MOUSE OF A DIFFERENT YAC

By ELIZABETH PENNISI

Yeast artificial chromosomes make possible bigger gene transfers

Strauss et al./Whitehead Institute

Spotted father passes his YAC to some offspring (brown) and not others (white).

Eleven years ago, scientists shocked the public when they produced larger-than-life mice by inserting a rat gene for growth hormone. Transgenic animals, then considered a miracle of genetic engineering, quickly took their place as laboratory workhorses essential in many types of research and in the production of medically useful compounds. Mice engineered to carry the gene responsible for cystic fibrosis, for instance, count as one of several animal models that researchers study as they pursue treatments for human diseases.

But until recently, transgenic mice carried only small pieces of foreign genetic material — genes no more than 50,000 base pairs long — in their cells. Genes responsible for many important disorders, such as muscular dystrophy and hemophilia, cover 1 million or more base pairs of nucleotides, the basic building blocks for DNA. Scientists just didn't know how to make enough copies of such giant strands of DNA; nor had they figured out how to get mammoth chunks of genetic material into mice.

Now, several research groups have pushed back the frontiers of transgenicity, creating mice that thrive not only with large genes but also, in some cases, with the entire genetic repertoire, or genome, of yeast inside the nucleus of each cell. These successes pave the way for a new generation of transgenic animals capable of mimicking human disease or producing proteins and antibodies that pass for our own. The new technology may also speed progress in understanding how genes work and in tying particular genes to specific biological problems.

"It gives you a way to assess genes on the level of a whole organism," says Roger H. Reeves, a geneticist at Johns Hopkins University School of Medicine in Baltimore. "It allows the information from the Human Genome Project [an ongoing effort to identify all human genes] to be applied across all areas of disease."

These advances make use of techniques developed six years ago at Washington University in St. Louis, when molecular geneticist Maynard V. Olson and student David Burke realized that yeast would readily make multiple copies of synthetic DNA inserted into them. "There had been a lot of work done during the preceding 10 years defining the components required for DNA molecules to function as chromosomes in yeast," recalls Olson, now at the University of Washington in Seattle. Burke put that knowledge to use and made the first yeast artificial chromosomes, dubbed YACs.

YACs contain tiny bits of DNA that make them look — and replicate — like the yeast's own genetic material. "You have all the elements of a natural chromosome," says Günther Schütz, a molecular biologist at the German Cancer Research Center in Heidelberg. In addition to these bits, extra DNA from other organisms, including mice and even people, can make up the core of each YAC. The core can extend for quite a stretch without upsetting the yeast's genetic operations, Schütz adds.

Olson's group and scientists at about 10 other research laboratories have since generated large YAC "libraries" by chopping up mouse or human chromosomes and splicing the pieces to yeast genetic material. Then the yeast cells do the work of making millions of copies of that YAC as they replicate. Scientists seeking to study a particular gene can borrow YACs from these libraries for use in their experiments.

But not until this spring have molecular geneticists used YACs for moving big genes into other organisms and shown that those genes pass on to succeeding

generations. In March, three teams independently reported successes with slightly different techniques; several others are close on the heels of these first achievements.

"This was the missing step — putting [YACs] into the mice," says Rudolf Jaenisch of the Whitehead Institute for Biomedical Research in Cambridge, Mass. And while genetic engineers have yet to transfer genes that number a million base pairs, researchers think such transfers are now possible.

Taking this step opens new doors in genetics research. Already, YAC technology is speeding the mapping of genes. Last year, using YACs, two research groups mapped the DNA on the entire Y chromosome and chromosome 21 (SN: 10/3/92, p.212). "Now, [YACs] are the mainstay method for doing large-scale physical mapping of chromosomes," Olson notes.

By sticking huge stretches of DNA into mice, molecular biologists hope to maintain the gene's native genetic environment and consequently invoke its normal expression. "It's now possible to introduce long gene complexes all together, so we have the possibility of analyzing [them] within their natural context," Schütz says. That native environment includes DNA distant from the gene but often essential to turning the gene on so that cells can use the information it contains.

From a practical perspective, for example, long YACs could contain gene complexes responsible for generating the immune system's antibodies. Thus, YACs offer biotechnology companies the potential for getting mice to churn out human antibodies, which would be less likely than mouse monoclonal antibodies to cause problems when used in people, says Jaenisch.

YACs also provide a different, functionally based way of homing in on new genes. With most gene-mapping strategies, molecular biologists can narrow the site of a gene to a section of chromosome, but then they must work incredibly hard to pinpoint its location to within less than a million base pairs. With YACs, they can now use some of the hundreds of mutant mouse strains to identify the exact genes responsible for these defects.

These mutants lack particular enzymes or other proteins and were created through the years, sometimes by accident and sometimes on purpose, by researchers studying mice for a variety of reasons. Scientists find the mutant gene by adding the right piece of DNA that corrects the mutation, thereby "rescuing" the mouse strain. YACs enable them to search for genes they couldn't find using other genetic engineering techniques.

To perform genetic rescues, researchers first use a large YAC whose DNA contains the gene somewhere along it. If that YAC causes the mouse to regain whatever it lost in the mutation, then the researchers make new YACs that include ever-smaller pieces of the original YAC's DNA until they locate the exact segment that carries the essential coding. "This gives you a way to identify a gene with specific biological activity," Reeves says.

YACs can serve other functions in genetic research as well. For studying Down's syndrome, in which extra chromosomal material causes a range of defects, other investigators hope to use YACs to introduce extra genetic material into mice. By sticking different parts of the extra chromosome into the mice, they hope to learn which genes lead to particular defects in Down's patients, explains John D. Gearhart of Johns Hopkins. These mice could then serve as models for testing ways to correct the defects.

In addition, both Gearhart and researchers at GenPharm International, a biotechnology company in Mountain View, Calif., seek to use YACs to give mice an extra gene for amyloid precursor protein. Scientists suspect that an overabundance of this protein can lead to the plaques found in the brains of people with Alzheimer's disease. Researchers have tried to verify this idea by adding the gene to mice, but the gene never worked right with conventional gene transfer techniques. So the two groups are now using 650,000-base-pair YACs that include more than just that gene.

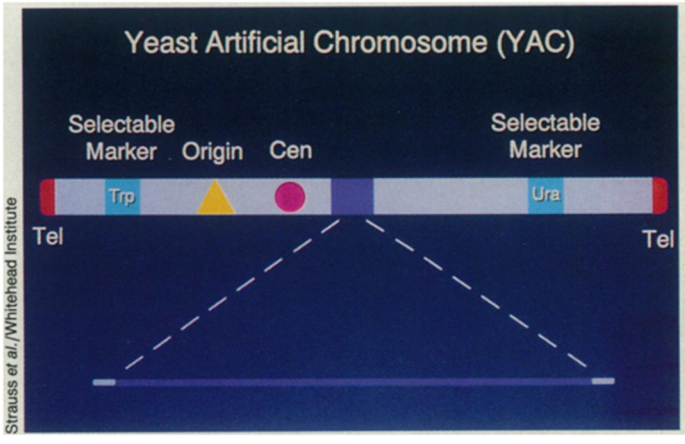
"We felt that if you went in with the whole genomic fragment, you'd get it to behave like the real thing," says Ted K. Choi, a geneticist at GenPharm. If that proves true, then the new generation of transgenic mice may not only help pinpoint a possible cause of the plaques but also provide a new model for researchers seeking to understand and prevent the disease.

This approach may work for many other diseases in which genetics plays a role. "That's the real power of using YACs," says Choi. "What used to be an extremely rare event—which is appropriate expression [of the gene] — we think will now become commonplace."

"[YAC gene transfer] is going to stimulate a lot of research," predicts Jeffrey M. Friedman, a geneticist at Rockefeller University in New York City. "It's an exciting prospect that this will become routine in [genetics] laboratories."

For the technology's pioneers, however, moving YACs into mice was no easy feat. Early YAC-movers spent many hours overcoming technical obstacles and solving them in different ways. Their efforts have provided molecular biologists with several techniques for incorporating YACs into mice, each with advantages and disadvantages. "Time will tell us which method will work the best," Friedman says.

YAC-mover Schütz and his colleagues at the German Cancer Research Center use a conventional method for transferring their YACs: They inject YACs into the nuclei of fertilized mouse eggs, in much the same way molecular biologists have transferred smaller DNA pieces for years. "I think simplicity is a strong attribute of the process we've been using," Schütz says.



Strauss *et al.*/Whitehead Institute

Diagram of YAC shows inserted gene (purple), yeast DNA components (red), markers (blue), and other DNA making up an artificial chromosome.

As part of their research, his team sought to correct a genetic defect in a particular type of albino mouse. These animals produce an aberrant form of tyrosinase, an enzyme essential in pigment production. Only upon receiving a gene that leads to the production of normal tyrosinase do newborn mice develop brown eyes and colored fur. "It's a simple, very sensitive, visual assay," Olson explains.

The gene itself was small enough that scientists could transfer it in other ways. But sometimes it restored coloration and sometimes it did not, depending on where the gene was inserted into the albino's chromosomes. Schütz assumed that this transferred DNA often became a second-class gene that resided too far from DNA that would help activate it.

Two years ago, he decided to make a YAC that contained not just the gene but also other DNA that he and his colleagues thought helped regulate that gene. An-

dreas Schedl, now at Western General Hospital in Edinburgh, Scotland, and Lluís Montoliu of the German Cancer Research Center began determining the experimental conditions necessary to produce, purify, and transfer YACs of ever-larger sizes.

To make their approach work, they needed to make many, many copies of the gene in the yeast cell to ensure that they would have enough YAC to work with after separating it from the yeast's own DNA. Their goal was to increase its concentration to 20 times that of the yeast genes, says Schütz.

Second, they needed to come up with ways to purify the DNA and to develop liquid mixtures that would help keep the DNA dissolved while in the injection needle. Long pieces of DNA, especially in high concentrations, tend to precipitate. "It sticks to the side of the needle, so you can't pipette it," Schütz explains. Also, even tiny clumps of precipitated DNA can clog the needle's narrow opening.

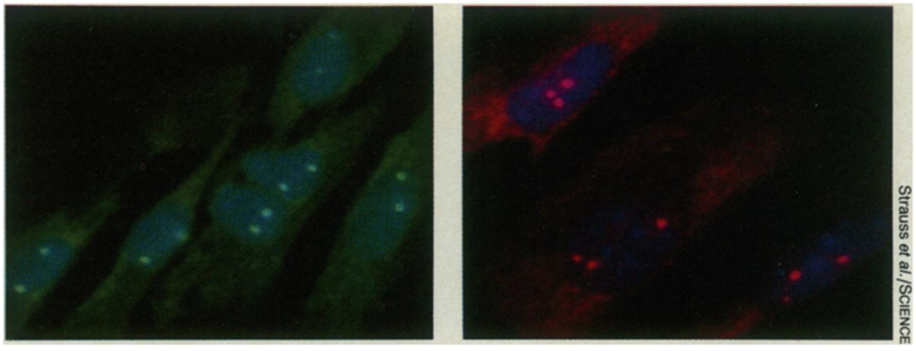
By 1992, Schedl succeeded in creating a transgenic mouse that contained a 35,000-base-pair YAC. And in the March 18 NATURE, the German group reports success with a 250,000-base-pair YAC. It contained the 80,000-base-pair-long tyrosinase gene as well as 150,000 base pairs that normally precede this gene.

For their experiment, Schedl and Montoliu injected YAC solution into 393 fertilized mouse eggs. They hoped that as each egg divided, replicating its chromosomes, it would incorporate and replicate the YAC as well. Of the 24 offspring, five bore brown eyes and colored skin, indications that these newborns possessed a working tyrosinase enzyme. "That was very surprising to us," Schütz recalls. "That was an excellent percentage."

One of the five mice died at birth and another proved sterile. The remaining three, however, survived to produce offspring of their own. Further analyses indicated that among these three, one mouse—which grew a mottled coat—had incorporated only one copy of the YAC into its genetic repertoire; another mouse had incorporated two copies; and the third mouse had incorporated eight.

The brown eyes and skin prove that at least 80,000 base pairs stayed together, but Schütz predicts that all three mice kept the entire 250,000 base pairs intact. "That's very pleasing to us," he says, adding that the YAC likely will persist intact through succeeding generations.

The German group then evaluated how well the YAC's tyrosinase gene took by examining the skin cells of descendants of these transgenic mice. The defective gene leads to the production of inactive tyrosinase, while the transferred gene, or transgene, causes production of the active enzyme. By measuring the amounts of RNA associated with good and bad genes, the researchers inferred the amount of tyrosinase produced per gene.



With fluorescence microscopy, glowing spots reveal just two foci of RNA for collagen in non-YAC mouse skin cells (left), but three in YAC mouse skin cells (right).

As they hoped, the experiment showed that transgenes need to be in the right "environment" to work best. By bringing with it a big enough chunk of neighboring DNA, the transgene functioned as well as the albino's original.

The mouse with one copy produced half as much active enzyme as inactive enzyme, the production of which was guided by a pair of defective genes. The mouse with eight copies produced four times as much active enzyme as inactive enzyme. The level of expression depends on the number of copies of the gene, Schütz concludes.

Moreover, because the gene probably inserted itself into different places on the normal chromosomes, these results indicate that each copy's activity was unaffected by neighboring DNA. "So we think we've introduced all the elements that are necessary for [normal] expression," says Schütz. "It allows us now to search for the specific regulatory sequence."

To do this, the researchers will delete some of the DNA that precedes the tyrosinase gene, make new YACs, and determine which deletions cause the gene to fail to work properly.

The German work illustrates not only that scientists can use YACs to "rescue" mice from defects, but also how vital other DNA can be in the proper functioning of a gene. Scientists at the Whitehead Institute sought to rescue a different type of defective mouse. But they developed a very different approach for transferring the YAC into the germline of mice. In doing so, they solved a 10-year-old predicament.

Twelve years ago, Whitehead geneticists created a mouse they thought carried a defective gene for collagen, a protein found in bone and cartilage. Mouse embryos with two defective collagen genes die early in development, and those born with only one normal gene develop bones that break easily. Several times over the course of a decade, the researchers tried to replace the defective gene with a normal one, but they failed to restore normal collagen levels.

So William M. Strauss, working with Jaenisch and his colleagues at the Whitehead Institute, made a 150,000-base-pair YAC that contained the normal collagen gene from a different species of mouse. Slight differences between this gene and that of the mutant mouse enabled the scientists to distinguish the introduced gene from the mutant's own.

This YAC included several parts: the collagen gene, DNA thought to turn the gene on or off, "markers" that allowed scientists to assess how well the YAC DNA stayed intact, and a gene that makes cells resistant to a particular antibiotic that would otherwise destroy them. After allowing the yeast to make many copies of this YAC, the Whitehead scientists separated these YAC copies from the rest of the yeast DNA.

That feat proved no trivial task. The researchers isolate the YAC from other yeast genetic material by allowing it to migrate down a wide agar gel column. DNA fragments of different sizes move at different rates and thus take up different positions along the gel. Then the researchers slice out the agar gel containing the YAC and dissolve the agar to retrieve the YAC. "You had to learn to do all these steps without breaking the DNA," Jaenisch says.

Next, they mix in fatty molecules called lipids, which surround the YAC. The lipid envelope readily fuses with the membrane of mouse cells called embryonic stem cells.

These cells come from a mouse embryo early in development, when it is no more than a ball of rapidly dividing cells. If removed, altered, and transplanted into this stage of an embryo, the stem cells become an integral part of the developing mouse. Descendants of stem cells specialize to form all the tissues needed to create a whole mouse. Thus, different tissues in the newborn mouse contain either its parents' genes or the genes from the embryonic stem cell. When the stem cells give rise to reproductive tissue, the stem cell's genes — and the YAC — will populate egg or sperm and pass on to that mouse's offspring.

For the YAC experiments, Jaenisch's group grew the YAC-exposed stem cells in a laboratory dish that contained the antibiotic. Only those cells that took in and actively used the YAC survived the antibiotic bath. The survivors provide cells for transplanting into an embryo.

Out of about 100 million cells exposed to these DNA-filled lipids, 35 survived, multiplying to form large populations of identical cells, or clones. Working with researchers from the University of Massachusetts Medical School in Worcester, the Whitehead group then looked for signs of markers they had stuck into different parts of the gene. Only seven clones kept the complete YAC, the scientists report in the March 26 SCIENCE. Five others incorporated a large portion of this guest genetic material, and 23 retained only a small bit.

The team then injected embryonic stem cells from three of the seven complete clones into very young mouse embryos. In descendants of two mice, the existence of RNA created from the new collagen gene proved that the YAC took and was working.

While the Whitehead Institute and German Cancer Research Center scientists struggled to separate their YACs from the yeast, scientists from a California biotechnology company skipped those steps altogether and still succeeded in making transgenic mice — much to the amazement of their colleagues.

Aya Jakobovits and her co-workers at Cell Genesys, Inc., in Foster City, Calif., simply allow whole YAC-filled yeast cells to fuse with mouse embryonic stem cells. All they do to the yeast cell is remove its tough outer walls, they report in the March 18 NATURE. Thus, the transgenic mice created by adding those stem cells to an embryo carry all the yeast's genes as well as the YAC in their nuclei. At the time, this YAC was a record-setting 670,000 base pairs long.

The YAC contained a human gene that could cause the production of an enzyme missing in a particular strain of mice. Mouse cells that fused with the yeast and accepted the YAC thrived because they started making the missing enzyme. Jakobovits then injected cells that arose from the survivors into early mouse embryos. Some of the embryos developed with the transgene and passed it on to their young.

Often, the process of inserting foreign DNA into cells leads to mutations in the native DNA. This has led Jaenisch and others to worry that the extra yeast DNA might make the YAC gene less stable or might alter the mouse's genetic material in some way.

But surprisingly, the cells don't seem to mind at all. "Even in the presence of yeast, there was total differentiation and development," says Jakobovits. "The yeast genome is there, but it doesn't really do much."

In addition, Jakobovits' data indicate that her YACs are more likely to remain intact than YACs transferred in other ways. "The nice thing about that is you don't have to isolate the [YAC] DNA," concurs Olson. Protected inside the yeast cell, this DNA appears less likely to break apart, he adds.

That protection will become increasingly important as the Cell Genesys scientists and others scale up their YACs. The longer the inserted gene, the more susceptible it becomes to changes that could alter its function. "Our belief is that there will be a size limitation using any other technique," Jakobovits says.

For the time being, size limitations will not hamper scientists concerned primarily with rescuing mice with defective enzymes. But Jakobovits' goals will require techniques capable of transferring genes a million or more base pairs long.

"That's another frontier of the technology," Olson says.

Already, Daniel Cohen of the Paris-based Center for the Study of Human Polymorphism has created a YAC library in which the YACs average a million base pairs. In 1992, Dutch scientists reported they had pieced together almost 3 million base pairs that come quite close to representing the DNA that codes for dystrophin, the protein involved in muscular dystrophy. Like the dystrophin look-alike YAC, these "mega-YACs" tend to include more mistakes in their sequences than shorter YACs. Scientists hope to solve that problem by developing yeast strains less capable of rearranging or otherwise manipulating DNA.

"Some bugs have to be worked out, but the promise is really great," comments Choi.

Researchers are anxious to put the mega-YACs to work in mice. Genes of that size include some responsible for generating antibodies, opening the door to many commercial applications. Both Choi and Jakobovits hope to use YACs to put the human genes essential to antibody production into mice. If they then deactivate the animals' own genes for producing antibodies, they can turn these mice into living factories for human antibodies.

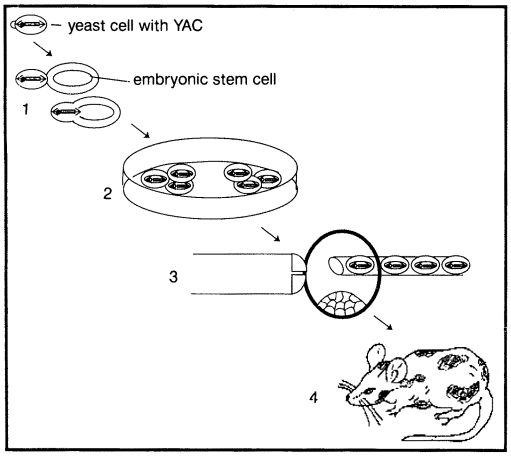
"That's going to revolutionize the way antibodies are produced," says Raju Kucherlapati, a molecular geneticist at Albert Einstein College of Medicine in New York City.

Toward that end, Choi and his GenPharm colleagues have made a YAC carrying a human gene that directs the production of an antibody segment called the heavy chain. Like the Whitehead group, they surround the YAC with lipids that fuse with the membranes of mouse embryonic stem cells. But their procedure differs from those of other teams in that they add a second piece of DNA to the soup from which the YAC-filled lipid envelope emerges. That second piece carries a gene that makes cells resistant to an antibiotic, enabling the researchers to select cells that successfully take up this foreign genetic material. While other groups also use this gene as a way to cull cells that have taken in the YAC, they make that gene part of the YAC itself. With the GenPharm approach, says Choi, "you don't have to spend a lot of time modifying your YAC. It saves a lot of work."

His YAC transfer went smoothly, and the mouse cells took in both the antibiotic-resistance gene and the YAC. The resulting transgenic animals carried human antibody fragments in their blood, Choi and his colleagues report in the June NATURE GENETICS. At first the GenPharm scientists detected only low concentrations of the fragments, but when they dismantled the animals' own machinery for making the mouse version of this molecule, the concentration of the human antibody fragment increased 10-fold.

Choi has not yet studied offspring of these mice to determine whether the animals use the transgene as much as they use their own genes. But he suspects that, thanks to being part of a YAC, the gene is doing exactly what it's supposed to do. That has been the case so far with the few genes transferred via YACs.

"People are very excited about finally getting transgenes to behave," Choi says. "I think that YACs will be quite successful." □



Jakobovits/Genesys, Inc.

(1) Naked yeast fuses with embryonic stem cell. (2) Only YAC-filled stem cells thrive in laboratory dish. (3) Stem cells are injected into early mouse embryo. (4) Spots show some tissue contains YAC.