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Review

Inhibition of gene expression in human cells using RNase P-derived ribozymes and external guide sequences

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Abstract

Ribonuclease P (RNase P) complexed with an external guide sequence (EGS) represents a novel nucleic acid-based gene interference approach to modulate gene expression. This enzyme is a ribonucleoprotein complex for tRNA processing. In *Escherichia coli*, RNase P contains a catalytic RNA subunit (M1 ribozyme) and a protein subunit (C5 cofactor). EGSs, which are RNAs derived from natural tRNAs, bind to a target mRNA and render the mRNA susceptible to hydrolysis by RNase P and M1 ribozyme. When covalently linked with a guide sequence, M1 can be engineered into a sequence-specific endonuclease, M1GS ribozyme, which cleaves any target RNAs that base pair with the guide sequence. Studies have demonstrated efficient cleavage of mRNAs by M1GS and RNase P complexed with EGSs in vitro. Moreover, highly active M1GS and EGSs were successfully engineered using in vitro selection procedures. EGSs and M1GS ribozymes are effective in blocking gene expression in both bacteria and human cells, and exhibit promising activity for antimicrobial, antiviral, and anticancer applications. In this review, we highlight some recent results using the RNase P-based technology, and offer new insights into the future of using EGS and M1GS RNA as tools for basic research and as gene-targeting agents for clinical applications.

Keywords: RNase P; External guide sequence; Ribozyme; Gene targeting; Antisense; Gene therapy

1. Introduction

1.1. Nucleic acid-based gene targeting approaches for modulating gene expression

Since their discovery, the idea of using catalytic nucleic acids for the purpose of gene inactivation has been highly touted [1,2]. Derived from the originally discovered function of self-cleaving and catalytic RNAs including group I introns and ribonuclease P (RNase P) RNAs [3,4], a wide variety of useful applications have been proposed and tested. Correcting genetic defects and targeted downregulation of pathogenic genes have been attempted, some with moderate success and other still at work. Of these strategies that range from commonly-used antisense oligonucleotides to over-expressing competitive RNA sequences to more recently discovered RNA interference technology, ribozymes stand out among the most extensively studied nucleic acid-based gene targeting approaches [1,2]. In fact, the abilities of these agents have been well characterized and engineered in a way that they can block expression of a wide variety of genes (any given targets) in a sequence-specific and highly potent manner, bringing them even closer to the clinic [1].

Ribozymes exist as naturally occurring catalytic RNAs, expressed in a wide range of living organisms [5,6]. In this review, we focus on RNase P and its derivatives and highlight some of the most recent studies in utilizing RNase P for the development of nucleic acid-based gene therapy, with a special focus on antiviral applications.

1.2. Ribonuclease P

RNase P, an essential cellular enzyme discovered about 35 years ago [7], is a key modulator in tRNA biogenesis; it is

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involved in the multistep processing of tRNA primary transcripts, being responsible for the removal of 5′ leader sequence from precursor tRNA transcripts (Fig. 1) [8–10]. Other reported substrates of RNase P include transfer-messenger RNA [11], bacterial operon RNAs [12,13], riboswitches [14], phage regulatory RNAs [15], and signal recognition particle RNAs [16]. Recently, RNase P has been shown to play an important role in RNA polymerase III transcription, suggesting that transcription and early processing of tRNA may be coordinated [17]. The fundamental nature of RNase P's presence is reflected by the fact that this enzyme has been found in every living cells across all kingdoms of life. The enzyme consists of a single RNA subunit providing the catalytic core and one protein subunit in bacteria, typically 4 in archaea and up to 10 protein subunits in eukarya [8,18]. The presence of ubiquitous homologues of RNase P RNA subunits found across the most primitive phylogenetic domains suggests that the RNA existed in some of the earliest forms of life, even before the differentiation of the phylogenetic kingdoms.

All bacterial RNase P enzymes consist of a single RNA and a single protein subunit. RNase P from *Escherichia coli* is composed of M1 RNA subunit of 377 nucleotides in length and a basic, 14 kDa C5 protein subunit [8,9]. While both RNA and protein subunits are required for *in vivo* activity, increased ionic concentration can activate catalysis by the RNA subunit alone *in vitro* [8]. Such phenomenon can be explained by the idea that a high concentration of salt facilitates screening of electrostatic repulsion in the RNA subunit, where the active structure is otherwise maintained by the presence of the protein subunit. The role of the protein subunit has been extensively investigated. Early experimental evidence indicated that the protein helped stabilization of tertiary structures, while later studies indicated that it could be involved in enhancing pre-tRNA specificity over that of mature tRNAs or pre-organizing metal ion binding sites relevant to catalysis and E–S formation [3,19–26].

Eurakyal RNase P enzymes are much more complex than their bacterial counterparts. The H1 RNA of human RNase P associates with at least ten different protein subunits [10,27].

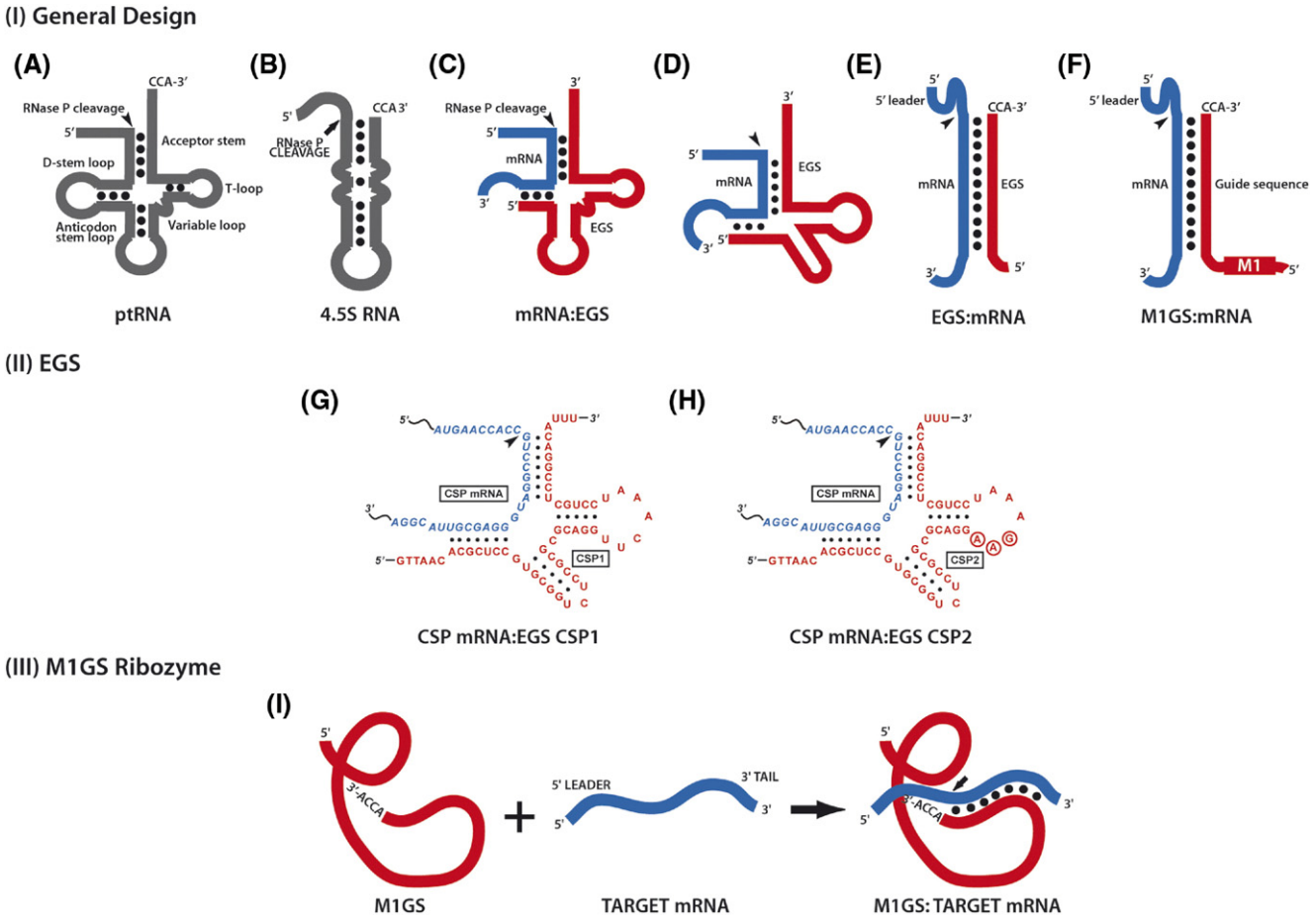

Fig. 1. (A, B) Representation of natural substrates (pre-tRNA (A) and 4.5S RNA (B)). (C–E) A hybridized complex of a target RNA (e.g. mRNA) and an EGS that resembles a part of structure of a tRNA and can be cleaved by RNase P. (D) results from (C) by deleting the anticodon domain of the EGS, which is dispensable for EGS targeting activity, while (E) results from (D) by further deleting the D stem/loop and variable regions. Substrates in panels C and D can be cleaved by human RNase P and M1 ribozyme. In contrast, the stem structure in panel E can only serve as a substrate for M1 RNA and cannot be cleaved by human RNase P. (F) A complex formed between an M1GS ribozyme and a target mRNA substrate. (G, H) Complexes between the HCMV capsid scaffolding protein (CSP) mRNA and EGS CSP1 and CSP2, respectively [82]. (I) Representation of an M1GS RNA construct to which a target RNA has hybridized. The arrow shows the site of the cleavage by RNase P and M1 RHA.