Down to the Wire for the NF Gene

The race for the NF gene has ended in a dead heat. And the gene the two groups found is a fascinating one, unlike any others turned up so far

LAST WEEK, newspapers around the world heralded the discovery, by two independent groups, of the gene that causes neurofibromatosis, or NF, a debilitating and disfiguring neurological disease that is one of the most prevalent of genetic disorders. What the papers didn't report, however, is that the simultaneous publication by these two groups, one led by Francis Collins of the University of Michigan, the other by Raymond White of the University of Utah, was less of a coincidence than it seemed.

The two investigators started out as collaborators but then parted ways about a year ago and ended up in a mad scramble to publish first. Collins submitted his group's paper to *Science* on 12 June. White submitted his to *Cell* 14 days later but almost beat Collins into print because *Cell* published the paper in a near record 17 days. But it ended in a dead-heat when *Science* moved up the schedule for Collins' paper; both were published on 13 July.

All this put the Howard Hughes Medical Institute, which funded both groups, in the rather awkward position of having one of its investigators trying to scoop the other. Still, it ended amicably enough, with a joint press conference where no word of the rivalry was mentioned.

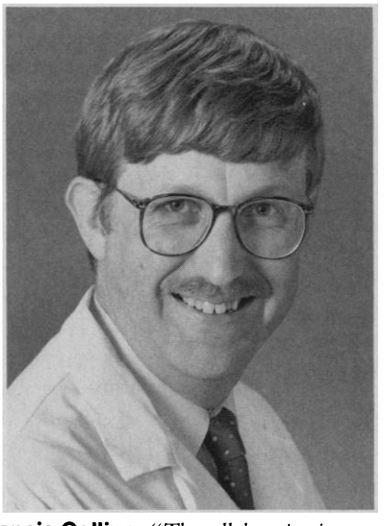
The search for the gene—one of a small set of "big," or highly visible, disease genes—was grueling and took some bizarre scientific twists. The prize turned out to be worth the effort, for the NF gene is an intriguing entity, unlike any human gene identified to date. It is a monster of a gene, anywhere from half a million to 2 million bases long. What's more, at least three other genes are embedded in this mega-gene. The only other published example of a nested gene in humans is one embedded within the Factor VIII hemophilia gene on the X chromosome.

"Three genes in an intron, that's really a fascinating story," says statistical geneticist Mark Skolnick of the University of Utah. "It is very exciting in itself."

What all this means for patients suffering from NF is difficult to say. The disease affects about 1 in 4000 newborns of all ethnic groups, and nearly 100,000 people in the United States now suffer its symptoms: café au lait spots, numerous benign tumors known as neurofibromas, and, less frequently, malignant tumors, learning disabilities, and other neurological symptoms. There is no treatment for the disease other than surgery to remove the tumors, which frequently return.

In the near term, having the gene should help with diagnosis, which can be tricky in children under 5. An improved prenatal test should also be possible. But neither test is likely to be as widely applicable as desired, since almost every family that has the disease carries a slightly different version of the gene. At best, speculates Collins, researchers may be able to come up with a test within the next several months that can detect, say, half of the mutations. Similarly, any therapeutic applications to correct the defect will be years away.

But the newly discovered gene is already providing the first tantalizing clues into the biological basis of the disease, which until now has been a black box. It looks as if the disease occurs when this gene, which may be as large as the gene for Duchenne muscular dystrophy, is somehow knocked out or inactivated. Thus, it may belong to the recently discovered class of tumor suppressor genes. But figuring out the function of the normal gene, and what happens when it is mutated, is likely to take years.



Francis Collins: *"The collaboration just never clicked."*

The scramble to pinpoint the gene began in earnest in 1987 when Skolnick at Utah, in collaboration with White, and James Gusella at Massachusetts General Hospital found the gene was located somewhere on chromosome 17. Almost as soon as the gene was mapped to chromosome 17, numerous investigators joined forces in a consortium sponsored by the National Neurofibromatosis Foundation, exchanging cell lines and DNA markers to fix its position.

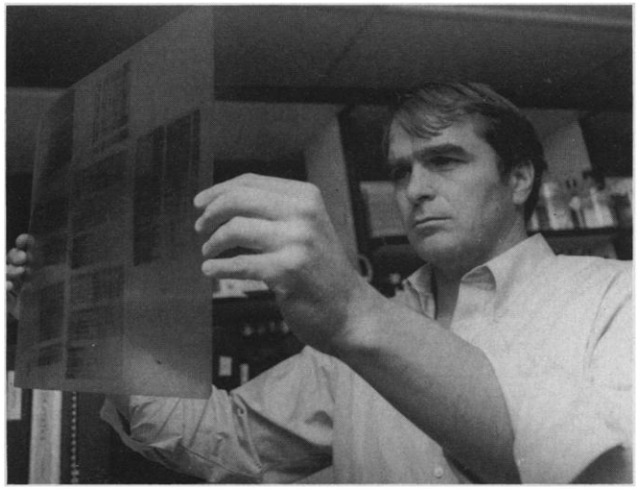
By early 1988 they had narrowed the search to a 3-million-base stretch, which they still might be slogging through today were it not for two NF patients who turned out to have distinctive mutations, known as chromosome translocations, right within that region. In one patient, chromosome 17 had broken and exchanged a piece with chromosome 1; in the other, the exchange was between chromosome 17 and chromosome 22. The simplest explanation was that these two translocations were disrupting the NF gene and thus causing the disease.

So all White and Collins, who were then collaborating, had to do was scour the area around the breakpoints, which turned out to be only about 60 kilobases apart, and look for the telltale sign of an expressed gene. The trouble is, they found several genes. The first came compliments of Art Buchberg, a National Cancer Institute researcher in Neal Copeland's lab who was studying mouse leukemia. Buchberg had found a gene, known as EVI2, on the part of mouse chromosome 11 that corresponds to human chromosome 17. He sent it to White's lab for mapping in the hope that it might turn out to be a leukemia gene. It wasn't, which was bad news for Buchberg. But it was good news for the NF investigators because the human counterpart of the mouse gene turned out to lie right between the two translocation breakpoints.

And that meant that, in a simple world at least, EVI2 was the NF gene. If so, the researchers would expect this gene to contain both of the two breakpoints and to extend beyond them, otherwise those mutations would not disrupt the function of the gene. And if it were indeed the NF gene, they would also expect to find subtle changes, known as point mutations, in the

DNA of patients who have the disease.

Both groups began doing the pivotal experiments to find out, and that is when things between them got "complicated and awkward," says Collins. Collins chalks most of their problems up to differences in style. "The collaboration just never clicked," he says. In his view, a collaboration meant pooling the effort and acting like one group. But to White, it meant sharing information and materials but proceeding more or less independently. "We worked mainly at arm's length, and it was not comfortable to me," says Collins. What's more, he adds, "EVI2 looked like the gene, and Ray felt strongly it was his story."



Ray White: *"Collaborations work best when two groups have something different and complementary to contribute."*

White agrees there were problems. "Collaborations work best when two groups have something different and complementary to contribute. And in the beginning, that was the case. Francis had that wonderful jumping technique [a method that enables investigators to span large regions of a chromosome in a single experiment] and I had mapping techniques. But when that stage was over and we were both looking hard for the gene, using the same techniques, it started to get a bit close." At Collins' request, they dissolved the collaboration. "I honestly thought it would be to our detriment," says Collins. "Odds were they had the gene."

But EVI2 turned out not to be the gene after all. Both groups found that it was wholly contained within the two chromosome breakpoints. And, look as they might, they could not find any mutations in that gene in NF patients.

Both groups resumed their separate hunts and soon bumped into another gene, just a few kilobases away. Hopes were dashed again when this new candidate gene, too, flunked both tests. White's group found yet another gene, and this one was expressed in brain tissue, making it the strongest candidate yet. But it, too, failed to pan out. "We were beginning to despair," recalls White.

By that time, Collins had begun to suspect that they were dealing with an embedded gene or genes. He likens it to looking for a particular fish and evaluating one after another, and then finally realizing that the rock you are perched on is a whale. But, while relatively common in lower organisms, embedded genes were hardly an accepted idea in humans. About that time Jane Gitschier of the University of California, San Francisco, reported her work on the Factor VIII hemophilia gene, which "made the idea a little less heretical," says Collins.

"Then the question was, if it was a large gene we were fumbling around in, how could we get outside of it and see what was going on?" Two postdocs in his lab tried two different strategies, both of which handed them overlapping pieces of the same big gene. But was it the gene? The Michigan group began a battery of tests to find out.

Unlike the earlier candidates, this gene did contain both translocation breakpoints. They found that it was switched on, or expressed, in brain tissue, which they would expect, but also in every other tissue they tested, which was something of a conundrum.

But what clinched the case for Collins' group was the mutation they found. You would expect the gene to be mutated in patients with NF—but the problem is, a gene that huge is likely to be mutated in lots of subtle ways that may have nothing at all to do with the disease. So how would you distinguish the disease-causing mutation from these otherwise harmless DNA changes? By looking at a patient with a new, as opposed to inherited, mutation, reasoned Collins.

The NF gene has an unusually high mutation rate; indeed, half the cases arise not from an inherited change but from a muta-

The First Piece in the NF Puzzle

With the neurofibromatosis (NF) gene in hand, researchers can finally get at the biology of this puzzling disease. Already, Francis Collins and Raymond White, who led the groups that identified the gene, are convinced that NF arises when the gene is inactivated by a mutation. To Collins that means that the normal gene probably plays a role in restraining cell growth and thus may be involved in other cancers as well.

Now they and nearly everyone else interested in NF want to study the gene for clues as to why the disease has such a high mutation rate—other than the fact that the huge gene is a very big target. And they want to know what accounts for the enormous variability in the disease's symptoms, which can range from mild to life-threatening.

Some patients have just a few tumors, while others are covered with them. Most, but not all, have learning disabilities. Two-thirds of NF patients can lead relatively normal lives, apart from the discrimination they all too often encounter because of their appearance. The other third have major problems, like malignant tumors, seizures, and sometimes, severe disfigurement. Indeed, for years, NF was falsely identified as the Elephant Man's disease—the name commonly given to the affliction suffered by Joseph Merrick, a 19th-century Englishman. Though Merrick's deformities resembled a severe case of NF, it is now believed that he suffered from another disease, Proteus syndrome.

If the gene really is a tumor suppressor gene, as Collins suspects, that may explain part of the variable manifestations of the disease. Perhaps two hits, or mutations, are needed to bring on the disease, as is the case with retinoblastoma, a rare eye cancer, Collins speculates. If so, a person may inherit a mutated, or inactivated, version of the gene from one parent, and then the normal copy of the gene on the other chromosome is hit with a second mutation during development. Exactly where that second mutation occurs, and at what stage in development, may determine the course of the disease. And what of those strange embedded genes—are they red herrings, or do they play a role in the disease? White suspects they might; indeed, he postulates that changes in these embedded genes may hold clues to the variability of NF.

Identifying the gene raises so many questions "that there is lots of room for all of us interested in the science," says White. More room, perhaps, than there was in the race to identify the gene. ■ L.R.

tion that occurs for the first time in that person. When this happens, the patient's NF gene must differ from those of his parents, who do not have the disease. By scanning the DNA in one such a patient, Collins found an extra half a kilobase of DNA stuck in the middle of the candidate gene. The patient's parents did not have that insert. "That's like catching it in the act," says Collins. Collins started writing.

White, meanwhile, had landed on the same gene and was furiously trying to characterize it. He knew Collins was "hot on the trail," he says. Indeed, following a mid-May meeting, sponsored by the National Neurofibromatosis Foundation, White surmised that they were closing in on the same gene. After that meeting, his lab redoubled its efforts. "These guys did not take a lot of vacations," says White.

They, too, found the gene was interrupted by at least one of the breakpoints. And while they found nothing as telling as Collins' new mutation, they did find strong circumstantial evidence implicating the new gene: three different patients all lacked pieces of this gene. Presumably, those deletions would knock out the gene and bring on the disease.

White still needed a mutation that would tie the gene to the disease, so he scoured the DNA of about 70 NF patients and 65 controls, looking for a single base pair change. He found six potential mutations in NF patients and none in the controls. What's more, two of the mutations would have a major effect on the gene's protein product. In fact, one mutation created a stop codon, which would stop gene transcription, creating a truncated protein.

That did it, says White. "We named it the NF gene and decided to write it up." And they "wrote as fast as they could," says White, who admits that by that time he had heard that Collins had already submitted a paper to *Science* describing the gene. "The grapevine is very efficient." White called *Cell*'s editor, Ben Lewin, who, he says, "appreciated the highly competitive nature of this research" and agreed to rush White's two papers into print.

By that same grapevine, Collins learned that White's paper, submitted 14 days after his own, was going to appear in *Cell* a week before his would be out in *Science*. He would have been scooped had not *Science* editor Dan Koshland agreed to remake the pages at the last moment and slip Collins' paper in a week earlier than planned, so at least they would appear simultaneously.

White and Collins did not see each others' papers until 3 days before last week's press conference. Only then were they sure that they had the same gene.

What makes these gene hunts so competitive, says Lap-Chee Tsui of the Hospital for Sick Children in Toronto, who has his own battle scars from his successful race for the cystic fibrosis gene, is simple: "It's public recognition. People don't come to you if you are second." Certainly, when two groups devote years of sweat and blood to tracking down a gene, their colleagues recognize their individual contributions. But the headlines go to whoever publishes first.

In this case, Collins and White both shared the glory in a front-page story in the *New York Times*. Indeed, their public personas were linked even more closely than the article intended: the paper ran a picture of White but identified him as Collins.

■ **LESLIE ROBERTS**

Shuttle Leaks: Good News for Science

Although the National Aeronautics and Space Administration continued to take a beating last week in Congress over a flaw in the Hubble Space Telescope, the space agency's engineers tempered the criticism with one piece of good news. If the latest test results hold up, two mysterious hydrogen fuel leaks that have grounded the shuttle fleet since 29 June (see *Science*, 13 June, p. 116) are probably not related, making it unlikely that the shuttle design itself is at fault.

One of the biggest beneficiaries should be the Ulysses solar spacecraft, which NASA officials say will fly on schedule through a narrow launch window on or around 5 October. Similarly, astronomers who have been waiting years to fly a package of telescopes known as Astro-1 (see *Science*, 22 June, p. 1486) may soon get their days in space. Astro-1 was scheduled for launch on 29 May, but was pulled off the launch pad when its shuttle sprang a leak. Now, if the leak repairs are straightforward, *Columbia* could fly again as early as 1 September. Failing that, NASA will launch Astro-1 immediately following the Ulysses mission.

In tests carried out last week on the launch pad and at Rocketdyne laboratories in Downey, California, NASA engineers exonerated their "prime suspect"—the main seal in the disconnect assembly that joins the shuttle orbiter to its external fuel tanks (ETs, in NASAspeak). Instead, engineers believe they've located two unrelated leaks in the shuttles *Atlantis* and *Columbia*. "It is not a generic problem, which would have been much more difficult to deal with," says shuttle program director Robert Crippen.

On *Atlantis*, the leak appears to be confined to the ET side of the disconnect assembly. After stripping away the foam insulation from the disconnect, engineers attached "baggies" at each likely leak location to capture escaping hydrogen. When they pressurized the fuel lines on 13 July, they found a substantial hydrogen leak in a flange seal on the ET side of the disconnect. William Lenoir, NASA associate administrator for space flight, told reporters that the leak could not have been detected by tests conducted when the disconnect was accepted because the seal isn't engaged until the orbiter is actually mated to an external tank—an action performed just before launch. While NASA may still decide to roll *Atlantis* back into its hangar for repair, it's possible that engineers could fix the leak on the pad by retightening or replacing the flange bolts.

Columbia, on the other hand, apparently leaks from both sides, although the leak from the ET side is ten times larger than that on the orbiter side. In the Downey laboratory, engineers discovered that the shuttle's disconnect assembly leaked at two seals where drive shafts pass through the 17-inch main fuel line. In a crude sense, these leaks have already been "repaired"; last week, engineers replaced *Columbia*'s disconnect assembly with one snitched from *Endeavor*, the replacement shuttle for *Challenger*. The *Endeavor* parts recently completed acceptance testing under liquid hydrogen conditions—which, according to Lenoir, should ensure that the parts will perform well. The *Columbia* parts are slated for failure analysis once NASA engineers complete their laboratory tests, which may be as early as this week.

Even if both problems are easily fixable, it's still likely that only one of the two shuttles will fly before Ulysses. "Our preference is to launch *Atlantis* first" with its classified military payload, Lenoir says. The reason? *Atlantis*'s next scheduled payload is the Gamma Ray Observatory, which will be considerably delayed if *Atlantis* is forced to wait until after Ulysses is launched. "Like Hubble, it's an expensive piece of hardware to have sitting on the ground," Lenoir says.

NASA officials are most clearly relieved not to have a major design problem on their hands. Although the test analysis is still tentative, Lenoir said the problem could be the result of an aging shuttle fleet. "You're always suspicious of coincidence, but we've had that before," says Lenoir. "Back on STS-5 [a 1982 *Columbia* mission on which Lenoir flew], what was the chance that we'd have two space suits fail in different ways? Well, it happened."

■ **DAVID P. HAMILTON**