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Review

Epithelial tube fusion as a mechanism for the development of complex lumen-containing organs

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ABSTRACT

Across both vertebrates and invertebrates, epithelial tissues fulfill a wide variety of necessary functions. These tissues frequently line the interior of organs involved in the exchange of matter, such as the lung or the intestine, but can also be responsible for secreting hormones or digestive agents as is the case with the pancreas. Because they are responsible for transporting fluids, many epithelial tissues contain an internal lumen and can have incredibly complex and branched architectures while still maintaining a continuous sheet. The epithelial tubes that comprise a single organ do not always develop together in the embryo; as such, mechanisms are needed to bring together and fuse distinct epithelial structures. These processes are observed notably in the *Drosophila* tracheal system, in the mammalian pancreas, and in the avian lung. Although the physical steps of epithelial fusion have been well described for select systems, the underlying molecular mechanisms remain unclear. This continues to be an active area of research with an abundance of potential health applications related to understanding developmental anomalies in these organs. By comparing how different organisms accomplish epithelial fusion, we hope to expand the engineering toolbox with which these anomalies can be treated.

KEYWORDS: morphodynamics, embryo, anastomosis, branching morphogenesis.

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ABBREVIATIONS

Arl3, Arf-like 3; Bmp, bone morphogenetic protein; Bnl, Branchless; Btl, Breathless; Cdc42, Cell division control protein 42; Dlt, Discs lost; Dpp, Decapentaplegic; DSRF, Drosophila serum response factor; Dys, Dysfusion; EGFR, Epidermal growth factor receptor; Esg, Escargot; FGF, fibroblast growth factor; Pnt, Pointed; Sas, Stranded at second; Sty, Sprouty; TGFβ, transforming growth factor-β; Trh, Trachealess; Wg,Wingless.

1. Introduction

Epithelial tissue, one of the four main types of animal tissue, is broadly conserved across vertebrates and invertebrates. Epithelial cells have a basal surface, which is in contact with the basement membrane separating the epithelial tissue from its surrounding mesenchyme, and an apical surface, which opposes the basal surface. These cells are tightly joined to each other, creating an impermeable tissue. Epithelial tissues can form diverse structures that provide a wide variety of functions including secretion of enzymes and hormones in organs such as the pancreas, gas exchange in the respiratory system, and nutrient exchange in the gut.

Epithelial morphogenesis is an essential part of development and has been well studied throughout the embryogenesis of many systems, ranging from sheet bending during gastrulation in *Drosophila* [1] and ascidians [2], to branching morphogenesis in the mammalian salivary gland [3], *Drosophila* tracheal system [4], and avian [5] and mammalian [6] lungs, and even postnatally in the pubertal

mammary gland [7]. These organs function in part by transporting fluids and thus they require a continuous lumen, which is sometimes generated through epithelial fusion. This process involves two distinct epithelial tissues coming together and forming new cellular junctions with each other, thus creating a single, unified tissue. These fusion events can vary from extensive remodeling of the epithelium to simple joining, or anastomosis, of branches. Examples include the fusion of secondary branches in the developing *Drosophila* trachea [8], anastomosis of airway parabronchi in the avian lung [9], and fusion of microlumens during branching of the mammalian pancreas [10, 11].

One system, the *Drosophila* trachea, has been extensively studied, in part due to its interesting resemblance to vascular branching, extension, and fusion in vertebrates [12]. In other systems, such as the avian lung, while some central features of the physical processes of the fusion events have been described, less is known about the underlying molecular cues. Gaining insight into these understudied systems could shed new light on developmental anomalies and improve the way we treat them. A crucial first step towards discovering these mechanisms is to obtain clues by comparing what is currently known about how various organisms accomplish epithelial fusion.

2. Fundamentals of epithelial fusion

Epithelial fusion is the process through which two distinct epithelial tissues become united into a continuous sheet or tube. While fusion can arise through different mechanisms, there are several conserved requirements for all epithelial systems undergoing fusion. First, the cells from either side of the future fusion site must move towards each other. A classic example of this type of process is wound healing, in which migration of the leading edge is induced by a combination of growth factors and chemoattractants [13] (Figure 1A). In this case,

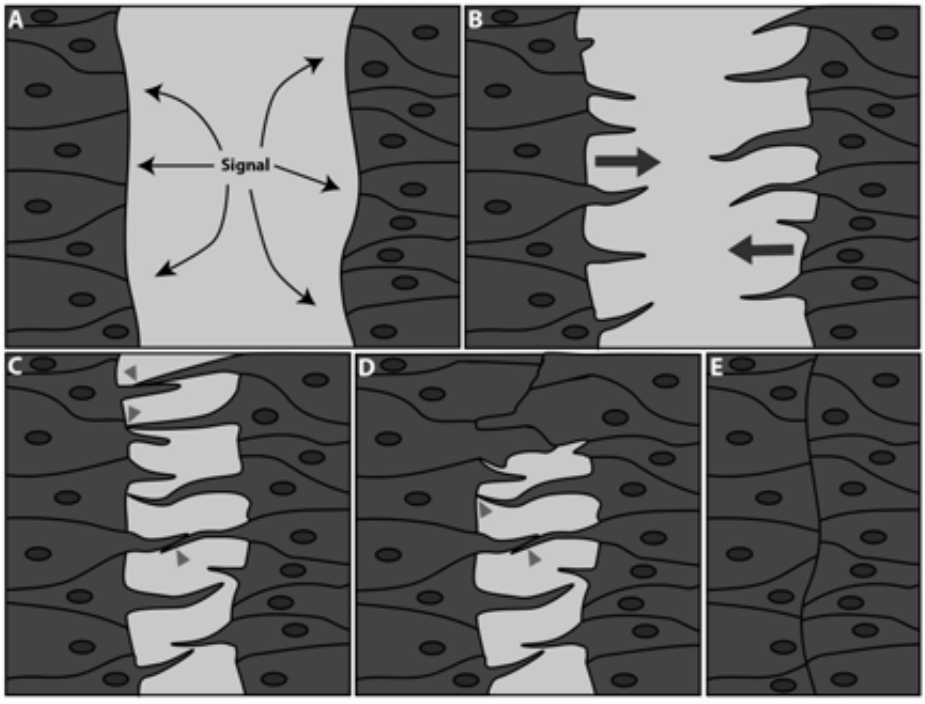

Figure 1. Overview of conserved requirements for epithelial fusion. (**A**) A signal is sent to the tissue to induce cellular changes involved in initiating the process of cell movement. (**B**) Cells begin to move towards each other (arrows indicate direction). In the case of wound healing, this is accomplished *via* lamellipodial extension. (**C**) Cells make contact (arrow heads) and cease movement. In wound healing, cells undergo 'contact inhibition' when they meet, which halts migration. (**D**) Cells begin to form new adhesions with each other. (**E**) Fusion is complete when all cells have finished forming new intercellular junctions, thus creating a continuous epithelium.

epithelial movement is driven by cell migration, which is governed by cytoskeletal reorganization and lamellipodial extension at the cellular front [14] (Figure 1B). Its role in this epithelial fusion process makes cytoskeleton-driven movement a promising candidate for the mechanism that drives motion in other systems.

In all cases of epithelial fusion, once the two cellular fronts have reached each other there must be some mechanism for initiating contact and halting cellular migration. Returning to our example of wound healing, when cells make contact they undergo 'contact inhibition', which is observed as a cellular spasm, and halt cell migration [15] (Figure 1C). This process ensures that the moving cells do not migrate past the cells they are advancing towards. This first contact is induced by filopodial extensions that act as the origins for the formation of nascent adherens junctions [15, 16]. The nascent junctions form along the leading edge of the epithelial sheets and unite the two epithelia (Figure 1D). Sensing contact and signaling for the production of new intercellular junctions is a complex phenomenon and understanding how this happens in other systems, as well as how to control it, could represent a significant advance in tissue engineering techniques.

This general process is used in many wound healing programs and is strikingly similar to the closure of the neural tube in the developing chordate [15, 17] and dorsal closure in the developing *Drosophila* embryo [18], which both also exhibit a migrating cell front followed by zippering of the two sides of what eventually becomes a continuous epithelial sheet. Considering these universal fusion requirements allows us to examine other systems and draw comparisons about how they function.

3. *Drosophila* tracheal system

In insects, the trachea is a system of epithelial tubules and sacs that transport gases and facilitate gas exchange with the tissues of the body [19]. Oxygen enters the system through spiracular openings and is transported by simple diffusion to body tissues [20]. While relying on diffusion alone may seem inefficient, this simple structure has supported the existence of insects through much of prehistory and into the modern era.

3.1. Overview of development

At stage 9 of embryonic development in *Drosophila*, the epithelial cells that are specified to become trachea are present as ectodermal placodes, and at stage 10 these placodes invaginate [19]. The cells in each placode undergo a final round of division during stage 11, after which they form incipient tubes called 'tracheal pits', which then undergo morphogenesis to become a defined stalk with six buds [19].

During stage 12 the buds extend into the body of the developing embryo to form primary branches [4, 19]. These branches extend towards target areas of the embryo, guided by fibroblast growth factor (FGF) signaling [4]. At stage 13, while most cells in the primary branch undergo extensive elongation and intercalation, two cells remain paired and eventually undergo cytoskeletal extension perpendicular to the primary branch. The extension of these two cells marks the initiation of the secondary branches off each primary branch [4].

These secondary branches continue to extend perpendicularly from the primary branch to meet up with complimentary branches from other hemi-segments to form a continuous anterior-posterior directed tube [19]. Terminal branches, which are simple cytoskeletal extensions, sprout from the fused secondary branches and ensure that all deep tissues receive oxygen [4]. Each tier of branching is characterized by a distinct set of genetic markers that regulate branch initiation, elongation, and fusion [4].

3.2. Branch fusion

Branching morphogenesis of the *Drosophila* trachea is highly stereotyped and guided by a strict developmental program [4]. Tracheal fusion is also highly stereotyped [8], thus ensuring that the tracheal network is continuous in the anterior-posterior direction, which is necessary to facilitate gas exchange to all the body tissues [19]. The fusion process is accomplished by modified tip cells, called 'fusion' cells, that are able to recognize and migrate towards their specific partner [8].

Initially, all tip cells in a migrating tracheal branch express the mitogen-activated protein kinase (MAPK) target genes *pointed* (*pnt*) and *sprouty* (*sty*) [4] (Figure 2A). At the onset of the overall fusion process

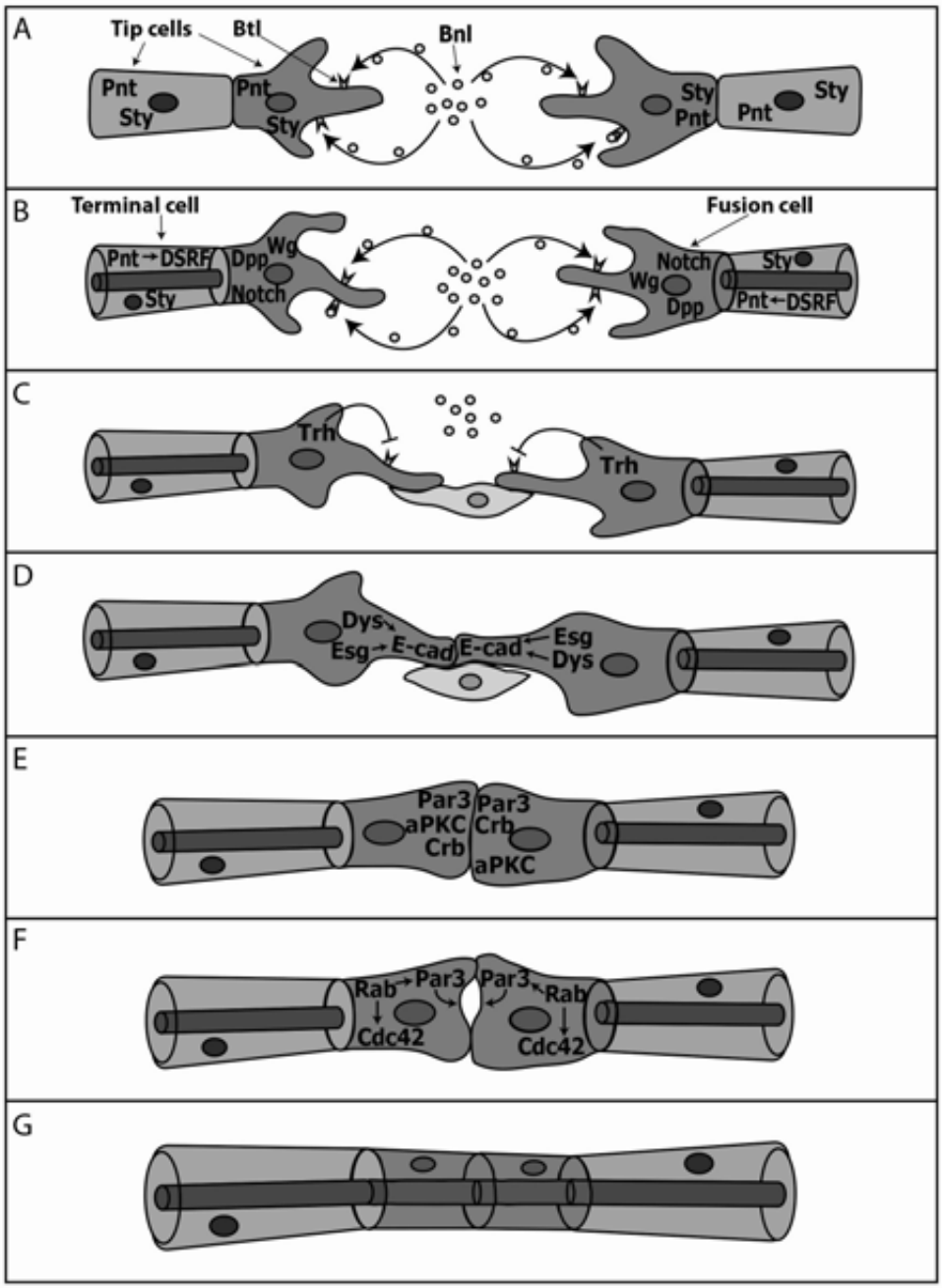

Figure 2. Tracheal fusion in *Drosophila*. (**A**) All tip cells express the MAPK target genes *pnt* and *sty* during outgrowth of trachea. Invasive branching of the trachea is guided by branchless (Bnl,) signaling through its receptor breathless (Btl, surface receptors). (**B**) Expression of *pnt* and *sty* becomes restricted to future terminal cells, where Pnt regulates DSRF which contributes to terminal cell determination. The terminal cell then forms a lumen by folding on itself and forming new adherens junctions. Fusion cell fate is specified by a signaling cascade of Wingless (Wg), Decapentaplegic (Dpp), and Notch. (**C**) When fusion cells approach each other, Bnl signaling is inhibited by trachealess (Trh), which downregulates Btl. The fusion cells also bind to the bridge cell. (**D**) The fusion cells contact each other and form an E-cadherin-stabilized connection regulated by Dysfusion (Dys) and Escargot (Esg). (**E**) Par3, aPKC, and Crumbs form an apical polarity complex at the site of fusion. (**F**) Lumen formation begins *via* Rab proteins that target Par3 to the apical surface and regulate Cdc42 activation. (**G**) The newly formed lumen quickly joins with the lumen of the terminal cells, creating a continuous lumen between the two branches.

this expression becomes restricted away from future fusion cells. Fusion cell fate is then further specified by a complex cascade of signaling through Wingless (Wg; *Drosophila* homologue of Wnt), Decapentaplegic (Dpp; *Drosophila* homologue of Bmp), and Notch [21-23] (Figure 2B). The fusion cells are closely followed by terminal cells that form lumenized cytoplasmic extensions to facilitate gas exchange with their target tissue [8].

Expression of *pnt* and *sty* is restricted to the cells fated to become terminal cells, where Pnt regulates the expression of other proteins including Drosophila serum response factor (DSRF), which contributes to the terminal cells becoming determined [4, 24] (Figure 2B). Sprouting and migration of tracheal branches is mediated by Branchless (Bnl; *Drosophila* homologue of FGF) signaling through its receptor Breathless (Btl), which is under the control of the transcription factor Trachealess (Trh) [25-27].

Once fusion cells approach each other, FGF signaling is downregulated by Trh [27] which regulates transcription of the FGF receptor Btl. Fusion is likely mediated by bridge cells that signal the fusion cells to come together by first binding to the bridge, then to each other [28, 29] (Figure 2C). The contact between the two fusion cells is stabilized by E-cadherin, which is upregulated under the control of Escargot (Esg) [30] and Dysfusion (Dys) [31] (Figure 2D). An apical polarity complex comprised of Par3, aPKC, and Crumbs forms at the site of fusion along with the proteins Stranded at second (Sas) and Discs lost (Dlt), which collectively render the fusion cell bipolar [32] (Figure 2E).

The first step in forming a continuous lumen between the fusion cells and the terminal cells is accumulation of membrane proteins necessary for lumen formation at the site of fusion cell contact, which is accomplished in part through the GTPase Arf-like 3 (Arl3) [33]. The new lumens initiate through the formation of an invagination in each fusion cell, which is accomplished *via* a network consisting of Rab proteins that target Par3 to the apical surface and control activation of the small GTPase Cdc42 [34] (Figure 2F). This lumen is quickly joined with that of the terminal cells and accounts for a relatively narrow section of the branch lumen [32] (Figure 2G). It is unclear how the fusion of the lumens themselves occurs, but Arl3 is required to transport the necessary components to the site of fusion [35].

The completion of lumen formation marks the end of this process of epithelial fusion and the generation of a continuous network of tubules. This example is somewhat unusual as it occurs between only two cells; the majority of epithelial fusion events observed during animal development occur between multiple cells.

4. Mammalian pancreas

The pancreas is essential for digestion as well as homeostasis of glucose [36]. These two very different roles make the pancreas an interesting organ to study for both its developmental biology and its physiology. Due to the multifaceted nature of pancreatic functions, it is simplest to consider the structures within the organ as two distinct functional groups: endocrine and exocrine.

The endocrine pancreas has significant medical relevance due to its involvement in insulin production and dysregulation during diabetes [36]. The endocrine function of the pancreas is carried out by cells in structures called the islets of Langerhans, which include α-cells, β-cells, δ-cells, and PP-cells [36]. β-cells are responsible for insulin synthesis and comprise roughly 80% of the islet cell mass [37].

The exocrine pancreas secretes digestive enzymes and is comprised primarily of acinar cells that form epithelial pouches at the ends of branches, called acini [36]. These cells contain a complex secretory apparatus on their apical surface that is responsible for secreting digestive enzymes into the duodenum [36]. Acinar cells are connected together *via* characteristically large gap junctions that facilitate transport of necessary components between cells [38]. These gap junctions are formed with the help of granules containing gap junction precursor proteins [38].

The exocrine and endocrine elements of the pancreas, though distinct in function, come together in a unique way to regulate essential processes in the organism. The development of this organ is equally complex and also requires that two distinct structures be united.

4.1. Overview of development

The pancreas stems from two buds that emerge from the embryonic foregut endoderm and give rise to the ventral and dorsal pancreas [36, 39]. Emergence of the dorsal bud is controlled by a

complex signaling cascade involving FGF, transforming growth factor-β (TGFβ), retinoic acid, vascular endothelial growth factor (VEGF), and Bmp inhibitors [40]. The ventral bud is connected to the cardiac mesenchyme and its growth is regulated by signaling through TGFβ, FGF, WNT, Hedgehog, and Notch [40, 41]. These protrusions are first observed in the mouse at embryonic day (*E*) 9.5 [39] (Figure 3A).

At *E*10 in the mouse, the mesodermal mesenchyme condenses around the pancreatic bud and accumulates on the left side of the gut tube, thus breaking the symmetry [36]. Islet structures also begin to form from the pancreatic epithelium at this time. At around *E*10.5, the dorsal bud starts proliferating and branching and continues to do so throughout the remainder of pancreatic development [36]. Epidermal growth factor receptor knockout ($EGFR^{-/-}$) mice show roughly a 50% decrease in the number of branches, indicating that EGFR signaling is necessary for normal pancreatic branching [42]. Between *E*11 and *E*12, the gut tube rotates and brings the growing ventral and dorsal buds close enough that they are able to fuse at around

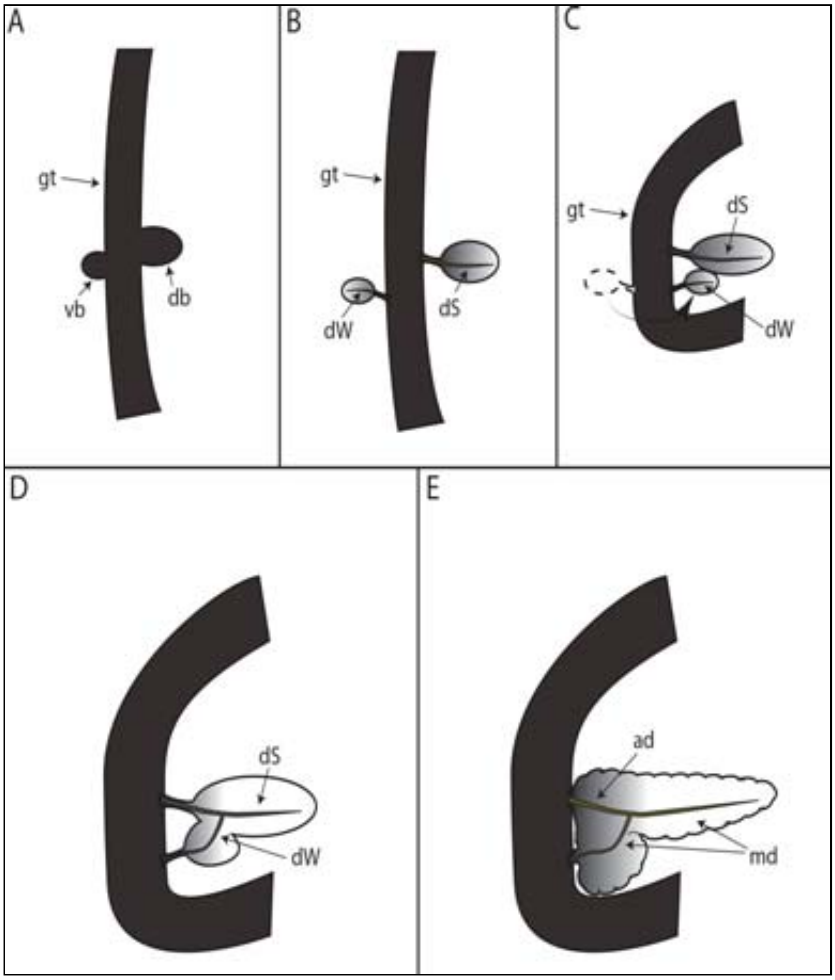

Figure 3. Fusion of dorsal and ventral pancreatic buds to create a continuous pancreatic duct. (**A**) Ventral bud (vb) and dorsal bud (db) emerge from the gut tube (gt) at *E*9.5. (**B**) By *E*10.5 the buds are proliferating rapidly and the ducts of Santorini (dS) and Wirsung (dW) begin to take shape. (**C**) Gut rotation occurs between *E*11 and *E*12, bringing the dorsal and ventral pancreatic buds into close proximity. (**D**) At around *E*13 the dorsal and ventral buds fuse and the ducts of Santorini and Wirsung join to create a continuous lumen. (**E**) Fully developed pancreas with the main pancreatic duct (md) and the accessory duct (ad).

*E*13 [36, 43]. Acini and ducts within the exocrine tissue become clearly visible by *E*14.5 [36].

4.2. Fusion of dorsal and ventral buds

During pancreatic development, the ventral and dorsal buds must come into contact and fuse in order to generate the main duct of the pancreas [44]. This occurs during the process of gut rotation [36] which takes place during the seventh week of gestation in humans [45] (Figure 3C). During this process, the gut tube in the region around the pancreatic buds, which will form the duodenum, physically twists and folds.

The fusion occurs between the superior branch of the ventral pancreatic duct and the dorsal pancreatic duct, which induces fusion of the duct of Wirsung and the duct of Santorini, thus generating the main pancreatic duct [45, 46] (Figure 3D, E). The duct of Wirsung fuses at a position midway along the duct of Santorini, the remainder of which forms the accessory duct [36] (Figure 3D, E).

The most common congenital pancreatic anomaly occurs when the ducts of Wirsung and Santorini fail to fuse correctly, leading to a condition called pancreas divisum [45, 46], which is associated with a higher incidence of pancreatitis [47] and increased risk for pancreaticobiliary tumors [48]. These potential health implications highlight the importance of proper epithelial fusion during pancreatic development. While the physical process of pancreatic fusion was described long ago [49], little is known about the underlying molecular signaling and cellular changes.

4.3. Fusion involved in pancreatic branching

4.3.1. Epithelial fusion is essential for normal pancreatic branching

Early studies suggest that branch and subsequent lumen formation in the mammalian pancreas occurs through fusion of microlumens [50, 51]. Microlumens are tiny enclosed cavities that densely occupy early pancreatic epithelial tissue at sites of future branching. This branching process was recently shown to be regulated by cell polarity [52].

In the mouse, pancreatic branches begin as wide protrusions studded with ‘tips’ [10]. During development, only short and long branches have been observed and no branches of intermediate or variable lengths. Instead, these short epithelial tips remain constant in size but increase in number over time [10, 11].

Using Muc1 immunostaining to label the lumens, it was revealed that prior to any appreciable branch formation, the epithelium has already formed a sophisticated luminal network of individual microlumens, called the luminal ‘plexus’ [10] (Figure 4A). This luminal plexus undergoes extensive remodeling in order to fuse the microlumens into larger lumens, and eventually a continuous luminal network [10] (Figure 4B, C).

These observations support a model wherein pancreatic branching occurs through an initial growth of the epithelium followed by dramatic remodeling and epithelial fusion. This is in contrast to branching in the mammalian lung or mammary gland, where a branch is initiated and then grows continuously and perpendicularly outward from the parent branch (Figure 4H). Remodeling in the pancreas is accompanied by a destratification of the epithelium, which is many cells thick during early development but reduces to just one cell in thickness once branches have formed [10]. The molecular cues for this remodeling are not fully understood, but some data suggest that it might be driven by interactions between proteins involved in neural migration and proteins involved in cell-matrix adhesion [53, 54].

4.3.2. Stratification process

The pancreatic bud originates from a polarized epithelium, but some cells undergo partial or complete loss of polarity during the stratification process that occurs prior to branching [10]. Upon the completion of stratification, the epithelial cells can be grouped by their polarity into three categories that are organized radially in the bud: ‘cap’ cells on the basal surface of the bud that maintain basal but not apical polarity, ‘lumen-lining’ cells on the luminal surface of the bud that lack basal polarity but maintain apical polarity, and ‘body’ cells located between the cap and lumen-lining cells that display weak basal polarity and no apical polarity [10] (Figure 4I). The process of stratification coincides with the period of pancreatic development in which the pool of multipotent progenitors is expanded, suggesting that stratification could play a role in ensuring the correct ratio of progenitors [10].

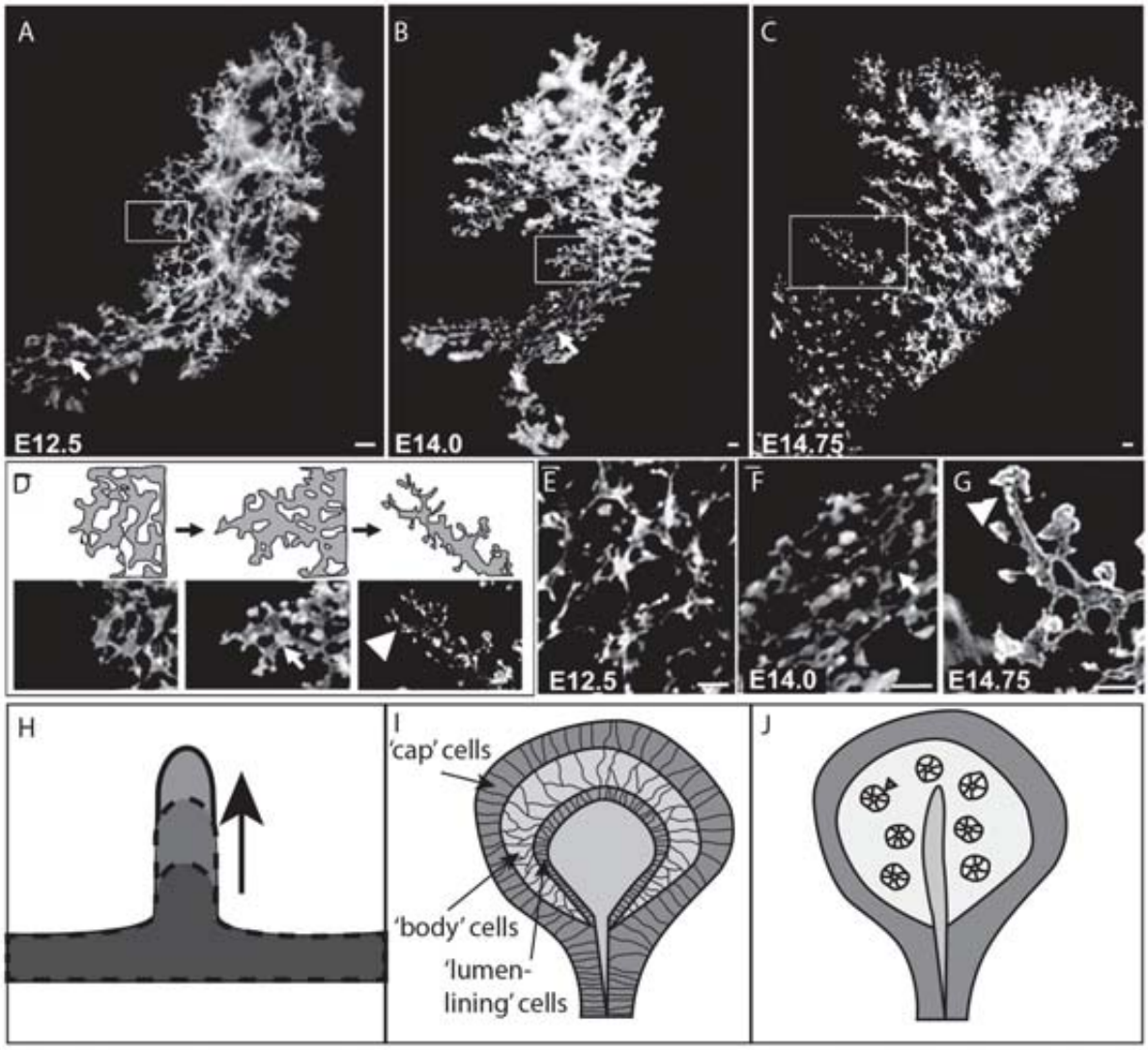

Figure 4. Reorganization of the lumen during epithelial fusion to facilitate branch initiation in the mammalian pancreas. (**A-C**) Muc1 immunofluorescence staining of the lumen during pancreatic branching. The pancreatic epithelium undergoes extensive remodeling to transition from a dense network of microlumens to fewer continuous lumens. (**D**) Magnified view of remodeling process with schematic. (**E**) Initial structure with thin lumens. (**F**) Lumens undergo noticeable widening prior to remodeling. (**G**) Epithelial remodeling and fusion create a single continuous lumen. (**H**) Schematic of parallel branch elongation, as occurs in other branched organs in mammals, including the lung. Transition from dark to light signifies advancement of time. (**I**) Stratified epithelial cells within a pancreatic bud. The cells can be sorted into three distinct groups based on polarity: 'cap' cells, 'body' cells, and 'lumen-lining' cells. (**J**) Cells orient themselves into a spherical 'rosette' and constrict their apical surfaces, causing a lumen to form at the center. (Panels A-G reprinted from Villasenor, A., Chong, D. C., Henkemeyer, M. and Cleaver, O. 2010, Development, 137(24), 4295-305 with permission from Company of Biologists).

4.3.3. Formation and fusion of microlumens

Some of the cells in the emerging bud alter their polarity and morphology [10]. The most notable morphological change is the visible apical constriction that occurs prior to the opening of a microlumen. The cells orient their apical ends at the center of what will become a spherical 'rosette', and as the apical surface constricts a lumen appears at the center [10] (Figure 4J). Microlumens later connect to each other *via* canals lined by polarized epithelial

cells [10]. This fusion is followed by extensive remodeling of the luminal plexus to transform the bud from a dense network of lumens to a branched structure with only a few continuous lumens. When this remodeling is complete, all cells have become destratified and returned to a polarized state [10].

This example of epithelial fusion differs somewhat in appearance from the classical examples of neural tube closure and *Drosophila* tracheal fusion described above. Instead of two fronts or branches coming together, the pancreatic branching process unites and reorganizes many cells in a much more complex geometry. However, if one considers each of the individual fusion events that occur during pancreatic branching in isolation, it becomes possible to draw some comparisons to fusion events in the *Drosophila* tracheal system. In both cases, cells must receive a signal that instructs them to migrate to their final position and to create new cell-cell junctions as necessary, thus uniting lumens. While there is no evidence for a conserved underlying molecular mechanism, reducing the process of epithelial fusion to general and basic requirements allows us to examine and compare the different ways in which systems have evolved to solve similar developmental problems.

5. Avian lung

All terrestrial vertebrates require a lung to facilitate gas exchange with the air. This organ transports oxygen from the surrounding environment into the blood and extracts toxic carbon dioxide to be exhaled. However, while all terrestrial vertebrates have lungs, the morphology differs dramatically between classes.

5.1. Overview of development

The development of the mammalian lung can be grouped into five main stages based on morphology: embryonic, pseudoglandular, canalicular, saccular, and alveolarization [55]. These stages are divided by the extent of branching. During the first four stages of mammalian lung development, the airways undergo extensive branching morphogenesis, each round creating a new generation of airways. This branching culminates in the formation of terminal buds that then undergo extensive folding during alveolarization to increase the surface area for gas exchange [55].

In contrast, the development of the avian lung is more continuous and cannot easily be divided into stages [56]. Additionally, avian lung development culminates in a network of connected airways as opposed to the terminally branched structures seen in the mammalian lung [56]. The epithelial network in the avian lung allows for unidirectional airflow and cross-current gas exchange and because of this, the avian respiratory system is more efficient than the mammalian lung [57, 58].

Early development of the avian lung is similar to that of mammals, with iterations of branching, but culminates with fusion, or anastomosis, of airway parabronchi to form a continuous network of tubes without terminal ends [9]. This connected architecture permits the unidirectional flow of air.

5.2. Avian airway anastomosis

Airway anastomosis in birds was first described in 1916 [9], but the underlying mechanisms remain enigmatic more than 100 years later. The process of airway fusion occurs between embryonic day 10 and 13 in the chicken. At *E*10, the airway parabronchi traverse the outer region of the lung parallel to each other and in two opposing fronts (Figure 5A). By *E*11 some of the airways have undergone anastomosis while many remain unfused (Figure 5B). At *E*12 more of the airways are fused and by *E*13 fusion is complete (Figure 5C).

We have observed that the tips of approaching parabronchi bend away from each other prior to fusion (Figure 5E-G). This observation suggests that tips may repel each other, possibly to keep the parabronchi parallel as they extend. This also suggests that fusion may result from either stalk-stalk interactions or stalk-tip interactions. However, the cellular and molecular cues that initiate these interactions remain unexplored.

Anastomosis has been observed to occur not only between parabronchi from opposite sides of the migration front but also between parabronchi on the same side (Figure 5F), suggesting that anastomosis in the avian lung is not limited to partner branches, as is the case in the *Drosophila* trachea.

6. Discussion and comparisons

Pancreatic bud fusion appears somewhat similar to the process of tracheal fusion, because both rely

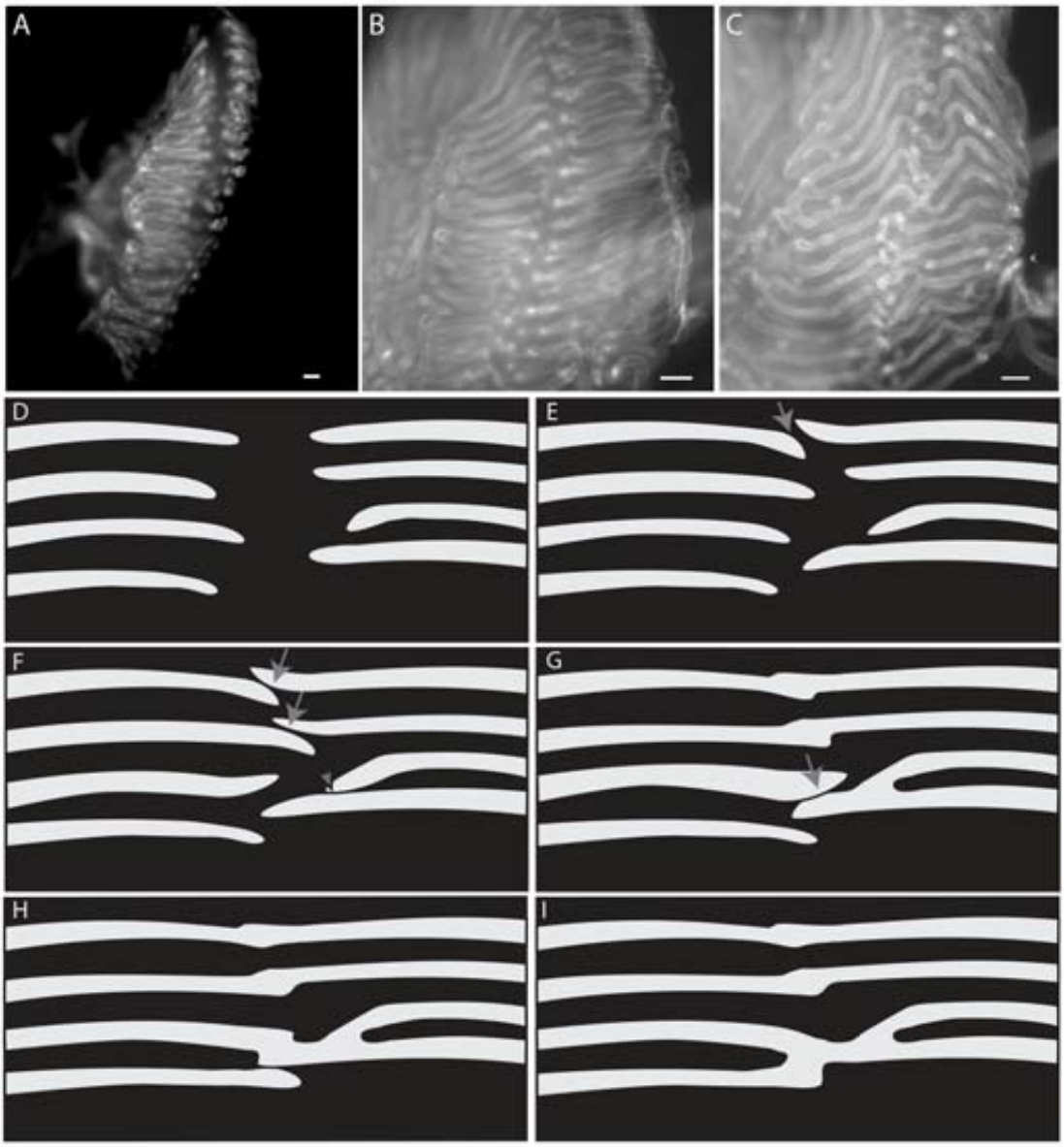

Figure 5. Overview of epithelial fusion in avian lung development. (**A**) Chicken lung stained for E-cadherin at *E*10. Parabronchi are organized into two approaching fronts. (**B**) Chicken lung at *E*11. The two fronts of parabronchi are nearing fusion at this stage. (**C**) Chicken lung at *E*12. Parabronchi have undergone fusion to create a continuous network. (**D**) Schematic of two opposing fronts of parabronchi moving towards each other. (**E**) As parabronchial fronts approach each other, tips that come into close proximity to other tips bend away (arrows). (**F**) Some parabronchi fuse with those in the same moving front (arrowhead). First contact is made between parabronchial stalks and not directly by tips. (**G-I**) Fusion occurs in some parabronchi before others and continues for approximately a day before all fusion events are completed. Scale bars, 100 μm.

on the fusion of two distinct branches, albeit on very different length scales. Similar to the tracheal system, the pancreatic buds require mechanisms for sensing each other, guiding cell movement, initiating fusion, and connecting lumens. The lumen-forming mechanism is likely more complicated in the pancreas than in the trachea because the pancreatic lumen is formed by many cells as opposed to the simple single-cell process in the trachea. However, it will be interesting to determine whether the signaling

pathways responsible for directing the movement and initial fusion of branches in the trachea are involved in guiding the pancreatic buds. Better understanding this process could shed light on whether animals have conserved mechanisms for epithelial fusion or whether these seemingly similar processes are simply examples of convergent evolution.

Despite differences in the number of branches involved, it is also unclear whether there could be any conserved mechanisms between tracheal fusion in insects and parabronchial fusion in birds, given the lack of available information regarding airway anastomosis. It is possible that the growth of airways towards one another and subsequent formation of new cell-cell junctions are guided by similar signaling pathways in both *Drosophila* and avian systems.

The steps of the fusion process can also be compared between avian lungs and pancreatic buds. Pancreatic bud fusion and airway anastomosis would be interesting phenomena to examine and compare for conserved mechanisms in vertebrates. These findings could then be used to compare to similar processes in more anciently evolved organisms, such as *Drosophila*, to gain insight into the evolutionary trajectory of these processes.

From a signaling standpoint there are some key pathways that are known to direct fusion in invertebrates such as *Drosophila* that could be conserved in the fusion events discussed in vertebrates. Ephrin receptor B2 (EphB2), a receptor tyrosine kinase, is known to be important for pancreatic branching in mice [10]. The Bnl pathway, which guides growth of the tracheal system of insects, is also a receptor tyrosine kinase pathway, indicating that this group of pathways might be conserved. Furthermore, FGF10, a vertebrate homologue of Bnl, is known to be expressed and to direct branching early in the development of the avian lung [59]. FGF10 is also present early in pancreatic development, and although not known to be involved in branching, it plays a role in maintaining the proliferative capacity of epithelial progenitor cells [60]. While there is a lack of information regarding gene expression at the fusion stages discussed in the mammalian pancreas and the avian lung, it is possible that FGF, along with other signaling pathways involved in tracheal fusion in insects, such as BMP and Notch, could play a role in the late-stage development of these vertebrate systems as well.

Another possible model system from which to obtain clues towards understanding epithelial fusion in the avian lung and mammalian pancreas is the process of blood vessel anastomosis in vertebrates. While the vascular system is comprised of endothelial cells, it shares many developmental characteristics with the epithelial *Drosophila* trachea, namely the use of tip cells for branching and guiding branches towards a chemoattractant [61]. When vascular branches undergo anastomosis, the cell junctions formed immediately following cell contact between two tip cells re-arrange into rings, which induces the formation of an intercellular surface of apical membrane compartments [62]. The subsequent unification of the lumens can occur by one of two processes: “membrane invagination”, which is similar to the process seen in the *Drosophila* trachea, and “cord hollowing”, during which the apical membrane compartments come together following cell rearrangement and extensive junctional remodeling, resulting in a multicellular tube [62]. While not a process in epithelial tissue, cord hollowing to form a multicellular lumen in the vascular system could shed some light on how epithelial fusion occurs in other multicellular luminal organs such as the avian lung and the mammalian pancreas. Combining what is known about the mechanisms driving branch fusion in both the vertebrate vascular system and the *Drosophila* trachea could help to illuminate the evolutionary trajectory of more complex fusion processes observed in vertebrates.

7. Conclusion

Organs that contain an internal lumen fulfill a wide range of functions in animals, from mass exchange involved in breathing to the production of enzymes that enable digestion. Although these organs require a continuous lumen, their morphogenesis frequently involves fusing two or more distinct luminal structures. This is accomplished in a variety of ways ranging from the fusion of two individual cells to form a single-cellular lumen, as occurs in *Drosophila*, to the fusion of large multicellular structures as occurs during the morphogenesis of the mammalian pancreas or avian lung.

Little is known about the molecular mechanisms that regulate fusion events in vertebrate systems, despite the fact that these processes were first observed roughly 100 years ago. In contrast, the molecular signaling pathways and morphological changes that drive the fusion of *Drosophila* tracheal branches have been well described. All of these processes involve the fusion of two lumens except for branching in the developing pancreas, in which many microlumens undergo extensive fusion during epithelial rearrangement to form branches. *Drosophila* tracheal fusion involves two partner cells that navigate through the embryo to connect with each other, whereas parabronchi in the avian lung are capable of fusing to parabronchi ahead of or adjacent to their migration trajectory.

In summary, the fusion of epithelia to create continuous lumens is a process observed in a variety of organisms but accomplished in multiple ways on different length scales. The cellular and molecular signaling that drives fusion events in the avian lung and murine pancreas are unknown and therefore this remains an active area of research. Better understanding epithelial fusion could greatly improve how we view and treat developmental anomalies involving similar processes in humans.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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