CELL BIOLOGY

The Immune System's Compact Genomic Counterpart

Small but powerful, piRNAs protect the genome and may have other functions as well

Parasitic DNA has infiltrated our genome and threatens our future. As in most other animals, much of the human genome derives from self-serving DNA strands known as transposons. These genetic gypsies often jump to new chromosome locations, sometimes disabling genes and even triggering cancer. In the germ line—sperm and eggs and the cells that spawn them—a transposon hopping to a new position can lead to sterility, a disaster from a Darwinian point of view. "Failure to control transposons in most animals is the surest path to extinction," says biochemical geneticist Phillip Zamore of the University of Massachusetts Medical School in Worcester.

For that reason, a specialized group of RNA molecules known as piRNAs (pronounced "pie-RNAs") are the superheroes of animal genomes. Discovered in the past decade, piRNAs team up with certain proteins to shackle transposons in animal germline cells. Together, these protein-RNA combos create a molecular defense that scientists liken to an immune system for the genome. Like our immune system, piRNAs and their partners can tell friend from foe, mobilize a response, and adapt to new invaders. Similarly, our genome guardians have a memory, a record of past threats.

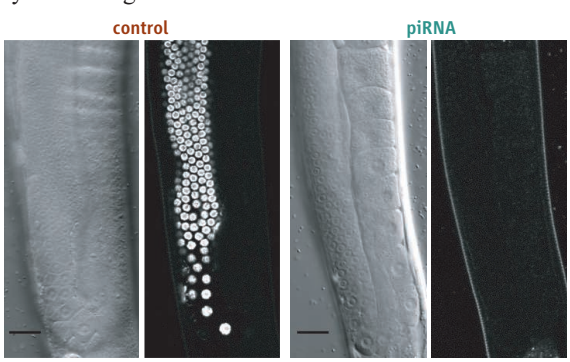
"The complexity of this [piRNA] pathway has exploded during evolution," says Julius Brennecke, a developmental geneticist at the Institute of Molecular Biotechnology in Vienna. The number of piRNA varieties that humans produce isn't clear, but the total could be in the millions. "It's not often that you discover something that is so abundant and that was missed for so long," Zamore says. "It's the perfect scientific problem."

Researchers intrigued by this problem have begun to sketch out details of how these small RNAs keep transposons in check. "We are starting to learn what's in the [piRNA] toolbox," says molecular biologist Ramesh Pillai of the European Molecular Biology Laboratory in Grenoble, France. But biologists still don't know how cells manufacture this type of RNA, or what piRNAs might do outside the cells of the germ line. "In mammals, the transposon-silencing function is just a small piece of what they do, but it's the only piece we understand," Zamore says. One recent study, for instance, raises the possibility that these molecules are important for learning. Researchers are also stumped as to why mice generate hundreds of thousands, even millions, of piRNA varieties that have no known transposon targets. "These are exciting times" in the field, Pillai says.

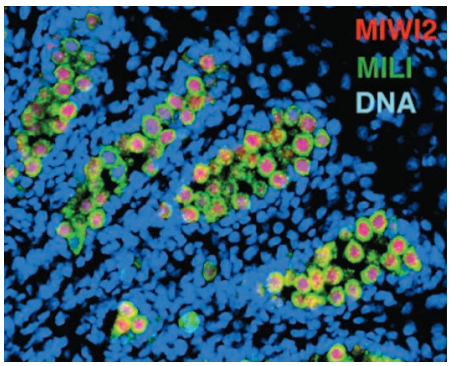
Discovery of a new RNA

When researchers first detected piRNAs in 2001, they were just beginning to grasp the importance of so-called small RNAs. These molecules, which are typically between 18 and 40 nucleotides long and don't code for proteins, were proving ubiquitous. "Small RNAs have been harnessed by almost every single life form we know," Pillai says. Organisms deploy some small RNAs to turn down the activity of their own genes, albeit indirectly. Before a cell synthesizes the protein encoded by a gene, it first makes an RNA version of the gene, known as messenger RNA (mRNA). The best-known types of small RNAs—small interfering RNAs (siRNAs) and microRNAs—target these mRNAs, destroying them or preventing the cell from translating them into proteins. Many organisms also enlist small RNAs to defend against pathogens. In plants and nematodes, for instance, small RNAs help destroy viral RNA.

Eleven years ago, Alexei Aravin, then a graduate student at Moscow State University, and colleagues discovered several small RNAs that shut down a transposonlike gene in fruit flies. At the time, the only hint that the molecules belonged to an unrecognized group of RNAs was that they were slightly longer than siRNAs, says Aravin, who is now a molecular biologist at the California Institute of Technology in Pasadena. In a follow-up fruit fly study 2 years later, however, he and colleagues identified more than 170 unique small RNAs that target transposons, suggesting that the insects have a specialized type of RNA for this function.



Shut down. Glowing egg cells stand out in worms whose piRNAs can't silence a foreign DNA sequence (*left panels*). But when the piRNAs recognize and shut down the sequence, the egg cells are dark (*right panels*).



Guarding the germ line. DNA (blue) and two kinds of protective Piwi proteins (red, green) are visible in this section of a mouse testis.

Aravin would soon come across this new class of small RNAs again, though by following a different research tack. He and other biologists were looking into the workings of Piwi proteins, which studies had indicated are necessary for fertility in several kinds of animals. Piwi proteins are part of the Argonaute family. siRNAs and microRNAs work by consorting with non-Piwi Argonaute proteins that slice up RNA molecules. Some researchers speculated that Piwis also functioned by partnering with RNAs. "It was only logical to imagine that these similar family members would also bind to small RNAs," says molecular biologist Gregory Hannon of the Cold Spring Harbor Laboratory in New York. In 2006, Aravin and colleagues, Hannon and coworkers, and two other groups independently confirmed this hypothesis, uncovering thousands of small RNAs that collaborate with Piwi proteins in mice. Researchers realized that these small RNAs resembled the ones Aravin and colleagues had initially identified in fruit flies and declared that all of them were an RNA family unto themselves, the Piwi-interacting RNAs, or piRNAs.

piRNAs differ from microRNAs and siRNAs in several ways (see table, p. 27). As Zamore's team first reported in 2006 in *Science* (21 July 2006, p. 320), cells don't need the enzyme Dicer to make piRNAs. However, Dicer is essential for the matu-

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ration of microRNAs and siRNAs. Also, unlike siRNAs and microRNAs, piRNAs are exclusive to animals, occurring even in ancient groups such as sponges.

The DNA sequences that code for piRNAs are bunched in a few so-called piRNA clusters. One of the field's big mysteries is how these clusters give rise to piRNAs, notes molecular geneticist Eric Miska of the University of Cambridge in the United Kingdom. "piRNA biogenesis is still very enigmatic." Cells likely make an RNA copy of an entire cluster and then dissect it, hewing the fragments into piRNAs. But the details of this processing remain obscure. "More than 10 proteins are involved, but we know very little about what steps they are doing," Aravin says.

Detecting danger

Although the workings of the piRNA system differ from those of our immune system, these two defenses face many of the same challenges. Their first job is detecting danger. piRNA clusters are crucial for this function. They contain partial and complete transposon sequences, and they serve as the memory banks for the piRNA system. "It's the way animals write down which transposons have invaded their genome," Zamore says. Each piRNA targets transposons that contain a matching sequence to its own RNA sequence. By making piRNAs that correspond to the transposon sequences stored in the clusters, animals can keep these selfish strands in check.

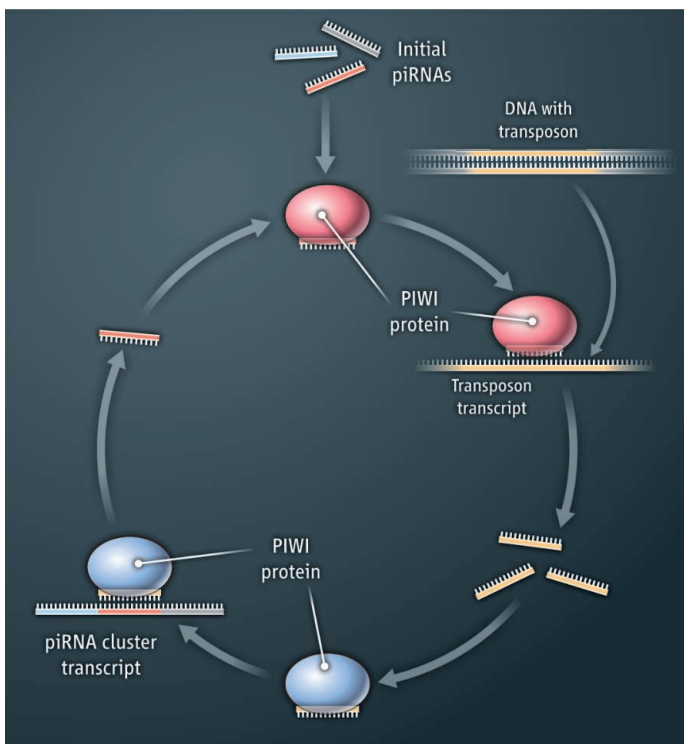
But what if an animal has to contend with a transposon that it hasn't encountered before? The piRNA system relies on a nifty trick in these situations. "It makes use of the only thing that [selfish] genetic elements have in common—they move around the genome," says molecular geneticist René Ketting of the Institute of Molecular Biology in Mainz, Germany.

As a new transposon migrates from location to location, it should eventually land in a piRNA cluster. When that happens, the transposon becomes part of the memory bank, and the animal will begin producing complementary, or matching, piRNAs to thwart the genomic interloper. Each piRNA cluster "is kind of a trap," Pillai says. "Once a transposon falls in, you have immunity."

Thanks to their genomic immune system, animals can recover from "infection" by a new transposon, much as you get over the flu because your immune system defeats the influenza virus. For example, in a study published in the 23 December 2011 issue of *Cell*, molecular geneticist William Theurkauf of the University of Massachusetts Medical School, Zamore, and colleagues followed what happened to young female flies that inherited a transposon called the *P* element, which they hadn't tangled with before. At first, the transposon got the jump on the insects. They were infertile and produced scant piRNAs that had any ability to control the *P* element. The genomic invader also unleashed other transposons that had been lurking in the flies' chromosomes. But as the flies grew older, they began to rein in the *P* element, cranking out piRNAs that targeted it. Moreover, the researchers found that other transposons released by the *P* element began falling into piRNA clusters, presumably allowing the flies to make piRNAs to counter them as well. As a result, the flies regained some of their egg-producing capability.

But how does a fly or another animal tell a transposon from its own DNA? If the immune system mistakes "self" for microbial invaders, its responses can trigger autoimmune diseases. One way that piRNAs avoid triggering genomic autoimmunity is their specificity; they key on transposons with complementary sequences. But a study of nematodes indicates that the piRNA system might deploy a second mechanism to prevent self-directed attacks, suggests molecular geneticist Craig Mello of the University of Massachusetts Medical School. He shared the 2006 Nobel Prize in physiology or medicine for discovering RNA interference: the ability of small RNAs to shut down gene activity.

To move, transposons often make an RNA copy, or transcript, of themselves that's converted back to DNA in a new place. In the 6 July 2012 issue of *Cell*, Mello's team proposed a novel way that piRNAs can avoid mistaking this transposon RNA for a cell's vital RNA, such as messenger RNAs. "People thought that piRNAs would target 'aberrant RNA,' " that is, any sequence that differed from the animal's own RNA sequences, Mello says. He has a different take: "Our findings suggest that a foreign sequence is recognized as foreign because it's never been expressed"—used to make protein.



No game for transposons. In the ping-pong loop, one Piwi protein and a piRNA slice up transposon RNA with a matching sequence. The transposon fragments then join a different Piwi protein to produce more matching piRNAs.

The researchers drew this conclusion after equipping nematodes with a fragment of worm DNA that also included a foreign sequence—instructions for making the fluorescent protein GFP. Mello and colleagues observed a curious pattern in the resulting mutant nematodes. In some of the worms, piRNAs ignored the inserted DNA, treating it as if it were a normal gene. Those worms made GFP and lit up.

But other worms remained dark because they reacted to the introduced DNA sequence as if it were a transposon and shut it down, preventing the production of GFP. These differences remained steady from generation to generation, Mello notes. "The ones that are on stay on, and the ones that are off stay off."

According to Mello, why some inserted DNA sequences are initially expressed and others are silenced is probably a matter of chance. But if the DNA snippet is accepted and used to make proteins, the animal thereafter treats it as "self," Mello suggests. He and his colleagues hypothesize that worms have a molecular pathway that keeps track of which DNA sequences have been active and prevents piRNAs and Piwi proteins from interfering with them. The researchers haven't pinpointed which molecules perform this job, Mello says,

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but they suspect an Argonaute protein called CSR-1 is the ringleader. Pillai describes this potential recognition mechanism as "an interesting idea and plausible," adding that the Mello group's paper is "the only one which might explain the available data."

Taking on transposons

Once immune cells meet an intruder, they counterattack. piRNAs do the same, using a variety of measures against transposons. Some piRNAs dive into the fray. They track down transposon RNAs, and the Piwi proteins they bring along slice up the rogue strands.

Some piRNAs let others do the dirty work. In the 3 August 2012 issue of *Science* (p. 574), Miska and colleagues described how piRNAs boost their power by enlisting siRNAs to stifle transposons. The reason, Miska suggests, might be that although piRNAs come in many varieties—more than 16,000 in nematodes—each germline cell harbors just a few copies of each one. "They can't do much on their own," he says. In contrast, siRNAs are plentiful.

Other animals bolster their piRNAs directly, relying on what's called the ping-pong amplification loop. Hannon's team and a group led by molecular biologist Mikiko Siomi, now at Keio University School of Medicine in Tokyo, independently described this mechanism in flies in 2007, but mice and zebrafish also take advantage of a similar process. In the ping-pong loop, piRNAs and Piwi proteins slice up transposon RNA. The resulting fragments undergo modification and join with other Piwi proteins to cut up RNA transcripts of piRNA clusters, thus making new piRNAs (see diagram, p. 26). The loop "only amplifies the useful piRNAs," those with a target available in the cell, says Brennecke, a co-author on one of the papers.

piRNAs can fight back even if a transposon remains quiescent and hides as a stretch of genomic DNA. Researchers have found that in mice, piRNAs spur germline cells to affix methyl groups to transposon DNA, preventing its transcription into RNA and thereby blocking the rogue strand's movement to a new location in the genome. Fruit fly piRNAs stymie transposon transcription using a similar mechanism that involves molecular modification of histones, the protein spools around which DNA coils, Brennecke and colleagues reported in the 21 November 2012 issue of *Cell*.

These strategies lead to long-term protection, and in that regard, piRNAs have our immune system beat. After you've recovered from an infectious disease, you often will be immune to the pathogen that caused it for life—but your children and grandchildren won't be. Animals' genomic guardians, by contrast, can suppress some transposons for multiple generations, Mello's and Miska's teams revealed in the 6 July 2012 issue of *Cell*. For example, this genomic resistance lasts for at least 20 generations in nematodes, Miska and colleagues showed. Persistent protection makes sense: Transposons in the germ line can reactivate each generation, so locking them down long-term is beneficial.

More than defense?

piRNAs may do more than thwart transposons. Some scientists suspect that they, like siRNAs and microRNAs, help adjust gene expression. For example, Mello and colleagues suggest that the targets of many nematode piRNAs are some of the worm's own genes, not transposons. They have shown that about 1000 of the roughly 20,000 nematode genes are under piRNA control. Many of the genes are normally turned off but switch on in worms that lack one kind of Piwi protein, Mello and colleagues reported in the 6 July 2012 issue of *Cell*. One possibility, Mello says, is that these genes perform functions that are useful in certain environmental conditions, such as when the worms are under stress. When times are tough, a cell in the germ line of a parent worm might rein in piRNAs, allowing the genes to switch on and helping the offspring cope with adversity.

The idea that piRNAs are tweaking gene activity gets mixed reviews from other researchers. Some remain skeptical that piRNAs ever silence genes. Even if they accept that possibility, other scientists question whether animals other than nematodes avail themselves of this gene-controlling mechanism. Few transposons trouble nematodes, so the worms might have the freedom to divert their piRNAs to new roles. "In most systems, the evidence favors transposons being the targets" of piRNAs, and not genes, Ketting says.

Also unclear is whether piRNAs function in nongermline cells. Most scientists have dismissed the possibility, as siRNAs quash transposons in these cells. Moreover, only germline cells seem to make Piwi proteins, piRNAs' collaborators.

But a discovery from neuroscientist Eric Kandel of Columbia University and colleagues suggests that piRNAs are active in the central nervous system, helping create memories. In the 27 April 2012 issue of *Cell*, the team reported that they had identified piRNAs in neurons from the sea slug *Aplysia*. The piRNAs help block the production of a protein called CREB2, which inhibits memory formation in these animals. Testing piRNAs' role in learning in other creatures shouldn't be difficult, Ketting says. If they do have a role, deleting Piwi proteins in animals such as mice or flies should cause memory lapses.

COMPARING DIFFERENT KINDS OF SMALL RNAs

	siRNA	microRNA	piRNA
Length	21–24 nucleotides	20–25 nucleotides	21–31 nucleotides
Organization	Double-stranded	Single-stranded	Single-stranded
Requires Dicer for maturation?	Yes	Yes	No
Found in	Animals, plants, fungi, protists	Animals, plants, protists	Only animals
Function	Controlling gene expression, blocking transposons	Controlling gene expression	Blocking transposons

The question that has researchers scratching their heads involves the pachytene piRNAs, which are named for the stage of meiosis—the process that produces eggs and sperm—in which they appear. Mammals generate a huge number of different pachytene piRNAs—one recent study estimated the total for mice at more than 800,000, but Zamore says that value is almost certainly too low. Yet the sequences of the pachytene piRNAs do not match those of any transposons, suggesting that they aren't targeting the rogue strands. "What these pachytene piRNAs are doing—nobody knows," Aravin says.

As piRNA researchers delve into such mysteries, some also wonder if these genomic superheroes sometimes take the day off to help a species adapt. We and other animals are alive today because, over hundreds of millions of years, piRNAs helped our recent and distant ancestors tamp down transposons. But transposons aren't necessarily all bad. They also create genetic variation in the germ line that is the raw material for natural selection. A few researchers speculate that when conditions are rough, animals might inhibit their piRNAs to unleash transposons and trigger more mutations, speeding up their evolution. That idea is "a very attractive hypothesis," Zamore says. "I'd like to think of a way to test it experimentally." **–MITCH LESLIE**

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