MEETING

Developmental Progress Fills the Air in Kyoto

KYOTO, JAPAN—Organizers of the 14th International Congress of Developmental Biology arranged for a special fireworks display to accent Kyoto's temples, shrines, and hot springs. But the 1400 biologists who gathered here already had plenty to get excited about, from new insights into limb and segmentation development to a heroic effort to define the regulatory network controlling early sea urchin development.

The End of the Progress Zone?

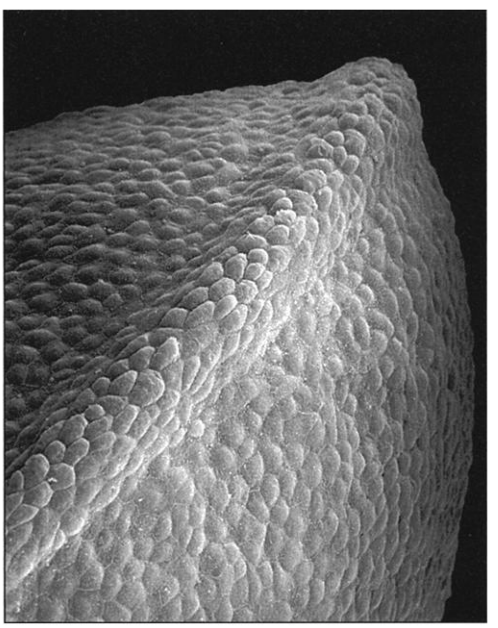
For the past 50 years, the progress zone model of limb development has been one of the most firmly entrenched notions of developmental biology. But now that model has come under fire.

The progress zone model is based on classic experiments in which scientists produced dramatic changes in limb structure by removing a specialized layer of tissue called the apical ectodermal ridge (AER) from the end of the developing front limb of the chick embryo. Cutting off the AER early produced a limb with a normal upper bone (humerus) but nothing farther out, while removing it progressively later in development allowed more and more of the limb to develop normally. The results were taken to indicate that the AER continually reprograms the cells nearest to it in the so-called progress zone, directing them to become first shoulder, then humerus, elbow, and so on. This model was strengthened by more recent experiments showing that replacing the AER with a bead soaked with fibroblast growth factors (FGFs), proteins involved in many developmental processes, produced normal limbs.

In Kyoto, however, Cliff Tabin, a developmental biologist at Harvard Medical School in Boston, described results from a postdoc, Andrew Dudley, that upset the progress zone model. "The term progress zone is very misleading," Tabin says. "There is no special zone, and it is not progressive."

Dudley set out to define the size of the progress zone by injecting dye and virus markers into the limb bud at different depths. The model predicts that markers placed in the zone should spread through the limb as cells proliferate. But Dudley never found a trail of markers. Instead, their final locations depended only on the depth at which they were first placed. Tabin says this leads to a very different model of limb bud development. Instead of having a progress zone, he suggests, the fate of the cells that produce the various limb structures is determined quite early; they then proliferate to produce the fully developed limb.

To confirm that the cells' fates were set early on, Dudley took the tips of limb buds from embryos of various ages and grafted them onto the stubs of limb buds that had been cut off. Under the progress zone model, the fate of the cells that produce the digits is determined last. But Dudley found that regardless of whether the tips came from early or late limb buds, they always produced digits—a strong indication that the fate of the cells that produced them had been determined early instead.



No progress? New results question the progress zone model of limb development involving the apical ectodermal ridge.

Tabin also offered a new interpretation of the older results. Building on work suggesting that removing the AER causes cell death to a depth of about 200 micrometers into the limb bud, the team analyzed the size of the limb bud at different stages of development. They concluded that the loss of 200 micrometers' worth of cells under the AER at an early stage would wipe out the entire limb. Later on, the loss of 200 micrometers would knock out just the digits.

Support for this idea came when Dudley again removed the AER of a number of limb buds at different stages of development. He then replaced it, either immediately or 10 hours later when the cells had died, with a bead containing FGFs, which were presumed to be involved in progress zone development. Only when the bead was implanted immediately did the full limb develop. That result suggests it is not the FGFs but the prevention of cell death achieved by covering the wound that allows normal development to continue. Further experiments tended to support the idea that cell death, rather than the absence of the AER, caused the limb deformations of the early experiments.

Other researchers had previously questioned the progress zone model. But Miguel Torres, a developmental biologist at Spain's National Center for Biotechnology in Madrid, says it "will be a shock to the community" if it needs to be discarded.

Segmentation Gets Pieced Together

Nature likes segmentation, the making of repetitive embryonic units that serve as the building blocks of all insect bodies and those of many higher animals as well. Although the mechanics are different, segmentation can be seen in the shells of crustaceans, the body rings of the earthworm, the stripes on the abdomens of bees, and the vertebrae of vertebrates. But developmental biologists have only just begun to understand what drives segment formation, particularly in higher organisms.

In previous work, Olivier Pourquié, a developmental biologist at the Université de la Méditerranée in Marseille, France, showed that in vertebrates many of the genes involved in forming somites, the segmental units that develop into the vertebrae and muscles of the torso, repeatedly cycle on and off. He suggested that a "segmentation clock" controls the timing of somite formation. Two presentations at the conference, the basis for papers appearing in the 27 July issue of *Cell*, shed further light on the interaction of this clock with other factors controlling segmentation.

This time Pourquié and his team focused on fibroblast growth factor 8 (FGF8). The somites form from head to tail by repeatedly budding off from groups of columnlike cells, called the presomitic mesoderm, that line up along either side of the neural tube, which runs along the posterior surface of the embryo and eventually forms the spinal cord. FGF8 is expressed at the posterior end of these columns, and the concentration of FGF8 surrounding the budding somites forms a gradient, decreasing with distance from the end of the columns toward the

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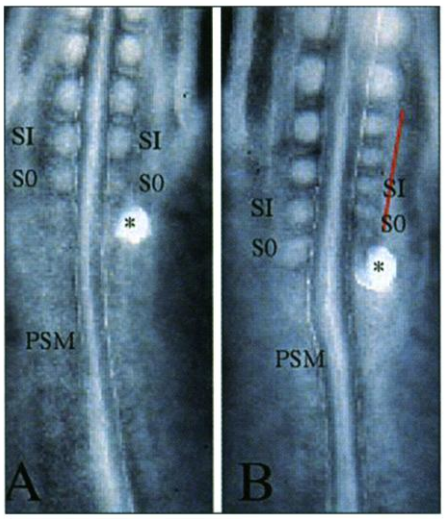

Beading up. A bead saturated with fibroblast growth factor 8 (B) interferes with differentiation in a chick embryo and leads to somites that are smaller than normal (A).

forming somites.

To test FGF8's role in somite formation, the team either blocked its activity by treating chick embryos with a drug that inhibits FGF binding to receptors or increased its concentration by inserting an FGF8-saturated bead into the embryos alongside the presomitic mesoderm. Reducing FGF8's concentration produced bigger somites, while increasing it led to smaller ones. These results suggest, Pourquié says, that FGF8 signaling, above a certain threshold, prevents the presomitic cells from beginning the differentiation that allows them to be incorporated into somites even as the segmentation clock keeps ticking. "The boundaries [between somites] are determined at a given time, and if fewer cells are available you get smaller somites," he says. In other words, the clock determines when the boundaries form, whereas the FGF8 signaling gradient controls where they form.

Another piece of the segmentation puzzle has been put in place by Denis Duboule and colleagues at the University of Geneva, Switzerland. Using mice, they closely monitored the timing and level of expression of *Hox* genes, which are known to be involved in giving each somite its identity, telling it where in the somite chain it is located so it can develop into the proper bone and muscle tissue. The researchers found that the same signaling that starts the formation of a somite triggers the expression of the *Hox* genes, a finding that suggests that the genes are linked to the segmentation clock.

Yoshiko Takahashi, a developmental biologist at Nara Institute of Science and Technology in Japan, calls the work "astonishing." The results from the two groups, she says, "are having a great impact on understanding a very important phenomenon." They also increase the need to better understand how the clock works and the role of somite formation in segmentation.

Coming to Grips With Gene Regulation

Most researchers content themselves with unraveling the functions of one gene or gene family, or maybe with tracing one signaling pathway. Not Eric Davidson, a developmental biologist at the California Institute of Technology in Pasadena. His team is trying to define the entire network of genes and regulatory signals needed to form the endomesoderm, the primordial cell layers that produce all the organs and tissues of the sea urchin.

Davidson is particularly interested in the cis-regulatory elements, DNA sequences associated with each gene that turn them on or off in response to the various developmental and environmental signals conveyed by the regulatory pathways. He believes that the different shapes and sizes of animals are due primarily to cis-regulatory activity, which controls where within an embryo and when the genes are active. "These regulatory networks are the key to really understanding development and evolution," Davidson says.

To identify the cis-regulatory elements, the researchers turned to comparative genomics. They performed a computer analysis of sequence data from two evolutionarily distant species of sea urchin, looking for evolutionarily conserved sequences that are likely to be cis-regulatory elements.

In the spotlight. Work with the embryos of sea urchins is providing insights into gene regulatory networks.

Another part of this Herculean task is identifying all the genes involved in the development of that endomesoderm. Because the organism is a popular experimental model, bits and pieces of the immense network of regulatory pathways that control its development were already known.

To fill in the rest, Davidson and his team disrupt the known regulatory pathways in a variety of ways. For example, they inject embryos with messenger RNAs that interfere with the expression of known genes or overexpress regulatory genes. Using microarrays bearing tens of thousands of clone complementary DNAs, they compare levels of gene expression between control embryos and embryos in which the pathways have been disrupted. In this way, they have identified nearly 100 genes not previously linked to endomesoderm specification. They also tied the expression of the genes to particular stages of development and areas of the embryo. They then knocked out or altered the expression of these genes to check their effect on other genes.

The end result of all these analyses is a computational model developed in collaboration with Hamid Bolouri of the University of Hertfordshire, U.K. (Continually updated diagrams of the regulatory networks can be seen at www.its.caltech.edu/~mirsky.) The model shows the flow of developmental regulatory information both in time and in the different spatial domains of the embryo. This makes it possible, Davidson says, to see which genes come on and when as the embryo forms first the endomesodermal precursor cells and then the endodermal, mesodermal, and skeletogenic domains. "It's a very complex diagram," he says, but one with "a tremendous amount of explanatory value."

Others aren't so sure. One researcher who doesn't want to be identified says the diagram is too complicated to be useful. But many others think Davidson is on to something. John Coleman, a developmental biologist at Brown University in Providence, Rhode Island, says it demonstrates how the use of microarrays "can lead to new insights rather than just masses of expression data." Noriyuki Satoh, a developmental biologist at Kyoto University, also likes the fact that Davidson has avoided the simplification that most developmental biologists do out of necessity. "To understand real evolutionary and developmental processes, we need to understand more of the details in gene networks," he says.

–DENNIS NORMILE

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