New technique gives precise picture of how regulatory RNA controls gene activity

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A new technique developed by researchers at the Stanford University School of Medicine allows researchers to identify the exact DNA sequences and locations bound by regulatory RNAs. This information is necessary to understand how the recently identified RNA molecules control the expression of neighboring and distant genes.

The study offers a startling glimpse into the intricate world of gene expression and how RNA, once thought to be only a lowly cellular messenger, actively unlocks our DNA-based genome. "We used to have to just infer where these RNAs were acting based on their biological effects," said Howard Chang, MD, PhD, professor of dermatology. "But now we can identify precisely where on the chromatin they are binding. We've found that these sites are focal, numerous and sequence-specific."

Deciphering the role of regulatory RNAs is critically important to understand many cellular functions, including those involved in development, cancer and regeneration.

Chang, who is also a member of the Stanford Cancer Institute, is the senior author of the work, which will be published Sept. 29 in *Molecular Cell*. He and his lab previously identified some of the first known regulatory RNAs, including one in 2007 they called HOTAIR. Graduate student Ci Chu is the first author of the research.

Fifty years ago, researchers first identified messenger RNAs, which serve to transcribe the genetic instructions encoded in the DNA from the

nucleus and deliver it to the cell's protein-building machinery. Over time, the unidirectional flow of information from DNA to RNA to protein became known as biology's "central dogma." Regulatory RNAs challenge that notion by binding to DNA and affecting which genes are selected to become proteins.

The existence of these regulatory RNAs, also called long intergenic non-coding RNAs, or lincRNAs, was first postulated decades ago by researchers who realized that DNA packaged into chromatin (a coiled complex of DNA and proteins) contains more than twice as much RNA by weight as DNA. They wondered if the RNA coating the chromatin actively regulates when and how specific genes are turned on and off. But technical difficulties have made it difficult to identify precise sites of binding until now.

"To capture this picture, you have to trap that interaction between lincRNAs and chromatin in living cells," said Chang. "It was not at all obvious how to do that."

In the end, Chu devised an innovative riff on a common assay used to identify the DNA sequences bound by a class of proteins called transcription factors. For that experiment, researchers mix protein and chromatin and then use antibodies to isolate, or immunoprecipitate, both the protein of interest and its preferred binding site.

Instead of using an antibody, Chu and Chang used two or three chemically tagged, complementary nucleotide sequences, or probes, to isolate the regulatory RNAs after they had bound to the DNA. However, they could capture only about 10 percent of the regulatory RNA using this method. Eventually Chu hit on the idea of using dozens of individually labeled nucleotide sequences that bind throughout the molecule.

"The problem was that RNA is a long, floppy molecule," said Chang. "During the experiment, it would get broken into pieces and the chemical tags would be lost. So, since we didn't know exactly which portion of the lincRNA binds to the chromatin, we made a series of tagged probes to bind to every part of the lincRNA." This "tiling" approach ensures that, even if the RNA molecule is fragmented during the procedure, the researchers will still be able to isolate the small portion that remains bound to chromatin.

Using the new technique, Chu and Chang were able to investigate the binding specificity of three lincRNAs — one from the fruit fly, and two from mammalian systems. They found that one, called roX2, seems to coat genes on the single X chromosome in males and facilitate gene expression.

"We showed that roX2 very precisely binds to the X chromosome only in male cells," said Chang, "and it does this in a gradient, binding more and more at the transcriptional end of the gene. This appears to help elongation and make transcription more efficient, which is important because male animals have only one copy of the X chromosome as compared to a female's two."

Another lincRNA from humans, a component of the telomerase complex called TERC, occupies not just a chromosome's telomeres (protective caps at the end of chromosomes), but also genes involved in the Wnt pathway, which has been shown to control self-renewal.

"This shows an interesting and direct connection between the machinery for chromosomal replication and self-renewal," said Chang. "It's possible this helps the cell monitor the status of its telomeres."

Finally, HOTAIR, first identified in Chang's lab, seems to recruit a gene-silencing protein complex called polycomb to bind broad swaths of

DNA.

"Taken together, our research shows that our technique is widely applicable and can vastly enrich our understanding of how regulatory RNAs unlock the genome in many very specific ways," said Chang. "It's very exciting."

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