

APRIL 27, 2001

## Researchers Unfold Single RNA Molecules Using Mechanical Force

By applying precise, mechanical forces to the ends of individual RNA molecules, researchers have successfully unfolded and refolded the molecules. According to the scientists, measurements of the forces needed to unfold and refold these molecules will yield new information about how RNA molecules achieve their stable three-dimensional structures.

In an article published in the April 27, 2001, issue of *Science*, Howard Hughes Medical Institute investigator [Carlos Bustamante](#) and colleagues Jan Liphardt, Bibiana Onoa, Steven B. Smith and Ignacio Tinoco, Jr., at the University of California, Berkeley, reported how they used mechanical force to unfold three types of RNA molecules.

"Researchers have been interested in the forces involved in folding proteins and nucleic acids such as RNA, because their three-dimensional structure is so critical to their function," said Bustamante. Additionally, cells appear to use mechanical forces to unfold both proteins and RNA molecules in the process of degrading them, and scientists have little idea how this process occurs.

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- **Carlos J. Bustamante**

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"Traditionally, scientists have tried to study unfolding by temperature melting or by denaturing the molecules with chemicals. But the problem with those approaches is that they were measuring massive numbers of molecules at a time and averaging over this vast population. Add to that the problem that every molecule might take a different pathway to unfolding," Bustamante said.

To measure the force needed to unfold a single RNA molecule, Bustamante and his colleagues attached each end of a test molecule to a single microscopic plastic bead via RNA/DNA "handles." They used an "optical trap" consisting of a laser beam that held and measured the force on one bead, as a piezoelectric actuator attached to the other bead supplied the minute forces required to unfold the molecule. The scientists also used the technique to measure the change in the length of the molecule as it was unfolded.

"This system eliminated both the problems of averaging large numbers of molecules and the multiple reaction pathways," said Bustamante, "because when we are pulling, we are following a single molecule unfolding along a particular pathway."

In their experiments, the scientists unfolded three kinds of RNA molecules, each more complex than the previous one:

- the simplest, a folded "hairpin" RNA structure
- an RNA that also contained a more complicated "helix junction" found in many folded RNAs
- the most complex, an RNA that had a "bulge" in its structure, and can attain tertiary structure

"Originally, we wanted to study a full ribozyme," said Bustamante, "but it became clear to us that the complexity we were observing when we pulled the ribozyme was going to make it impossible to understand everything from the beginning. Ultimately, we realized that because RNA molecules are hierarchical in their structure, with each domain being relatively independent, it would be possible to synthesize the different domains of the molecule and then pull on each type of domain to understand their characteristics."

The results of the scientists' experiments revealed that each of the three types of RNA molecules had distinctive characteristics. For example, the hairpin and helix junction RNAs both exhibited a phenomenon the scientists called "hopping." In these instances, if the molecules were held at a constant force that was great enough to cause the molecule to transition to the unfolded state, they would hop back and forth between the unfolded and folded states. From that hopping state, the scientists could pinpoint the forces required to unfold the molecules, measure the rates of folding and unfolding and the energy of the process.

They also found that the unfolding forces coincided with the refolding forces. "This means that all the work that we are doing mechanically, to pull the molecule, to unfold the molecule, is going to just break the bonds in the molecule that maintain the folding," said Bustamante. "This means you can

carry the process at equilibrium."

The scientists also investigated the folding characteristics in the presence of magnesium ions, which are intimately involved in RNA folding. "Once you allow the molecule now in the presence of magnesium to attain its three-dimensional structure, the molecule becomes much slower, both to unfold and refold, and you can no longer pull the molecule easily at equilibrium," said Bustamante.

In the most complex RNA with the added "bulge," the scientists observed a more complex unfolding that they called "ripping." In this process, the molecule may unfold partly, then pause, and only after a slight increase in force will it abruptly unfold the rest of the way.

"When you start looking at more complex molecules, you start seeing different pathways, with similar parts of the molecule unfolding at different forces," said Bustamante.

Such richness of measurements, said Bustamante, hints at the wealth of data on RNA folding that the new technique will yield.

"Now, when you ask a biochemist how stable a mutant molecule is, he can give you a number its melting temperature," said Bustamante. "We would like to give that biochemist an energy function curve that tells him the energy barriers that hold the molecule together and what is needed to unfold it." Eventually, he said, the mechanical unfolding technique could improve theoretical models of molecular folding by enabling theoreticians to compare their predictions of folding with experimental results from actual molecules.