CELL BIOLOGY

Getting a grip on RNA

By tagging RNA with aptamers, scientists isolated *in vivo*–formed complexes, consisting of single species of RNA and their regulatory proteins.

RNA is really the one calling the shots when it comes to regulation of translation. To be precise, it is specific sequence motifs in the RNA together with associated RNA binding proteins (RNP) and noncoding RNAs.

The composition of these RNP complexes is what in the end determines whether an RNA is transported to a specific location, translated or degraded. A major focus in RNA biology today is to determine how changes in RNP composition can alter the fate of an RNA. Reading this RNP code requires methods that allow the isolation of complexes specific to a single RNA species.

Shobha Vasudevan, a postdoc in Joan Steitz' lab at Yale University, was attracted by the complexity of this RNP code. In particular, she focused on an RNA motif called ARE, for (A+U)-rich element, which usually indicates that the RNA is destined for degradation. Under certain circumstances, however, ARE-containing mRNAs are highly expressed, and Vasudevan reasoned that AREs must be more than decay elements. She wanted to establish a system that allowed her to study the protein complexes formed on AREs *in vivo*, and compare proteins found on AREs in conditions that favor upregulation of translation versus those that lead to mRNA decay.

Traditional approaches to isolate RNP complexes are often limited to the detection of abundant RNPs; *in vitro* transcribed RNA is incubated with cell lysates and highly expressed proteins will crowd all others out. Vasudevan envisaged a method that allowed the RNA protein complex to form in the cell and therefore be sensitive enough to detect any *bona fide* binders. Subsequent isolation and analysis yielded the identity of the RNPs (Vasudevan & Steitz, 2007).

Steitz says that as they developed this technique two requirements became clear:

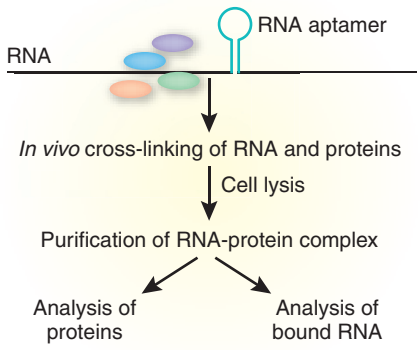

Figure 1 | Gripping RNA. An RNA sequence is tagged with an aptamer and expressed in a cell. After cross-linking and lysis, the RNA is purified with the binding partner of the aptamer and analyzed.

"What we needed to do was put a handle on the RNA and *in vivo* cross-link [RNA and RNPs] in order to avoid a problem of protein reassortment during lysis."

Vasudevan decided to use a streptavidin-binding aptamer as a handle for the RNA. She inserted the aptamer downstream of a reporter gene and the ARE motif, and grew transfected cells in full serum, a condition that favors inefficient translation, or in low serum, which favors upregulation of translation. She performed *in vivo* cross-linking with formaldehyde and then purified the RNA via the apatmer-streptavidin interaction (**Fig. 1**).

Analysis of the protein complex by mass spectrometry and western blot yielded very surprising results. One of the proteins that bound specifically in the condition that favored translational activation was Argonaute 2. This finding was unexpected as Argonaute 2, a central component of the RNA-induced silencing complex, is well known for its role in suppressing translation of mRNAs targeted by siRNAs or miRNAs.

A method to purify specific mRNP complexes *in vivo* meets with interest throughout the RNA research community. Georg Stoecklin from the German Cancer Research Center in Heidelberg, who also works on the regulation of mRNA by AREs, says, "This is where the field needs to go, to purify one species of RNA from the cell and determine the proteins that bind. To date, most approaches use *in vitro* synthesized 'naked' RNA, which does not take into account that the protein 'coat' is constantly remodeled."

Although this technique provides a nice proof of principle that the isolation of a complex formed *in vivo* can be done, Steitz also cautions that this is not an off-the-shelf protocol, and conditions need to be optimized for every application, in particular the cross-linking method and the choice of the aptamer.

A recent article in RNA by Hogg and Collins adds a new aptamer combination to the selection (Hogg & Collins, 2007). They devised a tandem RNA tag consisting of a binding site for a phage coat protein and a ligand-binding aptamer. With a double purification step, they identified several proteins of an RNP complex on a noncoding RNA from crude lysates, obviating the need for prefractionation.

These improved handles will allow researchers to pursue a variety of different goals. For Steitz, one goal is a more thorough analysis of the RNP complex to find the specificity determinant that recruits Argonaute 2. For Stoecklin it is the comprehensive analysis of the complexes. He says, "The goal is to identify all the proteins that bind to an RNA and determine how they cooperate in regulating the fate of this RNA."

The RNP code is indeed complex, and we have only begun to scratch the surface in deciphering it—but tools to tighten the grip on the RNA will be invaluable for the decoding process.

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RESEARCH PAPERS

Hogg, J.R. & Collins, K. RNA-based affinity purification reveals 7SK RNPs with distinct composition and regulation. *RNA*; published online 24 April 2007.

Vasudevan, S. & Steitz, J.A. AU-rich element–mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* **128**, 1105–1118 (2007).