Ribozymes as therapeutic agents

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Ribozymes were identified when an RNA precursor was found to be self-splicing; because this RNA was enzymatically active without the benefit of a protein component, it was termed a ribozyme. Ribozymes subsequently have been found not only to cleave themselves free from their RNA strand, but also to act *in trans* and cleave other RNA targets in a sequence-specific manner. This specificity, associated with Watson–Crick base pairing, coupled with catalytic activity to cleave the substrate RNA, makes them potentially effective agents against mRNA of viral or oncogenic origin.

Genes typically contain exons (expressed sequences) separated by introns (intervening sequences). When the RNA is transcribed, the intron sequences are spliced out and the exons are ligated in a transesterification reaction. The newly spliced series of exons is then translated. The protein encoded by a particular gene therefore normally corresponds to an RNA sequence shorter than the gene sequence.

In the early 1980s, Cech and Altman discovered that some RNA splicing reactions are catalyzed by RNA. Certain intervening sequences (group I) were inherently capable of catalyzing these splicing reactions to give rise to mature RNA (Figure 1). Cech termed these enzymatically active RNA molecules 'ribozymes'[1–3]. Ribozymes are found in many biological systems and can be engineered by chemical or molecular manipulation to bind to specific RNA sequence targets and cleave them, thereby inhibiting a gene function. Some also may be used to ligate new pieces of RNA onto the target by *trans*-splicing to create a new gene function.

Types of ribozyme

Ribozymes were identified when an RNA precursor of *Tetrahymena thermophila* was found to be self-splicing (Figure 1)[1,2]. Additional studies extended the kinds of self-splicing ribozymes from group I to group II introns[4–7], which are found predominantly in fungal mitochondria. Modification of the *Tetrahymena* group I intron converted the self-cleaving ribozyme into one acting on a sequence-specific external RNA (or DNA) substrate. The ribozyme acted *in trans*. Group I ribozymes range in size from 200 to 1,000 nucleotides (Figure 2). They require a U in the target sequence 5′ to the cleavage site, and bind four to six nucleotides at the 5′-side.

RNase P is a ribonucleoprotein consisting of an approximately 375-nucleotide RNA plus a small polypeptide. The RNA portion cleaves tRNA precursors to produce the mature tRNA[3].

The Varcud satellite (VS) ribozyme is derived from a satellite RNA of certain natural isolates of *Neurospora*. This ribozyme differs from the others shown in Figure 2; it will cleave double-stranded RNA[8]. These three ribozymes are relatively large, and applications must utilize gene therapy methods.

The 'hammerhead' (HH) and 'hairpin' (HP) ribozyme motifs (Figure 2) were originally identified in plant viroids and virusoids[9–13]; the hepatitis delta virus (HDV) ribozyme was found in a satellite RNA of human hepatitis B virus[14,15]. Each was identified in a self-cleaving form and has been modified to cleave external RNA substrates as well[12,16–21].

Design of ribozymes

The ability to design ribozymes against selected RNA targets has expanded their therapeutic potential. HH ribozymes can be modified in their binding arms to be complementary to any target RNA which contains a UH (where H is any nucleotide except guanosine). The optimum length of the binding arms appears to be 7/7 nucleotides on the 3′- and

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