Drosophila tracheal system formation involves FGF-dependent cell extensions contacting bridge-cells

Christian Wolf, Nina Gerlach & Reinhard Schuh⁺

Max-Planck-Institut für biophysikalische Chemie, Abt. Molekulare Entwicklungsbiologie, Am Fassberg, D-37077 Göttingen, Germany

Received August 24, 2001; revised March 7, 2002; accepted April 8, 2002

Development of the ectodermally derived *Drosophila* tracheal system is based on branch outgrowth and fusion that interconnect metamERICALLY arranged tracheal subunits into a highly stereotyped three-dimensional tubular structure. Recent studies have revealed that this process involves a specialized cell type of mesodermal origin, termed bridge-cell. Single bridge-cells are located between adjacent tracheal subunits and serve as guiding posts for the outgrowing dorsal trunk branches. We show that bridge-cell-approaching tracheal cells form filopodia-like cell extensions, which attach to the bridge-cell surface and are essential for the tracheal subunit interconnection. The results of both dominant-negative and gain-of-function experiments suggest that the formation of cell extensions require Cdc42-mediated *Drosophila* fibroblast growth factor activity.

INTRODUCTION

Tracheal system formation is initiated by the formation of 10 segmentally arranged tracheal metameres along each side of the *Drosophila* embryo. These metameres consist of ~80 ectodermal cells and begin to invaginate into the underlying mesoderm during stage 11 of embryogenesis (stages according to Campos-Ortega and Hartenstein, 1985). Concomitantly, they form a stereotyped pattern of finger-like branches in response to the *Drosophila* fibroblast growth factor (dFGF). dFGF, encoded by the gene *branchless* (*bnl*), is expressed in cell groups surrounding the individual metameres (see scheme in Figure 1; Sutherland *et al.*, 1996). The dFGF/Bnl signal is received by its receptor Breathless (Btl) in tracheal cells (Reichman-Fried *et al.*, 1994) and guides the tracheal cells towards the highest dFGF/Bnl concentration, resulting in a distinct pattern of primary branches

(Metzger and Krasnow, 1999). During stage 12, tracheal cells in a dorsolateral position of each placode migrate in anterior and posterior directions representing the dorsal trunk primary branches. These branches fuse with their counterparts of the adjacent metameres, forming the major tracheal tube along the longitudinal axis of the embryo, the dorsal trunk. In addition to dFGF/Bnl, a cellular guidance mechanism was identified to be essential for a continuous dorsal trunk formation. Single mesodermal bridge-cells, which are positioned between adjacent anterior and posterior dorsal trunk branches, serve as guidance posts to position correctly the outbudding dorsal trunk branches (see Figure 1; Wolf and Schuh, 2000). Subsequently, unicellular secondary branches are generated to form the lateral trunk and to interconnect the two halves of the embryonic tracheal network (reviewed in Shilo *et al.*, 1997; Metzger and Krasnow, 1999; Affolter and Shilo, 2000).

Here, we show that dFGF/Bnl controls not only cell migration processes during early periods of tracheal development, but also serves to induce filopodia-like tracheal cell extensions that contact the bridge-cell. Loss-of-function and gain-of-function experiments indicate that the formation of these cell extensions is a prerequisite for the dorsal trunk branch fusion.

RESULTS

Formation of cell extensions during tracheal branch outgrowth

Recent work has speculated that the dorsal trunk formation involves tracheal cell extensions contacting the bridge-cell (Wolf and Schuh, 2000). In order to visualize and demonstrate such possible structures, we used the enhancer trap fly line

⁺Corresponding author. Tel: +49 55 1201 1758; Fax: +49 55 1201 1755; E-mail: rschuh@gwdg.de

Fig. 1. Tracheal network formation. (A) Scheme of tracheal development during stage 12. Tracheal cells (red) and Branchless-expressing cells (green) are indicated. (B) Whole-mount *in situ* double hybridization of *I-eve-1* embryos at stage 12 using *lacZ* (red) and *hb* (blue) antisense RNA probes. *I-eve-1* embryos reveal *lacZ* marker gene expression in the tracheal cells (red). The bridge-cell (arrows; revealed by *hb* expression) serves as a guidance post for the dorsal trunk branches.

I-eve-1 to specifically mark the cytoplasm of tracheal cells by β -galactosidase expression (Perrimon *et al.*, 1991). We failed to detect cell extensions during the tracheal placode formation (stage 11; data not shown). However, after invagination of the tracheal cells when the primary branches begin to form during embryonic stage 12, cell extensions were found at the leading edges of the developing dorsal and lateral trunk branches (Figure 2A–C; data not shown). In mid stage 12, extensions of adjacent metameres fuse and form a continuous interconnection (Figure 2D). To exclude possible fixation artefacts, we also monitored the formation of cell extensions *in vivo* using transgenes expressing green fluorescent protein (GFP) fused to actin and tubulin, respectively (see Methods). Both GFP-labelled proteins were expressed specifically in the developing tracheal system using the UAS–Gal4 system (Brand and Perrimon, 1993). Such embryos form extensions, which interconnect adjacent metameres during mid stage 12 as found in fixed embryos (Figure 2E and F; compare with D). The cell extensions have a diameter of $\sim 0.5 \mu\text{m}$ during early stage 12; they both contain tubulin (Figure 2E) and filamentous actin cytoskeleton (Figure 2F).

The observation that the bridge-cells might be associated with the tracheal cell extensions led us to examine whether the bridge-cells are involved in their formation. In *hb* mutant embryos, which lack bridge-cells (Wolf and Schuh, 2000), normal cell extensions of the tracheal cells were found (compare Figure 2G with B). These results demonstrate that the proper formation of tracheal cell extensions does not require functional bridge-cells.

Tracheal cell extensions are FGF/Bnl-dependent

dFGF/Bnl signalling triggers primary branch outgrowth (Sutherland *et al.*, 1996). Thus, we asked whether dFGF signalling also participates in the formation of the tracheal cell extensions. To address this question, we made use of various loss-of-function and gain-of-function mutations affecting the dFGF/Bnl signal or the dFGF/Btl receptor. Figure 2H and I show that tracheal cellular extensions are absent in *bnl* and *btl* mutant embryos. Conversely, ectopic tracheal dFGF/Bnl expression (*UAS-bnl* via *btl-Gal4*) was able to induce ectopic cellular extensions found in fixed embryos (Figure 2J) and *in vivo* (compare Figure 2K with E). These results indicate that the formation of cellular extensions depends on dFGF/Bnl activity.

Tracheal cell extensions can be induced by activated Cdc42

Previous work has shown that the reorganization of cytoskeleton and the formation of filopodial cell extensions is triggered by Cdc42, a Rho-like GTPase (Hall, 1998). Cdc42 activity induces filopodial extensions in vertebrate fibroblasts (Nobes and Hall, 1995) and controls the cytoskeleton reorganization that is associated with cell migration processes during *Drosophila* development (Ricos *et al.*, 1999). In contrast, overexpression of a dominant-negative Cdc42 variant was shown to inhibit the formation of filopodial extensions and thereby prevents the appropriate cell–cell matching process necessary for cell migration in *Drosophila* (Jacinto *et al.*, 2000).

To examine whether Cdc42 participates in the formation of tracheal cell extensions, we misexpressed constitutively active Cdc42 in the tracheal cells (see Methods). Such cells developed ectopic tracheal extensions (Figure 2L and M) as had been observed in response to ectopic dFGF/Bnl (compare with Figure 2J). These results suggest that Cdc42 activity mediates dFGF/Bnl-dependent formation of tracheal cell extensions.

Cell extensions are essential for dorsal trunk branch fusion

In order to test whether tracheal cell extensions participate in dorsal trunk formation, we ectopically expressed dominant-negative forms of Cdc42 ubiquitously in the embryo during early tracheal development. We observed 32 and 9.8% dorsal trunk breaks, respectively, by ectopic expression of dominant-negative forms of Cdc42 with different strength (Table IA; Figure 3B). In control experiments (*hs-Gal4; UAS-GFP*), <1% of the dorsal trunk branches fail to interconnect properly (Table IA; Figure 3A). Thus, these data suggest that reduction of cell extensions by ectopic expression of dominant-negative Cdc42 interferes with dorsal trunk branch fusion.

In order to test if the induction of cell extensions mediates dorsal trunk fusion, we ectopically expressed activated Cdc42 specifically in tracheal cells of *bnl*-mutant embryos. Homozygous embryos of the strong *bnl^{P1}* allele not only lack tracheal cell extensions (see above), but also fail to develop an interconnected dorsal trunk (Sutherland *et al.*, 1996). Instead, these embryos show a segmental array of isolated metameric tracheal units with a lollipop-like structure in place of the dorsal trunk (Figure 3C). We never observed an interconnection of the

Fig. 2. Tracheal filopodia-like cell extensions are mediated by FGF signalling and activated Cdc42. (A) Scheme of a tracheal metamere at stage 12. Symbols indicate normal (open circles, closed circles) and ectopic (+, ‡, #) outgrowth of cell extensions. (B–D) Whole-mount antibody staining of *l-eve-1* embryos at early (B, C) and mid stage 12 (D) with anti- β -galactosidase antibodies. Primordial dorsal trunk branch cells form cellular extensions growing towards the adjacent tracheal metameres (B, C). In mid stage 12, extensions of adjacent metameres fuse and form a continuous interconnection (D). (E, F). Whole-mount GFP *in vivo* visualization of stage 12 embryos bearing *btl-Gal4* and *UAS-tau-myc-GFP* (E) or *UAS-actin-GFP* (F). *tau-myc-GFP* visualizes microtubules (E) and actin-GFP visualizes actin filaments (F); cytoskeleton connections between two adjacent metameres are indicated (arrows in E and F). Whole-mount antibody staining with anti- β -galactosidase antibodies of stage 12 *hb* (G), *bnl* (H) and *btl* (I) mutant embryos bearing the *l-eve-1* chromosome. *hb* mutant embryos form normal tracheal cell extensions (G). *bnl* and *btl* mutant embryos lack tracheal cell extensions (H, I). Whole-mount antibody staining with anti- β -galactosidase antibodies of stage 12 *l-eve-1* embryos bearing *btl-Gal4* and *UAS-bnl* (J). Ectopic tracheal cell extensions were found with ectopic tracheal expression of *bnl*. Whole-mount *in vivo* visualization of GFP of a stage 12 embryos bearing *btl-Gal4*, *UAS-tau-myc-GFP* and *UAS-bnl* (K); the focal plane is the developing dorsal trunk region and cytoskeleton interconnections between two adjacent metameres are indicated (arrows). Numerous tracheal cell extensions are formed by ectopic *bnl* expression (K) as compared to wild-type embryos (E). Whole-mount antibody staining with anti- β -galactosidase antibodies of stage 12 *l-eve-1* embryos bearing *btl-Gal4* and *UAS-Cdc42^{V12}* (L, M). Activated Cdc42 in tracheal cells induces ectopic tracheal cell extensions. The white bars indicate a size of 5 μ m.

lollipop-like structures in *bnl^{P1}* mutant embryos (Table IB). In contrast, upon expression of activated Cdc42, such embryos still lack coherent dorsal trunk structures but show occasional fusions (4.4%) of the adjacent lollipop-like dorsal trunk structures (Figure 3D). This result indicates that the formation of tracheal cell extensions is sufficient to partially rescue a dFGF/Bnl-dependent dorsal trunk phenotype.

Tracheal cell extensions are in contact with bridge-cells

Bridge-cells are likely to serve as guiding posts for the orientation of dorsal trunk branches (Wolf and Schuh, 2000). This observation and the finding that tracheal cell extensions are essential for proper dorsal trunk development suggest that the two components interact in the same process. In order to test this proposal, we examined the connection of tracheal cell extensions with the

bridge-cell surface by confocal microscopy combined with either optical Z-scan analysis or transversal sections (see Methods). Figure 4 shows that the tracheal cell extensions are in direct contact with the bridge-cells along their anterior–posterior axis. Between mid and late embryonic stage 12, the diameter of cell extensions increases by ~2-fold (2 μ m, Figure 4A2 and C; 4 μ m, Figure 4B2). These results indicate that the cell extensions are in direct contact with the bridge-cell and suggest that the bridge-cells serve as migration matrix for the extensions, which precede branch fusion along the same axis.

DISCUSSION

Drosophila dFGF signalling is used reiteratively during the different developmental steps of tracheal organogenesis. It triggers primary branch outgrowth, controls secondary branch sprouting and mediates terminal branching (Metzger and

Table I. Effects of ectopic constitutively active or dominant-negative Cdc42 expression on tracheal dorsal trunk formation

A. Induction of dorsal trunk breaks				
Genotype ^a	Heat shock ^b	<i>n</i> ^c	Dorsal trunk breaks	Dorsal trunk breaks (%) ^d
<i>UAS-Cdc42^{N17}; hs-Gal4</i>	yes	540	175	32
<i>UAS-Cdc42^{N17}; hs-Gal4</i>	no	720	0	0
<i>UAS-Cdc42^{S89}; hs-Gal4</i>	yes	540	53	9.8
<i>UAS-Cdc42^{S89}; hs-Gal4</i>	no	420	0	0
<i>UAS-GFP; hs-Gal4</i>	yes	720	4	0.56
B. Rescue of dorsal trunk fusion				
Genotype ^e	Temperature (°C)	<i>n</i> ^c	Fusion	Fusion (%) ^f
<i>bnl^{P1}/bnl^{P1}</i>	18	720	0	0
<i>UAS-Cdc42^{V12}/btl-Gal4; bnl^{P1}/bnl^{P1}</i>	18	1080	0	0
<i>bnl^{P1}/bnl^{P1}</i>	29	540	0	0
<i>UAS-Cdc42^{V12}/btl-Gal4; bnl^{P1}/bnl^{P1}</i>	29	250	11	4.4

^a*Cdc42^{N17}* exhibits a stronger dominant-negative form of Cdc42 than *Cdc42^{S89}* (Jacinto *et al.*, 2000).

^bEmbryos (4–6 h old; 25°C) were heat shocked at 37°C for 20 min. The embryos were fixed after 8 h recovery at 25°C. Embryos receiving no heat shock were fixed after 12–14 h of development at 25°C.

^cNumber of analysed tracheal metameres.

^dPercentage of dorsal trunk breaks caused by stalled and misguided dorsal trunk branches.

^e*Cdc42^{V12}* represents a constitutively active form of Cdc42 (Luo *et al.*, 1994).

^fPercentage of fusion between two adjacent tracheal metameres.

Fig. 3. Dominant negative and constitutively activated Cdc42 interferes with dorsal trunk development. Whole-mount 2A12 antibody staining of heat-induced (described in Table I) stage 15 embryos bearing *hs-Gal4* and *UAS-GFP* (A) or *hs-Gal4* and *UAS-Cdc42^{N17}* (B). Heat shock induced ectopic expression of GFP reveals a normal tracheal development (A), while ectopic expression of dominant-negative Cdc42 results in a lack of dorsal trunk fusion (B). Whole-mount 2A12 antibody staining of a stage 15 *bnl^{P1}* mutant embryo (C) and a *bnl^{P1}* embryo bearing *UAS-Cdc42^{V12}* and *btl-Gal4* (D). *bnl* mutant embryos, which express activated Cdc42 in tracheal cells, reveal rudimentary fusion of dorsal trunk structures (D). In contrast, *bnl* mutant embryos lack fusion of tracheal structures (C).

Krasnow, 1999) in response to the signals produced by oxygen-starved cells (Jarecki *et al.*, 1999). Here, we provide evidence that dFGF also acts as a growth factor that stimulates the development of tracheal cell extensions necessary for tracheal branch fusion. This conclusion is based on the observations that tracheal cell extensions are missing in dFGF-signalling mutants

while the formation of ectopic extensions is induced by ectopic dFGF/Bnl. Gain-of-function experiments suggest that the dFGF/Bnl-dependent cell extension formation is mediated via the Rho-like GTPase Cdc42.

What is the function of the tracheal cellular extensions? During early tracheal development, dFGF/Bnl is instructive for tracheal branch outgrowth. However, gain-of-function experiments indicate that the dorsal trunk forms independently of dFGF/Bnl-guidance, suggesting that dFGF/Bnl provides a permissive rather than an instructive signal for the dorsal trunk formation (Sutherland *et al.*, 1996). Previously, we identified the mesodermal bridge-cell, which guides the dorsal trunk branches (Wolf and Schuh, 2000). Our results establish that the dFGF/Bnl-induced tracheal cell extensions are necessary for bridge-cell-mediated dorsal trunk formation. Several observations support this conclusion. (i) Bridge-cells are in direct contact with the leading edges of the outgrowing dorsal trunk branches that form cell extensions. (ii) While the extensions grow out in an anterior or a posterior direction, they are in direct association with the bridge-cells. (iii) The cell extensions interconnect adjacent tracheal metameres ~2.5 h before the dorsal trunk branches fuse. (iv) Ectopic expression of dominant-negative Cdc42, which represses the formation of cell extensions (Jacinto *et al.*, 2000), frequently induces the lack of dorsal trunk branch interconnections. (v) Cdc42-activated ectopic extensions partially rescue fusion of dorsal trunk rudiments in embryos that lack dFGF/Bnl.

The ability of cell extensions to mediate branch fusion via bridge-cells is restricted to dorsal trunk formation. This specific function is likely to be essential since dorsal trunk branch fusion is a multicellular process while all other tracheal interconnections are single cell fusions. Furthermore, dorsal trunk fusion precedes the other fusion processes although all branches bud out at the

Fig. 4. Tracheal cell extensions grow along the bridge-cells. Whole-mount antibody staining and *in situ* hybridization of *1-eve-1* embryos at mid stage 12 (A) and late stage 12 (B) with anti- β -galactosidase antibodies (red) and *hb* anti-sense RNA (green). The dotted lines indicate optical Z-scan sections (see Methods) shown in A1–A3 and B1–B3, respectively. (C) Transversal section (see Methods) of a fluorescently stained embryo (as in B) visualizes the association of the bridge-cell (green) and the tracheal extensions (red). The white bars indicate a size of 2 μ m.

same time. In addition, different surrounding cell matrices may require various mechanisms for branch outgrowth. In fact, previous work has shown that dorsal branch cells follow a path along a pattern of grooves left between the muscle precursor cells of adjacent metameres, whereas the dorsal trunk branches remain in association with a contiguous population of mesodermal cells (Franch-Marro and Casanova, 2000).

Interestingly, dFGF signalling has also been implicated in the outgrowth of cytonemes, which are discussed to function in the distribution of morphogens during *Drosophila* imaginal disc development (Ramirez-Weber and Kornberg, 1999). However, cytonemes are remarkably long and microtubule-free cell extensions with a diameter of 0.2 μ m. Thus, they differ from the cell extensions described here in size and cytoskeletal composition, i.e. tracheal cell extensions are more than double in diameter and contain microtubules in addition to filamentous actin.

We speculate that the bridge-cell recognition, the sliding of the tracheal cell extensions along the bridge-cell surface and finally the fusion process are likely to involve extracellular matrix and/or cell adhesion molecules that are associated with the tubular cell extensions and the bridge-cell surface. The functional characterization of such proteins will provide further insights into the guidance mechanisms of cell extensions along specialized cells.

METHODS

Fly stocks. The following fly strains and alleles were used in this study: *hb^{FB}* (Tübingen Stock Centre), *bnl^{P1}* (Sutherland *et al.*, 1996) and *btl^{LG19}* (Klämbt *et al.*, 1992). To drive Gal4 ubiquitously in the tracheal system from stage 11 onwards, we used *btl-Gal4* driver fly lines (Shiga *et al.*, 1996). *UAS-tau-myc-gfp* and *UAS-actin-gfp* (Brand, 1999) were used to detect cytoskeletal-bound

GFP in the tracheal system. We also used *UAS-bnl* (Sutherland *et al.*, 1996), *UAS-Cdc42^{V12}*, *UAS-Cdc42^{N17}* (Luo *et al.*, 1994) and *UAS-Cdc42^{S89}* (Eaton *et al.*, 1995). The *lacZ* enhancer trap line *1-eve-1* reveals P-element integration in the *trh* gene and was used to mark tracheal cells by cytoplasmic β -galactosidase (Perrimon *et al.*, 1991).

Immunocytochemistry, *in situ* hybridization on whole-mount embryos and microscopy. Whole-mount immunostainings of fixed embryos (Goldstein and Fryberg, 1994) were performed with the monoclonal antibody 2A12 (DSHB, Iowa City, IA) and with rabbit anti- β -galactosidase antiserum (Cappel, Costa Mesa, CA). Secondary antibodies (Alexa 488 goat anti-rabbit and goat anti-mouse IgM antibodies, respectively) were obtained from Molecular Probes (Eugene, OR) and Vector Laboratories (Burlingame, CA). We used the horseradish peroxidase Elite ABC kit (Vector Laboratories) for signal amplification. Whole-mount *in situ* hybridizations on embryos (Goldstein and Fryberg, 1994) were performed with DIG-labelled antisense RNA probes of *lacZ* and *hb* (Tautz *et al.*, 1987). Phosphatase-conjugated anti-DIG antibodies were from Roche Diagnostics (Mannheim, Germany). Stained embryos were examined with a Zeiss Axiophot microscope. Fluorescent double immunostainings and RNA *in situ* hybridizations (Knirr *et al.*, 1999) were performed with TSA Cyanine 3 system (NEN, Boston, MA). Sheep anti-DIG and biotin-SP-conjugated affininure donkey anti-sheep antibodies were obtained from Roche Diagnostics and Jackson ImmunoResearch (West Grove, PA). To obtain transversal sections of fluorescently stained embryos, we first fixed the stained embryos in 10% paraformaldehyde, 50 mM EGTA pH 7 for 60 min. The fixed embryos were then cut in several slices using a hypodermic needle (0.4 $\times$ 21 mm; S. Roth, personal communication). The fluorescently stained transversal slices of embryos were examined with a Zeiss Laser Scan microscope LSM410 invert. Z-scan

C. Wolf, N. Gerlach & R. Schuh

images were performed with Zeiss LSM software 3.98 and images were edited using Adobe Photoshop 5.02.

ACKNOWLEDGEMENTS

We thank M. González-Gaitán, A. Jacinto and E. Wimmer for fly stocks and cDNAs. We are grateful to S. Roth for technical advice. We also thank H. Jäckle, R.P. Kühnlein and C. Krause for comments on the manuscript and for discussions. We give special thanks to H. Jäckle for providing a stimulating environment. This work was supported by the Max-Planck-Society (MPIIbpc Abt. 170) and the Deutsche Forschungsgemeinschaft (SFB 271).

REFERENCES

- Affolter, M. and Shilo, B.-Z. (2000) Genetic control of branching morphogenesis during *Drosophila* tracheal development. *Curr. Opin. Cell Biol.*, **12**, 731–735.
- Brand, A. (1999) GFP as a cell developmental marker in the *Drosophila* nervous system. *Meth. Cell Biol.*, **58**, 165–181.
- Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**, 401–415.
- Campos-Ortega, J.A. and Hartenstein, V. (1985) *The Embryonic Development of Drosophila melanogaster*. Springer, Berlin, Germany.
- Eaton, S., Auviven, P., Luo, L., Jan, Y.N. and Simons, K. (1995) Cdc42 and Rac1 control different actin-dependent processes in the *Drosophila* wing disc epithelium. *J. Cell Biol.*, **131**, 151–164.
- Franch-Marro, X. and Casanova, J. (2000) The alternative migratory pathways of the *Drosophila* tracheal cells are associated with distinct subsets of mesodermal cells. *Dev. Biol.*, **227**, 80–90.
- Goldstein, L.S.B. and Fryberg, E.A. (1994) *Drosophila melanogaster*: practical uses in cell and molecular biology. *Methods Cell Biol.*, **44**, 446–485.
- Hall, A. (1998) Rho GTPases and the actin cytoskeleton. *Science*, **279**, 509–514.
- Jacinto, A., Wood, W., Balayo, T., Turmaine, M., Martinez-Arias, A. and Martin, P. (2000) Dynamic actin-based epithelial adhesion and cell matching during *Drosophila* dorsal closure. *Curr. Biol.*, **10**, 1420–1426.
- Jarecki, J., Johnson, E. and Krasnow, M.A. (1999) Oxygen regulation of airway branching in *Drosophila* is mediated by Branchless FGF. *Cell*, **99**, 211–220.
- Klämbt, C., Glazer, L. and Shilo, B.Z. (1992) *breathless*, a *Drosophila* FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. *Genes Dev.*, **6**, 1668–1678.
- Knirr, S., Azpiazu, N. and Frasch, M. (1999) The role of the NK-homeobox gene *slouch* (*S59*) in somatic muscle patterning. *Development*, **126**, 4525–4535.
- Luo, L., Liao, L.Y., Jan, L.Y. and Jan, Y.N. (1994) Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes Dev.*, **8**, 1787–1802.
- Metzger, R.J. and Krasnow, M.A. (1999) Genetic control of branching morphogenesis. *Science*, **284**, 1635–1639.
- Nobes, C.D. and Hall, A. (1995) Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*, **81**, 53–62.
- Perrimon, N., Noll, E., McCall, K. and Brand, A. (1991) Generating lineage-specific markers to study *Drosophila* development. *Dev. Genet.*, **12**, 238–252.
- Ramirez-Weber, F.-A. and Kornberg, T.B. (1999) Cytotemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell*, **97**, 599–607.
- Reichman-Fried, M., Dickson, B., Hafen, E. and Shilo, B.-Z. (1994) Elucidation of the role of *breathless*, a *Drosophila* FGF receptor homolog, in tracheal cell migration. *Genes Dev.*, **8**, 428–439.
- Ricos, M.G., Harden, N., Sem, K.P., Lim, L. and Chia, W. (1999) *Dcdc42* acts in TGF- β signaling during *Drosophila* morphogenesis: distinct roles for the Drac1/JNK and Dcdc42/TGF- β cascades in cytoskeletal regulation. *J. Cell Sci.*, **112**, 1225–1235.
- Shiga, Y., Tanaka-Matakatsumi, M. and Hayashi, S. (1996) A nuclear GFP/ β -galactosidase fusion protein as a marker for morphogenesis in living *Drosophila*. *Dev. Growth Differ.*, **38**, 99–106.
- Shilo, B.-Z., Gabay, L., Glazer, L., Reichman-Fried, M., Wappner, P., Wilk, R. and Zelzer, E. (1997) Branching morphogenesis in the *Drosophila* tracheal system. *Cold Spring Harb. Symp. Quant. Biol.*, **62**, 241–247.
- Sutherland, D., Samakovlis, C. and Krasnow, M.A. (1996) *branchless* encodes a *Drosophila* FGF homolog that controls tracheal cell migration and the pattern of branching. *Cell*, **87**, 1091–1101.
- Tautz, D., Lehmann, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K. and Jäckle, H. (1987) Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature*, **327**, 383–389.
- Wolf, C. and Schuh, R. (2000) Single mesodermal cells guide outgrowth of ectodermal tubular structures in *Drosophila*. *Genes Dev.*, **14**, 2140–2145.

DOI: 10.1093/embo-reports/kvfl15