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Molecule Does More Than Slice and Dice RNA

A team of Howard Hughes Medical Institute (HHMI) scientists has peeled back some of the mystery of how cells are able to turn off genes selectively to control critical events of development. The new insights arise from the first clear molecular images of the structure of Dicer, an enzyme that enables cells to dissect genetic material precisely.

The finding, which is reported in the January 13, 2005, issue of the journal *Science* by an HHMI research team at the University of California, Berkeley, provides scientists with new information about a mechanism that enables cells to silence genes, a process that governs key developmental events ranging from brain development to stem cell differentiation.

The study was led by Jennifer A. Doudna, a Howard Hughes Medical Institute investigator at the University of California, Berkeley. Doudna's research team used x-ray crystallography to assemble a detailed three-dimensional picture of an enzyme known as Dicer. In cells, Dicer jumpstarts RNA interference, a process that causes genes to be turned off and which, in turn, prompts a host of key developmental events. With the structure of Dicer solved, Doudna's group showed that the enzyme is more than a molecular cleaver -- it also carefully measures and snips strands of RNA into precise increments. When Dicer cleaves large strands of RNA into smaller fragments, it initiates the process of RNA interference, which can turn genes off and thereby dictate key developmental events.

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- Jennifer A. Doudna

"The bottom line we've learned from the structure is that Dicer is a molecular ruler," Doudna explained. "It gives us a lot of insight into how the

mechanism works.”

Dicer, which is ubiquitous in the cells of higher animals, including humans, is a widely studied molecule. It was first discovered in 2001 by Gregory J. Hannon, an HHMI investigator at Cold Spring Harbor Laboratory, and has become a powerful laboratory tool to study cancer and other developmental events through its ability to harness the RNA interference pathway and selectively switch off genes.

“We knew what the protein did,” said Hannon. “We knew it was an enzyme and that it recognized double-stranded RNA and cut it into pieces. But we didn't have any clue how Dicer made the measurement and figured out where to make the cut.”

Doudna and her colleagues solved the structure of a Dicer enzyme obtained from the parasite *Giardia intestinalis* using x-ray crystallography, a technique that enables scientists to construct pictures of biological molecules in superb three-dimensional detail. When crystal samples of molecules like Dicer are exposed to x-rays, the x-ray beams are scattered in a way that helps researchers define the overall structure of the crystallized protein, as was the case with Dicer. Knowing how the atoms of specific molecules are arranged permits researchers to tease out their functional features and show how they go about their business inside a cell. In the case of Dicer, it shows how the enzyme recognizes RNA and snips it into precise increments.

“The fact that it makes these specific sized RNAs is important to the process,” Doudna said. The small RNA fragments created by Dicer are then assimilated into large multiprotein complexes and guide those molecules to destinations in the cell where they turn off genes.

“The size of these small RNAs is a determinant of their function,” Hannon explained. “If the RNAs are too big or too small, they don't make it into the effector complexes.”

Through its role in helping cells to turn off gene expression, Dicer is believed to be instrumental in initiating some of the critical processes of development. For example, scientists speculate that the RNA interference pathway plays a role in prodding blank-slate stem cells down developmental pathways to become specific kinds of cells or tissues. It may also play roles in maintaining cells, rearranging genomes and laying down the architecture of the brain, for example.

In the lab, Dicer is used in mice to switch off any combination of genes -- either in targeted or in random fashion -- to infer a gene's function, a capability that may be especially useful in understanding cancer and developing improved cancer therapies. Using the enzyme, it may be possible, Doudna explained, to “change silencing in cells to turn off genes that may be active in cancer development. Lots of companies and laboratories have been

betting on this.”

Dicer enzymes are found in all cells of higher animals, suggesting that it has an ancient evolutionary heritage. Because of its ability to recognize double-stranded RNA, scientists think Dicer's original function may have been to defend cells from certain kinds of viruses.

Having an image of Dicer from *Giardia intestinalis*, Doudna noted, will help scientists better understand its role across biology.

“From an evolutionary standpoint, it is very interesting,” said Doudna. “What is this enzyme used for in *Giardia*? We don't know. The *Giardia* Dicer is smaller than the Dicer found in other eukaryotes and we don't know why that is. What do the bells and whistles on the human enzyme do?”

Knowing Dicer's structure, scientists can now begin to tease out the mysteries of how Dicer functions, Doudna said. In particular, scientists would like to know how Dicer is involved in downstream events, how it hands off the cleaved RNA and directs it to the right gene targets.

Resolving the structure of Dicer, Doudna noted, was a technical challenge as the crystals are small. But she said that the work was facilitated by access to the HHMI-supported crystallography beam lines at the Lawrence Berkeley National Laboratory's Advanced Light Source. The synchrotron at the Advanced Light Source is capable of generating beams of x-rays to very specific wavelengths, which was critical in determining the structure of Dicer.

Additional authors of the new Science paper are Ian J. MacRae, Kaihong Zhou, Fei Li, Adrian Repic, Angela N. Brooks and W. Zacheus Cande of the University of California, Berkeley and Paul D. Adams of the Lawrence Berkeley National Laboratory.