Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1300–1304, February 1993
Biochemistry

Targeting RNA for degradation with a (2′-5′)oligoadenylate-antisense chimera

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Communicated by Bernhard Witkop, November 3, 1992

ABSTRACT **Antisense oligonucleotides hold considerable promise both as research tools for inhibiting gene expression and as agents for the treatment of a myriad of human diseases. However, targeted destruction of RNA has been difficult to achieve in a versatile, efficient, and reliable manner. We have developed an effective strategy for cleaving unique RNA sequences with 2-5A-dependent RNase, an endoribonuclease that mediates inhibitory effects of interferon on virus infection and is activated by 5′-phosphorylated 2′-5′-linked oligoadenylates known as 2-5A [p_n5′A2′(p5′A2′)$_m$p5′A], resulting in the cleavage of single-stranded RNA predominantly after UpUp and UpAp sequences. To direct 2-5A-dependent RNase to cleave unique RNA sequences, p5′A2′p5′A2′p5′A2′p5′A was covalently linked to an antisense oligonucleotide to yield a chimeric molecule (2-5A:AS). The antisense oligonucleotide component of 2-5A:AS bound a specific RNA sequence while the accompanying 2-5A component activated 2-5A-dependent RNase, thereby causing the cleavage of the RNA in the targeted sequence. This strategy was demonstrated by inducing specific cleavage within a modified human immunodeficiency virus type 1 vif mRNA in a cell-free system from human lymphoblastoid cells. Because 2-5A-dependent RNase is present in most mammalian cells, the control of gene expression based on this technology—including therapies for cancer, viral infections, and certain genetic diseases—can be envisioned.**

The potential of oligonucleotides as chemotherapeutic agents has long been appreciated. Levine and Stollar (1) found that tetra- and pentanucleotides partially inhibited binding of nucleic acids to systemic lupus erythematosus antibodies, while Shen (2) suggested the design of high-affinity oligonucleotide inhibitors of the formation of similar antigen–antibody complexes. Miller *et al.* (3) capitalized on the specific complementarity of nucleic acid hybridization (3) through the preparation of a series of modified trinucleotides. These short modified DNA sequences, complementary to tRNA anticodon regions, could inhibit translation. Zamecnik and Stephenson (4) used a similar reasoning in the synthesis of a 21-deoxyribonucleotide sequence to inhibit the replication of Rous sarcoma virus.

Human immunodeficiency virus (HIV) replication also can be inhibited by antisense oligonucleotides (5). Although the mechanism of action of such antisense reagents is complex and not well understood (6), complexes of target messenger RNA and the antisense complementary oligo-β-deoxynucleotides are degraded by the enzyme RNase H, which is present in both eukaryotes and prokaryotes (7). However many modified oligonucleotides, synthesized to improve delivery, cell penetration, and stability of the antisense sequence, form hybrids with RNA that fail to be substrates for RNase H (8) although they still can operate by the passive mechanism of hybridization arrest. Considerable effort therefore has been directed to the development of oligonucleotides which could induce chemical alteration or strand scission in their target mRNA. Thus, oligonucleotides have been modified with photoreactive agents such as psoralen or porphyrin (9, 10), oxidative nuclease metal ion complexes such as porphyrin·iron (11), phenanthroline·copper (12), or ethylenediaminetetraacetic acid·iron (13), nucleases such as staphylococcal nuclease (14) or RNase P (15), and catalytic RNA (ribozyme) (16).

2-5A-dependent RNase is a latent endonuclease that requires the unusual 2′-5′-phosphodiester-linked trimeric oligonucleotide ppp5′A2′p5′A2′p5′A for activation (17, 18). Many, if not all, cells and tissues examined from reptilian, avian, and mammalian species (19) contain basal levels of 2-5A-dependent endonuclease which cleave RNA specifically after UpNp sequences (20, 21). This enzyme is part of what has been termed the 2-5A system (22), which is believed to mediate certain actions of interferon such as inhibition of encephalomyocarditis virus replication (18, 23) and perhaps to play some role in the regulation of cell growth (24) and cell differentiation (25). Herein, we report the synthesis of chimeric molecules that link the antisense strategy with the 2-5A system, thus providing specificity to the 2-5A-dependent RNase. We demonstrate that such a construct can cleave, selectively and specifically, a modified HIV RNA.

MATERIALS AND METHODS

Synthesis and Characterization of 2-5A-Antisense Chimeras. Covalent coupling of a (2′-5′)oligoadenylate sequence [2-5A; general formula p_n5′A2′(p5′A2′)$_m$p5′A] through a linker moiety to a 3′-5′-linked antisense oligonucleotide creates a chimeric molecule (2-5A:AS). On the one hand, the antisense partner of this union could provide the means for the uptake and specificity missing from the exogenous application of 2-5A analogues (18, 26) (Fig. 1). On the other hand, the 2-5A component could provide a localized activation of the latent and potent RNA-degrading activity of the 2-5A-dependent endonuclease (Fig. 1). While there exists no theoretical barrier to the sequence and nature of the antisense component of the chimera, for prototype synthetic and *in vitro* evaluation experiments we chose to employ an oligo(dT) 18-mer as the antisense component because this provided a relatively simple model for synthesis and characterization. The tetrameric 2′-5′-phosphodiester-linked oligoadenylate p5′A2′p5′A2′p5′A2′p5′A was chosen as the 2-5A portion of

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Abbreviations: 2-5A, series of oligoadenylates of general formula p_n5′A2′(p5′A2′)$_m$p5′A; 2-5A:AS, chimera of 2-5A linked to an antisense sequence; HIV, human immunodeficiency virus; TAR, transactivation responsive.

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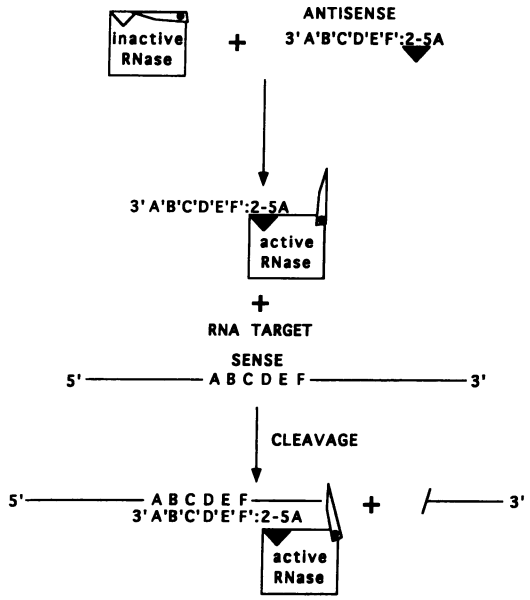

FIG. 1. The 2-5A-antisense strategy: Site-directed cleavage of RNA induced by 2-5A fused to antisense.

the construct, since the human 2-5A-dependent RNase requires only a 5′-monophosphorylated (2′-5′)oligoadenylate to be effectively activated (27). To link the two components, we used two 1,4-butanediol monomers joined to each other and to the antisense and 2-5A components by phosphodiester bonds. We chose to link the 5′ terminus of the antisense region of the chimera to the 2′ terminus of the (2′-5′)oligoadenylate; however, other linkage modes would be, in theory, equally workable, with the exception of linkages through the 5′ terminus of the 2-5A component, since incorporation of the 5′-phosphate into an internucleotide bond resulted in diminished binding to 2-5A-dependent RNase (28).

For this study we synthesized three compounds of the following type:

$$p5'A2'(p5'A2')_3p1(CH_2)_44p1(CH_2)_44(p5'dT3')_{17}p5'dT$$

1

The synthesis was based on the phosphoramidite method of solid-phase DNA synthesis (29) modified to accommodate incorporation of appropriate linkers and the synthesis of 2′-5′-internucleotide bonds (K.L., B. Uznanski, and P.F.T., unpublished results). Alkaline phosphatase digestion of crude synthetic oligonucleotide **1** (pA_4:T_{18}) removed the 5′-phosphate to yield oligonucleotide **2** (A_4:T_{18}). Oligonucleotide **3** (pA_4:T_4) is like **1** but has only 4 3′-5′-linked dT monomers. Details of the procedures, coupling cycles, and purification by HPLC will be published elsewhere. The assigned structures of **1–3** were corroborated by digestion with snake venom phosphodiesterase and characterization of the products by HPLC. Additionally, proton NMR supported the assigned structure **3** (K.L. and P.F.T., unpublished results).

Addition of the oligothymidylate antisense component to the 2′ terminus of the (2′-5′)oligoadenylate protected the 2-5A moiety from degradation by snake venom phosphodiesterase (data not shown), and presumably also the 2′-5′-phosphodiesterase (18, 30). Other chemical modifications at the 2′ terminus of 2-5A have been shown to impart considerable resistance to degradation by phosphodiesterases and to potentiate translational inhibition and antiviral activity (31–33).

Construction of Target RNA and Analysis of Its Degradation. Plasmid pSP6/TAR:A_{25}:vif (see upper construct in Fig. 2*A*) was made by first subcloning a portion of HIV-1 vif cDNA (*Hin*dIII and *Eco*RI fragment) from plasmid pSP2 (a gift from Klaus Strebel, National Institutes of Health) into a pSP64HIV+1→+80 vector obtained after *Hin*dIII and *Eco*RI digestion of the plasmid HIV+1→+231/IFNγ (34). An insert of $(dA)_{25}$·$(dT)_{25}$ was obtained by annealing two chemically synthesized complementary oligonucleotides having $(dA)_{25}$ and $(dT)_{25}$ sequences flanked by *Bss*HII and *Nde* I restriction site linkers. The $(dA)_{25}$·$(dT)_{25}$ insert was then inserted into the corresponding cohesive ends of the *Bss*HII- and *Nde* I-digested plasmid. The resulting plasmid, pSP6/TAR:A_{25}:vif, contains the SP6 promoter followed by 262 bp of the HIV-1 leader sequence (abbreviated as TAR because it begins with the trans-activation responsive sequence), followed by the $(dA)_{25}$ insert, followed by a 495-bp coding sequence for HIV-1 vif protein. Plasmid pSP6/TAR:vif (lower construct in Fig. 2*A*) was made by digesting plasmid pSP6/TAR:A_{25}:vif with *Bss*HII and *Nde* I and ligating the cut ends after filling in with the Klenow fragment of DNA polymerase.

Plasmids pSP6/TAR:A_{25}:vif and pSP6/TAR:vif were linearized with *Acc* I (within the vif coding sequence) and transcribed with SP6 RNA polymerase as described previously (34). *In vitro* synthesized RNAs were labeled at their 3′ termini with cytidine 3′,5′-[5′-^{32}P]bisphosphate with T4 RNA ligase (Pharmacia) and the RNAs were purified from sequencing gels (34). TAR:A_{25}:vif RNA and TAR:vif RNA are 500 and 471 nucleotides in length, respectively.

Daudi cell extracts were made by the method of Wreschner *et al.* (20) in a buffer containing 40 mM KCl, 10 mM Hepes at pH 7.5, 2.5 mM magnesium acetate, 0.5 mM ATP, 2.5% (vol/vol) glycerol, and 2.0 mM 2-mercaptoethanol. The extract used in the RNA cleavage assays was the supernatant of a 100,000 × *g* centrifugation of cell extract (20). Radiolabeled RNA, about 100 nM (approximately 25,000 cpm per assay), was incubated with oligonucleotides at 30°C in extract (10 μl) supplemented with 75 mM KCl in a final volume of 20 μl. RNA was extracted with phenol/chloroform and then chloroform, the RNA was precipitated with ethanol, and cleavage products were analyzed in 6% polyacrylamide/8 M urea gels (30 × 40 × 0.04 cm).

RESULTS

Interaction of 2-5A:AS With 2-5A-Dependent RNase and with Sense-Orientation RNA. The affinity of the 2-5A:AS constructs for 2-5A-dependent RNase was established with a highly specific radiobinding assay which measured the ability of 2-5A or 2-5A analogues to compete with a ^{32}P-labeled 2-5A probe, ppp5′A2′p5′A2′p5′A2′p5′A3′[^{32}P]p5′C3′p, for binding to 2-5A-dependent RNase (35). Chimeric 2-5A:AS constructs were undiminished in their ability to bind to the 2-5A-dependent RNase compared with unmodified 2-5A (results not shown). Constructs such as pA_4:T_{18} (**1**) formed stable complexes with the complementary poly(A) and had stability (as determined by melting temperature, T_m) similar to that of oligo(dT)·poly(A). Moreover, a complex of poly(A) with the oligonucleotide **1** bound to the 2-5A-dependent endonuclease as well as unmodified 2-5A.

The effect of the 2-5A moiety of the pA_4:T_{18} construct on the ability of the dT_{18} region to anneal with its complementary sequence in poly(A) was determined from UV absorbance–temperature profiles. A 3.2 μM solution of poly(A) mixed with a 2.9 μM solution of pA_4:T_{18} in a buffer of 40 mM KH_2PO_4 at pH 7.0 resulted in a hypochromicity of 17%, and the complex which formed had a T_m of approximately 38°C in the same buffer (data not shown). Under the same conditions, the complex $(dT)_{20}$·poly(A) had a T_m of 40°C, in good agreement with published values (36). When the antisense region of the chimera was annealed with its complementary sequence, there also was no significant effect on the ability of the construct to bind to 2-5A-dependent RNase. Thus, when

pA_4:dT_{18} was mixed in various concentrations with excess poly(A), there was no significant change in the binding affinity to 2-5A-dependent RNase as compared with the unannealed pA_4:dT_{18} or with 2-5A trimer (results not shown). In addition, when a radiobinding assay was performed on an aliquot of the solution that had been used to determine the T_m of the pA_4:dT_{18}·poly(A) complex, an identical binding curve was obtained (data not shown).

Site-Specific Cleavage of RNA Induced with 2-5A:AS. To determine the ability of the 2-5A:AS chimera pA_4:T_{18} (1) to induce site-directed RNA cleavage, a target RNA was constructed containing an internal (3′-5′)oligo(A) tract. Hybridization between the $(dT)_{18}$ in the 2-5A:AS (1) with the oligo(A) in the RNA would, according to our hypothesis, attract 2-5A-dependent RNase to the target RNA, resulting in a highly specific cleavage event (Fig. 1). The experimental target, TAR:A_{25}:vif RNA, was produced *in vitro* from a pSP64-derived plasmid containing a partial cDNA for the HIV vif protein interrupted with 25 adenylyl residues (upper construct in Fig. 2*A*). A control TAR:vif RNA was constructed with the A_{25} tract deleted (lower construct in Fig. 2*A*). These RNA species, which lack 3′-poly(A) tails, were radiolabeled at their 3′ termini with [5′-^{32}P]-pCp by T4 RNA ligase and gel purified prior to assay.

The 2-5A:AS strategy for site-directed RNA cleavage was tested in a cell-free system consisting of a postribosomal supernatant fraction of human lymphoblastoid Daudi cells (20), which exhibited basal levels of 2-5A-dependent RNase as detected by a radiobinding assay (35) (data not shown). The cleavage reaction was performed after adding pA_4:T_{18} and purified radiolabeled TAR:A_{25}:vif RNA to the Daudi cell extract (without preannealing) followed by incubation at 30°C. In the absence of added oligonucleotide, the TAR:A_{25}:vif RNA was degraded with a half-life of about 90 min as measured by image analysis of the autoradiogram shown in Fig. 2*B*, lanes 1 to 4. Remarkably, addition of the 2-5A:AS containing pA_4:T_{18} to the cell-free system resulted in the nearly quantitative conversion of the intact RNA to a specific cleavage product (Fig. 2*B*, lanes 5 to 11, lower arrow). Breakdown to the cleavage product could be seen clearly after as little as 5 min of incubation (Fig. 2*B*, lane 5). Quantitation of these results showed that the loss of the intact RNA was proportional to the production of the cleavage product, suggesting that the specific cleavage reaction accounted for essentially all of the breakdown reaction (data not shown). There was 50% breakdown to the specific cleavage product after 20 min of incubation in the presence of pA_4:T_{18}. Therefore, pA_4:T_{18} increased the rate of breakdown of the TAR:A_{25}:vif RNA more than 4-fold. To determine the optimal level of pA_4:T_{18} for the cleavage reaction, its concentration was varied against a constant amount of TAR:A_{25}:vif RNA. The cleavage reaction was observed with as little as 25 nM and was optimal at 250 nM of pA_4:T_{18} (data not shown).

To establish that the cleavage reaction was due to targeted degradation by 2-5A-dependent RNase and not to some other nuclease, a set of controls was devised and evaluated (Fig. 3). The specific cleavage product was obtained only after incubation with pA_4:T_{18} (Fig. 3, lane 3, lower arrow). The cleavage reaction was determined to be dependent on a functionally active species of 2-5A. Efficient activation of human 2-5A-dependent RNase requires (2′-5′)oligoadenylate with at least one 5′-phosphate group (27). Thus, the non-5′-phosphorylated compound A_4:T_{18} (2) failed to produce detectable amounts of the specific product (Fig. 3, lane 4). When an excess of $(dT)_{20}$ was added to the cell extract together with pA_4:T_{18} in an attempt to block specific breakdown of the TAR:A_{25}:vif RNA, the $(dT)_{20}$ completely prevented the cleavage reaction, suggesting that binding to the target RNA was a prerequisite for targeted cleavage (Fig. 3, lane 5). Another indication that targeting of the RNase was necessary for the specific cleavage reaction was that incubation of the TAR:A_{25}:vif RNA in the Daudi extract with (2′-5′)pA_4 plus unlinked $(dT)_{20}$ failed to produce the specific cleavage product (data not shown). Furthermore, p5′A2′p5′A2′p5′A2′p5′A fused to oligonucleotide sequences which were unrelated to those in the target RNA failed to produce the cleavage product (Fig. 3, lanes 6 and 7). Similarly, the chimera pA_4:T_4 failed to induce the specific cleavage reaction because the antisense chain was too short to permit a stable hybrid to form with the oligo(A) tract in the target RNA.¶ Finally, pA_4:T_{18} (at 0.2 or 1.0 μM) failed to yield a specific cleavage in the TAR:vif RNA, which lacks the oligo(A) tract (Fig. 3, lanes 16 and 17) demonstrating the need for hybridization to a complementary region on the target substrate. Thus competition, sequence variation, sequence deletion, and chain length experiments all pointed to the critical role of the T_{18} antisense portion of the chimera.

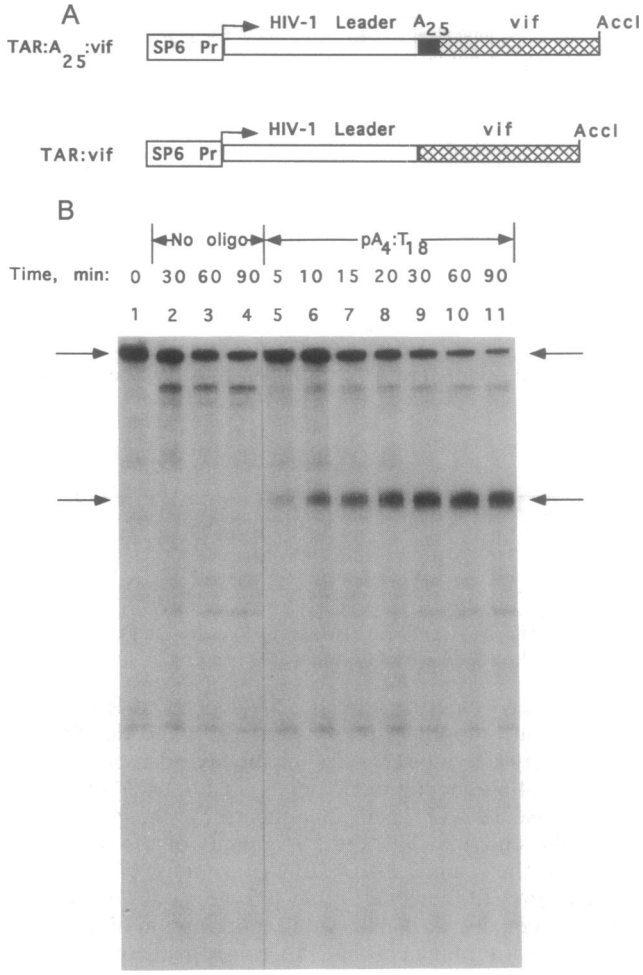

FIG. 2. (*A*) *Acc* I-digested plasmids containing TAR:A_{25}:vif and TAR:vif sequences. Transcription from the SP6 promoter is indicated by the arrow. (*B*) Specific cleavage of TAR:A_{25}:vif RNA induced by pA_4:T_{18} (100 nM) in an extract of Daudi cells as a function of time. An autoradiogram of the dried gel is shown. Upper arrow indicates intact RNA; lower arrow indicates specific cleavage product discussed in the text.

The role of the antisense portion of the 2-5A:AS molecule was further established in reactions involving unmodified 2-5A. Therefore, addition of 2-5A tetramer 5′-monophos-

¶Under conditions similar to those used to demonstrate hybridization between oligonucleotide pA_4:T_{18} and poly(A), we could not detect any interaction between poly(A) and pA_4:T_4.

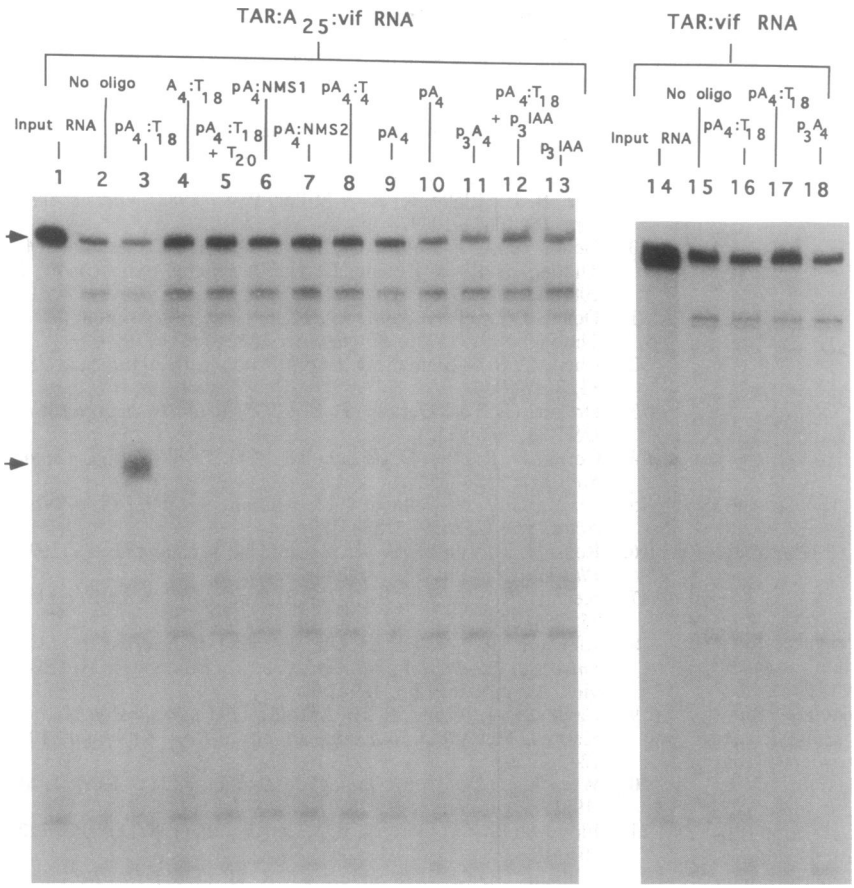

FIG. 3. Targeted cleavage of TAR:A_{25}:vif RNA induced by pA_4:T_{18} in Daudi cell extract was catalyzed by 2-5A-dependent RNase. Lanes 1–13 were with TAR:A_{25}:vif RNA and lanes 14–18 were with TAR:vif RNA as substrates. Lanes 1 and 14 were without Daudi extract. Oligonucleotides were present at 100 nM except for lane 5 [1.0 μM $(dT)_{20}$], lane 10 [1.0 μM (2′-5′) pA_4], lanes 11 and 18 [1.0 μM (2′-5′)p_3A_4], lanes 12 and 13 [2.0 μM (2′-5′)p_3IAA], and lanes 16 and 17 (0.2 μM and 1.0 μM pA_4:T_{18}, respectively). Incubation was for 30 min at 30°C in Daudi extract. NMS, nonmatched sequences (i.e., unrelated to the target RNA). These results were reproduced three times. Arrows as in Fig. 2*B*.

phate (pA_4) or tetramer 5′-triphosphate (p_3A_4) to the cell-free system (Fig. 3, lanes 9–11 and 18) did not induce the specific RNA cleavage observed with pA_4:T_{18}. The marginal degradation of RNA induced with the unmodified 2-5A indicated a low level of 2-5A-dependent RNase activity in the cell-free system and/or a restricted efficiency of action of these 2-5A-dependent RNase activators on these particular substrates. These results highlight the effective and efficient nature of targeted as compared with nontargeted degradation by the 2-5A-dependent RNase.

Additional proof for the critical role of the 2-5A-dependent RNase in the specific cleavage reaction was provided through the use of the 2-5A-dependent analogue/inhibitor ppp5′I2′p5′A2′p5′A (p_3IAA), an inosine-substituted derivative of 2-5A (39). When p_3IAA (2.0 μM) was present, it completely prevented the ability of pA_4:T_{18} to cause specific cleavage of the target RNA (Fig. 3, lane 12). Furthermore, the same specific RNA cleavage product was obtained by incubating pA_4:T_{18} and TAR:A_{25}:vif RNA with a partially purified fraction of Daudi 2-5A-dependent RNase prepared with $(A2'p)_3A$-cellulose as an affinity column (37) (data not shown).

To determine the exact sites of cleavage of the TAR:A_{25}:vif RNA induced by pA_4:T_{18}, a primer-extension DNA synthesis reaction was performed on the RNA cleavage products with reverse transcriptase (38) (Fig. 4). Consistent with previous results, levels of intact RNA, as measured by the full-length primer extension products, were greatly reduced after incubating the cell-free system in the presence of pA_4:T_{18} in comparison to incubating RNA in the absence of added oligonucleotides or with A_4:T_{18} or (2′-5′)p_3A_3 (data not shown). The RNA cleavage sites were determined by comparing the migration of the primer extension products and DNA sequencing products performed with the same primer annealed to plasmid containing the TAR:A_{25}:vif sequence (Fig. 4, lanes 4 and 5–8). Interestingly, the results showed that pA_4:T_{18} *induced* multiple cleavages within the oligo(A) tract of the RNA (Fig. 4, lane 4, see bracket). A_4:T_{18} appeared to be very weakly active in this assay compared with pA_4:T_{18}, once again indicating the importance of the 5′-phosphate moiety of 2-5A for activation of 2-5A-dependent RNase (Fig. 4, compare lanes 2 and 4), whereas there were no specific cleavage products in the absence of oligonucleotide or with p_3A_3 (lanes 1 and 3, respectively). Previous reports indicate that unmodified 2-5A stimulates 2-5A-dependent RNase to cleave RNA on the 3′ side of UpNp sequences (20, 21). It has been suggested that the sequence specificity of 2-5A-dependent RNase for uridine-rich RNA may be a function of the complementary adenosine residues in 2-5A (21). The results shown in Fig. 4 support this hypothesis and indicate that redirecting the binding of 2-5A-dependent RNase to a particular RNA sequence, in this case oligo(A), alters its cleavage specificity.

DISCUSSION

These results describe an approach to the selective and specific degradation of RNA and are consistent with the mechanism of action shown in Fig. 1; the 2-5A:AS directs 2-5A-dependent RNase to a specific RNA sequence complementary to the antisense domain of the chimera, resulting in localized cleavage of the target RNA. In theory, any RNA sequence could be targeted simply by changing the antisense cassette of the 2-5A:AS. The 2-5A:AS approach is an attractive strategy when compared with other methods for targeted RNA degradation. First, it relies upon a nuclease activity that is endogenous and ubiquitous in mammalian cells but is active only when bound to 2-5A (17–19). In this regard, it contrasts with methods that rely upon direct chemical (9–13) or nucleolytic action (14), which require that the RNA-degrading activity be introduced into the cell together with the tethered antisense oligonucleotide. Second, 2-5A:AS is of lower molecular weight than nuclease-oligonucleotide adducts (14) or ribozymes (16) and it is most probably more

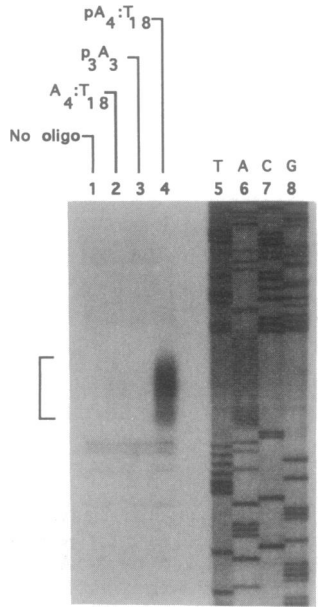

FIG. 4. Primer extension showed the specific cleavages to be within the oligo(A) tract of TAR:A_{25}:vif RNA. Primer extension reactions were performed on unlabeled TAR:A_{25}:vif RNA after incubation in Daudi extract without or with the oligonucleotides indicated above lanes 1–4 (each at 200 nM) at 30°C for 60 min. Primer extension assay was as described by Driscoll *et al.* (38) except all four dNTPs were included. DNA sequencing of plasmid pSP6/TAR:A_{25}:vif was with the same primer, using Sequenase version 2.0 (United States Biochemical) and deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate. Letters over lanes 5–8 indicate the nucleotides complementary to those which were sequenced. The sequence of the primer is 5′-TCCTGTATGCAGACCCCAATATGTTGTTAT-3′. The specific cleavage products are indicated by the bracket. Results were reproduced from two separate sets of RNA cleavage reactions.

stable than ribozymes. Third, in contrast to a number of other strategies, DNA chain modifications in antisense, such as methylphosphonate linkages, which eliminate RNase H-catalyzed scission (8), would not be expected to affect 2-5A-dependent RNase activation.

In essence, conjugation of 2-5A and antisense provides 2-5A-dependent RNase with a dramatically enhanced specificity of action by appending a new and specific substrate RNA binding domain to the endonuclease/2-5A complex. Viewed in this context, the 2-5A:AS strategy for the selective destruction of RNA species would have immediate and widespread application as an RNA equivalent of DNA restriction endonucleases. Although practical barriers to therapeutic usefulness, such as delivery and cell uptake, remain a problem with this concept, as with other related nucleic acid strategies, 2-5A:AS, because of its specificity, versatility, and potency, is a promising approach to the control of gene expression through targeted RNA destruction.

We thank Donna M. Driscoll for advice concerning the primer extension assay and Bryan R. G. Williams and Ganes Sen for valuable discussions. This investigation was supported in part by National Institute of Allergy and Infectious Diseases Grant 5 R01 AI28253 awarded to R.H.S. and by the National Institutes of Health Intramural AIDS Targeted Antiviral Program.

1. Levine, L. & Stollar, B. D. (1968) *Prog. Allergy* **12**, 161–191.
2. Shen, T. Y. (1970) *Angew. Chem. Int. Ed. Engl.* **9**, 678–688.
3. Miller, P. S., Barrett, J. C. & Ts'o, P. O. P. (1974) *Biochemistry* **13**, 4887–4896.
4. Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 280–284.
5. Zamecnik, P. C., Goodchild, J., Taguchi, Y. & Sarin, P. S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4143–4146.
6. Toulmé, J. J. & Hèléne, C. (1988) *Gene* **72**, 51–58.
7. Minshull, J. & Hunt, T. (1986) *Nucleic Acids Res.* **14**, 6433–6451.
8. Gagnor, C., Bertrand, J. R., Thenet, S., Lemaitre, M., Morvan, F., Rayner, B., Malvey, C., Lebleu, B., Imbach, J.-L. & Paoletti, C. (1987) *Nucleic Acids Res.* **15**, 10419–10436.
9. Lee, B. L., Blake, K. R. & Miller, P. S. (1988) *Nucleic Acids Res.* **16**, 10681–10697.
10. Doan, T. L., Praseuth, D., Perrouault, L., Chassignol, M., Thuong, N. T. & Helene, C. (1990) *Bioconjugate Chem.* **1**, 108–113.
11. Doan, T. L., Perrouault, L., Hèléne, C., Chassignol, M. & Thuong, N. T. (1986) *Biochemistry* **25**, 6736–6739.
12. Chen, C. B. & Sigman, D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7147–7151.
13. Dreyer, G. B. & Dervan, P. B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 963–972.
14. Corey, D. R., Pei, D. & Schultz, P. G. (1989) *J. Am. Chem. Soc.* **111**, 8523–8525.
15. Li, Y., Guerrier-Takada, C. & Altman, S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3185–3189.
16. Rossi, J. J., Cantin, E. M., Sarver, N. & Chang, P. F. (1991) *Pharmacol. Ther.* **50**, 245–254.
17. Kerr, I. M. & Brown, R. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 256–260.
18. Johnston, M. I. & Torrence, P. F. (1984) in *Interferon: Mechanisms of Production and Action*, ed. Friedman, R. M. (Elsevier, Amsterdam), pp. 189–298.
19. Cayley, P. J., White, R. F., Antoniw, J. F., Wallesby, N. J. & Kerr, I. M. (1982) *Biochem. Biophys. Res. Commun.* **108**, 1243–1250.
20. Wreschner, D. H., McCauley, J., Skehel, J. J. & Kerr, I. M. (1981) *Nature (London)* **289**, 414–417.
21. Floyd-Smith, G., Slattery, E. & Lengyel, P. (1981) *Science* **212**, 1030–1032.
22. Williams, B. R. G. & Silverman, R. H. (1985) *The 2-5A System: Molecular and Clinical Aspects of the Interferon-Related Pathway* (Liss, New York).
23. Chebath, J., Benech, P., Revel, M. & Vigneron, M. (1987) *Nature (London)* **330**, 587–588.
24. Etienne-Smekens, M., Vandenbussche, P., Content, J. & Dumont, J. E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4609–4613.
25. Krause, D., Silverman, R. H., Jacobsen, H., Leisy, S., Dieffenbach, C. W. & Friedman, R. M. (1985) *Eur. J. Biochem.* **146**, 611–618.
26. Williams, B. R. G. & Kerr, I. M. (1978) *Nature (London)* **276**, 88–90.
27. Haugh, M. C., Cayley, P. J., Serafinowska, H. T., Norman, D. G., Reeses, C. B. & Kerr, I. M. (1983) *Eur. J. Biochem.* **132**, 77–84.
28. Torrence, P. F., Imai, J., Lesiak, K., Jamoulle, J.-C. & Sawai, H. (1984) *J. Med. Chem.* **27**, 726–733.
29. Caruthers, M. H. (1991) *Acc. Chem. Res.* **24**, 278–284.
30. Johnston, M. I. & Hearl, W. G. (1987) *J. Biol. Chem.* **262**, 8377–8382.
31. Silverman, R. H., Wreschner, D. H., Gilbert, C. S. & Kerr, I. M. (1981) *Eur. J. Biochem.* **115**, 79–85.
32. Imai, J., Johnston, M. I. & Torrence, P. F. (1982) *J. Biol. Chem.* **257**, 12739–12745.
33. Defilippi, P., Huez, G., Verhaegen-Lewalle, M., DeClercq, E., Torrence, P. F. & Content, J. (1986) *FEBS Lett.* **198**, 326–332.
34. SenGupta, D. N., Berkhout, B., Gatignol, A., Zhou, A. & Silverman, R. H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7492–7496.
35. Silverman, R. H. & Krause, D. (1987) in *Lymphokines and Interferons: A Practical Approach*, eds. Clemens, M. J., Morris, A. G. & Gearing, A. J. H. (IRL, Washington, D.C.) pp. 149–193.
36. Janik, B. (1971) *Physicochemical Characteristics of Oligonucleotides and Polynucleotides* (Plenum, New York).
37. Silverman, R. H. (1985) *Anal. Biochem.* **144**, 450–460.
38. Driscoll, D. M., Wynne, J. K., Wallis, S. C. & Scott, J. (1989) *Cell* **58**, 519–525.
39. Imai, J., Lesiak, K. & Torrence, P. F. (1985) *J. Biol. Chem.* **260**, 1390–1393.