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Roundworm Studies Yield New Insight into Organ Formation

While studying the roundworm *C. elegans*, researchers have pinpointed an enzyme that controls the shape of a developing organ. This discovery opens the way for researchers to gain new understanding of the mechanisms by which organs blossom from single cells.

HHMI investigator Judith Kimble and colleague Robert Blelloch, both of the University of Wisconsin-Madison, report in the June 10, 1999, issue of the journal *Nature*, that a metal-containing enzyme called a metalloprotease guides the formation of gonads in the roundworm *C. elegans*. The enzyme is crucial for ensuring the proper elongation and curvature of the gonads.

"A pseudocolor enhanced view of the roundworm *Caenorhabditis elegans* as seen through a microscope."

The researchers began their experiments by isolating a gene that codes for GON-1, a protein that is required for normal gonad formation. Analysis of the protein revealed that it is a metalloprotease a type of metal-containing enzyme that snips apart other proteins. After tinkering with the enzyme to jam its operation, Kimble and Blelloch concluded that the protein-snipping activity is needed by the enzyme to perform its critical organ-forming functions.

To illuminate what else this protein was doing in the developing worm, the scientists constructed a fluorescently-labeled version of the enzyme that lights up when viewed under ultraviolet light. Then, using the modified enzyme, they traced the protein's expression within the developing worm.

"We found that the metalloprotease was expressed in leader cells (located at the tip of the gonad), which made a lot of sense because they are part of the developing gonad," said Kimble. "But we also found that it was expressed in the body wall muscle, which didn't make a lot of sense to us. So, we then asked what was important about these two distinct activities, since quite frankly, we were not expecting the body wall muscle expression to be important in gonad development."

Next, the investigators constructed two kinds of "mini-genes" containing the enzyme. One mini-gene included a switch, called a promoter, that drove production of the enzyme only in leader cells. The other mini-gene included a promoter that triggered the enzyme's production only in muscle wall cells. They then inserted these genes separately into mutant worms that were not able to produce the metalloprotease.

The mini-gene that worked in leader cells "rescued" the mutant worm, permitting the growing gonad to elongate into a near-normal u-shape. By contrast, the mini-gene that functioned in muscle wall cells did not support normal gonad development. "But we saw what we think was a dramatic and surprising effect," said Kimble. "We found that the developing gonad expanded in all directions into a discrete, football-like shape."

Thus, said Kimble, "the two activities together really define the shape. When you're making this organ, you not only need the directed elongation, but the organ also needs to expand."

To better understand the function of the worm metalloprotease, Blelloch and Kimble plan to study a similar enzyme in mice that has been implicated in advanced cancers.

"We speculate that just as the *C. elegans* metalloprotease is critical for the migration of a specific cell, the vertebrate counterpart may be important for the migration of cancer cells and their spread in mice and humans," said Kimble.

One theory is that the worm enzyme, like one of its vertebrate family members, snips apart a molecule called collagen that makes up the supporting structural matrix that plays a key role in the formation of many organs and tissues. The scientists' future experiments will include inserting the mouse gene for the metalloprotease into *C. elegans*, providing the researchers with another tool to explore the function of the mouse enzyme, and possibly new ways to thwart the cancer gene.

"If the mouse gene works in *C. elegans*, we can then look for inhibitors of the gene, and if we find them, ask whether these inhibitors affect cancer progression in mouse cells," said Kimble.

The scientists also plan to insert the worm gene into other tissues in the worm itself in order to determine whether and how the enzyme affects organ growth in those very different tissues. Such experiments may give further hints of the enzyme's action and regulation, said Kimble.

"Now that we know this enzyme activity can be used to manipulate organ shapes, we would like to see how far we can push it. While it's purely science fiction now, we can imagine someday understanding organ growth regulation well enough that we could put cells in a petri dish and use such regulatory substances to form the growing cells into a particular organ."