

DECEMBER 20, 2008

Same Genes Help Snails and Humans Tell Left from Right

In 1923, the distinguished British naturalist Captain Cyril Diver, who was not a trained scientist, published an influential article in *Proceedings of the Royal Society B* describing his discovery that the handedness of a snail's shell is dictated by the genetics of the snail's mother. Howard Hughes Medical Institute researcher Nipam Patel says that he often thinks of Diver's scientific contributions as a superb example of what amateur scientists can accomplish.

Admittedly, Diver's work has been on Patel's mind a lot lately. Patel studies the evolution of body patterning and segmentation at the molecular and genetic levels. His team is now reporting in *Nature* that they have uncovered evidence that the genetic mechanisms that establish asymmetry are more ancient than scientists suspected: the same genes that control asymmetry in humans and other vertebrates are also used to establish the characteristic swirl of a snail's shell.

The studies underscore an important point. While symmetry is pervasive in the natural world—reflected in the five arms that radiate from the center of a starfish and the mirrored patterns on a butterfly's wings—specific deviations from that symmetry are crucial to development.

Postdoctoral fellow Cristina Grande and Patel, who are at the University of California, Berkeley, reported their findings December 20, 2008, in advance online publication in the journal *Nature*.

Asymmetrical features are introduced early in the embryonic development of an organism. For humans and many other vertebrates, the external body is symmetrical, but a characteristic asymmetry is often found in the organization of internal organs.

Researchers had a good idea of how asymmetry was controlled in vertebrates, including chicks, mice, and humans. Two genes, *nodal* and *Pitx*, were known to be particularly important. *Nodal* has been known to be a key regulator of left-right asymmetry in vertebrates and echinoderms [like sea urchins and sea stars], said Patel. And we knew that *Pitx* is a downstream gene that is regulated by *nodal*. During development, they regulate asymmetry by being expressed only on one side of the embryo.

But researchers had come up empty-handed in searching for *nodal* in simpler organisms that are commonly studied in the laboratory—the fruitfly *Drosophila* and the nematode *C. elegans*. These organisms clearly display asymmetries, but they control asymmetry without the use of *nodal* and *Pitx*.

Patel, whose approach to understanding the origins of the diversity of life is to meld molecular biology with his love of the natural world, decided to broaden the search. He and Grande turned to two species of snails whose bodies are organized in opposing directions. The bloodfluke planorb (*Biomphalaria glabrata*) is a freshwater snail with a left-handed turn to its shell. Patel explains that this means if you were to place your left hand inside the bloodfluke's shell, it would be able to follow the shell's natural curve. The bloodfluke is best known as the carrier of the parasite that causes schistosomiasis. The owl limpet (*Lottia gigantean*) which clings to rocks in the ocean intertidal zone has a right-handed body organization. Although the owl limpet is cup-shaped and lacks the characteristic spiral shape of most snails, its internal organs are asymmetrical.

In studying how snails establish their asymmetry, Patel and his colleagues were building on work begun in the 1920s by naturalist Cyril Diver's discoveries regarding how handedness in snails is directed by the genetic endowment of the snail's mother. After Diver's studies, other researchers showed that this effect of the mother is exerted very early in development, when a four-celled embryo divides into eight cells. This all happens before the shell begins to form, Patel explains. Despite this crucial information, no one had been able to identify the molecules that drove the handedness decision.

Patel and Grande sought to gain a molecular foothold into the question, and their studies were jump-started by the recent availability of the fully sequenced genome for the owl limpet. Grande was able to trace the activity of the *nodal* and *Pitx* genes in the two types of snails as they developed. She found that those genes were active on the left side of the left-handed bloodfluke planorb larvae, and on the right side of right-handed owl limpets.

That finding was exciting because the fact that the genes were expressed on the opposite sides in these snails with opposite chiralities made it seem increasingly likely that they were involved in setting up left-right asymmetry, said Patel. However, we also wanted to demonstrate that functionally.

So Grande used a chemical to inhibit *nodal* signaling in the developing larvae of the bloodfluke planorb. Most of the treated snails died as embryos, but some of those that survived developed straight tubular shells, with no sign of coiling. What's more, the researchers found that chemically blocking *nodal* function also blocked *Pitx* from being activated asymmetrically, confirming the relationship of the two genes.

When Grande analyzed early embryos, she discovered that the *nodal* gene became active when the embryos reached the 32-cell development stage—after asymmetry was established.

Thus, we know that *nodal* is not symmetry-breaking, although it is a very early step in this pathway, said Patel. However, identifying this gene gives us the opportunity to use it as a molecular entry point to work our way upstream in this pathway to find out what this maternal factor [that originally establishes asymmetry] is, he said. And it gives us the opportunity to go downstream to understand how signaling molecules can set up very complex morphologies like the coiling of snail shells.

More broadly, this finding gives us insight into the evolution of breaking symmetry in all animals, said Patel. It suggests that the common ancestor of creatures including snails, humans, sea urchins and flies had a mechanism in place to generate left-right asymmetry that involved *nodal* and *Pitx*; and that mechanism was conserved in vertebrates and snails, but lost in animals like *Drosophila* and *C. elegans*.