

Mysteries of Development

DEVELOPMENT IS, LITERALLY, THE JOURNEY OF A LIFE time, and it is a trip still as mysterious as it is remarkable. Despite new methods to probe how an animal or plant forms from a single cell, biologists have much to learn about the unimaginably complex process. To identify some of the field's persistent riddles, Senior Editors Beverly Purnell and Stella Hurtley and the news staff of *Science* have consulted with developmental biologists on our Board of Reviewing Editors and elsewhere. The mysteries offered here are a humbling reminder that our knowledge of development remains to a great extent embryonic.

—JOHN TRAVIS

Fly balls. Images of fruit fly embryos, revealing locations of different microRNA molecules.

How Do Organs Know When They Have Reached the Right Size?

In the 1920s, biologists began exploring a new way of studying development: surgically removing rudimentary tissues that would form organs and limbs from embryos of one species and transplanting them into those of a related species. In one visually striking example, Yale University zoologists Victor C. Twitty and Joseph L. Schwind removed embryonic tissue that would become a leg in the large salamander species, *Ambystoma tigrinum*, and transplanted it into the embryo of a smaller species, *Ambystoma punctatum*. Despite the early stage of the transplant—before limb buds even appear in the subsequent larvae—the legs grew to the size that they would have on their original body; small salamanders ended up with a longer-than-normal leg, and large salamanders with a short leg. The result, published in 1931 and considered a classic experiment today, suggested that something intrinsic in the leg, rather than signals from the rest of the body, determines a limb's final size.

Since then, developmental biologists have found dozens of proteins and genes that play a role in the growth of plants and animals. But how growing organs and organisms can sense their size and know when to stop is still a mystery.

Developmental biologists continue to explore that mystery today, although most of their experiments now use fruit flies instead of salamanders. The current objects of their attention are imaginal discs, flattened sacs of cells that grow during the fly's larval stages. During the pupal stages and morphogenesis, specific discs differentiate to form the adult wings, legs, eyes, antennae, or other structures. Although they seem undifferentiated in the larval stages, the cells of the different discs are already destined to become a particular body part. Scientists can transplant a wing imaginal disc from a larva into the abdomen of an adult fly, leaving it there for months, and if it is transplanted back into a larva, it will still form wing tissue during pupation and morphogenesis.

A variety of experiments have shown that both the size of imaginal discs and the organs they form are very tightly controlled. When researchers transplant the wing imaginal disc from an early fly larva to a later one or vice versa, the wing still reaches normal size despite having different growing times. If researchers kill a portion of the imaginal disc cells with radiation or other techniques, the insect can boost cell division and still form a normal-size adult. If a fly receives just a fragment of a disc as a transplant, the animal won't move to the next stage of development until the disc has reached the correct size—pausing overall development to allow the disc to catch up. The transplanted disc “will know what size it should be,” says developmental biologist Savraj Grewal of the University of Calgary in Canada.

Scientists can also change the rate at which imaginal disc cells divide, prompting either too many or not enough cells to form, but the cell size adjusts so that organ size remains the same. “The organ isn't counting cell divisions. It's measuring something about dimension,” Grewal says. “What does the organ sense? I think the answer is still unclear.”

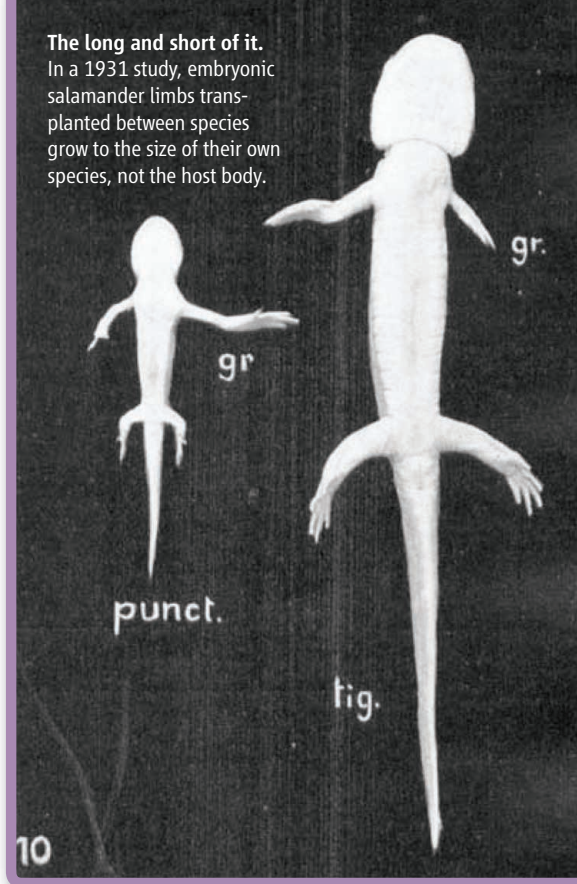
There are a few clues, however. A protein called Hippo is part of a cascade of signals that plays a role in the growth of insect imaginal discs and some mammalian organs. (The name comes from the appearance of flies that lack the gene for Hippo in their eye imaginal discs; their over-

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The long and short of it.

In a 1931 study, embryonic salamander limbs transplanted between species grow to the size of their own species, not the host body.



grown heads have a wrinkled surface that resembles a hippopotamus hide.) Disruptions in the Hippo signaling pathway can lead to overgrowth, suggesting that it plays some role in controlling size. But recent studies suggest that it probably isn't the whole answer, says developmental biologist Georg Halder of the KU Leuven in Belgium.

Another set of genes that help with size sensing are ones that produce proteins called morphogens. These molecules originate from a single source in an embryo and diffuse across cells. They are best known for helping determine patterns during development. But some also influence the size of organs, tissues, and limbs. One theory is that when a morphogen gradient is steep—that is, there is a large difference in its concentration from one cell to another—then cells continue to divide. As cells divide, the gradient becomes more gradual. Once it has flattened out to a particular level, cells stop dividing.

There is also evidence that a cell's sense of which direction is up—called planar cell polarity—helps control growth. When certain genes involved in determining cell polarity are disrupted or missing, body parts tend to grow larger, suggesting that they can't sense when they should stop. "It's no coincidence that some of these polarity genes may act as tumor suppressors," Grewal says, and are suspected of playing a role in some cancers.

Many researchers suspect that a developing organ somehow senses the mechanical forces on its growing and dividing cells. One theory is that relative crowding and stretching of cells helps determine whether a cell continues to divide or stops.

The size of an organ depends not only on how many cells it has, but also how big those cells are. Some developing organs—plant leaves, for example, and fruit fly wings—can compensate when fewer cells are available by making the individual cells larger. How a leaf knows when to expand its cells is also unclear, says Hirokazu Tsukaya, a developmental biologist at the University of Tokyo who was among the first to characterize the phenomenon in leaves. He and his team have evidence that some sort of cell-to-cell communication drives the process. Here, too, the evidence suggests that a plant doesn't count cells but can somehow assess the overall size of a leaf,

says plant biologist Beth Krizek of the University of South Carolina in Columbia. "But the mechanism of how that works is another mystery."

The size of tissues, and ultimately an overall organism, also clearly depends on signals from the environment, which researchers call extrinsic factors. Those size control systems are connected to, but different from, the intrinsic systems that help ensure an organism is correctly proportioned. In plants, growth can be especially sensitive to such outside factors, Krizek notes, because they can't move. Plants growing in shade, for example, concentrate on stem growth—to reach the sun—instead of leaf development. In animals, the amount of nutrition available can strongly influence the final size of some organs. One dramatic instance is the horn on a rhinoceros beetle. The horn is a sexually selected trait; males with bigger horns get access to more females. Recent studies have shown that the size of the horn is particularly sensitive to insulin signaling, which is related to the beetle's nutrition.

That, in turn, signals the animal's overall fitness (*Science*, 27 July 2012, p. 408).

The problem of size control is still a fundamental one for developmental biologists, says Peter Lawrence of the University of Cambridge in the United Kingdom. Together with shape, size "is the material that evolution largely works on." But the field is still mostly in the dark. Despite hundreds of papers on what happens when the Hippo signaling pathway is interrupted, Lawrence notes, what scientists really need to understand is what it does when it is working properly. "That is not something we know." —GRETCHEN VOGEL

Why Do So Many Neurons Commit Suicide During Brain Development?

With a few notable exceptions, the roughly 100 billion neurons we have at birth are the only ones we'll ever have. Unlike skin and immune cells, which continuously self-renew, once a neuron has differentiated from its parent stem cell it will never divide again. Given this finite supply, why do so many neurons—more than half in some brain regions—kill themselves during embryonic brain development?

For roughly 50 years, many scientists

focused on a single explanation for this rampant cellular suicide. Their hypothesis was rooted in research on the peripheral nervous system, which connects the nerves of the brain and spinal cord to limbs, organs, and sensory systems. To survive in the developing brain, researchers thought, neurons must compete for limited quantities of a chemical "trophic" factor released by the targets they aim to innervate. Without this signal, the cells self-destruct in a pro-

cess known as programmed cell death, or apoptosis. Called the neurotrophic hypothesis, the concept neatly explained how an overabundance of neurons could attach where needed, or be culled.

"We were all carried away by this observation," says neuroscientist Yves-Alain Barde of the University of Basel in Switzerland. Inspired by the discovery of nerve growth factor in the 1950s, a protein essential to the growth and survival of sensory and motor neurons in the peripheral nervous system, Barde hunted for a single "survival" molecule for neurons in