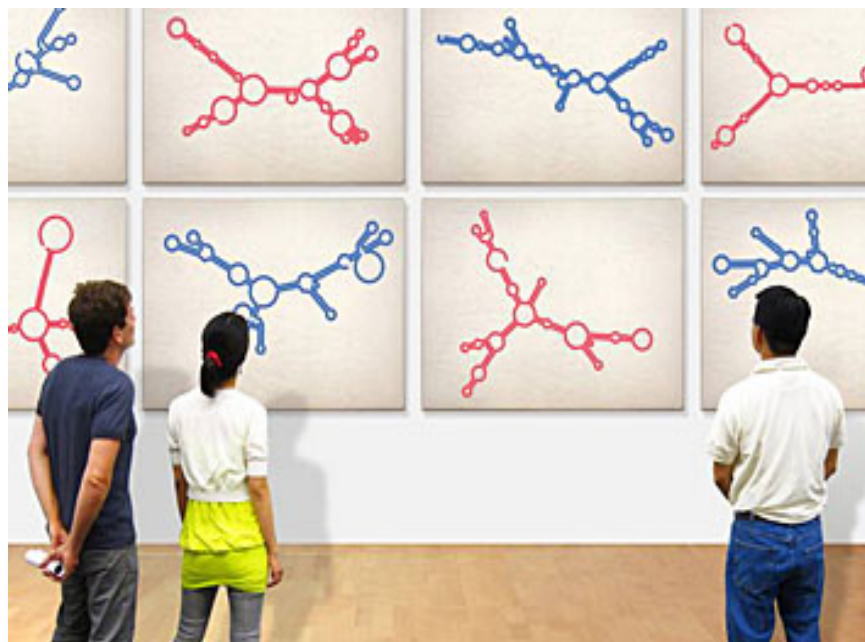


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## RNA Structure By Rapid Fire



**Image Title:** Members of Howard Chang's research team admire the structural beauty of RNA molecules. - Courtesy of Howard Y. Chang

A new rapid-fire technique for visualizing the structure of RNA in a cell could revolutionize the way scientists study this vital molecule. Unlike earlier techniques, which are labor-intensive and only yield the structure of one short strand of RNA at a time, the new process uses a parallel-processing approach to provide—for the first time—a global view of a species' diverse RNA structures.

An RNA molecule's structure provides crucial information about its function, its location within a cell, and even the proteins with which it interacts. It's clear the once underappreciated molecule has a lot of cellular contributions to reveal. Far from just helping to transcribe genes into proteins, RNA also helps regulate which genes are turned on and off.

Focusing on that common workhorse of cell biology, baker's yeast (*Saccharomyces cerevisiae*), HHMI early career scientist Howard Y. Chang

and his collaborators have determined the secondary structure of more than 3,000 RNA transcripts within the single-celled organism. The researchers describe their new technique and its first genome-wide application in the September 2, 2010, issue of *Nature*.

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if you don't really know what it looks like."**

**- Howard Y. Chang**

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Together with collaborator Eran Segal of the Weizmann Institute of Science in Israel, Chang capitalized on next-generation sequencing technology, which allowed them to do parallel and rapid sequencing of individual RNA fragments. The trick was determining not only which order the fragments had been in while inside the cell, but also their configuration. Single-stranded RNA has a penchant for doubling up and bonding to a complementary sequence on its own strand, creating a secondary structure of complex shapes rather like skeins of knotted yarn. Some regions of RNA remain free for translation -- single-stranded and accessible to the enzymes that will convert its information into protein -- while other sequences are unavailable, bound up into hairpins, twists and bulges.

Chang and Segal took advantage of RNA's structural eccentricities by choosing structure-specific enzymes, which snipped the molecule only at either double-stranded or single-stranded regions. Then, by sequencing each of the fragments and mapping them back onto the organism's genome, the researchers rapidly determined the secondary structure of thousands of the yeast's RNA molecules.

"For the first time, we have actual measurements on the secondary structure for thousands of transcripts," says Chang, a dermatologist and molecular biologist at Stanford University. "With this global view, we can see systematic features in different classes of RNAs and different kinds of activities."

Chang, who studies long noncoding RNAs (or lincRNAs), started investigating the technique because he felt hindered by the slow process of examining just one RNA molecule structure at a time—and even those were only short fragments of 100 to 200 bases. "It's hard to think about how something works if you don't really know what it looks like," he says. "And when it's long RNA, like lincRNA, you have to churn through one fragment at a time if you want to know the whole picture." Such a process is not only time- and energy-intensive, it can also be misleading: Breaking up a molecule can also change its behavior and, therefore, its shape.

But the structure-specific fragmentation allowed Chang and his colleagues to keep tabs on the RNA molecule's original structure, while the

high-throughput analysis allowed for simultaneous sequencing of millions of fragments.

The technique has already yielded information about global similarities in RNA structure, at least in yeast. The researchers found that the most accessible areas of messenger RNA (the type of RNA that transcribes DNA into proteins), the areas where it's single-stranded and least-likely to be folded up, are the sequences known as the start and stop codons—the genetic sequences that initiate and terminate protein production. “The transcript is already set up to say, ‘Play the tape right here. Track one starts here,’” Chang says.

The ability to synthesize such massive amounts of data into structural information is a boon for anyone interested in RNA function. Chang hopes that their results for yeast are the first step toward a multi-species database of RNA structures that researchers can access as needed. “Part of what's going to come out of this paper is a website where you can go look up the structure of your favorite gene,” he says. “We even plan to make an iPhone application where—if you want to know the structure while you're not at your computer—you can actually look up the structure of your favorite transcript.”

The more data that can be gathered about normal RNA structure, the more scientists can also learn about how RNA molecules interact with proteins to control gene regulation, and investigate the role that abnormal RNA structure may play in disease. The new technique help with all this, but another major step remains. Right now, Chang and his collaborators have only assessed molecular structure of RNA once it's been removed from cells—the removal process itself may impact some of its structural properties. Measuring RNA structure *in vivo* is a far more complex problem, but one that Chang hopes to attack soon.