



From telomere study, a new checkpoint gene

Perseverance and serendipity yield a gene that plays a critical role in the repair of damaged DNA

BY RENEE TWOMBLY

Tracking Rif1. In cells that have been exposed to radiation, scientists have discovered that a protein called Rif1 shows up at sites of DNA damage. Rif1, they say, is linked to a cell's ability to repair DNA and is part of a known cancer susceptibility pathway.

Joshua Silverman knew Edison's quote about genius, but he was approaching that 99 percent perspiration mark, and the one percent inspiration wasn't kicking in. His long, labored experiments, the basis of his doctoral thesis, were not paying off, and his goal of earning a Ph.D. from Rockefeller University was beginning to seem out of reach.

For his graduate studies in **Titia de Lange's** Laboratory of Cell Biology and Genetics, Silverman was exploring whether a gene that lengthens the ends of chromosomal caps called telomeres in yeast cells might have a counterpart that plays the same role in human cells. If so, it could lead to

clues about how an enzyme called telomerase can selectively add DNA back to chromosomes, lengthening the life span of a cell.

Over the years de Lange, who is Rockefeller's Leon Hess Professor, and the members of her lab have made fast progress in understanding telomeres, the specialized DNA complexes that, like the plastic sleeves on shoelaces, stop chromosomes from unraveling. To understand how chromosomes are controlled and how they are linked to aging and cancer, de Lange has characterized stuttering sequences, duplicitous enzymes and loopy hooks.

When she and Silverman embarked on their project, they expected a fairly uncomplicated process. To find the human counterpart to their yeast gene, Rif1, they would look through genetic databases. "Then Josh would make antibodies to Rif1, show that the protein is present at chromosome ends, study how it regulates telomeres, and he would get his Ph.D.," recalls de Lange. "We anticipated this would be a straightforward story."

But the protein wasn't on telomeres — and the scientists couldn't find any evidence it controlled telomere length.

After a year of experiments, Silverman and de Lange

Barcoding life

Short stretch of DNA sequence is a fast, accurate method for identifying species

BY BETSY HANSON

"For humans, birds are probably the easiest species to identify. They're big, they're colored differently, and they sing different songs. Yet even in that easy to identify group, there are hidden species that have been difficult to distinguish," says Mark Y. Stoeckle, M.D., guest investigator in the Program for the Human Environment at Rockefeller University.

If Stoeckle gets his way, different bird species — in fact, all species of plants and animals — may be as simple to recognize as apples and oranges. Stoeckle, working with scientists at two other institutions, has shown that small stretches of DNA can be read like

grocery-store barcodes to identify species.

Taxonomists traditionally have classified organisms on the basis of their physical characteristics. They use DNA too, but current techniques are labor intensive and difficult to compare.

Zoologist Paul Hebert, at the University of Guelph, last year proposed that a short DNA sequence from a gene found in all animals can be used to identify species because in each species the sequence varies slightly. He coined the term DNA barcode for this idea.

The technique depends on analyzing a portion of a gene called cytochrome c oxidase

I (COI) that is found in the power sources of cells. Most DNA is found in the nucleus of a cell. Mitochondria, however, the organelles within cells that are responsible for energy production, also contain DNA. Mitochondrial DNA (mtDNA) is known to accumulate mutations three to five times faster than DNA in the nucleus. As a result, the mtDNA of closely related species differs more than the nuclear DNA of those species.

When the researchers analyzed COI sequences from 260 bird species that breed in North America, they found that each had a distinct COI sequence. For 130 of these

species, the researchers looked at the DNA of two or more individuals. The variation in sequences between species — even closely related ones — was on average 18 times higher than the variation among individuals within the same species.

In a few cases, however, the scientists found two distinct COI barcodes within the species. In fact, the DNA barcoding technique has led to the discovery of four new species of North American birds. In the case of the solitary sandpiper, eastern meadowlark, marsh wren and warbling vireo, the scientists say there are actually two distinct species where there used

Genetic bug zapper

To prevent disease, scientists seek to keep mosquitoes from smelling

BY BETSY HANSON

“We’ve reached an impasse in the fight against bad insects,” says Rockefeller’s **Leslie Vosshall**. “Insect-transmitted diseases take a large toll, but most insect repellents are based on trial and error, or folk remedies.”

New research from Vosshall’s Laboratory of Neurogenetics and Behavior, however, is bringing hard science to the design of the lowly bug spray. In a study published in the September 2 issue of *Neuron*, Vosshall and her coworkers have discovered that insects’ ability to detect odors — and people whom they can bite — depends on a single gene. Fruit flies lacking the gene, known as Or83b, lack the sense of smell.

dish. When the researchers administered a dose of the Or83b receptor, delivered to the odor-sensing cells through a sort of fruit fly gene therapy, the fly larvae were “cured” — like the normal larvae, they crawled toward odors. (See illustration.)

To confirm that Or83b is necessary for sensing all odors, Vosshall and her colleagues also examined the larvae under a microscope, and found that in flies lacking Or83b receptors, the other smell receptors ended up in the wrong part of the nerve cells — in the body of the cell, instead of at the ends of the cell’s arm-like dendrites, where they would be exposed to odor molecules

were prepared to accept that Rif1 simply wasn’t where they thought it ought to be. In a last-ditch effort to salvage some knowledge from their work, de Lange suggested they see what would happen if they exposed human cells to radiation. “In yeast, Rif1 is part of a pathway that is also involved in a cell’s response to radiation, so we thought this might point us toward the function of Rif1 in human cells,” de Lange explains.

The experiment was a success. It turned out that human Rif1 did do something interesting; they’d just been looking in the wrong place. The radiation-damaged cells showed Rif1 on the sites where the DNA had been broken by the radiation. “We thought Rif1 would be sitting on natural chromosome ends, but we instead found it only binds to ends that are made when DNA is damaged,” de Lange says.

Thanks to that early failure, we now know that Rif1 is closely linked to a cell’s ability to repair DNA, and it is part of a pathway that includes several well-studied cancer susceptibility genes including BRCA1, implicated in breast and ovarian cancers.

“This is one of the most intensely studied pathways in the cell because of its importance to cancer biology, and through completely fortuitous ways, we bumped into an important component of that pathway,” says de Lange.

Encouraged, Silverman tested whether Rif1 was controlled by a master regulator of the DNA damage response known as Ataxia Telangiectasia Mutated (ATM) kinase, named after a faulty gene that predisposes people who inherit it to get cancer or other diseases.

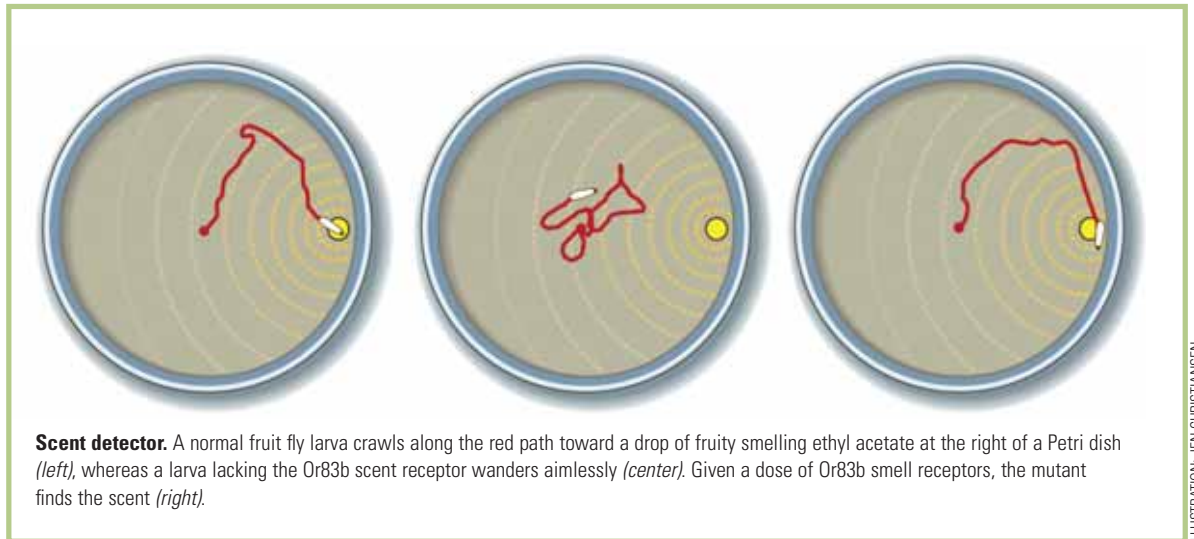
Researchers have found that the ATM kinase blocks a cell’s ability to divide when its DNA is damaged, and that mutations of a few key players within the ATM pathway are implicated in several human cancers, de Lange says.

“Downstream of ATM kinase are the disease genes BRCA1, BRCA2, Chk2, Mre11 and Nbs1,” she explains. “So if all the players in this little regulatory neighborhood in the cell are all involved in human cancer predisposition, it doesn’t take much to suspect that Rif1 might also be.”

When they examined cells that lacked ATM kinase, the Rockefeller researchers found that Rif1 had lost the ability to bind to damaged DNA, showing that Rif1 is a slave to ATM’s instructions. “There are several master regulators of the DNA damage response, and they all funnel through the same proteins, so we assumed that if we knocked out ATM, Rif1 might still respond,” says de Lange. “But that wasn’t so. Rif1 only listens to ATM, and is not regulated by anyone else. That makes it very unusual.”

The de Lange group also discovered that Rif1’s role was to halt the replication of a damaged cell so that the DNA could be repaired. Postdoctoral researcher Hiroyuki Takai used small strands of RNA to silence the Rif1 gene, and found that when these cells were exposed to radiation, they continued to synthesize their DNA. “And if chromosomes replicate when they are damaged, you get a real mess, a lot of replication mistakes that could potentially create a cancer cell,” de Lange says.

“The story turned out to be much more significant than we expected,” says de Lange. Not only did the scientists uncover a possible new player in the promotion of cancer, but de Lange expanded the focus of her lab to include this aspect of cancer biology and the project is now continued by postdoctoral fellow Sara Buonomo. And Silverman, for his part, got a cover article in the September 1 issue of *Genes & Development* and, finally, in June of this year, three new letters after his name.



Scent detector. A normal fruit fly larva crawls along the red path toward a drop of fruity smelling ethyl acetate at the right of a Petri dish (left), whereas a larva lacking the Or83b scent receptor wanders aimlessly (center). Given a dose of Or83b smell receptors, the mutant finds the scent (right).

ILLUSTRATION: JEN CHRISTIANSEN

Smell is very direct. In order for humans or fruit flies to smell bananas, for example, molecules from the fruit must waft through the air to specialized nerve cells that detect them. In humans these cells are at the top of the nasal passages. In the fruit fly they are located on the antennae and the maxillary palp, an appendage near the fly mouth. Odor-carrying molecules bind to receptors on brain cells, fitting like chemical keys into the lock-like receptors, and set off a series of signals that the brain perceives as an odor.

A different gene codes for each kind of receptor, and the Or83b receptor is unusual in that nearly all the neurons that enable a fly to smell have it. Other kinds of receptors are divided up among small groups of olfactory neurons.

The Rockefeller researchers wanted to determine whether Or83b receptors detected only one or a few scents, or if they were general odor detectors that underlie the fly’s ability to smell any scent. In the second case, knocking out the gene would render the insects unable to detect — and bite — humans.

The scientists used a technique called gene targeting to create a strain of fruit flies lacking the Or83b gene. Then they tested the flies’ sense of smell by placing larvae one at a time in the middle of a Petri dish, with a drop of fruity-smelling ethyl acetate near the edge. Because normal fly larvae sense odors via an organ on the top of the larvae’s head, they crawl toward the smell. The mutant larvae, however wandered aimlessly in the

in the air. Finally, using a tiny electrode attached to the antenna of a fly, they tested whether nerve cells there were activated in the presence of odors. The results confirmed their conclusion.

“For a fly to smell anything the Or83b receptor has to be present,” says Vosshall. “We still haven’t figured out how or why it works,” she adds. It could be that Or83b combines with other receptors to form different-shaped “locks” in which odorant molecules fit. Another possibility is that Or83b acts as a chaperone to direct other receptors to their proper placement in the cell. Or Or83b may be essential to the series of molecular signals that trigger perception of a scent.

Because the gene is found in a wide variety of insect species, repellents that block it, and thus prevent disease-carrying insects from smelling and finding human hosts, might eventually help fight malaria and other infectious diseases.

“Insects are the primary vectors for malaria, dengue fever, yellow fever, and West Nile encephalitis, and they locate human hosts largely through their exquisitely sensitive olfactory systems,” says Vosshall. “In regions where these diseases are endemic, bed nets could be impregnated with a repellent that blunts a mosquito’s olfactory response. For backyard barbecues, you might have candles that release a repellent. Having different tools — repellents to ward off insects as well as drugs to fight the diseases they spread — will help us prevent disease.”

to be one.

Based on traditional species identification methods such as morphology and behavior, some taxonomists had already suspected that these species should be split. The DNA barcode data confirmed the suspicions.

“New species won’t be determined by DNA analysis alone,” says Stoeckle, whose results are published in the September 28 issue of *Public Library of Science Biology*.

“Morphology, behavior and vocalization, for example, will still need to be taken into account. But barcoding will enable rapid screening of large numbers of organisms and highlight those that are likely to be new species.”

Beyond the intellectual satisfaction of naming species, DNA barcoding has practical applications. It requires only a small sample of tissue so that wildlife biologists could use it

to identify the stomach contents of animals and reconstruct food cycles. Other uses include identifying birds that fly into airplane engines and testing for protected fish species, for example, that sometimes make their way to market. It also works for identifying organisms at different stages of life, such as the eggs and larvae of insects. And it can easily distinguish between species that look alike.

As the cost of DNA sequencing goes down, the scientists envision developing a hand-held device that could be used for species identification in the field. They also picture building a library of barcodes to help taxonomists stay on the same page (see phe.rockefeller.edu/barcodeconference). “The first step toward a library is to show scientifically that a uniform approach can work. Then museums, ecologists and others can adopt it as a standard,” says Stoeckle.



Species scanner. Multicolored bands represent the DNA barcodes of the honey bee, bumblebee, american robin and hermit thrush. By comparing the barcodes scientists can distinguish between similar species and create evolutionary trees of the genetic distances between them.