Rules governing RNA's anatomy revealed

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(PhysOrg.com) -- University of Michigan researchers have discovered the rules that dictate the three-dimensional shapes of RNA molecules, rules that are based not on complex chemical interactions but simply on geometry.

The work, done by a team led by Hashim M. Al-Hashimi, is described in the Jan. 8, 2010, issue of the journal *Science*.

"RNA is a very floppy molecule that often functions by binding to something else and then radically changing shape," said Al-Hashimi, who is the Robert L. Kuczkowski Professor of Chemistry and a professor of biophysics. These shape changes, in turn, trigger other processes or cascades of events, such as turning specific genes on or off.

Because of the RNA molecule's mercurial nature, "you can't really define it as having a single structure," Al-Hashimi said. "It has many possible orientations, and different orientations are stabilized under different conditions, such as the presence of particular drug molecules."

A major goal in structural biology and biophysics is to be able to predict not only the complex three-dimensional shapes that RNA assumes (which are dictated by the order of its nucleic acid building blocks), but also the various shapes RNA takes on after binding to other molecules such as proteins and small-molecule drugs. Further, researchers would like to be able to manipulate the 3-D structure and resulting activity of RNA by tweaking the drug molecules with which it interacts. But to do that, they need to understand the rules that govern the anatomy of RNA.

The quest has parallels to the study of human anatomy, Al-Hashimi said. "Your body has a specific shape that changes predictably when you are walking or when you are catching a ball; we want to be able to understand these anatomical rules in RNA."

Manipulating RNA is a much sought-after goal, given the recent explosion in vital cellular roles ascribed to RNA and the growing number of diseases that are linked to RNA malfunction. RNA performs many of its roles by serving as a switch that changes shape in response to cellular signals, prompting appropriate reactions in response. The versatile molecule also is essential to retroviruses such as HIV, which have no DNA and instead rely on RNA to both transport and execute genetic instructions for everything the virus needs to invade and hijack its host.

In earlier work, Al-Hashimi's team determined that rather than changing shape in response to encounters with drug molecules, RNA goes through a predictable course of shape changes on its own. Drug molecules simply "wait for" the right shape and attach to RNA when the RNA assumes the particular drug's preferred orientation, Al Hashimi said.

But what rules control the predictable path of shapes the RNA molecule assumes? And are those rules the same for all sorts of RNA molecules? In the current work, Al-Hashimi's team investigated those questions.

"RNA is very similar to the human body in its construction, in that it's made up of limbs that are connected at joints," Al-Hashimi said. The limbs are the familiar, ladder-like double helix structures, and the joints are flexible junctions. The prevailing view was that interactions among loopy structures at the tips of the limbs played a role in defining the molecule's overall 3-D shape, much as a handshake defines the orientation of two arms, but Al-Hashimi's group decided to look at things from a different perspective.

"We wondered if the junctions themselves might provide the definition," Al-Hashimi said. "If you look at your arm, you'll notice that you can't move it, relative to your shoulder, in just any way; it's confined to a certain pathway because of the joint's geometry. We wondered if the same thing might be true of RNA."

To investigate that possibility, the researchers turned to a database of RNA structures and found that all structures with two helices linked by a particular type of junction called a trinucleotide bulge fell along the same pathway.

The team then went on to explore structures of RNA molecules with other kinds of junctions. All were confined to similar pathways, but the precise pathway of a given RNA depended upon structural features of its junction. Just as anatomical features of our shoulders, elbows, hips and knees define the range of motion of our arms and legs, the anatomy of RNA's junctions dictates the motion of its helices.

Next, Al-Hashimi and coworkers wanted to understand how drug molecules cause RNA molecules to freeze in specific positions. In earlier work with an RNA molecule known as TAR, which is critical for replication of HIV and thus a key target for anti-HIV drugs, the researchers had found that certain drug molecules froze the RNA molecule in a nearly straight position, while others trapped the molecule in a bent conformation and still others captured positions between the two extremes. But because that project involved a wide variety of drug molecules, it was hard to figure out why certain ones preferred certain orientations.

To explore the issue more methodically, Al-Hashimi's group used a series of aminoglycosides (antibiotics that are known to target RNA) that systematically differed from one another in charge, size and other chemical properties. Size turned out to be the key: bigger

aminoglycosides froze RNA in more bent positions; smaller ones favored straighter RNA structures. Looking more closely, the researchers discovered that the aminoglycoside molecule nestles between two helices and acts like wedge, forcing the helices apart. Examination of other RNA structures bound to small molecules revealed that this rule is not specific to TAR but a more general feature of RNA-small molecule interactions.

"With these findings, it now should be possible to predict gross features of RNA 3-D shapes based only on their secondary structure, which is far easier to determine than is 3-D structure," Al-Hashimi said. "This will make it possible to gain insights into the 3-D shapes of RNA structures that are too large or complicated to be visualized by experimental techniques such as X-ray crystallography and NMR spectroscopy. The anatomical rules also provide a blueprint for rationally manipulating the structure and thus the activity of RNA, using small molecules in drug design efforts and also for engineering RNA sensors that change structure in user-prescribed ways."

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