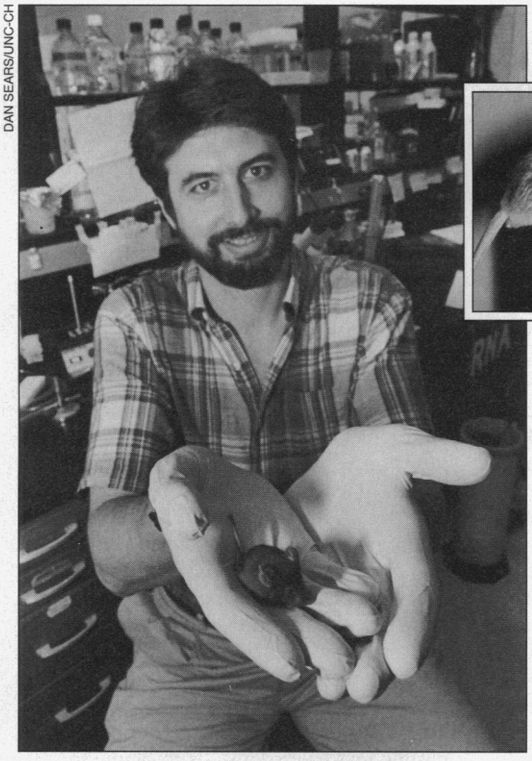
MOLECULAR GENETICS

Knockout Mice Offer First Animal Model for CF

When the gene for cystic fibrosis (CF) was cloned 3 years ago, it represented the biggest breakthrough to date in understanding the deadly disease, which is the most common genetic disorder afflicting Caucasians. Now researchers have taken the next big step: For the first time, they have produced an animal model that mimics many of the features of the human illness.



DAN SEARS/UNC-CH

J. SNOUWAERT, *ET AL.*

Mini-mouse. Postdoc John Snouwaert holds a knockout mouse, developed in the lab of Beverly Koller, that models some symptoms of cystic fibrosis. The CF abnomality causes the knockout mouse to be smaller than its normal littermates (inset); the color difference is not related to CF.

It's a welcome development. Analysis of the cystic fibrosis gene revealed that the protein it encodes is a chloride channel vital for proper secretion of salt and water by cells at mucus membranes. When the gene is defective, that apparently leads to flawed chloride ion transport and the characteristic cystic fibrosis symptoms: a thick mucus clogging the lungs and digestive tract, with accompanying lung infections and intestinal problems. But as important as it was, cloning the CF gene didn't answer all the questions researchers had. It didn't reveal, for example, the biological mechanisms that lead from a simple chloride channel defect to the disease's deadly conclusion.

Information about the progression of the disease is essential for designing therapies aimed at blocking that progression, and it is nearly impossible to get from studies of human subjects. "We don't have ready access to the lungs of little babies [in the early stages of] CF," says Tom Boat, a pediatric pulmonologist at the University of North Carolina. And the samples available from autopsies of patients with lungs ravaged by infection and scarring give few clues about the early stages of the disease.

Those problems—of understanding the mechanisms of the disease and designing therapies to combat them—can be overcome by having a good animal model that reproduces some, or all, of the symptoms of CF. "If you had an animal model," says CF researcher Michael Welsh of the University of Iowa, "you could study the tissues at various stages, early, late, and intermediate." And so several groups have been racing to produce an animal simulation of CF. The first group across the finish line is that of Beverly Koller, Oliver Smithies, and their colleagues at the University of North Carolina. In two papers in this issue of *Science*, they describe "knockout" mice that are not only missing the CF gene, but that also have some symptoms remarkably similar to those of human CF patients (see pages 1083 and 1125). "It's a pretty stunning development," says Francis Collins, of the University of Michigan, a member of the team that originally cloned the CF gene.

While the other groups in the race don't have papers in press yet—and aren't willing to say much about their work—their findings appear to be generally similar to Koller's. "Our results are consistent [with hers], and add to the picture," says David Porteous, a CF researcher at the University of Edinburgh whose group is about a month behind Koller's group in analyzing its batch of CF knockout mice. "The most important thing is that more than one group has got it to work, and it does seem to mimic important components of the human disease."

Once the CF gene was in hand, the conceptual path to a CF animal model seemed clear: use new gene-targeting methods to mutate or replace the CF gene in a line of mouse embryonic stem cells, then place those altered stem cells into early mouse embryos, and put the embryos into females to develop. The resulting mice, known as mosaics, would carry the mutant gene in some of their tissues, and could be bred to produce mice, known as "knockout mice," that have one copy of the altered gene in all their tissues. Those mice could then be bred again to produce offspring with two copies of the defective gene, which could be studied for the effects of the gene (*Science*, 5 June 1992, p. 1392).

But if the map of the race was so simple, why has it taken ace researchers years to reach the finish line? The reason is that, while the plan sounds straightforward, making knockout mice is far from a cookbook procedure. All the groups—which include in addition to Porteous's group, those of Mario Capecchi in Utah, Art Beaudet and Allan Bradley at Baylor, and Martin Evans and Bill Colledge in Cambridge, have been struggling to streamline the process. "It's been a heroic piece of work," says CF researcher Alan Smith, of Genzyme, in Cambridge, Massachusetts. "They've been working for two and a half years, and have had all kinds of problems."

The technical dilemmas for the Koller group began early, according to postdoc John Snouwaert. First, the CF gene proved very difficult to knock out in the embryonic stem (ES) cells. After many efforts produced no knockouts at all, the group switched to a new CF gene clone, and finally managed to obtain cell lines with the CF gene knocked out.

The next step in making the knockouts was to take those ES cells and use them to create mice carrying two copies of the altered CF gene. But as (bad) luck would have it, none of the mosaic mice carried the mutant CF gene in their eggs and sperm, so they couldn't pass it to their offspring. The group had to make more mutant ES cells and try again, before they got mice that could transmit the gene to their progeny.

Similar problems were shared by other groups in the race. "Last summer there was a mouse molecular genetics meeting and we calculated that over 100,000 different ES cell lines had been screened unsuccessfully for knocking out the [CF gene]," says Porteous. For other genes, researchers have typically been able to find knockouts after screening several hundred cell lines. Porteous adds that his group also had trouble producing mosaic mice that would pass the mutant gene to their offspring.

The first public report of the Koller group's lead came in early June at a Cystic Fibrosis Foundation meeting in Williamsburg, Vir-

ginia. There, Koller reported that her team was just about to produce its first mice with two copies of the mutant CTFR. Those reports only teased the community, however, because Koller didn't yet have the answer to the question on everyone's mind: Did those mice show CF-like symptoms?

Since that June meeting, CF researchers have been waiting on tenterhooks for the answer. "What we worried is that they would have no symptoms," says Lap-Chee Tsui, of the Hospital for Sick Children in Toronto, who was also part of the original CF gene-cloning team. It wasn't a groundless concern: Other mouse models have fallen flat for that very reason. In 1987, for example, researchers knocked out a gene for an enzyme called HPRT, whose loss in humans causes Lesch-Nyhan disease. But the mice had no symptoms, because they had another enzyme that could cover for the missing HPRT.

The news with the CF mice is much better. "The mouse didn't have any bypass system that would protect it," says Richard Boucher, a member of the North Carolina team. Boucher's lab did electrophysiological studies on cells taken from the intestinal tract and airways of the mice. They found that all the cells were abnormal in their chloride secretion, as are the cells of human CF patients. "The bottom line is there clearly is not another channel" that can substitute for this chloride channel in the mouse's airways and intestine, says Boucher.

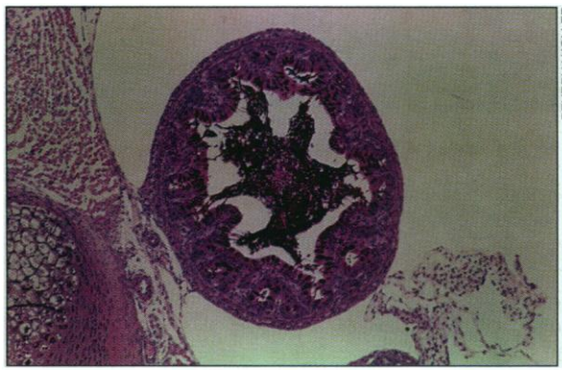
And most encouragingly of all, the lack of the chloride channel causes CF-like symptoms in the mouse. The most striking similarity is in the intestine, where the mice develop blockages like those in infants with the most severe cases of CF. Even before they develop the blockages, poor intestinal function prevents the CF mice from getting much nutrition from their food. That makes them small for their age, as are many CF children.

Despite their similarities, the symptoms of the CF mice are hardly identical to those of human CF. Indeed, says Koller, "it would be very unlikely to get a mouse model that is identical to humans," because there are just too many subtle physiological differences between the two species.

The most important contrast between the mice and human CF sufferers is in the lungs—the organ of greatest concern to CF researchers, since it's lung infections that kill 95% of people with CF. The lungs of CF mice have some of the same signs of illness as the lungs of CF patients, says Boucher, including a proliferation of mucus-secreting cells and enlargement of mucus glands. But despite these changes, the mice don't have the mucus-clogged lungs and persistent lung infections that plague human CF sufferers.

That could be because the lungs of mice are fundamentally different from those of humans—they have fewer mucus secreting glands and cells overall, for example. But the mice could also be dying of intestinal blockages before they have a chance to develop lung problems. "Most patients with CF have...normal lungs at birth," says North Carolina's Boat. "It's likely that it is after they get their first viral respiratory infections that they start having problems with excessive mucus secretion, and the pathology that ensues." The researchers hope to keep the mice alive longer—perhaps by dietary measures—so they have a chance to develop respiratory symptoms.

Most CF researchers agree that the mice will be useful in any case, but if they develop lung problems like those of human patients, they will be particularly valuable for understanding the course of the lung disease. If the mice do develop respiratory problems, says Iowa's Welsh, then researchers can begin to ask how the spiral of decline starts. "You could grow the mice in pathogen-free environments," he says, "and then expose them to various pathogens, and ask how does that influence the course of the lung disease."



BEVERLY KOLLER

Gut feeling. Mucus heavily blocks a section through the colon of a knockout mouse that models cystic fibrosis.

An animal model for CF lung disease would also help speed the development and testing of new treatments for CF, says Boucher. His group has been studying amiloride, a regulator of salt secretion, as a possible treatment for CF. "We studied amiloride in 15 patients, and asked if it looked promising." The answer was yes, he says, but "it took us a year and a half, and cost $700,000." Having a mouse model, he says, will bring down both the cost and the time of such trials, and will also make it possible to ask whether drugs are useful as preventive measures, before lung disease has begun. Without an animal model, he says, "you would have to do that with kids. And it's not only hard to give an unknown drug to a kid, but you're asking about something that will happen 5 years down the pike. It's a long study." A good mouse model, he says, "will really collapse our time frame."

And even if the mice don't develop lung disease that mimics human CF, they may prove useful to test treatments such as gene replacement or replacement of the faulty protein—therapies aimed at restoring normal chloride channel function. Such treatments have been shown to work in cell culture, but the next big question is whether they will correct the defect in a living animal. That question can be addressed now with the mice, says Tsui, since tests like those done by Boucher can be used to measure the presence of normal channels after treatment. "It's already possible to use [the mice] to see if we can deliver the gene and get correction of the chloride channel," says Tsui.

In fact, says Boucher, the differences between the knockout mice and human CF patients aren't all to the bad—some of them could actually shed light on the human disease. For example, CF babies that die of intestinal blockage usually have mucus-clogged pancreatic ducts, preventing the release of pancreatic enzymes needed for digestion. Researchers thought that the lack of those enzymes in the digestive tract was the main cause of the intestinal blockage. But the mice that die of intestinal blockage don't seem to have such severe pancreatic problems. "I think that is giving us a clue that there is more to the bowel problem than we thought," says Boucher.

Another important question the mice will help address is how the course of CF may be influenced by other, unknown genes that vary from person to person. "In the human case it's quite clear that [people] with the same primary defect can have [disease with] different degrees of severity," says Martin Evans of the University of Cambridge. "That means there is a very strong likelihood that genetic background is important." The mice will provide an excellent way to address that issue, says Evans, because the mice from different labs will have different genetic backgrounds, which may produce a range of symptoms.

With all the questions the upcoming crop of knockout mice may help answer, lots of researchers will undoubtedly be eager to get their hands on them. And Koller, for one, plans to make that as easy and inexpensive as possible. "We are going to make the animal available," she says, "and it will be very cheap." There has been negative publicity about the high prices being charged by private companies for some knockout mice (*Science*, 5 June 1992, p. 1393), but Koller says she is working with the CF foundation to get the mice transferred to the mouse breeding facility at Jackson Laboratory in Bar Harbor Maine, which will make them available for the cost of breeding. "The CF foundation has a real interest in seeing that [the mice] get out to as many investigators as possible," she says. And as mice from the other teams also become available, all those interested investigators should have quite a selection of mice to help them home in on the mechanisms by which CF does its deadly work.

–Marcia Barinaga