Race for Cystic Fibrosis Gene Nears End

As the race heats up, so, too, do the rivalries among the handful of labs in pursuit of the gene

A handful of investigators are closing in on the cystic fibrosis gene, bringing to an end one of the most intense races in human genetics. By 1985 the gene had been localized to a very small region of chromosome 7—an achievement that underlines the power of the "new genetics" now being brought to bear on a number of diseases. And since then the search has been narrowed to within 100,000 nucleotides, or perhaps less, of the gene itself. Now it is just a matter of time until the gene is isolated, opening up new possibilities for diagnosis, treatment, and perhaps cure.

All of these races in human genetics—such as those under way on Duchenne muscular dystrophy, Huntington's, and other diseases—typically have elements of both cooperation and competition. But in the race for the cystic fibrosis gene, the competition has, at times, been extreme.

There have been some notable fights, such as when Collaborative Research Inc., a biotechnology company involved in the hunt, found the first evidence of where the gene was located but, in the process, alienated its academic collaborators and competitors, who accused the company of withholding data.

Another flap followed close behind when Ray White of the University of Utah and Robert Williamson of St. Mary's Hospital in London, allegedly acting on abundant rumors of what Collaborative had found, overtook the company and nearly beat them into print with the same discovery.

Yet even during the worst episodes, there has been collaboration among trusted colleagues, if no one else. And sometimes, it is in everyone's best interest to cooperate. Such was the case in December 1986, after White and Williamson had found two markers—*met* and J3.11—to be tightly linked to the gene. To get from there to the gene itself, it would be enormously useful to know the positions of the two markers on the chromosome relative to the gene. But finding out would be a mammoth undertaking, involving extensive linkage analysis in numerous families.

At a hastily convened workshop in December, sponsored by the Cystic Fibrosis Foundation and held at Lap-Chee Tsui and Manuel Buchwald's lab at the Hospital for Sick Children in Toronto, almost every major group working on cystic fibrosis—seven in all—agreed to pool its data for a major collaborative study. They ended up with 211 families, in which they tested all the chromosome 7 probes.

This is the second of a two-part article on the high-stakes race for the cystic fibrosis gene. The first article appeared 8 April, page 141.

The results, published in the *American Journal of Human Genetics* in December 1986 and chiefly written by Jean-Marc Lalouel in White's group, were better than anyone could have hoped. It looked as if *met* and J3.11 flanked the gene, thus creating a defined area, albeit a large one, in which to concentrate the hunt. "It was amazingly fortuitous," says White. "It was a stroke of good fortune."

It was lucky, too, for diagnosis. Singly, each probe could be used in prenatal diagnosis with about 96% accuracy, in certain "informative" families, to detect whether a fetus is carrying the defective gene. But with flanking probes, the accuracy of screening jumped up considerably—to 99.9%. Such testing began right away in clinical centers around the world. At about that time Collaborative began working on its diagnostic test, which was made available in fall 1986, when the company had a panel of 12 probes, including *met* and J3.11, flanking and very close to the gene.

"They were heady days," remembers Williamson, "everything was moving so fast." One of the best things about the joint study, he says, is that it showed that "most groups can work together."

Most, but not all. Collaborative scientists had attended the Toronto meeting, but "no one wanted to talk to them," remembers Tsui, who, with Buchwald, had been working with the company. And Collaborative was conspicuously absent from the follow-up meeting held the next month in London. The reason the company was not invited, Williamson says, is that it was not willing to share probes and thus could not contribute to the joint study. Others say at least part of the reason was that tensions were escalating between Collaborative's Helen Donis-Keller and Williamson, who was perhaps the company's harshest critic.

"Helen [Donis-Keller] is a good and fine member of the scientific community," says Williamson, "but I don't particularly like her company's attitude. There is nothing personal in it." An ardent socialist, Williamson believes that, in general, no one should profit from publicly funded work on cystic fibrosis and that academic research should not be subject to commercial constraints.

The joint study essentially abolished any front-runners: now that the groups had exchanged probes and information, they all had a roughly equal shot at finding the gene. And they were within about 1 million bases of it—close in genetic terms, but still a formidable distance. The advantage would clearly belong to whoever found the best way to narrow the search. While the other

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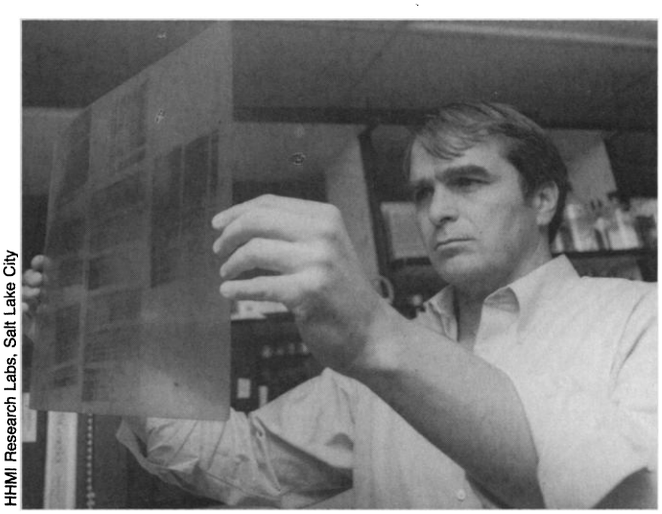

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groups planned their strategies, Williamson gambled on an untried approach that could backfire—but would give him a significant lead if it worked.

The problem was that, at 1 million bases, they were still too far to "walk" toward the gene by isolating overlapping DNA sequences, a technique geneticists use to cover up to about 200 kilobases along a long stretch of DNA. Nor did they know in which direction to go. Although the two probes flanked the gene, the orientation was still unknown; thus, starting from either landmark, it would still not be clear which way to walk.

Met, the oncogene White found to be tightly linked with the cystic fibrosis gene, provided a shortcut, but it would be risky. The idea is to take a special human cell line, with an activated *met* oncogene, and then break the chromosome into fragments and transfer them into mouse cells. Those cells that are transformed—on their way to becoming cancerous—would contain *met*.

And if those fragments also contained the other probe that flanks the gene, J3.11, they would almost certainly contain the cystic fibrosis gene as well. What's more, because of the special characteristics of *met*, related to the way it is activated, the experiment would also tell them on which side of *met* the gene lay, and thus which way to walk.

But as Williamson and several others were gearing up to do this experiment, George Vande Woude, who had isolated *met* and was working with it, was finding that the entire approach might be flawed. It turns out that *met* is switched on in a different way than they had expected, a way that essentially "scrambles" the cell line around the *met* locus and renders it useless for determining orientation. In fact, Vande Woude suspected that they might transfer *met* and not transfer the cystic fibrosis gene at all.

"I realized as soon as we found *met* that this approach was feasible," says White, "but we decided not to do it because it is too risky."

"We took it to mean the cell line was too bad to use," says Tsui. Instead, his group started looking for the gene the hard way, by generating more probes and mapping hundreds of them back to the chromosome in a strategy he calls saturation mapping. The idea is that if you generate enough probes, one of them is bound to be close to the gene, he says. "We call it systematic but it is actually very stupid. It is brute force."

Williamson, however, was already well down the path with *met* when the problem with the rearranged cell line came to light. He kept going anyway, hoping that, with luck, the rearrangement would not affect the cystic fibrosis locus. Apparently it did not.

When in April 1987 the Williamson group announced that they had isolated a gene from this region that was a "strong candidate" for the cystic fibrosis gene, some of their competitors kicked themselves for their timidity. Williamson's gamble, apparently, had paid off.

In his labyrinthine, cramped lab at St. Mary's, Williamson was "over the moon." Of all the investigators searching for the gene, Williamson had perhaps staked the most on it. He was one of the first to begin looking for the gene and had spent years amassing probes and families and looking for linkage. At first his group divided their efforts, working on Duchenne muscular dystrophy and other diseases. But over the years Williamson dropped those and concentrated almost exclusively on cystic fibrosis, committing his entire lab—15 people—and most of his time to it, and aggressively campaigning for research funds.

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"Bob is pretty single-minded," comments Arthur Beaudet of Baylor. "He wants to solve it. He wants to be famous for cloning the gene."

Some of it is pride—in a way Williamson has staked his reputation on finding the cystic fibrosis gene. But he also feels a deep obligation to the patients and their families who raise many of the funds for research. "One of the responsibilities of those of us who have done our research on behalf of the community is to ensure that profit comes back and is used for better treatment and better care," says Williamson. "That will not be the major interest of those interested in a quick return."

Over the years his group had pursued numerous false leads—chromosome 4 and chromosome 13, among others. And just a month before identifying this new "candidate," they had found another gene that looked promising. When it did not pan out, spirits flagged.

The new gene, however, looked, by all preliminary tests, as if it might be it. In the families they tested, the gene and the disease always appeared together—in other words, there was no "crossing over." What's more, the group found a phenomenon known as "linkage disequilibrium," which, though difficult to interpret, means you are very, very close. And the gene was switched on in the tissues affected by cystic fibrosis—the lung, pancreas, intestines, kidney, and placenta.

When Williamson and his colleagues published their results in the 30 April 1987 issue of *Nature*, they were careful to call it a "candidate" gene, but Williamson admits that "we really thought we had it." At meetings there were fewer qualifications, and others, picking up on his enthusiasm, were convinced as well. The media heralded the discovery, and the patients and their families were elated that, at last, a cure might be in sight.

But the curious thing, his competitors say, is that in his paper and his presentations Williamson provided little proof. All the same, he was acting very much like a man who had found the cystic fibrosis gene. They assumed he was holding back information that would let others catch up with him, trying to ensure his lead.

"Williamson's group was announcing the candidate gene very publicly, and with such conviction, that they seemed to know more than they were releasing," White recalls. "It turns out they knew less."

Williamson was so convincing that several groups, including White's, essentially stopped their search for the gene. And new groups that wanted to try to clone the gene, now that it had been localized to chromosome 7, found they could not get funds from the National Institutes of Health, where the peer reviewers cited Williamson's next-to-certain victory.

But by summer, when little new information was forthcoming from Williamson's lab, several of his competitors were beginning to question whether he actually had the gene. And they were increasingly frustrated by his reticence with his data.

At a Gordon conference in August, the tension was palpable. Williamson presented some evidence to suggest that it was not the gene, but he said that the data could be wrong. And as in the *Nature* paper announcing the candidate, he omitted information that would let others assess the candidate for themselves.

By all accounts, Donis-Keller was by that point steaming and was asking testy questions like, Why don't you release the sequence? She and Williamson tangled openly. White was prodding him too, others say, but they managed to avoid an outright fight.

What made things worse is that Williamson's talk at the Gordon conference followed close behind one by Louis Kunkel, which was, by all accounts, a study in contrasts. Kunkel was one of a number of investigators looking for the gene for Duchenne muscular dystrophy, a muscle-wasting disease as prevalent and lethal as cystic fibrosis. Although numerous labs were involved in the hunt, and five or so were competing in earnest,

that search has been notable, for the most part, for its collegiality and openness.

When the Duchenne researchers got close to the gene—and when it became apparent that the gene was either very large or the locus exceedingly complex—more than 20 groups pooled their data and exchanged materials to further the analysis. The paper reporting this work had 75 coauthors. Kunkel's group eventually found the gene, and when they announced their candidate, they published the partial gene sequence and made materials available to others.

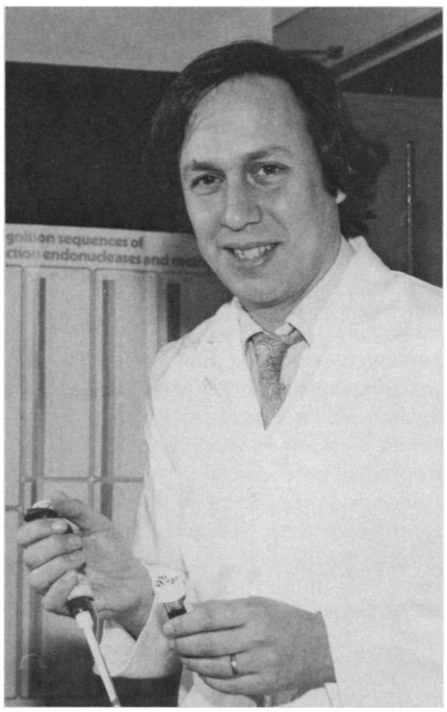
By the time of the Gordon conference Kunkel had had his first glimpse of the defective protein. He reported its location, what it looked like, and its size in advance of publication. Comments Kunkel: "That is the purpose of Gordon conferences—to release your brand new information."

Perhaps the comparison at the Gordon conference was unfair—Kunkel had won the race for the gene, so in a sense he could afford to be generous—but it was unavoidable: Williamson had yet to release the sequence or make his probe available, although he had published it 4 months earlier. And at the meeting, some of the cystic fibrosis researchers were barely civil to each other. Williamson and Donis-Keller were not the only source of tension: White and Donis-Keller were feuding as well. Relations between the two groups had been icy since their collaboration fell through in fall 1985, and they were now fiercely competing on developing a genetic linkage map.

One reason Williamson was so circumspect with his data is that things were beginning to unravel for him, evidence was accumulating that this was not, after all, the cystic fibrosis gene. Williamson says it came not as a blinding flash but as a gradual realization. In retrospect, he probably did not want to see the evidence lining up squarely against his candidate.

In April and May the London group had sequenced two copies of the candidate gene, now known as IRP, one from a normal cell and one from a cystic fibrosis cell. If a defect in this gene were the cause of cystic fibrosis, you would expect to see a difference between the two genes. They were identical. "It was an awful thing to face," Williamson recalls. But they told themselves—and their competitors—that they might have made a mistake, a likely possibility when sequencing some 2000 bases.

The first thing that got them "dead worried," Williamson says, was the protein encoded by the candidate gene—it did not seem right. To the best of anyone's knowledge, the cystic fibrosis protein is lodged in the membrane of epithelial cells, or at least within the cell. But from the sequence data, this looked like a protein that is secreted.



Robert Williamson: *"Even though I will defend our record on this, to be honest, I'm not sure of the right way to proceed."*

Early on there had been evidence of a crossover—a case in which the candidate gene was inherited separately from the disease. Yet this did not necessarily disqualify the gene because the family was unusual and there was some chance it had been misdiagnosed. Beaudet found another crossover in September, but again, there were questions.

"By then we were taking the problems more seriously," Williamson says. "We redid the sequence data, checked again that the protein was secreted. Then we learned of two more crossovers. One piece of data would not have made us despair. But the four together did."

Only in September 1987, at the Human Gene Mapping meeting in Paris, did Williamson come close to conceding defeat: he presented data on the protein, which he said was inconsistent with the cystic fibrosis phenotype. And by October, even Williamson could no longer deny the evidence.

"It was diabolical," Williamson remembers. "We were extremely depressed for a couple of months. Some of it is personal pride—we got a bit of egg on our face because we were pretty sure we had it and then we had to stand up and say we didn't. But there is a quite genuine feeling of having let the families down."

Others are harsher in their assessment. "The way he handled the candidate gene was inappropriate," says Kunkel. "He released little information that would have allowed others to reproduce or confirm his work. If you tell people you have a candidate gene, then others wonder if they should continue their work. I would."

And in retrospect, several say, there was little reason to think it was the gene. "There could be a gene there," says Tsui. "But to say, therefore, that it is the cystic fibrosis gene, that is a little weak," especially given the scrambled cell line.

Most attribute the episode to Williamson's abundant enthusiasm, but tensions are so bad that a few speculate that he misled people intentionally to scare off the competition, an accusation Williamson finds appalling. Intentional or not, the result was the same. White knows firsthand: "Having turned down the intensity of our effort, it is hard to bring it back up to speed 6 to 8 months later."

Williamson maintains that "we gave data in that paper that would have made it relatively simple for any group in the world to isolate that gene in a month or two." Nonetheless, he admits to some doubts of his own. "Even though I will defend how we handled this, to be honest, I'm still not sure of the right way to proceed. We really did think we had the cystic fibrosis gene. What do you do in that situation? In your heart of hearts you desperately want it to be the gene, you think it is the gene, then doubts begin to accumulate.

"Do you then, week by week, stand up and say we have six pieces of data, and four argue that it is the gene and two argue that it isn't? One reason we did not give data week by week is that it did not hang together until the end of August. But so far as timing is concerned, one has to acknowledge that if it had proven to be the gene we would have been a bit more eager to get it out."

Complaints aside, almost everyone acknowledges that Williamson's work constituted a major leap forward. IRP, Williamson's probe and former candidate, is very close to the cystic fibrosis gene—Williamson thinks within 20 to 40 kilobases, though others suspect it is considerably further.

The probe is so close to the gene that it removes the greatest limitation in prenatal diagnosis; couples without living, affected children can now be tested. And it also enables, for the first time, carrier exclusion—for example, to determine if an unaffected sibling harbors the gene.

The probe also puts Williamson within arm's reach of the cystic fibrosis gene, so that it is just a matter of time, as he walks and jumps down the chromosome, until he finds it. Perhaps not surprisingly, he is guarding the probe zealously. Although Williamson has sent the probe out to some 200 clinical centers for diagnostic use, his

competitors cannot touch it—a policy that draws mixed reactions.

According to Williamson, his probe is available to clinical research centers at no charge, and with no strings, and to companies in exchange for a contribution to the research fund. Stanley Rose at Collaborative, who bristles at the distinction, says the contribution Williamson asks for is substantial—more than $100,000.

Williamson also maintains that his probe is available to researchers who agree to collaborate, but those who have tried to get hold of it say otherwise. "IRP is not available to us for research," says Tsui. "I asked for it. Bob sent me a letter and said I could have his probe if I could think of something different to do with it. I wrote back and said that whatever I could think of, he was probably doing already. But if he would give me the probe, with no limitations, I would share the results with him. I never got an answer. I take that as 'no.' But I can understand why Bob would not let others use his probes for research."

Williamson's probes, however, were developed with public funds, a fact that several of his competitors point out. Says Beaudet: "Taxpayers, the government, did not intend this research to advance one lab's competitiveness only."

The problem is not unique to Williamson's lab. Most investigators have grappled with it, now that their competitors are set up to do this work and can catch up virtually overnight. In fact, compared with his brethren in human genetics, Williamson probably falls somewhere in the middle in terms of openness. "It's a tough one," says Kunkel. "You don't want to send a probe out to people doing the same thing, especially when you run a small lab. But if I have published a probe I make it available with no strings. You still have a bit of extra time, before the paper is published, in which to be clever in your experimental design."

"I suppose Kunkel is really the model of how we ought to behave," Peter Scambler, a postdoc in Williamson's lab, says somewhat wistfully. But after spending nearly 5 years looking for the gene, he is not inclined to further a competitor's quest. Scambler says that if a competitor, like Tsui or White, had an entirely new strategy for finding the gene, something the Williamson lab was not set up to do, then the probe would be theirs. But not otherwise.

This policy puts Beaudet in something of a bind. Williamson sent him the probe to help in his diagnostic work at Baylor. Having the probe is fantastic for prenatal diagnosis, says Beaudet, but now that he wants to resume his own quest for the gene, he finds his hands are tied by his agreement with Williamson. "We would like to clone the gene, but it is useful for us, for diagnostic purposes, to get probes from Bob," says Beaudet. "I'll follow his guidelines to play this diagnostic role. But it is not the way I do most things."

Now, after a 9-month hiatus when it looked as if Williamson had the gene, other investigators are resuming the hunt. "In the continued absence of the gene, there may be fresh approaches well worth pursuing," says White. "If we come up with an alternative approach, we'll jump on it. Or we will jump in if we think Williamson is not making enough progress—and frankly, we are reaching that point now."

Beaudet may start again, if he works out an agreement with Williamson. Collaborative is quietly pursuing the gene, as they have been for the past 2 years. "It is still a horse race," says Donis-Keller. "All of us have a chance of getting it."

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But the acknowledged front-runners are Williamson and Tsui, who never stopped his quest for the gene. "Bob may have had a candidate, but he still has to show me it is the cystic fibrosis gene before I close my case," says Tsui. "We are determined to use this method until we find the gene."

Williamson now has a new candidate, the next gene down the chromosome from the former candidate, IRP. The London group actually has just the tail end of a gene; getting out the whole sequence is tricky because they have hit an "unclonable" region. But this time, while they are doing all the tests that would indicate whether or not this new candidate is the cystic fibrosis gene, the group is uncharacteristically quiet.

"We know we have a gene in the right place, but we don't know if it is the right gene," says Williamson. Although the gene is expressed in the tissues that are affected by cystic fibrosis, "until you have a sequence, until you know there is a mutation, and until you know something of the physiology, you don't know that you have the gene," he says. "This time we are going to try hard not to get so excited until we are sure."

"Well, who would listen if he weren't?" snaps White.

The Toronto group is also very close to the gene and, Tsui suspects, in the exact same region, give or take 100 or 200 kilobases, as is Williamson. "We have a rough idea where they are and a very good idea where we are," says Tsui.

In the small region of the chromosome, about 100 kilobases in length, where both Tsui and Williamson are concentrating their hunt, Tsui has found two probes that show no recombination with the gene. From these probes, which sit right between *met* and J3.11, he and Francis Collins of the University of Michigan Medical School, with whom he is collaborating, are walking and jumping, looking for the cystic fibrosis gene.

They have already found two genes, which they have tentatively ruled out as candidates. There probably are no more than 10, or at most 20, genes in the region. And now 3, including Williamson's former candidate, have perhaps been eliminated, which further narrows the search.

Eventually, one of these investigators, or perhaps someone else, will pull out the gene, though how you know when you have it is not a trivial question. Most people think the gene will be found within the year, and perhaps much sooner. But tracking down the cystic fibrosis gene has proved more difficult than anyone expected, and predictions have been wrong in the past. "It's kind of depressing," says Donis-Keller. "We've been so close for so long."

There still will be the protein to isolate, the defect to understand. But if cystic fibrosis is caused by a single defect, a point mutation, as investigators suspect it is, then "the field might not accommodate too many people," says Tsui. This contrasts with Duchenne muscular dystrophy, in which the defect is so complex that numerous labs will be busy for years trying to sort it out.

"Maybe that is why the competition in cystic fibrosis is so stiff," speculates Tsui. "You have to be absolutely first or your contribution will not be that obvious."

Once the gene is in hand, Collaborative will begin working on a direct prenatal test, using the gene, and on strategies for carrier testing. And once the protein encoded by the gene is isolated and understood, physiologists, protein engineers, and drug designers will step in to see how this new knowledge can be translated into treatment. And most of the investigators involved in the quest will move on to a new gene, perhaps a less competitive one.

Are there lessons to be learned? Perhaps no more than that such quests are rarely as dispassionate and clearheaded as they seem from the outside, or with the benefit of hindsight. And that when the only reward is credit, and when only one can "win," emotions sometimes get the better of good intentions. ■ **LESLIE ROBERTS**