DEVELOPMENTAL BIOLOGY

Knocking Genes In Instead of Out

When it comes to studying gene function, the "knockout" mouse has proved to be an invaluable tool: Simply inactivate the gene you are studying and see what happens to the resulting animals. But all too often the technique delivers an uncomfortable jolt. The gene is inactivated all right, but little or nothing happens. The most frequent explanation advanced for this unpleasant surprise is that some other gene is taking over the knocked-out gene's job.

But proving that can be difficult. Now, by working a novel variation on the standard knockout methods, a team led by developmental geneticist Alexandra Joyner of New York University Medical Center in New York City has devised a method for replacing one gene with another that can be used to test whether two genes really are functionally equivalent (see p. 679). "It's a twist on what's been done, but it's an important twist. I think it's the first time anyone has actually replaced one [mouse] gene with another," says developmental biologist Gail Martin of the University of California, San Francisco. In addition to testing functional equivalency, the Joyner team's results are providing some intriguing insights into gene evolution.

Joyner, who moved her lab from Toronto's Mount Sinai Hospital late last year, says she developed the gene replacement method because of her own group's experiences with knockouts of two structurally related developmental control genes: *Engrailed* (*En*) *1* and *2*. Both *En-1* and *En-2* carry a conserved DNA sequence known as the homeobox. And while *En-1*'s range of action is wider, both are thought to play crucial roles in brain formation. The evidence for that includes observations that both genes become active early in the same regions of the developing brain, although *En-1* is turned on 8 to 10 hours before *En-2*.

To further pin down their roles, Joyner and her colleagues knocked out each gene separately. Mice lacking *En-1* turned out to have serious abnormalities, including a deleted midbrain and cerebellum, that caused them to die shortly after birth. In contrast, *En-2* knockouts had only minor problems. That might mean that *En-1*, the gene that comes on earlier, has a biochemical activity sufficiently similar to that of *En-2* to enable it to take over *En-2*'s functions, resulting in near-normal *En-2* knockouts.

One way to determine whether the two genes are functionally equivalent is to see whether the severe abnormalities of *En-1* knockouts are corrected by giving the animals an *En-2* gene that is expressed early like *En-1*. But Joyner notes that making such transgenic mice by conventional means is "notoriously difficult. The results are very variable" because there is no way to control where transferred genes insert in the genome.

Yet the knockout procedure itself suggested a way around the problem. Because it involves inactivating a target gene by inserting foreign DNA into the gene, it offered the opportunity to insert a functional gene into a specific site. So Joyner, with then-postdoc Mark Hanks, and their colleagues spliced the *En-2* gene into the DNA used to knock out *En-1*. As a result, *En-2* was inserted into the *En-1* gene, simultaneously inactivating it, while *En-2* itself was hooked up to *En-1*'s regulatory sequences. With *En-2* now expressed in *En-1*'s exact pattern, the animals thus produced turned out to be normal. "From a biological point of view, we can think of [*En-1* and *En-2*] as equivalent," Joyner concludes.

MARK HANKS

Mimic. A marker gene *(blue)* put in the *En-1* site is active in the same tissues of the developing mouse limb as *En-1*.

Developmental biologists predict that this new genetic sleight of hand will find widespread use. "I think people really appreciate the method's potential," says Robb Krumlauf, a homeobox gene expert at the National Institute for Medical Research in London. Krumlauf suggests it will be particularly useful for testing whether one gene can substitute for another, but could have other applications, such as dissecting how different parts of a protein contribute to its function by systematically altering the gene's structure.

Besides providing a hot new lab method, the Joyner group's results also have evolutionary implications. Earlier work with the fruit fly by Xuelin Li and Markus Noll of the University of Zurich, Switzerland, had shown that genes can gain new developmental functions not just through alterations in their protein-coding sequences but also by acquiring new regulatory sequences that alter where and when they are expressed. Joyner and her colleagues have found that something similar happens in vertebrates. Noll comments that this finding, while expected, is interesting nonetheless. "I think that this [change in regulation] is a general mechanism of evolution," he says, adding that he expects to see more examples. Indeed, they could well come from studies performed with the new gene-replacement method.

–Jean Marx

PHYSICS

Shrinking an Interferometer to Atom Size

It's a textbook physics experiment: Pass light through a pair of slits in a barrier, and an array of bright and dark lines will appear on a distant screen as light waves from the two sources interfere with each other. But Michael Noel and Carlos Stroud have given this familiar "interferometer" a different look—and a drastic downsizing. In place of the barrier and screen, the two University of Rochester physicists used a single potassium atom. For the two light sources, they substituted just one of the atom's electrons, shaped by laser pulses into a pair of distinct wave packets.

The work, to appear in the 14 August *Physical Review Letters*, is testimony to the ability of precise laser pulses to manipulate an atom, says John Yeazell of Pennsylvania State University. Tom Gallagher of the University of Virginia adds that such experiments also display physicists' ability to resurrect some of the certainties of classical physics from the hazy quantum world of the atom.

After all, an atom's electron ordinarily can't be pinned down precisely enough to make an interferometer, because it spreads out through space in a haze of probability. But by bumping the outermost electron of an atom with a laser of the right frequency, physicists can boost part of the electron's quantum wave into a well-defined shell of charge that behaves like a planet following a classic elliptical orbit. Just as the planet's orbital radius varies, this Rydberg packet, says Noel, "oscillates in and out, in a breathing motion." Unlike a planet, however, the packet has a phase, corresponding to the phase of the laser pulse that created it.

By bombarding potassium atoms with a pair of laser pulses, Noel and Stroud excite a single electron into a pair of Rydberg packets that "breathe" on exactly opposite schedules. In that way, says Noel, "we're localizing the electron in two different radial positions"—the equivalent of an interferometer's two slits. All that remains is to bring the wave packets together and watch them interfere.

Fortunately, the "orbits" of the Rydberg packets spread out as they age, eventually overlapping. Where they overlap, they interfere—and either cancel or reinforce each other, depending on the relative phase of the two laser pulses that created them. A third, probe pulse reveals the results and shows that the textbook rules of wave interference hold sway even in the realm of the atom.

–Tim Appenzeller