Discovery of functional genes by novel RNA-protein hybrid ribozyme libraries in the post genome era

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ABSTRACT

We constructed a novel RNA-protein hybrid ribozyme that have the site-specific cleavage activity of the hammerhead ribozyme and the unwinding activity of the endogenous RNA helicase. This hybrid ribozyme leads to extremely efficient cleavage of any target mRNA regardless of the secondary structure of the RNA. Since the novel hybrid ribozymes can attack any site within mRNA, libraries were made of the hybrid ribozymes with randomized binding arms and introduced into cells. This procedure made it possible to readily identify the relevant genes associated with phenotype in the apoptosis pathway. This application of a randomized library of hybrid ribozymes represents a simple powerful method for identification of genes associated with specific phenotypes in the post-genome era.

INTRODUCTION

Hammerhead ribozymes (Rz) are among the smallest catalytic RNAs. They have been tested as potential therapeutic agents and their mechanisms of action have been studied. These RNAs can cleave oligoribonucleotides at specific sites (namely, after the sequence NUX, where N and X can be A, G, C or U and A, C or U, respectively, with the most efficient cleavage occurring after a GUC triplet). Thus, RNA molecules consisting of only about thirty nucleotides can be generated for use as artificial endonucleases that can cleave specific RNA molecules. To date, numerous studies directed towards the application of ribozymes in vivo have been performed and many successful experiments, aimed at the exploitation of ribozymes for the suppression of gene expression in different organisms, have been reported (1,2).

RESULTS AND DISCUSSION

To connect the helicase to the ribozyme, we added a sequence of an RNA helicase binding motif (RBM) to the 3’ end of a tRNAVal-driven ribozyme (3,4,5). There are several RNA helicase binding motifs including a constitutive transport element (CTE) (3,4,5). In order to test the efficacy of hybrid ribozymes, we designed four hybrid ribozymes and conventional ribozymes aimed at specific targets. Three ribozymes, namely, Rz1, Rz2 and Rz3, were designed to target inaccessible sites that...
are located within the stable stem-structure. By contrast, Rz4 was designed, as a control, to target a relatively accessible site located in a loop region of the target mRNA.

To examine whether tRNA\textsuperscript{Val}-Rz-RBM-protein complexes can cleave target inaccessible sites, we performed in vitro cleavage assay by these ribozyme-protein complexes. At first, we generated duplexes as substrates by hybridizing partial target mRNAs and mixed with RBM-connected or -unconnected ribozyme-protein complexes as described above. As a result, RBM-unconnected Rz1, Rz2, Rz3 did not unwind the duplexes and, thus, they were unable to cleave the substrate. By contrast, Rz1-RBM, Rz2-RBM and Rz3-RBM were clearly capable of unwinding and cleaving the substrate. However, the inactive Rz2-RBM could unwind duplexes but did not cleave the substrate. Thus, these results clearly demonstrate that tRNA\textsuperscript{Val}-Rz-RBM-protein complexes had two activities such as unwinding and cleavage in vitro.

The sequence of the human genome has become available and it will be extremely valuable to have methods for the rapid identification of important genes. Since our hybrid ribozymes can attack any site, they can attack any mRNA. If libraries of hybrid ribozymes with randomized binding arms are introduced into cells, the genes associated with any changes in phenotypes can be readily identified by sequencing of the specific ribozyme clone (4-6).

This procedure was used to establish a novel functional gene screening system for the signal pathway of Fas or TNF-\(\alpha\)-induced apoptosis using the randomized Rz-RBM expression libraries. In this system, we randomized ten nucleotides in each substrate-binding arm of Rz- RBM. After treatment of the randomized Rz-RBM introduced cells with the Fas specific antibodies or TNF-\(\alpha\), cells that survived were collected and a respective genomic DNA was isolated from each clone. Sequencing of the randomized region of Rzs-RBM in each genomic DNA enabled us to rapidly identify genes that are responsible in the apoptotic pathway. Then we identified many pro-apoptotic genes and novel genes using this strategy. It should be emphasized that, in the absence of the RBM, we would not have identified many genes in our screening. Thus, our gene discovery system using hybrid-ribozyme libraries should be useful for the rapid identification of functional genes in the post-genome era.

REFERENCES