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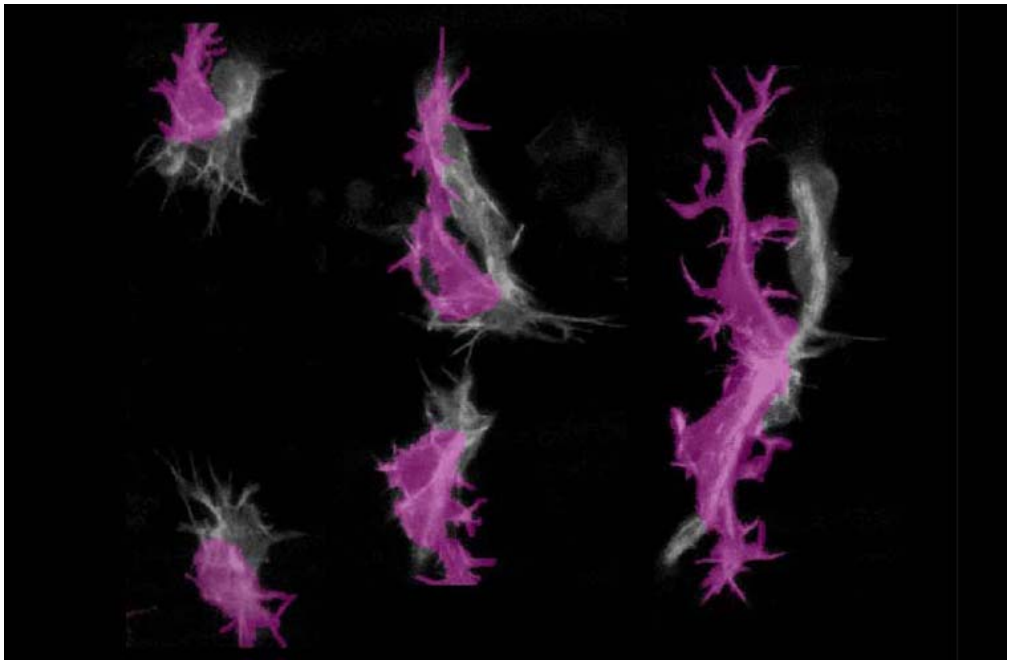
How Tracheal Cells Know Where to Grow

October 15, 2004 – The growing embryo is a hive of activity, with cells stretching, wandering and assembling to form the higher-order structures and networks that ultimately build the body. Some cells crawl along a matrix, making their way to distant locations, while others, such as some neurons, extend projections while the cell body remains in place. These types of shape change and migration are acknowledged as fundamentally important developmental phenomena, but scientists have long puzzled over the guidance mechanisms that make sure that cells and their processes end up in the right places. A number of migratory systems have been shown to rely on molecules known as morphogens, which can act as either attractors or repellants for migrating cells and steer them unerringly to their destinations.

During its embryonic development, the fruit fly, *Drosophila*, assembles a trachea – a tubular respiratory network which delivers oxygen to the rest of the body in the larva and adult. This organ arises from ten pairs of tracheal placodes in thoracic and abdominal segments, which send forth six primary branches that migrate in stereotypical patterns. The dorsal branches move to points on the inner surface of the epidermis on the medial axis of the embryo's dorsum (back) to fuse with their partner from the opposite side. Each dorsal branch is tipped with a specialized cell that leads the cells behind it, but the exact means by which these terminal cells find their way across the interior face of the epidermis to the dorsal midline has remained unknown.



Time-lapse images of the extending tracheal terminal branch (pseudocolored in purple) of the *Drosophila* embryo. Images of a dorsal branch fusion point at three successive time points (left to right) taken from GFP-labeled tracheal cells are shown.

In an effort to resolve this question published in the journal *Development*, Shigeo Hayashi (Group Director, Laboratory for Morphogenetic Signaling) and colleagues at the RIKEN Center for Developmental Biology and the National Institute of Genetics looked at molecules known to be involved in tracheal branching for their potential roles as cell migration path determinants. In order to study these molecular signals,

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Kagayaki Kato, a RIKEN special postdoctoral fellow in Hayashi's lab, first tracked cell movements during the process of tracheal development.

Watching pairs of GFP-tagged cells found at the tip of the dorsal branch, Kato saw that they migrated over the underside of the dorsal epidermis (DE), where they made contact with partner cells from the opposite side of the body. Throughout the process, these tip cells remained closely associated with the epidermis, indicating that guidance signals might be of epidermal origin. The team opted to focus on one of the tip cells, called the terminal cell, which stretches out, seemingly in response to directional signals.

At first, terminal cell filopodia project equally in all directions, but only those which extend ventrally (toward the belly of the embryo) stabilize; other filopodia tend to withdraw back into the cell body after a short time. It is this stabilization that allows the terminal cell to sprout its branch exclusively in a ventral direction. Hayashi et al. next looked at the epidermal region immediately adjacent to the spot where the dorsal branch tip cells congregate for specific patterns of gene expression and noted that their migration and subsequent activity seemed to home to a space underlying a dorsal epidermal region marked by the expression of a pair of morphogens: Decapentaplegic (Dpp), and Hedgehog (Hh), which are expressed in stripes in the DE. An experiment in which extra terminal cells were generated supported the idea that these cells display a preference for Hedgehog-positive zones, as terminal branch cells that were displaced from one such region would make their way through non-Hh-expressing territory to the closest Hh-positive segment.

Suspecting a role for *hedgehog* in directing the outgrowth of terminal branches, the team next made tests in which the gene was broadly misexpressed or its signal transduction interfered with, and obtained results that tended to confirm their hypothesis that external Hh influences the direction of terminal branch outgrowth. Cells rendered unresponsive to Hh signaling displayed aberrant dorsal branch migration, with filopodial extensions radiating in all directions unrestricted to their usual ventral orientation, indicating that tightly restricted expression of Hh is required for normal terminal branch migration.

Returning to the earliest stage of multidirectional outgrowth, the researchers examined whether Dpp, which is expressed in the overlying dorsal epidermis, might act as an inhibitor of terminal branch outgrowth, further ensuring that foraying branches ultimately only travel downward to the Hedgehog expressing regions. Dpp is already known to be important for dorsal branch specification, so Hayashi and colleagues designed tests to investigate whether it plays a specific role in branch migration. Experiments in which Dpp was overexpressed showed that branches failed to extend as normal, while Dpp downregulation resulted in misdirection of the terminal branch along the anterior-posterior axis.

The team has proposed a model to explain the localization to and subsequent behavior of dorsal branch cells at the intersection of expression of two developmentally crucial morphogens in which Hedgehog sets up a permissive environment allowing the cells to travel into the posterior compartment while Decapentaplegic exerts the opposite effect, posting molecular No Entry signs during the early exploratory phase of branch outgrowth. The team suspects that such coordinated permissive/repulsive mechanisms may be found in the patterning of organs and complex structures in other species as well and may represent a

relatively simple strategy by which the developing body lets its cells know where to grow.

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