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Review

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Contribution of mechanical forces in multiciliated cell differentiation

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Multiciliated cells, through the coordinated beating of motile cilia, are vital for moving fluids in the brain and mucus in the airways and in the reproductive organs. Their dysfunction leads to diseases, associated with chronic airway infections, subfertility and ventricular enlargement. A group of transcription factors and a cell cycle variant enable the differentiation of multiciliated cells. However, how the multiciliated differentiation programme is initiated, timed and spatially regulated within developing tissues remains unclear. In this review, we combine findings from airway, brain ventricles and *Xenopus* epidermal systems to reveal how mechanical cues may interact with transcriptional networks and cell cycle regulators during multiciliogenesis. We suggest a model where mechanical forces serve as contextual regulators that enable, time and control the execution of the multiciliated differentiation programme.

1. Introduction

Cilia are microtubule-based structures surrounded by a plasma membrane with a specific composition [1]. At their bases are basal bodies, derived from centrioles, which are docked to the plasma membrane. Several types of cilia exist: primary cilia, antennae-like structures that relay many chemico- or mechanopathways owing to the enrichment of receptors along their membranes, and motile cilia, which participate in fluid, mucus and unicellular organism movement, as well as in sensory signalling [2].

Most primary cilia are unique, immotile and composed of nine microtubule doublets. Most motile cilia are multiple, composed of nine microtubule doublets with an additional central pair, and contain microtubule motors and accessory proteins to ensure ciliary beating. Exceptions to these rules include, among others, the multiple non-motile cilia of the olfactory sensory neurons [3], though they are motile in amphibians and some fishes [4,5], or the single motile cilia of the node [6].

Motile cilia, likely present in primitive cells [7], are only found on some specific epithelial cells of specialized organs in mammals [8]. They are located in the brain (along ventricles, the spinal cord's central canal and the choroid plexuses), the upper (nasal passages, paranasal sinuses) and lower (bronchi, trachea) respiratory tracts [9] and genital organs [10] (fallopian tubes, uterus, endometrium and efferent ducts). They are essential for physiological processes in these tissues, including airway clearance, cerebrospinal fluid (CSF) circulation and reproductive function. During development, they are also observed on the node to establish the left–right asymmetry [11], on the oesophagus [12] and kidney [13,14], though their functions in these organs remain unclear. Impairment of cilia motility leads to primary ciliary dyskinesia (a disease associated with chronic airway infection, subfertility, laterality defects and ventricular enlargement) and to an increase in age-related dementias [8].

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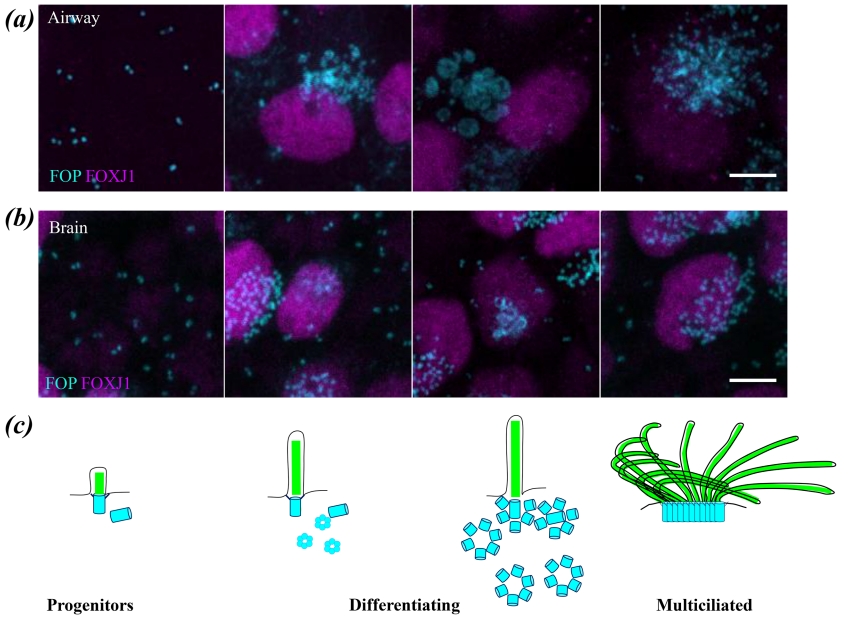


Figure 1. The different steps of centriole amplification. Cells were stained for a centriolar marker (cyan, FOP) and the transcription factor FOXJ1 (magenta) during the multiciliated cell differentiation process. (*a*) Human tracheal epithelial cells, 7 days after differentiation onset (Air Liquid, ALI7). (*b*) Whole mount of brain ventricles at post-natal day 5. (*c*) Drawing representing the centrioles/procentrioles (cyan), the cilia (green) and part of the plasma membrane (black) during cell differentiation. Scale bar, 5 µm.

Several model systems, in addition to mammalian multiciliated cells, are used to study the development and functions of these cells. These include the *Xenopus* (epidermis, ventricles, foregut, olfactory pit, pronephros and otic vesicle) and the zebrafish (ventricles, pronephros, olfactory pit and otic vesicle) multiciliated tissue [15], as well as multiciliated organisms, such as *Planaria*, *Paramecium* or *Tetrahymena* [16].

Multiciliated cell differentiation always occurs during intense tissue remodelling, such as airway branching morphogenesis [17], ventricular expansion in the brain [18] or radial intercalation in the *Xenopus* epidermis [19]. These developmental processes generate substantial mechanical forces, including tensile and compressive stresses, hydrostatic pressure and changes in tissue stiffness. Increasing evidence indicates that multiciliated cell progenitors not only are exposed to these forces but also actively respond to them (see below).

Over the past decade, major advances have defined the actors in centriole amplification, and the transcriptional hierarchy and cell cycle variant that enable multiciliated cell differentiation. Yet, a central question remains unresolved: how is the multiciliated differentiation programme initiated, timed and spatially restricted within developing tissues?

In this review, we propose a model in which mechanical forces act as contextual regulators that license, time and scale the execution of the multiciliated differentiation programme. We synthesize recent findings from airway, brain ventricles and *Xenopus* epidermal systems to illustrate how mechanical cues interface with transcriptional networks, cell cycle regulators and chromatin remodelling during multiciliogenesis. By integrating biomechanics with molecular control, this framework offers new perspectives on how multiciliated tissues achieve robust yet adaptable differentiation during development and homeostasis.

2. Centriole amplification

In vertebrates, centriole amplification pathways involved in generating multiciliated cells are mainly conserved. Progenitor cells, marked by tissue-specific markers (e.g. RC2 for radial glial cells in the brain and p63 for basal cells in the airway), possess two centrioles. At the onset of differentiation, pericentriolar material accumulates around these centrioles [20]. Deuterosomes, electron-dense platforms composed of proteins such as DEUP1 [21] and CCDC78 [22], form near the existing centrosome [23]. Procentrioles assemble around these platforms and centrosomal centrioles. They undertake simultaneous growth and are eventually released from the deuterosomes to migrate apically to the plasma membrane, where they anchor and form motile cilia [24] (figure 1). In the *Xenopus* epidermis, two waves of amplification may occur: one beginning in the lower layer of the tissue and finishing during cell intercalation in the upper layer and a second during expansion of the apical surface, after intercalation [25]. The mechanisms driving the amplification of centrioles during this putative second wave have not been described.

Proteins critical for centriole duplication, such as Plk4, Cep152 or Sas6, are involved in this amplification process [24]. However, while centriole duplication produces only two new centrioles, amplification results in dozens of centrioles (e.g. around 50 in ependymal cells and up to 300 in the airway cells). This number appears to be regulated by actin and apical surface size, though the mechanisms for this control remain unclear [26–28]. Notably, removing deuterosomes, parental centrioles or both does not prevent centriole amplification [29–31]. In their absence, centrioles assemble de novo within a cloud of pericentriolar material. The

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cell cycle machinery, which controls centriole duplication, is co-opted in these post-mitotic cells together with specific multiciliated cell regulators to activate the centriole amplification pathway [32,33].

3. Transcription factors and cell cycle actors

To differentiate into multiciliated cells, progenitors must exit their proliferative or quiescent state to commit to multiciliated cell fate. Thus, several pathways involved in this proliferation or quiescence are inhibited prior to differentiation. These include the Notch [34–39], bone morphogenetic protein (BMP) [40,41] and Wnt/β-catenin [42–54] pathways, depending on the tissue and area studied. Inhibition of the Notch [55] or Wnt/β-catenin [53] pathways or exit of the cell cycle [56–58] may depend on miR-34/449, which are expressed in all multiciliated tissues [59]. Accordingly, depletion of miR-449 in *Xenopus* epidermis or human airway ex vivo cells led to a decrease in the number of cells with centriole amplification [55]. However, mice lacking both miR-34b/c and miR-449a/b/c clusters still develop multiciliated cells, though with an abnormal number of cilia in the airways and reproductive tracts [53,57,60,61]. This suggests that these miRNAs may also regulate later steps of differentiation, such as centriole migration, through actin regulation or cilia elongation via CP110 (a centriolar cap protein restraining cilia formation) regulation [60,62]. In the brain, knockout mice exhibit microcephaly and disrupted development of some brain structures, but no clear alteration in multiciliation [60,63,64]. Thus, either the knockout mice have compensatory mechanisms that allow the fate transition in the absence of these miRNAs, or the function of the miRNA depends on the cellular context [55]. Notably, another transcription factor (transcription factor EB, TFEB) may activate the Notch pathway during brain development and be inactivated at differentiation onset by mammalian target of rapamycin (mTOR)-dependent phosphorylation [65], suggesting another possible regulation for the initiation of multiciliated cell differentiation. The regulation of miR-34/449 expression in multiciliated cells remains unclear. In other tissues, their expression depends on genotoxic stress, leading to p53 activation [66], or on changes in E2F1 expression [58,67]. However, p53 removal has little effect on miR-34a/b expression in the lung, brain and testis [68], and E2F1 levels do not correlate with miR-449a levels [69]. TAp73, an isoform of p73, a p53-related factor, induces miR-34b/c but inhibits miR-449 in some contexts [70,71]. Actually, TAp73 and miR449 cooperate to support multiciliogenesis in the brain [71], a regulation that may be important for centriole migration or cilia elongation. During spermatogenesis, cyclic adenosine monophosphate (cAMP)-responsive element modulator and SOX5 regulate miR-34/449 [56].

NOTCH inhibits the transcriptional co-factors GEMC1 (also known as Geminin coiled-coil domain-containing protein 1 (GMNC) and LYNKEAS) and MCIDAS (multiciliate differentiation and DNA synthesis associated cell cycle protein, also known as IDAS, MCI and MULTICILIN) [72–74], both of which are essential [72–78] and sufficient [72,74,79] for multiciliation, when expressed in multiciliated cell precursors. One study proposes that Notch signalling biases airway progenitors towards secretory versus multiciliated fate through opposing transcriptional regulators, such as HEY1 (hairy/enhancer-of-split related with YRPW motif protein 1) and MYCL (MYC proto-oncogene lung carcinoma-derived), which bind near genes encoding early regulators of multiciliated cell differentiation. The conservation of these factors in multiciliated cells of different origins remains to be fully established [80]. Chromatin remodellers participate in both GEMC1 and MCIDAS functions [81,82]. GEMC1 acts upstream of MCIDAS and triggers its expression [72,73,75,78]. GEMC1 and MCIDAS induce most of the genes important for multiciliation, including other transcription factors such as FOXJ1 [83], FOXN4 [84], RFX2 and RFX3 [85–87], p73 [70,71,88] and MYB (myeloblastosis) [89,90]. Depending on the system, GEMC1 is the main regulator of multiciliated cell specification, while MCIDAS is required for centriole amplification and ciliogenesis. Notably, in the MCIDAS mouse mutant trachea, multiciliated cells are specified but fail to assemble centrioles and cilia [91]. However, depending on the organisms, MCIDAS may also be dispensable or required for multiciliated cell specification [74,76,92].

In parallel with the expression of these transcription factors, a variant of the cell cycle is initiated [32,33,93], allowing centriole formation without DNA replication or cell division. Most cyclins, with the exception of Cyclin E2 and A2 replaced by Cyclin O and A1, respectively, Cyclin-dependent kinase (CDKs) and the anaphase-promoting complex/cyclosome (APC/C) are reused in a unique cell cycle iteration during differentiation. Cyclin O expression is controlled by MYB downstream of MCIDAS [89,91]. Loss of Cyclin O in the mouse brain and in human patients leads to a marked decrease in centriole number, although residual centrioles can still support motile cilia formation [94,95]. By contrast, partial multiciliation is observed in the mouse airway, suggesting a tissue/organism-specific requirement. The targets of Cyclin O and its CDK partner are unknown, although CDK13, a transcription-associated CDK, has been proposed in some cancer cell contexts [96].

Compared to the canonical cell cycle, the multiciliated cell cycle variant is characterized by expression of E2F7, which blocks DNA replication [32]. In cycling cells, E2F7 activation is regulated by G1/S transition factors (E2F1/RB1) and by DNA damage, and conversely [97]. Interestingly, DNA damage is observed during multiciliated cell differentiation [98,99], though whether this contributes to the high levels of E2F7 expression is unknown. Increasing DNA damage, and thus likely the DNA damage response, impairs multiciliated cell differentiation in the airway [98]. Yet, blocking the DNA damage response kinases also impairs multiciliated cell differentiation without inducing massive DNA damage in progenitor cells or affecting downstream transcription factors, such as FOXJ1 expression [98]. These results suggest that the DNA damage response kinase might not be required as a DNA damage responder. Still, how DNA damage and DNA damage response kinases inhibit and favour the differentiation, respectively, remains to be elucidated.

The multiciliated cell cycle variant also exhibits low levels of EMI1, thereby limiting Cyclin B1/CDK1 activity and preventing cell division [33]. EMI2, another APC/C inhibitor, is expressed at very low levels yet is necessary to ensure proper centriole

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amplification/maturation and the timed degradation of SECURIN to promote centriole disengagement from deuterosomes [100]. EMI1 and EMI2 are regulated by E2F transcription factors in other cell types, notably by E2F4, which is also present during multiciliated cell differentiation [101]. Additional DNA replication and cytokinesis factors are expressed at low levels in this multiciliated cell cycle variant, while the CDK inhibitor CDKN1A is co-expressed with the cell cycle actors, likely contributing to preventing complete cell division.

Links between the multiciliated cell cycle variant and the transcriptional activators of the multiciliated cell differentiation are emerging. GEMC1 and MCIDAS share homology with GMNN (also known as Geminin), which is important for DNA replication and for controlling cell fate choice, at least in the brain [79]. They interact with cell cycle regulators [102–104]. Notably, GEMC1, expressed in *Xenopus*, interacts with G1/S transition factors such as CDC45 and TopBP1 and is a substrate of Cdk2/Cyclin A or E [102]. These interactions were suggested *in vitro* to contribute to DNA replication re-firing, but have never been reinvestigated in the light of the importance of the G1/S transition factors for the initiation of multiciliated cell differentiation and notably for centriole amplification [105]. Interestingly, GEMC1 overexpression leads to multiciliated cells only during S-phase in cycling brain progenitors [79]. Other cell cycle-related factors involved in G1/S transition and differentiation include E2F/DP proteins, which act as co-factors/transcription factors for GEMC1 and MCIDAS, as these proteins lack DNA-binding domains. Both E2F4 and E2F5 are involved, with tissue- or organism-specific requirements [73,75,78,91,106–111]. Notably, fibroblasts, which do not normally assemble motile cilia, become competent when expressing both MCIDAS and E2F4 [112]. During G0 and G1 phases of the cell cycle, E2F proteins interact and are sequestered by proteins of the retinoblastoma family (RB1, RBL1 (also known as p107) and RBL2 (also known as p130)), inhibiting cell cycle progression. Sequential phosphorylations by Cdk4/6 and Cdk2 release E2F from retinoblastoma (RB) proteins [113]. Actually, RB1 is phosphorylated at the onset of brain multiciliated differentiation [99,114], and RBL2 or a non-phosphorylable RB1 represses MCIDAS transcriptional activity [114,115]. These suggest control of the E2F/MCIDAS interaction by RB phosphorylation and thus a control by the variant cell cycle over MCIDAS activation. Another transcription factor, FANK1, which is important for AP1 complex regulation (a complex involved in cell cycle initiation and apoptosis [116]) is also crucial for multiciliated differentiation in the airway [117]. This factor is observed in RNA sequencing data in differentiating cells in both the brain [33] and the airways [32]. Finally, p73, which is downstream of GEMC1 [118] and a direct regulator of FOXJ1 [88], controls some cell cycle events, including the cell cycle arrest gene p21 and factors of the G2/M transition [119]. An overview of these different pathways is represented in figure 2.

However, while these factors highlight the signalling pathways involved in centriole amplification and ciliogenesis, the mechanisms by which they are coordinated and initiated remain unclear. Moreover, other signalling pathways linked to mechanical forces have recently been implicated in some aspects of the differentiation process.

4. Multiciliated cell differentiation and mechanical forces

A complex interplay between physical forces applied to tissues and chemical signalization regulates development and fate choice [120,121]. While researches have primarily focused on understanding the genetic and biochemical mechanisms that shape life, mechanical forces have recently emerged as critical regulators in these processes [120]. Tissues experience a variety of forces, including tensile or compressive forces, shear stress, hydrostatic or osmotic pressure and changes in stiffness or viscoelasticity. These forces originate externally, such as changes in the stiffness of extracellular matrix (ECM), or internally, such as those generated by actomyosin networks.

To adapt cellular responses to mechanical forces, signalling pathways and transcriptional networks must be coordinated. Forces are detected by cells through specialized proteins, which then propagate the signal to physically linked structures and/or activate signalling molecules to modify cell behaviour or fate [122]. The initial level of force detection occurs at the plasma membrane, mediated by ECM receptors or mechanosensitive channels. Mechanosensitive channels, such as Piezo and TRPV (transient receptor potential vanilloid), open in response to stretch, enabling ion signalling and the activation of downstream signalling cascades [123]. ECM receptors, including integrins and cadherins, perceive changes in the mechanical properties of the matrix and relay this information to the cell interior via the actin cytoskeleton [124,125]. Forces can then be directly transmitted to the nucleus through the cytoskeleton [126]. The linker of nucleoskeleton and cytoskeleton (LINC) complexes, which span the nuclear envelope and link the nucleoskeleton (lamins) to the cytoskeleton (particularly actin), play a pivotal role in this transmission [127]. Following the application of force, nuclei deform, leading to several changes, such as the activation of phospholipase and Ca^{2+} signalling [128,129], the relocalization of Lamin A/C and alterations in its expression levels [130,131], chromatin modifications [132,133] and changes in the distribution and permeability of the nuclear pores. Changes in nuclear pores facilitate the entry of transcription factors such as YAP/WWTR1 (yes-associated protein/WW domain-containing transcription regulator protein 1) (also known as TAZ(transcriptional coActivator with PDZ-binding motif)) [134,135]. All these mechanisms contribute to modifications in gene expression and cell fate. In multiciliated tissues, ECM receptors, Piezo, nuclear deformation and YAP/WWTR1 are involved in various aspects of the differentiation, suggesting that this differentiation process may be mechanosensitive.

4.1. Extracellular matrix receptors

While integrins are the best-characterized ECM receptors, dystroglycan (DAG1 alpha and beta) proteins also form membrane-spanning complexes that interact with multiple ECM components, including laminins, and connect to the cytoskeleton [136,137].

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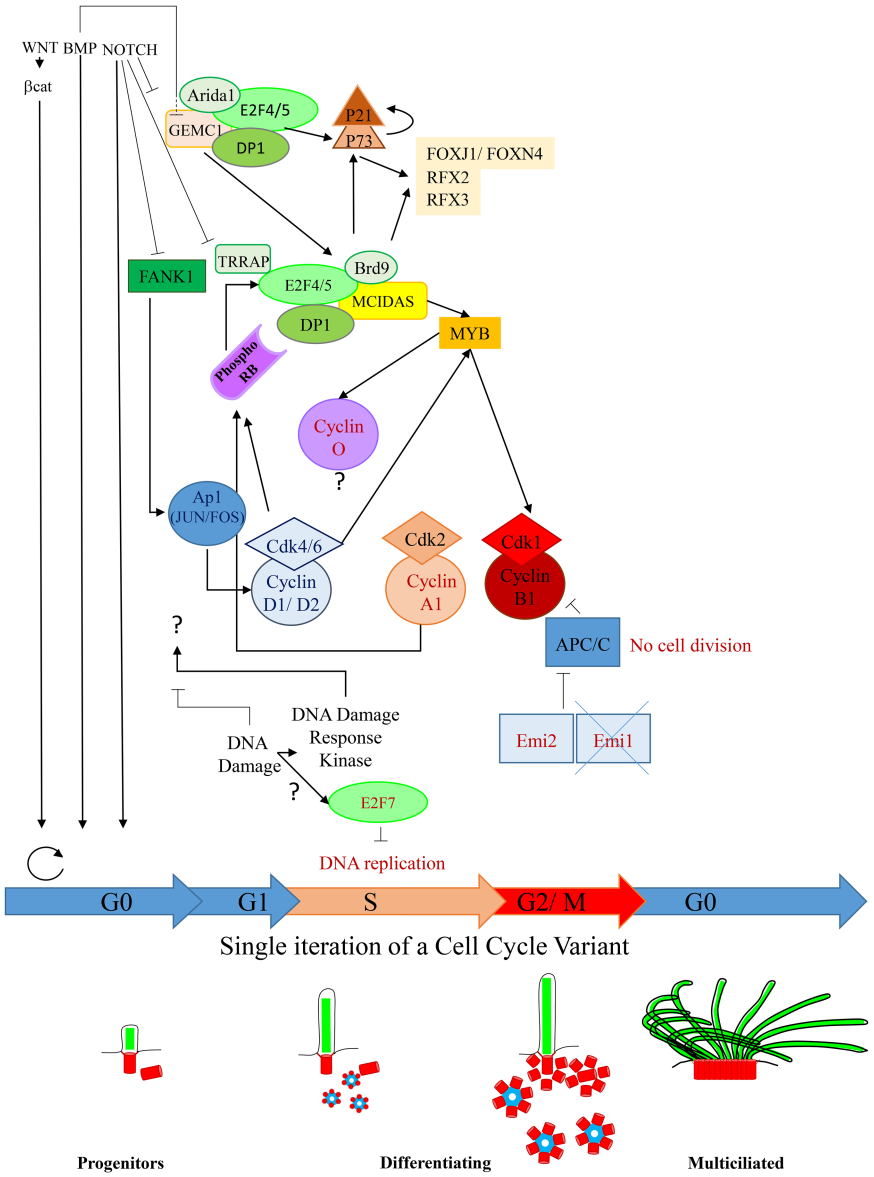


Figure 2. Transcription factors and cell cycle actors involved in multiciliated cell differentiation. Wingless (WNT), BMP or Notch signalling pathways promote either the proliferation or the deep quiescence of progenitor cells, depending on the tissue. Upon inhibition of these pathways, the master regulators of differentiation, GEMC1 followed by MCIDAS, are activated. These regulators interact with the cell cycle proteins E2F4 or E2F5 and DP1 to promote the transcription of other transcription factors important for differentiation including FOXJ1, FOXN4, RFX2, RFX3 and MYB. Components regulating chromatin organization, ARIDA1, BRD9 and TRRAP (transformation/transcription domain-associated protein) are also important. The different steps of differentiation are orchestrated by a variant of the cell cycle involving specialized cyclin (O and A1) as well as canonical cyclins. The targets and CDK partners of Cyclin O are unknown. The multiciliated cell cycle variant differs from the canonical cell cycle because DNA replication is prevented, through E2F7 expression, and cell division does not occur, owing to the absence of EMI1 and the low levels of EMI2, which keep mitotic cyclins low. Proteins shown in red are differentially expressed compared with the canonical cell cycle. Crosstalk between the cell cycle variant and the transcription factors is depicted. The schematic at the bottom depicts the successive steps of centriole amplification and ciliogenesis (red, centrioles and procentrioles; blue, deuterosomes; green, cilia; black, part of the plasma membrane) that is concomitant with the activation of the signalling pathway.

In mammals, DAG1 is expressed in most tissues where multiciliated cells develop, including the brain, the airway, certain regions of the genital tract and embryonic kidney [138,139].

In the *Xenopus* epidermis, DAG1 is also expressed by the cell surrounding the multiciliated cell progenitors. It interacts with the ECM and induces laminin and fibronectin accumulation [140]. Its depletion results in a reduced number of multiciliated cells and even suppresses the expression of p63, a marker of the multiciliated cell progenitors in this tissue. Notch promotes the expression of DAG1 that in turn activates multiciliated cell fate in the neighbouring cells. In the *Xenopus* epidermis, multiciliated cell progenitors intercalate into the upper tissue layer. Differentiation occurs during this intercalation as indicated by the expression of alpha-tubulin, an early marker of differentiation in this system [39]. DAG1 also contributes to this intercalation, potentially through regulation by the transcription factor RFX2 [141].

In the mammalian brain, DAG1 is expressed by multiciliated cell progenitors themselves and during the differentiation [142]. The removal of DAG1 decreases laminin around the radial glial cells, even if this decrease is compensated at later stage. The

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number of multiciliated cells is also decreased. Notch signalling is associated with DAG1 regulation, although it is not so clear whether DAG1 is regulated by Notch or regulates the Notch pathway.

Only a reduction in the number of multiciliated cells has been described; however, other ECM receptors may compensate the phenotypes, as suggested by the change in Laminin organization observed in mouse mutant at late stage of development, to allow the formation of the remaining multiciliated cells. In the mouse brain, DAG1 also contributes to the centriolar patch's polarity [142].

Similarly, Desmoplakin, a component of desmosomes that provides adhesion between cells and with the cytoskeleton, is important for the intercalation of multiciliated cells in the *Xenopus* epidermis [143].

4.2. Piezo/Ca^{2+} signalling

Piezo is a mechanoreceptor present at the plasma membrane in several multiciliated tissues. Piezo1 is expressed in *Xenopus* epidermis multiciliated cells, at the cell border and at the base of the multiple centrioles [25]. Piezo2 is observed in the multiciliated cells of the zebrafish chemosensory organs [144]. In mammals, Piezo1 is also expressed in neuroepithelial cells and human neural stem cells, and controls fate determination at least for some neurons [145,146].

In both the *Xenopus* epidermis and the mouse airway, the number of centrioles correlates with the apical surface area. Increasing tensile forces and apical cell surface through tissue stretching, genetic manipulation [25] or by increasing matrix rigidity [28,147] promote an increase in centriole number. However, the molecular mechanisms linking apical expansion to centriole amplification, and whether this relationship is mediated through activation of the multiciliated differentiation programme, remain unclear. Still, in the *Xenopus* epidermis, Piezo1 is essential for this coupling in a force-dependent manner.

Notably, Piezo1 is permeable to Ca^{2+}, and calcium bursts are critical for actin organization and apical extension [148,149]. The role of the actin cytoskeleton in the control of centriole amplification has not been directly addressed, but it is required for the activation of the differentiation pathway through nuclear deformation during brain multiciliated cell differentiation [114].

4.3. Transduction to the nucleus

Mechanical forces are relayed to the cell interior either through signalling pathways or by transmission to the cell nucleus via the cytoskeleton and the LINC complex. In the mouse brain, knockout of actin regulators (N-WASP or CDC42) or LINC complex components (double Sun1/2 or Syne1/2) leads to hydrocephalus or ventricular enlargement, respectively [150–152], conditions often linked to defects in multiciliated cells. Accordingly, recent findings indicate that the actin cytoskeleton plays a role in nuclear deformation via the LINC complex to trigger ependymal differentiation. We show that this regulation of the differentiation acts upstream of RB1 phosphorylation and MCIDAS activation [114]. The role of nuclear deformation has not been confirmed in other multiciliated tissues, although nuclear deformations are observed during multiciliated cell differentiation in the *Xenopus* epidermis [153]. Furthermore, the mRNA levels of LINC complex components increase during the differentiation of airway multiciliated cells [32,38]. Nuclear deformation drives DNA damage in many systems [154]. Notably, DNA damage occurs during ependymal and airway multiciliated cell differentiation [98,99]. Whether it is a consequence of nuclear deformation is not known, but it has consequences for the differentiation process.

Nuclear deformation has also been shown in other systems to induce chromatin reorganization, notably a change in histone methylation and nuclear fluidization, driven by SWI-SNF (SWItch/sucrose non-fermentable) components [132,155]. This protects the genome against damage and changes gene expression [133,155–157]. Notably, GEMC1 and MCIDAS, in human embryonic kidney cell overexpressing them, interact with components of the SWI-SNF family (ARID1A and BRD9, respectively). At least BRD9 is required for the transcriptional regulation of the differentiation in airway multiciliated culture [81]. Changes in histone methylation and DNA damage are also observed [98,99,114]. Importantly, some histone methylation events regulate the pool of multiciliated cell progenitors in the airway [158,159]. Thus, a plausible model is that nuclear deformation induces DNA damage and changes in chromatin organization to drive differentiation via GEMC1 or MCIDAS. However, further experiments are needed to validate this model.

4.4. YAP/WWTR1

Many phenotypes associated with nuclear deformation depend on the YAP/WWTR1 pathway [160] as these transcription factors enter the nucleus following the application of forces and the opening of nuclear pores [135]. The YAP pathway is involved in cell proliferation and differentiation in several tissues [161] and is linked to the G1/S transition [162,163]. Interestingly, overexpressed GEMC1 and MCIDAS interact with YAP in proximity labelling experiment (BioID) in cells that do not assemble motile cilia physiologically [81].

In the lung, depletion of YAP during cell specification favours the formation of progenitors and goblet cells at the expense of multiciliated cells. At the onset of differentiation, however, the expression of the active nuclear YAP inhibits lung multiciliated cell differentiation [164,165]. Similarly, YAP knockout in airway multiciliated progenitor cells (p63/Krt5+ cells) leads to premature differentiation into multiciliated and goblet cells [166]. These seemingly contradictory observations can be reconciled by a stage-specific model in which YAP promotes progenitor identity under high mechanical tension but must be downregulated for terminal multiciliated differentiation. Similarly, in the brain, YAP is involved in the specification and integrity of ependymal progenitor cells in the aqueduct [167], and together with its paralogue WWTR1, it regulates the proliferative capacity and structural organization of radial glial cells [168]. This suggests that, as in the airway, YAP/WWTR1 plays a role in progenitor specification.

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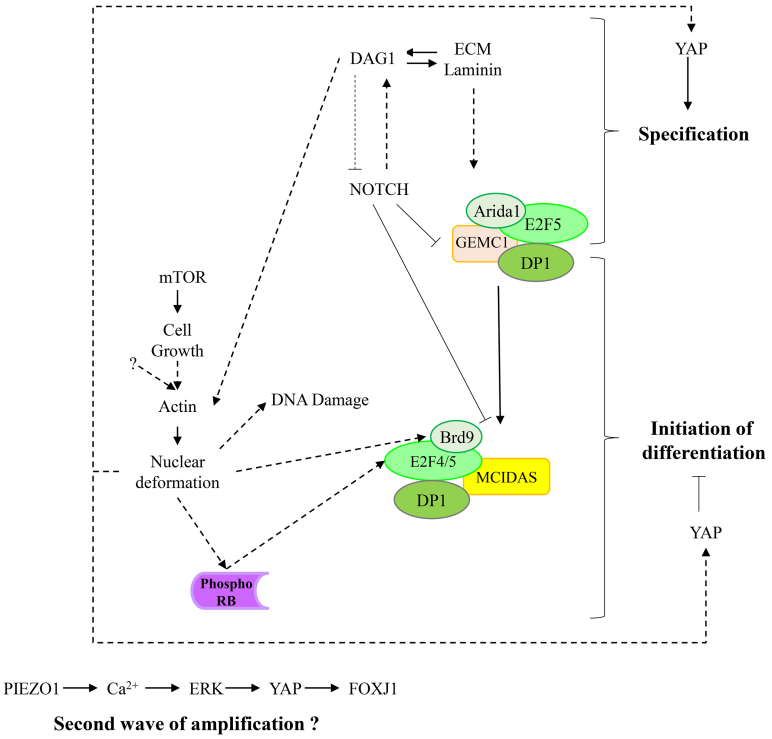


Figure 3. Hypothetical model integrating force-dependent pathways involved in multiciliated cell differentiation in different model organisms. Several factors involved in force detection are known to be important for multiciliated cell differentiation. This includes ECM receptors such as DAG1, in the *Xenopus* epidermis and mouse brain, as well as stretch-dependent receptors such as Piezo in the *Xenopus* epidermis. These signals may be transmitted to the cell interior through the actin cytoskeleton and promote nuclear deformation that is important, at least for multiciliated cell differentiation in the brain. This may be followed by chromatin reorganization involving factors such as Brd9, which are important for differentiation in the mouse airway, or by changes in the activity of the transcription factor YAP, as observed in the airway and brain for progenitor specification and in the airway for the initiation of differentiation.

However, removal of YAP alone during embryonic development (E11.5, Nestin-Cre-mediated removal) does not prevent ependymal differentiation [114]. Whether the depletion of both YAP and WWTR1 affects later brain multiciliated cell differentiation remains unknown. In the *Xenopus* epidermis, YAP may be involved in the control of centriole number following Piezo and calcium signalling [169].

4.5. Notch

The canonical Notch pathway is described as a lateral inhibition mechanism. Notch ligands interact with Notch receptors on juxtaposed cells, promoting Notch cleavage, translocation into the nucleus and regulation of transcription [170]. Notch proteolytic activation depends on a mechanical pulling force exerted by the Notch ligand, either directly through endocytosis of the ligands or indirectly through clustering of the receptors [171–175]. Mechanical forces also indirectly activate Notch through integrins, Piezo or YAP/TAZ [176,177]. In turn, Notch regulates tissue mechanics by altering cell proliferation and influencing ECM expression [176,177]. Whether mechanical forces regulate Notch during the specification of multiciliated cells or conversely is unknown. Given the roles of the YAP/TAZ and Notch pathways in the specification of multiciliated cell progenitors, it would be of interest to test whether YAP regulates the Notch pathway, as observed in other systems [178–183].

Collectively, these results suggest that mechanical forces influence multiple stages of multiciliated cell formation. They may play a role in progenitor specification upstream or at the level of Notch inhibition, as well as in differentiation itself. This includes ensuring the correct developmental set-up for differentiation upstream of cell cycle initiation through actin dynamics, nuclear deformation and YAP/WWTR1 activity, as well as controlling centriole number according to cell size downstream of the first centriolar amplification, via Piezo and calcium-dependent signalling (figure 3). Still, the linearity of the different molecular players described in this section has not been fully investigated making it complex to associate them with the mechanical forces observed during development. This would require investigating the players at precise developmental timing and might be quite challenging. Overall, mechanical forces may act as key integrators across multiple stages of multiciliated cell development. However, the mechanisms at play are far from understood, and further research is required to elucidate the precise molecular and mechanical interactions that govern these processes.

5. Forces applied on progenitors during differentiation

Mechanosignalling during epithelial differentiation results from physical forces acting on tissues. During development, differentiating multiciliated cells are exposed to various physical forces. Below, we review the forces involved in the mammalian airway,

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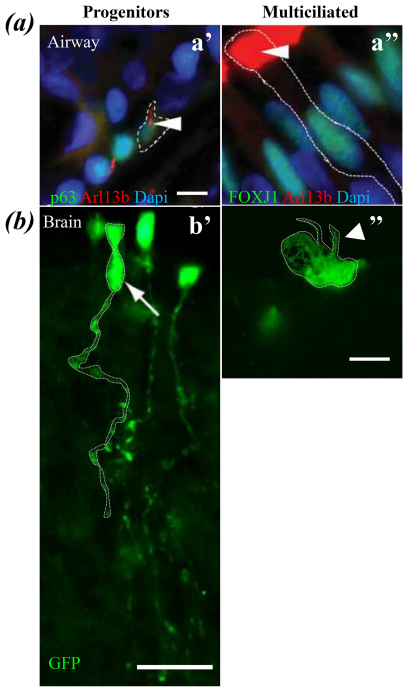


Figure 4. Cellular morphological differences between progenitors and multiciliated cells. (*a*) Human lung tissue formalin-fixed and paraffin-embedded showing progenitor basal cells (e.g. dashed line, a') and multiciliated cells (e.g. dashed line, a") stained for specific transcription factors (green, p63 for basal cells and FOXJ1 for multiciliated cells), cilia (red, Arl13b) and DNA (blue, dapi). Arrowheads indicate cilia (either primary or motile). Scale bar, 5 µm. Adapted from [184]. (*b*) Coronal sections of the lateral wall of enhanced green fluorescent protein (eGFP) (Z/EG) mice infected with Cre adenovirus at birth on the ventrolateral striatum to promote GFP expression in radial glial cells having their long processes in this region. (b') By P2, only radial glial progenitors (e.g. dashed line) are visible on the lateral wall. Cell bodies appear to undergo interkinetic nuclear migration (arrow). (b") By P30, multiciliated cells at the surface of the lateral ventricle are stained throughout their cell bodies, including their cilia (arrowhead). Scale bars, 25 and 5 µm. Adapted from [185]. (Panel (*a*) is reproduced with permission of the ERS 2026 [184]).

Xenopus epidermis and mammalian brain ventricular systems, the three principal model systems used for studying multiciliated differentiation. Figure 4 illustrates the drastic morphological changes observed between progenitor cells and multiciliated cells in the airway and brain ventricles.

5.1. Mammalian airway morphogenesis

The respiratory epithelium originates from the foregut endoderm and undergoes branching morphogenesis through reciprocal signalling between the epithelium and mesenchyme [186]. The lumen of developing airways is filled with fluid, and the pressure exerted by this fluid contributes to airway branching and tubulogenesis. Tissue tension and expanding mesenchyme are also essential for branching morphogenesis. Within this framework, the pseudostratified airway epithelium differentiates from multipotent progenitors.

Basal cells, marked by p63, act as progenitor cells and give rise to multiciliated and secretory cells (goblet and club cells) [17]. Secretory lineage, which secretes mucus, generally emerges before multiciliated cell lineage [187–189]. After birth, the airway epithelium further differentiates in response to environmental cues, including those involved in gas exchange [186]. Cell homeostasis is maintained throughout life [190]. Further differentiation of basal cells is also observed following injury [184,190]. Underlying tissues, like smooth muscle or interstitial fibroblasts, support the regulation of this regenerative airway epithelium [186].

Thus, throughout development and adult life, the airway epithelium experiences multiple mechanical forces: tensile and compressive forces from tissue growth and morphogenetic expansion, osmotic and hydrostatic forces from fluid secretion and mucus production, stretch and deformation associated with breathing and airway expansion.

5.2. *Xenopus* epidermis morphogenesis

During epiboly, cells from the inner and outer layers spread and intercalate to form a thin bi-layered epithelium [191]. Some inner cells then differentiate into basal precursors that give rise to multiciliated cells, ionocytes and small secretory cells (stages 12–14), while outer cells acquire goblet cell characteristics. The intercalation of differentiating basal precursors into the upper layer is time-dependent, beginning with multiciliated cells, then ionocytes and finally small secretory cells (stage 16–35) [192,193]. It relies on cytoskeletal dynamics and calcium bursts [148,194–197].

Cells change shape during the differentiation processes [153]. Multiciliated progenitors are already specified in the lower layer before or at the onset of intercalation, as evidenced by alpha-tubulin expression [39]. However, the timing of centriole amplification relative to intercalation remains unclear, and a second wave of centriole amplification may occur during apical extension [25].

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Multiciliated progenitors in the *Xenopus* epidermis are exposed to tensile and compressive forces inherent to morphogenetic movements and tissue rearrangements during intercalation.

5.3. Mammalian brain ventricle morphogenesis

Mechanical forces influence brain development, including neural crest migration, neural progenitor proliferation and differentiation into neurons or oligodendrocytes (for review, see [198]).

The brain ventricular system is located in the lumen of the neural tube and is initially filled with amniotic fluid that is progressively replaced by the CSF [199]. CSF production transitions from the neuroepithelium itself to the embryonic vasculature and choroid plexuses [200]. CSF exerts hydrostatic pressure on ventricular walls, influencing tissue deformation and growth during development [201]. Following neural tube closure, the ventricular system expands and regionalizes into the lateral ventricles, the third ventricle, the cerebral aqueducts and the fourth ventricle. This process is driven by the proliferation of neuroepithelial and radial glial cells [202]. These proliferative events generate mechanical forces contributing to shaping the ventricular architecture. The ventricular system continues to mature post-natally with the emergence of ependymal multiciliated cells. An increase in cell apical surface that is not contributed by cell division [185] generates tensile forces. This size increase is dependent on the mTOR pathway. Notably, blocking or overactivating mTOR respectively inhibits or enhances multiciliated differentiation [203]. Putative mTOR candidates in the brain are RBL1, ROCK1 [203] or TFEB [65,204,205].

Thus, during ependymal multiciliated cell differentiation, progenitors experience tensile and compressive forces from mTOR-dependent tissue expansion and fluid pressure.

6. Conclusion and perspectives

In all three models, significant mechanical forces accompany multiciliated differentiation. These include tensile, compressive, hydrostatic and shear forces. Mechanical forces appear to function as permissive and modulatory inputs, potentially licensing the initiation of multiciliogenesis, defining its developmental timing and scaling centriole number. A major challenge for the field is to determine how these mechanical cues are sensed and integrated with lineage-specific transcription factors and cell cycle regulators.

For example, does the YAP/WWTR1 pathway control Notch inhibition at the specification stages? What are the targets of this YAP pathway during differentiation in the airway, and is it conserved in other multiciliated cells? How does nuclear deformation induce the variant cell cycle, through YAP/WWTR1 or by altering chromatin organization and the expression of relevant genes? If so, what are the targets?

We could also check whether the mTOR-reported role in apical size extension is conserved beyond brain ventricles, and how this mTOR pathway is induced, and whether and how it controls the underlying nuclear deformation.

Addressing these questions will require approaches that combine developmental genetics with quantitative biomechanics and live imaging. Such efforts promise to uncover fundamental principles linking tissue mechanics to cell fate and may provide new insights into diseases associated with defective multiciliation.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. This article has no additional data.

Declaration of AI use. We have used AI-assisted technologies (Mistral and ChatGPT) to improve the readability and correct the grammar of the first draft. The authors reviewed and edited carefully the content and take full responsibility for the content of the publication.

Authors' contributions. N.S.: funding acquisition, validation, writing—review and editing; N.D.: conceptualization, funding acquisition, writing—original draft, writing—review and editing.

Both authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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References

1. Satir P, Christensen ST. 2007 Overview of structure and function of mammalian cilia. *Annu. Rev. Physiol.* **69**, 377–400. (doi:10.1146/annurev.physiol.69.040705.141236)
2. Jain R, Javidan-Nejad C, Alexander-Brett J, Horani A, Cabellon MC, Walter MJ, Brody SL. 2012 Sensory functions of motile cilia and implication for bronchiectasis. *Front. Biosci.* **4**, 1088–1098. (doi:10.2741/s320)
3. McEwen DP, Jenkins PM, Martens JR. 2008 Olfactory cilia: our direct neuronal connection to the external world. *Curr. Top. Dev. Biol.* **85**, 333–370. (doi:10.1016/s0070-2153(08)00812-0)
4. Lidow MS, Menco BP. 1984 Observations on axonemes and membranes of olfactory and respiratory cilia in frogs and rats using tannic acid-supplemented fixation and photographic rotation. *J. Ultrastruct. Res.* **86**, 18–30. (doi:10.1016/s0022-5320(84)90092-3)
5. Reese TS. 1965 Olfactory cilia in the frog. *J. Cell Biol.* **25**, 209–230. (doi:10.1083/jcb.25.2.209)

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6. Okada Y, Takeda S, Tanaka Y, Belmonte JCI, Hirokawa N. 2005 Mechanism of nodal flow: a conserved symmetry breaking event in left-right axis determination. *Cell* **121**, 633–644. (doi:10.1016/j.cell.2005.04.008)
7. Mitchell DR. 2017 Evolution of cilia. *Cold Spring Harb. Perspect. Biol.* **9**, a028290. (doi:10.1101/cshperspect.a028290)
8. Spassky N, Meunier A. 2017 The development and functions of multiciliated epithelia. *Nat. Rev. Mol. Cell. Biol.* **18**, 423–436. (doi:10.1038/nrm.2017.21)
9. Mercer RR, Russell ML, Roggli VL, Crapo JD. 1994 Cell number and distribution in human and rat airways. *Am. J. Respir. Cell Mol. Biol.* **10**, 613–624. (doi:10.1165/ajrcmb.10.6.8003339)
10. Wloga D, Joachimiak E, Osinka A, Ahmadi S, Majhi S. 2024 Motile cilia in female and male reproductive tracts and fertility. *Cells* **13**, 1974. (doi:10.3390/cells13231974)
11. Shinohara K, Hamada H. 2017 Cilia in left-right symmetry breaking. *Cold Spring Harb. Perspect. Biol.* **9**, a028282. (doi:10.1101/cshperspect.a028282)
12. Ménard D. 1995 Morphological studies of the developing human esophageal epithelium. *Microsc. Res. Tech.* **31**, 215–225. (doi:10.1002/jemt.1070310305)
13. Katz SM, Morgan JJ. 1984 Cilia in the human kidney. *Ultrastruct. Pathol.* **6**, 285–294. (doi:10.3109/01913128409018587)
14. Zimmermann HD. 1971 [Cilia in the fetal kidney of man]. *Beitr. Pathol.* **143**, 227–240.
15. Wesselman HM, Arceri L, Nguyen TK, Lara CM, Wingert RA. 2024 Genetic mechanisms of multiciliated cell development: from fate choice to differentiation in zebrafish and other models. *FEBS J.* **291**, 4159–4192. (doi:10.1111/febs.17012)
16. Brooks ER, Wallingford JB. 2014 Multiciliated cells. *Curr. Biol.* **24**, R973–R982. (doi:10.1016/j.cub.2014.08.047)
17. Rackley CR, Stripp BR. 2012 Building and maintaining the epithelium of the lung. *J. Clin. Investig.* **122**, 2724–2730. (doi:10.1172/jci6051)
18. Fame RM, Cortés-Campos C, Sive HL. 2020 Brain ventricular system and cerebrospinal fluid development and function: light at the end of the tube: a primer with latest insights. *Bioessays* **42**, e1900186. (doi:10.1002/bies.201900186)
19. Collins C, Ventrella R, Mitchell BJ. 2021 Building a ciliated epithelium: transcriptional regulation and radial intercalation of multiciliated cells. *Curr. Top. Dev. Biol.* **145**, 3–39. (doi:10.1016/bs.ctdb.2020.08.001)
20. Boudjema A-R *et al.* 2024 The cell cycle variant in multiciliated cells incorporates 2 centriole biogenesis cycles. *eLife* **13**, RP96584. (doi:10.7554/eLife.96584.2)
21. Zhao H *et al.* 2013 The Cep63 paralogue Deup1 enables massive de novo centriole biogenesis for vertebrate multiciliogenesis. *Nat. Cell Biol.* **15**, 1434–1444. (doi:10.1038/ncb2880)
22. Klos Dehring DA, Vladar EK, Werner ME, Mitchell JW, Hwang P, Mitchell BJ. 2013 Deuterosome-mediated centriole biogenesis. *Dev. Cell* **27**, 103–112. (doi:10.1016/j.devcel.2013.08.021)
23. Al Jord A, Lemaitre AI, Delgehyr N, Faucourt M, Spassky N, Meunier A. 2014 Centriole amplification by mother and daughter centrioles differs in multiciliated cells. *Nature* **516**, 104–107. (doi:10.1038/nature13770)
24. Rayamajhi D, Roy S. 2025 Multiciliated cells: development, functions and disease relevance. *Semin. Cell Dev. Biol.* **175**, 103660. (doi:10.1016/j.semcdb.2025.103660)
25. Kulkarni S, Marquez J, Date P, Ventrella R, Mitchell BJ, Khokha MK. 2021 Mechanical stretch scales centriole number to apical area via Piezo1 in multiciliated cells. *eLife* **10**, e66076. (doi:10.7554/eLife.66076)
26. Kulkarni SS, Griffin JN, Date PP, Liem KF Jr, Khokha MK. 2018 WDR5 stabilizes actin architecture to promote multiciliated cell formation. *Dev. Cell* **46**, 595–610. (doi:10.1016/j.devcel.2018.08.009)
27. LoMastro GM, Drown CG, Maryniak AL, Jewett CE, Strong MA, Holland AJ. 2022 PLK4 drives centriole amplification and apical surface area expansion in multiciliated cells. *eLife* **11**, e80643. (doi:10.7554/eLife.80643)
28. Nanjundappa R, Kong D, Shim K, Stearns T, Brody SL, Loncarek J, Mahjoub MR. 2019 Regulation of cilia abundance in multiciliated cells. *eLife* **8**, e44039. (doi:10.7554/eLife.44039)
29. Mercey O, Al Jord A, Rostaing P, Mahuzier A, Fortoul A, Boudjema A-R, Faucourt M, Spassky N, Meunier A. 2019 Dynamics of centriole amplification in centrosome-depleted brain multiciliated progenitors. *Sci. Rep.* **9**, 13060. (doi:10.1038/s41598-019-49416-2)
30. Mercey O *et al.* 2019 Massive centriole production can occur in the absence of deuterosomes in multiciliated cells. *Nat. Cell Biol.* **21**, 1544–1552. (doi:10.1038/s41556-019-0427-x)
31. Zhao H, Chen Q, Fang C, Huang Q, Zhou J, Yan X, Zhu X. 2019 Parental centrioles are dispensable for deuterosome formation and function during basal body amplification. *EMBO Rep.* **20**, EMBR201846735. (doi:10.15252/embr.201846735)
32. Choksi SP, Byrnes LE, Konjikusic MJ, Tsai BWH, Deleon R, Lu Q, Westlake CJ, Reiter JF. 2024 An alternative cell cycle coordinates multiciliated cell differentiation. *Nature* **630**, 214–221. (doi:10.1038/s41586-024-07476-z)
33. Serizay J *et al.* 2025 Cyclin switch tailors a cell cycle variant to orchestrate multiciliogenesis. *Cell Rep.* **44**, 116523. (doi:10.1016/j.celrep.2025.116523)
34. Kageyama R, Ohtsuka T, Shimojo H, Imayoshi I. 2009 Dynamic regulation of Notch signaling in neural progenitor cells. *Curr. Opin. Cell Biol.* **21**, 733–740. (doi:10.1016/j.ceb.2009.08.009)
35. Liu Y, Pathak N, Kramer-Zucker A, Drummond IA. 2007 Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros. *Development* **134**, 1111–1122. (doi:10.1242/dev.02806)
36. Mori M *et al.* 2015 Notch3-Jagged signaling controls the pool of undifferentiated airway progenitors. *Development* **142**, 258–267. (doi:10.1242/dev.116855)
37. Rock JR, Gao X, Xue Y, Randell SH, Kong YY, Hogan BLM. 2011 Notch-dependent differentiation of adult airway basal stem cells. *Cell Stem Cell* **8**, 639–648. (doi:10.1016/j.stem.2011.04.003)
38. Ruiz García S *et al.* 2019 Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. *Development* **146**, dev177428, (doi:10.1242/dev.177428)
39. Deblandre GA, Wettstein DA, Koyano-Nakagawa N, Kintner C. 1999 A two-step mechanism generates the spacing pattern of the ciliated cells in the skin of *Xenopus* embryos. *Development* **126**, 4715–4728. (doi:10.1242/dev.126.21.4715)
40. Cibois M *et al.* 2015 BMP signalling controls the construction of vertebrate mucociliary epithelia. *Development* **142**, 2352–2363. (doi:10.1242/dev.118679)
41. Omiya H, Yamaguchi S, Watanabe T, Kuniya T, Harada Y, Kawaguchi D, Gotoh Y. 2021 BMP signaling suppresses Gemc1 expression and ependymal differentiation of mouse telencephalic progenitors. *Sci. Rep.* **11**, 613. (doi:10.1038/s41598-020-79610-6)
42. Brechbuhl HM, Ghosh M, Smith MK, Smith RW, Li B, Hicks DA, Cole BB, Reynolds PR, Reynolds SD. 2011 β-catenin dosage is a critical determinant of tracheal basal cell fate determination. *Am. J. Pathol.* **179**, 367–379. (doi:10.1016/j.ajpath.2011.03.016)
43. Cooney RA *et al.* 2023 A WNT4- and DKK3-driven canonical to noncanonical Wnt signaling switch controls multiciliogenesis. *J. Cell Sci.* **136**, jcs260807. (doi:10.1242/jcs.260807)
44. Haas M, Gómez Vázquez JL, Sun DI, Tran HT, Brislinger M, Tasca A, Shomroni O, Vleminckx K, Walentek P. 2019 ΔN-Tp63 mediates Wnt/β-catenin-induced inhibition of differentiation in basal stem cells of mucociliary epithelia. *Cell Rep.* **28**, 3338–3352. (doi:10.1016/j.celrep.2019.08.063)

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45. Hashimoto S, Chen H, Que J, Brockway BL, Drake JA, Snyder JC, Randell SH, Stripp BR. 2012 β-catenin-SOX2 signaling regulates the fate of developing airway epithelium. *J. Cell Sci.* **125**, 932–942. (doi:10.1242/jcs.092734)
46. Hou Z *et al.* 2019 Wnt/Fgf crosstalk is required for the specification of basal cells in the mouse trachea. *Development* **146**, dev171496. (doi:10.1242/dev.171496)
47. Huang YL, Niehrs C. 2014 Polarized Wnt signaling regulates ectodermal cell fate in *Xenopus*. *Dev. Cell* **29**, 250–257. (doi:10.1016/j.devcel.2014.03.015)
48. Ma L *et al.* 2022 β-catenin deletion in regional neural progenitors leads to congenital hydrocephalus in mice. *Neurosci. Bull.* **38**, 81–94. (doi:10.1007/s12264-021-00763-z)
49. Malleske DT, Hayes D, Lallier SW, Hill CL, Reynolds SD. 2018 Regulation of human airway epithelial tissue stem cell differentiation by β-catenin, P300, and CBP. *Stem Cells* **36**, 1905–1916. (doi:10.1002/stem.2906)
50. Mucenski ML *et al.* 2005 Beta-catenin regulates differentiation of respiratory epithelial cells in vivo. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **289**, L971–979. (doi:10.1152/ajplung.00172.2005)
51. Reynolds SD *et al.* 2008 Conditional stabilization of beta-catenin expands the pool of lung stem cells. *Stem Cells* **26**, 1337–1346. (doi:10.1634/stemcells.2008-0053)
52. Schmid A, Sailland J, Novak L, Baumlin N, Fregien N, Salathe M. 2017 Modulation of Wnt signaling is essential for the differentiation of ciliated epithelial cells in human airways. *FEBS Lett.* **591**, 3493–3506. (doi:10.1002/1873-3468.12851)
53. Wu Q, Zhang Y, Bai F, Wu Y, Hu Y, Wu J. 2025 The miR-34/449 clusters regulate the differentiation of ciliated cells in the oviduct via the Wnt/β-catenin signaling pathway. *Cell Biosci.* **15**, 130. (doi:10.1186/s13578-025-01468-w)
54. Xing L, Anbarchian T, Tsai JM, Plant GW, Nusse R. 2018 Wnt/β-catenin signaling regulates ependymal cell development and adult homeostasis. *Proc. Natl Acad. Sci. USA* **115**, E5954–E5962. (doi:10.1073/pnas.1803297115)
55. Marcet B *et al.* 2011 Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. *Nat. Cell Biol.* **13**, 693–699. (doi:10.1038/ncb2241)
56. Bao J *et al.* 2012 MicroRNA-449 and microRNA-34b/c function redundantly in murine testes by targeting E2F transcription factor-retinoblastoma protein (E2F-pRb) pathway. *J. Biol. Chem.* **287**, 21 686–21 698. (doi:10.1074/jbc.M111.328054)
57. Wu YJ, Liu Y, Hu YQ, Wang L, Bai FR, Xu C, Wu JW. 2021 Control of multiciliogenesis by miR-34/449 in the male reproductive tract through enforcing cell cycle exit. *J. Cell Sci.* **134**, jcs253450. (doi:10.1242/jcs.253450)
58. Yang X, Feng M, Jiang X, Wu Z, Li Z, Aau M, Yu Q. 2009 miR-449a and miR-449b are direct transcriptional targets of E2F1 and negatively regulate pRb-E2F1 activity through a feedback loop by targeting CDK6 and CDC25A. *Genes Dev.* **23**, 2388–2393. (doi:10.1101/gad.1819009)
59. Loukas I, Skamnelou M, Tsaridou S, Bournaka S, Grigoriadis S, Taraviras S, Lygerou Z, Arbi M. 2021 Fine-tuning multiciliated cell differentiation at the post-transcriptional level: contribution of miR-34/449 family members. *Biol. Rev. Camb. Philos. Soc.* **96**, 2321–2332. (doi:10.1111/brv.12755)
60. Song R *et al.* 2014 miR-34/449 miRNAs are required for motile ciliogenesis by repressing *cp110*. *Nature* **510**, 115–120. (doi:10.1038/nature13413)
61. Yuan S *et al.* 2019 Motile cilia of the male reproductive system require miR-34/miR-449 for development and function to generate luminal turbulence. *Proc. Natl Acad. Sci. USA* **116**, 3584–3593. (doi:10.1073/pnas.1817018116)
62. Chevalier B, Adamiok A, Mercey O, Revinski DR, Zaragosi L-E, Pasini A, Kodjabachian L, Barbry P, Marcet B. 2015 miR-34/449 control apical actin network formation during multiciliogenesis through small GTPase pathways. *Nat. Commun.* **6**, 8386. (doi:10.1038/ncomms9386)
63. Fededa JP *et al.* 2016 MicroRNA-34/449 controls mitotic spindle orientation during mammalian cortex development. *EMBO J.* **35**, 2386–2398. (doi:10.15252/embj.201694056)
64. Wu J, Bao J, Kim M, Yuan S, Tang C, Zheng H, Mastick GS, Xu C, Yan W. 2014 Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. *Proc. Natl Acad. Sci. USA* **111**, E2851–E2857. (doi:10.1073/pnas.1407777111)
65. Zheng J, Chen Y, Hu Y, Zhu Y, Lin J, Xu M, Zhang Y, Song W, Chen X. 2025 Lineage trajectories and fate determinants of postnatal neural stem cells and ependymal cells in the developing ventricular zone. *PLoS Biol.* **23**, e3003318. (doi:10.1371/journal.pbio.3003318)
66. Kaller M, Hünten S, Siemens H, Hermeking H. 2022 Analysis of the p53/microRNA network in cancer. *Adv. Exp. Med. Biol.* **1385**, 187–228. (doi:10.1007/978-3-031-08356-3_7)
67. Lizé M, Pilarski S, Dobbelstein M. 2010 E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis. *Cell Death Differ.* **17**, 452–458. (doi:10.1038/cdd.2009.188)
68. Concepcion CP *et al.* 2012 Intact p53-dependent responses in miR-34-deficient mice. *PLoS Genet.* **8**, e1002797. (doi:10.1371/journal.pgen.1002797)
69. Lizé M, Herr C, Klimke A, Bals R, Dobbelstein M. 2010 MicroRNA-449a levels increase by several orders of magnitude during mucociliary differentiation of airway epithelia. *Cell Cycle* **9**, 4579–4583. (doi:10.4161/cc.9.22.13870)
70. Nemajerova A *et al.* 2016 TAp73 is a central transcriptional regulator of airway multiciliogenesis. *Genes Dev.* **30**, 1300–1312. (doi:10.1101/gad.279836.116)
71. Wildung M *et al.* 2019 Transcription factor TAp73 and microRNA-449 complement each other to support multiciliogenesis. *Cell Death Differ.* **26**, 2740–2757. (doi:10.1038/s41418-019-0332-7)
72. Kyrousi C, Arbi M, Pilz G-A, Pefani D-E, Lalioti M-E, Ninkovic J, Götz M, Lygerou Z, Taraviras S. 2015 Mcidas and GemC1 are key regulators for the generation of multiciliated ependymal cells in the adult neurogenic niche. *Development* **142**, 3661–3674. (doi:10.1242/dev.126342)
73. Zhou F, Narasimhan V, Shboul M, Chong YL, Reversade B, Roy S. 2015 Gmnc is a master regulator of the multiciliated cell differentiation program. *Curr. Biol.* **25**, 3267–3273. (doi:10.1016/j.cub.2015.10.062)
74. Stubbs JL, Vladar EK, Axelrod JD, Kintner C. 2012 Multicilin promotes centriole assembly and ciliogenesis during multiciliate cell differentiation. *Nat. Cell Biol.* **14**, 140–147. (doi:10.1038/ncb2406)
75. Arbi M, Pefani DE, Kyrousi C, Lalioti ME, Kalogeropoulou A, Papanastasiou AD, Taraviras S, Lygerou Z. 2016 GemC1 controls multiciliogenesis in the airway epithelium. *EMBO Rep.* **17**, 400–413. (doi:10.15252/embr.201540882)
76. Boon M *et al.* 2014 MCIDAS mutations result in a mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat. Commun.* **5**, 4418. (doi:10.1038/ncomms5418)
77. Lalioti ME *et al.* 2019 *GemC1* is a critical switch for neural stem cell generation in the postnatal brain. *GLIA* **67**, 2360–2373. (doi:10.1002/glia.23690)
78. Terré B *et al.* 2016 GEMC1 is a critical regulator of multiciliated cell differentiation. *EMBO J.* **35**, 942–960. (doi:10.15252/embj.201592821)
79. Ortiz-Álvarez G *et al.* 2019 Adult neural stem cells and multiciliated ependymal cells share a common lineage regulated by the Geminin family members. *Neuron* **102**, 159–172. (doi:10.1016/j.neuron.2019.01.051)
80. Byrnes LE, Deleon R, Reiter JF, Choksi SP. 2022 Opposing transcription factors MYCL and HEY1 mediate the Notch-dependent airway stem cell fate decision. *bioRxiv*. (doi:10.1101/2022.10.05.511009)

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81. Lewis M *et al.* 2023 GEMC1 and MCIDAS interactions with SWI/SNF complexes regulate the multiciliated cell-specific transcriptional program. *Cell Death Dis.* **14**, 201. (doi:10.1038/s41419-023-05720-4)
82. Wang Z *et al.* 2018 TRRAP is a central regulator of human multiciliated cell formation. *J. Cell Biol.* **217**, 1941–1955. (doi:10.1083/jcb.201706106)
83. Gomperts BN, Gong-Cooper X, Hackett BP. 2004 Foxj1 regulates basal body anchoring to the cytoskeleton of ciliated pulmonary epithelial cells. *J. Cell Sci.* **117**, 1329–1337. (doi:10.1242/jcs.00978)
84. Campbell EP, Quigley IK, Kintner C. 2016 Foxn4 promotes gene expression required for the formation of multiple motile cilia. *Development* **143**, 4654–4664. (doi:10.1242/dev.143859)
85. Chung MI, Peyrot SM, LeBoeuf S, Park TJ, McGary KL, Marcotte EM, Wallingford JB. 2012 RFX2 is broadly required for ciliogenesis during vertebrate development. *Dev. Biol.* **363**, 155–165. (doi:10.1016/j.ydbio.2011.12.029)
86. El Zein L, Ait-Lounis A, Morle L, Thomas J, Chhin B, Spassky N, Reith W, Durand B. 2009 RFX3 governs growth and beating efficiency of motile cilia in mouse and controls the expression of genes involved in human ciliopathies. *J. Cell Sci.* **122**, 3180–3189 (doi:10.1242/jcs.048348)
87. Lemeille S *et al.* 2020 Interplay of RFX transcription factors 1, 2 and 3 in motile ciliogenesis. *Nucleic Acids Res.* **48**, 9019–9036. (doi:10.1093/nar/gkaa625).
88. Marshall CB *et al.* 2016 p73 is required for multiciliogenesis and regulates the Foxj1-associated gene network. *Cell Rep.* **14**, 2289–2300. (doi:10.1016/j.celrep.2016.02.035)
89. Pan JH *et al.* 2014 Myb permits multilineage airway epithelial cell differentiation. *Stem Cells* **32**, 3245–3256. (doi:10.1002/stem.1814)
90. Tan FE *et al.* 2013 *Myb* promotes centriole amplification and later steps of the multiciliogenesis program. *Development* **140**, 4277–4286. (doi:10.1242/dev.094102)
91. Lu H, Anujan P, Zhou F, Zhang Y, Chong YL, Bingle CD, Roy S. 2019 *Mcidas* mutant mice reveal a two-step process for the specification and differentiation of multiciliated cells in mammals. *Development* **146**, dev172643. (doi:10.1242/dev.172643)
92. Zhou F, Rayamajhi D, Ravi V, Narasimhan V, Chong YL, Lu H, Venkatesh B, Roy S. 2020 Conservation as well as divergence in Mcidas function underlies the differentiation of multiciliated cells in vertebrates. *Dev. Biol.* **465**, 168–177. (doi:10.1016/j.ydbio.2020.07.005)
93. Vladar EK, Stratton MB, Saal ML, Salazar-De Simone G, Wang X, Wolgemuth D, Stearns T, Axelrod JD. 2018 Cyclin-dependent kinase control of motile ciliogenesis. *eLife* **7**, e36375. (doi:10.7554/eLife.36375)
94. Khoury Damaa M *et al.* 2025 Cyclin O controls entry into the cell-cycle variant required for multiciliated cell differentiation. *Cell Rep.* **44**, 115117. (doi:10.1016/j.celrep.2024.115117)
95. Wallmeier J *et al.* 2014 Mutations in *CCNO* result in congenital mucociliary clearance disorder with reduced generation of multiple motile cilia. *Nat. Genet.* **46**, 646–651. (doi:10.1038/ng.2961)
96. Jiang J, Yu W, Fang T, Li Y, Liang J, Zhao R, Feng Z, Wang J, Tian H. 2023 Cyclin O promotes lung cancer progression and cetuximab resistance via cell cycle regulation and *CDK13* interaction. *J. Thorac Dis.* **15**, 2167–2183. (doi:10.21037/jtd-23-437)
97. Lammens T, Li J, Leone G, De Veylder L. 2009 Atypical E2Fs: new players in the E2F transcription factor family. *Trends Cell Biol.* **19**, 111–118. (doi:10.1016/j.tcb.2009.01.002)
98. Jewett CE, Holland AJ, Pearson CG. 2026 The DNA damage response pathway is required for multiciliated cell differentiation. *Curr. Biol.* **36**, 2156–2166. (doi:10.1016/j.cub.2026.03.027)
99. Ortiz-Álvarez G *et al.* 2022 p53/p21 pathway activation contributes to the ependymal fate decision downstream of GemC1. *Cell Rep.* **41**, 111810. (doi:10.1016/j.celrep.2022.111810)
100. Kim S, Chien YH, Ryan A, Kintner C. 2022 Emi2 enables centriole amplification during multiciliated cell differentiation. *Sci. Adv.* **8**, eabm7538. (doi:10.1126/sciadv.abm7538)
101. Tategu M, Nakagawa H, Sasaki K, Yamauchi R, Sekimachi S, Suita Y, Watanabe N, Yoshid K. 2008 Transcriptional regulation of human polo-like kinases and early mitotic inhibitor. *J. Genet. Genomics* **35**, 215–224. (doi:10.1016/s1673-8527(08)60030-2)
102. Balestrini A, Cosentino C, Errico A, Garner E, Costanzo V. 2010 GEMC1 is a TopBP1-interacting protein required for chromosomal DNA replication. *Nat. Cell Biol.* **12**, 484–491. (doi:10.1038/ncb2050)
103. Caillat C, Pefani DE, Gillespie PJ, Taraviras S, Blow JJ, Lygerou Z, Perrakis A. 2013 The Geminin and Idas coiled coils preferentially form a heterodimer that inhibits Geminin function in DNA replication licensing. *J. Biol. Chem.* **288**, 31624. (doi:10.1074/jbc.M113.491928)
104. Pefani DE *et al.* 2011 Idas, a novel phylogenetically conserved Geminin-related protein, binds to Geminin and is required for cell cycle progression. *J. Biol. Chem.* **286**, 23234. (doi:10.1074/jbc.M110.207688)
105. Knockleby J, Lee H. 2010 Same partners, different dance: involvement of DNA replication proteins in centrosome regulation. *Cell Cycle* **9**, 4487–4491. (doi:10.4161/cc.9.22.14047)
106. Chong YL, Zhang Y, Zhou F, Roy S. 2018 Distinct requirements of E2f4 versus E2f5 activity for multiciliated cell development in the zebrafish embryo. *Dev. Biol.* **443**, 165–172. (doi:10.1016/j.ydbio.2018.09.013)
107. Danielian PS, Bender Kim CF, Caron AM, Vasile E, Bronson RT, Lees JA. 2007 *E2f4* is required for normal development of the airway epithelium. *Dev. Biol.* **305**, 564–576. (doi:10.1016/j.ydbio.2007.02.037)
108. Danielian PS, Hess RA, Lees JA. 2016 E2f4 and E2f5 are essential for the development of the male reproductive system. *Cell Cycle* **15**, 250–260. (doi:10.1080/15384101.2015.1121350)
109. Ma L, Quigley I, Omran H, Kintner C. 2014 Multicilin drives centriole biogenesis via E2f proteins. *Genes Dev.* **28**, 1461–1471. (doi:10.1101/gad.243832.114)
110. Stracker TH. 2019 E2F4/5-mediated transcriptional control of multiciliated cell differentiation: redundancy or fine-tuning? *Dev. Biol.* **446**, 20–21. (doi:10.1016/j.ydbio.2018.12.007)
111. Xie H, Kang Y, Wang S, Zheng P, Chen Z, Roy S, Zhao C. 2020 E2f5 is a versatile transcriptional activator required for spermatogenesis and multiciliated cell differentiation in zebrafish. *PLoS Genet.* **16**, e1008655. (doi:10.1371/journal.pgen.1008655)
112. Kim S, Ma L, Shokhirev MN, Quigley I, Kintner C. 2018 Multicilin and activated E2f4 induce multiciliated cell differentiation in primary fibroblasts. *Sci. Rep.* **8**, 12369. (doi:10.1038/s41598-018-30791-1)
113. Classon M, Harlow E. 2002 The retinoblastoma tumour suppressor in development and cancer. *Nat. Rev. Cancer* **2**, 910–917. (doi:10.1038/nrc950)
114. Basso M *et al.* 2025 Actin-based deformations of the nucleus control mouse multiciliated ependymal cell differentiation. *Dev. Cell* **60**, 749–761. (doi:10.1016/j.devcel.2024.11.008)
115. Quiroz EJ, Kim S, Gautam LK, Borok Z, Kintner C, Ryan AL. 2024 RBL2 represses the transcriptional activity of multicilin to inhibit multiciliogenesis. *Cell Death Dis.* **15**, 81. (doi:10.1038/s41419-024-06440-z)
116. Wang H, Song W, Hu T, Zhang N, Miao S, Zong S, Wang L. 2011 Fank1 interacts with Jab1 and regulates cell apoptosis via the AP-1 pathway. *Cell. Mol. Life Sci.* **68**, 2129–2139. (doi:10.1007/s00018-010-0559-4)
117. Johnson JA, Watson JK, Nikolić MZ, Rawlins EL. 2018 Fank1 and Jazf1 promote multiciliated cell differentiation in the mouse airway epithelium. *Biol. Open* **7**, bio033944. (doi:10.1242/bio.033944)
118. Lalioti ME *et al.* 2019 GemC1 governs multiciliogenesis through direct interaction with and transcriptional regulation of p73. *J. Cell Sci.* **132**, jcs228684. (doi:10.1242/jcs.228684)

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119. Allocati N, Di Ilio C, De Laurenzi V. 2012 p63/p73 in the control of cell cycle and cell death. *Exp. Cell Res.* **318**, 1285–1290. (doi:10.1016/j.yexcr.2012.01.023)
120. Alvarez Y, Smutny M. 2022 Emerging role of mechanical forces in cell fate acquisition. *Front. Cell Dev. Biol.* **10**, 864522. (doi:10.3389/fcell.2022.864522)
121. Vining KH, Mooney DJ. 2017 Mechanical forces direct stem cell behaviour in development and regeneration. *Nat. Rev. Mol. Cell Biol.* **18**, 728–742. (doi:10.1038/nrm.2017.108)
122. Dupont S, Wickström SA. 2022 Mechanical regulation of chromatin and transcription. *Nat. Rev. Genet.* **23**, 624–643 (doi:10.1038/s41576-022-00493-6)
123. Douguet D, Honoré E. 2019 Mammalian mechanoelectrical transduction: structure and function of force-gated ion channels. *Cell* **179**, 340–354. (doi:10.1016/j.cell.2019.08.049)
124. Charras G, Yap AS. 2018 Tensile forces and mechanotransduction at cell-cell junctions. *Curr. Biol.* **28**, R445–R457. (doi:10.1016/j.cub.2018.02.003)
125. Kechagia JZ, Ivaska J, Roca-Cusachs P. 2019 Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **20**, 457–473. (doi:10.1038/s41580-019-0134-2)
126. Heo S-J, Cosgrove BD, Dai EN, Mauck RL. 2018 Mechano-adaptation of the stem cell nucleus. *Nucleus* **9**, 9–19. (doi:10.1080/19491034.2017.1371398)
127. Alam S, Lovett DB, Dickinson RB, Roux KJ, Lele TP. 2014 Nuclear forces and cell mechanosensing. *Progr. Mol. Biol. Transl. Sci.* **126**, 205–215. (doi:10.1016/B978-0-12-394624-9.00008-7)
128. Lomakin AJ *et al.* 2020 The nucleus acts as a ruler tailoring cell responses to spatial constraints. *Science* **370**, eaba2894. (doi:10.1126/science.aba2894)
129. Venturini V *et al.* 2020 The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior. *Science* **370**, eaba2644. (doi:10.1126/science.aba2644)
130. Guilluy C, Osborne LD, Van Landeghem L, Sharek L, Superfine R, Garcia-Mata R, Burridge K. 2014 Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nat. Cell Biol.* **16**, 376–381. (doi:10.1038/ncb2927)
131. Swift J *et al.* 2013 Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**, 1240104. (doi:10.1126/science.1240104)
132. Le HQ *et al.* 2016 Mechanical regulation of transcription controls polycomb-mediated gene silencing during lineage commitment. *Nat. Cell Biol.* **18**, 864–875. (doi:10.1038/ncb3387)
133. Nava MM *et al.* 2020 Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage. *Cell* **181**, 800–817. (doi:10.1016/j.cell.2020.03.052)
134. Andreu I *et al.* 2022 Mechanical force application to the nucleus regulates nucleocytoplasmic transport. *Nat. Cell Biol.* **24**, 896–905. (doi:10.1038/s41556-022-00927-7).
135. Elosegui-Artola A *et al.* 2017 Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* **171**, 1397–1410. (doi:10.1016/j.cell.2017.10.008)
136. Barresi R, Campbell KP. 2006 Dystroglycan: from biosynthesis to pathogenesis of human disease. *J. Cell Sci.* **119**, 199–207. (doi:10.1242/jcs.02814)
137. Sciandra F, Bozzi M, Bigotti MG. 2023 From adhesion complex to signaling hub: the dual role of dystroglycan. *Front. Mol. Biosci.* **10**, 1325284. (doi:10.3389/fmolb.2023.1325284)
138. Durbeej M, Henry MD, Ferletta M, Campbell KP, Ekblom P. 1998 Distribution of dystroglycan in normal adult mouse tissues. *J. Histochem. Cytochem.* **46**, 449–457. (doi:10.1177/002215549804600404)
139. White SR, Wojcik KR, Gruenert D, Sun S, Dorscheid DR. 2001 Airway epithelial cell wound repair mediated by alpha-dystroglycan. *Am. J. Respir. Cell Mol. Biol.* **24**, 179–186. (doi:10.1165/ajrcmb.24.2.3993)
140. Sirour C, Hidalgo M, Bello V, Buisson N, Darribère T, Moreau N. 2011 Dystroglycan is involved in skin morphogenesis downstream of the Notch signaling pathway. *Mol. Biol. Cell* **22**, 2957–2969. (doi:10.1091/mbc.E11-01-0074)
141. Chung MI, Kwon T, Tu F, Brooks ER, Gupta R, Meyer M, Baker JC, Marcotte EM, Wallingford JB. 2014 Coordinated genomic control of ciliogenesis and cell movement by RFX2. *eLife* **3**, e01439. (doi:10.7554/eLife.01439)
142. McClenahan FK, Sharma H, Shan X, Eyermann C, Colognato H. 2016 Dystroglycan suppresses notch to regulate stem cell niche structure and function in the developing postnatal subventricular zone. *Dev. Cell* **38**, 548–566. (doi:10.1016/j.devcel.2016.07.017)
143. Bharathan NK, Dickinson AJG. 2019 Desmoplakin is required for epidermal integrity and morphogenesis in the *Xenopus laevis* embryo. *Dev. Biol.* **450**, 115–131. (doi:10.1016/j.ydbio.2019.03.010)
144. Aragona M *et al.* 2024 Piezo 1 and Piezo 2 in the chemosensory organs of zebrafish (*Danio rerio*). *Int. J. Mol. Sci.* **25**, 7404. (doi:10.3390/ijms25137404)
145. Nourse JL *et al.* 2022 Piezo1 regulates cholesterol biosynthesis to influence neural stem cell fate during brain development. *J. Gen. Physiol.* **154**, e202213084. (doi:10.1085/jgp.202213084)
146. Pathak MM, Nourse JL, Tran T, Hwe J, Arulmoli J, Le DT, Bernardis E, Flanagan LA, Tombola F. 2014 Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. *Proc. Natl Acad. Sci. USA* **111**, 16148–16153. (doi:10.1073/pnas.1409802111)
147. Valero C, Amaveda H, Mora M, García-Aznar JM. 2018 Combined experimental and computational characterization of crosslinked collagen-based hydrogels. *PLoS ONE* **13**, e0195820. (doi:10.1371/journal.pone.0195820)
148. Christodoulou N, Skourides PA. 2025 Calcium transients regulate the apical emergence of basally located progenitors during *Xenopus* skin development. *Nat. Commun.* **16**, 6650. (doi:10.1038/s41467-025-61610-7)
149. Wiegel J, Helmstädter M, Walz G, Bergen MD. 2025 Spontaneous calcium bursts organize the apical actin cytoskeleton of multiciliated cells. *Int. J. Mol. Sci.* **26**, 2507. (doi:10.3390/ijms26062507)
150. Jain N, Lim LW, Tan WT, George B, Makeyev E, Thanabalu T. 2014 Conditional N-WASP knockout in mouse brain implicates actin cytoskeleton regulation in hydrocephalus pathology. *Exp. Neurol.* **254**, 29–40. (doi:10.1016/j.expneurol.2014.01.011)
151. Peng X, Lin Q, Liu Y, Jin Y, Druso JE, Antonyak MA, Guan JL, Cerione RA. 2013 Inactivation of Cdc42 in embryonic brain results in hydrocephalus with ependymal cell defects in mice. *Protein Cell* **4**, 231–242. (doi:10.1007/s13238-012-2098-2)
152. Zhang X, Lei K, Yuan X, Wu X, Zhuang Y, Xu T, Xu R, Han M. 2009 SUN1/2 and Syne/Nesprin-1/2 complexes connect centrosome to the nucleus during neurogenesis and neuronal migration in mice. *Neuron* **64**, 173–187. (doi:10.1016/j.neuron.2009.08.018)
153. Tolonen M, Xu Z, Beker O, Kapoor V, Dumitrascu B, Sedzinski J. 2026 Single-cell morphodynamics predict cell fate decisions during mucociliary epithelial differentiation. *Mol. Syst. Biol.* **22**, 1040–1069 (doi:10.1038/s44320-026-00212-x)
154. Nader GPF, Williart A, Piel M. 2021 Nuclear deformations, from signaling to perturbation and damage. *Curr. Opin. Cell Biol.* **72**, 137–145. (doi:10.1016/j.ceb.2021.07.008)
155. Byfield FJ *et al.* 2025 Metabolically intact nuclei are fluidized by the activity of the chromatin remodeling motor BRG1. *Biophys. J.* **124**, 494–507. (doi:10.1016/j.bpj.2024.11.3322)
156. Shah P, Hobson CM, Cheng S, Colville MJ, Paszek MJ, Superfine R, Lammerding J. 2021 Nuclear deformation causes DNA damage by increasing replication stress. *Curr. Biol.* **31**, 753–765. (doi:10.1016/j.cub.2020.11.037)
157. Uhler C, Shivashankar GV. 2018 Nuclear mechanopathology and cancer diagnosis. *Trends Cancer* **4**, 320–331. (doi:10.1016/j.trecan.2018.02.009)

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158. Galvis LA, Holik AZ, Short KM, Pasquet J, Lun AT, Blewitt ME, Smyth IM, Ritchie ME, Asselin-Labat ML. 2015 Repression of *Igf1* expression by Ezh2 prevents basal cell differentiation in the developing lung. *Development* **142**, 1458–1469. (doi:10.1242/dev.122077)
159. Snitow ME, Li S, Morley MP, Rathi K, Lu MM, Kadzik RS, Stewart KM, Morrisey EE. 2015 Ezh2 represses the basal cell lineage during lung endoderm development. *Development* **142**, 108–117. (doi:10.1242/dev.116947)
160. Panciera T, Azzolin L, Cordenonsi M, Piccolo S. 2017 Mechanobiology of YAP and TAZ in physiology and disease. *Nat. Rev. Mol. Cell Biol.* **18**, 758–770. (doi:10.1038/nrm.2017.87)
161. Driskill JH, Pan D. 2023 Control of stem cell renewal and fate by YAP and TAZ. *Nat. Rev. Mol. Cell Biol.* **24**, 895–911. (doi:10.1038/s41580-023-00644-5)
162. He L, Pratt H, Gao M, Wei F, Weng Z, Struhl K. 2021 YAP and TAZ are transcriptional co-activators of AP-1 proteins and STAT3 during breast cellular transformation. *eLife* **10**, e67312. (doi:10.7554/eLife.67312)
163. Koo JH, Plouffe SW, Meng Z, Lee D-H, Yang D, Lim D-S, Wang C-Y, Guan K-L. 2020 Induction of AP-1 by YAP/TAZ contributes to cell proliferation and organ growth. *Genes Dev.* **34**, 72–86. (doi:10.1101/gad.331546.119)
164. Mahoney JE, Mori M, Szymaniak AD, Varelas X, Cardoso WV. 2014 The hippo pathway effector Yap controls patterning and differentiation of airway epithelial progenitors. *Dev. Cell* **30**, 137–150. (doi:10.1016/j.devcel.2014.06.003)
165. van Soldt BJ, Qian J, Li J, Tang N, Lu J, Cardoso WV. 2019 Yap and its subcellular localization have distinct compartment-specific roles in the developing lung. *Development* **146**, dev175810. (doi:10.1242/dev.175810)
166. Zhao R *et al.* 2014 Yap tunes airway epithelial size and architecture by regulating the identity, maintenance, and self-renewal of stem cells. *Dev. Cell* **30**, 151–165. (doi:10.1016/j.devcel.2014.06.004)
167. Park R, Moon UY, Park JY, Hughes LJ, Johnson RL, Cho S-H, Kim S. 2016 Yap is required for ependymal integrity and is suppressed in LPA-induced hydrocephalus. *Nat. Commun.* **7**, 10329. (doi:10.1038/ncomms10329)
168. Lavado A, Gangwar R, Paré J, Wan S, Fan Y, Cao X. 2021 YAP/TAZ maintain the proliferative capacity and structural organization of radial glial cells during brain development. *Dev. Biol.* **480**, 39–49. (doi:10.1016/j.ydbio.2021.08.010)
169. Narayanan V, Rao VG, Arrigo A, Kulkarni SS. 2025 Multiciliated cells adapt the mechanochemical Piezo1-Erk1/2-Yap1 cell proliferation axis to fine-tune centriole number. *bioRxiv*. (doi:10.1101/2025.01.21.634139)
170. Kopan R, Ilagan MXG. 2009 The canonical notch signaling pathway: unfolding the activation mechanism. *Cell* **137**, 216–233. (doi:10.1016/j.cell.2009.03.045)
171. Gordon WR *et al.* 2015 Mechanical allostery: evidence for a force requirement in the proteolytic activation of notch. *Dev. Cell* **33**, 729–736. (doi:10.1016/j.devcel.2015.05.004).
172. Langridge PD, Struhl G. 2017 Epsin-dependent ligand endocytosis activates notch by force. *Cell* **171**, 1383–1396. (doi:10.1016/j.cell.2017.10.048)
173. Luca VC *et al.* 2017 Notch-jagged complex structure implicates a catch bond in tuning ligand sensitivity. *Science* **355**, 1320–1324. (doi:10.1126/science.aaf9739)
174. Meloty-Kapella L, Shergill B, Kuon J, Botvinick E, Weinmaster G. 2012 Notch ligand endocytosis generates mechanical pulling force dependent on dynamin, epsins, and actin. *Dev. Cell* **22**, 1299–1312. (doi:10.1016/j.devcel.2012.04.005)
175. Parks AL, Klueg KM, Stout JR, Muskavitch MAT. 2000 Ligand endocytosis drives receptor dissociation and activation in the Notch pathway. *Development* **127**, 1373–1385. (doi:10.1242/dev.127.7.1373)
176. Kasirer S, Sprinzak D. 2024 Interplay between Notch signaling and mechanical forces during developmental patterning processes. *Curr. Opin. Cell Biol.* **91**, 102444. (doi:10.1016/j.ceb.2024.102444)
177. Stassen O, Ristori T, Sahlgren CM. 2020 Notch in mechanotransduction: from molecular mechanosensitivity to tissue mechanostasis. *J. Cell Sci.* **133**, jcs250738. (doi:10.1242/jcs.250738)
178. Esteves de Lima J, Bonnin MA, Birchmeier C, Duprez D. 2016 Muscle contraction is required to maintain the pool of muscle progenitors via YAP and NOTCH during fetal myogenesis. *eLife* **5**, e15593. (doi:10.7554/eLife.15593)
179. Grampa V *et al.* 2016 Novel *NEK8* mutations cause severe syndromic renal cystic dysplasia through YAP dysregulation. *PLoS Genet.* **12**, e1005894. (doi:10.1371/journal.pgen.1005894)
180. Totaro A, Castellan M, Battilana G, Zanconato F, Azzolin L, Giulitti S, Cordenonsi M, Piccolo S. 2017 YAP/TAZ link cell mechanics to Notch signalling to control epidermal stem cell fate. *Nat. Commun.* **8**, 15206. (doi:10.1038/ncomms15206)
181. Tschaharganeh DF *et al.* 2013 Yes-associated protein up-regulates Jagged-1 and activates the Notch pathway in human hepatocellular carcinoma. *Gastroenterology* **144**, 1530–1542. (doi:10.1053/j.gastro.2013.02.009)
182. Yimlamai D *et al.* 2014 Hippo pathway activity influences liver cell fate. *Cell* **157**, 1324–1338. (doi:10.1016/j.cell.2014.03.060).
183. Totaro A, Castellan M, Di Biagio D, Piccolo S. 2018 Crosstalk between YAP/TAZ and Notch signaling. *Trends Cell Biol.* **28**, 560–573. (doi:10.1016/j.tcb.2018.03.001)
184. Petit LMG *et al.* 2023 Airway ciliated cells in adult lung homeostasis and COPD. *Eur. Respir. Rev.* **32**, 230106. (doi:10.1183/16000617.0106-2023)
185. Spassky N, Merkle FT, Flames N, Tramontin AD, Garcia-Verdugo JM, Alvarez-Buylla A. 2005 Adult ependymal cells are postmitotic and are derived from radial glial cells during embryogenesis. *J. Neurosci.* **25**, 10–18. (doi:10.1523/JNEUROSCI.1108-04.2005)
186. Zepp JA, Morrisey EE. 2019 Cellular crosstalk in the development and regeneration of the respiratory system. *Nat. Rev. Mol. Cell Biol.* **20**, 551–566. (doi:10.1038/s41580-019-0141-3)
187. Guha A *et al.* 2012 Neuroepithelial body microenvironment is a niche for a distinct subset of Clara-like precursors in the developing airways. *Proc. Natl Acad. Sci. USA* **109**, 12 592–12 597. (doi:10.1073/pnas.1204710109)
188. Kurotani R, Tomita T, Yang Q, Carlson BA, Chen C, Kimura S. 2008 Role of secretoglobin 3A2 in lung development. *Am. J. Respir. Crit. Care Med.* **178**, 389–398. (doi:10.1164/rccm.200707-11040C)
189. Rawlins EL, Ostrowski LE, Randell SH, Hogan BLM. 2007 Lung development and repair: contribution of the ciliated lineage. *Proc. Natl Acad. Sci. USA* **104**, 410–417. (doi:10.1073/pnas.0610770104)
190. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, Randell SH, Hogan BL. 2009 Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc. Natl Acad. Sci. USA* **106**, 12 771–12 775. (doi:10.1073/pnas.0906850106)
191. Szabó A, Cobo I, Omara S, McLachlan S, Keller R, Mayor R. 2016 The molecular basis of radial intercalation during tissue spreading in early development. *Dev. Cell* **37**, 213–225. (doi:10.1016/j.devcel.2016.04.008)
192. Stubbs JL, Davidson L, Keller R, Kintner C. 2006 Radial intercalation of ciliated cells during *Xenopus* skin development. *Development* **133**, 2507–2515. (doi:10.1242/dev.02417)

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193. Walentek P. 2021 *Xenopus* epidermal and endodermal epithelia as models for mucociliary epithelial evolution, disease, and metaplasia. *Genesis* **59**, e23406. (doi:10.1002/dvg.23406)
194. Collins C *et al.* 2021 Tubulin acetylation promotes penetrative capacity of cells undergoing radial intercalation. *Cell Rep.* **36**, 109556. (doi:10.1016/j.celrep.2021.109556)
195. Sedzinski J, Hannezo E, Tu F, Biro M, Wallingford JB. 2016 Emergence of an apical epithelial cell surface in vivo. *Dev. Cell* **36**, 24–35. (doi:10.1016/j.devcel.2015.12.013)
196. Sedzinski J, Hannezo E, Tu F, Biro M, Wallingford JB. 2017 RhoA regulates actin network dynamics during apical surface emergence in multiciliated epithelial cells. *J. Cell Sci.* **130**, 420–428. (doi:10.1242/jcs.194704)
197. Ventura G, Amiri A, Thiagarajan R, Tolonen M, Doostmohammadi A, Sedzinski J. 2022 Multiciliated cells use filopodia to probe tissue mechanics during epithelial integration in vivo. *Nat. Commun.* **13**, 6423. (doi:10.1038/s41467-022-34165-0)
198. Abuwarda H, Pathak MM. 2020 Mechanobiology of neural development. *Curr. Opin. Cell Biol.* **66**, 104–111. (doi:10.1016/j.ceb.2020.05.012)
199. Chau KF *et al.* 2015 Progressive differentiation and instructive capacities of amniotic fluid and cerebrospinal fluid proteomes following neural tube closure. *Dev. Cell* **35**, 789–802. (doi:10.1016/j.devcel.2015.11.015)
200. Fame RM, Lehtinen MK. 2020 Emergence and developmental roles of the cerebrospinal fluid system. *Dev. Cell* **52**, 261–275. (doi:10.1016/j.devcel.2020.01.027)
201. Desmond ME, Jacobson AG. 1977 Embryonic brain enlargement requires cerebrospinal fluid pressure. *Dev. Biol.* **57**, 188–198. (doi:10.1016/0012-1606(77)90364-5)
202. Kriegstein A, Alvarez-Buylla A. 2009 The glial nature of embryonic and adult neural stem cells. *Annu. Rev. Neurosci.* **32**, 149–184. (doi:10.1146/annurev.neuro.051508.135600)
203. Bankolé A *et al.* 2025 mTOR controls ependymal cell differentiation by targeting the alternative cell cycle and centrosomal proteins. *EMBO Rep.* **26**, 3075–3105. (doi:10.1038/s44319-025-00460-2)
204. Roczniak-Ferguson A, Petit CS, Froehlich F, Qian S, Ky J, Angarola B, Walther TC, Ferguson SM. 2012 The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Sci. Signal.* **5**, ra42. (doi:10.1126/scisignal.2002790)
205. Settembre C *et al.* 2012 A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *EMBO J.* **31**, 1095–1108. (doi:10.1038/emboj.2012.32)

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