# Revising messages traveling along the cellular information superhighway

Cells use RNA messages to carry instructions from the genome to ribosomes about the types of proteins that should be made. These messages are generally revised by splicing before translation. Engineering ribozymes that can use splicing to repair mutant transcripts may be a useful approach to gene therapy of several genetic diseases.

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DNA genomes house instructions for building a vast array of biological molecules that are required to maintain life. This organization dictates that the conversion of these genetic instructions into needed macromolecules is a seminal event in cellular morphogenesis and metabolism. The molecular mechanism underlying this transformation has been extensively studied for the past several decades. Here, I will briefly review the role of RNA in this information conversion and speculate on how we may be able to employ RNA to rewrite genetic instructions for therapeutic ends.

### The RNA stretch of the cellular information superhighway

RNA molecules have been found to be key participants at several stages in the pathway used by eukaryotic cells to convert the warehouse of information stored in DNA genomes into needed biomolecules (Fig. 1). As every first-year biology student knows, the information contained in a given protein-encoding gene is directly copied into the corresponding pre-messenger RNA by transcription. The information embedded in this RNA is not fixed, however, and can be modified by splicing [1–4] or editing [5–7] to remove, add, or rewrite parts of the initial transcript. Interestingly, many of the molecules involved in revising the RNA messages are RNAs themselves. Small RNAs found in abundant ribonucleoprotein (RNP) particles are involved in pre-mRNA processing [1,8], and guide RNAs are thought to direct RNA editing in kinetoplastids [5,9]. Once revisions are complete, the rewritten messages are translated to produce the proteins

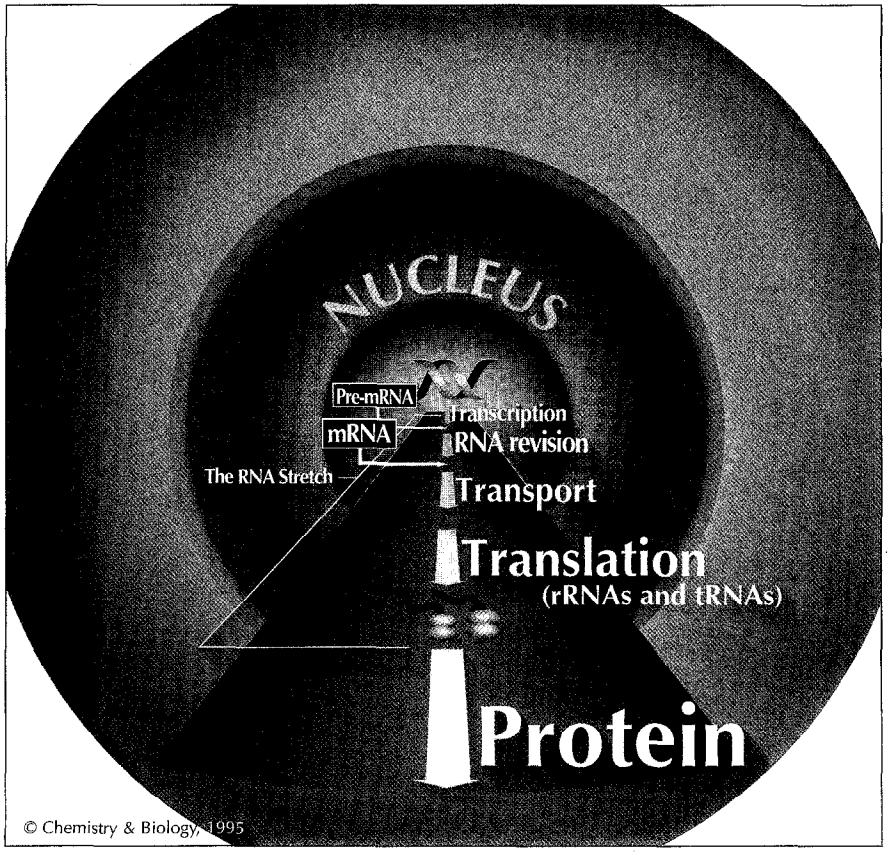


**Fig. 1.** The cellular information superhighway. DNA is transcribed into pre-messenger RNA (pre-mRNA) then revised by splicing, mediated by small nuclear RNAs (snRNAs) or self-splicing reactions, to give messenger RNA (mRNA). The mRNA is transported out of the nucleus before translation, which requires ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs).

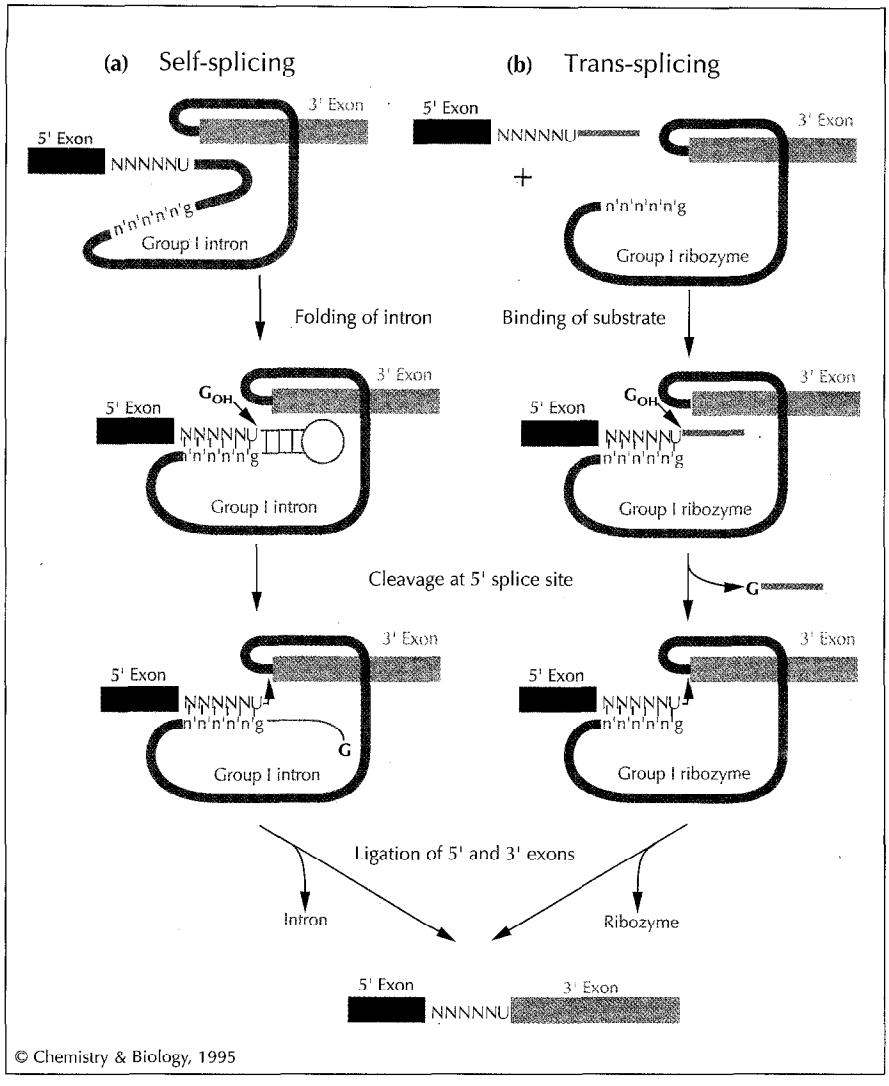


**Fig. 2.** Comparison of self-splicing and trans-splicing by the *Tetrahymena* group I ribozyme. **(a)** Self-splicing is initiated by attack of the 5' splice site with an intron-bound guanosine. This cleavage occurs just 3' of the uridine (shown in red) which is involved in a G–U base pair. The intron (green) holds onto the cleaved 5' exon via base pairing through its 5' exon binding sequence (green and in lower case: gn'n'n'n'n'). Ligation of the freed 5' exon (dark blue) to the 3' exon (yellow) splice site yields a processed RNA. **(b)** In trans-splicing, the ribozyme recognizes the substrate RNA by base pairing to the sequence (5'-NNNNNU-3') through the complementary 5' exon-binding site (5'-gn'n'n'n'n'-3') of the ribozyme. The ribozyme cleaves the bound substrate at the reactive uridine (red), releases the 3' cleavage product (light blue) and holds onto the 5' cleavage product (dark blue). Exon ligation then proceeds as in the self-splicing reaction.

that are needed at that moment. Two additional types of RNA molecules, ribosomal RNAs and transfer RNAs, are required for this decoding step [10].

Obviously this RNA-centered overview of cellular information flow is greatly oversimplified. Many proteins and other cellular factors are required to regulate gene expression. However, it serves to make the point that a fundamental role of RNA molecules in cells is to help manage the use of genetic information. RNA transcripts appear to be the sole carriers of instructions from the genome to the ribosome. Such RNA couriers can also be thought of as storage molecules; the content of what is stored can be easily modified by other RNAs, the molecular relatives of the couriers. Most of the modifying RNAs collaborate with proteins, forming RNPs.

### Ribozymes as revisionists

Not all message revisions require proteins. Certain group I and group II intron RNAs can excise themselves from precursor transcripts without the aid of protein [11–15]. In the process, the introns ligate flanking exon sequences together and produce a processed RNA with a revised primary sequence. These self-splicing reactions are accomplished by catalytic centers composed entirely of RNA residues that are formed by complex tertiary folding of the intron sequences. Examples of such self-splicing RNA enzymes or ribozymes have been found in the genomes of a range of eukaryotic organisms, although they have not yet been found in vertebrates.

The self-splicing reaction of the group I intron ribozyme from *Tetrahymena thermophila* is perhaps the most thoroughly understood reaction that revises RNA. The intron performs two consecutive trans-esterification reactions to liberate itself and join flanking exons (Fig. 2a) [15,16]. In the first reaction, an intron-bound guanosine attacks the phosphodiester bond at the 5' splice site that is defined by a conserved, unconventional G–U base pair [17]. In the second reaction, the free 3' hydroxyl group, now present on the end of the cleaved 5' exon, attacks the phosphorus atom at the 3' splice site to result in the liberation of the intron and the ligation of the 5' and 3' exons. Careful characterization of this reaction over the past decade has illustrated that the vast majority of sequence requirements for this self-splicing are contained within the intron. No specific sequence requirements exist for the 3' exon

[16], and the only specific sequence requirement for 5' exons is to have a uridine preceding the cleavage site [18]. In addition, base pairing must be maintained between the end of the 5' exon and the 5' exon-binding site present in the ribozyme so that the ribozyme can hold onto the 5' exon after cleavage. These base pairs can be composed of almost any sets of complementary nucleotides, however [18].

### Ribozyme-mediated trans-splicing

In addition to performing a self-splicing reaction, a slightly shortened version of the group I ribozyme from *Tetrahymena* can trans-splice an exon attached to its 3' end onto a targeted 5' exon RNA which is a separate RNA molecule [18–20] (Fig. 2b). In this reaction, the 5' exon is recognized by the group I ribozyme via base pairing through its 5' exon-binding site. In the process of pairing, a uridine is positioned across from the guanosine present at the 5' end of the 5' exon-binding site of the ribozyme. Once positioned, the ribozyme catalyzes the cleavage of the substrate RNA at the reconstructed 5' splice site and then ligates its 3' exon onto the 5' exon cleavage product.

Trans-splicing by group I ribozymes is extremely malleable because there are very few sequence requirements for the exons in this reaction. Virtually any uridine residue in a 5' exon can be targeted for splicing; it is only necessary to alter the nucleotide composition of the 5' exon-binding site to make it complementary to the target site. Because no specific 3' exon sequences are required, trans-splicing ribozymes of this kind could be designed that would be able to splice virtually any 3' exon sequence onto a targeted 5' exon.

### RNA revision by trans-splicing

Trans-splicing ribozymes can indeed be used to revise the sequence of targeted RNAs. In the first example of this application, the group I ribozyme from *Tetrahymena* was re-engineered to repair truncated *lacZ* transcripts via targeted trans-splicing [20]. In this system, the ribozyme's normal 3' exon was replaced with 200 nucleotides of *lacZ*. For trans-splicing to correct the defective *lacZ* messages, the ribozyme must recognize the truncated 5' *lacZ* RNA by base pairing, cleave off additional nucleotides, hold onto the 5' exon cleavage product by maintaining base pairing through the 5' exon-binding site of the ribozyme and ligate the *lacZ* 3' exon sequence onto the cleaved 5' product to yield the correct translational reading frame. It was shown that the ribozyme could faithfully accomplish such RNA revision both *in vitro* and in *Escherichia coli*.

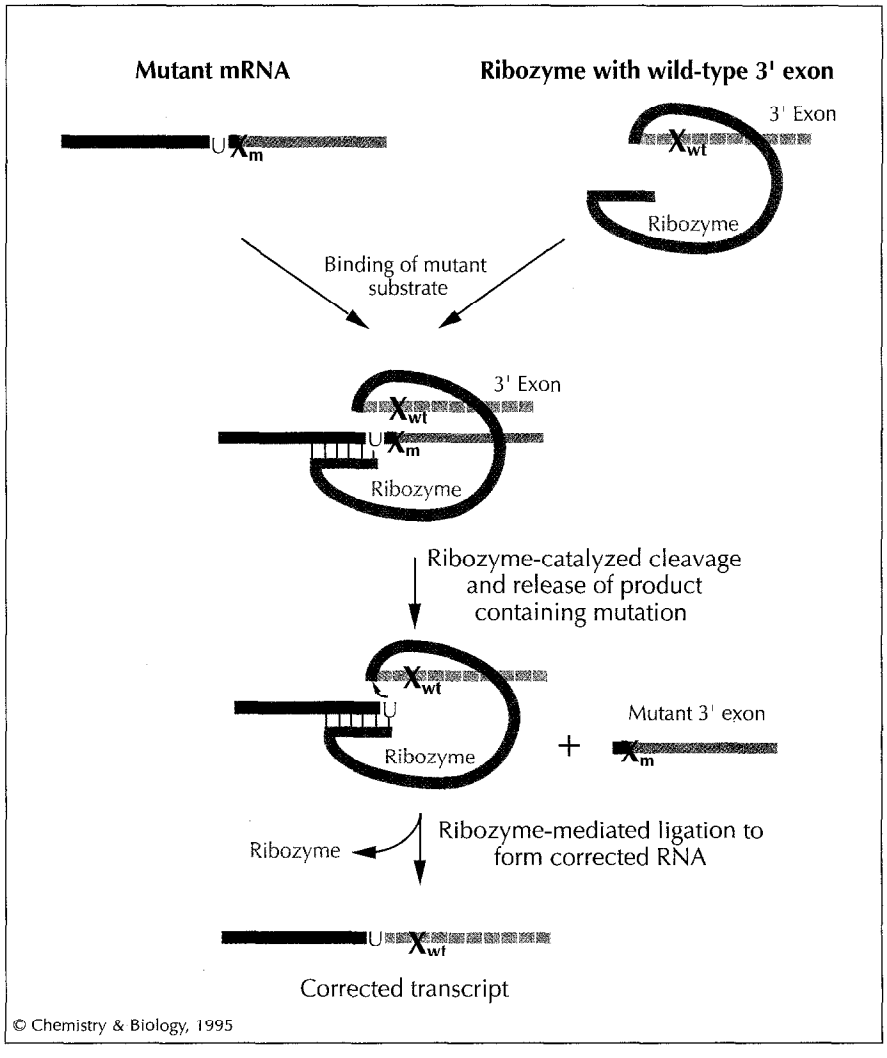


**Fig. 3.** Scheme to correct mutant transcripts with targeted trans-splicing. Mutant messages can be recognized by a ribozyme at any uridine (red) upstream of the mutation (marked by $X_m$). The ribozyme then removes the sequence containing the mutation (light blue) and replaces it with a 3' exon that encodes the correct sequence for the wild-type transcript ($X_{wt}$; correct sequence shown in yellow).

Furthermore, in *E. coli* the repaired RNAs went on to be translated to produce the correct protein product, the α-complement of β-galactosidase [20]. A generalization of this strategy is shown in Figure 3.

In this example, the properties of a group I RNA enzyme were exploited to revise mutant RNA messages in transit along the *E. coli* information superhighway. If similar revisions are possible in mammalian cells, these splicing ribozymes may represent very useful therapeutic agents. Altering genetic instructions at the RNA level for therapeutic ends is appealing intellectually because, as noted above, the information in most genetic messages is revised normally by similar cellular processes before translation.

### Therapeutic applications of RNA revision

In this era of molecular medicine, the genetic basis of an increasing number of inherited diseases is being discovered. Gene therapy represents a new and exciting approach to the treatment of such diseases [21,22]. Conceptually, gene therapy is quite simple. To treat a genetic deficiency, one would introduce a functional version of the defective gene into the cells of the deficient patient. To accomplish this in practice, most often a viral vector is used to transfer a cDNA copy of the wild-type gene, usually under the control of a heterologous promoter, to cells harboring a mutant version of the gene. If the human genome were a simple warehouse of information, this approach would probably be quite successful. Unfortunately for the gene therapist, our genome appears to be extremely complicated, and expression of the information contained within it appears to be highly regulated. This complexity may severely limit the utility of the simple 'add-back' approach to gene therapy. cDNA versions of genes that are integrated in incorrect locations in the genome and that are expressed from heterologous promoters will almost certainly not recapitulate the normal expression pattern of the endogenous gene. Therefore, unless significant technical advances are made, the add-back approach may only be useful for the treatment of genetic disorders associated with genes that do not require regulated expression to function properly.

RNA revision may offer a solution to this problem. Using trans-splicing ribozymes to alter RNA messages, defective transcripts can be emended to encode a wild-type version of a gene product (Fig. 3). This approach will mainly be useful for conditions resulting from a defective gene that contains a point mutation or deletion that does not alter the expression pattern of the gene; such defects occur in many genetic diseases, including sickle-cell anemia and cystic fibrosis. Trans-splicing in mutant cells of this kind should yield a corrected gene product only at the proper times, because repair can only occur when the targeted, mutant transcript is present. RNA repair may be especially appropriate for the treatment of genetic disorders characterized by gene defects that result in the production of deleterious or dominant-mutant proteins, such as sickle-cell anemia and certain cancers. Ideally, genetic treatment of these maladies should inhibit the production of the mutant protein and engender the production of the wild-type product. By repairing mutant messages, trans-splicing ribozymes might be able to accomplish both tasks simultaneously. RNA revision via trans-splicing or other means is thus a new and potentially very useful alternative approach to genetic therapy for inherited diseases.

### The significant hurdles ahead

The hypothesis that RNA revision can be useful in the clinic for the treatment of genetic deficiencies remains largely untested. Enzymologists have studied the *in vitro* trans-splicing reaction catalyzed by the group I intron for several years [15,16,18,19]. The scientific foundation laid by these studies has recently allowed biologists to begin to employ similar reactions to repair mutant RNAs in bacteria [20]. Now we must begin to determine if targeted trans-splicing can proceed in mammalian cells as well. Once *in vivo* experimental systems are available, biologists, enzymologists and chemists will have to work together to address the many unanswered questions about trans-splicing reactions in the mammalian cellular milieu. What limits the rate of ribozyme-mediated RNA repair inside eukaryotic cells? If necessary, can the overall reaction rate and efficiency be increased? Does the ribozyme react only with its targeted substrate *in vivo* or does it react with a large number of incorrect RNAs? If necessary, can specificity be increased? How does RNA trafficking and metabolism influence such reactions? These questions need to be answered before we can accurately assess how useful group-I-mediated RNA revision is likely to be. Even if a useful trans-splicing ribozyme can be made, improvements in gene transfer and expression systems will almost certainly be required to give stable expression of ribozymes inside cells, so that they can repair the mutant RNAs for extended periods of time. Nevertheless, the concept of directed RNA revision is intriguing and deserves attention. Other RNA revision strategies that employ different ribozymes, protein enzymes, or RNPs to modify RNA messages in a variety of ways should be explored, and the utility of RNA repair by trans-splicing ribozymes should be assessed.

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