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Review

Tube fusion: Making connections in branched tubular networks

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ABSTRACT

Organs like the vertebrate vascular system and the insect tracheal system develop from separate primordia that undergo fusion events to form interconnected tubular networks. Although the correct pattern of tubular connections (anastomoses) in these organs is crucial for their normal function, the cellular and molecular mechanisms that govern tube fusion are only beginning to be understood. The process of tube fusion involves tip cell specification, cell–cell recognition and contact formation, self-avoidance, changes in cell shape and topology, lumen formation, and luminal membrane fusion. Significant insights into the underlying cellular machinery have been provided by genetic studies of tracheal tube fusion in *Drosophila*. Here, we summarize these findings and we highlight similarities and differences between tube fusion processes in the *Drosophila* tracheae and in the vertebrate vascular system. We integrate the findings from studies *in vivo* with the important mechanistic insights that have been gained from the analysis of tubulogenesis in cultured cells to propose a mechanistic model of tube fusion, aspects of which are likely to apply to diverse organs and organisms.

Contents

1. Introduction

Tubular organs, such as the lungs, mammary glands, kidneys, and the vascular system perform vital body functions, such as gas exchange, excretion, and nutrient transport. The cellular and molecular principles underlying branching morphogenesis in these organs are being revealed by many studies in vertebrate and invertebrate model systems. Developmental programmes involving reiterative rounds of spatiotemporally controlled branching events may explain the complex structures of organs such as the mammalian lungs [1]. An additional layer of complexity is found in organs such as the vascular system, where vessels link up with each other to form anastomoses (connections between branches), thus giving rise to interconnected tubular networks [2–4]. Using terms from network theory, lungs and mammary glands represent tree-like structures, whereas the vascular system represents a mesh-like structure. Constructing a mesh-like network requires, in addition to branching events, the formation of connections between specific branches in the network. In biological terms, this involves the recognition and formation of contacts between specific cells, followed by morphogenetic events that generate a patent lumen for

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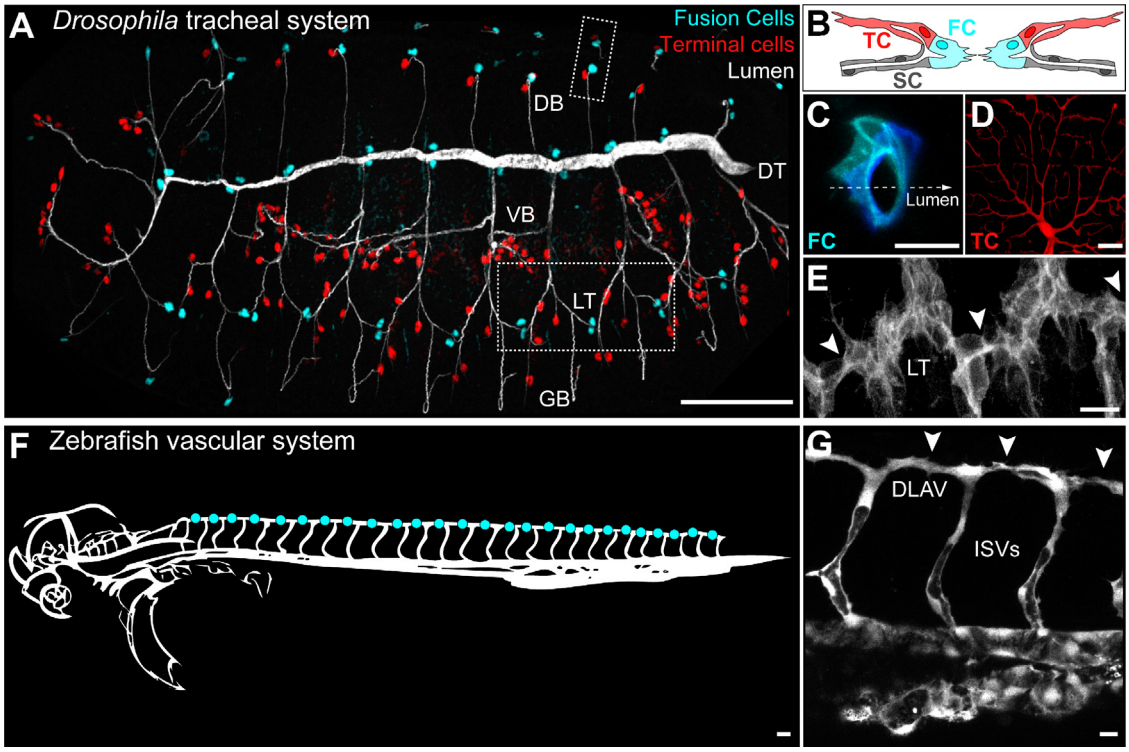

Fig. 1. Anastomoses in the *Drosophila* tracheal system and in the Zebrafish vascular system. (A) Lateral view of the tracheal system in a *Drosophila* embryo (15 h after egg lay). Each branch contains a stereotyped set of fusion cells (FCs; cyan nuclei) and terminal cells (TCs; red nuclei). The tracheal lumen is shown in white. Abbreviations indicate primary branches: DT, dorsal trunk; LT, lateral trunk; DB, dorsal branches; GB, ganglionic branches; VB, visceral branches. (B) Schematic representation of one DB anastomosis (region marked by small box in (A)). Each DB contains one FC (cyan) and one TC (red) connected to a stalk cell (SC; grey). Contralateral FCs meet at the dorsal midline. (C) Confocal image of a FC pair in the DT. Plasma membranes of the two cells are labelled in blue and cyan, respectively. FCs are doughnut-shaped cells lacking autocellular junctions. The arrow marks the luminal axis. (D) Confocal image of a larval TC expressing cytoplasmic GFP (red). TCs are ramified cells containing seamless lumina. (E) Confocal image of fused LT branches (large rectangle in (A)). Cells are labelled with a membrane marker. Arrowheads indicate fusion points. (F) Schematic lateral view of vascular system in larval Zebrafish (36 hpf). Positions of intersegmental vessel (ISV) anastomoses in the trunk are indicated by cyan dots. Note that anastomoses also occur in many other positions not marked in this image.(G) Confocal image of ISVs in the trunk region of a Zebrafish embryo expressing GFP controlled by an endothelial-specific promoter. Arrowheads indicate anastomosis sites in the dorsal longitudinal anastomotic vessel (DLAV). Images in panels (F) and (G) were kindly provided by Anna Lenard and Markus Affolter. Scale bars: (A, D, F), 50 μm; (C, E, G), 10 μm.

efficient liquid transport through the tubular connection. This process, which we refer to as tube fusion, takes place in organs, which derive from initially separate tubular units that get connected during subsequent development. During angiogenesis, the vascular network is remodelled through the fusion of certain endothelial sprouts with each other or with pre-existing vessels [5,6]. Despite the fundamental role of this process in angiogenesis, only few studies have investigated the mechanism of blood vessel fusion [5,7–10]. Notably, live imaging studies in zebrafish have revealed a first view on blood vessel fusion at cellular resolution [5,7,9]. Besides angiogenesis, tube fusion also occurs during vertebrate kidney development. The kidney originates from separate precursors, the metanephric mesenchyme and the ureteric bud, each of which form tubes that subsequently fuse to generate a functional excretory system [11,12]. Despite their key role in nephron formation, the cellular and molecular basis of these fusion events is not understood. Tubular networks are also found in invertebrates. The insect tracheal system is a network of gas-filled epithelial tubes that delivers oxygen to the tissues (Fig. 1A; [13–16]). Tracheal development shares considerable similarities with angiogenesis: in both cases branched tubular trees are built through tip-cell-guided collective cell migration, and tube fusion events generate anastomoses between branches in the tree (Fig. 1F and G; [7,17]). Genetic studies of tracheal tube fusion in *Drosophila* have revealed insights into the underlying cellular and molecular machinery. In this review, we summarize these findings, highlighting similarities and differences between flies and vertebrates.

2. Tube fusion: what has to be accomplished

The formation of connections in epithelial or endothelial tubular networks is orchestrated by specialized cells located at the tips of the branches undergoing fusion. Tip cells were first described in the developing vascular system of quails and in the tracheal system of *Drosophila melanogaster* nearly 20 years ago [18,19], although their significance in leading angiogenic sprouts was only demonstrated by studies of angiogenesis in the mouse retina some years later [20]. In the following, we first discuss the mechanisms of tip cell selection and of tip cell-guided migration of fusion sprouts towards each other. Next, we review the mechanisms of cell–cell recognition, contact formation, and repolarization involved in tube fusion. Finally, we discuss roles of the cytoskeleton and of the membrane trafficking machinery in generating, expanding and joining luminal spaces. In this context, it is important to emphasize that tip cells actually do not fuse during tube fusion, unlike, for instance, myoblasts, which form syncytia during muscle development. Instead, the term tube fusion [21] refers to a cell hollowing mechanism employed by tip cells to connect ("fuse") pre-existing lumina formed by the stalk cells (SCs) adjacent to the tip cells.

3. The *Drosophila* tracheal system as a model for studying tube fusion

The tracheal tree develops from twenty epidermal placodes, ten on each side of the embryo, each composed of approximately 80

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