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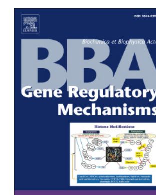

Review

Metabolite-binding ribozymes ☆

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ABSTRACT

Catalysis in the biological context was largely thought to be a protein-based phenomenon until the discovery of RNA catalysts called ribozymes. These discoveries demonstrated that many RNA molecules exhibit remarkable structural and functional versatility. By virtue of these features, naturally occurring ribozymes have been found to be involved in catalyzing reactions for fundamentally important cellular processes such as translation and RNA processing. Another class of RNAs called riboswitches directly binds ligands to control downstream gene expression. Most riboswitches regulate downstream gene expression by controlling premature transcription termination or by affecting the efficiency of translation initiation. However, one riboswitch class couples ligand-sensing to ribozyme activity. Specifically, the *glmS* riboswitch is a nucleolytic ribozyme, whose self-cleavage activity is triggered by the binding of GlcN6P. The products of this self-cleavage reaction are then targeted by cellular RNases for rapid degradation, thereby reducing *glmS* expression under conditions of sufficient GlcN6P. Since the discovery of the *glmS* ribozyme, other metabolite-binding ribozymes have been identified. Together, these discoveries have expanded the general understanding of noncoding RNAs and provided insights that will assist future development of synthetic riboswitch–ribozymes. A very broad overview of natural and synthetic ribozymes is presented herein with an emphasis on the structure and function of the *glmS* ribozyme as a paradigm for metabolite-binding ribozymes that control gene expression. This article is part of a Special Issue entitled: Riboswitches.

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1. Introduction

1.1. Ribozymes: RNA enzymes at the cutting edge

The “RNA world” hypothesis posits that primordial microorganisms relied entirely on RNA molecules for both genomic and catalytic functions [1,2]. This theory has been strengthened by discoveries of ribozymes [3,4], which are RNA macromolecules exhibiting catalytic activity, and riboswitches (reviewed in [5]), which are RNA elements that directly bind metabolic ligands to control gene expression. RNA molecules have also been subjected to two decades of molecular engineering and directed evolution experiments. A key contribution of these latter experiments was the creation of ribozymes whose enzymatic activity depends on allosteric control by small molecule ligands. Subsequently, *glmS*, a natural ribozyme with metabolite-responsive self-cleavage activity, was identified, demonstrating that such ribozymes still reside in living organisms. In general, the investigation of these different RNA molecules will both shed light on the many functions of RNAs in present-day organisms and assist future development of synthetic, “designer” ribozymes and riboswitches.

2. The long and short of natural ribozymes

Natural ribozymes can be grouped into two overall categories: “large” and “small”. The former category includes, among other members, ribonuclease P (RNase P). RNase P is a ubiquitous ribonucleoprotein that catalyzes the removal of an extraneous sequence from the 5′ ends of precursor tRNAs. Enzymatic inactivation of RNase P by treatment with micrococcal nuclease or pancreatic RNase A indicated an essential role for RNA, and the enzyme was later shown to consist of an RNA subunit and at least one protein subunit [6]. After its discovery, the RNA component was speculated to coordinate substrate recognition by site-specific hydrogen bonding to the pre-tRNAs, but was not expected to participate in catalytic cleavage. However, subsequent *in vitro* experiments with both bacterial [4] and eukaryotic [7] RNase P have revealed that the RNA subunits can catalyze pre-tRNA cleavage in the absence of the protein subunit(s). RNase P catalyzes the hydrolysis of pre-tRNA by facilitating a nucleophilic attack of water on the connecting phosphate, thereby breaking the phosphodiester linkage. Two magnesium ions reside in the catalytic core of the enzyme. One of these cations stabilizes the transition state of the enzyme–substrate complex, while

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the other activates cleavage by the enzyme [8]. The arrangement of these divalents is consistent with the two-magnesium arrangement commonly found in many enzymes that perform catalysis on nucleic acids, including all DNA and RNA polymerases and many nucleases [9]. In all such instances, two magnesium ions are coordinated both by the nucleic acid substrate and amino acid residues involved in catalysis. Where amino acid carboxylate groups coordinate and position the metals in protein enzymes, the negatively charged phosphate backbone is utilized by the M1 RNA of RNase P to site-specifically coordinate the divalent cations in a similar overall configuration. RNase P is a true enzyme in that it catalyzes multiple turnovers of pre-tRNA substrates. However, in addition to its central role in tRNA processing, it has also been proposed to affect gene expression patterns by recognizing certain endogenous mRNA substrates [10].

Another class of “large” ribozymes is that of self-splicing group I introns, found in bacteriophages, bacteria, chloroplasts, mitochondria and nuclei of eukaryotic microorganisms (reviewed in [11]). These ribozymes catalyze their excision from other RNAs through two sequential transesterification reactions [11,12] (Fig. 1). In the first reaction, an exogenous guanosine is transiently stabilized within a specific binding pocket through a combination of hydrogen bonding and base stacking interactions. This positions the guanosine 3′ hydroxyl group for nucleophilic attack of the phosphodiester bond at the 5′ splice site, resulting in attachment of the guanosine to the 5′ end of the intron and a free 3′ hydroxyl group on the upstream exon. In the second step, this 3′ hydroxyl attacks the phosphodiester bond at the 3′ splice site, resulting in exon ligation and concomitant release of the intron. A two-metal-ion mechanism, similar to the one found in the RNase P catalytic site, is utilized by group I introns for these catalytic reactions [13]. The selective binding of guanosines by group I introns resembles the selective binding of nucleobase-containing ligands to riboswitch RNAs. Indeed, several classes of riboswitches function as direct sensors of guanine-containing ligands, albeit through structural mechanisms that are distinct from introns [5,14].

The requirement for exogenous guanosine or GTP by group I introns is bypassed in another class of large ribozymes—the Group II introns [15,16]. This class of ribozymes is present across bacteria and in certain eukaryotic organelles. Here, the nucleophilic attack on the 5′ splice site is initiated by a conserved internal adenine, resulting in the formation of an exon–3′ intron intermediate. In a second step, the newly formed 3′ OH of the 5′ exon attacks the 3′ splice site to form ligated exons, releasing the cleaved intron. The presence of a lariat shaped structure and similarities to the spliceosomal RNAs suggest that the group II introns may in fact be ancestors of the eukaryotic spliceosomal machinery. Similar to the group I introns, this class of RNAs also have an absolute requirement for metal ions. A two metal center catalyzes the phosphotransfer reactions resulting in splicing. Sequence and structural features of the ribozyme stabilize the exons in the catalytic site, proximal to the two metal center.

3. A metabolite-binding large ribozyme

One remarkable set of closely related group I introns combine characteristics of both riboswitches and ribozymes [17] (Fig. 1). Observed in *Clostridium difficile*, these introns co-occur with a riboswitch that senses the second messenger molecule, cyclic diguanylate (c-di-GMP). The

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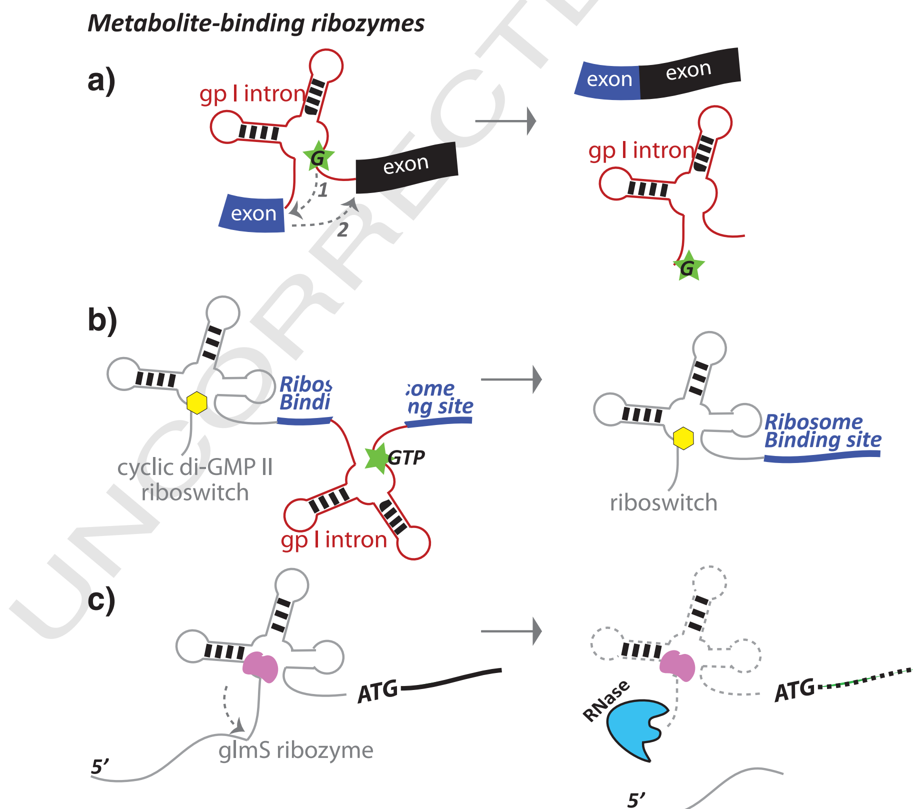

Fig. 1. Mechanisms of gene regulation by metabolite-responsive ribozymes. a) The group I intron (red) binds guanosine or its derivatives, such as GTP (green), to catalyze two successive transesterification reactions (1 and 2). This results in the joining of exons from its 5′ and 3′ termini and subsequent release of the intron. b) The cyclic diguanylate (c-di-GMP) class II riboswitch selectively binds c-di-GMP (yellow), allowing formation of a catalytically-competent structure of a downstream group I intron. Association of c-di-GMP with the riboswitch, along with association of GTP (green) with the group I intron, accelerates catalysis. The product of this reaction is a ligated mRNA that contains a functional ribosome-binding site, which is absent in the precursor RNA. c) The *glmS* ribozyme selectively binds glucosamine-6-phosphate (pink) to stimulate site-specific self-cleavage at an upstream position. The newly formed 5′ terminus is then targeted by an RNase for degradation of the mRNA, thus decreasing expression of downstream genes.

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