Researchers make first direct observation of 3-D molecule folding in real time

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All the crucial proteins in our bodies must fold into complex shapes to do their jobs. These snarled molecules grip other molecules to move them around, to speed up important chemical reactions or to grab onto our genes, turning them "on" and "off" to affect which proteins our cells make.

Recently, scientists have discovered that RNA-the stringy molecule that translates our genetic code into protein-can act a lot like a protein itself. RNA can form loopy bundles that shut genes down or start them up without the help of proteins. Since the discovery of these RNA clumps, called "riboswitches," in 2002, scientists have been striving to understand how they work and how they form. Now, researchers at Stanford University are looking closer than ever at how the three-dimensional twists and turns in a riboswitch come together by grabbing it and tugging it straight. By physically pulling on this loopy RNA, they have determined for the first time how a three-dimensional molecular structure folds, step by step.

The researchers used a machine called an "optical trap" to grab and hold the ends of an RNA molecule with laser beams. Based on technology developed by Bell Labs researchers in 1986, the machine was designed by a team led by Steven Block, the Stanford W. Ascherman, M.D., Professor and a professor of applied physics and of biology. The optical trap allows them to hold the ends of the RNA tightly, so they can pull it pin-straight, then let it curl up again. In the Feb. 1 issue of *Science*, their paper, of which Block is senior author, describes the development of

every loop and fold in one particular RNA riboswitch, and the energy it takes to form or straighten each one-an unprecedented achievement that opens the door for equally thorough studies of other molecules and their behaviors.

The researchers are the first to study the energy and folding behavior of a riboswitch in this detailed, physical way. More important, they are the first to use directly applied force to determine how a molecule makes a three-dimensional bundle, a tertiary structure. No other research has tracked the formation of such a complex structure, fold by fold.

Previous studies typically have used biochemical techniques rather than lasers, which can directly grab and tug the RNA. Biochemical techniques give less clear estimates of how molecules fold in real time. They often give a description of the molecule's average folding behavior, which must be interpreted by mathematical models. Crystallography-a technique involving freezing the molecule in place-provides a good picture of its shape, but not how it forms or the energy involved.

"What we're interested in is understanding, in a very fundamental way, how biomolecules take the shapes they do, and how they perform the functions they do," Block said. "No one has been able to explore in great detail tertiary structure yet." RNA riboswitches must have this tertiary structure to work.

"Most RNAs just make secondary [two-dimensional] structure. But the ones that really do stuff," he added, "those all have tertiary structure."

What RNA can do

RNA has the job of copying the genetic code from DNA (transcription), and using that code to build the proteins organisms need to live (translation). To make RNA, a protein called RNA polymerase moves

along the length of a strand of DNA. It reads a pattern in the building blocks of DNA, nucleic acids whose names are abbreviated A, C, G and T, and it makes RNA with a complementary pattern. This long strand of RNA is then the recipe for a specific protein. Another structure called a "ribosome," which is also made of RNA, then reads this recipe and makes a protein to order.

The RNA copied from DNA generally does not twist up very much, often only forming two-dimensional loops or tight bends called "hairpins." Occasionally, its loops and hairpins form a three-dimensional structure that does nothing. Sometimes, though, this snarl of loops and hairpins works as a riboswitch. The RNA begins to bundle up while it is being made, so the jumbled portion is attached to a tail still under construction. The riboswitch must have a tertiary structure, because it likes to make a pocket and grab small molecules. When a riboswitch clutches the right molecule, it folds up even more tightly, tugging on its own incipient long tail and changing its shape in a way that will affect its eventual protein product. That RNA tail usually has a hairpin fold that straightens out when pulled. By tugging out this kink in the RNA, a riboswitch changes how the RNA is translated into protein, effectively turning the gene on or off.

The riboswitch Block's team studied grabbed onto a molecule called adenine, the nucleic acid dubbed "A." Whenever the riboswitch gripped a free-floating adenine, a gene that makes a protein crucial to adenine production stopped working correctly. The RNA responsible for translating it to the protein had changed shape. The riboswitch regulated how much adenine was available in the cell; when there was plenty, it shut down the adenine factory. Before scientists discovered riboswitches, they thought only proteins controlled genes this way. "Your average RNA at random is not going to do that," Block said. "These are highly evolved things."

The closest look

The researchers who study molecular folding in Block's lab cannot actually see an RNA molecule under the microscope, but they can see two polystyrene beads; they attach one on either end, and that creates a dumbbell shape the laser beams can manipulate. Their largest beads are 1,000 nanometers across, so 1,000 of them lined up would be a millimeter long. The beads are enormous relative to the RNA, and so are the lasers holding them. To keep the lasers from coming too close together and merging their light into a single beam, the researchers need to attach some extra length to the RNA. To do this, they tack a long strand of DNA on one side.

Under the microscope, the two plastic beads look like tiny pearls against a gray backdrop. The researchers pull the beads apart, taking into account two factors: force and extension. By understanding how much force it takes to cause a certain amount of extension of the RNA, they can describe with unsurpassed accuracy how the folds form and the energy needed to make each fold happen.

"When you pull it apart, different structures will pop open-pop, pop, pop-and you can see the order in which different structural elements get pulled apart," Block said. "You can map out the order in which the pieces come together, for both folding and unfolding."

Learning by force

To build a clear picture of how their riboswitch folded in real time, the researchers mapped out the energy of the molecule's folding based on the forces required to uncurl it and the time the RNA took to re-curl. Block calls the energy graph the "crown jewel of the work," adding that "all the numbers you'd like to know about this folding sequence are right

in front of you in that diagram."

Block's team could only attain this detailed "energy landscape" of the RNA's folding by physically toying with the molecule. The particular RNA they studied folds four times, and each time it adopts a more stable, more comfortable configuration with lower energy. If it grabs an adenine, it hangs on tightly because it is in its most stable state. But because molecules are always jiggling, sometimes a fold pops open briefly. The more stable each fold is, the less likely it is to come undone. The researchers stretched out the RNA to study all four folded states, noting how stable each one was.

Using force, Block's team described not only the energy of each fold in the RNA, but the energy it needed to go from one folded state to the next, and how often the folds popped open and closed in real time. The researchers watching little white beads move under the microscope got the closest look yet at how a molecule with a three-dimensional structure behaves in life, thanks to a pair of keen, green lasers and a little judicious tugging. "It's so cool to be able to take a single molecule and bend it to your will," Block said.

Source: Stanford University