NON-CODING RNA

Hunting for human ribozymes

An approach based on enrichment of 5′ hydroxylated RNAs reveals a new self-cleaving ribozyme that maps to a very long non-protein-coding RNA in simians. The ribozyme is active only among hominin sequences, suggesting a recent acquisition of the activity.

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Self-cleaving ribozymes comprise a group of structurally diverse, small (~50–150 nucleotides) RNAs that site-specifically cleave their phosphodiester backbone by activating a 2′ hydroxyl group and promoting its attack on the adjacent phosphate, yielding a 2′-3′ cyclic phosphate and a 5′ hydroxyl group[1]. Most of these ribozymes have been biochemically characterized, yielding a comprehensive view of the structural diversity and catalytic prowess of RNA. In this issue, Chen et al. present a novel biochemical approach to the mapping of cleaved RNAs and reveal a new class of human self-cleaving ribozymes[2].

To identify the ribozyme, the authors sophisticatedly use the property of the 5′-OH-terminated RNA produced by self-cleaving ribozymes. They produced an enriched RNA library containing 5′-OH termini through in vitro transcription of a human genomic library, which was followed by treatment with RppH 5′ pyrophosphohydrolase and XRN-1 exoribonuclease (Fig. 1a). In vitro-generated transcripts begin with a 5′ triphosphate, and RppH cleaves these to yield 5′-monophosphorylated RNAs, which are substrates for processive degradation by the XRN-1 exonuclease. Notably, 5′ OH-terminated RNAs are not substrates for exonucleolytic digestion by XRN-1. By analyzing the digested library by high-throughput sequencing, the authors found that the second most frequent sequence was the previously-described self-cleaved CPEB3 ribozyme[3], thus validating the accuracy of the approach. The most abundant sequence mapped to a very long intergenic non-coding RNA (vlincRNA). A comparative sequence analysis showed that the sequence is conserved among simian genomes, but self-scission was only observed in the hominin variants, comprising the human, chimp, and bonobo sequences. This observation suggests that self-scission is a relatively new function embedded within an older RNA. Because the ribozyme appears to be active only in hominin variants of the vlincRNA, the authors named it the hovlinc ribozyme.

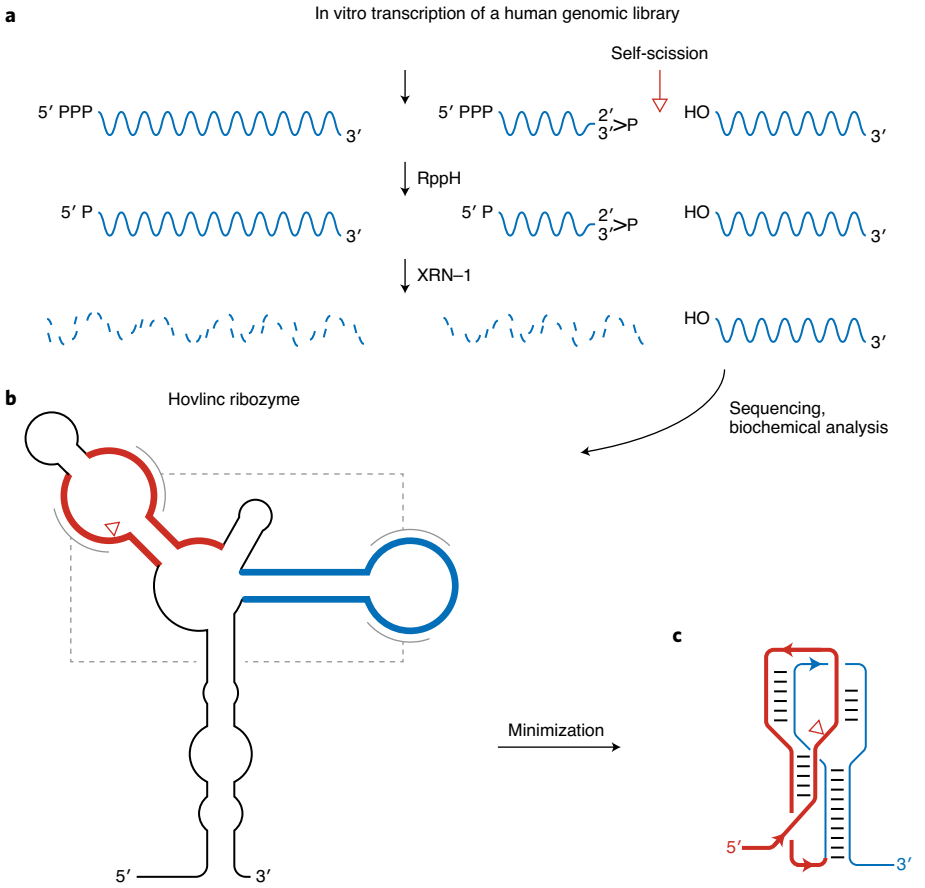

Fig. 1 | Discovery of the hovlinc ribozyme. a, To reveal self-cleaving ribozymes, a human-genomic library was transcribed in vitro and processed using RppH and XRN-1 enzymes, leaving only RNAs terminated with 5′ hydroxyls, which are hallmarks of self-scission. **b**, Sequencing of the library revealed a new type of self-cleaving ribozyme that maps to a hominin vlincRNA. Gray dotted line indicates base-pairing interactions that give rise to the pseudoknot secondary structure shown in **c**. **c**, Minimization and covariation analysis of the ribozyme sequence suggests that the secondary structure consists of two stem-loops, with extensive base-pairing between the two loops (red and blue). Red-outlined triangle marks the self-cleavage site.

Biochemical characterization of the ribozyme showed a distinct activity profile that categorizes the hovlinc RNA as a new class of small self-cleaving ribozymes, a status supported by secondary structure prediction and mutational analysis (Fig. 1b). The authors modeled its minimal, ~70-nucleotide secondary structure as two stems capped by large loops that base-pair with each other in two separate

segments, making it a unique kind of a double-pseudoknot (Fig. 1c). The cleavage site maps to the base of one of the proposed kissing-loop interactions, and the ribozyme appears to be most active at high pH and at high Mg^{2+} concentrations, although Mn^{2+} and Ca^{2+} also support its activity. Further structural and biochemical studies will be needed to pinpoint the functional groups that organize the active site and catalyze the self-cleavage reaction. Since the ribozyme appears to assemble around the cleavage site, it may be possible that the reverse reaction (RNA ligation) will also be observed, particularly at neutral or low pH.

The biological role of this ribozyme remains unknown. Self-cleaving ribozymes were initially discovered in the 1980s as autocatalytic motifs promoting self-scission (and sometimes ligation) during rolling-circle replication of single-stranded RNAs encoded in viroids, the human hepatitis delta virus (HDV), and some genetic satellites. More recently, they have been identified in a variety of genomic contexts[1,4], including in mammals: an in vitro selection from a human genomic library yielded an HDV-like ribozyme in the cytoplasmic polyadenylation element binding protein 3 (*CPEB3*) gene[3], and bioinformatic searches revealed hammerhead ribozymes in reversion-inducing cysteine-rich protein with Kazal motifs (*RECK*) and *C10orf118* (now called *CCDC186*) genes[5,6], as well as a discontinuous hammerhead ribozyme in the 3′ untranslated region (UTR) of C-type lectin type II (*Clec2*) gene in rodents[7]. Last year, an analysis of mammalian short interspersed nuclear element (SINE) retrotransposons showed an epigenetic ribozyme in the murine B2 RNA and the human ALU elements, although these ribozymes appear to be highly activated by the Polycomb protein EZH2 (ref. [8]). The first three ribozymes map to introns of mammalian-conserved genes. In the first description of a biological role for one of these sequences, inhibition of the CPEB3 ribozyme using an antisense oligonucleotide was recently shown to result in higher levels of spliced CPEB3 mRNA and protein in mouse hippocampus, strengthening long-term memory[9]. Intronic ribozymes may therefore be used for fine-tuning of co-transcriptional splicing of their host pre-mRNAs.

In contrast, hovlinc maps to a long intergenic non-coding RNA with unknown function. This family of RNAs plays diverse roles in molecular and cellular processes, including chromatin remodeling, transcriptional regulation, and RNA stability[10]. A self-cleaving sequence may play a part in defining or inhibiting the activity of one of these RNAs. The vlincRNA harboring the hovlinc ribozyme is only detectable in a single cell line out of more than 30 with available RNA sequencing data tested by the Encyclopedia of DNA Elements (ENCODE) consortium, and so the ribozyme may be expressed in only a limited set of tissues or conditions. Future studies aimed at controlling the activity of this ribozyme will be needed to define the biological function of not only the ribozyme, but also the RNA that harbors it. If the ribozyme is indeed scarcely expressed, but compatible with expression in human cells, it may find applications in processing of synthetic RNAs. Further insights into its unusual secondary structure will surely offer ways to engineer this catalytic RNA into a regulatory tool. ❐

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Competing interests

The authors declare no competing interests.