



Yin and yang

How can RNAs shut
down the activity
of a gene?



In the days of computers, it's easy to delete a word from a text, a book, a dictionary. Programs like *Word* or *QuarkXPress* (used in writing this book) have a search-and-replace function that can easily be turned into search-and-delete. Back in the era of hard copies, accomplishing the same thing would have been a miserable, messy job involving scissors, paste, a black magic marker, white-out, a hair drier, or all of the above.

It used to be much harder and messier to knock out a gene in an organism, too. You had to physically remove it from the creature's genome, or paste in something that disturbed the process by which DNA is transcribed into useful RNA molecules.

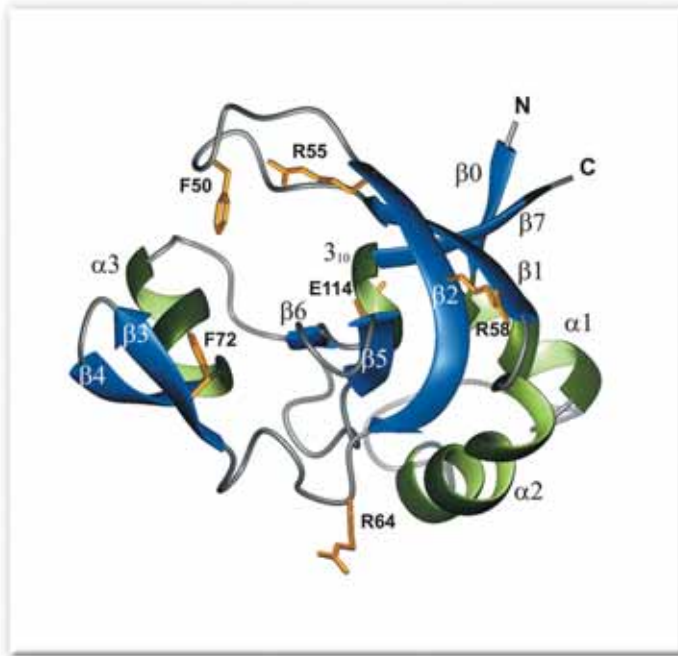
But a few years ago a much simpler method was developed; it works in the mid-field of the passage of information from genes to proteins, by interfering with RNA. The method is called *RNA interference*, or RNAi. Scientists have tinkered with it to the point that laboratories across the world, including many groups at EMBL, are now using it to study a wide range of organisms, from worms to mosquitoes to mammals.

The groups of Elisa Izaurralde and Michael Sattler have now gotten a look at the structure of part of a protein called *Argonaute 2* (Ago2). This molecule is a key figure in a molecular machine that carries out RNAi in the fruitfly. The building plan of the protein tells a story about how it functions in flies and, probably, in most other species.

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Andreas Lingel steadies an aluminum ladder as Bernd Simon climbs to the top of a huge silver tank mounted on legs. I'm suddenly worried about the one credit card in my wallet, my video club membership, my wrist-

Left: Bernd Simon, Elisa Izaurralde, Andreas Lingel, and Michael Sattler in the NMR room.



The structure of the Ago2 PAZ domain. Amino acids of the protein that were mutated are colored gold.

watch, although Bernd says I don't need to be. Andreas, a PhD student in Elisa's lab, has brought over samples of a part of the Ago2 protein to be examined using *nuclear magnetic resonance* (NMR). Bernd is loading it into the machine, which is basically an enormous magnet, capable of wiping the credit off your EMBL canteen card, data from your memory stick. "It's all right," Bernd promises, "I come in here every day."

Michael's group uses NMR to create pictures of the structures of molecules. The magnetic field of the machine aligns the nuclei of atoms, as if they were billions of tiny compasses pointing true north. Then they reorient themselves into positions that are affected by the identity and locations of other nearby atoms. From the readout of the experiment, scientists can draw a three-dimensional coordinate map of the atoms in a protein. Since a molecule's behavior is determined by its physical and chemical properties, this often lets researchers connect the dots on a biochemical event.

That's what they were doing with a part of the Ago2 protein.

"DNA is a double-stranded helix," Elisa says. "RNA is made of nucleotides and it can also form a double strand. But the RNA in a cell ought to come in a single strand." It should come as a yin without a yang. If a cell detects double-stranded RNA, it thinks it's a bad mistake, or possibly something from a virus, and chops it up into small fragments called *small interfering RNAs* (siRNAs).

The process doesn't stop there. A protein machine called *RISC* scours the cell for messenger RNAs with the same code – finding even those that are supposed to be there – and prompts their destruction, like a global "search-and-delete" function. If an RNA disappears, the cell will no longer synthesize the corresponding proteins. Ago2 is

a part of the RISC machine, and a part of Ago2 is called *PAZ*. Elisa thinks PAZ is one of the stars of the show.

"What we see in the structure is a fold that looks like it locks onto nucleotides," Michael says. "We knew that when there are certain mutations in this area of the molecule, RNAi won't work in the organism. That was probably because something in the shape of the fold was changing, and PAZ could no longer hold onto RNA."

The researchers verified this hypothesis by proving that PAZ can capture RNAs like the fragments produced in RNAi. Using NMR, they also showed exactly where a string of nucleotides could fit into the fold. Additional experiments by Elisa demonstrated that two specific amino acid changes in the part of PAZ that comes in contact with the RNA did not disrupt PAZ's architecture, but could seriously disturb the binding.

How does the RISC machine recognize siRNAs and why doesn't it bind to other RNA molecules? "The fragments are still double-stranded, and after they have been cut they are left with a jagged edge," Michael says. "It seems that the PAZ domain may recognize these characteristic features of siRNAs and clearly mark them as something special to be incorporated into the RISC complex." Once incorporated, these siRNAs serve as guides to detect mRNAs that have similar sequences. If a match is found the mRNA is degraded.

The mechanism by which this happens is still unknown. But RNAi is such an important new technology, it's doubtful that will remain the case for long.