PERSPECTIVES: MOLECULAR BIOLOGY

Ribozymes in the Nucleolus

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Ribozymes are RNA molecules that behave as enzymes, severing other RNAs at specific sites into smaller pieces. They may be valuable therapeutic tools for repairing cellular RNAs transcribed from mutated genes or for destroying unwanted viral RNA transcripts in the cell. However, targeting ribozymes to the cellular compartment containing their target RNAs has proved a challenge. Now, Samarsky *et al.* (*1*) report that a family of small RNAs in the nucleolus (snoRNAs) can readily transport ribozymes into this subcellular organelle.

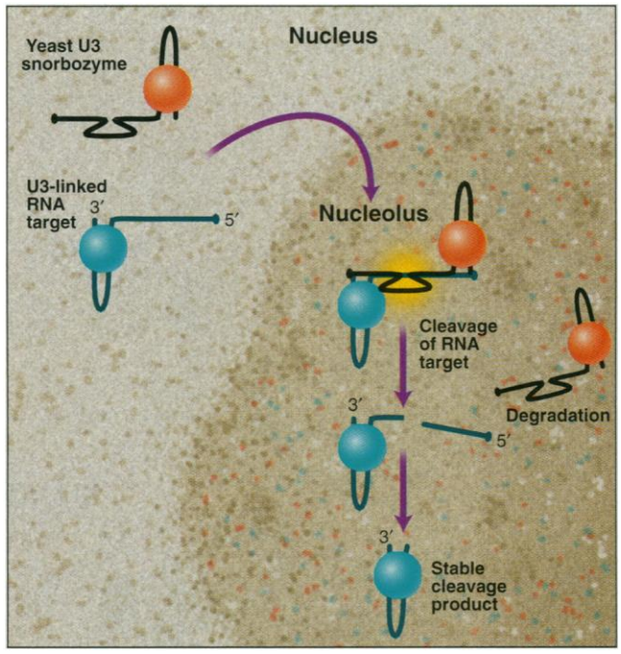
There are two major classes of snoRNA, each with its own highly conserved sequence motif. The C/D box snoRNAs regulate 2′-*O*-methylation of the ribose sugars of ribosomal RNAs (rRNAs), and the H/ACA box snoRNAs guide pseudouridylation of rRNA uridine bases. A few snoRNAs also participate in processing precursor rRNA transcripts (*2–4*). Most snoRNAs are transcribed and processed in the nucleus, although some may be synthesized in the nucleolus (the nuclear site of rRNA synthesis).

Samarsky *et al.* chose yeast for their experiments because the requirements for trafficking of a specific snoRNA (called U3) are well understood in this organism. They showed that nucleolar localization of the yeast U3 snoRNA was primarily dependent on the presence of the C/D box motif (*5*). The investigators appended a test ribozyme to the 5′ end of U3, and then inserted its RNA target sequence into the same location in a separate U3 construct. So, both the ribozyme and its target were expressed in separate, modified U3 snoRNAs. The snoRNA-ribozyme molecule (called a snorbozyme) and its U3-tethered target were transported into the nucleolus. Here the ribozyme cleaved its target RNA with almost 100% efficiency.

Three crucial prerequisites for effective ribozyme action are (i) colocalization of the ribozyme and its RNA target in the same place, (ii) accessibility of the cleavage site in the target RNA to pairing with the ribozyme, and (iii) high levels of ribozyme relative to target RNA (*6, 7*). The importance of colocalization was first demonstrated by tethering a ribozyme to the packaging signal (psi) of a murine retroviral vector and showing that copackaging of the ribozyme with a psi-tethered target resulted in greater than 90% reduction in viral infectivity (*6*).

Samarsky and colleagues used a clever method to assay ribozyme activity based on the rate of appearance of one of the two cleavage products (see the figure). The RNA target tethered to U3 is stable, with a half-life of over 90 min, and its cleavage by the ribozyme generates two products: a short, rapidly degraded 5′ fragment and a 5′ extended form of the U3 snoRNA. The 5′ extension itself gets degraded, leaving intact the U3 hairpin, which is quite stable and easily distinguished from endogenous U3. Taking advantage of the accumulation of this stable product, the investigators were able to measure the kinetics of ribozyme cleavage in vivo. By using similar assay systems, it should be possible to analyze ribozyme cleavage kinetics for virtually any ribozyme-substrate combination under physiological conditions.

There should be plenty of applications for snorbozymes, particularly as the nucleolus is proving to be more than just the place where rRNA is synthesized. For example, precursor transfer RNAs (*8*), RNA encoding the enzyme telomerase, signal recognition particle RNAs, and U6 snRNAs all pass through the nucleolus where they are either processed or receive base and/or backbone modifications (*3*). Messenger RNAs for the c-myc, N-myc, and myoD oncogenes (*9*), and sense-strand RNAs of the neurotropic Borna virus, also travel through the nucleolus (*10*). Viral proteins such as HIV's Rev and Tat and HTLV-1's Rex accumulate in this subcellular organelle (*11–13*). Rev is a crucial regulatory protein that shuttles unspliced viral RNA from the nucleolus into the cytoplasm. Recent findings show that Rev itself is transported out of the nucleolus by binding to a Rev-binding element in a U16 snoRNA (*14*). Using a snoRNA to localize a ribozyme that targets either viral RNA or the Rev-binding element to the nucleolus may be an effective therapeutic strategy to combat HIV. Ribozymes, antisense RNAs, and RNA decoys that bind Rev or Tat may be more effective in the nucleolus than in other regions of the nucleus or cytoplasm. SnoRNA chimeras harboring ribozymes or protein-binding elements should prove valuable not only therapeutically but also for elucidating why certain RNAs and proteins traffic through the nucleolus.



Snorbozyme express. A yeast U3 snoRNA transports a ribozyme (snorbozyme) into the nucleolus where it colocalizes with its RNA target also linked to a modified U3 molecule. The ribozyme cleaves the target into two pieces: The 5′ product is rapidly degraded, whereas the 3′ product is stable and can be readily assayed.

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