TRANSGENIC ANIMALS

Infant Monkey Carries Jellyfish Gene

Efforts to make a fluorescent green monkey are not quite a glowing success—yet.

In an attempt to create the first transgenic primate, scientists at the Oregon Regional Primate Research Center in Beaverton have produced a rhesus monkey that carries the gene coding for green fluorescent protein (GFP). This gene, first isolated from glowing jellyfish, has been inserted into a host of experimental species, including plants, frogs, and mice. Although he is not green, the 3-month-old monkey named ANDi, described on page 309 of this issue, is something of a proof of principle. The achievement could lead to valuable experimental models for certain diseases and a better understanding of primate and human development, say other biologists. But the cumbersome technique is not likely to lead to transgenic humans, green or otherwise.

To produce ANDi, reproductive biologists Anthony Chan, Gerald Schatten, and colleagues injected a genetically modified virus into the unfertilized eggs of rhesus monkeys. A few hours later, they injected sperm into the oocytes to fertilize them. As with other in vitro fertilization (IVF) procedures in nonhuman primates, this one was relatively inefficient. Half of the fertilized eggs developed into embryos, and five pregnancies resulted from 20 embryo transfers, including one set of twins, which were miscarried.

Close, but no glow. ANDi, the first transgenic rhesus monkey, carries the gene for green fluorescent protein but does not glow green.

Three healthy monkeys were born, but the team has detected the GFP gene only in ANDi. The miscarried twins also carried the GFP gene, but unlike ANDi, their hair follicles and toenails did glow under fluorescent light. Schatten attributes the miscarriage to the fact that rhesus twins are rare, but the team is investigating whether it might be related to the inserted gene. So far, the team doesn't know whether ANDi's cells are expressing the protein. But Schatten says other transgenic animals have delayed producing their transgene for up to a year after birth.

Although the gene transfer techniques the researchers used are routine in other organisms, reproductive biologist Ted Golos of the Wisconsin Regional Primate Research Center in Madison says the birth of ANDi is the first demonstration that a primate egg can develop normally after such manipulations.

"We've made an incremental step from one species to another," Schatten says. And even that small step involved multiple hurdles. Whereas the experiment "is essentially several days' work in transgenic mice," Golos notes, monkey eggs are difficult to collect, and primatologists do not know how to artificially control a monkey's reproductive cycle. That meant the researchers had to time the experiment precisely so that an embryo was ready when a surrogate mother was at the right stage of her reproductive cycle. In fact, ethics considerations aside, the project might have been easier to achieve in humans, for whom IVF technology is much more advanced.

Even so, the work will not inspire fertility doctors to try the technique with human embryos anytime soon, Schatten predicts. Scientists can't control where the modified virus enters the genome, so the risk of an inserted gene interrupting an important gene would be relatively high. "I don't see an immediate therapeutic application," says bioethicist LeRoy Walters of the Kennedy Institute of Ethics at Georgetown University.

And until researchers find more efficient ways to create specific genetic changes, says Schatten, transgenic monkeys will not be common research tools. Even if those techniques were feasible, expense and ethical considerations would limit the use of transgenic monkeys as medical models, he says: "We don't need a knockout monkey for every disease."

But for questions that are difficult to study in rodents, such as those related to aging, neurodegenerative diseases, immunology, and behavior, transgenic primates could prove a plus, Golos says. Schatten predicts that genetically altered monkeys could be a boon to developmental biologists as well. Because monkeys are large enough to fit into magnetic resonance imaging machines, researchers might be able to introduce gene markers and track organ development by noninvasive means. "ANDi and his future cousins and brothers and sisters will help us bridge that gap between what we know in the mouse and what we're keenly interested in in human development," he says.

–GRETCHEN VOGEL

CIRCADIAN RHYTHMS

Mutant Gene Speeds Up the Human Clock

"Early to bed and early to rise, makes a man healthy, wealthy, and wise," advised Benjamin Franklin in his *Poor Richard's Almanack for the Year 1757*. Today, as then, people assume that self-discipline is the key to following such counsel. But in a paper published online today by *Science* (www.sciencexpress.org), a team led by Ying-Hui Fu and Louis Ptáček at the University of Utah in Salt Lake City shows that a person's genes may be more important. The researchers have identified a mutation that causes people to be extreme early birds, rising, say, at 4:00 a.m. The discovery opens a window into the genetic basis of the human circadian clock, which keeps body activities such as sleeping and eating running on a roughly 24-hour rhythm.

Researchers have identified genes that drive the circadian clocks in fruit flies, mice, and other species. They have also found what seem to be the human equivalents of some of those genes, but they've had no direct proof that those genes are in fact part of the human clock machinery. The Utah team has now provided that proof for one of the human genes, known as h*Per2*, by showing that a mutation in the gene speeds up the circadian clock. "It's the first example of a circadian clock gene in a human," says Joseph Takahashi, a geneticist at Northwestern University in Evanston, Illinois.

It's also one of the first times that researchers have linked a single gene to a complex human behavior. Ultimately, the work could lead to treatments for patients affected by the mutation, which causes a disease called familial advanced sleep-phase syndrome (FASPS), and perhaps even for people with more common sleep disturbances.

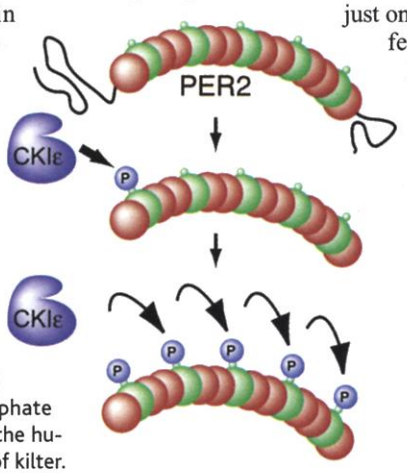
FASPS is an inherited disorder discovered just last year by Utah's Christopher Jones and colleagues. The internal clocks of these patients appear to run fast, shifting their sleep schedules ahead by about 4 hours. Fu and Ptáček's team set out to find the genetic cause of this circadian shift by combing through the DNA of members of a large family afflicted by the disorder, searching for genetic variations associated with the disease.

They ultimately homed in on the end of chromosome 2 as the likely site of the defec-

CREDIT: CHAN *ET AL.*

tive gene—an intriguing finding, as that's where the human equivalent of one of the animal circadian clock genes is located. The researchers soon discovered that most of the family members afflicted with FASPS, but none of those unaffected, carry a single base-pair mutation in one copy of this gene, known as h*Per2*. In addition to fingering h*Per2* as the culprit in FASPS, the finding also provided a clue as to how the mutation might be exerting its effects.

The mutation maps to a region of the h*Per2* protein, known as PER2, that looks like it might be a target for phosphate addition by an enzyme called casein kinase Iε. Last year, Takahashi's group discovered that hamsters suffering from a FASPS-like disorder carry a mutation in the gene encoding just that enzyme (*Science*, 21 April 2000, p. 483). The kinase seems to help maintain the proper 24-hour cycling of the mammalian circadian clock by phosphorylating PER proteins.



Roadblock. When human PER2 is mutated, casein kinase Iε (CKIε) can't add the first phosphate to the protein, throwing the human circadian cycle out of kilter.

Clock researchers have found that PER and other clock proteins accumulate during the 24-hour circadian cycle until they reach a concentration that acts to shut down genes, including the *per* genes themselves. The proteins' concentrations then decline until this inhibition is relieved and they start accumulating again in the next day's cycle. The correct timing of the gene down-regulation seems to depend, in part, on phosphate addition to the PER proteins by a kinase enzyme, possibly casein kinase Iε. Takahashi's team found that the mutant kinase does not phosphorylate PER proteins as well as the normal enzyme does. Combined with evidence from PER studies in fruit flies, this led the researchers to propose that, as a result, PER might build up faster than it should, shortening the circadian cycle.

The new work by the Fu and Ptáček team now provides evidence for a similar scenario in humans. They've shown that mutated fragments of PER2 are not phosphorylated as readily by casein kinase Iε as are fragments from the normal protein. So even though the human mutation differs from the hamster mutation, its similar effects on PER phosphorylation are likely to result in similar consequences: early PER2 buildup and an accelerated cycle. "This paper fits in so beautifully with [the hamster] story," says Takahashi.

But the story is far from over. Still uncertain, for example, is whether the h*Per2* mutation has other effects that might contribute to the acceleration of the body's circadian clock. In addition, the mutation is probably just one among many that can affect human clocks. Indeed, Fu and Ptáček have identified two dozen families who suffer from FASPS without carrying the h*Per2* mutation.

Ptáček now wants to search for small molecules that affect the phosphorylation state of PER2 as a first step toward developing drugs that either speed up or slow down the clock—helping not only FASPS patients but perhaps run-of-the-mill early birds or night owls, as well as jet-lagged travelers and night-shift workers. In the future, a pill may be all it takes to fulfill Franklin's sage advice.

–MARINA CHICUREL

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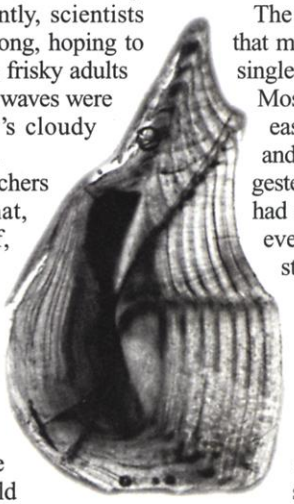
FISHERIES SCIENCE

Ear Bones Reveal Homing Tendencies

For centuries, anglers along the marshy Delaware Bay have eagerly awaited the annual return of the weakfish, a blue-gray finned delicacy that crowds into the estuary each spring to spawn. Recently, scientists have joined the expectant throng, hoping to test a hunch: that many of the frisky adults making whoopee beneath the waves were themselves born in the bay's cloudy waters just a few years earlier.

Now, on page 297, researchers offer intriguing evidence that, contrary to common belief, weakfish do indeed have a strong homing instinct. The finding, based on a study of chemical isotopes bound up in the fishes' tiny ear bones, suggests that many marine fish populations may be more complex than once envisioned. The research could also prompt fisheries managers to rethink how they regulate catches and help conservationists design more effective marine reserves.



Lend me your ears. Weakfish otoliths, or ear bones, provide geochemical clues to the fish's birthplace.

Biologists have long known from tagging and genetic studies that salmon and some other anadromous fish have a remarkable ability to navigate back to their birth rivers after years at sea. But documenting similar "natal homing" in fish that spend their entire lives in salt water has proved difficult. One problem is that these fish generally don't display telltale genetic differences; they also produce young that are too small, too numerous, or too dispersed for easy tagging or recapture.

To get around these difficulties, Simon Thorrold, currently of the Woods Hole Oceanographic Institution in Massachusetts, and his team looked to chemical clues contained in otoliths, tiny concretions that form in the ears of many fish. As the otolith grows, each new layer of calcium carbonate captures the chemical signature of the surrounding water. As a result, the bony pebble "acts like a flight recorder, encoding time-specific information about the waters through which the fish passes, from birth to death," says Robert Warner, a fish biologist at the University of California, Santa Barbara.

To begin their study, in 1996 Thorrold's team analyzed otoliths from hundreds of juvenile weakfish caught in Delaware Bay and four other major estuaries along the East Coast of the United States. Each estuary, they discovered, has a unique geochemical signature created by different ratios of chemical isotopes, including carbon-13, oxygen-18, and various forms of magnesium, barium, and strontium. The team returned to the same areas in 1998 and captured 2-year-old weakfish fresh from their wintering grounds off Cape Hatteras, North Carolina. The researchers then analyzed the central cores of the adult fishes' otoliths, formed when the half-meter-long spawners were mere minnows, to see where they originated.

The results were "surprising," given that most biologists view weakfish as a single coastwide stock, says Thorrold. Most of the 2-year-old fish could be easily matched to their home waters, and subsequent statistical work suggested that up to 81% of the spawners had found their way back home. And even the 19% to 40% of fish that strayed to new spawning grounds didn't miss by much, typically ending up in adjacent estuaries.

That mixing probably explains why weakfish up and down the coast are genetically similar, Thorrold says. But it may be a mistake, he adds, for state and federal fisheries managers to ignore the geographically distinct spawning populations even if they are not genetically different. "You can't assume that vagrants from one estuary can

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