Hairpin ribozyme-antisense RNA constructs can act as molecular lassos

郭泰志

Dallas A;Balastkaya SV;Kuo TC;Ilves H;Vlassov V;Kaspar RL;Kisich KO;Kazakov SA;Johnston BH

摘要.

Abstract

We have developed a novel class of antisense agents, RNA Lassos, which are capable of binding to and circularizing around complementary target RNAs. The RNA Lasso consists of a fixed sequence derived from the hairpin ribozyme and an antisense segment whose size and sequence can be varied to base pair with accessible sites in the target RNA. The ribozyme catalyzes self-processing of the 5'- and 3'-ends of a transcribed Lasso precursor and ligates the processed ends to produce a circular RNA. The circular and linear forms of the self-processed Lasso coexist in an equilibrium that is dependent on both the Lasso sequence and the solution conditions. Lassos form strong, noncovalent complexes with linear target RNAs and form true topological linkages with circular targets. Lasso complexes with linear RNA targets were detected by denaturing gel electrophoresis and were found to be more stable than ordinary RNA duplexes. We show that expression of a fusion mRNA consisting of a sequence from the murine tumor necrosis factor- (TNF-) gene linked to luciferase reporter can be specifically and efficiently blocked by an anti-TNF Lasso. We also show in cell culture experiments that Lassos directed against Fas pre-mRNA were able to induce a change in alternative splicing patterns.