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Mechanisms of the inhibition of reverse transcription by antisense oligonucleotides

(synthetic oligodeoxynucleotides/reverse transcriptase/retroviruses)

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ABSTRACT **We have demonstrated that the synthesis of cDNA by avian myeloblastosis virus and Moloney murine leukemia virus reverse transcriptases can be prevented by oligonucleotides bound to the RNA template ≈100 nucleotides remote from the 3′ end of the primer. The RNA was truncated at the level of the antisense oligonucleotide–RNA duplex during the reverse transcription. The key role played by the reverse transcriptase-associated RNase H activity in the inhibition process was shown by the use of (*i*) inhibitors of RNase H (NaF or dAMP), (*ii*) Moloney murine leukemia virus reverse transcriptase devoid of RNase H activity, or (*iii*) α-analogues of oligomers that do not elicit RNase H-catalyzed RNA degradation. In all three cases the inhibitory effect was either reduced (NaF, dAMP) or totally abolished. However, an α-oligomer bound to the sequence immediately adjacent to the primer-binding site prevented reverse transcription. Therefore, initiation of polymerization can be blocked by means of an RNase H-independent mechanism, whereas arrest of a growing cDNA strand can be achieved only by an oligonucleotide mediating cleavage of the template RNA.**

The replication cycle of retroviruses requires conversion of the single-stranded RNA genome into double-stranded DNA. This conversion is achieved by reverse transcriptase (RT), a multifunctional viral enzyme. The RTs of avian myeloblastosis virus (AMV) and Moloney murine leukemia virus (MMLV) exhibit significant structural differences: the AMV enzyme is a heterodimer composed of 95- and 63-kDa subunits, whereas the MMLV RT is an 80-kDa monomer (1). Both enzymes, which contain DNA polymerase and RNase H, are capable of endonucleolytic cleavage of the RNA template, even though the specificity of cleavage and the size of the remaining hybrid differ after digestion (2). The polymerase and RNase H functions can operate independently (3). Indeed, proteins that display polymerase activity with no RNase H activity can be produced by recombinant DNA techniques (4). Inhibition of cDNA synthesis would prevent the integration of viral information into the host genome and, therefore, disrupt the viral replication cycle. This inhibition might be achieved by complementary oligonucleotides.

Since the use of synthetic oligonucleotides to inhibit the *in vitro* development of Rous sarcoma virus (5), the potential therapeutic interest of the antisense strategy has been recognized (6). The emergence of AIDS and the need for different approaches to control retroviruses have led to numerous studies in the field. The antiviral efficiency of both unmodified and modified antisense oligomers has been tested (7–12). Successful results have been reported with oligomers targeted to different sites of viral RNA or mRNA. However, in most cases, the mechanism by which the antisense oligonucleotide inhibited syncytia formation, human immunodeficiency virus protein synthesis, or RT activity was not elucidated.

We anticipated that the production of cDNA by retroviral polymerase could be inhibited by an oligonucleotide bound to the RNA downstream from the primer: the RT molecule traveling on the template would be blocked by the hybrid formed between the RNA and the antisense oligonucleotide (Fig. 1). In a preliminary report we showed that an unmodified 17-mer, indeed, arrested cDNA synthesis by AMV RT (13). We present here a detailed analysis of the effect of antisense oligonucleotides on DNA polymerization by AMV and MMLV RTs. We have been able to demonstrate that the RNase H activity of AMV and MMLV enzymes was involved in the process because a truncated RNA template was produced. α-Oligomers that are nuclease-resistant analogues of oligonucleotides (14, 15) revealed a second way to prevent reverse transcription: an α-17-mer bound to the sequence adjacent to the primer-binding site arrested polymerization by means of an RNase H-independent mechanism.

MATERIALS AND METHODS

Oligonucleotides. Unmodified β- and α-oligodeoxynucleotides listed in Table 1 were synthesized either on a Pharmacia or on a Biosearch automatic synthesizer. According to previous studies α-oligomers were designed to bind in a parallel orientation with respect to the RNA strand (16, 17). Oligonucleotides linked at their 5′ end to 2-methoxy-6-chloro-9-aminoacridine through a pentamethylene linker were prepared as described (18). All oligomers were purified in one step by HPLC on a reverse-phase column eluted by an acetonitrile gradient (10–50%) in a 10 mM ammonium acetate (pH 7.2) buffer. Purity of the oligomers was evaluated by running ^{32}P-labeled samples on a 20% polyacrylamide/7 M urea gel. Unmodified and α-oligomers with a 5′-OH group were 5′ end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Acridine-linked oligomers were 3′ end-labeled with [α-^{32}P]ddATP by terminal nucleotidyltransferase. Although the yield of 3′ end-labeling was very low, the products could be detected after autoradiography. All preparations contained mainly (>95%) a single species.

RNAs and Enzymes. T4 polynucleotide kinase was from Boehringer Mannheim. *Escherichia coli* RNase H and RNasin were from Promega. AMV, MMLV RT with RNase H (MMLV H$^+$) and without RNase H (MMLV H$^-$) as well as rabbit globin mRNA, were purchased from GIBCO/BRL. Intact RNA was used without any treatment. A fragment,

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Abbreviations: RT, reverse transcriptase; AMV, avian myeloblastosis virus; MMLV, Moloney murine leukemia virus; MMLV H$^+$ RT, MMLV RT with RNase H; MMLV H$^-$ RT, MMLV RT without RNase H; nt, nucleotide(s).

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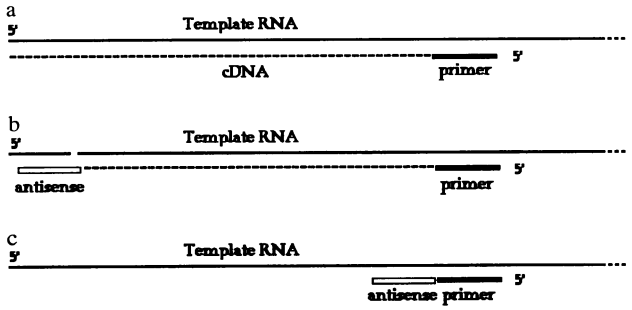

FIG. 1. Scheme of inhibition of reverse transcription by complementary oligonucleotides. cDNA synthesis from the primer oligonucleotide (*a*) could be prevented by an antisense oligonucleotide bound to a template sequence remote (*b*) or adjacent (*c*) to the primer-binding site. In *b*, inhibition leads to cleavage of the template, whereas in *c* the oligonucleotide competes directly with the polymerase.

≈150 nucleotides (nt) long, was obtained by oligonucleotide-directed cleavage of rabbit β-globin mRNA by *E. coli* RNase H: 0.5 μg of β-globin mRNA was incubated with 10 units of *E. coli* RNase H in the presence of 100 pmol of a 15-mer complementary to nt 147–161 of the β-globin mRNA. The reaction was done for 2 hr at 37°C in 20 μl of a 20 mM Tris·HCl, pH 7.5, buffer containing 10 mM $MgCl_2$, 100 mM KCl, and 0.1 mM dithiothreitol. After incubation, RNA was phenol-extracted, ethanol-precipitated, and dissolved in 25 μl of sterile water. For reverse transcription, one-tenth of the RNA solution (corresponding to 50 ng of intact RNA) was used in each reaction as described below.

Primer Extension and RNA Analysis. The standard reaction procedure for cDNA synthesis was as follows: 50 ng of globin RNA (containing 0.3 pmol of intact β-globin mRNA), 50 pmol of primer, and the desired amount of antisense oligodeoxynucleotide were preincubated for 30 min at 39°C. After addition of 1 μl of 10× RT buffer (1 M Tris·HCl, pH 8.3/720 mM KCl/100 mM $MgCl_2$/100 mM dithiothreitol) containing 8 units of RNasin, 2 pmol of [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham), and 5 nmol of the four dNTPs, the volume of the samples was adjusted to 10 μl with sterile H_2O. AMV RT (1–10 units—i.e., 0.13–1.3 pmol) was then added. Reaction with the MMLV RT was done with 10 or 50 units of MMLV H^- or MMLV H^+, respectively. The reaction was incubated for 1 hr at 39°C (except for RNA blot analysis, for which incubation time was reduced to 15 min). cDNA was chloroform-extracted, ethanol-precipitated according to standard procedures, and loaded on a 10% polyacrylamide gel.

RNAs were analyzed on 10% polyacrylamide/7 M urea gels and electroblotted on a nylon membrane (Pall) according to the supplier's instructions. Blots were probed with a 17-mer complementary to nt 51–67 of β-globin mRNA.

Gels (cDNA) and blots (RNA) were autoradiographed, and the films were analyzed by video densitometry. Quantitative results were confirmed by scintillation counting of the sliced gels. Results obtained for cDNA synthesis were corrected for the number of labels incorporated into each fragment.

Table 1. Characteristics of synthetic oligodeoxynucleotides

Abbreviation	Sequence	Target	Use
17cap	TTGTGTCAAAAGCAAGT	3–19	Antisense
17αcap	TGAACGAAAACTGTGTT	3–19	Antisense
17αAcrcap	TGAACGAAAACTGTGTT	3–19	Antisense
12cap	TCAAAAGCAAGT	3–14	Antisense
22cap	CACAGTTGTGTCAAAAG-CAAGT	3–24	Antisense
17aug	GCAGATGCACCATTCT	51–67	Probe
17sc	CACCAACTTCTTCCACA	113–129	Primer
17αsc	ACACCTTCTTCAACCAC	113–129	Antisense
17αAcrsc	ACACCTTCTTCAACCAC	113–129	Antisense
15sc	TGCCCAGGGCCTCAC	130–144	Primer
15sc	GTAGACAACCAGCAG	147–161	Primer
19sc	AAGGACTCGAAGAACCTCCT	172–190	Primer

Abbreviations used are listed in the first column; sequences are written in the 5′ → 3′ direction (from left to right) for both β- and α-oligonucleotides. Position of the target on rabbit β-globin mRNA is indicated (+1 is the first nucleotide after the 7-methylguanosine residue).

RESULTS

Unmodified Oligonucleotides Inhibit cDNA Synthesis and Mediate Cleavage of RNA Template. Reverse transcription of rabbit β-globin mRNA by AMV RT was primed with 17sc, a 17-mer complementary to nt 113–129 (see Table 1 for oligonucleotide sequences), giving rise to the expected cDNA fragment. In the presence of 17cap, an oligomer targeted to the cap region of the mRNA, a shortened DNA fragment was synthesized, at the expense of the full-length product. The size of the DNA fragment corresponded to the distance between primer and binding site of the antisense oligonucleotide. A dose-dependent effect was seen—50% aborted reverse transcription being achieved at 0.3 μM oligonucleotide (Fig. 2*a*). Antisense oligonucleotides targeted to the same region of the template, but of different lengths, led to similar results: oligomers 12cap and 22cap inhibited the full-length product by 50% at 30 and 0.1 μM, respectively (data not shown). This result might indicate a relationship between the oligonucleotide length and its inhibitory efficiency. It should be pointed out that the antisense oligonucleotides we used had a 3′-OH group and could, therefore, function as primers. However, due to the location of their binding sites (the 3′ end of the oligonucleotides was complementary to the third nucleotide of rabbit β-globin mRNA), they could not be lengthened by more than a few nucleotides.

Northern (RNA) blot analysis of a 150-nt-long β-globin RNA fragment, incubated in the presence of oligomers 12cap, 17cap, or 22cap, showed that the template was truncated during the reverse transcription reaction. Length of the break-down product indicated that cleavage occurred at the level of the oligomer hybridization site (Fig. 2*b*). In this experiment concentration of antisense oligonucleotides was adjusted to induce half reduction of the full-length cDNA product. Interestingly, cleavage of ≈50% of the template was induced under these conditions by all three oligomers (Fig. 2*b*). This result strongly suggested that cleavage of the RNA template was the major, if not the only, mechanism of arrested reverse transcription.

Reduction of RNase H Activity Decreases Inhibition Efficiency. NaF (3) and deoxyadenosine monophosphate (19) can inhibit the RNase H activity of AMV RT without perturbing the polymerase function of the retroviral enzyme. We reverse-transcribed the rabbit β-globin mRNA with both the antisense oligomer 17cap and RNase H inhibitors. Under the conditions indicated for Fig. 3, the oligomer 17cap induced the formation of 59% of truncated transcript. Addition of 20 mM NaF reduced the amount of truncated cDNA to 43% and concomitantly decreased the yield of RNA cleavage product to the same level. Identical behavior was seen with 20 mM dAMP (Fig. 3). Therefore, aborted reverse transcription seemed to correlate with the attack of the antisense oligonucleotide–RNA hybrid by the RNase H activity of RT.

MMLV RT Devoid of RNase H Activity Is Not Arrested by a Complementary Oligonucleotide. We tested the ability of an antisense oligonucleotide to block reverse transcription of β-globin mRNA by the MMLV enzyme. This enzyme was commercially available, either as a monomer bearing both the

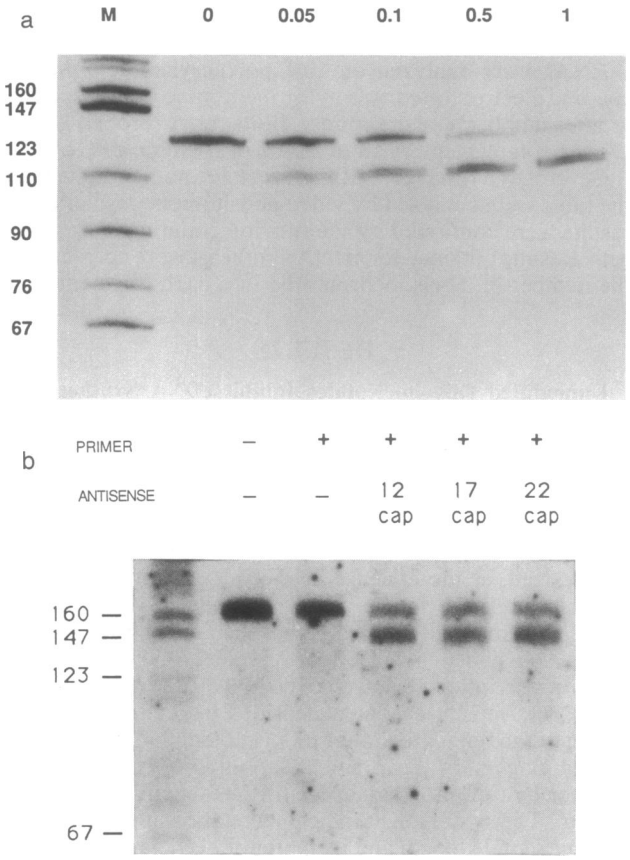

FIG. 2. Effect of antisense oligonucleotides on cDNA synthesis (*a*) and template RNA (*b*). (*a*) Reverse transcription done as indicated by 10 units of AMV RT was primed by 5 μM oligomer 17sc without (0) or with the amount (in μM) of oligomer 17cap indicated above each lane. Analysis was done on a 10% polyacrylamide/7 M urea gel. (*b*) RNA blot analysis of a 150-nt-long fragment of rabbit β-globin RNA incubated with 10 units of AMV RT with the primer (17sc, 5 μM) and antisense oligonucleotides indicated above each lane (30 μM 12cap; 0.1 μM 17cap; and 0.1 μM 22cap). RNA fragments were run on a 10% polyacrylamide/7 M urea gel, electroblotted on a nylon membrane, and probed with a ^{32}P-labeled 17-mer complementary to nt 51–67. Left lanes in *a* and *b* correspond to size markers.

polymerase and the RNase H activity (MMLV H^+) or as a truncated protein devoid of its COOH-terminal end that comprises the RNase H catalytic site (MMLV H^-). Reverse transcription of rabbit β-globin mRNA was done, with 17sc and 17cap as primer and antisense oligonucleotides, respectively, by the H^+ and H^- forms of MMLV RT. We had to use larger amounts of MMLV H^+ than of MMLV H^- because the RNase H activity aborts synthesis (20). Fig. 4 shows that oligomer 17cap induced the characteristic shortened cDNA fragment when the reaction was done with MMLV H^+ RT, thus indicating that the antisense oligomer inhibited reverse transcription by the intact enzyme. In contrast, the MMLV H^- form was not arrested by the oligonucleotide: only the full-length cDNA fragment was obtained with 1 μM oligomer 17cap (Fig. 4). Indeed, we checked that the template RNA was cut during the reaction with MMLV H^+ RT but remained intact when MMLV H^- enzyme was used (data not shown). Therefore, the antisense oligomer did not compete directly with the enzyme. This result unambiguously demonstrated the central role played by RNase H in inhibiting full-length cDNA synthesis by oligonucleotides.

α-Analogues of Antisense Oligonucleotides Do Not Block Reverse Transcription. Were the above conclusion correct, we might expect that oligonucleotides that do not elicit

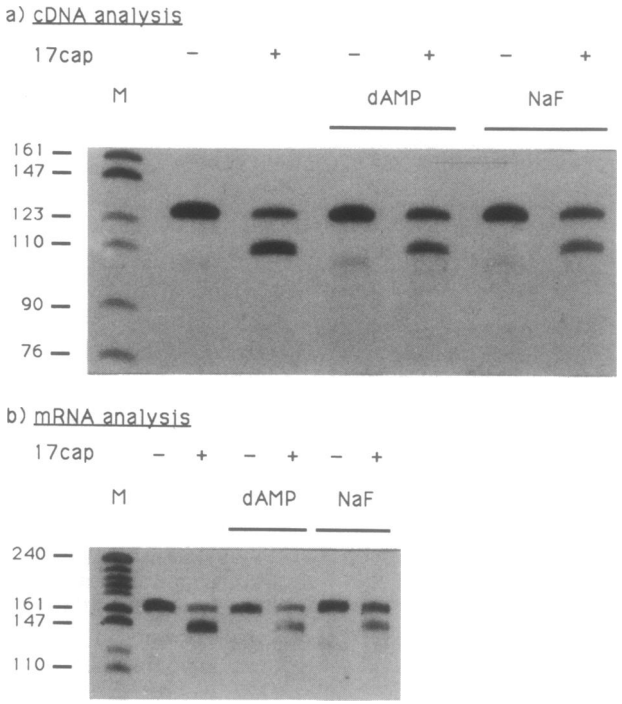

FIG. 3. Effect of RNase H inhibitors on inhibition of reverse transcription by complementary oligonucleotides. (*a*) cDNA synthesis, primed by 5 μM oligomer 17sc, was done by 1 unit of AMV RT either without (−) or with (+) 1 μM 17cap antisense oligonucleotide. Reverse transcription was done with 20 mM NaF (right lanes), 20 mM dAMP (middle lanes), or without any RNase H inhibitor (left lanes). cDNA fragments were run on a 10% polyacrylamide/7 M urea gel. (*b*) RNA blot analysis of the 150-nt-long RNA template used in the reaction described in *a*. RNA fragments were run and analyzed as described for Fig. 2*b*. First lanes on left (M) in both *a* and *b* correspond to size markers.

RNase H activity will not display inhibitory capacity. α-Derivatives of oligomers, which bind in a parallel orientation to their complementary sequence, are known not to induce RNA cleavage by either *E. coli* or eukaryotic RNases H (16, 21). We used 17αcap, the α-analogue of the 17cap antisense oligonucleotide, in the reverse transcription reaction of rabbit β-globin mRNA by AMV RT. cDNA synthesis primed by oligomer 19sc (Table 1) led to the expected transcript 190 nt long, which could no longer be seen when the reaction was done with oligomer 17cap. Instead a shortened fragment $\approx$170 nt long was detected (Fig. 5). In contrast, oligomer 17αcap did not affect synthesis of the full-length product, despite the fact that this oligonucleotide could compete with its unmodified analogue for binding to its target sequence. Addition of increased concentrations of oligomer 17αcap to a reverse transcription mixture containing unmodified 17cap led to the disappearance of the 170-nt-long transcript. The 190-nt-long transcript reappeared concomitantly (data not

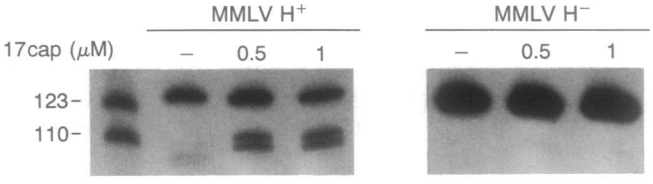

FIG. 4. Contribution of RNase H to inhibition of reverse transcription by MMLV RT. Analysis of cDNA fragments, primed by 5 μM oligomer 17sc, synthesized by 50 units of MMLV H^+ RT (*Left*) or 10 units of MMLV H^- RT (*Right*) without (−) or with 0.5 or 1 μM antisense oligomer 17cap. Transcripts were analyzed as indicated in the legend for Fig. 2*a*. Left lane contains size markers.

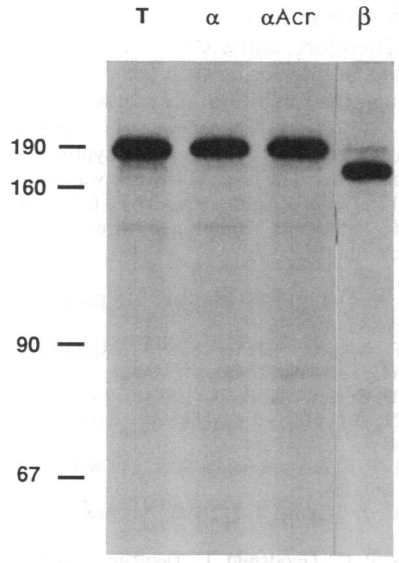

FIG. 5. Effect of α-oligonucleotides on reverse transcription. Analysis of cDNA, primed by 5 μM oligomer 19sc, synthesized by 10 units of AMV RT without antisense oligonucleotide (T) or with 5 μM oligomer 17αcap (α), 5 μM oligomer 17αAcrcap (αAcr), or 15 μM oligomer 17cap (β). Position of size markers is indicated on left. DNA fragments were analyzed as described for Fig. 2*a*.

shown). Linking an acridine derivative at the 5′ end of 17αcap, thereby increasing affinity of the oligonucleotide for its target RNA (23), did not endow this oligomer with inhibitory properties (Fig. 5). Therefore, cDNA synthesis cannot be blocked by direct competition between the RT and an oligonucleotide that does not induce cleavage of the template RNA.

α-Oligomers, Adjacent to the Primer, Arrest cDNA Synthesis. Unmodified oligonucleotides are rapidly degraded by DNases present both inside cells and in growth medium (15, 24). Antisense oligomers resistant to nucleases have to be synthesized for use in cultured cells or even in intact organisms. α-Oligomers fulfill this requirement, but unfortunately do not induce abortive reverse transcription, as they do not form substrates for RNase H (see above). We speculated that an oligomer adjacent to the primer (see Fig. 1*c*) could work in a different way: the primer–antisense tandem may be viewed as a single complementary sequence by the priming RT molecule. As α-oligonucleotides cannot be lengthened by RT (25), such an α-oligomer could inhibit reverse transcription. To test this hypothesis we synthesized 17αsc, an α-oligonucleotide complementary to nt 113–129 of rabbit β-globin mRNA and an unmodified 15-mer, 15sc complementary to nt 130–144. Fig. 6 shows that addition of 17αsc reduced synthesis of the 144-nt-long transcript—50% inhibition being achieved at <0.5 μM. This inhibition was specific, as the antisense α-oligomer had no inhibitory effect on reverse transcription primed by a 15-mer complementary to nt 147–161 (Fig. 6), in good agreement with results presented in Fig. 5. It should be noted that α-oligonucleotide 17αsc linked to an acridine derivative at its 5′ end was a significantly less efficient inhibitor than the parent compound, despite the additional binding energy provided by dye intercalation into the oligonucleotide–RNA duplex (23). Template analysis at the end of the reaction did not reveal increased cleavage of β-globin RNA with α-oligonucleotide 17αsc (data not shown). Therefore, we could arrest reverse transcription by an oligonucleotide that did not elicit RNase H activity.

DISCUSSION

We have used synthetic oligonucleotides to prevent reverse transcription. This result can be achieved in at least three

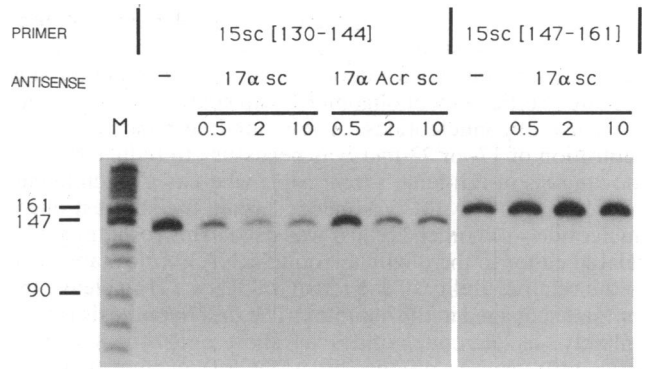

FIG. 6. Effect of the relative location of antisense and primer-binding sites. Analysis of cDNA fragments synthesized by 1 unit of AMV RT. The reaction, primed either by a 15-mer complementary to nt 130–144 (*Left*) or to nt 147–161 (*Right*), was done without (–) or with antisense oligonucleotides 17αsc or 17αAcrsc at the concentration (μM) indicated above each lane. DNA fragments were analyzed as indicated for Fig. 2*a*. M, size markers.

different ways. Competitive binding of phosphorothioate oligomers on the enzyme was shown to inhibit polymerization and to result in control of the development of human immunodeficiency virus in *de novo*-infected lymphocytes (26, 27). This process was essentially sequence-independent and rested on the preferential binding of the modified oligomers to the retroviral enzyme compared with mammalian DNA polymerases. Synthesis of cDNA by MMLV RT was also blocked by complementary oligonucleotides through a direct competition with the primer (17, 28). In this case, the competitor, an α-oligonucleotide analogue, and the primer had the same sequence, but the former could not prime reverse transcription—hence, the inhibition. Our results describe another strategy: the polymerization was inhibited by a complementary oligonucleotide bound to the template RNA downstream of the primer (Fig. 1*b*).

We have demonstrated that the inhibitory process involved the attack of the antisense oligonucleotide–RNA hybrid by RNase H activity borne by the RT enzyme; this holds true both for AMV and MMLV enzymes, despite their structural differences. Reduction of the nucleolytic capacity of the retroviral transcriptase decreased inhibitory efficiency. On the one hand, addition of dAMP or NaF, which reduces cleavage of the template at the hybrid level, also reduced the amount of arrested transcript. On the other hand, unmodified antisense oligonucleotides failed to prevent cDNA synthesis by MMLV H^- RT.

Analysis of the truncated template RNA resulting from the aborted reverse transcription reaction indicated that the cleavage occurred in the vicinity of the 5′ end of the antisense oligonucleotide. Previous studies showed that RNase H cleaved the template 10–15 nt back from the nucleotide complementary to the 3′ end of the DNA strand, suggesting that this corresponded to the distance between RNase H and polymerase catalytic sites (2, 29). Were the transcribing RT arrested by the oligonucleotide–template hybrid, we would have rather expected a cleavage site located 10–15 nt from the nucleotide facing the 5′ end of the oligomer. Therefore, it is difficult to imagine that the RNA strand could be cleaved by the incoming RT molecule that started DNA synthesis from the upstream primer. We suggest an alternative mechanism: an RT molecule bound to the antisense oligonucleotide–RNA hybrid could be responsible for the cleavage event. Subsequently, the truncated template could no longer support polymerization beyond the antisense-binding site. In this case, the DNA fragment would extend up to a position located within the antisense complementary sequence.

We might expect a minimal length for an oligonucleotide to induce the RNA-strand cleavage leading to aborted reverse transcription; this length dependence could explain the low inhibitory efficiency of oligomer 12cap compared with longer antisense oligonucleotides. A few hundred nanomolar concentration of 17- or 22-mer was necessary to reduce by 50% the amount of full-length transcript, whereas a much higher concentration (30 μM) was needed when the shortest oligonucleotide—oligomer 12cap—was used. This result might be related either to the oligonucleotide–RNA hybrid stability or to the relative ability of the retroviral RNase H to cleave the template engaged in the complex. The first hypothesis is very unlikely, as oligonucleotides as short as 11-mers can be efficiently used as primers by AMV RT, even at 45°C (2). Alternatively, the hybrid formed between oligomer 12cap (even converted to a 14-mer) and the template would be too short to be efficiently recognized as a substrate by RNase H of a bound RT molecule. Indeed, an 11-mer linked at its 3′ end to an acridine derivative did not significantly block reverse transcription, although the hybrid it formed with a complementary RNA strand was cleaved by the *E. coli* RNase H (N. Loreau and J.-J.T., unpublished results); this result merits further investigation.

No significant cleavage of the template RNA was detected either at the level of the primer oligonucleotide or along the transcription product (compare lanes 1 and 2 in Fig. 2*b*), in fair agreement with a recent report showing that AMV and MMLV RTs left most potentially degradable hybrid undigested (30). In contrast, due to its location at the end of the template RNA, the oligomer 17cap could not be extended by more than a few nucleotides, allowing RNase H to cleave at the antisense-binding site. Indeed, selective inhibition of cDNA synthesis was also achieved by modified analogues of oligomer 17sc, the modification consisting of either a 3′ terminal dideoxynucleotide residue or a 3′-linked dodecanol tail. The process of aborted transcription involved specific cleavage of the RNA template bound to antisense oligonucleotides (32). These results indicate that RNase H is less active than DNA polymerase during cDNA synthesis.

α-Analogues of oligonucleotides bind in a parallel orientation to their complementary sequence and do not elicit RNase H activity, as shown by previous studies (16, 17). Consequently, the 17-mer α-analogue of oligomer 17cap did not arrest cDNA synthesis by AMV RT, even though affinity of the two oligomers for their target sequence is similar, as shown by thermal-elution experiments of filter-bound complexes (22). Similarly, unmodified oligodeoxynucleotides have been reported not to prevent DNA-dependent DNA synthesis by AMV RT (31). We also observed that methylphosphonate analogues, which do not give rise to an RNase H substrate once bound to the complementary RNA, did not arrest reverse transcription either (C.B. and J.-J.T., unpublished results). Therefore, antisense oligonucleotides are brushed aside by a polymerizing RT, and cDNA is synthesized through the melted oligonucleotide–RNA hybrid. A different situation was encountered with an α-oligomer complementary to a site immediately adjacent to the primer-binding site (Fig. 1*c*). We showed that such an oligomer prevented the regular oligonucleotide being used as a primer by AMV RT. As expected, the inhibition did not involve degradation of the template by RNase H. The mechanism by which such an α-oligonucleotide impedes reverse transcription is unknown, but we speculate that the 3′-OH group of the primer could be masked by the contiguous stopper. The cooperatively interacting stopper–primer oligomers could then act as a pseudo primer, which would not be lengthened by the polymerase due to the reverse polarity of the α-oligonucleotide. Although efficiency of such an α-oligomer is lower than that of an antisense oligonucleotide acting by means of an RNase H-dependent mechanism, nuclease resistance of the former could confer an advantage for use with intact cells. Therefore, antisense oligonucleotides might constitute a means of interfering with the development of retroviruses through the inhibition of reverse transcription.

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