Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 7219–7224, July 1996
Medical Sciences

U1 small nuclear RNA chimeric ribozymes with substrate specificity for the Rev pre-mRNA of human immunodeficiency virus

(catalytic RNA/hammerhead/splicing/*Xenopus laevis*)

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Communicated by Robert P. Perry, Fox Chase Cancer Center, Philadelphia, PA, April 4, 1996 (received for review December 18, 1995)

ABSTRACT **The *in vivo* effectiveness of ribozymes strongly depends on the correct choice of the vector molecule. High levels of expression, stability, active conformation, and correct cellular localization are the most important features for a ribozyme vector. We have exploited the utilization of the U1 small nuclear RNA (snRNA) as a vector for specifically targeting a ribozyme into the nucleus. The Rev pre-mRNA of human immunodeficiency virus type 1 was chosen as target for testing the activity of the U_1-ribozyme. The catalytic core of the hammerhead motif, plus the recognition sequences, substituted the stem-loop III of the U1 snRNA. The resulting construct displays efficient cleavage activity *in vitro*. In addition, in the *in vivo* system of *Xenopus laevis* oocytes, the U1-chimeric ribozyme accumulates in large amounts in the nucleus and produces a considerable reduction of Rev pre-mRNA levels. The Rev-specific ribozyme was also inserted in a derivative of the U1 snRNA mutated in the region of pairing with the 5′ splice site, such as to match it with the suboptimal splice junction of the Rev precursor. This construct shows more efficient reduction of Rev pre-mRNA *in vivo* than the wild-type U1 vector.**

The ability to target ribozymes to specific RNAs *in vitro* (1–3) has lead to the idea of exploiting their potential therapeutic value as antiviral agents *in vivo*. Indeed, several examples have been reported in which ribozymes have been used as anti-human immunodeficiency virus (HIV) molecules (4–6), and in some cases protection against virus replication has been described in different human T-cell populations (7–10). One major problem related to the effectiveness of ribozymes is that while in test tubes, these molecules and their substrates can diffuse freely, whereas in cells RNAs are sorted to specific cellular locations. Viral RNAs are also specifically compartmentalized *in vivo*, and this feature may reduce their availability for ribozyme interaction. It has been demonstrated that the delivery of a ribozyme to the same cellular location as its target substrate strongly increases the effectiveness of the ribozyme (11). For this reason many efforts are now devoted to setting up new strategies for directing ribozymes to specific cellular compartments.

In this paper we have developed a strategy for the efficient expression and compartmentalization of an hammerhead ribozyme inside the nucleus and for its specific targeting to a pre-mRNA. The target chosen for testing ribozyme activity is the Rev pre-mRNA of HIV, which undergoes a very peculiar posttranscriptional regulation (12–15). U1 small nuclear RNA (snRNA) was chosen as vector because it has nuclear localization and it is known to participate in the splicing reaction. The specificity of U1 snRNA interaction inside the spliceosome resides in its base pairing with the 5′ splice junction of the substrate pre-mRNA. In Rev pre-mRNA this sequence is suboptimal (12) and, together with the 3′ splice site (13), reduces splicing efficiency. To increase the specificity of the U_1-ribozyme for the Rev pre-mRNA, mutations were introduced in the 5′ splice site pairing region of U1 snRNA such as to match it with the suboptimal 5′ junction of the Rev precursor. Altogether this strategy should confer three features to the ribozyme: (*i*) stability, (*ii*) nuclear localization, and (*iii*) increased substrate specificity for the Rev precursor. U1 RNA should carry the ribozyme on the Rev pre-mRNA, increasing the relative concentration of ribozyme and substrate, thus allowing efficient cleavage. In addition, the use of a Rev-specific U1 snRNA should increase the specificity of the U_1-ribozyme for the Rev precursor in comparison with all the other endogenous unmodified U1 RNA.

We have tested the activity of these ribozymes *in vitro* and in *Xenopus laevis* oocytes, which represent an ideal *in vivo* system for the study of RNA compartmentalization and accumulation. We have found that the chimeric ribozymes are stable and correctly compartmentalize inside the nucleus where they produce a considerable reduction of Rev pre-mRNA levels. The use of disabled ribozymes has allowed us to show that degradation is mainly due to catalytic cleavage rather than antisense effect.

MATERIALS AND METHODS

Construction of Ribozymes. The sequence of the hammerhead active and inactive core where derived from the studies of Uhlenbeck and Haseloff (2, 16, 17). The cleavage site of the Rev sequence corresponds to the C at position 5597 of the HIV genome (18).

The U1 chimeric ribozymes (U1-Rz) were obtained by inverse PCR on the XlU1.340 clone containing the entire U1 RNA gene (19) or on its T7 derivative which allows *in vitro* transcription under the T7 promoter. Both clones were kindly provided by I. Mattaj (European Molecular Biology Laboratory, Heidelberg). The oligonucleotides utilized are as follows: A, 5′-GGACTCATCA(G/C)AGACTCATCACGCAGGGGTCAGCACAACCGGA-3′; and B, 5′-GTGAGGACGAAACTGCCTTGACGACTGCATAATTTCTGGTAGT-3′. The degeneration of the A oligonucleotide allows the simultaneous construction of both the active (U1-Rz) and the inactive (U1-Rz_m) ribozymes. The following oligonucleotides were used for constructing the human chimeric ribozymes: A′, 5′-GGACTCATCA(G/C)AGACTCATCGCAGGGGTCAGCACATCCGGA-3′; and B′, 5′-GTGAGGACGAAA-

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Abbreviations: snRNA, small nuclear RNA; U1-Rz, active U1 chimeric ribozymes; U1-Rz_m, inactive U1 chimeric ribozymes; AdML, major late adenovirus.

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CTGCCTTCGACTGCATAATTTGTGGTAGT-3′. U1-Rz-5′ were obtained by inverse PCR on the U1-Rz and U1-Rz_m constructs by using oligonucleotides C (5′-TGGGCAGGGGAGATACCATG-3′) and D (5′-GTAAGT-ATGAGTTGAACAAG-3′). The ribozyme substitutes nucleotides 95–116 of the U1 snRNA sequence. The tRNA ribozyme was obtained by inverse PCR on a tRNApro gene (20) cloned into the *Eco*RI site of the Bluescript vector. The ribozyme substitutes nucleotides 30–33 of the tRNA. The oligonucleotides used are (*i*) 5′-GGACTCATCAGAGACT-CATGAGAATCATACCACTAGACCAT-3′, and (*ii*) 5′-GTGAGGACGAAACTGCCTTTGGGTGCGAGA-GGTCCCGGGTT-3′.

Construction of Rev Substrates. A 2510-nt-long fragment, covering the first coding exon (76 nt), the entire intron (2330 nt), and 104 nt of the second coding exon of Rev was generated by PCR amplification of HIV clone BH10 (18). The oligonucleotides used are as follows: Rev-A, 5′-CCGAATTCATG-GCAGGAAGAAGCGGA-3′; and Rev-B, 5′-AAGGATC-CGTTCACTAATCGAATGGA-3′. The primers included the *Eco*RI and *Bam*HI sites that allow the cloning in the corresponding sites of the pGEM-4Z (Promega) vector to generate clone pGEM-Rev. The *Bam*HI digestion of this clone allows the *in vitro* transcription of the entire cloned sequences, while the *Ava*II digestion produces a template for the transcription of a 166-nt-long RNA (Rev–*Ava*II) that was used for the *in vitro* analysis of ribozyme activity.

The same 2510-nt-long Rev region was inserted in the *Bam*HI site of the −166pTCAT vector (21), between the *X. laevis* L14 r-protein gene promoter and the simian virus 40 polyadenylylation site; the insert was obtained by PCR using the Rev-B and Rev-C (5′-CCGGATCCATGGCAGGAA-GAAGCGGA-3′) oligonucleotides, both including a *Bam*HI site. This construct (L14–Rev) allows efficient expression of the fused sequences in a (poly)A^+ RNA in *X. laevis* oocytes (21).

The template used for T7 transcription of the U6 snRNA was obtained by PCR amplification of the U6 *X. laevis* gene kindly provided by E. Lund (University of Wisconsin, Madison).

***In Vitro* Ribozyme Activity.** Ribozymes and ^{32}P-labeled substrate RNAs were *in vitro* transcribed from the corresponding T7 constructs. Ribozyme and substrate (150 nM and 50 nM, respectively, or 400 nM and 100 nM) in 50 mM Tris·HCl (pH 7.5) were heated at 95°C for 2 min and cooled 5 min in ice. $MgCl_2$ (10 mM) was added, and the reactions were allowed to proceed at 37°C or when specified at different temperature, for the indicated times. The RNA was analyzed on 6% polyacrylamide-7 M urea gels.

Oocyte Microinjections. Plasmid DNAs, after purification on CsCl gradients, were dissolved in 80 mM NaCl and 10 mM Tris (pH 7.0) at a concentration of 400 ng/μl and 10 nl were injected into the germinal vesicles of stage VI *X. laevis* oocytes according to Caffarelli *et al.* (22). ^{32}P-labeled U6 snRNA (300,000 cpm/μl) was coinjected as internal control. After incubation, RNA was extracted from total oocytes or from manually dissected nuclei (23). Northern blot analysis was performed with probes specific for Rev RNA and U1 snRNA (18, 19).

AdML pre-mRNA is 253 nt long and covers part of the major late transcript of adenovirus; it is obtained by *in vitro* transcription of plasmid pAdML-Δi1 digested with *Sca*I (24). ^{32}P-labeled AdML and U6 RNAs (respectively, 1.5×10^6 and 0.4×10^6 cpm/μl) were coinjected in oocytes that were injected the night before with U1-Rz or U1 plasmid DNAs (4 ng/oocyte). Incubation was allowed to proceed for an additional hour; RNA was extracted and analyzed on a 6% polyacrylamide-urea gel.

Immunoprecipitations with Sm antibodies (25 μl) were performed as described by Hamm *et al.* (25).

RESULTS

Construction of U1 snRNA Ribozymes. A 42-nt-long fragment, containing the catalytic core of the hammerhead ribozyme (17) plus flanking sequences complementary to the target site, was replaced to a 22-nt-long region of the stem-loop III of *X. laevis* U1 snRNA (position 95–116; see ref. 26). The choice of this region was dictated by the following reasons: (*i*) it does not participate in the binding of Sm proteins and of U1-specific proteins that contribute to the correct compartmentalization of the mature U1 snRNP particle (26, 27), and (*ii*) it is not essential for the stability of the U1 snRNA (28). The target sequence is represented by 20 nts of the first coding exon of the Rev pre-mRNA and the cleavage site corresponds to the C at position 5597 of the BH10 sequence (Fig. 1 and ref. 18). From this construct (U1-Rz) a second one was derived (U1-Rz-5′) in which the 5′ terminus of U1 snRNA (5′-GAUACUUACCUGGC-3′) was changed into 5′-GAUACU-UACUGGGC-3′. These substitutions (boldface type), in the region of complementarity to the 5′ splice junction (underlined), produce a U1 snRNA with perfect complementarity to the suboptimal 5′ splice site of the Rev pre-mRNA (see Fig. 1).

Similar constructs were also made in the human U1 snRNA gene and in plasmids containing the T7 promoter. The same sequence containing the hammerhead catalytic core plus flanking sequences was also cloned in a tRNA vector (20), upstream of the anticodon loop (tRNA-Rz).

To control for antisense effect of ribozymes, derivatives of U1-Rz and U1-Rz-5′, which have the conserved C, present in the catalytic core of the hammerhead (CUGAUGA) substituted with a G known to completely abolish cleavage activity (17), were also produced. These constructs, named U1-Rz_m and U1-Rz_m-5′, should display the same antisense effect as their active counterpart but lack catalytic activity.

***In Vitro* Activity of the U1 Chimeric Ribozymes.** T7 transcripts of U1-Rz and U1-Rz_m (185 nt) were coincubated with ^{32}P-labeled Rev–*Ava*II target. Fig. 2 shows a time course of incubation of 150 nM of ribozyme and 50 nM of substrate at 37°C in 10 mM $MgCl_2$ and 50 mM Tris·HCl (pH 7.5). It appears that the U1-Rz converts the substrate (band S, 166 nt long) very efficiently into the expected cleavage products (bands 5′ and 3′), whereas in the same conditions no cleavage is produced by the mutant ribozyme (lanes U1-Rz_m). Interestingly, the tRNA-Rz works less efficiently than the U1-Rz, as demonstrated by the reduced conversion of the substrate into truncated products (lane tRNA-Rz). These data indicate that inside the U1 vector the ribozyme sequences are available for interaction with the substrate and that U1 sequences do not interfere with catalytic activity.

Fig. 2*b* shows the temperature-dependent activity of the U1-Rz. In this case, 400 nM of ribozyme and 100 nM of substrate was used. It appears that at 25°C the activity of the ribozyme is strongly reduced with respect to 37°C. No difference was found between 25°C and 20°C (not shown). U_1-ribozyme has shown substrate-specific cleavage also when incubated in HeLa nuclear extract, indicating that also in the presence of proteins U1-Rz is able to interact with the target sequence (not shown). The enzyme and substrate concentrations used in these two experiments are in the range of those used in other similar cases (29).

Expression and Localization of the U1-Rz Transcripts. The genes encoding for the U1 chimeric ribozymes were purified on ethidium bromide–CsCl gradients and injected into oocyte nuclei (4 ng/oocyte). After overnight incubation, the nuclei were manually separated from the cytoplasms and RNA extracted from the two different compartments. Fig. 3 shows a Northern blot analysis performed with control oocytes (lanes control) and with oocytes injected with the U1-Rz (lanes U1-Rz) and U1-Rz-5′ (lanes U1-Rz-5′) genes. The picture shows that both U1 chimeric ribozymes are efficiently ex-

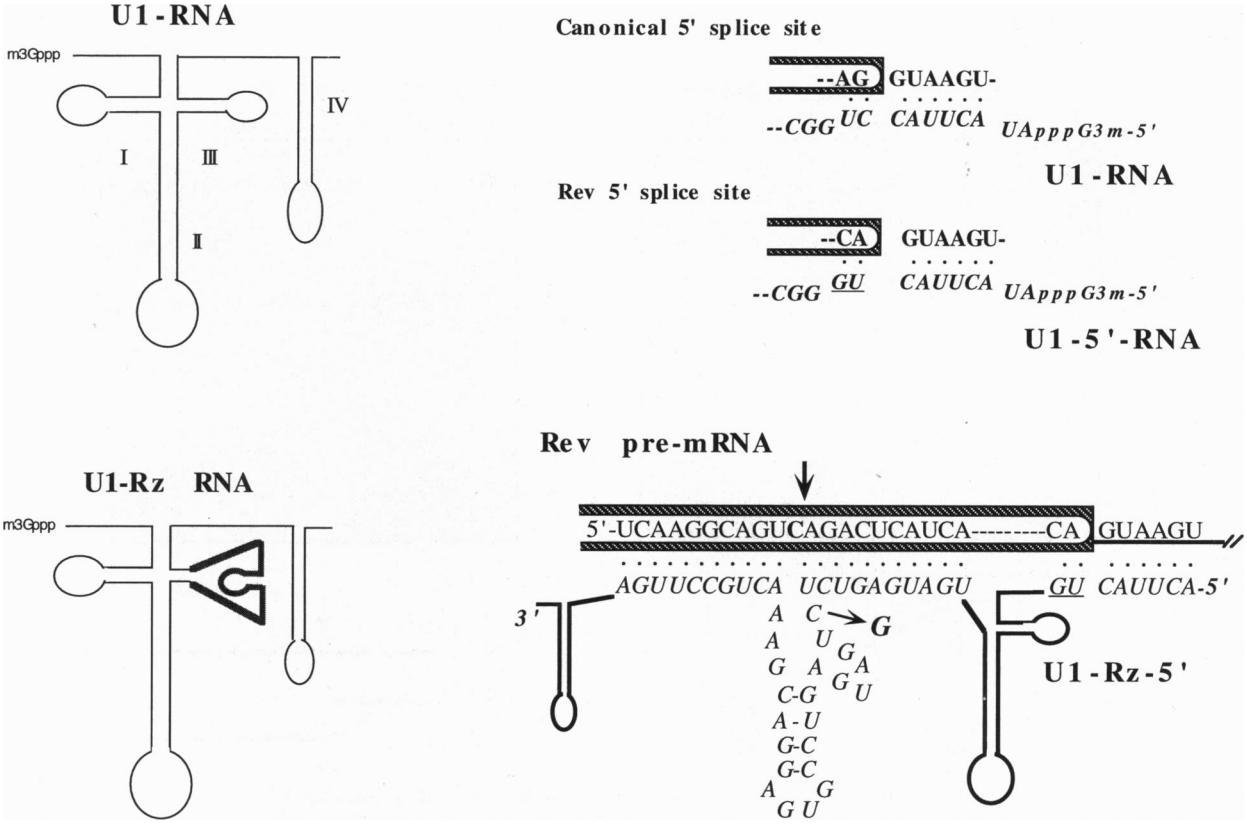

FIG. 1. Construction and sequences of the U1 chimeric ribozymes. (*Left*) Schematic representation of the structure of wild-type and U1-Rz snRNAs. The hammerhead sequence (bold) substitutes part of stem-loop III (nt 95–116; see ref. 26). (*Right*) Sequences of the canonical and Rev 5′ splice sites are shown together with the U1 snRNA sequences. Exons are represented as open boxes; underlined nucleotides indicate the substitutions introduced in the U1 snRNA. The pairing of Rev pre-mRNA and U1-Rz-5′ is shown below; the arrows indicate the cleavage site and the C to G substitution of the disabled ribozymes.

pressed and stably accumulate inside the nucleus. High levels of expression and correct nuclear compartmentalization are observed for the human (lanes U1h-Rz) and mutant constructs (not shown).

U1 snRNA is known to be associated with a specific set of proteins; in particular, the proteins constituting the core particle are in common with other snRNAs and are recognized by Sm antibodies (30). Immunoprecipitation with these antibodies was used to test whether the U1 chimeric ribozymes are able to form U1 snRNP-like complexes. Fig. 3 shows that both ribozymes react to some extent with Sm antibodies: U1-Rz and U1-Rz-5′ are immunoprecipitated one-half and one-fifth as efficiently as wild-type U1 RNA. These results indicate that U1-chimeric ribozymes are able to assemble into Sm-containing particles, even though less efficiently than wild-type U1 snRNA.

***In Vivo* Ribozyme-Catalyzed Cleavage of Rev pre-mRNA.** The *in vivo* activity of the chimeric ribozymes was tested by coinjection of plasmid DNAs coding for the Rev pre-mRNA (clone L14-Rev) and for the different U_1-ribozymes into the nuclei of *X. laevis* oocytes. After overnight incubation, the levels of accumulation of the different transcripts were analyzed by Northern blotting. The transcription of U_1-ribozymes is driven by the homologous U1 snRNA gene promoter, while Rev transcription is driven by the promoter of the *X. laevis* L14 ribosomal protein gene of plasmid −166pTCAT (see *Materials and Methods*). This vector, in *X. laevis* oocytes, ensures efficient expression of the fused sequences in (poly)A^+ RNA (21). Following this protocol, U_1-ribozyme RNAs (185 nt) and Rev pre-mRNA [2510 nt plus ≈200 nt of (poly)A tail] are expressed simultaneously. The molar excess of U1 plasmid with respect to the Rev plasmid (5:1) and the expected higher transcriptional activity of the U1 promoter, should ensure an excess of U1 chimeric ribozymes over the Rev transcripts. ^{32}P-labeled U6 snRNA, known to be specifically retained inside the nucleus (31), was also coinjected. This RNA represents an internal control for successful nuclear injections of plasmid DNAs and for normalization of different injections.

Single injected oocytes (or pools of them) of three independent experiments were analyzed by Northern blot for the accumulation of Rev, U_1-ribozymes, and U6 RNAs. Densitometric analysis was performed on a total of 30 oocytes and the results are diagrammed in Fig. 4*b*. An example of the Northern analysis is shown in Fig. 4*a*).

In comparison with control injections (lanes −), the Rev pre-mRNA signal is 5 times lower in oocytes where plasmid L14-Rev was coinjected with U1-Rz (lanes U1-Rz) and 10 times lower in injections of U1-Rz-5′ (lanes U1-Rz-5′). The inactive versions of U_1-ribozymes produce a 2-fold reduction of the Rev pre-mRNA (lanes U1-Rz_m and U1-Rz-5′$_m$); this effect has not been analyzed in more detail, but it is very likely due to antisense activity (5). The comparison of the effect of the disabled ribozymes with that of the wild types indicates that catalytic activity is higher than antisense effect and strongly contributes to the degradation of Rev pre-mRNA. It is important to remember that oocytes grow at 20°C, which is not the optimal temperature for hammerhead activity. In addition, low temperatures favor antisense effect, especially when the pairing region is long such as in our constructs (20 nt).

The fact that U1-Rz-5′ shows the strongest effect indicates that the mutation introduced in the U1 vector increases the recognition of the substrate, thus enhancing catalytic activity.

Differently from the *in vitro* situation, the 5′ and 3′ cut-off molecules originating from ribozyme cleavage are not ex-

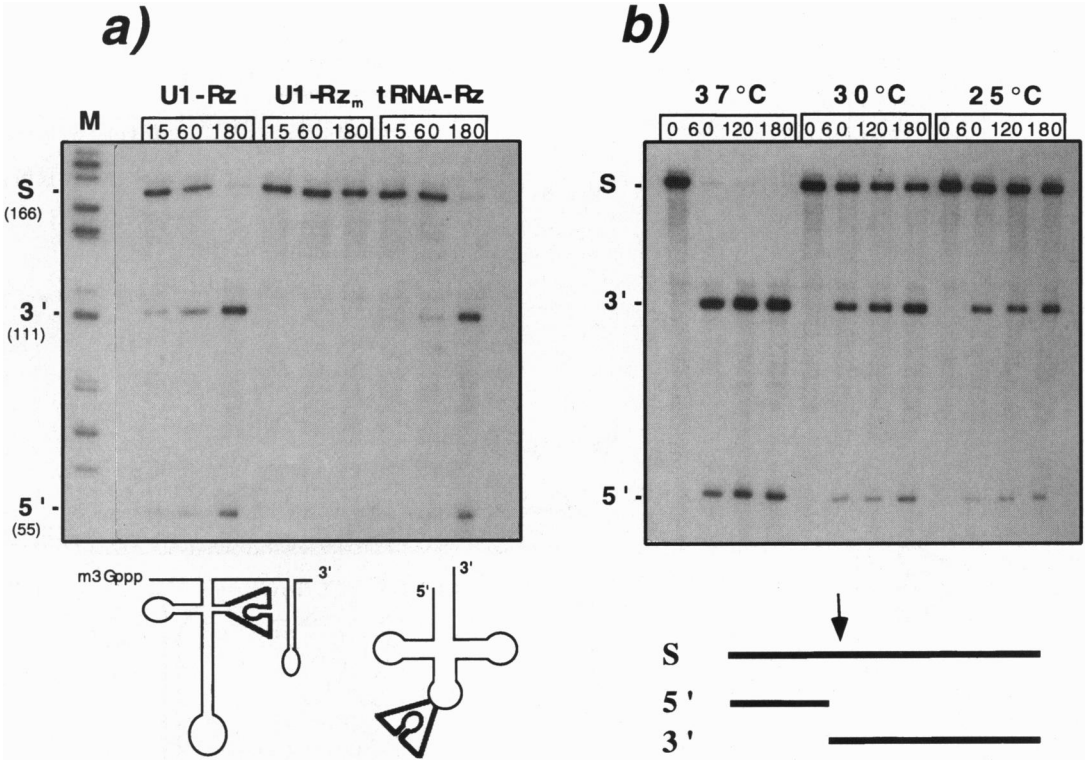

FIG. 2. *In vitro* analysis of ribozyme activity. (*a*) Unlabeled ribozymes (0.15 μM; U1-Rz, U1-Rz$_m$, and tRNA-Rz) were incubated for the indicated time (in minutes) with 0.05 μM of ^{32}P-labeled Rev substrate (166 nt long). The products of the reaction (5′, 55 nt long; and 3′, 111 nt long, schematically represented below) were analyzed on a 6% acrylamide-urea gel. Lane M, molecular weight markers (pBR322 plasmid DNA, *Msp*I digested). The structures of the U1 and tRNA ribozymes are represented below. (*b*) Unlabeled U1-Rz (0.4 μM) and of ^{32}P-labeled Rev (0.1 μM) substrate were coincubated at three different temperatures (25°C, 30°C, and 37°C) for the indicated time (in minutes).

pected to accumulate *in vivo* because their unprotected 5′ and 3′ termini are rapidly degraded by endogenous 5′-3′ and 3′-5′ exonucleases. Rapid turnover of cleaved RNA molecules has been indeed observed in *X. laevis* oocytes (23, 32). On the other hand, mature Rev RNA is not visualized since Rev pre-mRNA is a very inefficient splicing substrate (13) and, in the oocyte, produces very little amounts of mRNA even at long incubation times (not shown), in analogy with other cases of inefficient splicing substrates (22, 23).

Fig. 4*a* (*Middle*) shows that the injected U1 chimeric genes are efficiently expressed in all the different oocytes.

The U6 signals in the lower panel derive from the injected ^{32}P-labeled U6 snRNA and witness that the same amount of plasmid DNA was correctly injected inside the nucleus. Oocyte

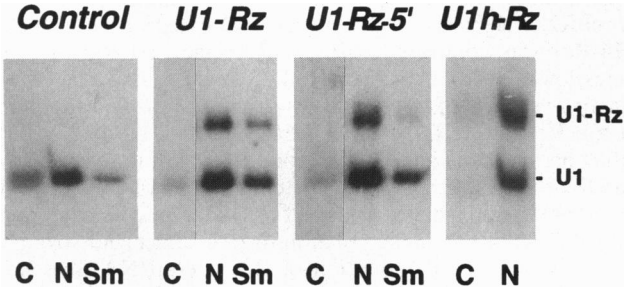

FIG. 3. Cellular compartmentalization of the U1 chimeric ribozymes. Plasmid DNAs coding for U1-Rz, U1-Rz-5′, and U1h-Rz were injected into the nuclei of *X. laevis* oocytes and incubated overnight. Two nuclei (lanes N) and the corresponding cytoplasms (lanes C) were manually dissected and the RNA analyzed by Northern blot with a U1 snRNA specific probe. Two additional nuclei were utilized for immunoprecipitation with Sm antibodies before RNA extraction (lanes Sm). The endogenous U1 snRNA and the U$_1$-ribozymes (20 nt longer) are indicated.

injections of the tRNA-ribozyme have shown that this construct is less active than the U1 constructs even if the expression driven by the pol III promoter is higher than that of the pol II promoter of the U1 gene (not shown).

Effect of U$_1$-Ribozymes on Splicing. To analyze whether the excess of a modified form of U1 snRNA could affect the efficiency of the cellular splicing machinery, we tested the accumulation of splicing products derived from a ^{32}P-labeled AdML pre-mRNA (clone pAdML-Δi1; see refs. 21 and 23) injected in oocytes previously incubated with wild-type U1 snRNA (lanes U1) or U1-Rz (lanes U1-Rz) genes. Also in this case ^{32}P-labeled U6 snRNA was coinjected as an internal control. Fig. 5 shows that only a minimal effect on the efficiency of splicing is observed when U1 chimeric ribozymes are expressed in the nucleus of *X. laevis* oocytes in comparison with overexpression of wild-type U1 snRNA. This is shown by the slightly lower conversion of pre-mRNA into spliced products in lanes U1-Rz. In the lower panel of Fig. 5, the Northern analysis of the same oocytes is shown; it indicates that a very high level of expression of the exogenous genes is obtained. In conclusion, the overexpression of U1-Rz only does not visibly affect the overall efficiency of the splicing machinery.

DISCUSSION

In this paper we have used the oocyte system in order to analyze whether the U1 snRNA gene can be utilized as a vector system for expressing high levels of chimeric ribozymes and for their delivery to the nuclear compartment. U1 snRNA genes would also provide appropriate cassettes for the expression of ribozyme sequences in a stable and tissue nonspecific manner. Constructs were specifically designed with the idea of targeting the ribozyme to a pre-mRNA before it is spliced and the mRNA transported to the cytoplasm. This strategy is well

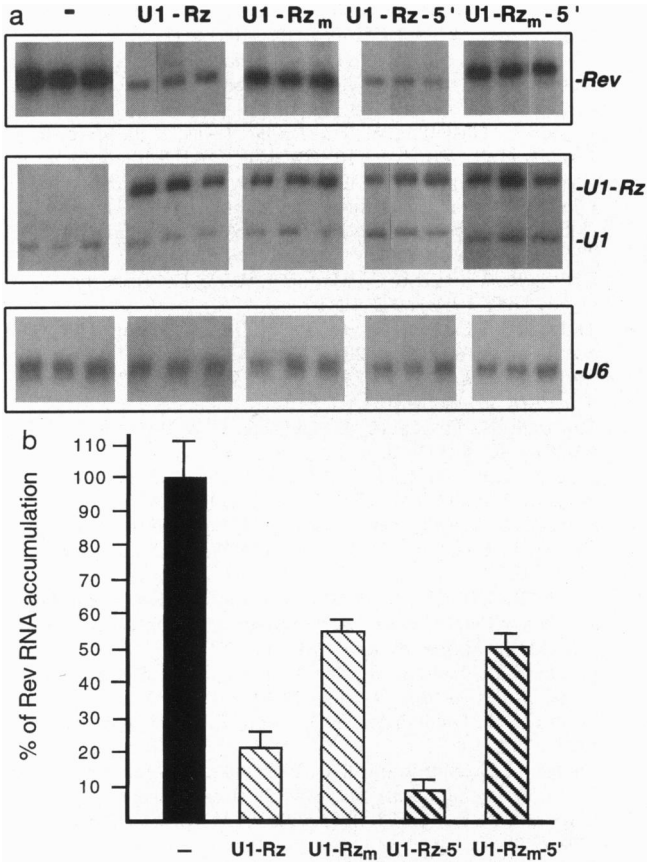

FIG. 4. *In vivo* cleavage activity. (*a*) A solution (10 nl) containing 1 ng of L14-Rev plasmid was injected alone (lanes −) or in combination with 3 ng of each one of the different U1 constructs (U1-Rz, U1-Rz$_m$, U1-Rz-5′, and U1-Rz-5′$_m$) into the nuclei of *X. laevis* oocytes and incubated overnight. As an internal control, *in vitro* transcribed ^{32}P-labeled U6 snRNA was also coinjected. RNA from single oocytes was extracted and analyzed by Northern blotting on: 1% agarose-formaldeyde gel [hybridization with Rev probe (*Top*)] and 6% polyacrylamide-urea gel [hybridization with the U1 probe (*Middle*)]. The direct autoradiography of the polyacrylamide gel allows the visualization of the U6 RNA signal (*Bottom*). (*b*) Autoradiograms of Rev hybridizations from three independent experiments were analyzed by densitometric scanning, and the values were expressed as percentage of the Rev pre-mRNA values obtained in controls.

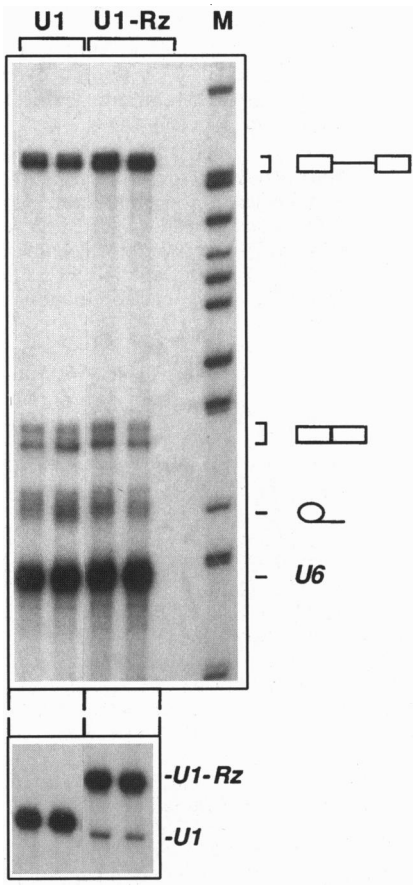

FIG. 5. Effect of U$_1$-ribozymes on splicing. Oocytes were injected with wild-type U1 (lanes U1) or U1-Rz (lanes U1-Rz) plasmids; after overnight incubation, 10 nl of a solution containing 1.5×10^6 cpm/μl of ^{32}P-labeled AdML pre-mRNA and 0.4×10^6 cpm/μl of ^{32}P-labeled U6 snRNA were injected into the germinal vesicles and incubation allowed to proceed for an additional hour. Nuclei from single oocytes were manually isolated and RNA extracted. Samples were run on a 6% polyacrylamide-urea gel and blotted. Direct autoradiography of the filter allows the visualization of ^{32}P-labeled RNAs, while hybridization with U1 probe allows to analyze the expression of the injected plasmids. The different splicing products are indicated on the side. Lane M, molecular weight markers (pBR322 plasmid DNA, *Msp*I digested).

suited for those pre-mRNAs that are not efficient splicing substrates such as the Rev pre-mRNA, which is poorly spliced (12, 13). The colocalization of the Rev pre-mRNA and its specific ribozyme should increase the relative concentration of enzyme and substrate and the effectiveness of the ribozyme activity. To increase the specificity of the U1 chimeric ribozyme for the Rev precursor, mutations were introduced in the region of U1 complementarity with the 5′ splice site such as to have a perfect match with the Rev 5′ splice site.

Experiments of oocyte microinjection have shown that the U1 chimeric ribozymes are efficiently expressed and stably accumulated; in addition, their pathway of expression reflects that of the wild-type U1 snRNA in that it accumulates in the nucleus and it is assembled with Sm antigens.

The U1 chimeric ribozymes display a specific effect on the accumulation of Rev pre-mRNA: U1-Rz reduces 5 times the level of Rev pre-mRNA, while U1-Rz-5′ displays an additional 2-fold greater effectiveness relative to the control. This indicates that the increased specificity of the ribozyme vector for the Rev substrate favors catalytic activity.

Ribozymes have all the properties of antisense RNA with the additional feature of catalytic cleavage. To separate antisense from cleavage effect, we created inactive ribozymes by substituting an essential nucleotide of the catalytic core with an inactive one. Because the inactive ribozymes showed only a partial effect relative to the wild types, we could conclude that the additional activity of the active ribozymes was indeed due to catalytic cleavage. This activity is expected to be much higher in cells growing at 37°C than in oocytes since this temperature is optimal for hammerhead cleavage.

We have shown that the U1 chimeric ribozymes only slightly reduce the splicing efficiency of the oocytes. This is very likely due to the large nonphysiological excess of the U1-Rz RNA, obtained in the oocyte expression system, that competes with the endogenous U1 snRNA. This excess is unlikely to occur in experiments of gene transfer in human cells.

In conclusion, these results show that U1 snRNA is an effective vector for the efficient expression and delivery of ribozymes in the nuclear compartment, pointing out to its general use in cases where nuclear targets should be hit. The choice of substituting stem-loop III with ribozyme sequences has resulted in efficient *in vitro* and *in vivo* cleavage demonstrating that the ribozyme sequences do not markedly affect the stability and compartmentalization of the chimeric construct and that they are exposed and accessible for substrate interaction. The efficient activity of the U1 chimeric ri-

bozymes, raised against the Rev pre-mRNA, justify their utilization and test in HIV-infectable T lymphocytes; in addition, the ribozyme activity in these systems is expected to be strongly enhanced by the growth temperature of 37°C, which is optimal for hammerhead cleavage.

We are grateful to Massimo Arceci for skillful technical help and to Fabio Riccobono and M-medical for oligonucleotide facilities. S.P. is the recipient of an AIDS fellowship and A.M. is the recipient of a Cenci-Bolognetti fellowship. This work has been supported by grants from Ministero della Sanità, Istituto Superiore di Sanità, Progetto A.I.D.S., Rome, and from P.S. "Oligonucleotidi antisenso" of Consiglio Nazionale delle Ricerche.

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