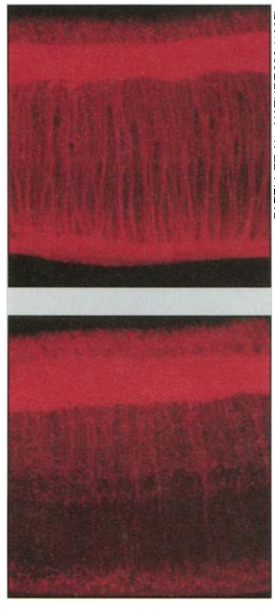
HUMAN GENETICS

Putative Cancer Gene Shows Up in Development Instead

Seven years ago, a team at Johns Hopkins University used a new chromosome-walking technique to locate a long-sought suspect in colon cancer. They announced in *Science* the identification of a gene called *Deleted in Colorectal Cancer*, or *DCC* (*Science*, 5 January 1990, p. 49). As the name implies, this gene is part of a long stretch of DNA on human chromosome 18 that was already known to be missing in tumor cells from many patients with colon cancer. Finding an actual gene in this stretch helped shore up the then-emerging theory that cancer results in part from inactivation of tumor-suppressor genes. And since then, nearly 150 papers have probed *DCC*'s function and clinical significance. Today, its absence in tumor cells is used as an important diagnostic marker, helping to identify patients who need aggressive treatment, explains Ian Summerhayes, a cancer biologist at Beth Israel Deaconess Medical Center in Boston.

But what exactly does normal *DCC* do in cells? Eric Fearon and Bert Vogelstein, the leaders of the Johns Hopkins team that identified *DCC*, hypothesized that it suppresses tumors by regulating cell growth or differentiation. In this week's issue of *Nature*, however, three independent groups report that *DCC* and newly discovered relatives in rats and mice have an entirely different function: They encode cell surface receptor proteins that interpret directional signals used by migrating cells or developing neurons. One group also reports that mice lacking *DCC* have brain and spinal cord defects, but no excess of gastrointestinal tumors. This raises doubts about whether *DCC* itself helps regulate normal cell growth or simply lies close to an unknown tumor-suppressor gene and is usually deleted along with it. "It could be useful as a marker and have nothing to do with [cancer] progression," says Summerhayes. As one leading researcher jokes, *DCC* should perhaps be renamed "Deleted as a Cause of Cancer."

Still, closing arguments in the case are yet to come. Vogelstein calls the papers "terrific studies, very thorough," and says they "very convincingly show that *DCC* [and its cousins] are important components of nervous-system development." But the absence of tumors in mice with *DCC* knocked out, he says, "is a negative result, and unfortunately those are much more difficult to interpret than positive results." And Fearon, now at the University of Michigan, suggests *DCC* could have other roles in addition to directing axonal growth. He remains convinced that it is the leading candidate for the role of tumor suppressor in cells lining the colon and rectum. It may be years before researchers map enough of the genetic sequence around *DCC* to flush out other candidate tumor suppressors, so many researchers say a faster way to incriminate or exonerate the gene may be to learn its exact function in development and whether disrupting that function can accelerate tumor growth.



FAZELI *ET AL.*, *NATURE* 386 (1997)

Lost in the spinal cord. In a normal embryo, axons grow from one side of the spinal cord to the other *(top)*; in those lacking *DCC*, the axons never make it to the other side *(above)*.

Developmental biologists have been citing their work's links to cancer in grant proposals for years, but *DCC* offers a striking case. Indeed, only one of the teams behind the trio of *Nature* papers originally set out to learn something about cancer. In 1990, Amin Fazeli, a Yale University medical student working in the laboratory of cancer biologist Robert Weinberg at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, decided to delve further into Fearon and colleagues' discovery that 18q21, a region on the long arm of chromosome 18, is missing in more than 70% of human colon and rectal tumors. Hundreds of genes could fit inside the large 18q21 region, but *DCC* was the first one detected, and other studies rapidly began to link it to cancer. Fazeli put this to the test by genetically engineering several strains of transgenic mice, including one with a single working copy of the mouse version of *DCC* (labeled $DCC^{+/-}$) and another with no working copies of the gene ($DCC^{-/-}$).

All the $DCC^{-/-}$ mice perished shortly after birth, although the problem was not in their gastrointestinal tracts, which were normal. Fazeli expected the $DCC^{+/-}$ mice to show an increase in gastrointestinal tumors as a result of spontaneous mutations in the lone working copy of *DCC*. But he was puzzled to find that of nearly 200 $DCC^{+/-}$ mice, only one developed such a tumor. "We could not come up with the smoking gun," he says. "We were very disappointed, and surprised."

With Fazeli's studies stalled, the next batch of clues to *DCC*'s character came from an unexpected source: the tiny worm known as *Caenorhabditis elegans*. In developing worms, as in most animal embryos, neurons and their threadlike axons—the cellular telephone lines that connect them to other neurons—travel quite a distance before settling into their assigned positions; their route is guided by nearby cells, which either attract or repel them. One vertebrate protein that cells secrete to attract axons is netrin-1, first discovered by developmental geneticist Marc Tessier-Lavigne and colleagues at the University of California, San Francisco. Mice lacking netrin-1, they found, exhibit nervous-system defects. Joseph Culotti at the University of Toronto, Ed Hedgecock at Johns Hopkins, and colleagues, meanwhile, discovered a *C. elegans* homolog for netrin-1, called UNC-6, that is secreted by neurons along the main trunk of the worm's nervous system; they also found that UNC-6 seems to pass along the attractant signal by binding to a receptor protein called UNC-40 on the axons' leading tips.

Then, late last year, Culotti and Hedgecock announced a surprising discovery: The worm gene encoding the netrin receptor UNC-40 shares 30% of its amino acid sequence with vertebrate DCC (*DCC*'s protein product), indicating that the two genes are distant evolutionary cousins and raising the possibility that DCC is also a netrin receptor, Culotti says.

That's where the three new *Nature* studies come in. When Fazeli, now completing his Ph.D. in biology at the Massachusetts Institute of Technology, heard of the Toronto team's result, he reexamined his ill-fated $DCC^{-/-}$ mice, this time focusing on their nervous systems. "We were very pleasantly surprised," he says. The mice had brain and spinal cord defects "strikingly similar" to those seen in the netrin-1 knockouts. Fazeli and the other Whitehead researchers teamed up with Tessier-Lavigne's group and, in the first *Nature* paper, report careful anatomical studies of $DCC^{-/-}$ embryos showing that without DCC, axons get lost. In normal cells, netrin-1 is emitted on the inner or ventral side of the developing spinal cord and attracts axons from the outer side. Without DCC, those axons never make their ventral journey (see photo). "That provided a very strong piece of evidence supporting the hypothesis that DCC is a receptor for netrin-1

in vertebrates," says Fazeli.

And that's not all. Culotti's group had previously found another netrin receptor in worms, called UNC-5, that apparently switches an axon's response, turning UNC-6 into a repellent rather than an attractant. Now, proteins homologous to this netrin receptor are turning up in vertebrates. The two other *Nature* papers report such homologs in rats and mice, and both are active in the brain and spinal-cord regions.

All this suggests that netrins and their receptors make up a genetic module so powerful that it is conserved across worms, rats, mice, and even humans—and that DCC itself is a netrin receptor, expressed at the tips of growing axons and given the job of guiding them to the right position, says Culotti. The accumulating evidence makes it more and more difficult to see how *DCC*'s absence in colorectal tumor cells could be the crucial factor permitting cancerous growth, says Ray White, a human geneticist at the University of Utah.

Another gene in the 18q21 region could be the real culprit, with DCC as an innocent neighbor, says White. At least two other candidate tumor suppressors, called *Smad2* and *Smad4*, have already been traced to the area; *Smad4* loss is suspected as a leading cause of pancreatic cancer (*Science*, 19 January 1996, pp. 294 and 350). Both genes seem to modulate signals carried by a protein called TGF-β and its relatives, which among other functions are thought to direct the development of colon cells. All three genes, however, could merely be carried along for the ride when the entire 18q21 region is deleted. So, although all the candidate genes appear to have links to the colon, "somebody could find a [tumor-suppressor] gene that's an even better target than these," explains Vogelstein.

But Fearon notes that *DCC* might be more than just a receptor for netrins, perhaps interacting with other signaling molecules in some other pathway regulating cell movement or cell fate. *DCC* appears to be expressed at low levels in many cells that might need directional cues, Fearon says, including the colonocytes that slowly migrate up the lining of the colon, then slough off. "I think most of the data still weigh in for *DCC* as the most likely candidate," he says.

And the new data on *DCC* don't diminish its diagnostic usefulness as a marker for aggressive colorectal tumors, adds Beth Israel Deaconess's Summerhayes, because it seems clear that DCC is at least close to the actual tumor suppressor. Whichever gene proves to be the crucial target of deletion, *DCC*'s story illustrates that developmental biology has a role to play in cancer research, says Johns Hopkins cancer researcher Scott Kern: "There's no better place to apply [the growing knowledge of development] than in human cancer biology."

–Wade Roush

MEETING BRIEFS

Ideas on Human Origins Evolve At Anthropology Gathering

ST. LOUIS More than 1000 anthropologists gathered here from 1 to 5 April for the 66th annual meeting of the American Association of Physical Anthropologists and associated groups. Researchers presented new genetic and fossil findings marking key milestones in long-running debates on such topics as the ancestors of apes (*Science*, 18 April, p. 355), the origins of modern humans, and the evolution of menopause.

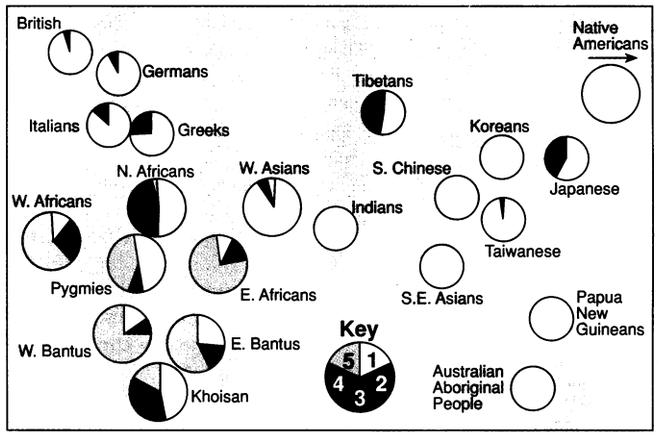
Back to Africa

Since the mid-1980s, two diametrically opposed hypotheses for the origin of modern humans have been battling for primacy. One, called Regional Continuity, holds that our earliest ancestors arose in Africa and spread around the world more than 1 million years ago. Modern humans then arose in many different regions through separate evolution and interbreeding. The other—the favored contender—is the theory known as Out of Africa, which suggests that our ancestors arose in Africa and swept around the globe 100,000 years ago, completely replacing existing human populations on other continents. This model hasn't been proven, but a series of genetic and fossil studies have suggested to many researchers that, as Stanford University geneticist Neil Risch put it last year, "the rest of the world emerged from the northeast corner of Africa" (*Science*, 8 March 1996, pp. 1364 and 1380).

Now, a middle ground may be emerging, as new data from two international teams of geneticists challenge the most extreme version of Out of Africa. That theory predicts that all genes in modern humans were inherited from a small number of Africans, but the new data suggest that some modern human genes come from ancestors in Asia, not Africa. The new evidence falls far short, however, of proving Regional Continuity; rather, it shows that both of these leading models of how modern humans emerged have been overly simplistic. "There's more than one migration out of Africa," says Michael Hammer, a geneticist at the University of Arizona, Tucson. "And the direction is not just one way. Some are moving back to Africa."

Hammer came to that conclusion after studying DNA in 1500 males from 60 populations around the world. One region he focused on was a 2600-base-pair segment of the Y chromosome: the YAP region, which is passed from fathers to sons. This segment varies among individuals, but the sequences cluster in five major groups, known as haplotypes (shown in five colors; see map). The haplotypes occur in different frequencies in different populations, and Hammer's team found that one—YAP haplotype 3 (shown in red)—shows up far more often in Asians than Africans. Its sequence shows more diversity in Asians, implying that the haplotype had more time to acquire mutations in Asia than in Africa—and, therefore, that it arose in Asia. Its presence in some Africans hints that human ancestors migrated back from Asia into Africa at some point, says Hammer.

What's more, Hammer's team thinks that



SOURCE: M. F. HAMMER *ET AL.*, *GENETICS* 145, 787 (1997)

Male routes. A marker on the Y chromosome comes in five types (pie charts). One version (red) is found chiefly in Asians but also in a few Africans, suggesting that it arose in Asia and was later carried back to Africa by migrating (male) human ancestors. This marker then gave rise to others in Africa (blue, yellow).