

DEVELOPMENTAL BIOLOGY

Fly Gene Discovery Gets at Root of Branching Structures

Imagine a tree with hollow trunk and limbs, and you'll have a good picture of the branching tubular structures, such as the bronchi of the lungs or the arteries of the circulatory system, that deliver vital gases and fluids to animal tissues. These structures usually follow a stereotypical pattern—for example, everyone's bronchi divide to enter two lobes in the left lung and three in the right—suggesting that such motifs arise from fixed developmental programs encoded in our genes. But just how this occurs in development—how an embryonic tube of cells “knows” where to sprout branches—has long left biologists stumped.

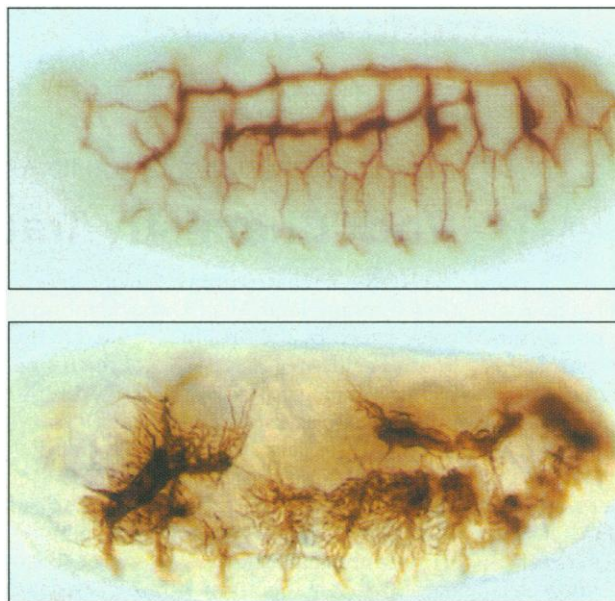
Now, researchers have extracted new clues to the enigma of branching structures from the old standby of developmental genetics, the fruit fly *Drosophila melanogaster*. As a fly embryo develops into a larva, it acquires an intricately branched network of respiratory tubes called trachea that supply oxygen directly to interior tissues. A group led by Stanford University biochemist Mark Krasnow has now found the signal that controls the sprouting. Clumps of cells around the budding trachea express a newly discovered gene called *branchless* (*bnl*), the group reports in the 13 December issue of *Cell*—and new tracheal branches grow out just where Branchless protein (Bnl) is concentrated.

What's more, Bnl's amino acid sequence shows that, as predicted, this protein is closely related to the powerful mammalian fibroblast growth factor (FGF) clan of messenger proteins, which direct cells to divide, differentiate, or migrate. Bnl is the first FGF family member characterized in an invertebrate—a kinship suggesting that some FGFs may have equivalent branch-building roles in mice and men. “I'm sure the whole story is going to turn out to be very parallel in mice and *Drosophila*,” says Gail Martin, developmental biologist at the University of California, San Francisco (UCSF), who studies FGFs in mice. “It's really exciting work.”

A fly larva's tracheal system starts out as 10 clumps of ectodermal cells spaced along each flank of the embryo. These clusters of about 80 cells each fold inward to form sacs pressing up against the epidermis. Each sac becomes the “trunk” of a tracheal system. It forms six buds that develop into primary branches reaching into the larva's innards, and these branches later produce smaller secondary and terminal offshoots. No cell division takes place: Each of the 10 complex

tracheal trees is built solely through cell migration and shape changes.

The molecular signals regulating these movements remained completely mysterious until 1991, when developmental geneticist Benny Shilo at the Weizmann Institute of



Bushed. Normal fruit fly larvae develop treelike trachea (top), while larvae with a mutation in the *branchless* gene sprout bushy tracheal branches everywhere (bottom).

Science in Rehovot, Israel, discovered a fly gene called *breathless* (*btl*) and noted that mutant flies without the gene don't form tracheal branches. Shilo found that *btl* encodes a protein resembling cell-surface receptors for the FGF family in mammals, suggesting that normal tracheal branching in the fly requires an FGF-like signal.

Now, building on work by biochemists Tzumin Lee and Denise Montell at Johns Hopkins University, Krasnow and Stanford graduate student David Sutherland and postdoc Christos Samakovlis have found the signal that the *btl* receptor is designed to receive—and sketched a picture of how this signaling system controls branch navigation. As the trio reports in *Cell*, a search for flies with telltale tracheal defects uncovered some 50 mutants, and several strongly resembled the *btl* mutants. The affected gene in one of these, which the group named *branchless*, turned out to encode an FGF family member—the missing key, or ligand, to the *btl* receptor's lock.

When the researchers examined embryos

to see where *bnl* is active, they saw six droplets of *bnl*-expressing cells beside each tracheal sac, in positions that exactly presage where the six primary branches will grow. What's more, when the group engineered flies to manufacture Bnl protein in specific places, branches sprouted in those positions. And when they provoked Bnl synthesis throughout the embryo, it filled with a spaghetti-like tangle of trachea (see photos). All this offers definitive proof that Bnl not only turns on branch outgrowth, but guides it. Says Shilo, “It's a wonderful demonstration of the fact that most aspects of directionality are provided by the ligand for the FGF receptor.”

Of course, these elegant results raise new questions, such as how Bnl tells cells where to go. UCSF's Martin theorizes that Bnl may directly influence the cells' cytoskeletons, the internal frameworks that lead remodeling as cells migrate and change shape. Another question is what controls the sites of *bnl* expression. The answer may lie in already-identified patterning molecules, says Krasnow. Previous work has shown that each body segment of the fly embryo is crisscrossed by stripes of cells expressing other pattern-forming proteins such as Wingless and Engrailed.

Different combinations of those proteins could act as “volume knobs” for the *bnl* gene, precisely controlling where high concentrations of Bnl will accumulate, Krasnow speculates: “The same kind of thing—key morphological regulators tapping into the simpler, gridlike spatial patterns of the earlier regulators—may be true for many different kinds of organ structures.”

Indeed, with the fly work buzzing along, an understanding of branching tubular structures in mammals may not be far behind. Already, Rusty Williams at UCSF and Kevin Peters at Duke University have found that mouse embryos with genetically engineered defects in their FGF receptors have dramatically reduced lung branching—a finding that hints at future methods for regenerating the tissue damaged by emphysema, atherosclerosis, and other diseases affecting branching structures in humans. “If you start to understand what controls the branching of those kinds of tissues,” explains Krasnow, “you can begin to think of ways to restore them.”

—Wade Roush