of PCP is exemplified in some chronic human inflammatory airway diseases, where core PCP proteins are mislocalized, overproduced or absent [24–26]. Moreover, some genetic diseases cause defects in cilia polarity that impair mucociliary clearance. For example, the gene, growth-arrest specific protein-2-like-2 (GAS2L2), has been found to

be mutated in some individuals and knockdown of this gene in *Xenopus* tadpole skin led to polarity defects in ciliated cells [27].

Ciliary motility

Axonemal dynein motor proteins form outer and inner dynein arms and generate the force to slide adjacent microtubules with respect to each other, effecting the movement of cilia[28]. Indeed, motile ciliopathies such as primary ciliary dyskinesias (PCD), which cause defective mucociliary clearance in the human airways, are frequently caused by mutations in genes that encode axonemal dyneins[29]. Genes such as DNAH5 (commonly mutated in PCD) were identified as downstream targets of FoxJ1 and associated transcription factors in *Xenopus* MCCs[14,30]. A recent study in *Xenopus* described a liquid-like organelle (dynein axonemal particles or DynAPs) containing axonemal dyneins, dynein axonemal assembly factors (DNAAFs) and chaperone proteins[31]. Significantly, depletion of individual DNAAFs that have been implicated in PCD[32], caused disruption of the whole DynAP complex and dynein arms were not localized to the cilia, resulting in immotile cilia[31]. The authors hypothesised that the DynAPs exist to facilitate fast assembly and movement of dyneins to ciliary axonemes. Meanwhile, human mutations in another DNAAF, ZMYND10, causes PCD due to loss of axonemal dynein arms[33]. The identification of DynAPs provides a plausible explanation for this phenomenon. As for many of the studies highlighted, the ZMYND10 study used the tadpole model to further understand the clinical phenotype – immotile cilia - of mutations in this protein.

Secretory cells

The tadpole skin has two types of secretory cells that structurally resemble the human airway mucin-secreting goblet cells (Fig. 2B). Indeed, the first secretory cells to be identified in the tadpole skin were named goblet cells by analogy to the human cell type[34]. However, the other secretory cell type – the small secretory cells (SSCs) – actually bear more resemblance to human goblet cells, with large, apically-localized electron-dense vesicles[35]. Irrespective of their names, both cell types secrete the same gel-forming mucin, MucXS, with properties reminiscent of the human airway mucins, MUC5AC and MUC5B [36,37]. These large, polymeric heavily O-glycosylated proteins underpin the characteristic viscoelastic properties of mucus[38]. Mucin polymers form through disulphide bonding between specific domains (VWF and cysteine knot domains) and have central (mucin) domains rich in threonine and serine residues that become O-glycosylated[39]. In both the human airways and the tadpole skin, mucins are condensed inside vesicles until secretion, when they decondense and rapidly expand in size to provide the structural framework of the protective mucus barrier [40]. Just as the airway mucins provide protection against infection in the airways[41], knock down of MucXS in the tadpole skin led to a loss of the mucus barrier and increased susceptibility to infection[36].

It remains unclear why the tadpole skin requires two mucin-secreting cell types, but in fact airway mucins can also be secreted by other cell types in addition to goblet cells[42]. Very little else is known about the tadpole skin goblet cells, other than that they secrete a lectin called Intelectin-2[43]. Intelectin-2 is secreted by human intestinal goblet cells and has

also been identified in the airways[44]. In the tadpole, Intelectin-2 is believed to bind and agglutinate bacteria[43].

The SSCs appear to be most similar to the human goblet cells and this extends to their key markers. The SSCs express the forkhead box A transcription factor, FoxA1, and knockdown of FoxA1 led to their depletion[35] which caused an increased susceptibility to infection, similar to when MucXS was depleted[36]. The related transcription factor, FOXA3, promotes gene expression in human and mouse airway goblet cells[45]. FoxA1 is also an important transcription factor driving the gene expression of Muc2 in mouse intestinal goblet cells[46].

As demonstrated for the ciliated cells, as more becomes known about the tadpole skin secretory cells, this has the potential to enhance our understanding of human airway goblet cells. This includes aspects such as the gene regulatory network that stimulates their differentiation, mucin synthesis and secretion and the factors involved in regulating these processes. Ultimately, this can facilitate the development of new therapeutics since mucin hypersecretion and goblet cell hyperplasia are common features of airway diseases such as asthma, COPD and CF[47].

Ionocytes

Ionocytes were originally identified in the developing *Xenopus* embryonic skin, appearing shortly after the ciliated cells[4,48]. These cells are rich in mitochondria and often have apical microvilli (Fig. 2C). The genes identified as markers of this cell type include ion channels, proton pumps and enzymes such as carbonic anhydrases[4]. Two subtypes of ionocytes have been identified in the tadpole skin[48]. The v-ATPase proton pump is expressed in both, but shows different subcellular localization[4]. In some cells, v-ATPase localizes apically, whilst in others it localizes basolaterally. Apical localization of v-ATPase corresponds to expression of the anion exchanger, AE1, whilst basolateral expression of v-ATPase corresponds with the expression of the chloride-bicarbonate exchanger, Pendrin, and the transmembrane enzyme carbonic anhydrase 12[4,48]. It is thought that the *Xenopus* tadpole, which is found in freshwater environments, requires v-ATPase to pump out protons, in order to take in sodium ions (against the concentration gradient), by generating a local electrochemical gradient [49]. The transcription factor Foxi1 was found to be an early marker of the tadpole ionocytes[48]. Knockdown of Foxi1 led to loss of ionocytes, whilst its overexpression led to increased numbers of ionocytes[4]. Depletion of ionocytes also had a corresponding non-cell autonomous effect on neighbouring MCCs, which still developed, but had fewer cilia and numerous basal bodies were found within the cytoplasm rather than docked to the apical membrane[4].

It is known that regulation of ionic balance is needed in the human airways in order to maintain hydration of the mucus. In CF, mutations in CFTR leads to defective chloride/bicarbonate transport and airway surface dehydration[50]. This results in thick and sticky mucus that cannot be cleared effectively by cilia[51]. Until recently, CFTR was believed to be predominantly expressed in ciliated cells, with other ion channels found in secretory cells[52]. However, two independent single cell RNAseq studies of mouse and human airways revealed the additional presence of pulmonary ionocytes, named after those

identified in the tadpole skin[8,9]. Like the tadpole ionocytes, the pulmonary ionocytes express genes for v-ATPase and the transcription factor, Foxi1. Knockout of Foxi1 led to loss of pulmonary ionocytes, leading to increased viscosity of airway mucus (like in CF) [9]. Intriguingly, more than 50% of the CFTR transcripts in the airway cells were found clustered with the pulmonary ionocytes, indicating that this cell type could be a crucial target for treatment of CF airway disease[9]. Whilst it is unclear if the tadpole skin ionocytes express CFTR and no subtypes of ionocytes have yet been identified in the human airways, it seems certain that this cell type will receive more attention in the coming years. Greater understanding of the tadpole ionocytes could provide insight of importance to human airway physiology and ultimately to the treatment of disease.

Basal stem cells

The tadpole skin is a bilayered epithelium (Fig. 1B). Precursors of MCCs, ionocytes and SSCs originally reside in the inner layer and then intercalate into the outer layer to fully differentiate alongside the goblet cells[4,35,53]. However, the inner layer of the epithelium remains and contains undifferentiated basal cells. These cells strongly express the transcription factor tumour-related protein 63 (P63; Fig. 2D), which is also expressed in human airway basal stem cells[5,54]. P63 is essential for the maintenance and survival of airway basal cells[55]. Knockout of P63 in mouse airways resulted in a highly ciliated airway with loss of basal cells[55], whilst P63 overexpression in the tadpole led to a depletion in the number of MCCs, SSCs and ionocytes[56]. Injury models in the mouse airways have shown that the basal cells can become both secretory cells and ciliated cells, and they can also self-renew to maintain the basal cell population[57]. The pulmonary ionocytes were also shown to derive from the basal stem cells[9]. Interestingly, isolated human airway basal stem cells can differentiate into a mucociliary epithelium at an air-liquid interface, demonstrating that the basal cells are *bona fide* stem cells[58]. It is unclear if the tadpole basal cells are also capable of converting into the other cell types, but a recent study of isolated basal cells (which are likely the P63$^+$ stem cells) shows that they are capable of regenerating a mucociliary epithelium [59]. A similar transcriptional profile between airway basal cells and the tadpole skin basal cells has been described, indicating that they have a conserved function[56]. Airway basal cells are altered in disease and are a target for gene therapy. For example, in COPD, basal cells are depleted[60], whilst a proof-of-principle study showed correction of CFTR mutations in basal cells isolated from CF patients[61]. Understanding the tadpole basal cells in greater detail could facilitate further development of therapeutics to generate lasting changes in airway basal stem cells to treat disease.

Conserved signalling pathways in the development and repair of mucociliary epithelia: Notch, Wnt and BMP signalling

The key signalling pathways required for the differentiation of the tadpole skin and the human airway mucociliary epithelium are also conserved[62]. Notch signalling controls the balance of secretory and ciliated cell fate in the development of the airways and the tadpole skin[53,63,64]. In the tadpole skin, SSCs increase in response to Notch activation[64], whilst ciliated cells and ionocytes decrease[48,53]. Moreover, Notch activation in the developing

mouse airways causes an increase in secretory cells at the expense of ciliated cells[65], whilst pulmonary ionocytes have also been shown to express downstream targets of Notch[8]. It is not clear whether Notch signalling is required for the initial formation of basal stem cells in the developing airways, but in the tadpole skin, Notch activation increases the numbers of P63-positive basal cells[6]. However, after development, adult airway basal stem cells do require Notch signalling to differentiate into secretory or ciliated cells in the steady-state and upon injury[66].

A side-by-side comparison of the developing tadpole skin and regenerating human airway epithelial cells showed similar responses to BMP activation and inhibition[67]. BMP inhibition is required for the specification of ciliated cells, ionocytes and SSCs in the tadpole skin and for differentiation of goblet cells and ciliated cells in human airway epithelia. It is hypothesised that, in *Xenopus*, attenuation of BMP signalling is needed prior to downstream Notch signalling to stimulate differentiation of the different cell types. Addition of exogenous BMP4 ligand to *Xenopus* embryos led to a reduction in ciliated cells, ionocytes and SSCs, but also to P63-positive basal cells[67]. In mouse tracheal basal cells, addition of BMP4 inhibited the proliferation and differentiation of P63-positive cells[68]. Meanwhile, *in vivo* injury to the mouse trachea led to upregulation of BMP antagonists indicating that attenuation of BMP signalling is required for proliferation and subsequent regeneration of the tracheal epithelium[68]. Defects in BMP signalling have been described in mild asthma and in COPD induced by cigarette smoking[69,70].

Canonical Wnt signalling, via activation of b-catenin-induced gene expression, is also important in the development of both the airways and tadpole skin. Wnt-β catenin has been implicated in expression of FoxJ1 and ciliogenesis in ciliated cells of the tadpole skin[71]. The same study also showed that Wnt signalling regulates expression of FoxA1 in SSCs and inhibition leads to loss of secretory vesicles. Meanwhile, activating β-catenin in mouse airways led to goblet cell hyperplasia with enhanced expression of the mucin, Muc5ac[72]. Meanwhile, P63 is directly regulated by β-catenin at its promoter in both mammalian cells and *Xenopus*[73,74]. Indeed, Wnt/ β-catenin has recently been shown to upregulate P63 in basal cells of the tadpole and isolated human airway epithelial cells, preventing differentiation to other cell types[56]. b-catenin knockdown led to an increase in ciliated cells at the expense of basal cells, whilst chronic Wnt activation caused basal cell hyperplasia[56]. As for the other signalling pathways, several airway diseases have been associated with defective Wnt signalling. For example, asthmatic airway remodelling has been shown to stem from aberrant activation of Wnt/ β-catenin signalling[75], which may also drive cigarette-induced COPD[76]. Since the major signalling pathways in the tadpole skin and the airways are conserved, it may be possible to test out drug targets of these pathways to treat airway disease using the tadpole model before moving into mammalian models and ultimately for the treatment of human patients.

Conclusions and Future outlook

The research findings indicate that the tadpole skin is similar in composition and function to the mucociliary epithelium of the large airways in humans. Its small size, genetic tractability and accessibility makes it a powerful *in vivo* model to understand this epithelium in health

and disease. This has been most clearly demonstrated for the ciliated cells, where many studies have used *Xenopus* to understand the function of genes or signalling pathways; findings which are mostly conserved in human airway ciliated cells. The other cell types in the tadpole skin also have equivalents in the human airways, but these are at an earlier stage of research. As we look to the future, understanding more about the role of ionocytes in the tadpole skin will provide great insight into the function of these cells in the human airways, including their role in diseases such as CF. Likewise, the SSCs appear to be highly similar to human airway goblet cells so a thorough understanding of their function is warranted. As single cell technologies continue to develop rapidly, the specific functions of genes in each cell type will need to be understood. The ability to quickly identify, knockdown and overexpress genes in the *Xenopus* model make it an ideal candidate for this task.

New genome-editing technologies such as CRISPR-Cas9 have proven to be successful in *Xenopus*[77] and open up a pathway towards mimicking human genetic mutations in airway diseases, as well as endogenous tagging of target genes with fluorophores. The fact that the tadpole is small and its skin is accessible means that live *in vivo* imaging is feasible, unlike for mammalian airways. Fluorescently labelling mucins and cilia would enable study of processes such as mucin packaging into secretory granules and their subsequent secretion, and cilia interactions with mucus during clearance. The ability to manipulate the local environment of the tadpole together with the capability to generate large amounts of material lends this system to rapid high-throughput drug screening programmes to initially identify novel drugs affecting mucin secretion or cilia beating. In addition, the introduction of pathogens to the media can enable studies of host-pathogen interactions at a mucociliary surface. In fact, the tadpole model has already been employed to screen muco-active agents and study the impact of particulate matter from diesel engines[78,79]. Other areas where the tadpole model could be used in future include studies of drug delivery across mucus barriers, which hinders the bioavailability of drugs and testing of gene therapy approaches following mimicking of human genetic mutations[38].

I envisage that this model will be increasingly used alongside and, in some cases, as a replacement for, other models of mucociliary epithelia. It is ideally suited as a tool to understand the function of particular genes, to mimic genetic mutations and for the pre-screening of drugs. It could be used for hypothesis generation and testing, with key findings validated in mammalian models. Furthermore, as the tadpole skin is an accessible larval model it does not require some of the invasive techniques required for mammalian *in vivo* studies of the airways, making it an ideal candidate for the reduction, refinement and replacement of animals in research.

Perspectives

- The *Xenopus* tadpole has a mucociliary skin surface with cell types similar to the human airways; including motile multiciliated cells, mucin-secreting cells, ionocytes and basal stem cells. The *Xenopus* model is ideally suited to researching mucociliary epithelia and their role in airway disease.

- Each cell type has conserved gene regulatory networks and functions, as well as signalling pathways that control their differentiation. The *Xenopus* model has been used to further our understanding of these aspects as well as to understand the clinical phenotypes of airway disease, particularly for ciliated cell dysfunction.

- The unique combination of conserved cell types, genetic tractability and ease of accessibility makes the tadpole skin model ideally suited for live imaging of mucociliary epithelia. By employing the latest genome editing technologies to mimic disease and label proteins, in combination with environmental challenge, there is great potential to employ the tadpole model in future, perhaps even in place of some more established mammalian models.

Abbreviations

CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
COPD	chronic obstructive pulmonary disease
MCC	multi-ciliated cell
PCP	planar cell polarity
Dvl	Dishevelled
PCD	primary ciliary dyskinesia
DNAAF	dynein axonemal assembly factor
DynAP	dynein axonemal particle
SSC	small secretory cell
BMP	bone morphogenetic protein

Acknowledgements

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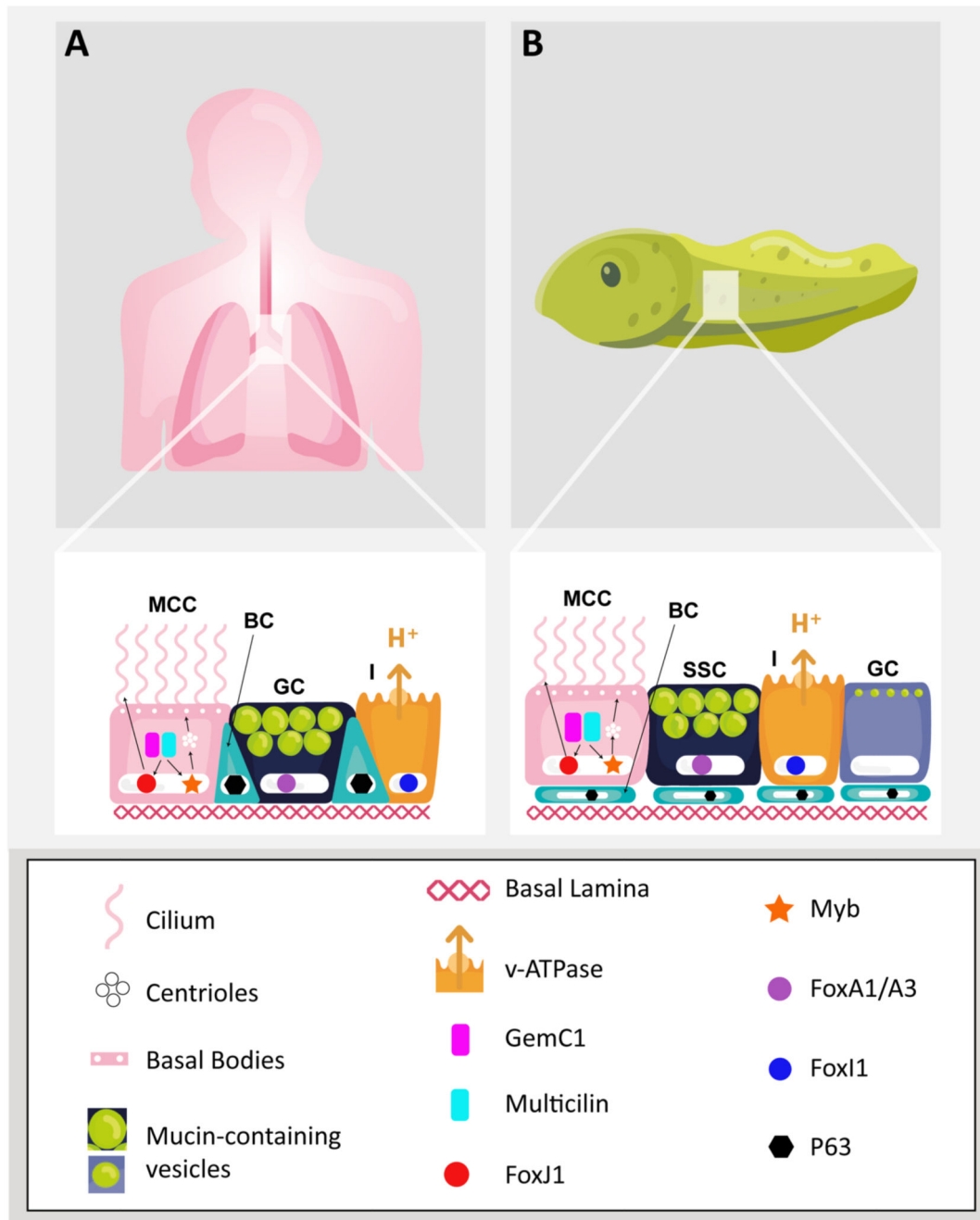
Figure 1. Comparison of human large airway epithelium and tadpole skin

(A) A mucociliary epithelium extends from the human nasal cavity to the trachea and bronchioles. Details of the tracheobronchial epithelial cell types are shown. It is a pseudostratified epithelium with basal stem cells (BC; turquoise) residing slightly below and in between multiciliated cells (MCC; pink), mucin-secreting goblet cells (GC; dark blue) and ionocytes (I; orange). A fibronectin-rich basal lamina is also shown (B) The tadpole skin is a mucociliary epithelium with multiciliated cells (MCC; pink), small secretory cells (SSC; dark blue), goblet cells (GC; purple) and ionocytes (I; orange), residing above basal stem

cells (BC; turquoise) and a fibronectin-rich basal lamina. Key features that are conserved in the human epithelium and the tadpole skin are highlighted. For the ciliated cells, the conserved gene regulatory network is shown and arrows indicate how GemC1/Multicilin drive centriole biogenesis and basal body formation through Myb, whilst ciliogenesis is driven through FoxJ1.
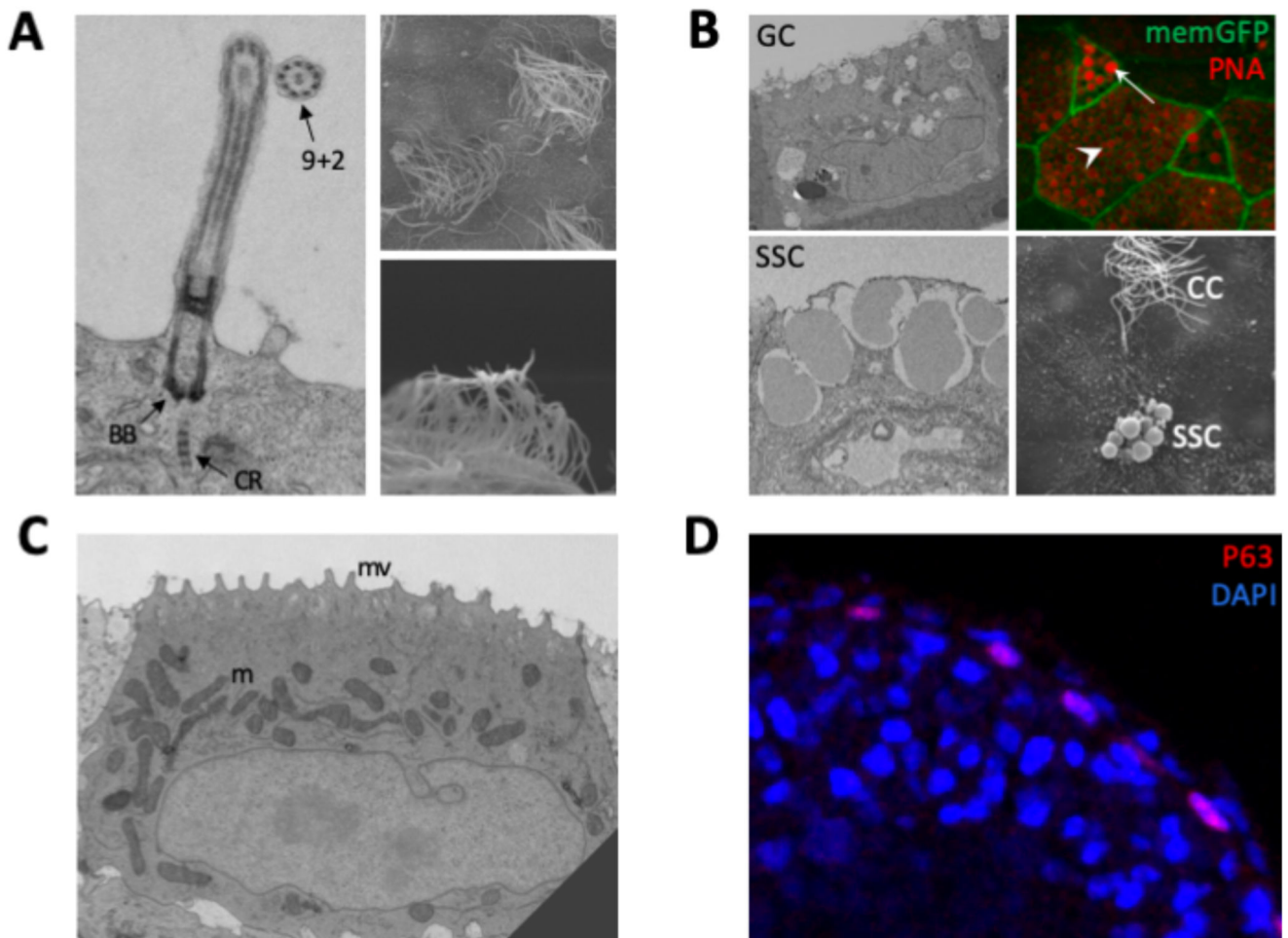
Figure 2. The tadpole skin cell types

(A) Multi-ciliated cells. Transmission electron micrograph (left) shows a single cilium with key features of ciliary rootlet (CR), Basal Body (BB) and '9+2' arrangement of microtubules highlighted. Scanning electron micrographs (right) show multiple cilia. (B) Secretory cells. Goblet cells (GC) and small secretory cells (SSC) shown in transmission electron micrographs of skin sections. Apical vesicles containing mucins are evident. Immunofluorescence shows peanut agglutinin (PNA) binding to mucins in SSCs (arrow) and goblet cells (arrowhead). Membrane-GFP marks the plasma membrane. Scanning electron micrograph of tadpole skin shows an SSC near to a ciliated cell (CC). (C) Transmission electron micrograph of an ionocyte, which is mitochondria-rich (m) and has apical microvilli (mv). (D) Immunofluorescence of a section of tadpole skin showing P63 (a marker of stem cells) staining. DAPI marks the nuclei. P63 positive basal cells are found in the inner layer of the skin. Images in (B) and (C) reproduced from Dubaissi et al (2011), Fig. 3[4]