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DEVELOPMENT AT A GLANCE

Branching morphogenesis

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ABSTRACT

Over the past 5 years, several studies have begun to uncover the links between the classical signal transduction pathways and the physical mechanisms that are used to sculpt branched tissues. These advances have been made, in part, thanks to innovations in live imaging and reporter animals. With modern research tools, our conceptual models of branching morphogenesis are rapidly evolving, and the differences in branching mechanisms between each organ are becoming increasingly apparent. Here, we highlight four branched epithelia that develop at different spatial scales, within different surrounding tissues and via divergent physical mechanisms. Each of these organs has evolved to employ unique branching strategies to achieve a specialized final architecture.

KEY WORDS: Collective migration, Morphodynamics, Morphogen gradients, Mechanical stress, Smooth muscle

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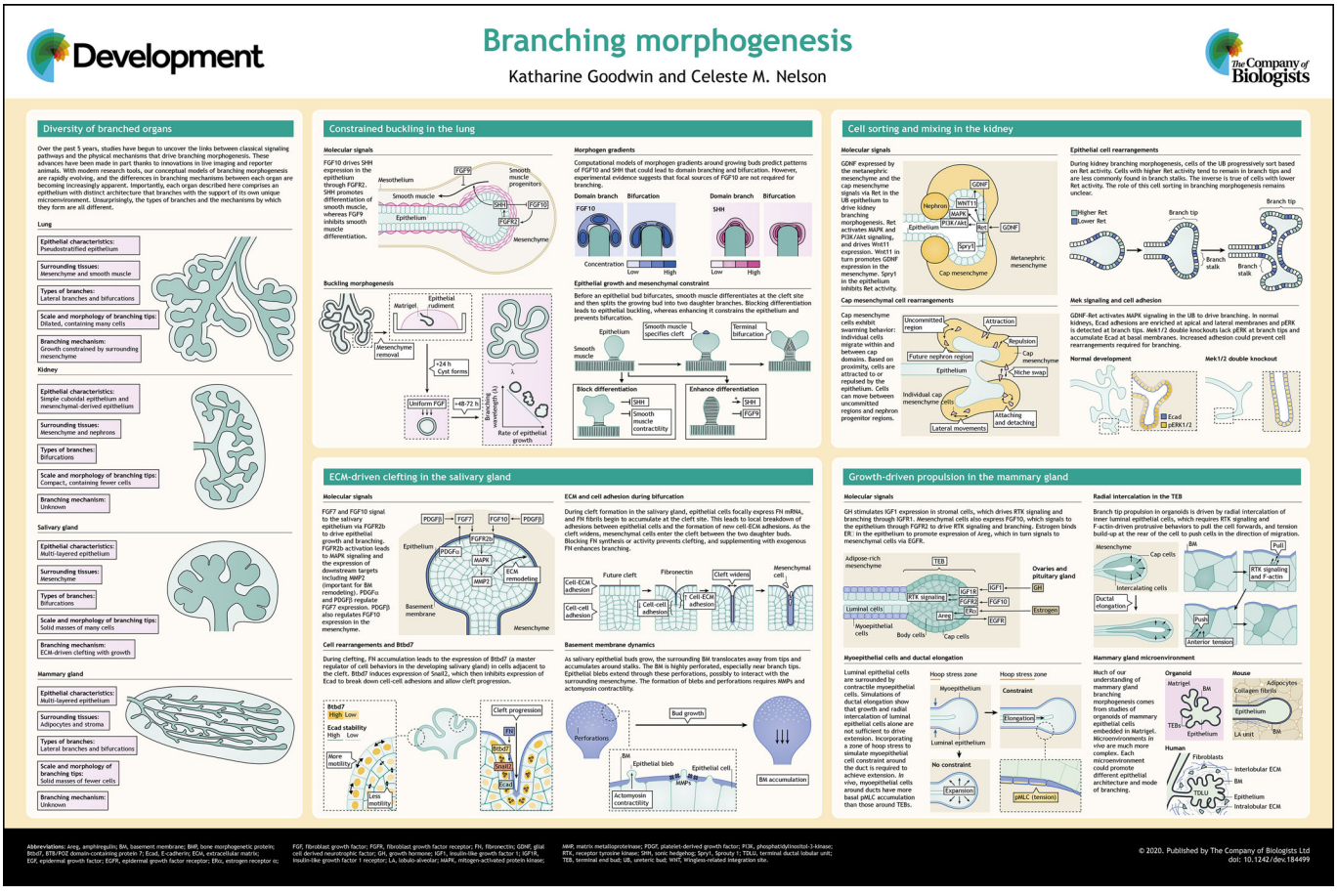
Introduction

The airways of the lung develop as relatively wide, single-layered epithelial tubes that form lateral branches and bifurcations. Branching of the mammalian lung epithelium is guided by molecular and physical signals from the surrounding mesenchyme and airway smooth muscle (Herriges and Morrisey, 2014; McCulley et al., 2015; Morrisey and Hogan, 2010; Spurlin and Nelson, 2017). The kidneys also contain single-layered epithelial tubes (albeit at a much smaller scale than those in the lung), which form primarily as a result of bifurcations *in vivo* and trifurcations during *ex vivo* culture (Short et al., 2014). Kidney branching is influenced by factors from the cap mesenchyme but, unlike the lung, occurs in the absence of a surrounding smooth muscle layer (Costantini and Kopan, 2010;



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Short and Smyth, 2016). Branching morphogenesis of the mammalian salivary gland occurs again by different mechanisms, in which the extracellular matrix (ECM) drives clefting of a multi-layered epithelium. Salivary epithelial bud elongation and clefting occur in the presence of important signals from the surrounding mesenchyme, but in the absence of smooth muscle (Myllymaki and Mikkola, 2019; Wang et al., 2017). Finally, the mammary gland uses yet another set of strategies to generate a branched architecture. During puberty, mammary branches are thought to be created primarily via bifurcation of multi-layered epithelial tips or from side-branching off existing ducts (Fata et al., 2004; Gjorevski and Nelson, 2011; Huebner and Ewald, 2014). Branching events are influenced by the surrounding stroma, and the elongation of ducts is aided by a smooth muscle-like population of myoepithelial cells that surround the basal surface of the luminal epithelium (Fata et al., 2004; Neumann et al., 2018). Importantly, although we can often describe the molecular requirements for branching, the physical mechanisms that initiate branches are largely unknown, especially in the kidney and the mammary gland.

Here, we review the biochemical signaling and physical processes that drive branching morphogenesis, and we emphasize how they are integrated to build current conceptual models of the development of the mammalian lung, kidney, salivary gland and mammary gland.

Constrained buckling in the lung

Branching morphogenesis of the murine lung begins from a simple, wishbone-shaped epithelial structure at embryonic day (E) 11.5 and culminates in a complex, arborized network at E15.5. Murine lung development is so highly stereotyped that the same sequence of branching events can be observed in every embryo. Each branching event is classified as either a domain branch or a bifurcation; domain branches establish the underlying architecture of the airways and bifurcations generate a space-filling tree (Metzger et al., 2008). In the mammalian lung, the branching epithelium is surrounded by the pulmonary mesenchyme, and the entire organ is surrounded by a thin layer of cells called the mesothelium. Mesenchymal cells differentiate into a variety of cell types required to support the form and function of the lung, including airway and vascular smooth muscle cells, myofibroblasts and lipofibroblasts (McCulley et al., 2015; Morrisey and Hogan, 2010).

Genetic manipulations have demonstrated that several diffusible signals are necessary for lung development, including fibroblast growth factor (FGF), sonic hedgehog (SHH), Wingless-related integration site (WNT) and bone morphogenetic protein (BMP), and that many of these are involved in crosstalk between the airway epithelium and the surrounding mesenchyme and mesothelium (McCulley et al., 2015; Morrisey and Hogan, 2010). In particular, the FGF10-SHH signaling loop is crucial for branching morphogenesis in the mouse lung. FGF10 expressed in the distal mesenchyme signals through fibroblast growth factor receptor 2 (FGFR2) in the epithelium to drive the expression of SHH by cells at the branch tips (Morrisey and Hogan, 2010). SHH released by the epithelium then signals to the mesenchyme to downregulate FGF10 expression locally and to drive smooth muscle differentiation (Morrisey and Hogan, 2010). Smooth muscle fate is suppressed by FGF9 signaling from the mesothelium, which maintains mesenchymal cells in a progenitor state (McCulley et al., 2015). In human embryonic lungs, FGF10 has different effects than in the mouse: instead of stimulating branching morphogenesis, treatment of explants with recombinant FGF10 leads to cystic, hypoplastic lungs, indicating that FGF ligands may have divergent roles in mouse and human (Danopoulos et al., 2019).

Computational models have been used to investigate how concentration gradients of FGF10 and SHH could generate branches and be shaped by airway epithelial geometry. Assuming focal sources of FGF10 and gradients that are regulated by FGF10-SHH feedback, these models predict the regions of the epithelium that will form new branches or bifurcations (Menshykau et al., 2012). However, genetic experiments in which FGF10-null mice are rescued by ubiquitous expression of FGF10 in the lung mesenchyme have revealed that branching morphogenesis does not require focal sources of this growth factor (Volckaert et al., 2013). Turing models of FGF10 and SHH ligand-receptor signaling combined with epithelial geometry can predict patterns of epithelial growth even without focal sources of FGF10 (Menshykau et al., 2014), but these models do not reveal anything about how the epithelium grows in response to activation of these pathways.

Although the molecular signals that are required for branching morphogenesis are well defined, it remains unclear how these stimuli synergize with mechanical signals and elicit physical changes in epithelial morphology. Growth of the airway is regulated in part by orientated divisions of airway epithelial cells, downstream of extracellular signal-regulated kinase (ERK) activity and mechanical forces (Tang et al., 2011; Tang et al., 2018). Divisions parallel to the long axis of the tissue drive tube elongation and are predominant in the earliest stages of branching morphogenesis, whereas divisions perpendicular to the long axis lead to tube widening. In mouse mutants with increased ERK signaling, control over cell division angle is lost and tube morphogenesis is impaired (Tang et al., 2011). Applying mechanical strain to the whole lung along the axis of the primary bronchi rescues the effects of overactive ERK signaling on division angles, restores the bias along the direction of strain, and partially rescues tube morphogenesis (Tang et al., 2018). These studies convincingly demonstrate that control over epithelial division angles regulates tube development in the lung, but it is unclear whether the orientation of cell division also contributes to domain branching or bifurcation (although some spatial correlations in division angle and branching events have been reported; Schnatwinkel and Niswander, 2013).

The airway epithelium is highly proliferative and is under outward pressure generated by its fluid-filled lumen. Luminal pressure promotes branching morphogenesis in microfluidic chest cavities (Nelson et al., 2017), and developmental defects in which luminal pressure is abnormal can impair lung development. Isolated epithelial rudiments embedded in Matrigel and cultured in the presence of a uniform FGF signal buckle owing to mechanical compression and growth-induced instabilities, resulting in the formation of branched structures (Varner et al., 2015). Within the intact lung, the epithelium retains this capacity for growth and buckling, but is subject to forces from the surrounding mesenchyme (Jaslove and Nelson, 2018; Short and Smyth, 2016). In particular, airway smooth muscle derived from the pulmonary mesenchyme physically constrains the epithelium. During bifurcation, epithelial buds are split into two new daughter branches by local smooth muscle differentiation at the cleft site (Kim et al., 2015). Similarly, smooth muscle differentiates on either side of emerging domain branches to regulate their position and morphology (Goodwin et al., 2019). Without patterned smooth muscle differentiation, the epithelium dilates and buckles; conversely, ectopic smooth muscle wrapping impedes domain branching and bifurcation (Goodwin et al., 2019; Kim et al., 2015). Epithelial buckling in the absence of mesenchymal or smooth muscle constraint has only been observed in culture models; it is currently unknown whether mechanical buckling could occur *in vivo* either under normal conditions or in response to smooth muscle perturbation. It is possible

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that the higher luminal pressure in lungs within the embryo precludes buckling and instead promotes the dilated, cystic phenotype observed in embryos with mutations that disrupt airway smooth muscle differentiation (Boucherat et al., 2015; He et al., 2017).

Integrating all of these findings, we propose a working model wherein molecular signals that drive epithelial growth and smooth muscle differentiation help to generate the necessary physical changes for branching morphogenesis. FGF10 promotes epithelial growth and production of SHH, which in turn stimulates local changes in mesenchymal gene expression and smooth muscle differentiation, leading to sculpting of the epithelium into the correct branched morphology. To fully elucidate the mechanisms that drive branching morphogenesis in the mouse lung, we must integrate our understanding of molecular and physical stimuli; precise spatiotemporal control of lung branching likely requires a combination of patterned activation of molecular signaling pathways and physical changes in morphology and tissue mechanical properties.

Cell rearrangements and cell sorting in the kidney

The mouse kidney initiates from the ureteric bud (UB) at E10.5-E11 and undergoes approximately ten rounds of branching until about E15.5 (Costantini and Kopan, 2010; Short and Smyth, 2016). The UB epithelium evaginates from the nephrogenic cord into the metanephric mesenchyme, which provides important signals for initiation and branching of the UB and contains cells that will eventually differentiate into nephrons (Costantini and Kopan, 2010; Short and Smyth, 2016). Epithelial branching in the kidney occurs via T-shaped bifurcations (*in vivo*) and trifurcations (in *ex vivo* organ culture), and nephrons form in the 'armpits' of these branches (Short and Smyth, 2016). Throughout the branching process, pools of mesenchymal cells at the tips of UB branches form the cap mesenchyme and adopt specific morphological and molecular characteristics. A subset of these cells remains as cap mesenchyme whereas another subset leaves the tip region and differentiates into nephrons (Short and Smyth, 2016).

The master molecular regulator of kidney development is glial cell-derived neurotrophic factor (GDNF) produced by the metanephric and cap mesenchyme. GDNF signaling through Ret in UB cells activates, among others, the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)-Akt signaling pathways, which are important for epithelial cell proliferation and kidney branching morphogenesis (Costantini and Kopan, 2010; Short and Smyth, 2016). Ret activity is suppressed by epithelial expression of sprouty 1 (Spry1) and induces the production of WNT11, which signals to the cap mesenchyme to maintain the expression of GDNF (Costantini and Kopan, 2010; Short and Smyth, 2016). These signaling networks may help establish feedback loops that regulate branching patterns, but this remains to be shown. Similar to its role in the lung, FGF signaling can also influence branching morphogenesis of the kidney in specific scenarios, albeit to compensate for loss of GDNF and perhaps not as a primary regulator. In kidneys that lack GDNF, eliminating Spry1 can rescue UB outgrowth, but further elimination of FGF10 abrogates this effect (Michos et al., 2010). Therefore, in the absence of GDNF and Spry1, other signaling pathways can compensate to rescue kidney branching morphogenesis.

Imaging and fluorescent labeling techniques have been developed to visualize the complex architecture of the kidneys and to track the dynamics of kidney morphogenesis in culture *ex vivo* (Riccio et al., 2016; Short et al., 2014; Watanabe and Costantini, 2004). These studies have revealed some of the differences between kidney branching *in vivo* and *ex vivo*; for example, lateral branches form off of the UB stalks during *ex vivo* culture, but these are not detected *in vivo* (Short and Smyth, 2016; Watanabe and Costantini, 2004). Combined with *in vivo* studies of reporter animals, live imaging of kidney explants has revealed that extensive cell rearrangements and sorting occur during UB outgrowth and branching.

Mosaic mouse models have been used to show that Ret-null cells are excluded from the UB outgrowth, whereas Spry1-null cells (with enhanced Ret activity) are preferentially found within the outgrowth. During branching morphogenesis, similar biases exist for cells with reduced Ret activity. Tracking wild-type clones showed that UB epithelial cells contribute approximately equally to tip and stalk regions, whereas Ret-null cells are progressively excluded from branch tips and accumulate only in stalks (Riccio et al., 2016). These data suggest that the UB may rearrange its constituent cells such that cells that are more responsive to GDNF or that have higher Ret activity are preferentially maintained at the branch tips. Epithelial cells of the UB also actively rearrange during mitosis; the mitotic cell delaminates from the basement membrane to divide and its daughters re-insert into the epithelial layer several cell lengths apart from their original location (Packard et al., 2013). It remains unclear how or whether these striking cell rearrangements contribute to branching morphogenesis of the UB. Dispersal and mixing of cells with slightly different gene expression programs could cause the formation of local 'instabilities' in the UB epithelium, leading to branching events (Short and Smyth, 2016). Cell sorting could also limit the number of proliferating tip cells to maintain smaller, refined branch tips (Costantini and Kopan, 2010). Similar sorting behaviors have been observed during the migration of *Drosophila* tracheal branches: the cells that respond most strongly to FGF overtake their neighbors to lead the collective (Ghabrial and Krasnow, 2006).

Dramatic cell rearrangements, or 'swarming' behaviors, also occur in the cap mesenchyme, where highly motile mesenchymal cells migrate between cap domains and establish transient contacts with the UB tip epithelium (Combes et al., 2016). These migrations are likely to be important for maintaining the cap mesenchymal pool, but it is unclear whether these behaviors also play a role in epithelial bifurcation or growth.

Ex vivo culture experiments and *in vivo* studies of mutant mice have shown that targeting Mek1, a core component of MAPK signaling, can shift relative growth due to tip splitting versus stalk elongation. When exposed to a Mek1 inhibitor, kidney explants exhibit fewer bifurcations but normal branch elongation (Watanabe and Costantini, 2004). Similar defects in organ morphology are observed in Mek1/2 double mutant mice, and closer examination of cell-level phenotypes revealed that loss of Mek1/2 leads to aberrant E-cadherin (also known as cadherin 1) accumulation in the UB epithelium (Ihermann-Hella et al., 2014). These findings suggest that bifurcation of the UB requires Mek activity and regulation of epithelial cell-cell adhesions. Furthermore, they provide a possible connection between GDNF or other growth factors upstream of MAPK signaling and cell-level behaviors and physical properties.

The physical mechanisms that drive bifurcation in the embryonic kidney will likely be elucidated using a combination of live-imaging experiments, genetic perturbations, and physical or mechanical computational models. In particular, we hope to see future investigations into the specific cellular behaviors (changes in morphology, contractility, etc.) within the UB epithelium that accompany branching events, and into possible mechanical stimuli from the cap mesenchyme that could induce or shape bifurcations.

ECM-driven clefting in the salivary gland

At E11, the salivary epithelial placode enlarges and invaginates into the surrounding mesenchyme to form the nascent salivary gland

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(Hauser and Hoffman, 2015). Unlike the lung and the kidney, the salivary gland is initiated and undergoes branching morphogenesis as a solid epithelial outgrowth. New branches are generated by clefting, wherein epithelial cells separate from one another as the result of localized deposition of ECM. The lumen forms later through a cavitation or hollowing-out process, leaving a bi-layered epithelium of luminal cells on the inside and basal cells on the outside (Myllymaki and Mikkola, 2019). Given these differences in tissue architecture, it is unsurprising that the exact mechanisms of branching appear to be different from those observed in the lung or kidney. However, there are several overlapping molecular signals that govern branching morphogenesis in each case, such as FGF signaling (Hauser and Hoffman, 2015).

Salivary gland initiation and branching in the mouse requires mesenchymal expression of FGF10 that signals through epithelial FGFR2b, similar to the lung (Hauser and Hoffman, 2015). In organ culture, FGF7 also signals to the epithelium through FGFRs to promote growth and branching, but the precise effects of FGF7 stimulation appear to be distinct from those of FGF10 and have yet to be confirmed *in vivo* (Steinberg et al., 2005). In the absence of mesenchyme, FGF10 promotes elongation of existing salivary epithelial buds, whereas FGF7 promotes formation of new buds (Steinberg et al., 2005). The effects of FGF7 and FGF10 on mesenchyme-free branching depend on matrix metalloproteinase (MMP) activity (Steinberg et al., 2005), which may be involved in localized ECM remodeling during clefting. Expression of FGF10 and FGF7 in the mesenchyme are regulated by platelet-derived growth factor (PDGF) signaling (Patel and Hoffman, 2014). Epidermal growth factor (EGF) signaling is also important for growth, branching and maturation of salivary glands (Patel and Hoffman, 2014).

The physical mechanisms of branching morphogenesis in the salivary gland have been well characterized using reporter mice, explant culture and live imaging. New branches in the salivary gland are formed through clefting. Fibronectin (FN) accumulates within newly forming clefts, leading to a breakdown of cell-cell adhesion and increase in cell-ECM adhesion in the adjacent epithelial cells (Sakai et al., 2003; Wang et al., 2017). Epithelial cells of the nascent cleft respond to the increase in FN by expressing BTB/POZ domain-containing protein 7 (Btbd7), a transcription factor that drives the expression of Snail2 (Snai2). Cleft cells that express Snail2 then downregulate E-cadherin, leading to a breakdown of cell-cell adhesions that promotes cleft progression (Onodera et al., 2010). Btbd7 also regulates the migratory behaviors of the epithelial cells that make up the surface of the salivary gland bud by regulating the extent of E-cadherin turnover (Daley et al., 2017).

The entire salivary gland is surrounded by a basement membrane, and ECM remodeling throughout this layer (not just in clefts) may play an important role in branching morphogenesis. Rho kinase signaling is crucial for maintaining the basement membrane layer around salivary branch tips and thus establishing correct epithelial polarity (Daley et al., 2012). The basement membrane is highly perforated, particularly around branch tips, and epithelial blebs protrude through these holes (Harunaga et al., 2014). Perforations depend on actomyosin contractility (which is likely necessary to physically remodel the ECM) and on protease-mediated degradation of the matrix (Harunaga et al., 2014). Photobleaching experiments revealed that the basement membrane translocates away from branch tips during elongation, and the number of perforations decreases steadily with increasing distance from the branch tip (Harunaga et al., 2014). Overall, these findings suggest that local basement membrane remodeling at branch tips may facilitate branch extension.

It remains to be determined how the signaling pathways involved in salivary gland development influence the cell behaviors and ECM remodeling that physically drive branching morphogenesis. Given the requirement for MMP activity downstream of FGF, these growth factors could activate a program of proteolytic and mechanical ECM remodeling by epithelial cells located at the tips of the buds. The links between growth factor signaling and clefting are less clear, but we anticipate that these will be uncovered soon through the use of live imaging and genetic analyses.

Growth-driven propulsion in the mammary gland

The earliest stages of murine mammary gland development are similar to those of the salivary gland; the mammary epidermal placode first invaginates into the primary mammary mesenchyme as a solid epithelial mass at E12 and forms a rudimentary branched network in the secondary mammary fat pad mesenchyme between E15 and E16, which hollows out shortly after birth (Gjorevski and Nelson, 2011; Huebner and Ewald, 2014). This structure continues to branch slowly until puberty (week 3) when a robust branching morphogenesis program commences (Howard and Lu, 2014). In the mouse, the onset of branching is marked by the formation of large, multi-layered branch tips called terminal end buds (TEBs) that extend into the adipocyte-rich stroma of the fat pad, leaving behind ducts that resolve into bi-layered epithelial tubes (Fata et al., 2004; Huebner and Ewald, 2014). TEBs comprise body cells surrounded by cap cells, whereas ducts comprise luminal epithelial cells surrounded by myoepithelial cells (Gjorevski and Nelson, 2011).

The molecular programs that initiate branching morphogenesis of the mammary gland are primarily downstream of hormones from the ovaries and the pituitary gland, specifically estrogen and growth hormone (GH) (Gjorevski and Nelson, 2011). GH activates the release of insulin-like growth factor 1 (IGF1) from stromal cells of the mammary fat pad, which then signals to epithelial cells via IGF1 receptor (IGF1R) to promote growth and branching (Gjorevski and Nelson, 2011). FGF10 signaling through FGFR2 is again important for mammary gland development, similar to the lung and salivary gland. The initial embryonic rudiment does not form in the absence of FGF10 or FGFR2, and FGFR2-null cells are outcompeted in the TEB by their wild-type counterparts during branching morphogenesis (Gjorevski and Nelson, 2011). Receptor tyrosine kinase activity is essential for TEB growth and ductal elongation (Myllymaki and Mikkola, 2019). EGF signaling is also induced by estrogen binding to estrogen receptor α (ERα; ESR1) during puberty; epithelial cells of the mammary gland express an EGF ligand called amphiregulin (Areg) that signals via epidermal growth factor receptor (EGFR) to stromal cells (Gjorevski and Nelson, 2011). Loss of Areg or EGF impairs branching, but the downstream mediators of this signaling pathway in mammary gland development are still unclear (Gjorevski and Nelson, 2011).

The mammary fat pad is relatively large and optically inaccessible, making live imaging of intact mammary glands challenging. Therefore, much of our understanding of mammary gland branching morphogenesis comes from organoid models in which dissociated mouse mammary epithelial cells that are embedded in reconstituted ECM (usually a combination of Matrigel and collagen) form clusters that undergo robust branching and ductal elongation (Huebner and Ewald, 2014; Neumann et al., 2018). Live imaging of mammary organoids has revealed that TEB elongation occurs via a collective, non-invasive, migration process (Huebner and Ewald, 2014). TEBs within organoids resemble those *in vivo* in that they are multi-layered agglomerations of highly proliferative and loosely connected cells that barrel forward into the surrounding matrix.

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Although the mechanisms by which TEBs bifurcate or by which side branches form are still unknown, studies have more extensively elucidated the mechanisms of branch elongation. In organoids, cells at the tips of branches polarize and radially intercalate towards the basement membrane at the leading edge of the bud under the control of receptor tyrosine kinase signaling, providing a directed force for collective migration (Neumann et al., 2018). Meanwhile, the ductal regions just behind the branch tips are surrounded by a myoepithelial layer that provides mechanical constraint and allows the TEB-like branch tip to propel itself forward (Neumann et al., 2018). It is still unknown whether myoepithelial cell-mediated constraint drives branch elongation *in vivo*. Unlike in organoids, where myoepithelial cell coverage around branch tips is sparse, TEBs *in vivo* are surrounded by myoepithelial-like cap cells. Increased accumulation of phosphomyosin light chain in ductal myoepithelial cells compared with tip myoepithelial cells has been observed (Neumann et al., 2018), but whether this leads to increased tension and sufficient constraint to propel the TEB forward is unclear.

Whereas the insights into mammary gland development gained using organoid models are invaluable, these behaviors have been evaluated in the absence of fat (adipocytes) – the actual gland may form into a tree using entirely different mechanisms within its native microenvironment. Studies *in vivo* and in culture have demonstrated that modulating the amount or composition of the ECM or manipulating cell-ECM receptors can impede mammary epithelial branching morphogenesis (Fata et al., 2004; Linnemann et al., 2015; Nguyen-Ngoc et al., 2012), demonstrating the importance of the native tissue microenvironment for mammary gland development. Furthermore, the microenvironment of the developing mammary gland is different in mouse (adipose-rich) than in human (dense, fibroblastic), which raises the possibility that branching morphogenesis proceeds differently in each species. Indeed, the functional unit of the human mammary gland, called the terminal ductal lobular unit, is morphologically distinct from the functional unit of the mouse mammary gland, called the lobulo-alveolar unit (Cardiff and Wellings, 1999). Differences in the morphogenesis of these structures may be related to their distinct microenvironments.

The mechanisms that control branching morphogenesis in the mammary gland are being elucidated using powerful combinations of organoids, genetics, reporter animals and computational models. These approaches, along with more challenging techniques such as intravital imaging (Scheele et al., 2017), will be key to uncovering how bifurcations and side branches are physically achieved, and to determining the role of the microenvironment in sculpting the mammary epithelial tree.

Conclusions

Significant progress has been made over recent years in uncovering the physical mechanisms that drive branching morphogenesis. There are of course many other branched organs that may employ similar mechanisms of branching morphogenesis to those reviewed here, or that (likely) have their own unique set of branching behaviors to achieve their specialized morphologies and functions. Tissues containing fewer cells at branch tips, including vascular networks and *Drosophila* tracheae, use more protrusive and invasive branching modes (Varner and Nelson, 2014). Prostate branching morphogenesis is accomplished by a solid epithelium that hollows out to form a lumen in the final stages of organogenesis, and may be guided by smooth muscle derived from the surrounding stroma (Toivanen and Shen, 2017). Even within specific branched organs, there are marked differences between the mechanisms that drive branching across species, including the examples discussed above in which human and mouse branching morphogenesis differ. For example, whereas branching of the mouse lung appears to occur by constrained growth, branch initiation in the chicken lung is achieved by apical constriction and active tissue folding (Kim et al., 2013).

Nature has developed several solutions to the problem of building branched tissues. By studying each of these unique strategies, we are moving towards more comprehensive models of branching morphogenesis that integrate genetic information and soluble factors with cell behaviors and mechanical signals.

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Competing interests

The authors declare no competing or financial interests.

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