Effect of RNA secondary structure and modified bases on the inhibition of trypanosomatid protein synthesis in cell free extracts by antisense oligodeoxynucleotides

Philippe Verspieren, Nadine Loreau, Nguyen T.Thuong1, David Shire2 and Jean-Jacques Toulmé*
Laboratoire de Biophysique, INSERM U201, Muséum National d'Histoire Naturelle, 43 rue Cuvier, F-75005 Paris, 1Centre de Biophysique Moléculaire, CNRS, F-45071 Orléans cédex and 2Sanofi Elf BioRecherches, 31328 Labège, France

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ABSTRACT
Every messenger RNA from leishmanias and trypanosomes has at its 5' end a conserved region termed the mini-exon sequence which, however, varies from species to species. In a systematic study mRNAs from Trypanosoma brucei, Trypanosoma vivax, and Leishmania enriettii were translated in cell-free extracts in the presence of oligodeoxynucleotides complementary to part of the mini-exon sequence. The affinity of the same oligonucleotides for target and non-target mRNAs was determined by thermal elution of filter-bound complexes showing that the critical temperature of half-dissociation of the complexes was linearly related to log (l + x), where l is the length of the oligomer and x its G + C content. A few oligomers exhibited a lower Tc value than expected which was ascribed to the presence of modified RNA bases or to the existence of a hairpin structure in the L. enriettii mini-exon. In most cases the efficiency of translation inhibition by the oligonucleotides was clearly correlated to their affinity for the target RNA. The modified bases weakened the inhibition of protein synthesis by oligonucleotides complementary to these regions.

INTRODUCTION
Trypanosomes and leishmanias are protozoan parasites responsible for severe diseases in animals and in man. In these organisms the synthesis of mRNA involves discontinuous transcription (1,2). Every mature mRNA contains at its 5'-end a conserved nucleotide sequence termed spliced-leader or mini-exon (3,4). This short RNA fragment is contributed by a small capped RNA, having the mini-exon at its 5'-end immediately followed by a consensus 5' splice site sequence (5–8). The mini-exon RNA is trans-spliced onto the major-exon bearing the coding sequence through a process analogous to cis-splicing in higher eukaryotes (9–11). The length of the mini-exon sequence in mature RNAs of various trypanosomatids was initially reported to be 35 nucleotides. However more detailed analysis of Trypanosoma brucei and Leptomonas collosoma mRNAs revealed the presence of four extra modified nucleotides at the 5'-end of the mini-exon (12,13). The mini-exon sequence is identical for all mRNAs extracted from a given parasite but varies from species to species (14–18).

The mini-exon sequence is a very attractive target for antisense oligonucleotides. This strategy is now widely used to prevent translation of mRNA in a highly selective sequence-dependent manner. The formation of mRNA-oligodeoxynucleotide hybrids is assumed either to prevent the reading of the message by the ribosome or to induce its degradation by RNase-H. Successful experiments were reported both in cell-free extracts, in microinjected and in cultured cells (19–21). Oligonucleotides complementary to mini-exon sequences were previously used to demonstrate their universal occurrence in kinetoplastida mRNA (16,22,23) and their presence at the 5'-end of a few percent of nematod mRNAs (24). Moreover chemically-modified oligonucleotides complementary to the T. brucei mini-exon were shown to kill parasites in culture (25).

Our increasing knowledge about mini-exon sequences and the potential interest of this sequence from the therapeutic point of view prompted us to investigate systematically in more detail the effect of oligodeoxynucleotides on in vitro protein synthesis in order to determine the key parameters for hybrid-arrested translation and to optimalize the target on trypanosomatid mRNA. We therefore studied a series of oligomers, complementary to various parts of the mini-exon sequence of three different parasite species: T. brucei, T. vivax and Leishmania enriettii. Besides their capacity to prevent translation in cell-free extracts we determined their affinity for target and non-target RNA by thermal elution of filter-bound complexes. Using this technique we determined for every oligonucleotide/RNA hybrid the temperature at which 50% of the complexes were dissociated. This parameter, equivalent to the melting temperature of double-stranded nucleic acids in solution, takes into account the peculiarities of the target RNA molecules. We demonstrate that the antisense efficiency of oligomers is clearly correlated to the stability of oligonucleotide/RNA hybrids: mismatches and modified bases

* To whom correspondence should be addressed
which weaken complexes also reduce the amplitude of the antisense effect.

**MATERIAL AND METHODS**

**Oligonucleotides.** Oligodeoxyribonucleotides listed in figure 1 were synthesized on Applied Biosystems or Biosearch synthesizers. They were purified via high pressure liquid chromatography on a C18 reverse phase column eluted by an acetonitrile gradient (from 15 to 45% in 0.1M ammonium acetate pH 7.3). They were then precipitated by acetone in the presence of LiCl, and dissolved in distilled water. Oligomer purity was controlled by electrophoresis on a 20% polyacrylamide/7M urea gel of 5’ [32P]-end labelled aliquots. Concentrations of the solutions were measured by UV absorbance using a molar extinction coefficient \( \varepsilon \) (at 260nm) calculated using equation (1) according to (26). For an oligonucleotide of chain length \( l \) and sequence \( \nu P w x y z \ldots \nu P z \), \( \varepsilon (M^{-1} \times cm^{-1}) \) is equal to:

\[
1^{-1}(2\varepsilon_{wp} + \varepsilon_{w}px + \ldots + \varepsilon_{ypz}) - (\varepsilon_{w} + \varepsilon_{x} + \ldots + \varepsilon_{z})(1)
\]

where \( \varepsilon_{i} \) and \( \varepsilon_{jat} \) are the molar extinction coefficients at 260nm for mono- and dinucleotides, respectively.

Electrophoretic analysis of the two oligodeoxynucleotides MEX Le and MEX Tb having the DNA sequence corresponding to the mini-exon RNAs from *L. enriettii* and *T. brucei*, respectively, (Figure 1) was performed on a non-denaturing 20% polyacrylamide gel (14 x 10 x 0.15 cm) with a TBE (0.089M Tris, boric acid, pH 8.3, 1mM EDTA) buffer run at 150 volts at room temperature.

**Melting experiments.** These experiments were performed on a Uvikon 820 spectrophotometer equipped with a six compartment thermostated holder. Temperature was adjusted by a Haake D8 thermostated holder. Temperature was increased at a rate of 0.25°C/mn and absorbance was measured every minute on each sample at both 340 and 260nm.

**RNA preparation.** The trypanosome clone 118 (MITat 1.5) of *T. brucei* strain IL1392 (stock STIB 731-A) were used. Trypanosomes were grown on SDM79 medium (27). Cells were pelleted by centrifugation at 2000 g for 10 minutes at room temperature. RNAs from parasites were extracted with 3M LiCl/6M urea (28) either from total blood of infected mice (trypanosomes) or from cultured cells resuspended in a phosphate glucose buffer (leishmanias). RNA was either stored in ethanol at −20°C until use or dissolved in sterile water at a concentration of 1mg/ml.

**Filter hybridization.** Nitrocellulose filters were loaded with 3μg of total RNA which was irreversibly bound by heating at 80°C for 2 hours. In general, RNA was not treated prior to loading: comparative experiments performed with non-denatured and heat-denatured RNA did not show any difference. Filters were hybridized overnight, with about 5 x 10^6 cpm (5–20 pmoles) of oligonucleotides 5’-end labelled with [32P]-γ-ATP (Amersham) and polynucleotide kinase (Boehringer) and purified from residual label by gel filtration on a G50 Sephadex column (30 cm; \( \Phi \) 1.3 cm). The description of the device and technical details for thermal elution will be published elsewhere. Typically, a first hybridization was performed in 1ml of 8xSSC/10xDenhardt’s solution (1xSSC = 0.15M NaCl, 0.015M sodium citrate ; 10xDenhardt’s is 0.2% bovine serum albumine, 0.2% Ficoll, 0.2% polyvinyl- pyrolidone) at 4°C. Filters were rinsed with 6 ml of 8xSSC at the hybridization temperature and then eluted by 8xSSC at temperatures increasing by 1.2°C/mn. The eluted radioactivity was collected in scintillation vials (300μl/fraction) and counted. Thermal elution profiles were drawn and the temperature of mid-transition was determined. A second hybridization was performed at temperature about 15°C below this value. Under these conditions partial duplexes did not form, the peak was symetrical and \( T_e \) the critical temperature of half-dissociation was taken as that temperature at which radioactivity peaked. No significant dissociation of RNA from the filter was observed during elution; the same filter was re-used up to three times without any \( T_e \) variation. At least three different elutions were carried out for each oligonucleotide/RNA complex. Reproducibility was better than ±1.5°C.

**In vitro translation.** Nuclease-treated wheat germ extract and rabbit reticulocyte lysate were purchased from Genofit (Geneva). 1μg of total RNA was added to 25μl of the translation mixture containing 0.55MBq of [35S]-methionine (Amersham) and the oligonucleotide at the desired concentration. Mixtures were incubated either at 25°C for 45 mn (wheat germ extract) or at 30°C for 1h (rabbit reticulocyte lysate). [35S]-labelled proteins were analyzed either by trichloroacetic acid (TCA) precipitation of a 2 μl aliquot or by gel electrophoresis on 0.5% SDS/13.5% polyacrylamide gels. Gels were soaked in Amplify (Amersham) and dried prior to autoradiography.

The relative protein synthesis in the presence of an antisense oligonucleotide was quantitated from TCA measurements according to formula:

\[
100 \frac{(P_1-b)}{(P_0-b)}
\]

where \( P_1 \) and \( P_0 \) are the activities of samples (in cpm) programmed with mRNA in the presence and in the absence of oligomers, respectively. \( b \) is the radioactivity of the control (without added mRNA). \( P_0 \) was 4–6 times higher than \( b \).

**RESULTS**

Mini-exon sequences of *T. brucei* (Tb), *T. vivax* (Tv) and *L. enriettii* (Le) are highly conserved (Fig. 1). *T. brucei* and *T. vivax* sequences differ only in two positions, in the 5’ part of the mini-exon. Eight differences are found between *T. brucei* and *L. enriettii* mini-exons, including six contiguous bases in the 3’ half. A block of 13 nucleotides in the 3’ half of the sequence is identical in all the three species. Oligomers, 9 to 34 nucleotides in length, complementary to different parts of the RNA sequences, were synthesized. They will be termed according to the abbreviations listed in figure 1. The 13-mer (13MEX), complementary to the conserved motif, has therefore a target in every mRNA which weaken complexes also reduce the amplitude of the antisense effect.

**Thermal stability of oligonucleotide/mRNA hybrids**

We determined the affinity of oligonucleotides for either complementary or non-complementary RNA, using thermal elution of filter bound complexes, as described in ‘Material and Methods’. The temperature \( T_e \) was used to characterize the
According to the abbreviations indicated in Fig. 1, synthesized, in wheat germ extracts, from T. brucei mRNA in the presence of trichloroacetic acid precipitation of 35S-methionine-labelled proteins. The oligomers were designated exon sequences (bases 4-39), respectively. RNA mini-exon, MEX Tb and MEX Le are oligodeoxynucleotides, 35 nucleotides long, whose sequences are analogue to the L. enriettii mini-exon sequence. The abbreviations of the antisense oligomers are listed on the right of the figure. MEX Tb and MEX Le are oligodeoxynucleotides, 35 nucleotides long, whose sequences are analogue to the L. enriettii mini-exon, and the T. brucei mini-exon.

Figure 1: Mini-exon sequences of T. brucei, T. vivax (14) and L. enriettii (17) (top box) and of complementary synthetic oligodeoxynucleotides. Underlined bases in the L. enriettii and T. vivax mini-exon oligonucleotides indicate differences from the T. brucei sequence. The abbreviations of the antisense oligomers are listed on the right of the figure. MEX Tb and MEX Le are oligodeoxynucleotides, 35 nucleotides long, whose sequences are analogue to the T. brucei and the L. enriettii mini-exon sequences (bases 4–39), respectively. RNA mini-exon, MEX Tb and MEX Le sequences are written with the 5' end on the right. Antisense oligonucleotide sequences are written in the 3' to 5' orientation (from left to right).

The stability of the oligonucleotide/RNA complex. Tc values obtained for various oligonucleotide/mRNA complexes are listed in Table 1.

The stability of perfect duplexes i.e. of duplexes formed between oligonucleotides and complementary RNAs is related to both the length l and the (G+C) content x of the oligomer. When plotted against log (1+x) most of the Tc values fall on a straight line (fig. 2) whose equation under our experimental conditions is:

\[ T_c = 111 \log (1+x) - 103 \]  

However, a few oligomers (12Tb cap, 15Tb cap and 12Tv cap) did not satisfy equation (3) : their characteristic Tc is significantly lower than expected (see Table 1). For the 9-mer, 9Tb no Tc value could be determined although we expected a Tc of about 16.8°C (Table 1). The target of these low affinity oligomers is located close to the 5' end of the mini-exon and includes the adenine in the sixth position of the T. brucei or the second one in the T. vivax sequence (Fig. 1). Significantly, this residue has been reported to be a modified base of unknown structure in the T. brucei mini-exon (12). The oligonucleotide 34Tb also gave a low Tc value. Although the target RNA sequence includes the modified A residue, the contribution of this terminal unstable base pair should not decrease the Tc of a 34 base pair duplex by 7°C (the difference between the theoretical and the experimental Tc). It is probable that equation (3) is no longer valid at high (1+x) values (represented by a dashed line in figure 2).

Table 1. Stability of oligodeoxynucleotide/RNA hybrids and inhibition of T. brucei protein synthesis in wheat germ extract. The theoretical Tc value was calculated from equation (3). The temperature Tc of half dissociation of filter-bound complexes formed between T. brucei (Tb), T. vivax (Tv) and L. enriettii (Le) RNA and synthetic oligodeoxynucleotides was determined as indicated in ‘Material and Methods’. The sign < indicates that no signal was detected from these hybrids (Tc lower than ±1.5°C). The inhibition of translation was calculated from the measurement of incorporation of [35S]-methionine-labelled proteins synthesized, in wheat germ extracts, from T. brucei mRNA in the presence of 35M oligonucleotides (see ‘Material and Methods’). The oligomers were designated according to the abbreviations indicated in Fig. 1.

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duplex (not shown): in the presence of 80mM K+, Tms were 34°C, of a structure far less stable than that of the full 35 base pair transition (Fig. 4A). This transition characterized the denaturation of the T. brucei mini-exons, respectively. Melting of the MEX Le oligomer, followed by UV absorption, resulted in a cooperative transition was observed with MEX Tb (Fig. 4D).

However the structure given in figure 3 for the L. enriettii mini-exon with six contiguous matched base pairs, including a G:U wobble, in the corresponding DNA sequence results in decreased protein synthesis (22, 23). This sequence-dependent process is generally assumed, although hitherto not formally demonstrated, that at a given oligonucleotide concentration the Tm is shifted by 9°C compared to the perfect 12Tb mid:7, RNA hybrid whereas an internal T:U mismatch completely prevented the binding of 12Tb ter to L. enriettii mRNA (Table 1).

Effect of mismatches

As expected the presence of mismatches in oligonucleotide/RNA complexes led to low Tc values compared to fully paired duplexes (Table 1). For instance no signal was detected following elution of filters loaded with T. vivax RNA and hybridized with 12Tb ter. This undoubtedly reflected the presence of two mismatches out of 12 base pairs. The presence of a single terminal T:U mismatch in 12Tb mid/T. vivax RNA complexes reduced the Tc by 9°C compared to the perfect 12Tb mid/T. brucei RNA hybrid whereas an internal T:U mismatch completely prevented the binding of 12Tb ter to L. enriettii mRNA (Table 1).

Inhibition of in vitro translation

As previously reported, the translation of T. brucei mRNA in a cell-free system in the presence of oligonucleotides complementary to the mini-exon sequence results in decreased protein synthesis (22, 23). This sequence-dependent process results from the formation of a hybrid between the RNA and the oligodeoxynucleotide. The extent of inhibition is expected to be correlated with the concentration of the oligonucleotide/RNA complex. Therefore, increasing oligonucleotide concentration should decrease protein synthesis. Conversely, it is generally assumed, although hitherto not formally demonstrated, that at a given oligonucleotide concentration the amplitude of the antisense effect will increase with the binding constant of the oligonucleotide for its target, i.e. with its (1+x) value.

**Figure 3.** Computed structures of the T. brucei (T. vivax) and the L. enriettii mini-exons (see paragraph 2).

**Figure 4.** Melting curves determined from UV absorbance measurements (λ=260nm) of 0.38mM MEX Le in A) low salt (80mM potassium acetate), in B) high salt (200mM potassium acetate + 1mM MgCl2), of 3.8mM MEX Le in C) low salt, of 0.38mM MEX Tb in D) low salt. Numbers above the arrows indicate the melting temperatures Tm.

Figure 5: Electrophoretic properties of mini-exon DNA sequences. [32P]-labelled MEX Le in the absence (lane 1) or in the presence of the complementary 35-mer DNA (lane 2) and MEX Tb (lane 3), were submitted to electrophoresis on a non-denaturing (a) or on a denaturing (b) 20% polyacrylamide gel. Oligonucleotides were kept at room temperature in the presence of 0.4 M sodium acetate prior to loading.
Figure 6: Effect of antisense oligonucleotides on the translation of *T. brucei* and *L. enriettii* mRNA in cell free extracts. A) Relative *T. brucei* protein synthesis in wheat germ extract in the presence of 11Tb (○), 12Tb mid (△), 13MEX (■), 16Tb (□) and 34Tb (○) on the translation of *T. brucei* (○, ■) and *L. enriettii* mRNA (□, △) in rabbit reticulocyte lysate. B) Effect of 19Le (○, △) and 34 Tb (■, □) on *T. brucei* mRNA synthesis in wheat germ extracts. Relative synthesis of 35S-labelled proteins was determined from TCA precipitations as indicated in 'Material and Methods'.

The presence of mismatches in an oligonucleotide/RNA hybrid would be expected to result in a decreased or even an abolished antisense effect. Indeed, 34 Tb induced 50% inhibition of *T. brucei* and *L. enriettii* protein synthesis in rabbit reticulocyte lysate at 3 and 11 µM respectively (Fig. 6b). In the latter case eight mismatches occur in the hybrid. Nevertheless, inhibition of *L. enriettii* mRNA translation was complete at about 30 µM. Another example is given by the effect of 12Tb cap on the translation of *T. brucei* and *T. vivax* mRNAs in rabbit reticulocyte lysate (Fig. 7). *T. brucei* protein synthesis in a rabbit reticulocyte lysate was inhibited in a dose-dependent manner by this 12-mer. This oligomer had a much weaker effect on *T. vivax* protein synthesis, undoubtedly reflecting the fact that the imperfect 12Tb cap/T. vivax mRNA hybrid, which did not give rise to a signal upon thermal elution of filter-bound complexes, was not stable enough to efficiently interfere with translation. In control experiments no significant inhibition of translation (<10%) of non target RNA (BMV or TMV mRNA) was observed in the presence of any oligonucleotide over the concentration range used in our studies (data not shown). No significant homology (<70%) was detected by a computer search between any of the oligonucleotides used and the TMV or BMV genomes.

It should also be noted that the presence of the modified adenine residue in the sixth position of the *T. brucei* mini-exon sequence led to a lower antisense efficiency of 12Tb cap compared to 12Tb mid and 12Tb ter, in good agreement with the Tc values (Fig. 6c and Table 1).

The effect of the hairpin structure in the *L. enriettii* mini-exon sequence on the inhibition of protein synthesis was also investigated. As shown in figure 6b, the oligomer 19Le reduced protein synthesis from *T. brucei* and *L. enriettii* mRNAs, in rabbit reticulocyte lysate, to a similar extent. The two types of complexes could be destabilized in two different ways: a secondary structure in the case of *L. enriettii* and an 'A-A' mismatch in the case of *T. brucei*. However, for *L. enriettii* the destabilization was probably limited as pre-heating the oligonucleotide with the RNA prior to translation to eliminate possible secondary structures had no effect on the amplitude of the inhibition. In addition translation of *T. brucei* and *L. enriettii* mRNAs was reduced to the same extent by 13MEX (not shown) also indicating the unimportance of a putative hairpin structure in the *L. enriettii* mini-exon.

**DISCUSSION**

Our results show that the binding of oligonucleotides to RNA can be monitored by thermal elution of filter-bound complexes. The association of an antisense oligonucleotide with its target is usually performed in solution using a complementary oligodeoxyribonucleotide of the same sequence as the target. In
contrast to our technique this does not take into account the peculiarities of RNA. As demonstrated here this can be relevant: the consequence of the presence of modified bases at the 5'-end on the stability of oligonucleotide/RNA complexes would have escaped detection. Moreover, the formation of specific complexes can be monitored within a heterogeneous population of RNA.

We used the thermal elution technique to follow the behavior of a series of unmodified oligonucleotides complementary to the mini-exon sequence of various trypanosomatids. The critical temperature \( T_C \) of half-dissociation of hybrids correlated both of a series of unmodified oligonucleotides complementary to the mini-exon sequence of various trypanosomatids. The critical temperature \( T_C \) was plotted vs log (1 + x). These deviations could be ascribed to the presence of anomalies either in the primary or in the secondary structure of the target regions for the corresponding oligonucleotides.

Oligomers whose target included the sixth nucleotide of the \( T. brucei \) mini-exon gave low \( T_C \)s compared to oligonucleotides having an identical (1+x) parameter but complementary to other regions of the sequence. Five modified bases of unknown structure have been detected at the 5'-end of the \( T. brucei \) mini-exon sequence (12,13), including a modified ‘A’ in the sixth position. This suggests that the presence of this modified base did not allow efficient pairing of oligonucleotides in this 5’ part of the mini-exon sequence. This could also account for the low \( T_C \) values obtained with 9Tb, 12Tb cap and 15Tb cap. It is not known whether the \( T. vivax \) mini-exon structure also contains such modified nucleotides in the homologous area. However, binding of 12Tv to \( T. vivax \) mRNA gave a low \( T_C \) (15°C) compared to the one (26°C) obtained for the complex 12Tb mid/\( T. brucei \) mRNA. The former value is strikingly close to the one (17°C) characterizing the hybrid 12Tb mid/\( T. vivax \) RNA which has a terminal T:U mismatch, suggesting that the 3'-terminal ‘T’ of 12Tv is not hydrogen-bonded to its vis-a-vis ‘A’ in complexes formed with \( T. vivax \) mRNA. Therefore it is very likely that the particular ‘cap’ structure described for \( T. brucei \) and \( Leptomonas collosoma \) mRNA (12,13) is present at the 5’ end of any trypanosomatid messenger. However, 12Tb cap, complementary to the region homologous to the 12Tv target had a \( T_C \) value only slightly lower than the expected one.

Folding calculations indicated the presence of a hairpin structure in the \( L. enriettii \) mini-exon sequence. Absorbance-monitored melting experiment and electrophoretic analyses of the 35-mer DNAs, whose sequences were analogous to those of the mini-exon Le and Tb RNAs seemed to confirm the calculations. A folded structure would account for the low \( T_C \)s characterizing the hybrids formed between 13MEX or 19Le and \( L. enriettii \) mRNA. Moreover, \( T_C \) values obtained for these complexes (23 and 46°C, respectively) were highly dependent on the temperature conditions we used during the hybridization step. These values were shifted up to the expected values (38 and 52°C, respectively) when hybridization was performed at high temperatures (25 and 35°C, respectively), suggesting unfolding of the hairpin and formation of perfect duplexes under these conditions. Low or high \( T_C \) could be obtained when the same filter was re-used under different conditions indicating that equilibrium between linear and hairpin forms took place on the immobilized filter.

It should be pointed out that the high salt conditions used for the melting experiment shown in figure 4B are close to the ionic concentrations in cell-free extracts. \textit{In vitro} translation experiments were performed either at 25°C (in wheat germ extracts) or at 30°C (in rabbit reticulocyte lysate).

Antisense oligonucleotides targeted to the mini-exon sequence could interfere with the binding of the 40S ribosomal subunit to the cap region. Another mechanism can account for the inhibition of protein synthesis by complementary oligomers: it has been demonstrated that binding of the oligodeoxynucleotide can induce the degradation of the target mRNA by RNase-H (31–34). Whatever the mechanism by which antisense oligonucleotides inhibit translation, the key parameter is the concentration of the oligonucleotide/RNA hybrid. This is directly related to the affinity of the oligonucleotide for the RNA sequence. Indeed the longer the oligonucleotide, the more important the effect: in wheat germ extract, 50% inhibition of \( T. brucei \) protein synthesis was reached at 0.2, 0.7, and 5µM of 34Tb, 16Tb and 13MEX, respectively. The presence of the weak (if any) pair formed by the 3' terminal ‘T’ in 12Tb cap not only gave a low \( T_C \) value but led to an antisense oligomer of lower efficiency than the analogues 12Tb mid and 12Tb ter.

Mismatches weaken hybrids, resulting in decreased antisense efficiency. This constitutes the basis for the specificity of translation inhibition. From our results the presence of non-paired bases drastically affected the stability of short duplexes, a terminal mismatch being less destabilizing than an internal one. The effect of a mismatch in long hybrids was less drastic than in short ones: 34Tb was still able to bind to \( L. enriettii \) RNA despite 8 unpaired internal residues. This was even more striking with 19Le: despite an internal A-A mismatch this oligomer reduced the translation of \( T. brucei \) mRNA. The formation of imperfect duplexes with long oligomers can result in hybrids stable enough to partially prevent translation. This non-specific effect could be amplified by RNase-H if the partial hybrid were recognized by this enzyme.

An acridine-linked 9-mer has been demonstrated to have a trypanocidal activity against \( T. brucei \) in culture (25). The target for this oligonucleotide was located at the 5’ end of the mini-exon sequence and included the modified ‘A’ residue in the sixth position. From the results reported here we might expect to increase the efficiency of an acridine-linked 9-mer if the target were shifted downstream.

As shown above, the mini-exon sequence is an excellent target for antisense oligonucleotides. Due to its presence at the end of every mRNA of the parasite, expression of all genes can be affected at once by a single oligonucleotide sequence. Moreover, the presence in various trypanosomatid species of both a conserved block of 13 nucleotides and divergent sequences at the 5’ end of the mini-exon allows one to target either all the trypanosomatids or a unique parasite species selectively. This could be of particular interest for diagnosis and therapeutic applications.
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