GENETICS

Zebrafish Earns Its Stripes In Genetic Screens

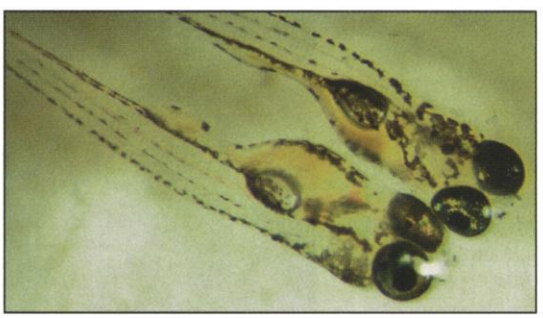
Researchers are using the zebrafish to search for a variety of genes involved in everything from obesity to bone diseases

COLD SPRING HARBOR, NEW YORK—In biology, as in mechanics, one of the best ways to figure out how something works is to break it. For decades, biologists have followed that principle, randomly mutating genes in experimental animals to discover the roles of those altered genes and the proteins they encode. Until recently, most of these studies have been performed on fruit flies, nematode worms, or mice—each of which has its own staunch advocates. But in the last few years a new creature has joined the cadre of laboratory mutants: the zebrafish.

Originally from the tropical rivers of India, the zebrafish has long been a favorite of aquarium keepers. In the scientific arena, developmental biologists first took a shine to the animal because its clear embryos offer an unparalleled opportunity to watch the development of vertebrate tissues and organs not present in the simpler worm or fly. Now, it seems, biologists of all stripes are clamoring for this lab-friendly creature, as was evident at a recent meeting.* Using a variety of clever techniques to identify mutants, scientists are using the zebrafish to probe the genes involved in a wide variety of human maladies, from bone diseases to obesity.

Because it is a vertebrate, the zebrafish is genetically closer to humans than flies or worms, and its small size, quick generation time, and inexpensive care make it possible to keep thousands of fish in a single lab, says Marnie Halpern of the Carnegie Institution of Washington in Baltimore, Maryland. Add to that the transparency of its young, and you have what some consider an ideal lab animal. "The only limit is the creativity and imagination of the scientists," says Halpern. Hoping to inspire that creativity, officials at the U.S. National Institutes of Health are offering $4.5 million dollars next year to fund efforts to devise new screens for mutants in zebrafish.

The slender fish might not seem like an obvious choice as a model for obesity research, but molecular neuroscientist Wolfgang Liedtke of The Rockefeller University in New York City believes it is. Liedtke is a postdoctoral fellow in the laboratory of geneticist Jeffrey Friedman, who with his colleagues originally identified the leptin protein in mutant mice. Leptin is a key part of the body's system of weight regulation. Although the mouse has been "a tremendous tool" for understanding weight regulation, Liedtke says, the fish is a welcome addition. Because an investigator can create so many mutants in a single laboratory, he or she is likely to turn up new genes involved in the complex pathway, he says, whereas for a similar effort in the mouse "you would need a factory."



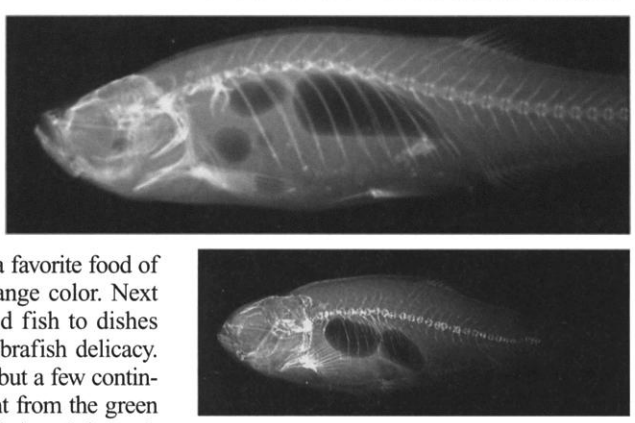
Fill 'er up. Zebrafish larvae fed orange brine shrimp show characteristic full stomachs (arrow).

Mice missing the leptin protein or its receptor never seem to feel full. They continue eating indefinitely, growing obese. To find mutant fish with similar behavior, the researchers took advantage of the animals' clear bodies to identify abnormal feeding patterns. They created mutants by exposing zebrafish males to a chemical that causes mutations in sperm-producing cells. They placed the males' inbred offspring in petri dishes with an unlimited supply of brine shrimp, a favorite food of zebrafish with a distinct orange color. Next they transferred the well-fed fish to dishes containing algae, another zebrafish delicacy. Most fish ignored the algae, but a few continued to munch, as was evident from the green algae on top of the orange brine shrimp already visible in their stomachs. The Rockefeller team has identified three families of fish that seem insatiable. One of the mutants, dubbed *jumbo*, grows considerably larger than normal zebrafish. Another, called *fressack* (a German term for a hearty eater), is no larger than normal despite its gluttony.

Because mice missing leptin or its receptor also have problems regulating their body temperature, the scientists designed a screen for mutant zebrafish that might have similar defects. Fish are cold-blooded; they regulate their body temperature by swimming in warmer or cooler water. Liedtke and his colleagues rigged a tank with a temperature gradient. Normal zebrafish remain tightly clustered in one region of the tank—between 27° and 28°C. However, one mutant family, dubbed *hot body*, prefers water several degrees warmer. It also lacks satiety. Friedman cautions that it may be difficult to identify the mutated genes responsible for the abnormal behavior. He notes that in mouse mutants, appetite and satiety characteristics are notoriously variable and sometimes prove difficult to track down. Liedtke agrees, but he is optimistic that his zebrafish work will pay off.

When Shannon Fisher, a developmental biologist at The Johns Hopkins University School of Medicine in Baltimore, began looking for bone mutations in zebrafish, the standard screen for bone structure required killing each potential mutant—often valuable fish that had required careful tending to survive to adulthood. Her training as an M.D., however, gave her an alternative: x-rays. With some initial help from her dentist and Purdur Jagadeeswaran at the University of Texas Health Science Center in San Antonio, and a bit of trial and error, she has designed a screen in which she anesthetizes the fish, takes a quick x-ray, and then returns them to their tank, where they revive in a few minutes. "It's easy and convenient," she says, and it has already paid off.

One of the first mutants she identified, called *chihuahua* for its rounded forehead and small jaw, has some of the same symptoms as humans with the disease osteogene



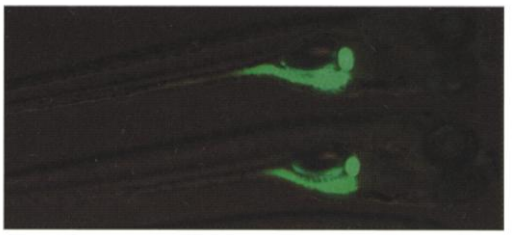
Fragile bones. An x-ray of the *chihuahua* mutant *(above)* reveals broken bones and a misshapen face compared to normal zebrafish *(top)*.

CREDITS: (TOP) S. FARBER/THOMAS JEFFERSON UNIV. AND M. PACK/PENN SCHOOL OF MEDICINE; (BOTTOM) S. FISHER/JOHNS HOPKINS SCHOOL OF MEDICINE

* Zebrafish Development and Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 26 to 30 April.

sis imperfecta. The mutant initially caught her attention with its small size. When she x-rayed it, she found a mass of broken ribs. On closer inspection of the mutant strain, she found that it forms cartilage normally during early development, but its bones in adulthood are fragile. Although she has not identified the exact mutation at fault, it seems to be near a collagen-encoding gene fingered in human cases of osteogenesis imperfecta. Fisher suspects that this mutant strain could prove valuable for research into collagen's role in bone formation and maintenance.

Whereas broken bones are relatively easy to spot, problems in biochemistry can be harder to detect, even in the see-through zebrafish. But here, too, scientists are hoping the animal will help answer some difficult questions. Steven Farber of Thomas Jefferson University in Philadelphia and Michael Pack of the University of Pennsylvania School of Medicine in Philadelphia and their colleagues have devised a way to observe the biochemical reactions of digestion in living zebrafish. To identify genes that regulate one part of digestion, lipid processing—known to influence the development of colon cancer, heart disease, and other human ills—the team has designed lipid molecules that glow when they are digested by a key enzyme in the intestine. When the scientists feed this molecule to zebrafish larvae, they can see the molecule light up in the digestive tract and liver and then travel to the gallbladder. Although the screen is in its early stages and the scientists have only begun to identify potential mutants, developmental biologist Didier Stainier of the University of California, San Francisco, is impressed. "If you can actually ask questions about how these intestinal cells are processing a substrate, that's very powerful," he says. The team hopes to design other molecules to probe the digestion of carbohydrates and other molecules. "We're visualizing biochemical processes in living vertebrates," says Farber. "Zebrafish is the only game in town where you can do that."



Healthy glow. A custom-made lipid, designed to identify fish with faulty digestion, lights up in the digestive tract of zebrafish larvae.

The most difficult part of the process is still tracking down the mutant gene itself, but scientists say that ongoing work in zebrafish genomics is making that task easier. And the likely launch of an effort to sequence the zebrafish genome (*Science*, 5 May, p. 787) will also ease that task. Genome projects in the mouse and human, says developmental biologist Nancy Hopkins of the Massachusetts Institute of Technology, will only make the zebrafish more important. Those projects will turn up thousands of unknown genes, she says, and it is likely to be easier to figure out what they do in the fish. Says Hopkins: "We've barely begun to tap" the potential of zebrafish. **–GRETCHEN VOGEL**

DIGITAL ENCRYPTION

Algorithmic Gladiators Vie For Digital Glory

As NIST zeroes in on a new cryptographic standard, the competitors scramble to face an unforeseen threat—from lawyers

And then there were five. For 2 years, glory-seeking cryptographers from across the globe have been cracking one another's ciphers, trying to establish their own algorithms as the new standard in encryption. In mid-April the five finalist teams faced off in New York. They subjected each other's algorithms to the withering fire of cryptographic attack after cryptographic attack, while judges observed the melee. But as the smoke cleared, the contestants found themselves facing a menace from a new and unexpected quarter: the realm of patent law.

The five finalist algorithms—MARS, Twofish, Rijndael, RC6, and Serpent—are vying to be the new standard in encryption, replacing the aging Digital Encryption Standard (DES), endorsed by the National Bureau of Standards in the mid-'70s. Thanks to the government's stamp of approval, DES has become perhaps the most widely used encryption system in the world. The new algorithm, selected by the National Institute of Standards and Technology (NIST)—the Bureau of Standards' successor—will replace DES and should assume its mantle of preeminence. No money is at stake in the competition; under NIST's licensing terms, the inventor of Advanced Encryption Standard (AES) will not benefit financially. "The big thing, personally, is the fun of doing it," says John Kelsey of Counterpane Internet Security in San Jose, California. "If you're in block ciphers, it's the coolest thing you can do, as far as I can tell."

Outside the arena, however, the stakes are serious indeed. If someone were to crack the AES a few years down the line, all the reams of data encrypted with NIST's standard could be compromised. Medical records, bank transactions, and other confidential information would potentially be wide open to anyone with the know-how, and it would take years for engineers to replace the cracked algorithm in smart cards, computers, and descrambler boxes.

Fears of such a breach are what drove officials to seek a replacement for DES in the first place. DES was designed to take a stream of digital data, split it into 64-bit chunks, and encipher it. In theory, an eavesdropper could not decipher the data without guessing the 56-bit cryptographic "key" that opens the cipher—a secret shared by only the sender and intended receiver.

By the early 1990s, fissures had begun to show in DES's security. Cryptographers such as Eli Biham and Adi Shamir of the Technion–Israel Institute of Technology in Haifa developed new attacks such as "differential" cryptanalysis, in which a cryptographer tries to crack an algorithm by feeding very slightly different data into it and comparing how the encrypted outputs differ. As a result, instead of having to guess 56 bits of a key (which requires a search through 2^{56} possible keys), would-be crackers could decipher the message after trying only 2^{46} keys or so—a 1000-fold improvement. More important, computers got faster. DES was being cracked by brute-force searches in which speedy computers simply tried every possible key. Last year, in response to a challenge by the San Jose–based cryptography company RSA Security, volunteers yoked nearly 100,000 PCs together via the Internet to decipher a DES-encrypted message. They succeeded in less than a day. To beef up security, wary DES users started running the algorithm three times with three different keys. NIST, however, decided that patches were not enough. In 1997, the institute called for a new standard; the AES contest was born.

Cryptographers from all over the world submitted candidate algorithms, from which NIST selected 15. Two years and a lot of code-cracking and skirmishing later, NIST narrowed the field to five finalists—and the international cryptographic community turned its attention to testing, and breaking, them.

On 13 and 14 April, participants in the

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