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Notch signalling: sensor and instructor of the microenvironment to coordinate cell fate and organ morphogenesis

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During development, stem cells give rise to specialised cell types in a tightly regulated, spatiotemporal manner to drive the formation of complex three-dimensional tissues. While mechanistic insights into the gene regulatory pathways that guide cell fate choices are emerging, how morphogenetic changes are coordinated with cell fate specification remains a fundamental question in organogenesis and adult tissue homeostasis. The requirement of cell contacts for Notch signalling makes it a central pathway capable of linking dynamic cellular rearrangements during tissue morphogenesis with stem cell function. Here, we highlight recent studies that support a critical role for the Notch pathway in translating microenvironmental cues into cell fate decisions, guiding the development of diverse organ systems.

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Introduction

The construction of precise cellular ensembles during tissue development relies on an intricate interplay between cell proliferation, differentiation, communication, migration and death. Among the signalling cues that coordinate these cellular programs, the Notch pathway is widely recognised as a major determinant of cell fate across all metazoans. First discovered in *Drosophila melanogaster* a century ago, the Notch receptor is a central element of an evolutionarily conserved pathway that controls a broad spectrum of cell fate decisions through local cell communication [1].

Notch signalling is triggered by interactions between Notch receptors and their ligands on adjacent cells (Box 1). Receptor activation results in Notch target gene induction, including genes of the *Hairy-Enhancer of Split* (HES) family, which act as repressors of lineage-specific determinants. In turn, this juxtacrine signalling mechanism dynamically regulates lineage specification according to the position of a cell and the composition of its neighbours. Its simplicity in design — a direct route from the membrane to the nucleus lacking second messenger amplification and regulation — belies exceptional complexity, as Notch activation guides cells towards opposing developmental paths in a tissue and time-dependent manner. Integration with coincident signalling events and mechanical cues also shape Notch pathway activity, generating the diverse biological outcomes required for each context [2]. Notch signalling, therefore, provides an ideal paradigm to examine how cells combine multiple inputs from neighbouring cells and the physical extracellular environment to coordinate cell fate specification with tissue morphogenesis.

Notch signalling: bridging spatiotemporal control of stem cell specification with organ morphogenesis

The role of Notch in determining cell fate during development is well-recognised, and has been extensively reviewed elsewhere [2–4]. While Notch promotes cellular differentiation in some contexts (e.g. in skin keratinocytes [5] and in the lung [6]), signal activation is often associated with stem cell maintenance and proliferation, including in muscular, intestinal, hematopoietic and neural stem cells [7–12]. Indeed, the developmental outcome of Notch signals depends on their integration with a multiplicity of regulatory factors that vary across morphogenetic systems [2]. Cell shape [13], cellular movements, proximity to local cues (e.g. basement membrane (BM) attachment) [14] and mechanical stimuli associated with local tissue deformations [15] can all contribute to cell fate determination [16,17]. Thus, dynamic changes in cellular composition and tissue architecture during organ growth and repair expose stem cells to evolving niche environments, instructing gene regulatory networks such as Notch to guide lineage decisions in a highly regulated, spatiotemporal manner. Below, we outline designs of Notch signal modulations between stem cells and their surrounding cellular and non-cellular microenvironment, and highlight recent

Box 1 Notch signalling in brief

The central element of the pathway is the plasma membrane protein Notch, which acts both as a receptor and a transcription factor. Notch is initially cleaved in the *trans*-Golgi network and is presented on the cell surface in a heterodimeric form, tethered together via non-covalent interactions. In mammals, the Notch receptor has four paralogues, Notch 1 to Notch 4. Molecularly, the extracellular domain of either of the transmembrane ligands, Delta-like-1, Delta-like-2 and Delta-like-4, and Jagged-1 and Jagged-2 (Delta and Serrate in *Drosophila*) on the surface of one cell, interacts with the extracellular domain of the Notch receptor on an adjacent cell. A series of post-translational modifications modulate the affinity and activity of the Notch receptor and its ligands (reviewed in Ref. [48]). Ligand binding triggers two proteolytic cleavages by ADAM and γ-secretase (juxtamembrane and intracellular, respectively) that result in the release of the Notch intracellular domain (NICD) from its plasma membrane tether. NICD is subsequently translocated into the nucleus where it forms a complex with the DNA-binding factor RBPJ and the co-activator Mastermind-Like (Su(H) and Mastermind in *Drosophila*). This nuclear complex induces the expression of Notch target genes, among which the most conserved belong to the HES gene family [49,50]. HES proteins are basic Helix-Loop-Helix (bHLH) DNA-binding transcription factors that suppress expression of lineage-specifying bHLH genes, such as *Mash-1* and *Math-1* (neurogenesis, endocrine lineages), *Myogenin* (myogenesis) and *E2A* (B lymphopoiesis), controlling cell differentiation in diverse organs, including the nervous system, heart, skeletal muscle, pancreas, endodermal endocrine organs and hematocytes [51].

studies that describe how spatial arrangements of cells underpin cell fate decisions during tissue morphogenesis (Figure 1).

Notch signalling responds to dynamic reorganisation of the cellular niche

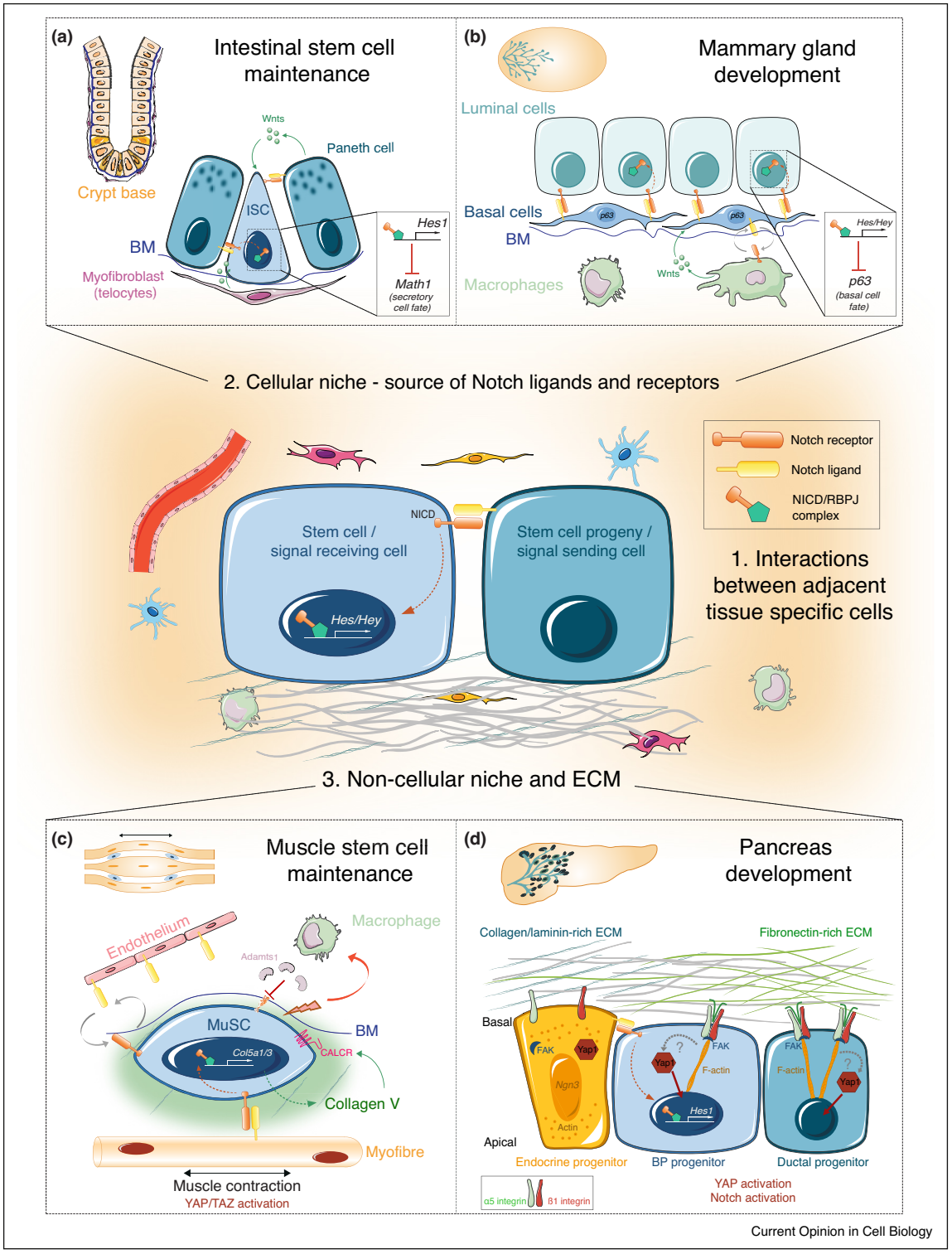
The source and availability of Notch ligands are essential for defining how Notch determines cell fate. In the context of directional Notch signalling, cellular rearrangements can position a given cell in proximity to a Notch ligand-expressing cell that, in turn, determines its neighbour's destiny. In the developing mammary gland, for example, Notch signalling is well-established to be a critical determinant of luminal cell differentiation [18,19•], one of the two epithelial lineages that constitute the mammary ductal tree [20]. Pathway activation in luminal cells, triggered by neighbouring Dll1-bearing basal cells, suppresses the transcription factor p63, a key mammary basal cell determinant [18,21,22] (Figure 1b). In agreement, a recent study demonstrated that forced Notch activation during embryonic mammogenesis, and in the adult lineage-committed basal compartment, drives the obligatory specification of luminal cells [19•]. Intriguingly, cell fate specification in the embryonic mammary gland coincides with the initial morphogenetic sprouting events that give rise to the branched epithelium present at birth [19•]. Thus, it is tempting to speculate that, during the initial stages of tubulogenesis, differential cell contacts establish basal (Dll1) to luminal (Notch) signalling, with some embryonic mammary cells exposed to the BM, while others face the forming lumen; an intriguing hypothesis that warrants further investigation.

Similarly, coordinated morphogenesis and Notch-mediated lineage diversification was recently described in the developing pancreas [23,24•]. Indeed, excessive endocrine differentiation in Hes1 mutant embryos resulted in ectopic pancreas formation [24•]. This study supports a model where the extension of the dorsal pancreatic bud perpendicularly into the associated mesenchyme is ensured by the repressive action of Hes1 on the endocrine determinant Neurogenin3 (Neurog3). A second report also examined the coordination between pancreas plexus morphogenesis and endocrine fate allocation [23]. In this case, morphogenetic cues within the epithelial plexus niche, where pancreatic progenitors reside, initiated endocrine commitment. The integration between Neurog3-driven endocrine differentiation, Notch-stimulated pancreatic progenitor maintenance and epithelial remodelling ensures the correct balance between cell differentiation and organ morphogenesis. Precisely how transcription-factor determinants feedback and are coordinated with pancreatic morphogenetic programs remains to be elucidated, although it is likely influenced by concomitant biochemical and biomechanical cues (discussed below).

Distinct temporal and spatial patterns of cell differentiation are also evident during the development of other tissues. For example, precise regionalisation of ligand expression in thymic epithelial cells was recently shown to be necessary for establishing discrete Notch niches that instruct T cell specification in the developing thymus [25]. Notch-mediated binary cell fate decisions are also required for mammalian nephrogenesis, where the necessary cell-to-cell interactions are established through a morphogenetic process that maintains nephron progenitors in aggregates during tubule formation [26,27]. The requirement for positional cues to generate diverse and specialised cell types during organ morphogenesis is evolutionarily conserved, as similar signal regionalisation is necessary for nephrogenesis in zebrafish [28]. Moreover, a recent study in *Drosophila* reported that distinct glial precursors are found in specialised regions of the fly central nervous system, and that Notch-mediated glial cell diversity can be tracked back to their anatomical position [29].

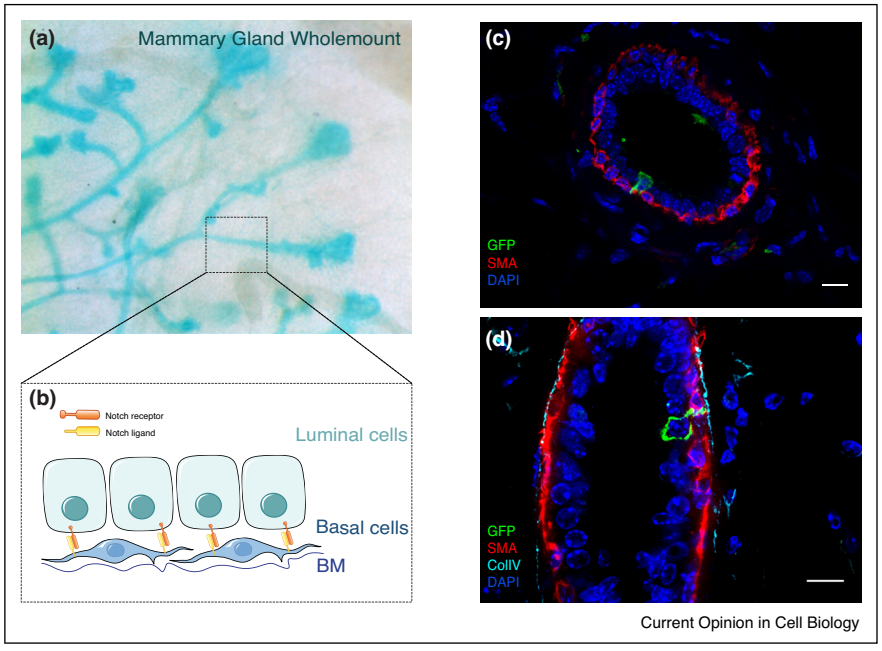
In addition to signalling between stem/progenitor cells and differentiated progeny, interactions with other cell types within the niche can modulate Notch activity. For example, a recent study revealed that Dll1-expressing mammary basal cells communicate with resident Notch-expressing macrophages during mammary gland development. Here, Notch activation in macrophages was shown to result in Wnt ligand secretion, defining a niche for mammary basal cells in the postnatal gland [30•]

Figure 1



Notch integrates niche signals to direct stem cell specification during organ morphogenesis and homeostasis.
Dynamic changes in cellular composition and tissue architecture during organ growth and regeneration expose stem cells to evolving niche environments, instructing gene regulatory networks such as Notch to guide lineage decisions in a highly regulated spatiotemporal manner. This includes communication between stem cells and their progeny (1), other neighbouring cell types (2), and the extracellular matrix (ECM) (3), in addition to mechanical cues associated with tissue morphogenesis. **(a)** In the intestinal crypt, Notch activation in intestinal stem cells (ISCs) by Dll1/4 ligand-expressing Paneth cells is crucial for their maintenance and differentiation. Notch activation leads to Hes1 expression which, in turn,

Figure 2



Cellular protrusions in Notch-expressing mammary luminal cells during mammary gland morphogenesis.
(a) Wholemount image of a mammary epithelial tree at puberty, stained with methyl green. **(b)** Schematic representation of the mammary epithelial bilayer, consisting of an inner layer of Notch-expressing luminal cells, and an outer layer of Delta-like-expressing basal cells adjacent to the basement membrane (BM). **(c–d)** Immunostaining of pubertal mammary gland tissues demonstrating that luminal Notch1-expressing cells (c) and Notch3-expressing cells (d) (marked by membrane GFP in N1-Cre^{ERT2}/R26^{mTmG} and N3-Cre^{ERT2}/R26^{mTmG} mice respectively) extend cellular protrusions that traverse the basal layer (marked by smooth muscle actin (SMA) in red). These protrusions allow luminal cells to contact the BM (marked by Collagen IV (ColIV) in cyan in d), and to be exposed to microenvironmental signals. Scale bar: 10 μm. Panel (d) reproduced from: @2013 LAFKAS *et al.* Originally published in the Journal of Cell Biology, https://doi.org/10.1083/jcb.201307046.

(Figure 1b). Intriguingly, Notch-expressing luminal cells extend cellular protrusions that cross the basal layer, also exposing them to mammary stromal signals (Figure 2). The functional significance of this behaviour, however, remains unclear. A similar heterologous niche was recently reported in skeletal muscle, where interstitial endothelial Dll4-expressing cells were suggested to stimulate Notch signalling in muscle stem cells (MuSC) situated under the BM [31]. It is noteworthy, however, that physical Notch-triggering cell contact across the BM remains to be demonstrated experimentally in both contexts [30•,31]. Indeed, soluble factors secreted by interstitial cells may modulate Notch signalling instead, as was recently demonstrated for the metalloproteinase, Adamts1. Adamts1 produced by macrophages at sites of muscle injury was shown to bind and degrade intracellular Notch1 in MuSC, promoting their activation [32] (Figure 1c).

(Figure 1 Legend Continued) represses the secretory cell determinant Math1 [33]. Communication between ISCs and Wnt-producing myofibroblasts (telocytes) across the basement membrane (BM) also regulates stem cell fate; however, a role for Notch in this context has yet to be identified. **(b)** During mammary gland development, interactions between ligand-bearing basal cells and Notch-expressing luminal cells ensure correct fate allocation [18,19•]. Luminal differentiation is specified by Notch through Hes/Hey-mediated repression of the basal determinant p63. Basal cells were also reported to induce Notch signalling in surrounding stromal macrophages, leading to Wnt ligand secretion to support basal cell maintenance [30•]. **(c)** Mechano-dependent activation of YAP by muscle contraction induces Jag2 ligand expression in chick myofibres that triggers Notch activation in muscle stem cells (MuSC), preventing their differentiation [43]. In addition, Notch activation induces the production of collagen V that acts as a surrogate ligand of the calcitonin receptor (CALCR) to maintain MuSC quiescence [39••]. Other cell types in the niche have been suggested to modulate Notch signalling in MuSC, including Dll4^{+} endothelial cells [31] and macrophages that secrete the metalloproteinase Adamts1 to degrade Notch receptors in response to damage (red arrow) [32]. **(d)** In the pancreas, a collagen/laminin rich ECM reduces integrin-FAK signalling, promoting endocrine specification [42••], and is associated with apical cell narrowing, basalward cell movement and eventual cell-rear detachment [23]. Concomitant with these morphogenetic changes, reduced Notch and YAP signalling leads to increased Neurog 3 (Ngn3) expression and endocrine differentiation. In contrast, exposure to a fibronectin-rich ECM maintains integrin production, resulting in F-actin bundling and increased cellular tension. This stimulates a F-actin–YAP1–Notch mechano-signalling axis that promotes ductal differentiation [42••]. Thus, coordination between mechanical cues and cell fate allocation is fundamental for pancreas development. Cell drawings were reproduced and/or modified from Servier Medical Art (http://smart.servier.com).

Collectively, these studies strongly imply that cellular flows during morphogenesis generate spatially restricted cues at precise developmental time points, dictating the preferential expression, or engagement, of a Notch ligand or receptor. In turn, this establishes directional signalling via well-established lateral inhibition mechanisms that impose differential cell fate to the progeny of stem and progenitor cells during development [4]. Moreover, this fundamental mechanism of action appears to be re-employed during tissue renewal, ensuring homeostasis of regenerative tissues throughout life. In the intestinal epithelium, for example, Notch safeguards that the correct ratio of absorptive and secretory cells are generated from multipotent stem cells throughout tissue homeostasis (reviewed in Ref. [33]). As dynamic and spatial deployment of niche signals intimately regulate cell fate commitment across metazoans, a systems-level approach that integrates morphogenetic and gene-regulatory programs into a larger 'niche framework' is necessary to unravel complex developmental patterning processes.

Notch signalling: a responder and constructor of the non-cellular niche

Alongside facilitating stem cells to sense and respond to their immediate neighbours, Notch also acts as a molecular bridge between stem cells and their non-cellular microenvironment. Indeed, the juxtacrine nature of Notch signalling ensures a spatially delimiting mechanism for localised and reciprocal connections between stem cells and the surrounding extracellular matrix (ECM). In addition to direct interactions between ECM proteins and Notch components, integration with other matrix-stimulated signalling networks, including integrins, also regulate Notch activity during morphogenesis [34•,35]. Basement membrane laminins, for example, stimulate Notch signalling by inducing β1-integrin mediated expression of Dll4 to regulate tip cell development during sprouting angiogenesis [36]. Conversely, during chick embryo somitogenesis, β1-integrin was shown to regulate Notch activity in a Wnt-dependent manner via integrin-linked kinase [37]. However, cross-regulation of Wnt and Notch signalling by integrins remains controversial, and further studies are needed to clarify the mechanisms underlying complex Wnt-Notch crosstalk. In contrast, recent biochemical analyses suggest that β3-integrin attenuates Notch responsive transcriptional activity by inducing c-Src-mediated phosphorylation of intracellular Notch (NICD) [34•]. The *in vivo* relevance of these results to physiological tissue morphogenesis, however, has yet to be established.

Notch signalling can also feedback to the ECM in a number of ways throughout tissue development and homeostasis. For example, a recent study revealed that Notch1 activity in the developing heart promotes ECM degradation (by inducing Adamts1 expression), driving the formation of endocardial projections that are critical for cardiac trabeculation. Here, antagonistic Notch1 and Neuregulin1 signalling spatially and temporally coordinate cardiomyocyte lineage specification with the complex morphogenetic processes necessary for establishing normal trabecular architecture [38•]. Notch signalling in adult skeletal muscle stem cells, however, has an opposing role, as it directly induces the secretion of extracellular collagens. Notch activation in MuSC, likely triggered by Dll-bearing myofibres, drives the expression of ECM collagen type V, which binds to Calcitonin receptor on MuSC to maintain their quiescent state [39••,40] (Figure 1c). Similarly, Notch signalling ensures the anchoring (homing) of emerging MuSC during development by regulating the expression of basal lamina components and adhesion molecules [41].

The topological architecture and physical constraints of the stem cell niche also profoundly influence cellular differentiation dynamics during organogenesis [16,17]. Mechanical stimuli during tissue shaping can control cell shape, localisation and spatial relationships with other cells [16], providing another dimension in Notch-mediated cell fate regulation. Indeed, by combining micropatterning with receptor *trans*-endocytosis assays and theoretical modelling, a recent study showed that the magnitude of juxtacrine Notch signalling was dependent on the cell–cell contact area, with smaller cells more likely to become signal-sending cells [13]. While recapitulated during early chick inner ear development, further *in vivo* studies are required to ascertain the generality of these intriguing results to other tissues.

Alternatively, the physical properties of the cell microenvironment may also regulate Notch activity through the YAP/TAZ mechanotransduction pathway (reviewed in Ref. [15]). In the developing pancreas, for example, interactions between integrins and fibronectin-rich ECM stimulates an F-actin–YAP1–Notch mechano-signalling axis that promotes ductal differentiation of bipotent pancreatic progenitors [42••] (Figure 1d). In this context, both cell extrinsic and intrinsic mechano-transduction pathways are coordinated to dictate the lineage decisions of pancreatic progenitor cells during organogenesis. In epidermal stem cells, however, mechano-activation of YAP/TAZ promotes epidermal stemness by inhibiting Notch-mediated keratinocyte differentiation [15]. In contrast, contraction-stimulated YAP/TAZ in myofibres induces Jag2 expression, triggering Notch activation in adjoining MuSC that prevents their myogenic differentiation [43]. Collectively, these recent studies highlight a role for Notch as a molecular link between YAP/TAZ mechano-transduction signalling and the cell microenvironment, guiding lineage decisions in response to structural changes during tissue morphogenesis. Finally, as ligand-applied force is required to induce proteolytic cleavage and activation of the Notch receptor [44], tissue mechanics could conceivably regulate Notch-driven cell

fate decisions directly during tissue shaping, an intriguing possibility that warrants further investigation.

Conclusions

The role of Notch signalling in determining cell fate throughout development is well established. How diverse intrinsic and extrinsic signals converge on Notch signalling to coordinate cell fate specification and tissue morphogenesis, however, is less clear. In light of the recent studies discussed above, we propose that Notch acts as a biological kapellmeister (orchestra conductor), coordinating spatial cues generated by cell flows during morphogenesis to dictate cell fate decisions at specific developmental times. Precise spatiotemporal integration of environmental cues likely drives the preferential expression of a Notch ligand or receptor, establishing directional signalling via lateral inhibition mechanisms that impose differential cell fate to the progeny of tissue stem cells.

An additional parameter that contributes to the complexity of Notch signalling is gene oscillations, a well-recognised mechanism of converting temporal information into spatial patterns during morphogenesis. Notably, Notch activity is known to oscillate during somitogenesis and brain development [45,46]. A recent study suggested that oscillation dynamics may couple different signalling outputs, demonstrating that the timing and rhythm of asynchronous Notch-driven and Wnt-driven gene oscillations are essential for correct presomitic vertebrate development [47••]. Whether oscillatory gene expression patterns drive the development of other tissues, however, remains unknown. It is tempting to speculate that gene oscillatory dynamics represents a mechanism of generating periodic bursts of signalling that are integral and, possibly, necessary for cell fate commitment during organogenesis.

The recent studies briefly summarised herein exemplify how direct links between transcriptional cell fate determinants and regulation of tissue morphogenesis are necessary for establishing the form and function of diverse tissues. Further work is needed to associate dynamic cell behaviours with fate acquisition, both during development and in tissue regeneration, where cells are exposed to new neighbours and niche signals. The emergence of tools that facilitate non-invasive spatiotemporal mapping of tissue mechanics, combined with improved lineage tracing and *in vivo* 4D imaging approaches, will undoubtedly yield exciting new insights into Notch-mediated control of cell fate specification and morphogenesis.

Conflict of interest statement

Nothing declared.

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