Developmental Biology 451 (2019) 5–15

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review article

Single-cell branching morphogenesis in the Drosophila trachea

Benedikt T. Best[a,b]

[a] Director's Research Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstr. 1, 69117 Heidelberg, Germany
[b] Collaboration for Joint PhD degree from EMBL and Heidelberg University, Faculty of Biosciences, Germany

ARTICLE INFO

Keywords:
Branching
Cytoskeleton
Actin
Microtubules
Hypoxia
Modelling
Angiogenesis

ABSTRACT

The terminal cells of the tracheal epithelium in Drosophila melanogaster are one of the few known cell types that undergo subcellular morphogenesis to achieve a stable, branched shape. During the animal's larval stages, the cells repeatedly sprout new cytoplasmic processes. These grow very long, wrapping around target tissues to which the terminal cells adhere, and are hollowed by a gas-filled subcellular tube for oxygen delivery. Our understanding of this ramification process remains rudimentary. This review aims to provide a comprehensive summary of studies on terminal cells to date, and attempts to extrapolate how terminal branches might be formed based on the known genetic and molecular components. Next to this cell-intrinsic branching mechanism, we examine the extrinsic regulation of terminal branching by the target tissue and the animal's environment. Finally, we assess the degree of similarity between the patterns established by the branching programs of terminal cells and other branched cells and tissues from a mathematical and conceptual point of view.

1. Introduction

Among branch-forming cell types, terminal cells (TCs) of the respiratory system in fruit flies must overcome a unique challenge: To fulfill their function as the oxygen source for internal organs, they combine the extension of branched cellular processes with the growth of a subcellular tube hollowing each branch for gas transport. These terminal tracheal branches can exceed lengths of 200 µm, leading to cell sizes few other cell types approach (Fig. 1A).

In *Drosophila melanogaster*, around 300 cells adopt the TC fate during the late stages of embryogenesis in stereotypical locations within the tracheal system. Tracheal cells stop dividing at the beginning of the tissue's development (Djabrayan et al., 2014; Nishimura et al., 2007). Consequently, instead of dividing asymetrically, cells are assigned their fates and specific location within the tracheal anatomy by switching on and off regulatory transcription factors (TFs) in response to positioning signals such as *hedgehog* and *notch* (Kato et al., 2004; Petit et al., 2002). The mechanisms determining the TC fate decision are well-understood for TCs of the dorsal tracheal branches and involve an interplay of *fgf*, *sprouty* and *escargot*. This process has been reviewed previously and will not be discussed further here (Ghabrial et al., 2003; Schottenfeld et al., 2010). Once their cell fate is specified, TCs follow chemotactic guidance tracks laid down by cells of other tissues (Uv et al., 2003) to reach the different target tissues such as muscles, gut, imaginal discs, or parts of the nervous system. After establishing contact with their target, TCs attach and continue maturing their subcellular tube. By the time the larva hatches from the egg, TCs are typically elongated in shape, containing a single, unbranched tube. As part of the tracheal epithelium, TCs maintain apical-basal cell polarity (Fig. 1B). In this case, the apical membrane is invaginated into the cell to form the tube, and the basal membrane forms the outside of the cell's branches and is in contact with the underlying tissue (Sundaram and Cohen, 2017). A ring of adherens and septate junctions keeps the TC attached to its neighbouring tracheal cell, and its subcellular tube is continuous with the tracheal lumen network.

While the initial cell elongation and tube growth during embryogenesis is under guidance of stereotypical signals, terminal cell growth during larval stages follows non-stereotypical expression of the fibroblast growth factor (FGF) *branchless* (Bnl), which is secreted to attract terminal branches by most cells (Jarecki et al., 1999), presumably when their oxygen requirements are not met. Throughout the three larval stages, the cells thus ramify in response to signalling from surrounding tissues, with most growth occurring in the second and third instar (L2 and L3 stages) (JayaNandanan et al., 2014). The resulting branching pattern of each TC consequently depends strongly on the underlying tissue; both due to the surface it provides as a growth substrate for the TC, and its specific behaviour of Bnl secretion. Yet, all TCs have a distinctly "vasculature-like" branching appearance, creating patterns strikingly reminiscent of vertebrate blood vessels regardless of each cell's location within the body.

This review aims to provide a comprehensive collection of the intrinsic and extrinsic factors known to influence branch formation in terminal cells particularly during the larval stages. The first section provides an

Abbreviations: FGF, fibroblast growth factor; TC, terminal cell; TF, transcription factor; CA, constitutively-active; RNAi, RNA interference; MT, microtubule; GTP, guanosine triphosphate

https://doi.org/10.1016/j.ydbio.2018.12.001
Received 19 August 2018; Received in revised form 23 November 2018; Accepted 1 December 2018
Available online 04 December 2018
0012-1606/

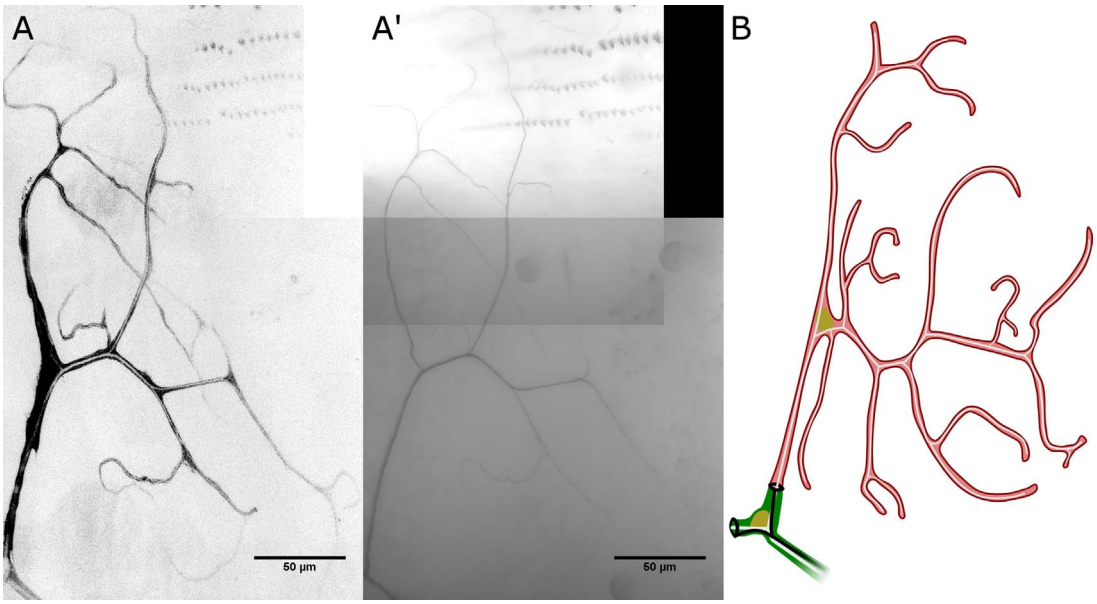

Fig. 1. Larval TC morphology and membrane domain organisation. Confocal fluorescence micrograph of a dorsal terminal cell in a heat-fixed third-instar *Drosophila melanogaster* larva expressing DsRed. Genotype: w*;Btl-Gal4, UAS-DsRed. The image is stitched from two separate acquisitions using FIJI (Schindelin et al., 2012). 2D-projection of a 40 µm deep optical slice stack. (A′) Transmitted light channel of (A), revealing gas-filled subcellular tubes. (B) Terminal cell schematic loosely based on a manual tracing. The basal membrane (dark red) outlines the cell (pink) and is separated from the apical membrane domain by a ring of adherens and septate junctions (thick black lines) at the interface with the stalk cell (green). The subcellular tube (white) is shaped by the apical membrane and contains an apical extracellular matrix which forms spiralling ridges known as taenidia (not illustrated). The nucleus (ochre) is usually located near the first branching point. Scale bars, 50 µm. Larval TC morphology and membrane domain organisation.

overview of transcription factors involved in TC morphogenesis (Fig. 2). The second section focuses on molecular pathways that play a part in branch growth, subdivided into three parts. It first summarises evidence on the embryonic TC's elongation mechanism and relevant details on tube formation, second discusses the scarce data on branching during the larval stages (Fig. 3), and finally proposes a working model for larval branch induction and growth (Fig. 4), combining what is known from both stages under the assumption that the processes are more or less analogous. The third section discusses recent evidence regarding the influence of the animal's environment on TC morphogenesis. Finally, we look at terminal branching from an interdisciplinary perspective and consider to what extent this process might share mechanisms with other branch-forming tissues from a mathematical and algorithmic point of view (Fig. 5).

2. Genetic control of terminal cell morphogenesis

2.1. Blistered / Serum Response Factor

First identified around 1990 (Affolter et al., 1994, 1989), *blistered / Drosophila serum response factor* (DSRF) was shortly after demonstrated to be an important transcription factor for terminal cell growth (Guillemin et al., 1996). It is the most specific known marker for TC fate and DSRF-Gal4 constructs are a commonly used tool for driving transgene expression specifically in TCs. Guillemin and colleagues suggested it regulates transcription of genes that modify the cytoskeleton for long-lasting shape changes. In *dsrf* mutant TCs, the subcellular tube invaginates, but then does not extend (Guillemin et al., 1996; Wong et al., 2015). Non-terminal tracheal cells expressing constitutively-active (CA) human SRF can exhibit TC behaviour if a Bnl source is close by, elongating and sprouting a subcellular tube (Gervais and Casanova, 2011; Guillemin et al., 1996). Moreover, exessive transgenic Bnl signalling can induce terminal branches even in *dsrf* mutant embryos (Gervais and Casanova, 2011), suggesting that DSRF serves a supportive function at wildtype Bnl signalling levels rather than regulating genes which the cells would not otherwise express. In mammalian cells, SRF typically functions in complex with either a ternary complex factor or a myocardin. A cell type can express cofactors of both groups, though in this case ternaries and myocardins compete for SRF binding (Olson and Nordheim, 2010). This mode of interaction is conserved in TCs, where Myocardin-related Transcription Factor (MRTF, formerly known as Mal-d) acts as a DSRF cofactor (Han et al., 2004). No cofactor of the ternary category has been identified in TCs, although DSRF is receptive to regulation by transgenically expressed mammalian ternary complex factor Elk-1 (Guillemin et al., 1996), which has no *Drosophila* homologue. Migrating border cells in *Drosophila* egg chambers require the DSRF/MRTF complex to express genes that stabilise the cytoskeleton after migratory shape changes (Somogyi and Rørth, 2004). Mechanical tension is sufficient to trigger translocation of MRTF to the nucleus in these cells. Particularly terminal branches attached to muscles and gut are subject to severe mechanical strain throughout their lifetime. This could mean that in larval TCs, DSRF/MRTF might receive feedback from

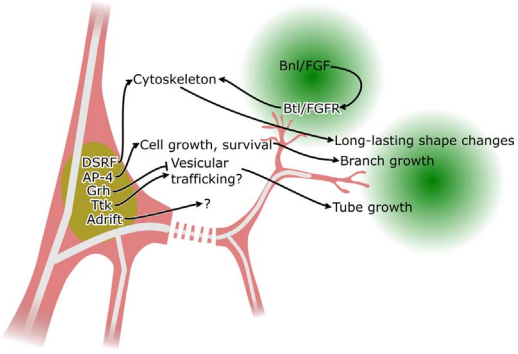

Fig. 2. Genetic control of terminal cell branching. Transcription factors expressed by terminal cell and their proposed roles in branch formation. Blistered/DSRF provides cytoskeletal material both for the formation of new branches and stabilisation of existing ones. Excessive Btl signalling can circumvent the need for DSRF-dependent gene expression. Cropped/AP-4 regulates cell size in other tracheal cells; it is proposed to support the vast growth in cell volume particularly during the third larval instar. Grainy head/Grh negatively regulates apical membrane growth, possibly through vesicular trafficking. Grh may be induced by Btl to fine-tune the amount of tube growth to the branch's need. Tramtrack/Ttk is needed for tube extension, possibly due to an involvement in endocytosis. Adrift plays a role in guiding the embryonic terminal cell elongation; whether and how it is involved in larval terminal branching is unknown. Genetic control of terminal cell branching.

cellular processes to maintain expression of genes that increase the branch's mechanical stability in turn. The gene *enabled* / *vasodilator-stimulated phosphoprotein* has been identified as a transcriptional target of DSRF in TCs (Gervais and Casanova, 2011, 2010). Its role in branch formation remains unknown, although its human homologue has been associated with filopodial and lamellipodial actin dynamics as well as cell migration (UniProt, 2017). The transcriptional targets of mammalian SRF have been extensively characterised (Miano et al., 2007). In mouse neurons, axon growth relies on SRF-mediated expression of microtubule stabilisers such as *microtubule-associated protein 1b* and basal adhesion genes, particularly *vinculin* (Li et al., 2014). For DSRF, *actin* has been proposed as the primary target based on data from *Drosophila* ovaries and the broader myocardin/SRF literature context (Knöll, 2010; Salvany et al., 2014). Considering the tube-related phenotypes associated with DSRF in TCs, and the role of microtubules in tube formation detailed later, it seems likely that DSRF target genes include microtubule-stabilising genes.

2.2. Cropped / AP-4

Cropped (AP-4) presents a curious case because both a lack of AP-4 activity and excessive AP-4 in the trachea cause the same TC phenotype of forming fewer gas-filled tubes, or none (Wong et al., 2015). The study used a fluorescent cytoplasmic protein to visualise terminal cells in *ap-4* mutant larvae, but for all other experiments only transmitted light images were shown, which visualises the gas in the subcellular tube (similar to Fig. 1A and A′). As elaborated later, an absence of gas-filling does not indicate an absence of TC branches. Therefore, the reduction of branching in *ap-4* mutant larvae is conclusive, but it remains unclear whether TCs overexpressing AP-4 formed branches without a gas-filled lumen, or failed to form branches altogether. By contrast, non-terminal tracheal cells showed no abnormalities with excessive AP-4. However, interfering with AP-4 activity by expressing dominant-negative AP-4 reduced the cell number in the tracheal system.

Endogenous AP-4 expression was found in "almost every cell in the larvae of *Drosophila*" (Wong et al., 2015), making it unlikely to be a specifically branching-related TF. Its roles in other organs suggest instead that it supports TCs in survival and size increase. Salivary glands overexpressing AP-4 increased in length, while those expressing dominant-negative AP-4 were drastically shorter (Wong et al., 2015). The former was mostly due to an enlargement of the cells, and the latter due to apoptosis. AP-4 expression can be induced in *Drosophila* larvae by *myc* (Wong et al., 2015), a well-known proto-oncogene (Di Giacomo et al., 2017) which functions through AP-4 in colorectal cancer cells (Jackstadt et al., 2013). *Ap-4* itself is considered an oncogene (Jung and Hermeking, 2009) and a candidate genetic marker for various epithelial cancers (Gong et al., 2014; Sun et al., 2017; Xinghua et al., 2012). Specific genes regulated by AP-4 are not known in TCs. In colorectal cancer cell lines its target genes include cell motility and adhesion genes, with AP-4 overexpression downregulating *e-cadherin* and upregulating *actin* as well as translocating *β-catenin* to the nucleus (Jackstadt et al., 2013). The observations of AP-4 overexpression, where TCs form no gas-filled branches, could thus be explained by a loss of cell polarity akin to epithelial-to-mesenchymal transition. Even with tissue-wide AP-4 overexpression, only TCs were abnormal, suggesting either that AP-4 functions differently in TCs or that neighbouring cells can regulate each other's growth in the rest of the tracheal system.

2.3. Grainyhead

TCs mutant for the TF *grainy head* (Grh) develop normally during early and mid-embryonic stages, but after the extension during late embryogenesis, their subcellular tube becomes tortuous as a result of apical membrane domain expansion (Hemphälä et al., 2003). In the converse experiment, TCs overexpressing Grh elongated but failed to form a subcellular tube. Apical membrane growth therefore seems to scale inversely with Grh activity. This is not TC-specific, as other tracheal cells showed similar phenotypes related to their apical membranes. Tracheal Grh activity can be induced by Bnl overexpression (Hemphälä et al., 2003), contradicting the primary role of Bnl to stimulate both basal (branch) and apical (tube) membrane growth. The authors proposed that Bnl activates Grh to fine-tune the amount of tube growth. Embryos mutant for *grh* produce a weak cuticle (Wang and Samakovlis, 2012), ultimately leading to death. This cuticle-related phenotype suggests a secretory defect in epidermal cells, supporting the hypothesis put forward by Hemphälä and colleagues that Grh controls apical membrane size by modulating membrane trafficking to and/or from the apical domain. Grh transcriptional target genes have been identified in whole *Drosophila* embryos (Nevil et al., 2017; Yao et al., 2017) and screened for tracheal phenotypes by RNA interference (RNAi) (Hosono et al., 2015). The resulting candidate genes' functions cover all major cellular mechanisms and will require further investigation to assess their relevance to terminal branching.

2.4. Tramtrack and Adrift

Embryos mutant for the TF *tramtrack* (Ttk) develop various tracheal and non-tracheal abnormalities (Araújo et al., 2007). Most relevant for TCs, *bnl* expression by target tissues is reduced or lost, causing defects in TC specification (lack of DSRF expression) and migration. Those TCs which express DSRF in *ttk* mutants still fail to extend their tube, and overactivation of the Bnl/Btl pathway could not rescue this defect. This phenotype could be due to a deficiency of *shibire* / *dynamin*, which is a target gene of Ttk in dorsal appendage tube formation during *Drosophila* oogenesis (Peters et al., 2013). TCs mutant for *ttk* had fewer apical-material-containing vesicles (Araújo et al., 2007), consistent with Dynamin's role of catalysing vesicle scission during endocytosis.

Lastly, the transcription factor Adrift shows a unique expression pattern, being present initially in all tracheal cells and then gradually restricted to only TCs by stage 15 (Englund et al., 1999). Its expression in TCs appears to be induced by both Bnl and Dpp signalling (Ebner et al., 2002), and mutant embryos have defects in terminal branch guidance. No further data have been reported about this gene since.

3. Molecular mechanisms of branch outgrowth

The term "terminal branch formation" is commonly used for two distinct, but related, processes: on the one hand, TC elongation and *de novo* subcellular tube formation during late embryogenesis (Sigurbjornsdottir et al., 2014), and on the other hand, the repeated subcellular branch sprouting and elongation during the larval stages. To distinguish these two, I will refer to the embryonic process as "elongation" throughout this review. Most published studies focus only on one of these two aspects of TC development, and it is frequently assumed that data from one process are applicable to the other. The actin cytoskeleton and microtubules are the two systems that have been characterised both in embryonic (Gervais and Casanova, 2010) and larval TCs (Schottenfeld-Roames and Ghabrial, 2012). In most other cases, the degree of similarity between embryonic and larval TC morphogenesis has not been addressed. Many studies on the larval stages investigated only the TCs that ramify on the surface of dorsal muscles, and it is presumed that TCs on other target tissues form branches through the same molecular mechanisms.

TC growth is primarily controlled by the fibroblast growth factor *branchless* (Bnl), which TCs sense using the corresponding receptor tyrosine kinase *breathless* (Btl) (Hayashi and Kondo, 2018). Bnl induces TCs to extend branches towards the source of secretion, either by rerouting and extending an existing branch tip, or by undergoing a branching event to form a new process. The molecular mechanisms of

cell and tube extension are best understood in embryonic TCs during the first, stereotypically guided, elongation event. This is owed to the relative ease of imaging access, particularly live imaging. Branching events and growth of new cell processes during the larval ramification stage so far have not been observed live, or at least not reported. The second subsection on the larval TC stage thus mainly summarises where different components localise in fixed samples.

3.1. Embryonic terminal cell elongation

Embryonic TC elongation begins with the formation of filopodia from the basal membrane in random directions. This activity quickly polarises, with more and longer filopodia extending ventrally (towards the chemoattractant signal source) as opposed to anterior and dorsal (Gervais and Casanova, 2010; Oshima et al., 2006). The cell then elongates towards the larger filopodia and simultaneously begins to invaginate the apical membrane into the cytoplasm to form the future tube. Local accumulations of actin can occur anywhere on the basal membrane during the early stage, and they then shift along with the filopodial activity to the growing basal tip. An actin meshwork containing Moesin lines the entire length of the apical membrane. Throughout the elongation process, the cell and tube extend in concert, keeping a more or less constant distance between the cell's and the tube's growing tips. This alignment of the tube with the axis of cell elongation is probably mediated by basal-filopodial actin, as the tube can grow at odd angles or in a U-turn in TCs mutant for filopodial actin organising genes (Box 1). For basal actin to mechanically influence the apical membrane, there must be communication between the basal and apical actin pools. This could be effected by direct actin bridges across the basal-apical axis, though this has not been shown. If not actin itself, microtubules (MTs) are likely to provide this link: MTs line the tube and bridge the gap between the apical and basal-tip actin meshworks (Gervais and Casanova, 2010). The tube membrane is also where MTs can be nucleated as evidenced by the localisation of centrosomes (Ricolo et al., 2016) and γ-Tubulin (Gervais and Casanova, 2010; Ricolo et al., 2016), both of which might act as MT organising centres at different points in time (Box 1). Experiments inducing MT depolymerisation (Gervais and Casanova, 2010) or loss of centrosomes (Ricolo et al., 2016) showed that tube growth depends more strongly on MTs, as the cells were still able to elongate while failing to form a tube. Yet, an increase in centrosome number due to loss of *regulator of cyclin A1* was able to induce not only the growth of additional tubes branching off the existing one, but also concomitant cytoplasmic processes branching off into abnormal directions. This will be discussed later in the context of a potential branch sprouting mechanism. The coordinated growth of both basal and apical membrane domains requires substantial amounts of new membrane material as well as targeted secretion of new lipids and proteins to the correct membrane compartments. Nothing is known regarding basal membrane trafficking in embryonic TCs. The polarity and trafficking systems involved in supplying the apical membrane rely on MTs and have been reviewed elsewhere (Sigurbjornsdottir et al., 2014).

3.2. Larval terminal cell branching

On a gross level, the localisation of cytoskeletal markers in larval TCs suggests that the overall principles of growth that pertain to the embryo are maintained in the larva (Fig. 3A): Acetylated MTs line the now gas-filled tube and extend beyond it or branch off it into filopodia (JayaNandanan et al., 2014). γ-Tubulin remains restricted to the apical membrane, indicating that MT orientation remains the same. An overexpressed EB1-GFP fusion protein, a marker for polymerising MT tips, showed growth of MTs throughout branches, primarily close to the tube and parallel to it, in both directions (towards and away from the nucleus) (Schottenfeld-Roames and Ghabrial, 2012). The positioning of centrosomes at this stage is unknown. The tube maintains its apical identity and its actin cortex (Schottenfeld-Roames and Ghabrial, 2012). Filopodial activity is markedly different. Instead of short, usually straight protrusions, the structures that most closely resemble filopodia in larval TCs are trees of thin cellular processes, sometimes elaborately branched (Fig. 3B). Some of these presumably later mature into tube-bearing branches. Filopodial trees are commonly seen at branch tips, but often also between two branching points, although this has not been quantified. Filopodial actin pools appear connected to the apical actin at the tube in larval TCs (Schottenfeld-Roames and Ghabrial, 2012). The remaining basal membrane is smooth and devoid of actin labelling besides occassional enriched patches. Within immature branches, multivesicular bodies that seem to contain material destined for the tube have been identified using electron microscopy (Nikolova and Metzstein, 2015). Tubulin immunostaining ahead of the tube sometimes colocalises with small patches of apical markers (Schottenfeld-Roames and Ghabrial, 2012), which could be the same

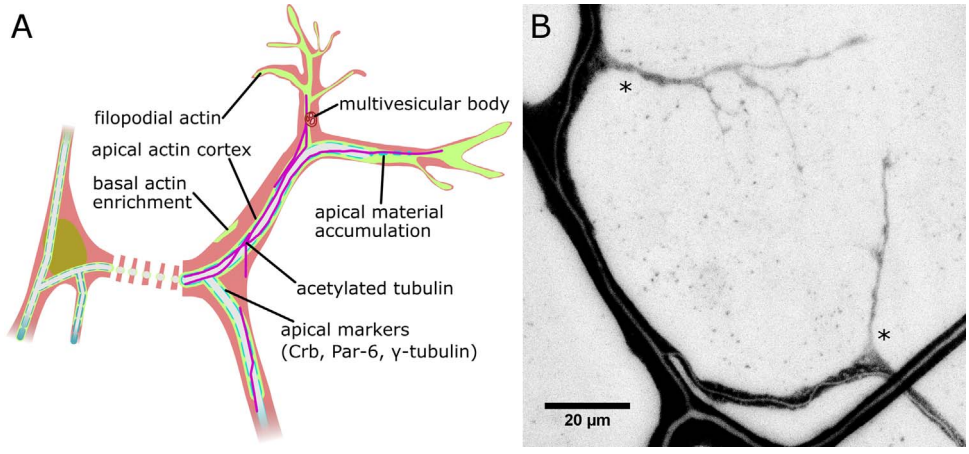

Fig. 3. Molecular components of branching in larval terminal cells. (A) Schematic of a terminal branch illustrating subcellular localisation of structures with known involvement in larval branch formation. Actin (lime) lines the tube, but also prominently labels filopodial trees. Filopodial actin is seen connected to the apical actin cortex. Occassional actin patches can be found near the basal membrane. Acetylated tubulin (purple) is found in tracts along the tube and extending partway into filopodia. Live imaging of EB1-GFP, which marks growing microtubule ends, showed that microtubules polymerise primarily parallel to the tube, both towards the nucleus and away from it. γ-Tubulin, a microtubule nucleator and minus-end marker, is found exclusively on the tube. Apical markers such as Crumbs and Par-6 (teal) are distributed along the tube in a punctate manner. Occasionally, apical markers can be seen in small patches near microtubules ahead of the tube. Electron microscopy has been used to demonstrate the existence of multivesicular bodies in immature branches, which sometimes contain vesicles with a lumenal matrix reminiscent of the tube's apical extracellular matrix (Nikolova and Metzstein, 2015). (B) Example of filopodial trees visualised by cytoplasmic DsRed in a terminal cell as in Fig. 1A. Asterisks mark roots of filopodial trees. Scale bar, 20 µm. Molecular components of branching in larval terminal cells.

multivesicular bodies. An analogue for these has not been shown in embryonic TCs so far.

Perhaps the best-described difference between the embryonic and larval TC growth programs, as mentioned above, is the regulation of the attracting signal to which the TC responds. The embryonic signal is stereotypically determined, whereas in the larva, cells seem to secrete the FGF Branchless (Bnl) according to their oxygen needs, which attracts TC branch growth towards them. The signal is received by the FGF receptor Breathless (Btl), which transduces it in the TC cytoplasm at least in part through Zinc finger protein 1 (Zpr1) (Ruiz et al., 2012). Bnl/Btl signalling is sufficient for inducing branching of both cytoplasm and tube. Transgenic induction of Bnl secretion in the salivary gland resulted in tracheation of this tissue, which normally does not receive any terminal branches (Jarecki et al., 1999). Conversely, TCs expressing a constitutively-active Btl receptor (Btl-CA) grow more branches (Schottenfeld-Roames and Ghabrial, 2012; Ukken et al., 2014). The equilibrium between branch and tube growth is disturbed in these cells, resulting in branches with more than one tube and bundles of tube both near the nucleus and at branch tips. At abnormally high levels, Btl signalling thus coordinates the branching of the cell body and subcellular tube branching independently. Perhaps Grh-dependent apical growth inhibition meets a saturation point, allowing the apical membrane to grow in excess.

The relationship between branch and tube is less well understood in larval than in embryonic TCs. The tube does not need to be gas-filled for the cell to reach normal branch lengths and numbers (Baer et al., 2012; Hemphälä et al., 2003; Ríos-Barrera et al., 2017). There are also various known conditions where the apical extracellular matrix is defective or absent but the TC is able to form an extensive branch network (Nikolova and Metzstein, 2015; Rosa et al., 2018; Schottenfeld-Roames and Ghabrial, 2012). The extracellular contents of the tube therefore do not seem to be relevant for branch growth. On the other hand, there have been no reports of TCs ramifying in the absence of a membranous tube structure labelled by cytoplasmic apical markers. Additionally, many conditions which should only affect the apical membrane, i.e. the tube, also result in reduced branch numbers (Box 2). The emerging picture thus supports the notion that the basal membrane can only grow when the apical membrane is doing the same. The presence of the subcellular tube is the defining difference between a filipodial tree and stable branches. The dependence of branch growth on the apical membrane therefore implies that actin-based filopodial trees have a limit of size that they can only transgress with support from the apical membrane, its associated cytoskeleton, or both. Curiously, in some contexts in which a TC is unable to grow sufficient amounts of apical membrane, the neighbouring tracheal cell's apical membrane can invade the TC and supply a fraction of the subcellular tube (Francis and Ghabrial, 2015). In contrast, phenotypes like that of Btl-CA expression and the "collapsed branches" phenotype of *talin* and *integrin* mutant TCs (Box 2) demonstrate that the apical membrane can grow and branch independently of basal membrane growth.

3.3. Working model of branching induction and branch formation

Given the available evidence, it is possible to synthesise a working model for the formation of new larval TC branches. The first step in this process would be determining when and where a new branch will form. Although this mechanism is crucial to understanding terminal cell branching, very little is known about it. Bnl/Btl signalling undoubtedly plays a role in this process (Fig. 4A), but how has so far not been determined. Btl signalling is first of all responsible for the genetic programs allowing TCs to express cytoskeletal components which other tracheal cells do not require (recently reviewed (Sundaram and Cohen, 2017)). Embryonic TCs respond to their migratory cue as one unit, deforming the entire cell towards the signal source. Larval TCs instead respond to their signal with local deformations, and potentially respond to multiple signals from different directions at the same time. For a Bnl secreting source to act as a chemoattractant in such a localised manner, one would therefore have to propose that Btl at this stage directly induces formation of filopodia. Alternatively, the cell could stochastically form filopodia at random places along its surface, which subsequently collapse unless a threshold of Btl activation is met at their membrane. Either option requires a Btl function interacting

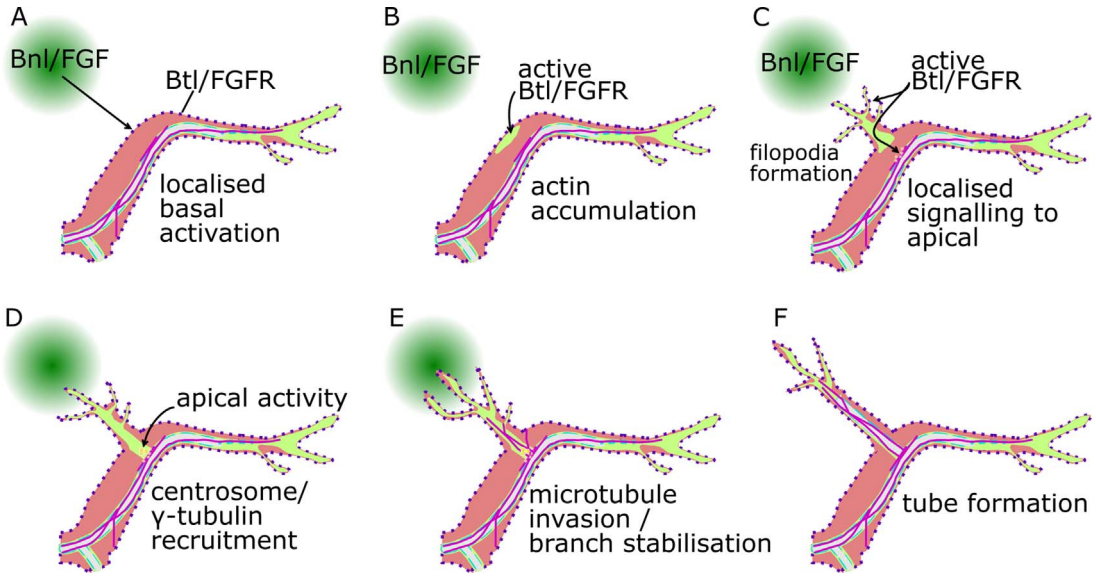

Fig. 4. Working model for larval terminal branch formation. A working model for sprouting and extension of a new terminal branch, integrating evidence from embryonic and larval terminal cells. (A) The first step is induction of a new filopodial sprout site. This may be a stochastic event or triggered by localised activation of Btl. (B) Once the site has been determined, Btl stimulates actin accumulation into a basally localised meshwork to provide physical support for actin bundle polymerisation. (C) Actin bundles deform the membrane and form filopodia. These continue moving towards the Bnl source and thereby sustain Btl signalling. Next, the Btl signal is transduced to the closest apical membrane surface. This may occur through cytoplasmic diffusion or connections between the basal and apical actin meshworks. As a result, an unknown factor - possibly Par-6, aPKC or Cdc42 - at the apical membrane gets activated. (D) The basal and apical actin are now connected and allow recruitment of e.g. actin-anchored vesicles to supply membrane material. The apically activated proteins recruit microtubule nucleating structures (here represented by a centrosome). (E) The centrosomes and/or γ-Tubulin polymerise microtubules into the filopodial sprout and stabilise it. Apical secretory vesicles bind to the microtubules and begin formation of a multivesicular body to induce branching of the subcellular tube. (F) A new branch with subcellular tube has been initiated and continues to grow or shrink depending on Bnl availability. Working model for larval terminal branch formation.

with the local actin cytoskeleton independent of transcriptional regulation. The small GTPases Rac1 and Rac2 are part of the Btl signalling network in tracheal cells (Chihara et al., 2003) and could be a candidate for coupling Btl to actin (Muha and Müller, 2013), though no data on Rac in TCs has been published. Whether Btl-induced or not, the earliest marker of an incipient filopodial sprouting site could be the patches of actin sometimes seen localised at the basal membrane (Fig. 4B) (Schottenfeld-Roames and Ghabrial, 2012). Such a patch could be a mesh of actin filaments, capable of providing physical support for actin bundles to protrude into the basal membrane and form filopodia. The most promising avenue towards understanding this mechanism better would be optogenetic control of potential components of the pathway. For example, a photoactivated FGFR1 has been tested in HeLa cells (Kim et al., 2014). A similar construct adapted for expression in *Drosophila* could provide valuable insights into TC branching.

Considering the role of MTs, γ-Tubulin localisation and centrosomes in embryonic TC tube formation (Gervais and Casanova, 2010; Ricolo et al., 2016), the recruitment of centrosomes to an apical membrane surface could be the instructive step determining which basal processes are invaded by MTs and consequently mature into stabilised branches with a tube. The same interpretation has recently been offered by independent reviewers (Hayashi and Kondo, 2018). The next step triggered by sustained local Btl activity, after a filopodial sprout site has been initiated, should thus be the transduction of the signal from the basal to the apical membrane surface (Fig. 4C). This step could be related to the connections seen between the filopodial and apical actin pools (Schottenfeld-Roames and Ghabrial, 2012). An actin-bound signalling component activated by Btl could use this to bridge the apical-basal gap and activate apical signalling systems, perhaps Par-6, aPKC or Cdc42 as suggested previously (Jones et al., 2014). Alternatively, simple diffusion of a cytoplasmic Btl effector could mediate the basal-to-apical signal transduction and the actin bridge could form as a consequence. To elucidate how this takes place, it is first necessary to identify the downstream targets of Btl in larval TC morphogenesis. A screen for FGFR signal modifiers in *Drosophila* eye development provides a shortlist of candidates for this (Zhu et al., 2005). Ultimately this process would result in recruitment of MT nucleating structures such as centrosomes or γ-Tubulin to the site of apical activation (Fig. 4D).

Excess centrosomes can induce additional branches ignoring the direction of the migratory signal (Ricolo et al., 2016). This suggests that once MTs are nucleated and invade a filopodial sprout (Fig. 4E), localised or directional Btl activity might no longer be required to promote the following steps of tube formation and extension into the new branch, but only for branch guidance (Fig. 4F). It is also possible that centrosomes recruited at the apical membrane can induce branching by deforming the basal membrane via MTs, independent of filopodial activity. An experiment where TCs form excess centrosomes but also receive no Btl signal could shed light on this question. The Btl-CA phenotype certainly shows that continuous and excessive Btl signalling can promote exuberant tube growth after this step. Once MT tracts have been established, transport of new membrane and secretory material could be targeted to the MTs in a similar manner as in fusion cells (Caviglia et al., 2016; Kato et al., 2016) to initiate a multivesicular body for maturing pre-lumenal compartments (Nikolova and Metzstein, 2015). These subsequently direct material to the tube to promote budding of a new lumenal branch.

4. Plasticity of terminal branching in response to environmental factors

TC morphology reflects the environmental conditions in which the larva develops in the number of branches formed until the wandering third larval stage. It is influenced by the availability of oxygen, nutrients, and possibly other factors throughout the larva's lifetime. The effect of ambient oxygen on TC branch number is mediated directly through Bnl/Btl signalling and has been investigated and reviewed extensively (Cabernard et al., 2004; Gorr et al., 2006; Horowitz and Simons, 2008; Romero et al., 2007). Briefly, most tissues sense hypoxia through *similar / hypoxia inducible factor α* (*sima/HIFα*), which is expressed but quickly degraded at normal oxygen levels. When insufficient oxygen is available, Sima protein levels increase due to the inactivation of various factors that mediate its degradation (Box 3). Sima then cooperates with Tango / HIFβ to upregulate Bnl expression and secretion. Correspondingly, TCs respond by growing more branches (Jarecki et al., 1999). This simple view is complicated by the response of tracheal cells themselves to hypoxia. On the one hand, the additional Bnl secreted by other tissues is detected by tracheal cells. On the other hand, larval TCs sense hypoxia themselves, and respond by upregulating *btl* expression (Centanin et al., 2008). Their threshold for activating a transgenic hypoxia reporter (Ldh-Gal4) is uniquely low; with decreasing oxygen levels, TCs are the first to switch on Ldh-Gal4 within the larval body (Centanin et al., 2008). The mechanisms of sensing hypoxia seem to be the same in the trachea and other tissues (Box 3), and only the consequences of Sima stabilisation differ - tracheal cells upregulate *btl*, all other cells *bnl*. This genetic network begins functioning during late embryogenesis (Mortimer and Moberg, 2009), and has been shown to remain functional throughout larval stages. A recent report complements this with the first evidence from adult *Drosophila* flight muscles, which also receive a higher number of tracheoles if the fly grew up under hypoxia (throughout its embryonic and larval life in this study) (VandenBrooks et al., 2017). These tracheoles have a larger diameter than at normoxia, a feature that studies in larvae did not assess.

Besides hypoxia, an emerging environmental factor governing TC branching behaviour is nutrition. First results show that the percentage of yeast in the nutritional medium correlates with the number of branches on dorsal TCs and those targeting the gut, but not TCs in the central nervous system (Linneweber et al., 2014). This effect relates to the sensitivity of TCs to insulin-like peptides (ilp), which are secreted by neurons in response to nutrient availability and function as ligands for insulin receptor (InR) (Rulifson et al., 2002). Interferening with InR or *phosphoinositide-3-kinase* (PI3K), one downstream component that integrates signal from InR, did not affect dorsal TCs but strongly reduced branches in gut-TCs (Linneweber et al., 2014). This contrasts with larvae mutant for the InR ligands *ilp2*, *3* and *5*, where dorsal TCs had severely reduced branch numbers, while the TCs in most parts of the gut were unaffected. This suggested that dorsal TCs are more sensitive to the absence of the InR ligands than interference with their own expression of InR. Yet, overexpressing InR increased dorsal TC branching by around 20% in another study (Wong et al., 2014). The finer details of insulin signal reception by these cells thus require further experiments to dissect, perhaps using *inR* mutant clones. For those TCs that tracheate the hindgut, a neuronal population was identified which directly regulates TC branching in this region by secreting Ilp7 (Linneweber et al., 2014). Exogenous over- or inactivation of these neurons by expressing different ion channels resulted in increased or decreased TC branching in the area respectively. Additionally, the neurons increased activity both in response to yeast and hypoxia as reflected by a fluorescent Ca^{2+}-reporter, implying regulation of TC branching depending on both conditions. Terminal cells appear to maintain their plasticity in response to nutrition also in adult life, as suggested by an increase in intestinal tracheation of adults after a switch in diet (Linneweber et al., 2014).

The finding that tracheal cells express receptors for octopamine (OctR-α1b, β1R and β2R (El-Kholy et al., 2015)), a chemical relative and functional insect analogue of noradrenaline in mammals, could mean that the trachea responds to behavioural states such as stress or aggression, but this has not been investigated so far. Preliminary data suggest that TC branching may additionally be modulated by ambient temperature, with branch numbers being higher at 29 °C compared to 18 °C rearing temperatures (Best, B., Leptin, M., unpublished).

5. Mechanisms underlying branching pattern formation

Branching morphogenesis and the logical and algorithmic problems it solves have long fascinated researchers not only in biology but also in the informational, mathematical and computational sciences (Lasser and Katifori, 2017). So far no formal attempts at approximating the TC branching pattern with mathematical or computational models have been reported. In the first study of hypoxia-induced TC branch formation, the authors proposed an informal model where throughout larval development, cells further away from TC branches become locally hypoxic as they grow and increase their oxygen consumption. This would then lead to Bnl secretion, attracting TC growth towards the source and thereby correcting the local oxygen deficiency (Jarecki et al., 1999). Some issues with this model have arisen in the meantime: First, the hypoxic master regulator Sima is dispensible for larval terminal branching in a normoxic environment, and Sima-overexpressing cells reportedly do not attract terminal branches (Centanin et al., 2008). The Sima-dependent hypoxic response is thus unlikely to contribute to a certain baseline Bnl secretion. An oxygen-dependent model of TC branching would therefore require a second mechanism for sensing oxygen which is active around atmospheric ambient oxygen tension, or perhaps which differentiates between systemic hypoxia and localised oxygen need. The second issue relates to the mode of Bnl-Btl signalling. Recent evidence shows that Bnl-secreting cells throughout the embryonic stages and later in the wing disc have direct physical contact with the tracheal cells they are guiding (Du et al., 2017; Sato and Kornberg, 2002), whereas the above model implies contact-independent signalling. Transgenically expressed Bnl is able to attract terminal branches over long distance (Centanin et al., 2008; Jarecki et al., 1999), suggesting that contact might be a consequence of Bnl/Btl signalling rather than a prerequisite. However, the signalling range of endogenous Bnl levels and the effective oxygen diffusion range out of terminal branches have not been determined. These would be critical parameters for testing the feasibility of such a model. Finally, a particular challenge to the model is presented by TCs near the body wall. These ramify on syncytial muscles, usually with only one TC per muscle and two or three muscles covered by one TC's branches. *In situ* hybridisation detecting *bnl* mRNA suggests that at least in the third larval instar, all somatic muscle nuclei express *bnl* (Jarecki et al., 1999). A model where branches are guided by local oxygen need would require the muscle to sense oxygen demand within physically continuous subdomains of its cytoplasm, and then direct Bnl secretion to the corresponding fraction of its surface membrane. In the flight muscle, a mechanism somewhat like this exists: the T-system tubules are subcellular invaginations into the flight muscle, forming a canal system. During pupation, the muscle secretes Bnl into their lumen, attracting terminal tracheal branches to invade them (Peterson and Krasnow, 2015). This process depends on the T-tubule's basolateral membrane identity. In the adult fly, this tubular system juxtaposes tracheal branches with "virtually every mitochondrion" in the flight muscles (Wigglesworth and Lee, 1982). In the larva, TC branches remain on the surface of the muscle, which eliminates cell polarity cues as a mechanism for subcellular targeting of Bnl secretion at this stage. The current state of evidence also permits the possibility that Bnl might not act as a directional cue during physiological terminal branching, though transgenic experiments show that it has the capacity to guide directionally at abnormally high levels. Nevertheless, the substrate tissue on which the TC ramifies plays a major role in orchestrating TC branching. This is evidenced by the distinct branch numbers per TC and branching patterns found on different target tissues. Even an individual TC's branches can form distinguishable patterns. Most TCs only tracheate one type of tissue. However, the most posterior dorsal TCs often attach some branches to the heart, in addition to their stereotypical target muscle. In these cases, the branches that tracheate the heart form a much denser pattern than those on the skeletal muscle (Fig. 5A). However, based on the present literature on the interaction between growth substrate and TC, deriving a formal model based on molecular mechanisms is hampered by too many unknowns. The following section therefore aims to integrate context from branching morphogenesis in other species.

While in *Drosophila*, the trachea and nervous system are the only extensively ramified organs, the list is longer in mammals: vascular system, lungs, kidneys, mammary glands, lymph system, bile ducts and prostate all form highly branched structures. Among these, only the nervous system contains cells that form stable and numerous branches on a subcellular level. The growing tip of TCs has been compared to the axonal growth cone (Affolter and Caussinus, 2008), and some molecular components are shared between neuronal and TC branching. Substantial efforts have been made to build computational models that recapitulate natural neuronal branching patterns (Tsaneva-Atanasova et al., 2009; van Ooyen, 2011), and some of these are based on mechanisms that might be transferable to TC branching. Namely, filopodial pulling force (O'Toole et al., 2008), tubulin (Diehl et al., 2016; Hely et al., 2001) and actin dynamics, and vesicle exocytosis

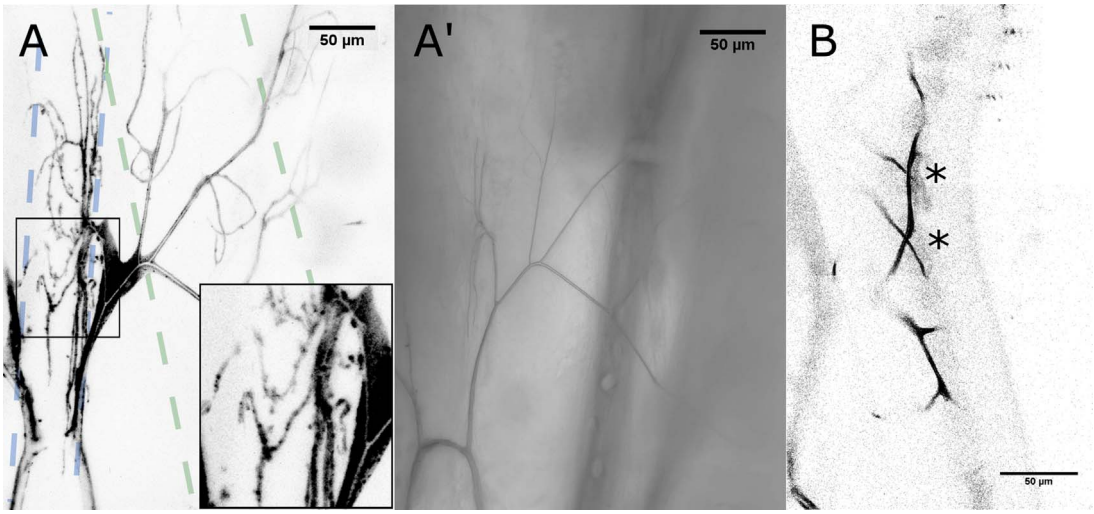

Fig. 5. Dual-type branching pattern in a dorsal terminal cell. (A) Confocal fluorescence micrograph of a dorsal terminal cell in segment A6 of a heat-fixed third-instar larva expressing DsRed, acquired as in Fig. 1A. One terminal cell branch has attached to the heart (location outlined by blue dashed lines) instead of the dorsal muscle (marked by green dashed lines). Its subsequent branching is much denser and numerous than the cell's other branches. (A′) Transmitted light channel of (A), showing gas-filling of heart-attached branches. (B) A single 1.5 µm thick optical slice of the terminal cell in Fig. 1A, showing crossed-over branches (asterisks). Scale bars, 50 µm. Dual-type branching pattern in a dorsal terminal cell.

Box 1. Genes in embryonic terminal cell morphogenesis.

The filopodial polarisation at the beginning of TC morphogenesis is mediated at least in part by I-kappaB kinase ε (IKKε) (Oshima et al., 2006). In TCs deficient for *singed* / *fascin* or *forked* / *espin*, the number of filopodia is reduced because a fraction of them bend, then break and are retracted (Okenve-Ramos and Llimargas, 2014). Mutations in these actin-organising proteins can cause an angled or u-turning tube while the cell itself elongates normally. This suggests independent functions for the basal-filopodial and the apical actin pools. However, it is the basal actin pool that is affected in these mutant cells, indicating that the two pools interact to direct the tube towards the basal membrane region closest to the chemoattractant signal. As far as immunostainings detect, all MTs in TCs are acetylated. They are found on the tube and protruding ahead of it into the actin meshwork of the growing basal tip (Gervais and Casanova, 2010). The MTs near the growing tip of the tube and ahead of it up to the basal tip are also tyrosinated. Detyrosination is a consequence of MT stabilisation and occurs over time (Fukushima et al., 2009). This suggests that in embryonic TCs the primary site of MT polymerisation is at the growing tip, and these newer MTs form a connecting tract between the basal and apical sites of elongation. The rest of the tube is lined with older, stabilised MTs. Consistent with this idea, centrosomes were found to localise near the apical membrane of the incipient tube (Ricolo et al., 2016). γ-Tubulin immunostaining (a marker for MT nucleation sites) has been reported near centrosomes at stage 14 (Ricolo et al., 2016) and all along the tube at stage 16 (Gervais and Casanova, 2010), which could hint at a shift from centrosomal to noncentrosomal organisation of MTs once the tube has been initiated. cGMP signalling is involved in embryonic TC tube growth. When *guanylyl cyclase at 76C* or *cGMP-dependent protein kinase at 21D* were knocked down by expressing an RNAi transgene in the trachea, tube elongation arrested after the initial invagination (Myat and Patel, 2016).

Box 2. Genes in larval terminal cell morphogenesis.

Subcellular TC branches are formed by the basal membrane. However, many genetic perturbations which should impact exclusively the apical membrane also result in decreased total branch numbers per TC. One simple explanation for these observations is a requirement by the basal membrane for a growing apical membrane.

- Most notably, defects in the apical polarity complex composed of Atypical protein kinase C (aPKC), Par6, and Cdc42 severely reduce TC branch number but do not abolish branching as such (Jones and Metzstein, 2011), indicating that the cell maintains correct polarity and the capacity to form branches, but the defects of the apical membrane domain also prohibit the basal domain from growing.
- The Bitesize protein is present at the apical membrane and recruits phospho-Moesin to modify the physical properties of the apical actin meshwork (JayaNandanan et al., 2014). Opposing this, the endocytic and early endosomal systems function at the apical membrane to reduce levels of phospho-Moesin (Schottenfeld-Roames et al., 2014). Mutations of Bitesize, Moesin or endocytic components like Shibire (Dynamin) or Syntaxin 7 (with the consequence of Moesin hyperactivity) all affect primarily the apical actin cortex, and yet cause drastic reductions in TC branch numbers. As with the TC's need for the DSRF transcription factor, also the need for Moesin can be overridden by excessive Btl signalling, even though enriching phospho-Moesin in the apical actin cortex is part of the programs downstream of Btl signalling (Ukken et al., 2014). This particular function of Btl seems to be independent of the canonical MAP-kinase pathway, as interfering with Ras, Raf, Mek or Erk did not affect phospho-Moesin localisation.
- The exocyst complex, a component of the machinery tethering vesicles for fusion to a target membrane (Wu and Guo, 2015), similarly impacts both branch number and gas-filling of the tube in TCs deficient for one of its subunits. While its function at the apical membrane is so far not clear, a Cdc42- and Par6-dependent enrichment at the tube has been demonstrated for some of its subunits (Jones et al., 2014), suggesting that also here the branching defect might be a consequence of defunct tube growth.
- The Tango1 protein is present at the interface between endoplasmatic reticulum (ER) and Golgi compartments, where it is required for the export of large proteins such as Dumpy from the ER (Ríos-Barrera et al., 2017). In *tango1* mutant TCs, gas-filling fails and the branch number is reduced as a consequence of ER stress, which interferes with secretory pathways.
- Arl3 and Staccato / Unc-13-4 are proteins involved in formation of apical tube material in tracheal fusion cells (Caviglia et al., 2016). Arl3 is not normally expressed by TCs, but inducing its misexpression still reduced branch number and length.
- Mutations in *germinal centre kinase III* and *N-ethylmaleimide-sensitive factor 2* cause dilations of the tube at the TC stalk, and also reduce the number of branches (Song et al., 2013).
- The only known gene related to the basal membrane is *rhea* / *talin,* whose protein product links actin filaments to integrin-based focal adhesions (Klapholz and Brown, 2017). *Talin* mutant TCs can ramify normally up to the early second larval stage. Only during the third larval stage, their cytoplasmic branches collapse and their tubes bundle up in the remaining cell soma (Levi et al., 2006). This phenotype is commonly thought to indicate that basal adhesion is needed for branch growth. The same defects arise in *β-integrin* or combined *α1-* and *α2-integrin* deficiency, but not with additional factors that interact with Talin and Integrins to mature adhesions, such as Vinculin, Integrin-linked kinase and Steamer duck / Pinch. The time window during which *talin* and integrin defects develop coincides with the period in which more than 75% of TC branches grow (JayaNandanan et al., 2014). Thus, only the core components of integrin-based adhesion cause this phenotype, and it arises only during the most intense period of cell growth. Integrin-based adhesion is therefore not essential to branch growth per se, but rather to reaching and maintaining the final size of the cell.

(Tsaneva-Atanasova et al., 2009) could impose similar constraints on subcellular branching in both cell types. Further data on the interaction between these components during TC branch growth will be needed to determine the degree of overlap quantitatively. On the larger scale, neuronal and TC branching patterns are easily distinguishable to a human observer, suggesting that substantial parts of the underlying mechanisms differ.

The capillaries of the vascular system on the other hand form very similar ramification patterns as TCs, a hardly surprising observation considering the analogous function of oxygen delivery to tissues. Blood capillaries employ two types of branching mechanism, of which only one – sprouting angiogenesis – results in patterns similar to TCs. Sprouting angiogenesis is a collective migration mechanism similar to the multicellular movements that form the primary branches in the *Drosophila*

Box 3. Sima regulation in the larval tracheal system compared to other tissues.

In tracheal target tissues, the degradation of Sima protein at normal oxygen levels is mediated by *fatiga* / *HIF prolyl hydroxylase* (Centanin et al., 2008), *Drosophila Von Hippel-Lindau* protein (dVHL) and the ubiquitin ligase *archipelago* (Mortimer and Moberg, 2013). Inactivation of any of these factors leads to a stabilisation of Sima protein. Regarding the hypoxic response of TCs, an increase of Sima protein has not strictly been shown, but two observations strongly suggest that Sima is conserved as the master regulator in TCs as in the target tissues: First, TCs overexpressing non-degradable Sima grow more branches (Centanin et al., 2008). Second, knockdown of *musashi* in TCs causes excess branching - Musashi being a repressor of *sima* mRNA translation (Bertolin et al., 2016). Out of the three Sima-degrading factors known in non-tracheal cells, only dVHL has been shown to act in TCs. Like Musashi, knocking down dVHL in the tracheal system causes TCs to grow excess branches (Mortimer and Moberg, 2009). Fatiga is probably also active in TCs, and itself regulated by *miR-190*. This micro-RNA can bind *fatiga* mRNA in the 3′ untranslated region and mediate its decay (De Lella Ezcurra et al., 2016). Larvae upregulate *miR-190* under hypoxia, and its overexpression induces excess TC branching. If it acts by degrading *fatiga* mRNA, its effect on TC branch number implies that TCs also express *fatiga*. Whether *miR-190* is normally active in TCs was not tested, but its genomic location in an intron of the *rhea* gene (*talin*), which the larva upregulates under hypoxia (De Lella Ezcurra et al., 2016), suggests that TCs express it as part of their own hypoxic response.

trachea, preceding TC development (Ochoa-Espinosa and Affolter, 2012). Modelling this process is an active area of research due to its relevance to tumour growth (Chaplain, 1995; Suzuki et al., 2018). Some models have reached a high degree of sophistication, taking into account signalling kinetics, migration dynamics of different cell populations and intravascular fluid effects (McDougall et al., 2012). What makes the similarity between TC and vascular branching intriguing from a mathematical point of view is that the latter involves several cell types, and branches grow from coordinated multicellular migration. In contrast, to establish similar patterns, TCs need to implement an equivalent algorithm within a single cell. The *Drosophila* tracheal system therefore offers the opportunity to identify the underlying conceptual basis of vascular-type branching independently of cell-cell interactions. Aligning models with experimental data should furthermore be simpler for TCs because they execute their morphogenetic process on two-dimensional (albeit curved) tissue surfaces rather than into three-dimensional volumes. Vice versa, known principles from more abstract models of angiogenesis may reveal mathematical constraints on terminal branching. In this regard, the distribution of hypoxic signal sources and their secretion rate of the chemoattractant appear to be the primary factors influencing the final structure in sprouting angiogenesis (Moreira-Soares et al., 2018). Dissecting the conflicts discussed above of the TC branching model regarding the role of hypoxic signalling could therefore yield valuable advances for both fields.

For the mammary gland, kidney and prostate, a unified theory of branching has recently been introduced (Hannezo et al., 2017). This proposes that the mechanisms governing their branching processes can be reduced to a relatively simple paradigm of "branching and annihilating random walks". In this process, growing buds elongate with random directionality and stochastically bifurcate, creating two growing daughter tips. Branch tips stop growing when they enter within a certain distance of an existing branch. This process continues until the space provided by the growth substrate is filled and all tips are annihilated. This model recapitulated high-resolution captures of mouse mammary glands and kidneys remarkably well. The same principle could also apply to TCs, although there are aspects of TC morphology that distinguish them from the mammalian branched organs and contradict this branching theory at least in its most basic form. TCs characteristically leave a lot of tissue surface uncovered, and branches are often seen growing very close to or in contact with one another, or even crossing over (Fig. 5B). However, modifications to the specific conditions of growth velocity, branching probability and tip annihilation might allow the model to also fit TC branching patterns. This would demonstrate an evolutionary conservation of a core principle of branching morphogenesis from insects to mammals and multicellular organs to single cells.

6. Outlook

Evidently we know quite a few details about the genetic makeup of terminal cells, the cellular pathways involved in their morphogenesis and their communication with other tissues. Yet, our answer to the question "How does an epithelial cell form long and stable branches from its cytoplasm?" remains hypothetical. On the molecular scale, understanding terminal cell branching will require further studies on subcellular tube growth, given that the branches probably depend on the tube but not vice versa. In particular, identifying the signal carrier that communicates the Btl signal from the basal plasma membrane to the apical membrane that forms the tube would allow us to discover how a receptor tyrosine kinase can orchestrate shape changes in both membrane domains of an epithelial cell simultaneously. The key to the terminal cell's intrinsic branching decision making might lie in the same mechanism, if indeed there is an apical signalling network that is activated by Btl and in turn nucleates microtubules to stabilise a new branch. Terminal branching is a collaborative effort between the target tissue and the tracheal cell. Compared to the inner workings of the terminal cell, our understanding of its extrinsic regulation is rudimentary. To progress here, it will be necessary to dissect the role of hypoxic signalling in branch guidance. This might also reveal new regulatory mechanisms of paracrine secretion in tracheal target tissues.

Acknowledgments

Work in the author's laboratory is supported by Deutsche Forschungsgemeinschaft Grant LE 546/7-1. All literature review and conceptual synthesis was done by B.T.B in the laboratory of M. Leptin, who supervised the writing process and approved the manuscript. The author is supported by the EMBL International PhD Programme. My gratitude goes to L.-D. Rios-Barrera and A. Cepraga for comments on the manuscript and discussion.

References

Affolter, M., Caussinus, E., 2008. Tracheal branching morphogenesis in Drosophila: new insights into cell behaviour and organ architecture. Development 135, 2055–2064. http://dx.doi.org/10.1242/dev.014498.

Affolter, M., Montagne, J., Walldorf, U., Groppe, J., Kloter, U., LaRosa, M., Gehring, W.J., 1994. The Drosophila SRF homolog is expressed in a subset of tracheal cells and maps within a genomic region required for tracheal development. Development 120, 743–753.

Affolter, M., Walldorf, U., de la Rosa, M.E., Kloter, U., Gehring, W., 1989. Characterization of a serum response factor-like gene of Drosophila. ForschBer. Biozentrum Univ. Basel, 26.

Araújo, S.J., Cela, C., Llimargas, M., 2007. Tramtrack regulates different morphogenetic events during Drosophila tracheal development. Development 134, 3665–3676 https://doi.org/10.1242/dev.007328.

Baer, M.M., Palm, W., Eaton, S., Leptin, M., Affolter, M., 2012. Microsomal triacylglycerol transfer protein (MTP) is required to expand tracheal lumen in Drosophila in a cell-autonomous manner. J. Cell Sci. 125, 6038–6048. http://dx.doi.org/10.1242/jcs.110452.

Bertolin, A.P., Katz, M.J., Yano, M., Pozzi, B., Acevedo, J.M., Blanco-Obregón, D., Gándara, L., Sorianello, E., Kanda, H., Okano, H., Srebrow, A., Wappner, P., 2016. Musashi mediates translational repression of the Drosophila hypoxia inducible factor. Nucleic Acids Res. 44, 7555–7567. http://dx.doi.org/10.1093/nar/gkw372.

Cabernard, C., Neumann, M., Affolter, M., 2004. Cellular and molecular mechanisms involved in branching morphogenesis of the Drosophila tracheal system. J. Appl. Physiol. 97, 2347–2353. http://dx.doi.org/10.1152/japplphysiol.00435.2004.

Caviglia, S., Brankatschk, M., Fischer, E.J., Eaton, S., Luschnig, S., 2016. Staccato/Unc-13-4 controls secretory lysosome-mediated lumen fusion during epithelial tube anastomosis. Nat. Cell Biol. 18, 727–739. http://dx.doi.org/10.1038/ncb3374.
Centanin, L., Dekanty, A., Romero, N., Irisarri, M., Gorr, T.A., Wappner, P., 2008. Cell autonomy of HIF effects in Drosophila: tracheal cells sense hypoxia and induce terminal branch sprouting. Dev. Cell 14, 547–558. http://dx.doi.org/10.1016/j.devcel.2008.01.020.
Chaplain, M.A., 1995. The mathematical modelling of tumour angiogenesis and invasion. Acta Biotheor. 43, 387–402.
Chihara, T., Kato, K., Taniguchi, M., Ng, J., Hayashi, S., 2003. Rac promotes epithelial cell rearrangement during tracheal tubulogenesis in Drosophila. Development 130, 1419–1428.
De Lella Ezcurra, A.L., Bertolin, A.P., Kim, K., Katz, M.J., Gándara, L., Misra, T., Luschnig, S., Perrimon, N., Melani, M., Wappner, P., 2016. miR-190 enhances HIF-dependent responses to hypoxia in drosophila by inhibiting the prolyl-4-hydroxylase Fatiga. PLoS Genet. 12, e1006073. http://dx.doi.org/10.1371/journal.pgen.1006073.
Di Giacomo, S., Sollazzo, M., Paglia, S., Grifoni, D., 2017. MYC, cell competition, and cell death in cancer: the inseparable triad. Genes (Basel) 8, 120. http://dx.doi.org/10.3390/genes8040120.
Diehl, S., Henningsson, E., Heyden, A., 2016. Efficient simulations of tubulin-driven axonal growth. J. Comput. Neurosci. 41, 45–63. http://dx.doi.org/10.1007/s10827-016-0604-x.
Djabrayan, N.J.-V., Cruz, J., de Miguel, C., Franch-Marro, X., Casanova, J., 2014. Specification of differentiated adult progenitors via inhibition of endocycle entry in the Drosophila trachea. Cell Rep. 9, 859–865. http://dx.doi.org/10.1016/j.celrep.2014.09.043.
Du, L., Zhou, A., Patel, A., Rao, M., Anderson, K., Roy, S., 2017. Unique patterns of organization and migration of FGF-expressing cells during Drosophila morphogenesis. Dev. Biol. 427, 35–48. http://dx.doi.org/10.1016/j.ydbio.2017.05.009.
Ebner, A., Kiefer, F.N., Ribeiro, C., Petit, V., Nussbaumer, U., Affolter, M., 2002. Tracheal development in Drosophila melanogaster as a model system for studying the development of a branched organ. Gene 287, 55–66.
El-Kholy, S., Stephano, F., Li, Y., Bhandari, A., Fink, C., Roeder, T., 2015. Expression analysis of octopamine and tyramine receptors in Drosophila. Cell Tissue Res. 361, 669–684. http://dx.doi.org/10.1007/s00441-015-2137-4.
Englund, C., Uv, A.E., Cantera, R., Mathies, L.D., Krasnow, M.A., Samakovlis, C., 1999. adrift, a novel bnl-induced Drosophila gene, required for tracheal pathfinding into the CNS. Development 126, 1505–1514.
Francis, D., Ghabrial, A.S., 2015. Compensatory branching morphogenesis of stalk cells in the Drosophila trachea. Development 142, 2048–2057. http://dx.doi.org/10.1242/dev.119602.
Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R., Tsujiuchi, T., 2009. Post-translational modifications of tubulin in the nervous system. J. Neurochem. 109, 683–693. http://dx.doi.org/10.1111/j.1471-4159.2009.06013.x.
Gervais, L., Casanova, J., 2011. The Drosophila homologue of SRF acts as a boosting mechanism to sustain FGF-induced terminal branching in the tracheal system. Development 138, 1269–1274. http://dx.doi.org/10.1242/dev.059188.
Gervais, L., Casanova, J., 2010. In vivo coupling of cell elongation and lumen formation in a single cell. Curr. Biol. 20, 359–366. http://dx.doi.org/10.1016/j.cub.2009.12.043.
Ghabrial, A., Luschnig, S., Metzstein, M.M., Krasnow, M.A., 2003. Branching morphogenesis of the Drosophila tracheal system. Annu. Rev. Cell Dev. Biol. 19, 623–647. http://dx.doi.org/10.1146/annurev.cellbio.19.031403.160043.
Gong, H., Han, S., Yao, H., Zhao, H., Wang, Y., 2014. AP-4 predicts poor prognosis in non-small cell lung cancer. Mol. Med. Rep. 10, 336–340. http://dx.doi.org/10.3892/mmr.2014.2209.
Gorr, T.A., Gassmann, M., Wappner, P., 2006. Sensing and responding to hypoxia via HIF in model invertebrates. J. Insect Physiol. 52, 349–364. http://dx.doi.org/10.1016/j.jinsphys.2006.01.002.
Guillemin, K., Groppe, J., Ducker, K., Treisman, R., Hafen, E., Affolter, M., Krasnow, M.A., 1996. The pruned gene encodes the Drosophila serum response factor and regulates cytoplasmic outgrowth during terminal branching of the tracheal system. Development 122, 1353–1362.
Han, Z., Li, X., Wu, J., Olson, E.N., 2004. A myocardin-related transcription factor regulates activity of serum response factor in Drosophila. Proc. Natl. Acad. Sci. USA 101, 12567–12572. http://dx.doi.org/10.1073/pnas.0405085101.
Hannezo, E., Scheele, C.L.G.J., Moad, M., Drogo, N., Heer, R., Sampogna, R.V., van Rheenen, J., Simons, B.D., 2017. A unifying theory of branching morphogenesis. Cell 171 (242–255), e27. http://dx.doi.org/10.1016/j.cell.2017.08.026.
Hayashi, S., Kondo, T., 2018. Development and function of the Drosophila tracheal system. Genetics 209, 367–380. http://dx.doi.org/10.1534/genetics.117.300167.
Hely, T.A., Graham, B., Ooyen, A. Van, 2001. A computational model of dendrite elongation and branching based on MAP2 phosphorylation. J. Theor. Biol. 210, 375–384. http://dx.doi.org/10.1006/JTBI.2001.2314.
Hemphälä, J., Uv, A., Cantera, R., Bray, S., Samakovlis, C., 2003. Grainy head controls apical membrane growth and tube elongation in response to Branchless/FGF signalling. Development 130, 249–258.
Horowitz, A., Simons, M., 2008. Branching morphogenesis. Circ. Res. 103, 784–795. http://dx.doi.org/10.1161/CIRCRESAHA.108.181818.
Hosono, C., Matsuda, R., Adryan, B., Samakovlis, C., 2015. Transient junction anisotropies orient annular cell polarization in the Drosophila airway tubes. Nat. Cell Biol. 17, 1569–1576. http://dx.doi.org/10.1038/ncb3267.
Jackstadt, R., Röh, S., Neumann, J., Jung, P., Hoffmann, R., Horst, D., Berens, C., Bornkamm, G.W., Kirchner, T., Menssen, A., Hermeking, H., 2013. AP4 is a mediator of epithelial–mesenchymal transition and metastasis in colorectal cancer. J. Exp. Med. 210, 1331–1350. http://dx.doi.org/10.1084/jem.20120812.
Jarecki, J., Johnson, E., Krasnow, M.A., 1999. Oxygen regulation of airway branching in Drosophila is mediated by branchless FGF. Cell 99, 211–220. http://dx.doi.org/10.1016/S0092-8674(00)81652-9.
JayaNandanan, N., Mathew, R., Leptin, M., 2014. Guidance of subcellular tubulogenesis by actin under the control of a synaptotagmin-like protein and Moesin. Nat. Commun. 5, 3036. http://dx.doi.org/10.1038/ncomms4036.
Jones, T.A., Metzstein, M.M., 2011. A novel function for the PAR complex in subcellular morphogenesis of tracheal terminal cells in Drosophila melanogaster. Genetics 189, 153–164. http://dx.doi.org/10.1534/genetics.111.130351.
Jones, T.A., Nikolova, L.S., Schjelderup, A., Metzstein, M.M., 2014. Exocyst-mediated membrane trafficking is required for branch outgrowth in Drosophila tracheal terminal cells. Dev. Biol. 390, 41–50. http://dx.doi.org/10.1016/j.ydbio.2014.02.021.
Jung, P., Hermeking, H., 2009. The c-MYC-AP4-p21 cascade. Cell Cycle 8, 982–989. http://dx.doi.org/10.4161/cc.8.7.7949.
Kato, K., Chihara, T., Hayashi, S., 2004. Hedgehog and Decapentaplegic instruct polarized growth of cell extensions in the Drosophila trachea. Development 131, 5253–5261. http://dx.doi.org/10.1242/dev.01404.
Kato, K., Dong, B., Wada, H., Tanaka-Matakatsu, M., Yagi, Y., Hayashi, S., 2016. Microtubule-dependent balanced cell contraction and luminal-matrix modification accelerate epithelial tube fusion. Nat. Commun. 7, 11141. http://dx.doi.org/10.1038/ncomms11141.
Kim, N., Kim, J.M., Lee, M., Kim, C.Y., Chang, K.-Y., Heo, W.D., 2014. Spatiotemporal control of fibroblast growth factor receptor signals by blue light. Chem. Biol. 21, 903–912. http://dx.doi.org/10.1016/j.chembiol.2014.05.013.
Klapholz, B., Brown, N.H., 2017. Talin – the master of integrin adhesions. J. Cell Sci. 130, 2435–2446. http://dx.doi.org/10.1242/jcs.190991.
Knöll, B., 2010. Actin-mediated gene expression in neurons: the MRTF-SRF connection. Biol. Chem. 391, 591–597. http://dx.doi.org/10.1515/BC.2010.061.
Lasser, J., Katifori, E., 2017. NET: a new framework for the vectorization and examination of network data. Source Code Biol. Med. 12, 4. http://dx.doi.org/10.1186/s13029-017-0064-3.
Levi, B.P., Ghabrial, A.S., Krasnow, M.A., 2006. Drosophila talin and integrin genes are required for maintenance of tracheal terminal branches and luminal organization. Development 133, 2383–2393. http://dx.doi.org/10.1242/dev.02404.
Li, C.L., Sathyamurthy, A., Oldenborg, A., Tank, D., Ramanan, N., 2014. SRF phosphorylation by glycogen synthase Kinase-3 promotes axon growth in hippocampal neurons. J. Neurosci. 34, 4027–4042. http://dx.doi.org/10.1523/JNEUROSCI.4677-12.2014.
Linneweber, G.A., Jacobson, J., Busch, K.E., Hudry, B., Christov, C.P., Dormann, D., Yuan, M., Otani, T., Knust, E., de Bono, M., Miguel-Aliaga, I., 2014. Neuronal control of metabolism through nutrient-dependent modulation of tracheal branching. Cell 156, 69–83. http://dx.doi.org/10.1016/j.cell.2013.12.008.
McDougall, S.R., Watson, M.G., Devlin, A.H., Mitchell, C.A., Chaplain, M.A.J., 2012. A hybrid discrete-continuum mathematical model of pattern prediction in the developing retinal vasculature. Bull. Math. Biol. 74, 2272–2314. http://dx.doi.org/10.1007/s11538-012-9754-9.
Miano, J.M., Long, X., Fujiwara, K., 2007. Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus. Am. J. Physiol. Physiol. 292, C70–C81. http://dx.doi.org/10.1152/ajpcell.00386.2006.
Moreira-Soares, M., Coimbra, R., Rebelo, L., Carvalho, J., D M Travasso, R., 2018. Angiogenic factors produced by hypoxic cells are a leading driver of anastomoses in sprouting angiogenesis-a computational study. Sci. Rep. 8, 8726. http://dx.doi.org/10.1038/s41598-018-27034-8.
Mortimer, N.T., Moberg, K.H., 2013. The archipelago ubiquitin ligase subunit acts in target tissue to restrict tracheal terminal cell branching and hypoxic-induced gene expression. PLoS Genet. 9, e1003314. http://dx.doi.org/10.1371/journal.pgen.1003314.
Mortimer, N.T., Moberg, K.H., 2009. Regulation of Drosophila embryonic tracheogenesis by dVHL and hypoxia. Dev. Biol. 329, 294–305. http://dx.doi.org/10.1016/j.ydbio.2009.03.001.
Muha, V., Müller, H.-A.J., 2013. Functions and mechanisms of Fibroblast Growth Factor (FGF) signalling in Drosophila melanogaster. Int. J. Mol. Sci. 14, 5920–5937. http://dx.doi.org/10.3390/ijms14035920.
Myat, M.M., Patel, U., 2016. Receptor-type guanylyl cyclase at 76C (Gyc76C) regulates de novo lumen formation during drosophila tracheal development. PLoS One 11, e0161865. http://dx.doi.org/10.1371/journal.pone.0161865.
Nevil, M., Bondra, E.R., Schulz, K.N., Kaplan, T., Harrison, M.M., 2017. Stable binding of the conserved transcription factor grainy head to its target genes throughout *Drosophila melanogaster* development. Genetics 205, 605–620. http://dx.doi.org/10.1534/genetics.116.195685.
Nikolova, L.S., Metzstein, M.M., 2015. Intracellular lumen formation in Drosophila proceeds via a novel subcellular compartment. Development 142, 3964–3973. http://dx.doi.org/10.1242/dev.127902.
Nishimura, M., Inoue, Y., Hayashi, S., 2007. A wave of EGFR signaling determines cell alignment and intercalation in the Drosophila tracheal placode. Development 134, 4273–4282. http://dx.doi.org/10.1242/dev.010397.
O'Toole, M., Lamoureux, P., Miller, K.E., 2008. A physical model of axonal elongation: force, viscosity, and adhesions govern the mode of outgrowth. Biophys. J. 94, 2610–2620. http://dx.doi.org/10.1529/biophysj.107.117424.
Ochoa-Espinosa, A., Affolter, M., 2012. Branching morphogenesis: from cells to organs and back. Cold Spring Harb. Perspect. Biol., 4. http://dx.doi.org/10.1101/

cshperspect.a008243.
Okenve-Ramos, P., Llimargas, M., 2014. Fascin links Btl/FGFR signalling to the actin cytoskeleton during Drosophila tracheal morphogenesis. Development 141, 929–939. http://dx.doi.org/10.1242/dev.103218.
Olson, E.N., Nordheim, A., 2010. Linking actin dynamics and gene transcription to drive cellular motile functions. Nat. Rev. Mol. Cell Biol. 11, 353–365.
Oshima, K., Takeda, M., Kuranaga, E., Ueda, R., Aigaki, T., Miura, M., Hayashi, S., 2006. IKK epsilon regulates F actin assembly and interacts with Drosophila IAP1 in cellular morphogenesis. Curr. Biol. 16, 1531–1537. http://dx.doi.org/10.1016/j.cub.2006.06.032.
Peters, N.C., Thayer, N.H., Kerr, S.A., Tompa, M., Berg, C.A., 2013. Following the 'tracks': Tramtrack69 regulates epithelial tube expansion in the Drosophila ovary through Paxillin, Dynamin, and the homeobox protein Mirror. Dev. Biol. 378, 154–169. http://dx.doi.org/10.1016/j.ydbio.2013.03.017.
Peterson, S.J., Krasnow, M.A., 2015. Subcellular trafficking of FGF controls tracheal invasion of Drosophila flight muscle. Cell 160, 313–323. http://dx.doi.org/10.1016/j.cell.2014.11.043.
Petit, V., Ribeiro, C., Ebner, A., Affolter, M., 2002. Regulation of cell migration during tracheal development in Drosophila melanogaster. Int. J. Dev. Biol. 46, 125–132.
Ricolo, D., Deligiannaki, M., Casanova, J., Araújo, S.J., 2016. Centrosome amplification increases single-cell branching in post-mitotic cells. Curr. Biol. 26, 2805–2813. http://dx.doi.org/10.1016/j.cub.2016.08.020.
Ríos-Barrera, L.D., Sigurbjörnsdóttir, S., Baer, M., Leptin, M., 2017. Dual function for Tango1 in secretion of bulky cargo and in ER-Golgi morphology. Proc. Natl. Acad. Sci. USA 114, E10389–E10398. http://dx.doi.org/10.1073/pnas.1711408114.
Romero, N.M., Dekanty, A., Wappner, P., 2007. Cellular and developmental adaptations to hypoxia: a Drosophila perspective. Methods Enzymol. 435, 123–144. http://dx.doi.org/10.1016/S0076-6879(07)35007-6.
Rosa, J.B., Metzstein, M.M., Ghabrial, A.S., 2018. An Ichor-dependent apical extracellular matrix regulates seamless tube shape and integrity. PLOS Genet. 14, e1007146. http://dx.doi.org/10.1371/journal.pgen.1007146.
Ruiz, O.E., Nikolova, L.S., Metzstein, M.M., 2012. Drosophila Zpr1 (Zinc finger protein 1) is required downstream of both EGFR and FGFR signaling in tracheal subcellular lumen formation. PLoS One 7, e45649. http://dx.doi.org/10.1371/journal.pone.0045649.
Rulifson, E.J., Kim, S.K., Nusse, R., 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 80-. (296), 1118–1120. http://dx.doi.org/10.1126/science.1070058.
Salvany, L., Muller, J., Guccione, E., Rorth, P., 2014. The core and conserved role of MAL is homeostatic regulation of actin levels. Genes Dev. 28, 1048–1053. http://dx.doi.org/10.1101/gad.237743.114.
Sato, M., Kornberg, T.B., 2002. FGF is an essential mitogen and chemoattractant for the air sacs of the drosophila tracheal system. Dev. Cell 3, 195–207.
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. http://dx.doi.org/10.1038/nmeth.2019.
Schottenfeld-Roames, J., Ghabrial, A.S., 2012. Whacked and Rab35 polarize dynein-motor-complex-dependent seamless tube growth. Nat. Cell Biol. 14, 386–393. http://dx.doi.org/10.1038/ncb2454.
Schottenfeld-Roames, J., Rosa, J.B., Ghabrial, A.S., 2014. Seamless tube shape is constrained by endocytosis-dependent regulation of active Moesin. Curr. Biol. 24, 1756–1764. http://dx.doi.org/10.1016/j.cub.2014.06.029.
Schottenfeld, J., Song, Y., Ghabrial, A.S., 2010. Tube continued: morphogenesis of the Drosophila tracheal system. Curr. Opin. Cell Biol. 22, 633–639. http://dx.doi.org/10.1016/j.ceb.2010.07.016.
Sigurbjornsdottir, S., Mathew, R., Leptin, M., 2014. Molecular mechanisms of de novo lumen formation. Nat. Rev. Mol. Cell Biol. 15, 665–676. http://dx.doi.org/10.1038/nrm3871.
Somogyi, K., Rørth, P., 2004. Evidence for tension-based regulation of Drosophila MAL and SRF during invasive cell migration. Dev. Cell 7, 85–93. http://dx.doi.org/10.1016/j.devcel.2004.05.020.
Song, Y., Eng, M., Ghabrial, A.S., 2013. Focal defects in single-celled tubes mutant for Cerebral cavernous malformation 3, GCKIII, or NSF2. Dev. Cell 25, 507–519. http://dx.doi.org/10.1016/j.devcel.2013.05.002.
Sun, X., Feng, Z., Qu, Y., Shao, J., Wei, N., 2017. Expression of Activating Protein 4 and Its Relationships with Prognosis in Gastric Cancer. Chemotherapy 62, 181–186. http://dx.doi.org/10.1159/000455004.
Sundaram, M.V., Cohen, J.D., 2017. Time to make the doughnuts: building and shaping seamless tubes. Semin. Cell Dev. Biol. 67, 123–131. http://dx.doi.org/10.1016/j.semcdb.2016.05.006.
Suzuki, T., Minerva, D., Nishiyama, K., Koshikawa, N., Chaplain, M.A.J., 2018. Study on the tumor-induced angiogenesis using mathematical models. Cancer Sci. 109, 15–23. http://dx.doi.org/10.1111/cas.13395.
Tsaneva-Atanasova, K., Burgo, A., Galli, T., Holcman, D., 2009. Quantifying neurite growth mediated by interactions among secretory vesicles, microtubules, and actin networks. Biophys. J. 96, 840–857. http://dx.doi.org/10.1016/j.bpj.2008.10.036.
Ukken, F.P., Aprill, I., JayaNandanan, N., Leptin, M., 2014. Slik and the receptor tyrosine kinase Breathless mediate localized activation of Moesin in terminal tracheal cells. PLoS One 9, e103323. http://dx.doi.org/10.1371/journal.pone.0103323.
UniProt, 2017. UniProt: the universal protein knowledgebase. (Entry P50552)Nucleic Acids Res. 45. http://dx.doi.org/10.1093/nar/gkw1099.
Uv, A., Cantera, R., Samakovlis, C., 2003. Drosophila tracheal morphogenesis: intricate cellular solutions to basic plumbing problems. Trends Cell Biol. 13, 301–309.
van Ooyen, A., 2011. Using theoretical models to analyse neural development. Nat. Rev. Neurosci. 12, 311–326. http://dx.doi.org/10.1038/nrn3031.
VandenBrooks, J.M., Gstrein, G., Harmon, J., Friedman, J., Olsen, M., Ward, A., Parker, G., 2017. Supply and demand: How does variation in atmospheric oxygen during development affect insect tracheal and mitochondrial networks? J. Insect Physiol.. http://dx.doi.org/10.1016/j.jinsphys.2017.11.001.
Wang, S., Samakovlis, C., 2012. Grainy head and its target genes in epithelial morphogenesis and wound healing. Curr. Top. Dev. Biol., 35–63. http://dx.doi.org/10.1016/B978-0-12-386499-4.00002-1.
Wigglesworth, V.B., Lee, W.M., 1982. The supply of oxygen to the flight muscles of insects: a theory of tracheole physiology. Tissue Cell 14, 501–518.
Wong, D.M., Shen, Z., Owyang, K.E., Martinez-Agosto, J.A., 2014. Insulin- and warts-dependent regulation of tracheal plasticity modulates systemic larval growth during hypoxia in Drosophila melanogaster. PLoS One 9, e115297. http://dx.doi.org/10.1371/journal.pone.0115297.
Wong, M.M.-K., Liu, M.-F., Chiu, S.K., 2015. Cropped, Drosophila transcription factor AP-4, controls tracheal terminal branching and cell growth. BMC Dev. Biol. 15, 20. http://dx.doi.org/10.1186/s12861-015-0069-6.
Wu, B., Guo, W., 2015. The exocyst at a glance. J. Cell Sci. 128, 2957–2964. http://dx.doi.org/10.1242/jcs.156398.
Xinghua, L., Bo, Z., Yan, G., Lei, W., Changyao, W., Qi, L., Lin, Y., Kaixiong, T., Guobin, W., Jianying, C., 2012. The overexpression of AP-4 as a prognostic indicator for gastric carcinoma. Med. Oncol. 29, 871–877. http://dx.doi.org/10.1007/s12032-011-9845-8.
Yao, L., Wang, S., Westholm, J.O., Dai, Q., Matsuda, R., Hosono, C., Bray, S., Lai, E.C., Samakovlis, C., 2017. Genome-wide identification of Grainy head targets in Drosophila reveals regulatory interactions with the POU domain transcription factor Vvl. Development 144, 3145–3155. http://dx.doi.org/10.1242/dev.143297.
Zhu, M.Y., Wilson, R., Leptin, M., 2005. A screen for genes that influence fibroblast growth factor signal transduction in Drosophila. Genetics 170, 767–777. http://dx.doi.org/10.1534/genetics.104.039750.