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Ribozymes Come Ready for Action

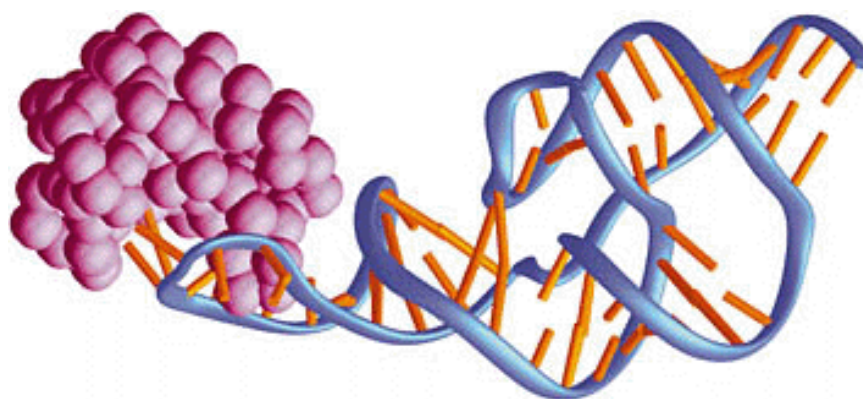


Image Title: A structural model of the ribozyme from the human pathogen hepatitis delta virus. - (Top) Adrian Ferr-D'Amar/Jennifer Doudna, HHMI at Yale University, (Bottom) Thomas Cech, HHMI at University of Colorado, Boulder.

Using x-ray crystallography, two research teams from the Howard Hughes Medical Institute (HHMI), working independently, have found that catalytic RNA, like its protein cousins, is ready to function as soon as it is produced.

Ever since HHMI investigator Thomas Cech at the University of Colorado in Boulder uncovered the catalytic properties of RNA in 1982, researchers have been diligently studying these ribozymes. Scientists have since discovered more than 500 ribozymes in a diverse range of organisms and have found that they share many similarities with their more widespread protein cousins, enzymes.

"The finding of an enzyme-like active site fits right in with the story we've been building about ribozymes. But a picture is worth a thousand words and now we've got the picture," says Cech, who won the 1989 Nobel Prize in Chemistry for his discovery of ribozymes.

The results of Cech's group, spearheaded by HHMI associate Barbara Golden, appear in the October 9, 1998, issue of *Science*. They describe the three-dimensional structure of the largest ribozyme ever crystallized, which comes from the organism *Tetrahymena thermophila*. In the October 8, 1998, issue of *Nature*, HHMI investigator Jennifer Doudna and colleagues from Yale University detail the structure of a smaller ribozyme from the human pathogen, hepatitis delta virus (HDV).

Crystallizing RNAs is notoriously tricky. Crystals contain billions of molecules that must be aligned precisely if they are to provide a sharp image of a molecule's three-dimensional structure. But RNA molecules are difficult to align because they don't usually have many surface "nooks and crannies" to help the molecules stick together within the crystal. While Doudna's group could easily crystallize the viral ribozyme, most of the crystals they created didn't interact well with x rays. Inspired by "desperation," Doudna's team came up with a nifty trick for overcoming the problem.

Doudna's group attached an RNA-binding protein onto a region of the viral ribozyme where it wouldn't interfere with the ribozyme's activity. The protein provides surface contacts that enable the ribozyme-protein complex to pack snugly into a crystal, allowing the researchers to create a much higher resolution image than they could have with the RNA alone. Using their new technique, Doudna's group attained an image with a resolution of 2.3 angstroms, which is good enough to see the exact position of every nucleotide in the ribozyme.

Cech's group crystallized the *Tetrahymena* ribozyme without help from proteins, but their molecules didn't pack as tightly as those from HDV. The looser packing resulted in an image resolution of 5 angstroms, high enough to see the molecule's overall architecture while leaving the picture "a little blurry around the edges," says Cech. He's confident that future efforts will produce an image with greater resolution.

Despite the difference in resolution, both groups produced images revealing active sites that are largely preorganized—they don't require major shape changes in order to catalyze reactions. The active sites of both the *Tetrahymena* ribozyme and the viral ribozyme have tight binding pockets that sit ready and waiting for the right RNA molecules to come along. This preorganized structure wasn't seen in the hammerhead ribozyme, the only other ribozyme crystallized previously. The hammerhead has an active site that is more like a folding chair in that it must change shape before it can do its job.

The latest x-ray pictures will help scientists in their search for nature's rules for building RNA scaffolds. Doudna says that given RNA's diversity, it's a sure bet that ribozymes use a variety of catalytic strategies. She hopes future studies will uncover some of these strategies. "Having structures available will allow us to see what those active sites really look like and then we're able to go in and really ask much more specific questions using other techniques," Doudna says.

Doudna's work provides a new tool for creating RNA crystals, and together with Cech's results, shows that crystallizing RNA is feasible despite previous doubts that RNA might be too "floppy" to crystallize well. "There are a lot of RNAs out there that are of biological interest, and I think this will be an incentive for more people to work on RNA crystallography," says Cech.