Genetic 'Master Switch' for Left-Right Symmetry Found

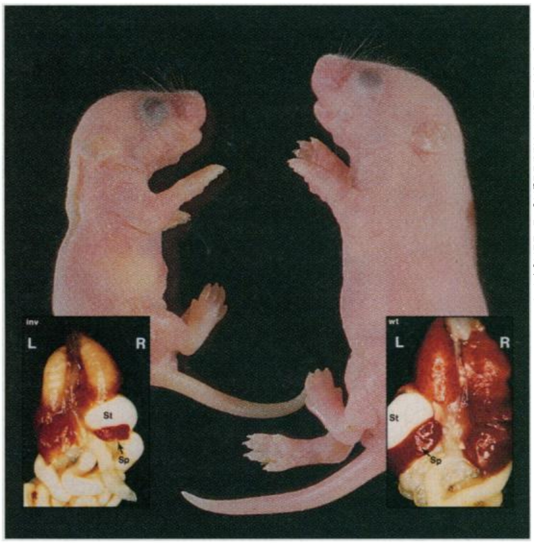
Location, location, location. It's as important to students of vertebrate development as it is to real-estate developers. In mammals, for example, one of the most important steps in the development of the embryo is to assign the heart to the left side of the chest cavity. And that isn't the only left-right arranging that gets done as the embryo matures. The major lobe of the liver gets put on the right side of the abdomen, the spleen on the left side of the abdomen, and the stomach in a precise position in between. Usually, that is, but not always. About one in 10,000 people are born with situs inversus: complete reversal of the left-right positions of these organs. Some experience no life-threatening consequences, but most suffer cardiovascular problems, some experience pulmonary problems, still others become infertile. What causes these devastating inversions?

Developmental biologists have observed the phenomenon they call asymmetrical positioning of the visceral organs from the earliest days of their profession. But centuries later, while biologists have teased out reams of data on the molecular mechanisms that cause muscle cells, say, to become differentiated from bone cells in the embryo—and while they know a lot about positioning in invertebrates—embryologists have remained in the dark about the mechanisms controlling vertebrate organ positioning. Until now.

By pure serendipity, researchers at Baylor College of Medicine in Houston have identified a gene that appears to play a major role in the mysterious process of positioning—at least in mice. They detail their work on page 679 of this issue of *Science*. That gene alone will not explain the entire process, and the finding may not apply directly to human beings, but it certainly will speed research into how we wind up with our parts in the right (or left) places.

Says biologist Lewis Wolpert of University College in London, one of the first to propose models of how the developmental axes of an embryo form: "It's a remarkable finding. We know a lot about anterior-posterior positioning in vertebrates yet we have so little understanding of left-right polarity. This is a great opportunity to work out the mechanisms responsible for left-right positioning of visceral organs. In principle, it could hold the key to understanding how such problems as cardiac defects are associated with reversal of internal organs."

The spatial positioning of the heart emerges early in development. Early on, the heart reveals signs of left-right asymmetry as the cardiac tubes, precursors of the heart's mature structures, mature. At first, the tubes are symmetrical along the midline; then they bend toward the right. The bending forms a loop, placing the left ventricle on the left side and the right ventricle to



L. LASUPP, P. OVERBEEK, AND T. YOKOYAMA

Organ swap. After making pigmented mice *(left)* from normal albinos, Baylor College of Medicine researchers were surprised to discover that internal organs *(inset)* had reversed along the left-right axis.

the right and anterior. Where the bending happens in the reverse, babies (human as well as mice) are born with reverse cardiac polarity, accompanied in many instances by heart problems.

Helping those who suffer from conditions like this requires understanding the genetics that controls the left-right positioning of the organs. And that has proved difficult, since left-right asymmetry has been one of the more intractable problems in developmental biology. While there have been many papers exploring the molecular mechanisms of axis determination in nematodes and *Drosophila*, and, as Wolpert notes, lots of work on the anterior-posterior (or the head-tail) axis in vertebrate embryos, breakthroughs in vertebrate left-right polarization have been rare.

There have been a few, however. In 1959 a mutation (called *iv* for *inversus viscerum*) was discovered by Katherine Hummel and Dorothy Chapman at the Jackson Laboratory in Maine. That mutation causes organs to switch positions—but in only half of the mice that carry two copies of the mutation. By the time this mutation was mapped in 1989, by a group at Yale University, embryo manipulation experiments in chickens and frogs had been found to result in random organ re-positioning. Like *iv*, however, those changes did not consistently reverse organ positioning, and developmental biologists began to think there was no single "master switch" gene that would always reverse normal positioning if it were mutated.

Enter the new work by Paul Overbeek and his colleagues, including postdoc Takahiko Yokoyama. Overbeek and Yokoyama didn't start out to study left-right asymmetry. On the contrary, they were trying to create a stock of pigmented mice by injecting albino mouse embryos at the one-cell stage with the gene that carries the genetic code for the first enzyme (called tyrosinase) in the pathway to melanin synthesis.

The plan was to breed carriers of one copy of the gene to get homozygous pigmented mice (which carry two identical gene copies). As it happened, the homozygous offspring lived no more than 7 days because of kidney failure, and when the researchers autopsied each animal, they made a startling finding: The internal organs in each were reversed across the left-right axis. Says Overbeek: "When we turned the newborn mice over, we could see, through the skin, that their milk-filled stomachs were on the wrong side. We weren't looking for a particular mutation that would reverse internal organs—it was a serendipitous, rather than an intentional, finding. We got lucky." As the researchers autopsied more and more of the mice, they found that their stomachs were always on the wrong side and, in 95% of cases, their hearts and spleens were also reversed.

How could that remarkable change, which reverses developmental biologists' prediction that there would be no single "master switch" for left-right symmetry, have come about? When a gene is inserted into a cell (such as the mouse embryo), the inserted gene generally positions itself at random in the target cell's genome. In this case, it seems, the inserted tyrosinase gene (in a manner that is not yet fully understood) inserted itself in a position that somehow interferes with the normal function of what Overbeek's group came to call the *inv* gene. The result: a reversal of the left/right polarities of 100% of the homozygous carriers.

As exciting as the Overbeek team's finding is, it doesn't mean that everything about left-right asymmetry will now fall into place. One reason is that the determination of where things develop in the body is clearly a multi-gene process. Overbeek and his colleagues report their newly discovered *inv* mutation maps to chromosome 4. The mutation discovered three decades ago by the Jackson Labs team of Hummel and Chapin, which alters organ position 50% of the time, on the other hand, lies on chromosome 12, indicating that it isn't in the *inv* gene. And there are probably other genes as well that are involved in the determination of the left-right patterning of the organism.

That means that the genetics of this process won't be unraveled immediately. And even when it is, it may not be immediately applicable to human beings. While the discovery of the effect the *inv* mutation has on mouse embryo organ alignment has insiders excited, all are quick to point out that it may ultimately have little direct relevance to human development. Even Overbeek says: "It's not clear from previous evidence that human embryos undergo the same embryonic turning as mice—although humans obviously have definite sidedness." Still, one of the first researchers to map the Hummel-Chapin mutation, Martina Brueckner of the Yale University Medical School's Department of Pediatric Cardiology, says: "I would bet on the same gene being found in man." That would be good news indeed.

–**Tania Ewing**

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ASTRONOMY

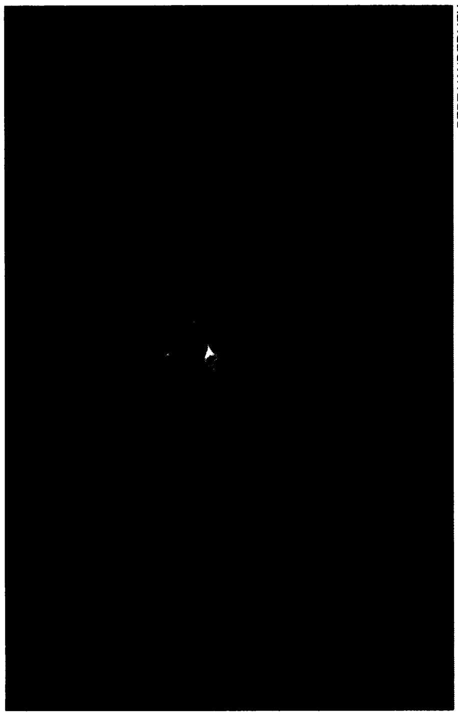
Quasars: Double Darkness in Draco

The conventional picture of a quasar is more than enough to strain credibility. A black hole as massive as millions of suns lurks at the center of a galaxy, sucking in stars and gas. As they whirl inward toward the black hole, the stars and gas are heated to incandescence, generating brilliance that makes a quasar visible from the far corners of the universe. But even that outlandish picture isn't enough to explain the antics of a quasar 3 billion light-years away in the constellation Draco. After 5 years of observing wiggles in a jet of material that squirts from the heart of the quasar 4C 73.18, a group of radio astronomers in the Netherlands and the United States has concluded that the quasar may harbor not one but two giant black holes, locked in a tight orbit around each other.

The new evidence, described by Nico Roos of Leiden Observatory, Jelle Kaastra of the Leiden Laboratory for Space Research, and Christian Hummel of the U.S. Naval Observatory in a forthcoming paper in *Astrophysical Journal*, helps to bear out a hypothesis advanced more than a decade ago by astronomers Mitchell Begelman of the University of Colorado, Roger Blandford of the California Institute of Technology, and Martin Rees of Cambridge University. They proposed that at least some of the hundreds of known quasars are home to "binary" black holes, created when their host galaxies merged with other galaxies. Even before the latest observations, astronomers had begun to see hints of black hole pairs in other quasars. But Rees thinks the new observations make "a better case than most" for a binary black hole.

Begelman and his colleagues based their 1980 proposal on an idea, put forward by other theorists, that many quasars and "active" galaxies may be ignited when galaxies collide. The scenario holds that such mergers hurl material into the heart of the merged galaxy, where it rains down into the quasar and stokes its brilliance. Begelman, Blandford, and Rees took the next step: They realized that if the second galaxy also contains a quasar, the collision will lob a second black hole into the heart of the merged galaxy, creating a binary.

According to Sterl Phinney of Caltech, some of the first evidence supporting this picture came from objects like the active galaxy 3C 75, which has two visible nuclei, each presumably harboring a black hole. Then, in 1988, a group of Scandinavian and U.S. astronomers reported new evidence for a black hole binary in the quasar-like object OJ 287 based on the pattern of its outbursts, which implied that the tidal pull of a massive companion is disrupting the inflow of material. And last year, Kaastra and Roos saw clues in the jets of radio-emitting plasma that spurt from the quasar 3C 273 and the giant elliptical galaxy M87. Both jets seemed to precess, or wander, slowly around the axis of the galactic nucleus, as if something—perhaps another black hole—were slowly tugging each quasar into a new orientation.


BERT VANDERMEY

Two to tango. In an artist's conception of the heart of the quasar 4C 73.18, two black holes do a slow dance.

The jet of 4C 73.18 has now yielded more vivid evidence for the binary black hole scenario, as the British magazine *New Scientist* first reported earlier this month. Monitoring it through radio telescopes, a team of observers led by Hummel detected wiggles, much like the wiggles that appear in water spouting from a garden hose when you shake the end. After 5 years of observations, Hummel, Roos, and Kaastra say the signs are unmistakable: Something as massive as the black hole itself must be tugging it back and forth.

The culprit, Roos and his colleagues conclude, is another giant black hole, circling the one emitting the jet every 3 years. To explain the 3-year period, the pair would have to be separated by only about 30 times the average distance between the sun and Pluto. From the tightness of the black holes' orbit and the quasar's brilliance, the researchers estimate that each black hole must pack about 100 million times the sun's mass.

The wiggly jet of 4C 73.18 may add weight to the merger scenario for the birth of some quasars. But it's also a sign of mortality, at least for the twin black holes themselves. Theorists including Caltech's Phinney have suggested that the paired black holes would gradually spiral closer together as they lose energy through encounters with gas and stars. If their slow dance gets close enough, they may even roil the fabric of space and time and give rise to the gravity waves predicted by Einstein's theory of general relativity. The gravity waves would carry off still more energy from the black holes, and before long they would actually merge.

The two black holes at the heart of 4C 73.18, Roos thinks, are already close enough to be generating gravity waves. In about a million years, he calculates, the black holes will collide in a titanic burst of gravitational radiation—a powerful, though invisible, culmination to their brilliant double career as a quasar powerhouse.

–**Ray Jayawardhana**

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