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Progenitor Outgrowth from the Niche in *Drosophila* Trachea Is Guided by FGF from Decaying Branches

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Abstract

Although there has been progress identifying adult stem and progenitor cells and the signals that control their proliferation and differentiation, little is known about the substrates and signals that guide them out of their niche. By examining *Drosophila* tracheal outgrowth during metamorphosis, we show that progenitors follow a stereotyped path out of the niche, tracking along a subset of tracheal branches destined for destruction. The embryonic tracheal inducer *branchless* FGF (fibroblast growth factor) is expressed dynamically just ahead of progenitor outgrowth in decaying branches. Knockdown of *branchless* abrogates progenitor outgrowth, whereas misexpression redirects it. Thus, reactivation of an embryonic tracheal inducer in decaying branches directs outgrowth of progenitors that replace them. This explains how the structure of a newly generated tissue is coordinated with that of the old.

Many adult stem cells reside in specific anatomical locations, or niches, and are activated during tissue homeostasis and after injury (1–4). Although considerable effort has been made to identify factors that control stem cell proliferation and differentiation, how stem or progenitor cells move out of the niche and how they form new tissue are not well understood (4–6). Tissue formation in mature animals faces challenges not present in the embryo (7, 8). The new cells migrate longer distances and navigate around and integrate into a complex milieu of differentiated tissues. In this work, we investigated the substratum and signals that guide *Drosophila* tracheal imaginal progenitor cells into the posterior during metamorphosis to form the pupal abdominal tracheae (PAT) that replace the posterior half of the larval tracheal system (tracheal metameres Tr6 to Tr10), which decays at this time (9, 10) (Fig. 1A).

The PAT extend from the transverse connective (TC) branches in Tr4 and Tr5 (Fig. 1A). Each PAT consists of a multicellular stalk with many secondary branches, each of which has dozens of terminal cells that form numerous fine terminal branches (tracheoles) (10). There

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Supplementary Materials

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Materials and Methods

Figs. S1 to S10

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are two known tracheal progenitor populations at metamorphosis: dedifferentiated larval tracheal cells and spiracular branch (SB) imaginal tracheal cells set aside during embryonic tracheal development (11–14). Lineage tracing showed that PAT derive from imaginal progenitors (fig. S1, B and C).

To determine how progenitors in Tr4 and Tr5 reach the posterior, we used a *btl-RFP-moe* transgene (15) (RFP, red fluorescent protein) to label activated progenitor cells, and *ppk4>GFP* (16) (GFP, green fluorescent protein) to label larval tracheal branches (fig. S2A). Before metamorphosis, there are 7 to 10 quiescent progenitor cells in each SB niche (Fig. 1B and fig. S1A) (11, 13). In early third larval instar (L3), progenitors proliferate but remain in the niche (Fig. 1C). Later in L3, progenitors leave the niche, moving onto the larval TC branches toward the dorsal trunk (DT) (Fig. 1D), while progenitors within the niche continue to proliferate (13). Progenitors in other metameres also proliferate but do not move out of the niche (fig. S2B). Migrating progenitors in Tr4 and Tr5 crawl along the basal surface of larval tracheal cells, with cytoplasmic projections emanating from cells at the leading edges of the progenitor cluster (fig. S3C). Progenitors maintain epithelial polarity and a lumen continuous with the SB and TC branches, forming a saclike structure (fig. S3, A and B) (9). By wandering L3, progenitors reach the DT (Fig. 1E), where they pause (~12 hours) until the onset of puparium formation (Fig. 1F).

Around 1 hour after puparium formation (APF), progenitors move onto the DT and turn posteriorly (Fig. 1G). Posterior migration continues for 9 hours, extending half the animal's length (~0.8 mm) past Tr9 (Fig. 1, H to J). Live imaging showed that progenitors move at ~1.7 $\mu\text{m}/\text{min}$, crawling along and wrapping around the DT as they migrate (movie S1).

Differentiation begins as progenitors migrate. At the beginning of puparium formation (0 hours APF, Fig. 1F), a subset of progenitors that have exited the niche begins to express the terminal cell master regulator Pruned SRF (serum response factor) (17), initiating cell specialization (fig. S4A). As progenitors migrate along the DT, budlike structures composed of Pruned-expressing cells are detected at the tips of progenitor clusters, whereas Pruned-negative cells form the stalks of new trachea (fig. S4B). By 6 hours APF, Pruned-expressing progenitors in the tips adopt an elongated and differentiated morphology (fig. S4, C and D), flattening along the DT as they extend further posteriorly (movie S1). Around 13 hours APF, the PAT mature and fill with gas as posterior tracheal branches collapse (Fig. 1, A and J).

What guides tracheal progenitors on their stereotyped path along specific branches of the larval tracheal system? Expression of *breathless* (*btl*) FGFR (fibroblast growth factor receptor) is induced in PAT progenitors (11, 13), as shown by the *btl-RFP-moe* reporter (Fig. 1, B to J). We tested whether the Btl pathway, which directs tracheal branch outgrowth in embryos (18–20) and larvae (21) and induces adult air-sac primordium formation (22), is involved. Expression of dominant-negative Btl FGFR (19) in the progenitors and their descendants (23) blocked migration and diminished or eliminated PAT formation (Fig. 2 and fig. S5). To determine the source of the only known Btl ligand, Branchless (Bnl) FGF (20), we used a *bnl* reporter, *bnl-Gal4* enhancer trap line NP2211 (24) driving *UAS-GFP*. Unlike previously described examples of tracheal outgrowth (20–22), *bnl* was not expressed in surrounding tissue. Instead, it was expressed within the tracheal system, specifically by larval tracheal cells along which progenitors migrate. The expression pattern is dynamic and precise, almost perfectly matching the positions and timing of progenitor migration (Fig. 3A and fig. S6A). In L3 animals, when progenitors are observed along the TC branches, *bnl>GFP* was expressed in TC larval cells in Tr4 and Tr5, but not in other metameres. Shortly after puparium formation, when PAT progenitors turn to migrate toward the posterior, DT larval cells in the segment just posterior to PAT progenitors express *bnl>GFP*.

As progenitors continue along the DT, DT larval cells activate *bnl>GFP* expression one segment at a time from anterior to posterior, matching progenitor movement.

This dynamic *bnl* expression along the migration path is required for progenitor outgrowth. Knockdown of *bnl* expression by RNA interference (RNAi) in larval tracheal cells blocked migration and resulted in diminished or absent PAT (Fig. 3, B and C; fig. S7, A to C; and movie S2). Mosaic expression of *bnl RNAi* in small patches along the path (23) also arrested migration, so long as the patch encompassed the full DT circumference (Fig. 3D; fig. S7, D and E; and movies S3 and S4). Thus, Bnl is required all along the migration path, and the signal does not cross even short gaps.

Ectopic *bnl* expression in GFP-labeled clones of larval tracheal cells induced by *dfr-FLP* (23) redirected progenitor migration. Depending on the location of the clones, ectopic *bnl* caused incorrect exit from the niche, premature entry onto the DT, or wrong turns on the DT (Fig. 4, B to D). Dual clones induced bifurcation with groups of progenitors moving toward each ectopic *bnl* source (Fig. 4E). Clones in Tr3 and posterior metameres caused progenitors in these regions to leave the niche, even though they do not normally do so (Fig. 4, G and H, and fig. S8, D and E). When there was a large clone, progenitors migrated throughout the clone (Fig. 4F), implying that progenitors do not require a gradient and will spread to cover an entire region of cells expressing *bnl* at equivalent levels. When *bnl*-expressing clones failed to induce migration, the clones appeared to be too far from the progenitors or there was competition from another clone close by (fig. S8, A and B). Ectopic *bnl* expression within the progenitor cluster arrested migration (fig. S8C).

The results show that the embryonic tracheal inducer Bnl FGF guides tracheal progenitors out of the niche and into the posterior during tracheal metamorphosis. The source of Bnl is the larval tracheal branches destined for destruction, which serve both as the source of the chemoattractant and as the substratum for progenitor migration. Several days earlier in embryos, these larval tracheal branches were themselves induced by Bnl provided by neighboring tissues. But after embryonic development, most tracheal cells, including those in the decaying larval branches, down-regulate *btl* FGFR expression (fig. S2A) and thus do not respond to (or sequester) the Bnl signal they later express. One of the most notable aspects of this larval Bnl is its exquisitely specific pattern in decaying larval branches, which presages progenitor outgrowth. It is unclear how Bnl expression is controlled, though it does not appear to require signals from migrating progenitors because the *bnl* reporter expression front progressed normally when progenitor outgrowth was stalled by a tracheal break (fig. S6C). Perhaps expression of Bnl involves gradients in the tracheal system or spatial patterning cues established during embryonic development in conjunction with temporal signals mediated by molting hormones.

Because the signal guiding progenitor migration is provided by tracheae destined for destruction, progenitors become positioned along the larval branches they replace (Fig. 4I). Perhaps during tissue repair and homeostasis, recruitment of adult stem or progenitor cells from the niche is similarly guided by signals from decaying tissue, thereby ensuring that new tissue is directed to the appropriate sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Progenitor outgrowth during *Drosophila* tracheal metamorphosis

(A) Air-filled trachea (reflected light) (left) and schematics (right) in L3 larva and pupa ~13 hours after puparium formation. Tr, tracheal metamere; DB, dorsal branch; LT, lateral trunk; VB, visceral branch. Circles denote spiracles. Tracheal branches in the posterior (Tr6 to Tr10) are lost (dashed lines) during metamorphosis and are replaced by PAT from Tr4 and Tr5. (B to E) Fluorescence micrographs and schematics of Tr5 of *ppk4-Gal4>UAS-GFP*; *btl-RFP-moe* larva of the indicated ages stained to show activated tracheal progenitors (anti-RFP, red), larval tracheal cells (anti-GFP, green), and nuclei (4',6-diamidino-2-phenylindole, blue). BPF, approximate time before puparium formation; W3L, wandering third-instar larva. (B) Quiescent progenitors in a Tr5 SB niche. Progenitors are also present in the SB niche of Tr2 to Tr4 and Tr6 to Tr9, but only those in Tr4 and Tr5 are activated to form PAT. P6 to P10, progenitors 6 to 10; the other five progenitors (P1 to P5) are shown in fig. S1A. NE, niche exit site (SB-TC junction). (C) Activated progenitors proliferating in the niche. (Inset) Progenitors near the niche exit site (dash), which have begun expressing *btl-RFP-moe* (red). (D) Progenitors exiting the niche. *btl-RFP-moe* expression increases and *ppk4>GFP* decreases as progenitors leave the niche and migrate dorsally along TC branches. Arrowhead, progenitor migration front. (E) Progenitors paused at the DT (arrowhead). Several progenitors have extended onto the VB (arrow). (F to J) Schematics and micrographs [as in (B) to (E)] of trachea (Tr4 to Tr9) of pupa of indicated ages APF. Paused progenitors (F) move onto the DT and turn posteriorly (G), extending along the DT (H and I) before ramifying into the PAT (J). Posterior tracheal branches start to collapse 9 hours APF and are fully collapsed and no longer conduct air by 13 hours APF. The larval cells die during metamorphosis (10), although we did not detect the apoptosis marker anticleaved caspase-3 during collapse (fig. S9). Scale bars: 100 μ m, (A) and (F) to (J); 50 μ m, (B) to (E).

Fig. 2. Progenitor out-growth requires *breathless* FGFR

(A) PAT progenitor migration in a control *esg^{P127}-Gal4, UAS-GFP/act5c>Y>Gal4, UAS-GFP; UAS-FLP* pupa 6 hours APF at 18°C with progenitors marked with GFP (green) and tracheal lumens stained (red). Brackets show position of progenitor migration front in the four phenotypic classes (0, I, II, III) scored in control and in *esg^{P127}-Gal4, UAS-GFP/act5c>Y>Gal4, UAS-GFP; UAS-FLP/UAS-DN-btl* pupae, in which dominant-negative *breathless* (*DN-btl*) is selectively expressed in progenitors. Examples of each phenotypic class can be found in fig. S5, A to D. Arrowhead, progenitor migration front. The graph at right shows quantification. n, number of branches. (B) PAT formation in control (top) and *DN-btl* pupae (bottom) as above, except reared for 1 to 2 additional days to allow formation of mature, air-filled pupal tracheae (reflected light). No PAT have formed in the *DN-btl* pupa. The graph shows quantification, with phenotypes classified as in fig. S5, E to H. Scale bars, 100 μ m.

Fig. 3. Expression and requirement of *branchless* FGF during progenitor outgrowth

A) *branchless* reporter expression during progenitor outgrowth visualized by GFP immunostaining (white) of *UAS-GFP; bnl-Gal4 NP2211/btl-RFP-moe* larvae and pupae of indicated ages. Reporter expression dynamically expands along the progenitor outgrowth path, initially turning on in isolated larval cells in each area. Arrowheads denote furthest detected reporter expression. **(B and C)** Quantification (as in Fig. 2) of progenitor migration 3 hours APF (B) and PAT formation (C) phenotypes in control (*ppk4-Gal4, UAS-GFP; btl-RFP-moe*) and *ppk4-Gal4, UAS-GFP; btl-RFP-moe/UAS-bnl RNAi* pupae, in which *bnl* was inactivated in larval tracheal cells. For examples of the phenotypic classes, see fig. S7, A and B. **(D)** Frames at indicated times (hours:minutes) APF from live imaging of an *act5c>Y>Gal4, UAS-GFP/UAS-FLP; prd-Gal4, btl-RFP-moe/UAS-bnl RNAi* pupa in which *bnl* expression was inactivated in a DT patch (brackets). Tr5 progenitors (*btl-RFP-moe*) exit the niche (dashes) and move onto the DT but never pass the patch. Scale bars, 100 μ m.

Fig. 4. Effect of ectopic *bnl* on progenitor migration

Tracheal progenitors (red, anti-RFP) in Tr4 or Tr5 [(A) to (F)] and Tr8 [(G) and (H)] of control (*dfr-FLP/act5c>Y>Gal4, UAS-GFP; btl-RFP-moe*) and experimental (*dfr-FLP/act5c>Y>Gal4, UAS-GFP; btl-RFP-moe/UAS-bnl*) wandering third-instar larvae showing control clones of larval tracheal cells expressing GFP alone (green) [(A) and (G)] or experimental clones expressing GFP and ectopic *bnl* FGF [(B) to (F) and (H)]. Blue, tracheal lumen (Alexa Fluor 350-conjugated wheat germ agglutinin). Arrowheads denote progenitor migration fronts. (A) Progenitors have exited the niche and reached the TC-DT junction (arrowhead). Control clones (green) have no effect. (B) Some progenitors have exited the niche in the wrong direction along TC branches (open arrowhead), extending toward the *bnl*-expressing clone ventral to the niche exit. Other progenitors (solid arrowhead) have exited the niche normally toward DT. (C) Progenitors have prematurely moved onto DT, extending toward a single larval DT cell expressing *bnl*. (D) Inappropriate anterior migration of progenitors along DT (arrowhead) toward the *bnl*-expressing clone. (E) Bifurcation of progenitor cluster (arrowheads) toward a pair of *bnl*-expressing clones located anterior and posterior to the TC-DT junction. (F) Bifurcation (arrowheads) where progenitors extend to fill the shape of a large *bnl*-expressing clone (open arrowhead). (G) Progenitors in Tr8 (as well as Tr3 and Tr6 to Tr9) normally remain within the niche and are unaffected by control clones. (H) Tr8 progenitors exit the niche toward a clone of *bnl*-expressing cells on TC branches. Scale bars [(A) to (H)], 100 μ m. (I) Model of progenitor outgrowth guided by a signal produced by decaying tissue (green). Progenitors (red) are attracted to and form new tissue at the site of decay.