DEVELOPMENT

Missized Mutants Help Identify Organ Tailors

When is an organ big enough? Two studies published this week point to genes that might optimize size in parts of the nervous system

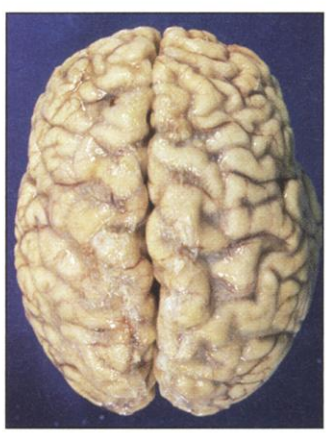
Of all the mysteries of developmental biology, few are as perplexing as how tissues know when to stop growing. How does a mouse's heart or a horse's lung attain a form big enough to do its job but small enough not to crowd other organs? Scientists have a few theories about what mechanisms the body uses to grow perfectly proportioned fingers, stomachs, and hearts, but most remain untested.

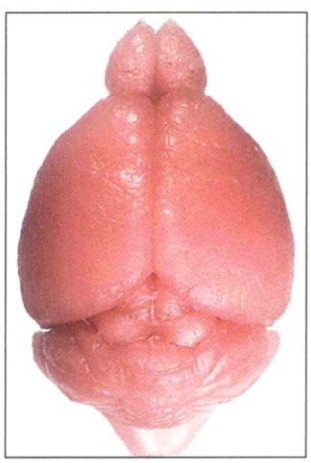
This week, two groups add pieces to the puzzle. On page 365, Anjen Chenn and Christopher Walsh of Brigham and Women's Hospital and Beth Israel Deaconess Medical Center in Boston describe the effects of a ubiquitous protein called β-catenin on the size of the brain's cerebral cortex. And in a paper published online this week by *Science* (www.sciencemag.org/cgi/content/abstract/1073263), Xue Li, Michael Rosenfeld, and their colleagues at the University of California, San Diego, School of Medicine explain how a protein called Six6 interacts with key cell-division genes to control the size of the developing retina and pituitary gland.

The cerebral cortex is the outermost layer of the brain, responsible for the higher order thoughts that allow humans to read, speak, and solve problems. In primates, the cerebral cortex hasn't gotten much thicker over the course of evolution, but its surface area has expanded enormously: The human cerebral cortex has 1000 times the surface area of a mouse's, but it is only about twice as thick. To fit the expanded surface area into a reasonably sized skull, the cortex of primates is wrinkled and creased, like a carpet that's much too large for its room, whereas that of rodents is smooth. No one knows what genetic changes prompted the primate cortex's expansion. Now Walsh and Chenn's experiments point to one gene that might have played a role.

The pair created transgenic mice that carried an engineered form of β-catenin. They connected the gene to a promoter active in developing cells of the central nervous system, thus allowing mutant β-catenin to be expressed in these cells. Because the designer protein was resistant to breakdown, it accumulated in the target cells. The resulting embryos had dramatically enlarged brains, and the cerebral cortex was especially striking. It had a normal thickness but an increased surface area, as well as folds and cavities somewhat similar to those seen in monkeys or humans.

The embryos had an abnormally large number of neural precursor cells, which give rise to several types of brain cells. The overexpressed protein apparently increases the number of precursor cells by telling cells that would normally differentiate to keep dividing. That process produces a bigger cortical "sheet," Walsh says, "and folds seem to be a passive response to the bigger sheet."





Deep thoughts. The human cerebral cortex (*above*) buckles and folds with 1000 times the surface area of a mouse's smooth cortex (*right*).

The scientists are not sure exactly how an excess of β-catenin spurs the neural precursor cells to proliferate. It might act by means of proteins called Wnts that are known to interact with β-catenin and have been shown to influence the multiplication of neural precursor cells during development. But Walsh and Chenn suspect that β-catenin might also work through structures on the cell surface called adherens junctions, where β-catenin concentrates. They seem to play a role in the asymmetric cell divisions that determine which daughter cells will continue to divide as precursor cells and which will stop dividing and differentiate.

However, cautions Martin Raff, a developmental biologist at University College London, when a protein is forced to accumulate in such an artificial way, "it's very hard to tell what its normal role is." It is possible that increased production of β-catenin was involved in increasing brain size during evolution, he says. But that is difficult to prove, given its complex role in so many cells, such as helping an early embryo determine its back from its front.

In the retina, meanwhile, scientists have now pieced together a more precise picture of how precursor cells know when to stop dividing. A gene called *Six6* is expressed for a short time in the retina, hypothalamus, and pituitary gland as these tissues develop. Li, Rosenfeld, and their colleagues suspected that the gene might play a role in regulating cell division. When they created genetically engineered mice lacking the gene, they found that the retina and the pituitary were smaller than in normal mice.

Curious to see how the gene influenced the organs' size, the team tested the Six6 protein's behavior in cultured cells. They found that it could turn off expression of genes in part by interacting with another key regulator of eye development called Dach. Suspecting that the pair might help regulate another well-known set of proteins that tell cells to stop dividing, the researchers looked at expression levels of several of these proteins in their mutant animals. In mice lacking Six6, the amounts of two proteins, p27Kip1 and p19Ink4d, were two to four times higher than normal—suggesting that Six6 keeps the genes encoding these proteins in check, thereby allowing cells to continue to divide. When Six6 levels decrease, the researchers suspect, proteins such as p27Kip1 abound, leading cells to stop dividing and begin differentiating. Further experiments in cell culture showed that Dach and Six6 bind directly to an on switch for the *p27Kip1* gene.

The find is an important insight into the complex web of regulations that controls growth-related proteins, says Raff. "This is one of the few clues we have" about the mechanisms that help regulate the size of a specific tissue, he says. "One of the big questions in size control is the process that stops cell proliferation," and although Six6 seems to be limited to controlling the process in the retina and pituitary, there are likely similar mechanisms in other tissues, he says.

Nevertheless, both papers leave the larger question of overall size regulation unanswered. Says Allan Spradling of the Carnegie Institution of Washington's branch in Baltimore: "When you start to hear this absurd talk about how much biology understands after [the sequencing of] the human genome, just consider size regulation. There's a lot we don't understand." –GRETCHEN VOGEL

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