EVOLUTIONARY BIOLOGY

Twinned Genes Live Life in the Fast Lane

The financial rewards of genome sequencing may go to the companies, but the intellectual fruits of this multimillion-dollar enterprise are going to the likes of evolutionary biologist Mike Lynch and computer scientist John Conery. On page 1151, this duo at the University of Oregon, Eugene, describes new insights into how genes arise and fuel evolution. By trolling through sequence data for nine very distinct organisms, they have uncovered evidence that genes are copied far more frequently—and the duplicates are lost from the genome far faster—than researchers had thought. What's more, the work suggests that some duplicate genes play a key role in the evolution of new traits and in speciation.

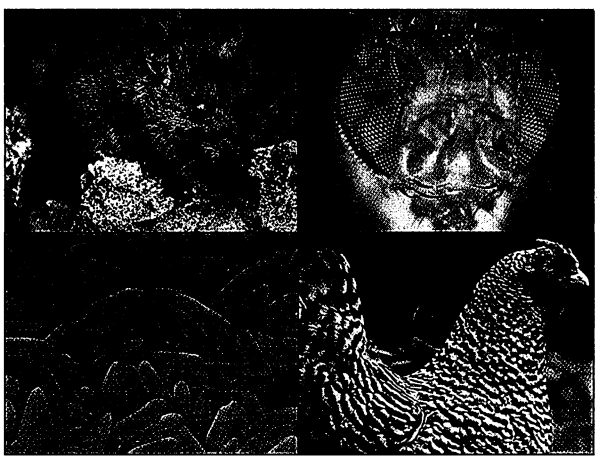
Although some researchers question the Oregon team's conclusions, the report is nevertheless "a very nice example of how the creative analyses of genomic databases can provide valuable but previously inaccessible information about evolution," says Loren Rieseberg, an evolutionary biologist at Indiana University, Bloomington.

More than 30 years ago, geneticist Susumo Ohno of the City of Hope Hospital in Los Angeles proposed that genomes grow and diversify by gene duplication, an idea that most evolutionary biologists have since come to accept. It seems that when—by some quirk of DNA replication—a gene, piece of a chromosome, or whole genome is copied twice, the "extra" genes can take on a new function and expand the organism's genetic repertoire. This extra copy might become active at a different time in development or in a different tissue, or it may undergo base changes that alter the properties of the protein encoded by that gene. Yet until recently, researchers had no good way to determine whether Ohno was right. "They have had only a few duplicates" with which to try to estimate the life-spans of twinned genes, explains Andreas Wagner, an evolutionary biologist at the University of New Mexico, Albuquerque, and the Santa Fe Institute.

But thanks to the recent flurry of genome sequencing—the genomes of the fruit fly, yeast, and nematode are virtually complete, and others are close behind—Lynch and Conery were able to get a more comprehensive view of the potential of duplicate genes for furthering evolution. To do so, Lynch and Conery used a computer program to find duplicate genes in the three completed genome sequences and among all the protein-coding sequences available for the mouse, chicken, human, rice, and the plant *Arabidopsis thaliana*.

The researchers relied on sequence differences in the matched copies to estimate the age of each copy, as differences accumulate through time. Specifically, they counted the number of silent nucleotide base changes—those that didn't alter the protein code—to date the duplication event. Then they compared the number of silent changes to the number of base changes that caused protein alterations. This ratio told them whether the copy was changing faster or slower than expected.

Lynch and Conery found that most of the duplicated genes are relatively young and that extra genes disappear quickly, at least on an evolutionary time scale. In the human and mouse, for example, about half of new copies disappear within 7.3 million years.



No matter the species. Genes in organisms as diverse as the mouse, fruit fly, chicken, and rice *(clockwise from upper left)* undergo frequent duplications.

Perhaps most surprising, the two found an "astronomical rate of gene duplication," says Sally Otto, an evolutionary biologist at the University of British Columbia in Vancouver, Canada. In fact, duplications occur as often as single-base changes within genes, which have long been considered the primary means by which genomes evolve. The rates are similar among such disparate organisms as fruit fly and yeast, notes Lynch; a genome with 15,000 genes could acquire between 60 and 600 duplicate genes over a million years as fodder for speciation. "Gene duplications are so frequent that we really need to take them into account as an important source of genetic variation," says Wagner.

Nor do genes need to morph much before they begin to divide one species into two, Lynch suggests. For example, if a population carrying a recently twinned gene

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Silver Lining Advocates of more controls on human subjects research will be getting help from Paul Gelsinger, father of 18-year-old Jesse Gelsinger, who died last year in a gene therapy trial at the University of Pennsylvania in Philadelphia (*Science*, 12 May, p. 951). Gelsinger received a "significant" financial settlement from Penn last week, his attorney says, after agreeing to end a malpractice suit. As part of the deal, Gelsinger dropped two defendants—former medical school dean William Kelley and Penn bioethicist Arthur Caplan, who gave informal advice on the trial's design. Caplan says, "It would be horrible to have anyone sued for expressing an opinion to a colleague."

Gelsinger intends to use the funds to form "a private foundation to support a few organizations that we consider ethical," including the National Organization for Rare Disorders in New Fairfield, Connecticut, and Citizens for Responsible Care & Research in New York City. Gelsinger adds: "We need legislation to protect research subjects by imposing stiff fines and jail time for violators."

Planning Ahead When you are shelling out $2.4 million per day, it pays to plan ahead. That is the conclusion of Britain's mammoth biomedical charity, the Wellcome Trust, which this week released its first-ever 5-year plan. The roadmap will guide the $22 billion charity's increasing spending, which has tripled over the last 3 years to about $900 million per year, says trust director Mike Dexter.

According to the 14-page document, the trust will spend nearly $4.5 billion by September 2005 on a wide variety of projects around the globe, including research grants, lab construction, education, and its share of constructing the new Diamond synchrotron near Oxford. The trust will also create a $390 million fund to support unexpected "emerging research opportunities." Wellcome, however, will not feel bound by the document if priorities change, Dexter says: "The plan is not written in stone. Every year we will be evaluating things."

Drilling Denunciation Scientists have taken a stand against drilling in Alaska's oil- and wildlife-rich Arctic National Wildlife Refuge. More than 240 scientists and resource managers released a letter to President Clinton on 1 November asking him to permanently protect the refuge. The long-running issue came up again in this year's presidential election, with candidate George W. Bush saying he would consider drilling and Al Gore vowing to bar it. The impacts of drilling, the letter signers say, have not "been adequately considered."

Rosenfeld of the University of California, San Diego, and Aneel Aggarwal of Mount Sinai School of Medicine in New York City and their colleagues points to an answer.

To exert its effects, Pit-1, like other transcription factors, has to bind to a regulatory sequence on its target genes. The Rosenfeld-Aggarwal team has shown that a small sequence variation between the regulatory elements of the prolactin and growth-hormone genes causes Pit-1 to bind very differently to the two. As a result, when Pit-1 binds to the regulatory region of the growth-hormone gene in prolactin-producing cells, it apparently attracts proteins that suppress the gene's activity, whereas on the prolactin gene it attracts activating coregulators. "They're basically arguing that a given regulatory factor can act as a switch, in some cases causing activation and in others repression," says developmental biologist Michael Levine of the University of California, Berkeley (UCB).

It's not unusual for transcription factors to have dual functions, but until now Pit-1 was thought to be an activator only. Beyond that, the finding supports the idea that the sequences that bind transcription factors are more than just docking sites. Instead, as work with another set of transcription factors, the so-called nuclear receptors, has already shown, small changes in these sequences can influence the three-dimensional structure of the bound factor. And that structural change can in turn influence which other proteins bind to the transcription factor on the regulatory site—and ultimately, whether genes are turned on or off. "The information is in the primary DNA sequence, as Watson and Crick told us," Rosenfeld says.

The Rosenfeld team started their experiments by asking what role Pit-1 binding sites might play in selective expression of a target gene. To find out, Scully introduced two genes separately into mice: one with the normal regulatory region for growth hormone and one in which the Pit-1 binding regions were replaced by a comparable element from the prolactin gene.

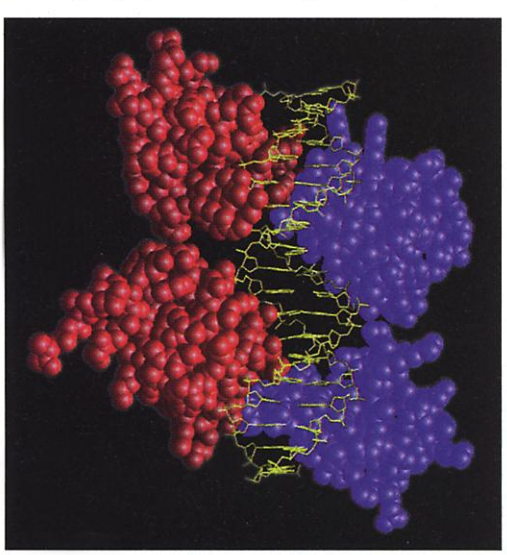
As expected, the gene with the normal regulatory element was expressed only in the growth hormone–producing cells (somatotropes) in the animals' pituitaries. But the gene with the mutant sequences was expressed in both the growth hormone– and prolactin-producing cells (lactotropes). These results indicate that Pit-1, when bound to the normal growth-hormone sequence, somehow keeps the gene "off" in the prolactin-producing cells while allowing it to be "on" in the growth-hormone cells. Indeed, cell biologist Keith Yamamoto, also of UCB, notes that the Rosenfeld team's results imply that gene repression is Pit-1's default activity. That finding, he says, "is a real surprise," and suggests that "the growth hormone and prolactin elements evoke distinct configurations of Pit-1 that somehow produce distinct patterns of activity."

X-ray crystallographic studies performed by Aggarwal and his Mount Sinai colleague Eric Jacobsen in collaboration with the Rosenfeld group support that idea. They showed, for example, that two of the protein's characteristic regions, or "domains," end up on perpendicular faces of the DNA of the prolactin element but are on the same face of the DNA of the growth-hormone sequence. This difference can be traced to the presence of two extra bases in the Pit-1 binding site of the growth-hormone gene. Further work indicates that the shape change induced in Pit-1 by this difference enables it to recruit repressive proteins to the growth-hormone gene.

These results help explain why growth hormone is "off" in lactotropes; what is still unclear is how the gene is turned on in somatotropes. "That's the crucial question," Rosenfeld says. "You have to have an override mechanism" to eliminate the repressive effects of the gene's regulatory sequences.

Despite the unanswered questions, Levine is impressed that the team has gone so far—"from transgenic animals to crystallography"—in explaining the cell specificity of Pit-1's effects. Given that a similar mechanism has already been found by Yamamoto and others for nuclear receptors, which regulate gene expression in response to steroid hormones and retinoids, Levine suspects it may be widespread: "You can envision many proteins where this can happen."

–JEAN MARX



CREDITS: (LEFT TO RIGHT) H. VIADIU; USGS

Off site. When bound to the regulatory element of the growth hormone gene, as shown here, Pit-1 represses the gene expression in lactotropes.

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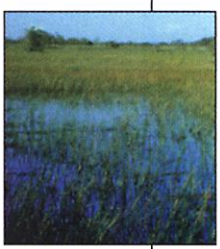
Everglades Green Light Environmentalists last week celebrated congressional approval of the first phase of a $7.8 billion Everglades restoration plan, but some scientists are withholding their applause until outside experts review the project.

Under the Everglades "restudy," the U.S. Army Corps of Engineers would undo one of its main engineering feats: a system of pumps and levees that since 1948 has diverted water that once flowed south from Lake Okeechobee to Florida Bay. To restore water needed by wildlife, the corps now plans to rip out levees and canals and store water in aquifers and reservoirs.



The bill, headed for President Clinton's signature, allots the first $1.4 billion for the 20-year project. But some scientists say the plan relies too much on engineering solutions and should have been peer-reviewed. Until a National Academy of Sciences advisory committee weighs in, says Columbia University ecologist Stuart Pimm, "it's an open question whether this plan [will] have any ecological benefit."

Next Question? The U.S. Equal Employment Opportunity Commission (EEOC), which has been investigating alleged discrimination against minorities and women at Lawrence Livermore National Laboratory (LLNL) in California (see p. 1072), is catching some flak from lab officials over a survey.

After the EEOC e-mailed the questions to many nonwhite and female Livermore employees on 13 October, lab managers shot back with an e-gram of their own. The survey was "a unilateral action taken by EEOC without our knowledge," LLNL's public affairs office wrote. "We have serious concerns about their methodology and we don't believe the confidentiality of the survey responses can be maintained." It added that staff were "not obligated to respond."

Several Asian-American staff protested, saying the lab e-mail amounted to intimidation. And the EEOC warned survey recipients not to answer electronically, as "email from LLNL may be read by LLNL." But Livermore managers say they were merely answering employees' questions about the survey. And one says the EEOC was sloppy: "They missed half the Asian Americans in the lab." Since the flap, lab director Bruce Tarter has met with some Asian-American employees, assuring them that the lab will be working closely with EEOC investigators.

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