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The Alternative Migratory Pathways of the *Drosophila* Tracheal Cells Are Associated with Distinct Subsets of Mesodermal Cells

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The *Drosophila* tracheal system is a model for the study of the mechanisms that guide cell migration. The general conclusion from many studies is that migration of tracheal cells relies on directional cues provided by nearby cells. However, very little is known about which paths are followed by the migrating tracheal cells and what kind of interactions they establish to move in the appropriate direction. Here we analyze how tracheal cells migrate relative to their surroundings and which tissues participate in tracheal cell migration. We find that cells in different branches exploit different strategies for their migration; while some migrate through preexisting grooves, others make their way through homogeneous cell populations. We also find that alternative migratory pathways of tracheal cells are associated with distinct subsets of mesodermal cells and propose a model for the allocation of groups of tracheal cells to different branches. These results show how adjacent tissues influence morphogenesis of the tracheal system and offer a model for understanding how organ formation is determined by its genetic program and by the surrounding topological constraints. © 2000 Academic Press

Key Words: Drosophila; **migration; morphogenesis; trachea; mesoderm; cell interactions.**

INTRODUCTION

Cell migration plays an important role in the development of many organisms and is involved in different steps in morphogenesis, from gastrulation to the development of complex structures such as the nervous system. The *Drosophila* tracheal system has become an appropriate model for the study of the mechanisms that guide cell migration (reviewed in Hogan and Yingling, 1998; Metzger and Krasnow, 1999). The *Drosophila* tracheal system arises from the tracheal placodes, clusters of ectodermal cells that invaginate and migrate in different and stereotyped directions to originate each of the primary tracheal branches (reviewed in Manning and Krasnow, 1993).

The ordered migration of tracheal cells depends on the integration of different signaling pathways. On one hand, the Branchless (Bnl)–Breathless (Btl) pathway acts as a motogen that stimulates and guides tracheal cell migration of all primary branches (Klämbt *et al.*, 1992; Sutherland *et al.*, 1996); Bnl, an FGF homologue, is expressed in clusters of cells surrounding the developing tracheal system at each position where a new branch will form and grow out (Sutherland *et al.*, 1996). On the other hand, the Dpp and EGF pathways are also required for tracheal morphogenesis and appear to influence the choice between alternative paths of migration (Llimargas and Casanova, 1997; Vincent *et al.*, 1997; Wappner *et al.*, 1997). For instance, those cells receiving the Dpp signal will migrate in the dorsoventral axis and not in the anteroposterior axis.

The general conclusion from these studies is that the direction of migration of tracheal cells relies on positional cues provided by nearby cells. However, very little is known about how tracheal cells migrate and what kind of cell interactions they establish to move in the appropriate direction. In addition, it is not clear how subsets of tracheal cells are specified to adopt particular migratory pathways. Here we analyze how tracheal cells migrate relative to their surrounding cells and which tissues participate in tracheal cell migration to form the different tracheal branches. We find that cells on different branches exploit different strategies for their migration; while some migrate through preexisting grooves, others make their way through homogeneous cell populations. We also find that migrating cells establish distinct interactions with particular subsets of

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mesodermal cells according to the branch they will generate. These specific mesodermal derivatives are required for the development of particular branches and constrain the final morphogenesis of the tracheal tree. We propose that formation of different tracheal branches depends on specific interactions between tracheal and distinct subsets of mesodermal cells and offer a model for understanding how organ formation is determined by an intrinsic genetic program and the surrounding topological constraints.

MATERIALS AND METHODS

Fly Strains

We have used the following alleles: btl^{LG19} (Glazer and Shilo, 1991), cli^{1} (Boyle *et al.*, 1997), srp^{9L06} and srp^{6G54} (Reuter, 1994), tin^{EC40} (Bodmer, 1993), trh^{3} (Wilk *et al.*, 1996), twi^{ID96} (Simpson, 1983), $zfh\text{-}1^{75.26}$, $zfh\text{-}1^{65.34}$, and double mutants tin^{GC14} $zfh\text{-}1^{75.26}$ and tin^{GC14} $zfh\text{-}1^{65.34}$ (Moore *et al.*, 1998). A *twi*-CD2 construct (Dunin-Borwoski and Brown, 1995) was used to mark mesoderm tissues. The UAS-*trk* line was provided by A. Casali (unpublished). The *btl*-Gal4 (Shiga *et al.*, 1996) was used to drive expression of different genes in the tracheal system. We also used a UAS-*tkv** line (Lecuit *et al.*, 1996). To identify homozygous mutant embryos we used the CyO-*hb*-lacZ, TM3-*abdA*-lacZ, and TM3-*hb*-lacZ chromosomes.

Immunochemistry and in Situ Hybridization

Embryos were staged according to Campos-Ortega and Hartenstein (1985) and stained according to standard protocols. We used the following primary antibodies: mAb2A12 monoclonal antibody (1:5–1:10, from the DSHB, University of Iowa), which recognizes the lumen of the tracheal tree; anti-Srp rabbit antibody (1:1000, from R. Reuter); anti-Trh rat antibody (1:200, from S. Crews); anti-CD2 monoclonal antibody (1:1000, from Serotec); and anti-Trk rabbit antibody provided by A. Casali (1:1000, unpublished). Detection of β-gal was performed with a specific antibody (Cappel; 1:2000).

Embryos were stained according to standard protocols using the Vectastain Elite ABC kit. For double labeling we used 8% $NiCl_2$ in the first immunostaining to obtain a darker color. Whole-mount *in situ* hybridizations were done following the method of Tautz and Pfeifle (1989) with minor modifications. *bnl* and *trh* probes were generated from clones from M. Krasnow and from D. Andrew. To double label for *srp* and *trh* we followed the procedure described by Manoukian and Krause (1992). Photographs were taken using Nomarski optics.

RESULTS

Migration of the Tracheal Cells Relative to the Surrounding Mesoderm

Migration of tracheal cells begins with the formation of six major buds from the tracheal placodes; the expression of the *bnl* gene in small groups of cells surrounding the placodes prefigures the position where these buds will form and the future direction of migration of the tracheal cells

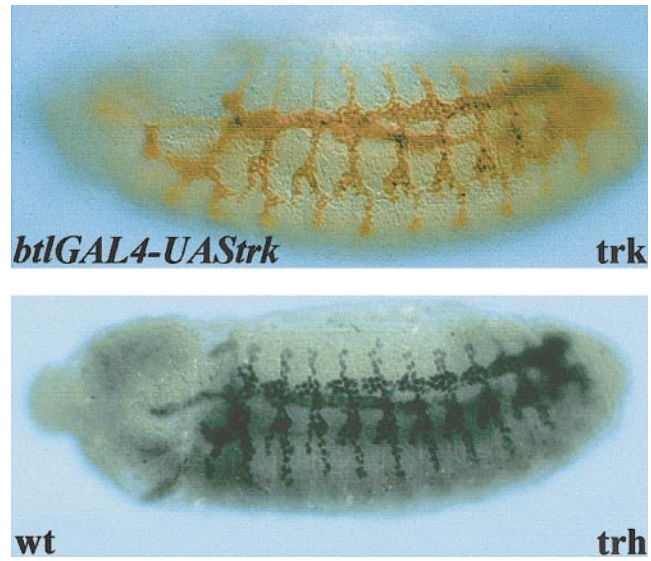

FIG. 1. Identification of the tracheal cell bodies. (Top) Embryos carrying a *btl*-GAL4 and a UAS-*trk* construct stained with an antibody against Trk. In those embryos, *trk* is expressed in all the tracheal cells compared with the expression of *trh* (bottom). Note that the anti-Trh antibody detects nuclei while the anti-Trk antibody reveals cell shapes.

(Sutherland *et al.*, 1996). To unveil the path followed by the migrating tracheal cells and to define the tissues that may participate in their migration we have used different constructs that specifically mark the migrating tracheal cells and the surrounding mesoderm. To mark the tracheal cell bodies we have used the GAL4 system (Brand and Perrimon, 1993) with a *btl*-Gal4 construct (Shiga *et al.*, 1996) that is expressed specifically in tracheal cells to drive a UAS-*trk* construct (Casali and Casanova, unpublished). In embryos with both constructs, accumulation of the trk protein outlines the tracheal cell shape without disturbing their normal migration. In addition the flies that carry both the *btl*-Gal4 and the UAS-*trk* are perfectly viable. We detect the same tracheal pattern with this system or with an antibody against a tracheal marker, the product of the *trachealess* (*trh*) gene, with the difference that the staining with the Trh antibody appears earlier and detects nuclei while our construct reveals cell shapes (Fig. 1). To visualize the embryonic mesoderm we have used a genetic construct in which the *twist* (*twi*) promoter directs the synthesis of the cell surface protein CD2 (Dunin-Borkowski and Brown, 1995). In this section, we will present a general description of the pattern of tracheal cell migration in relation to the embryonic mesoderm. Muscle precursors will be named according to Dunin-Borkowski *et al.* (1995).

Tracheal cells are first specified as clusters of ectodermal cells at the embryonic surface. As tracheal cells invaginate and form the tracheal pits they occupy the grooves between the muscle precursors of adjacent metameres (Figs. 2A–2D).

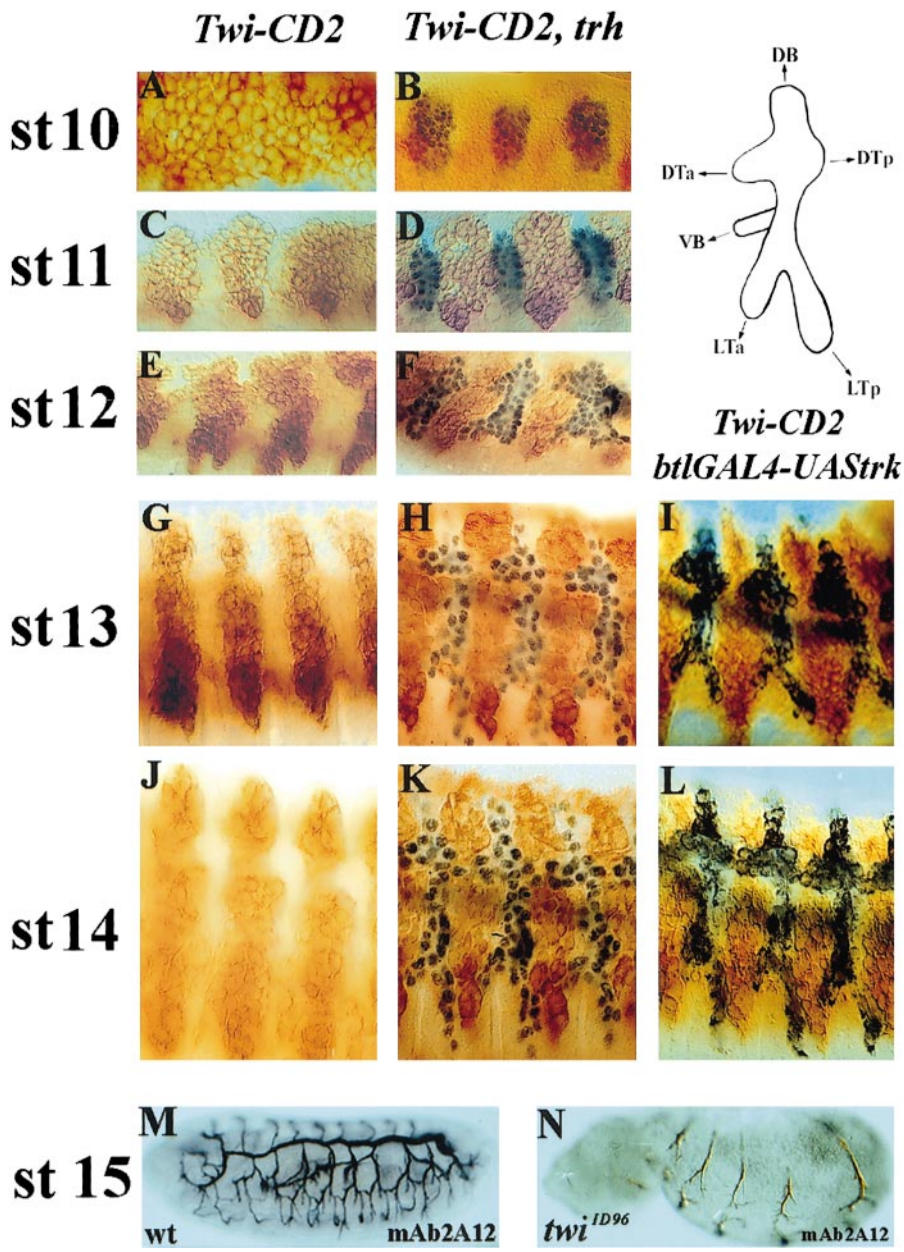

FIG. 2. (A–L) Migration of the tracheal cells relative to the surrounding mesoderm. In the upper right is a schematic representation of the initial development of the tracheal branches (DB, dorsal branch; DTa, dorsal trunk anterior; DTp, dorsal trunk posterior; LTa, lateral trunk anterior; LTp, lateral trunk posterior; VB, visceral branch). (A–L) Mesoderm and tracheal cells from three central metameres of the embryo. Mesoderm cells are visualized in embryos carrying the *twi*-CD2 gene stained with an antibody against CD2. Tracheal cells are visualized either with an antibody against Trh (B, D, F, H, K) or with the antibody against Trk in embryos carrying *btl*-GAL4 and UAS-*trk* constructs (I, L). At early stage 10, the mesoderm appears as a continuous layer (A) while the Trh antibody marks the cells of the tracheal placode at the ectoderm (B). By stage 11, there is an invagination of some mesodermal cells (C) and the tracheal pit forms in those regions of mesoderm invagination (D). At stage 12, the six primary branches begin to bud (F) and the mesodermal cells outline the shape of the tracheal pits (E). At stage 13, the tracheal cells occupy the groove between the mesodermal cells (G). The dorsal and ventral cells are migrating along the groove while the cells that migrate anteriorly and posteriorly move across the precursors of the somatic muscles (H). Note the close apposition between mesodermal and tracheal cells (I). At stage 14, cells of the dorsal trunk from one metamere have reached the cells from the adjacent metameres (K, L) and separate the precursors of the most dorsal muscles from the precursors of more ventral ones (J). (M and N) Tracheal morphogenesis is disrupted in embryos that do not form mesodermal derivatives. (M) Lateral view of a wild-type stage 15 embryo labeled with mAb2A12 specific for the tracheal lumen. (N) In *twi* mutant embryos only some abnormal dorsolateral branches form.

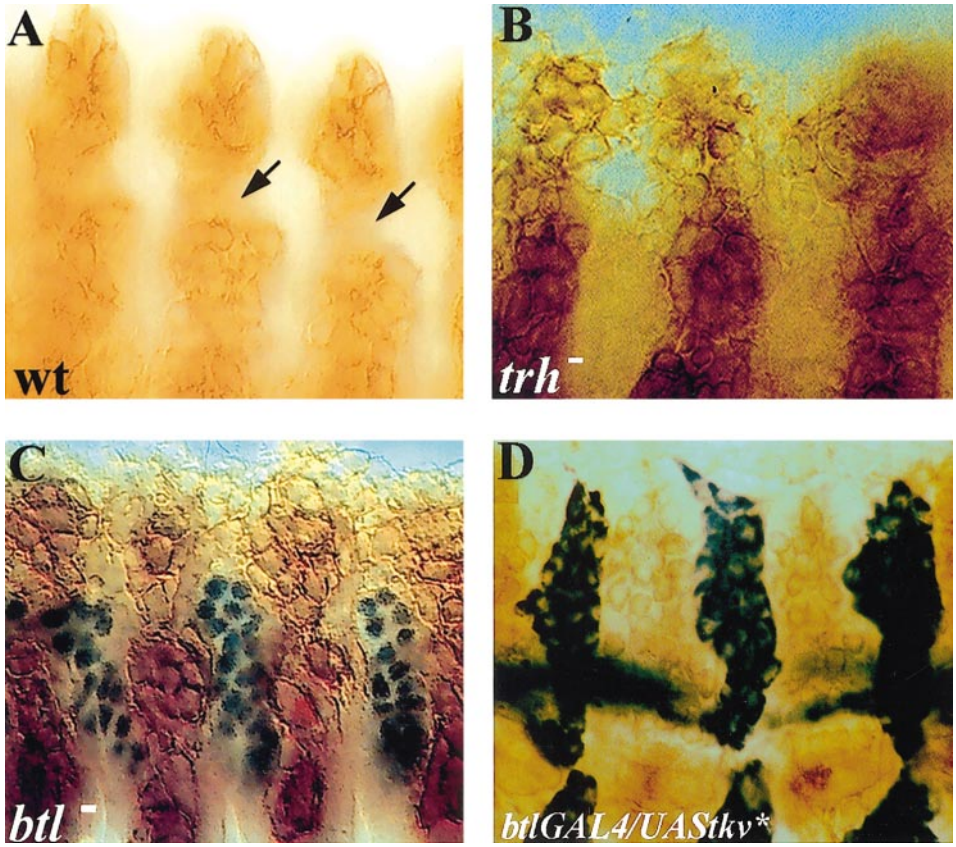

FIG. 3. The invagination of the mesoderm is independent of formation of tracheal pits but the segregation of dorsal muscle precursors depends on migration of the dorsal trunk. The metameric invagination of the mesoderm occurs in wild-type embryos (A) as well as in *trh* mutant embryos in which tracheal pits do not form (B). Conversely, the gap between the most dorsal muscle precursors and the more ventral ones (arrows in A) depends on the formation of the dorsal trunk. This gap is absent in *trh* mutant embryos (B), in *btl* mutant embryos in which the tracheal cells are confined to elongated sacs that sometimes have occasional extensions (C), or in embryos in which a constitutive form of the Tkv receptor expressed in the tracheal cells induces them to migrate in the dorsoventral axis (D). Mesoderm and tracheal cells are visualized as in Fig. 2. Details of embryos around stage 14.

The formation of this groove is independent of tracheal invagination as it also forms between metameres that do not have tracheal placodes (not shown) and it also develops in *trh* mutant embryos, which do not undergo tracheal invagination (Fig. 3B).

A subset of the tracheal cells moves anteriorly, whereas another subset moves posteriorly until they reach the cells from the adjacent placodes. These cells will form the dorsal trunk, the most prominent tracheal branch that spans the embryo longitudinally (Figs. 2E–2L). We observe that those cells migrate across the adjacent precursors of somatic muscles and separate the precursors of the most dorsal muscles from the precursors of more ventral muscles, as described by Dunin-Borkowski *et al.* (1995) (Fig. 2J). Other cells, those from the dorsal side of the tracheal pit, move dorsally along the longitudinal groove to form the dorsal branches (Figs. 2E–2L) that will end up fusing with the dorsal branches coming from the contralateral hemisegments. In the ventral side, the tracheal cells follow two different paths along the two clusters of lateral muscle precursors at each side of the groove. Anterior ventral cells will form the anterior lateral trunk while the posterior ventral cells will form the posterior lateral trunk (Figs. 2E–2L). Finally, another group of cells from a midposition in the tracheal pit will migrate inward and will form the visceral branch.

To ascertain the role of the mesoderm in tracheal cell migration, we have examined what tracheal structures develop in embryos that lack mesoderm derivatives. In *twi* mutant embryos, formation of the tracheal tree is severely perturbed: there is no dorsal trunk and there is only some development of dorsal and ventral branches (Fig. 2N). Although *twi* mutant embryos are severely affected, their tracheal phenotype suggests an important role of the mesoderm in tracheal cell migration. In the next sections we will examine the distinct interactions between specific subsets

of mesodermal derivatives and the tracheal cells as they migrate to form the different tracheal branches.

The Tracheal Cells of the Dorsal Trunk Migrate between the Dorsal Muscle Precursors

To elucidate how the cells of the dorsal trunk migrate across the precursors of somatic muscles we first determined whether they recognized some kind of preexisting gap between the most dorsal and the remaining muscle precursors. Thus, we have examined whether the gap between these muscle precursors is also established in the absence of migration of the cells of the dorsal trunk or, alternatively, is a consequence of the migration of these tracheal cells. First, we have analyzed the situation of muscle precursors in *breathless* (*btl*) mutant embryos in which there is no tracheal migration. Second, we have studied embryos in which a constitutive form of the Tkv receptor was induced specifically in the tracheal cells; in these embryos, the cells that would normally migrate anteriorly or posteriorly to form the tracheal trunk are instead forced to migrate in the dorsoventral axis. In both cases, we do not observe a topological segregation between the most dorsal and the remaining precursor cells, indicating that migration of the tracheal cells separates the two subset of muscle precursors cells (Figs. 3C and 3D), at a stage at which the somatic muscle precursors have been specified. Thus, the cells of the dorsal trunk appear to open up their way through a contiguous population of cells.

The Lateral Mesoderm as a Cue for the Migration of the Tracheal Cells of the Dorsal Trunk

The above results indicate that the migrating cells of the dorsal trunk do not recognize any preexisting gap between the muscle precursor cells. However, we noticed that the cells of the dorsal trunk appear to be just over the cells of the fat body (Figs. 4A–4D). The fat body develops from discrete regions of the lateral mesoderm. After germ-band retraction the fat body cells proliferate and the primordia fuse to form a continuous mesodermal layer between the midgut visceral mesoderm and the somatic mesoderm (Riechmann *et al.,* 1998). We therefore reasoned that the tracheal cells of the dorsal trunk could be using the fat body cells as a cue for their migration. We tested this possibility by disrupting the development of the fat body and examining the effect on dorsal trunk formation. We first studied mutant embryos for *serpent* (*srp*), a gene required for fat body development, and we found that formation of the tracheal dorsal trunk is basically normal (data not shown). However, in *srp* mutant embryos the fat body precursors do not disappear; instead they are partially transformed into another derivative of the lateral mesoderm, the somatic gonadal precursors (Moore *et al.,* 1998; Riechmann *et al.,* 1998). Therefore in *srp* mutant embryos the gonadal cells, also of lateral mesoderm origin, could sustain migration of the cells of the dorsal trunk.

Thus, we examined the role of the lateral mesoderm in the migration of tracheal cells. The lateral mesoderm, which comprises the fat body and the somatic gonadal precursors, is determined by the early functions of *tinman* (*tin*) and *zinc-finger homeodomain protein-1* (*zfh1*) (Moore *et al.,* 1998). In embryos mutant for either *zfh-1* or *tin,* the number of somatic gonadal and fat body precursors is reduced (Broihier *et al.,* 1998; Moore *et al.,* 1998). Consistent with a role for the lateral mesoderm in the migration of the dorsal trunk cells, we observe that the development of the dorsal trunk is impaired in these mutants. This is a nonautonomous effect since *tin* and *zfh-1* are not expressed in the tracheal cells (Lai *et al.,* 1991; Bodmer, 1993). The effect is quite mild, however (Fig. 4E). As *tin* and *zfh-1* have overlapping and partially redundant functions (Moore *et al.,* 1998) we analyzed mutant embryos for both genes. In those embryos, the somatic gonadal mesoderm and the fat body precursors are virtually absent (Broihier *et al.,* 1998; Moore *et al.,* 1998) and we find that the tracheal dorsal trunk is almost completely absent, but formation of the dorsal and ventral branches is not impaired (Fig. 4F). This defect is not due to a failure of *bnl* expression since it appears at the right position between the tracheal pits (Fig. 4I). However, structures other than the lateral mesoderm are also affected in *tin zfh-1* double mutant embryos. Thus, to specifically correlate the formation of the dorsal trunk with development of the lateral mesoderm we studied *clift* (*cli*) mutant embryos; in *cli* mutant embryos, fat body and somatic gonadal precursors form, but the fat body fails to differentiate its structure (Boyle *et al.,* 1997; Moore *et al.,* 1998). We find that formation of the dorsal trunk is also impaired in *cli* mutant embryos, although the effect is weaker (Fig. 4G), consistent with the milder effect on fat body development. Nevertheless, in *cli* mutant embryos we can correlate lack of dorsal trunk formation with those regions where the lateral mesoderm derivatives fail to form a continuous layer (Fig. 4H). Altogether, our results indicate that proper migration of the dorsal trunk cells requires the presence of the derivatives of the lateral mesoderm.

The Tracheal Cells of the Dorsal Branch Migrate between the Metameric Clusters of Muscle Precursors

Unlike the dorsal trunk cells that migrate through a contiguous population of muscle precursor cells, the dorsal branch cells appear to exploit the groove separating the muscle precursor cells of adjacent metameres. These grooves precede migration (Fig. 3) and seem to track the cells of the dorsal branch. Moreover, the width of the groove could have a role in defining the morphology of the dorsal branch. We observe that migration of the dorsal branch begins as a group of cells that protrude into the groove between the dorsal muscle precursors. At this stage, we can observe several cells in the same row (Figs. 5C and 5D). However, the cells appear to become highly ordered in an end-to-end arrangement precisely when they are inside the

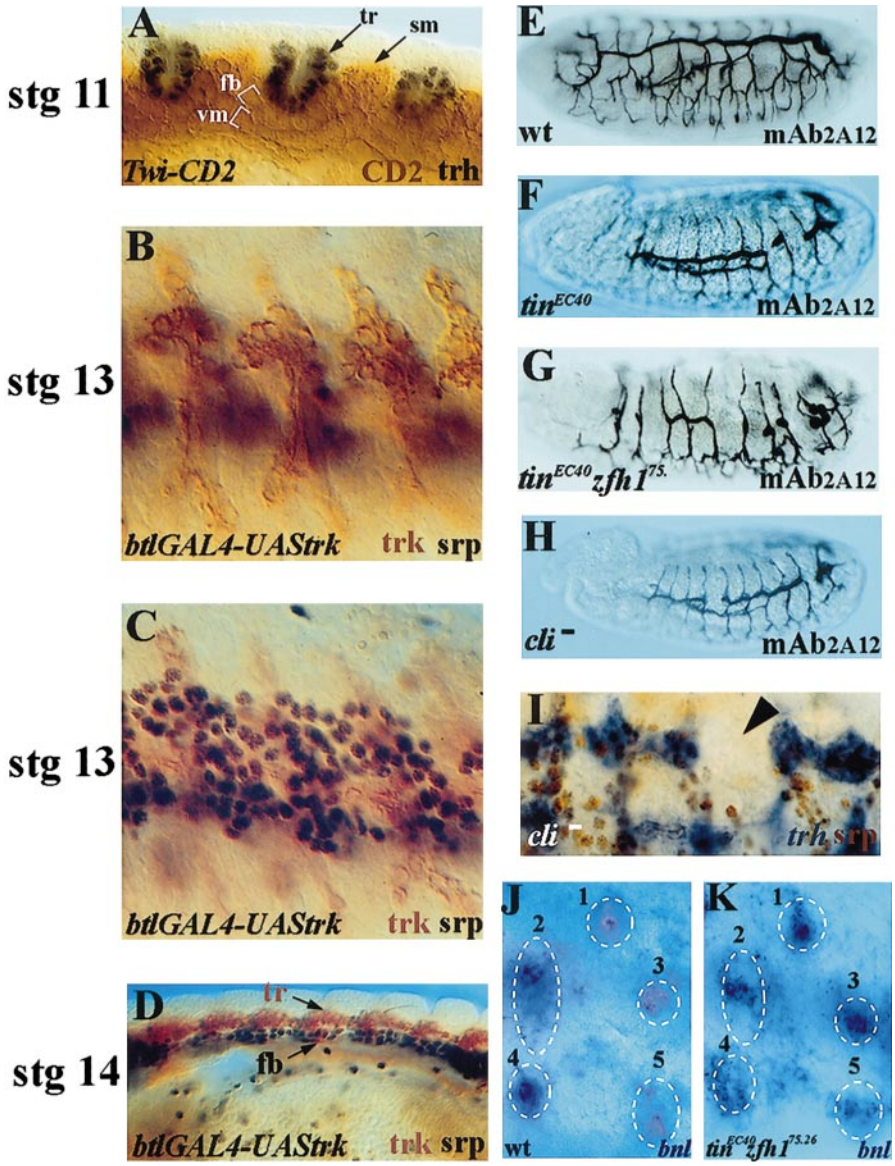

FIG. 4. The lateral mesoderm as a cue for the migration of the tracheal cells of the dorsal trunk. (A) Spatial organization of the invaginating tracheal pit relative to the underlying mesoderm. Tracheal cells are detected with the anti-Trh antibody (in black) and mesodermal cells with an anti-CD2 antibody (in brown) in embryos with the *twi*-CD2 gene. (B and C) Detail of a lateral view of the same embryo at two focal planes. A more external view shows the tracheal cells marked with the *btl*-GAL4/UAS-*trk* constructs and stained with an anti-Trk antibody (in brown) (B). A more internal view shows the fat body cells as detected with an anti-Srp antibody (in black) (C). Note how the cells of the budding dorsal trunk lie over fat body cells. (D) Detail of a dorsal view of a stage 14 embryo. The tracheal dorsal trunk lies over the contiguous layer of fat body cells. The tracheal cells are marked with the *btl*-GAL4/UAS-*trk* constructs and stained with an anti-Trk antibody (in brown) and the fat body cells are detected with an anti-Srp antibody (in black). (E, F, G, and H) Lateral view of stage 15 embryos labeled with mAb2A12 specific for the tracheal lumen. In *tin* (F) and *cli* (G) mutant embryos there are gaps in the tracheal dorsal trunk; in *tin zfh-1* double mutant embryos the dorsal trunk is almost completely disrupted while dorsal and ventral branches develop normally (H). (I) Detail of a lateral view of a *cli* mutant embryo. Note a gap in the dorsal trunk in a region where the fat body fails to form a continuous layer (arrowhead). (We have scored 67 gaps in the dorsal trunk of 22 *cli* mutant embryos and have found a perfect correlation in 83% of the gaps). Tracheal cells are detected with a *trh* probe (in blue) and fat body cells are stained with an anti-Srp antibody (in brown). (J and K) Detail of a lateral view around a tracheal placode in a wild-type and a *tin zfh-1* double mutant embryo (stage 11) indicating the five clusters of Bnl expression as detected with a *bnl* probe. fb, fat body; sm, somatic mesoderm; tr, tracheal cells; vm, visceral mesoderm.

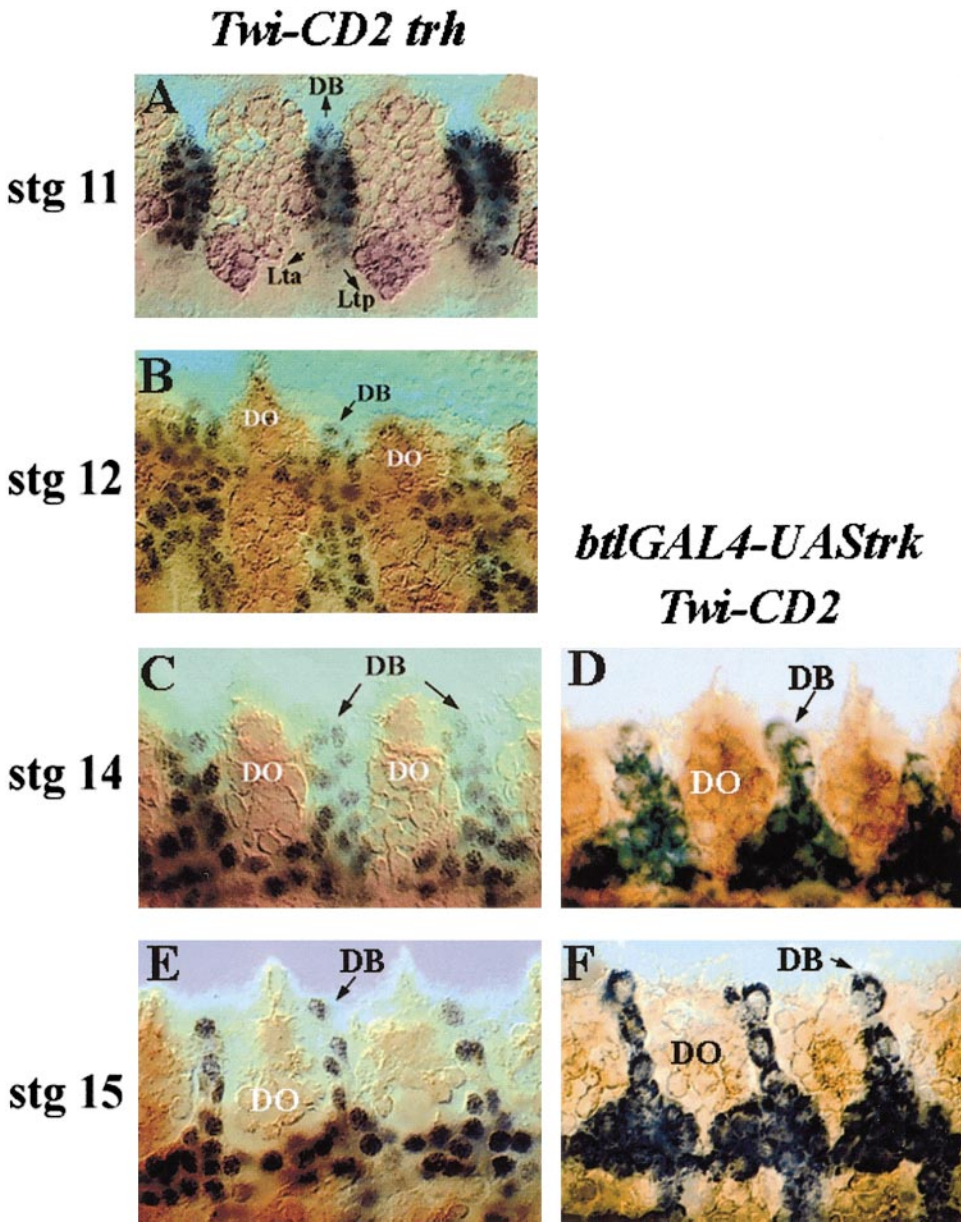

FIG. 5. The tracheal cells of the dorsal branch migrate between the metameric clusters of muscle precursors. (A and B) Cells that form the dorsal branches migrate dorsally. (C and D) By stage 14, the cells of the dorsal branches are located between the precursors of the most dorsal muscles; we can observe several cells in the same row. (E and F) By stage 15, the cells of the dorsal branches acquire an end-to-end arrangement corresponding to the narrower width of the groove between the muscles precursors. Mesoderm and tracheal cells are as in Fig. 2. DB, dorsal branch; DO, dorsal oblique muscles; LTa, lateral trunk anterior; LTp, lateral trunk posterior.

narrower part of the groove (Figs. 5E and 5F). Samakovlis *et al.* (1996) had already described that the dorsal branches become narrow as they grow and that even one or two cells at the base of the dorsal branch appear to recede into the dorsal trunk. Our results suggest that the constraints of the migratory path can have a role in the final morphology of the dorsal branch.

The Tracheal Cells of Ventral Branches Migrate in Apposition to the Lateral and Ventral Muscle Precursors

Upon invagination, the ventral region of the tracheal pit is located just at a position where the groove becomes wider (Figs. 6A and 6B). At this location, ventral tracheal cells

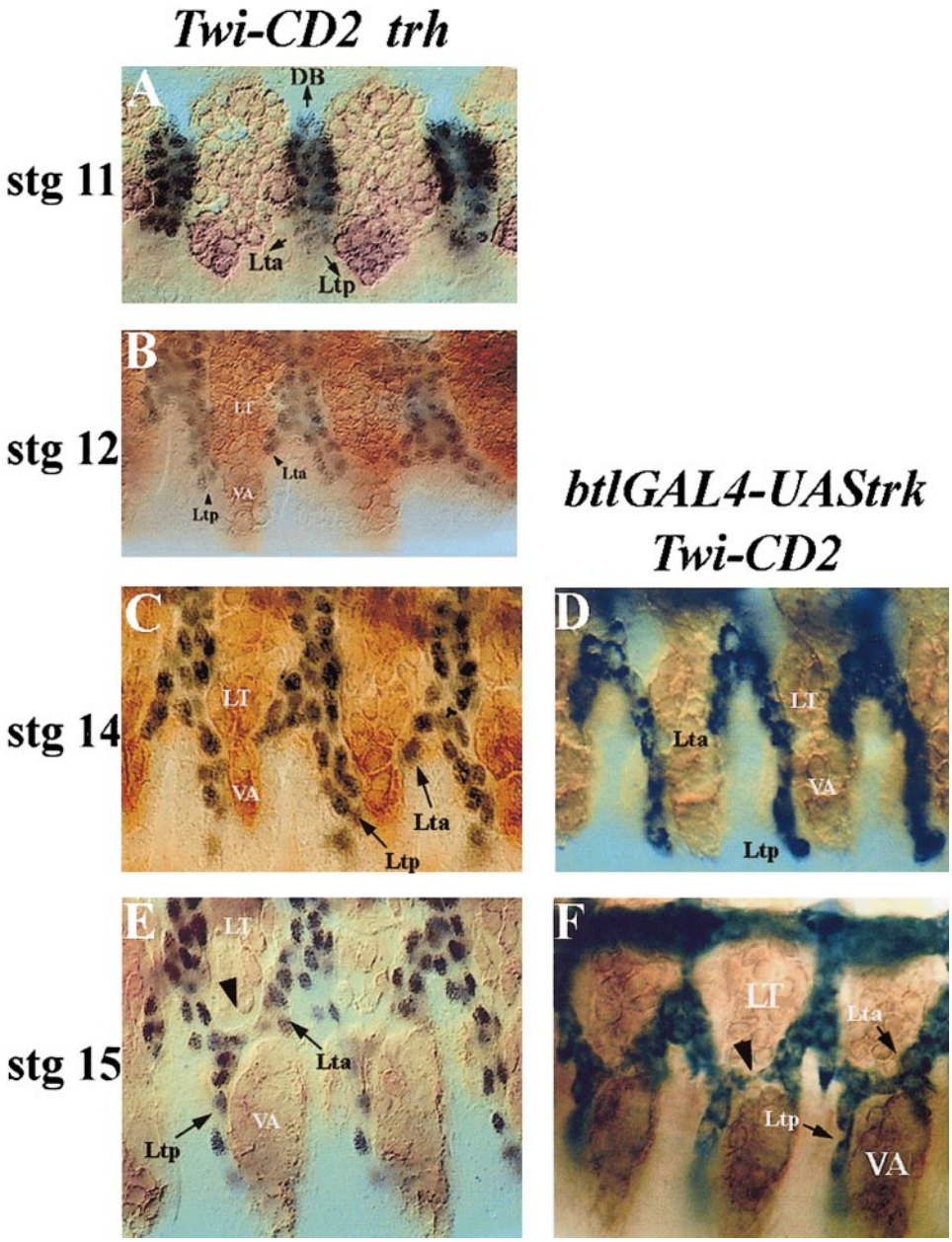

FIG. 6. The migratory pathways of the cells of the ventral branches. (A and B) Upon invagination, ventral tracheal cells follow two migratory pathways in close apposition to the lateral muscle precursors. (C and D) The cells that form the lateral trunk posterior continue to move until they reach the ventral acute muscle precursors. (E and F) Conversely, the cells that form the lateral trunk anterior change their direction and migrate between the lateral and the ventral precursors (arrowheads) and merge with the cells of the lateral trunk posterior of the adjacent metamere. Note the close apposition between mesodermal and tracheal cells. Mesoderm and tracheal cells are visualized as in Fig. 2. DB, dorsal branch; LT, lateral transverse muscles; LTa, lateral trunk anterior; LTp, lateral trunk posterior; VA, ventral acute muscles.

follow two different paths in close apposition to the two populations of lateral muscle precursors at each side of the widening groove. Anterior cells in the tracheal pit migrate along the posterior side of the lateral muscle precursors of one segment and form the anterior lateral trunk (LTa). Posterior cells migrate along the anterior side of the lateral muscle precursors of the posterior adjacent segment and form the posterior lateral trunk (LTp) (Figs. 6C and 6F).

However, the path followed by the two branches is not symmetrical. While the cells of the LTp migrate along the lateral and ventral muscle precursors, the cells of the LTa migrate only halfway and then change their direction to migrate between the lateral and the ventral muscle precursors (Figs. 6E and 6F) through a preexisting groove that separates the two clusters. As a result, the LTa of one segment connects with the LTp of the anterior adjacent segment.

DISCUSSION

Many studies on cell movement have been carried out using *in vitro* cultures and the mechanisms discovered probably also apply *in vivo*. However, a very specific point of migration in the whole animal is the different kinds of interactions that migrating cells have to establish with the distinct types of neighboring cells. In particular, cells often migrate over a particular substrate and move across a given population of unrelated cells. Therefore, cell migration does not depend only on the existence of spatial cues that could act as guiding factors but also on the physical constraints by surrounding tissues.

Our analysis has identified different pathways followed by the tracheal cells, allowing us to recognize which are the surrounding tissues that may participate in their migration. We have also found that the migratory behavior of the tracheal cells differs according to their pathway. For instance, cells that form the dorsal branches take advantage of preexisting grooves between muscle precursors of adjacent metameres. Conversely, cells that form the dorsal trunk migrate across a contiguous population of mesodermal cells. This suggests that cells in different pathways use different mechanisms to migrate according to the interactions that they establish with other cells. In support of this idea, the morphology of the tracheal cells undergoes specific changes depending on the chosen path. Thus, cells that migrate along preexisting grooves are those of elongated shape, whereas cells that migrate across a contiguous population of mesodermal cells are those that do not elongate and remain columnar (Llimargas and Casanova, 1999). Furthermore, subsets of cells that will follow different paths express distinct genes (see for example Kühnlein and Schuh, 1996; Chen *et al.*, 1998).

Distinct Subsets of Mesodermal Cells Are Associated with the Migratory Pathways for Each Tracheal Branch

Previous work has shown that the expression of *bnl* in clusters of cells surrounding the tracheal cells marks the positions where new branches will grow out (Sutherland *et al.*, 1996). However, how are these migratory paths established? Our results indicate that they are associated with different mesodermal derivatives. Accordingly, the distribution of specific subsets of mesodermal cells anticipates the migratory path that the tracheal cells will follow. For example, the outline of the precursors of the lateral and ventral muscles prefigures the migratory path of the tracheal cells that form the ventral branches. Similarly, the lateral mesoderm derivatives identify the migratory path of the cells that form the dorsal trunk. The role of these subsets of mesodermal cells as migratory cues is highlighted by our observation that formation of a particular branch, the dorsal trunk, is disrupted in mutations that prevent the development of a specific mesodermal derivative.

A Model for the Role of Mesoderm in Tracheal Cell Migration

While Bnl signaling marks the position where new branches will grow out (Sutherland *et al.,* 1996), Dpp and EGF have a role in deciding which tracheal cells will migrate in one direction or another. Only some cells in the tracheal placode would be reached by the Dpp and/or EGF signals from nearby tissues, assigning distinct specifications to subpopulations of the tracheal cells (Llimargas and Casanova, 1997; Vincent *et al.,* 1997; Wappner *et al.,* 1997). This led us to propose that Dpp could instruct some tracheal cells to migrate in the dorsoventral axis by enabling them to recognize a localized cue that would not be recognized by the cells from the center of the placode (Llimargas and Casanova, 1997). However, there was no indication about the nature of these putative cues. Our present analysis indicates that specific subsets of mesodermal cells could act as those localized cues that would trace the migratory pathways for groups of cells to form specific branches. In this model, appropriate combinations of distinct cell membrane proteins in tracheal and mesodermal cells could mediate the choice between different migratory pathways (see Fig. 7). Interestingly, it has been found that different genes coding for transcription factors, *spalt* (*sal*) and *knirps* (*kni*), are transcribed in subsets of tracheal cells (Affolter *et al.,* 1994; Kühnlein and Schuh, 1996; Chen *et al.,* 1998). These transcription factors could activate the expression of genes encoding membrane proteins. Moreover, expression of *sal* and *kni* is regulated by the EGF and Dpp pathways (Vincent *et al.,* 1997; Wappner *et al.,* 1997), indicating a possible link between the activity of those signaling pathways and the presence of specific cell surface proteins. In summary, we propose that signaling by transduction pathways, such as the Dpp and the EGF pathway, would control the choice between alternative migratory paths of the tracheal cells by inducing the expression of distinct cell surface proteins that would allow specific interactions with subsets of mesodermal derivatives. Clearly, further support of this model would have to await the characterization of those cell membrane proteins in tracheal and mesodermal cells.

Topological Constraints and Morphogenesis

The identification of the migratory pathways of the tracheal cells clearly indicates that the formation of the

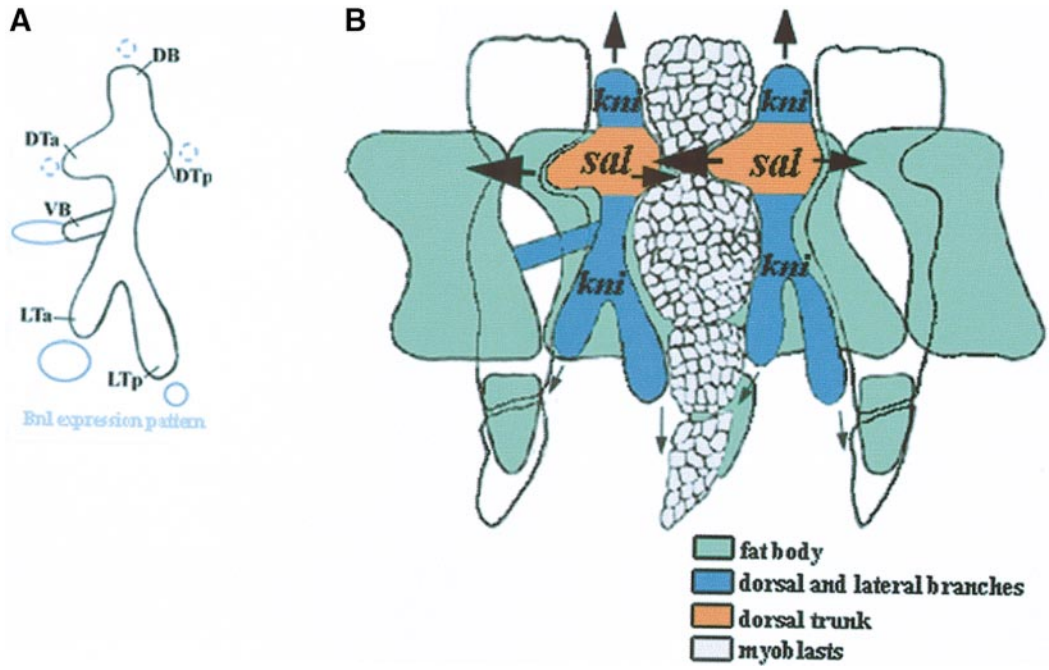

FIG. 7. A model for the role of mesoderm in tracheal cell migration. (A) Schematic representation of the clusters of Bnl expression around the tracheal sac at the positions where the primary branches will form. (B) Schematic representation of the tracheal and mesodermal precursors. The tracheal pits are located between the muscle precursors and above the fat body precursors. Two populations of muscle precursors are only outlined to allow visualization of the fat body cells at a more internal level. Signaling from nearby cells specifies different subpopulations among the tracheal cells; these populations can be identified by the domains of gene expression. Cells in a central position express the *sal* gene and migrate in the anteroposterior axis. Cells in a dorsal and ventral position are reached by the Dpp signal and express *kni;* thus, dorsal and ventral cells in the tracheal pit would be able to recognize a cue different from that of those cells from a central position. Other cells from the tracheal pit would migrate inward and form the visceral branch. In this model, the different subsets of mesodermal cells would act as the localized cues tracing the migratory pathways for groups of cells to form specific branches.

tracheal system is physically constrained by surrounding structures. For instance, this is very conspicuous in the case of the dorsal branch in which migration is channeled by the groove between muscle precursors of adjacent metameres. In addition, even the final diameter of the dorsal branch appears to be conditioned by the width of the mesodermal groove: the dorsal branch initiates as a small bud but becomes a one-cell row as the cells move across the groove. Thus, while there are signals that can direct branch outgrowth to defined positions it is clear that tracheal cells cannot migrate to every possible position. Consistent with this idea, redirection of tracheal migration toward ectopic patches of *bnl* expression is restricted in the embryo (Sutherland *et al.*, 1996). A clear case is illustrated by *tin zfh-1* double mutant embryos: in the absence of lateral mesoderm, there is no proper migration of the cells that form the dorsal trunk, despite appropriate *bnl* expression. Therefore, migration of tracheal cells requires both a signal acting as a guidance molecule and a distinct and recognizable surrounding tissue. Thus, as we show for the tracheal system, understanding organ formation requires unveiling both its genetic program and the surrounding topological constraints.

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