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Inhibition of Rabbit Globin mRNA Translation by Sequence-Specific Oligodeoxyribonucleotides†

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ABSTRACT: Oligodeoxyribonucleotides 8–12 nucleotides in length whose sequences are complementary to the 5′ end, the initiation codon regions, or the coding regions of rabbit globin mRNA were tested for their ability to inhibit translation in a rabbit reticulocyte lysate and in a wheat germ extract. The oligomers interact specifically with their target mRNAs as shown by their ability to serve as primers with reverse transcriptase. In the reticulocyte lysate, oligomers complementary to the 5′ end or the initiation codon regions inhibit translation of both α- and β-globin mRNA, whereas oligomers complementary to the coding regions have little or no effect. This suggests that reticulocyte ribosomes are able to displace the oligomers from the mRNA during the elongation but not the initiation step of translation. In the wheat germ system, translation was effectively inhibited by all oligomers regardless of their binding site on the message. In contrast to their behavior in the reticulocyte system, the oligomers inhibited α- and β-globin synthesis in a specific manner. This observation suggests that control of α- and β-globin mRNA translation is coordinated in the reticulocyte lysate system but not in the wheat germ extract. The results of our studies indicate that oligodeoxyribonucleotides may be useful probes for studying control of mRNA translation in cell-free systems.

Messenger RNA translation can be specifically inhibited in vitro by hybridization to complementary DNA (cDNA).[1] This procedure, called hybridization arrest (Paterson et al., 1977), has been used to study the location and arrangement of adenovirus 2 genes within the viral genome and to analyze mRNA populations in mouse liver (Hastie & Held, 1978). Hybridization arrest has also been used to study the function of the 3′-noncoding region of globin mRNA in a cell-free translating system (Kronenberg et al., 1979).

Arrest of translation by complementary ribonucleic acids appears to occur naturally during osmoregulation of the Omp F protein of *Escherichia coli* (Mizuno et al., 1984). In this case, mRNA complementary to the 5′ end of Omp F mRNA is produced, and this complementary RNA inhibits translation of Omp F mRNA. Anti-sense mRNAs transcribed from plasmid DNA can be used to inhibit translation of specifically targeted mRNA in bacterial and mammalian cells. For example, Coleman et al. (1984) find reductions in the amount of Lpp protein or Omp C protein in *E. coli* cells transfected with plasmids that code for anti-sense Lpp mRNA or anti-sense Omp C mRNA. Production of Herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) is reduced dramatically in HSV-1-infected TK$^-$ mouse L cells which have been transformed by a plasmid which encodes anti-sense HSV-1 TK mRNA (Izant & Weintraub, 1984).

Oligodeoxyribonucleotides and oligodeoxyribonucleoside methylphosphonates have also been used to inhibit mRNA translation. A tridecanucleotide complementary to part of the 3′ and 5′ redundant sequence of Rous sarcoma virus (RSV) 35S RNA inhibits translation of the viral mRNA in a wheat

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[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; Cl_3CCOOH, trichloroacetic acid; VSV, vesicular stomatitis virus; cDNA, complementary DNA; HSV-1, Herpes simplex virus type 1; TK, thymidine kinase; RSV, Rous sarcoma virus; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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germ cell-free translating system (Stephenson & Zamecnik, 1978). This oligomer was also reported to inhibit RSV replication and cell transformation in chicken embryo fibroblasts (Zamecnik & Stephenson, 1978). We have shown that an oligodeoxyribonucleoside methylphosphonate which is complementary to the Shine/Dalgarno sequence of *E. coli* 16S RNA prevents translation of mRNA in a bacterial cell-free translating system and in bacterial cells by inhibiting initiation of protein synthesis (Jayaraman et al., 1981).

To better understand hybridization arrest by oligonucleotides, and as part of our studies on the effects of oligonucleoside methylphosphonates on mRNA translation (Blake et al., 1985), we have investigated the effects of various oligodeoxyribonucleotides on the cell-free translation of globin mRNA in rabbit reticulocyte lysates and in wheat germ extracts. The relation between the binding site of the oligomer and its effectiveness and specificity in inhibiting translation has been examined. Our studies indicate that sequence-specific oligonucleotides are useful probes for exploring mechanisms of mRNA interaction with the translation apparatus in cell-free systems.

EXPERIMENTAL PROCEDURES

Rabbit globin mRNA, rabbit reticulocyte lysate, wheat germ extract, AMV reverse transcriptase, and T_4 polynucleotide kinase were purchased from Bethesda Research Laboratories, Inc. [^{35}S]Methionine, (1000 Ci/mmol), thymidine [α-^{32}P]triphosphate (800 Ci/mmol), and adenosine [γ-^{32}P]triphosphate (3000 Ci/mmol) were purchased from New England Nuclear, Inc., or Amersham, Inc. All reagents used were either reagent grade or electrophoresis grade. Buffer solutions were prepared by using autoclaved water.

Preparation of Oligodeoxyribonucleotides. The oligodeoxyribonucleotides were prepared by a phosphotriester method on a 1% cross-linked polystyrene support as described by Ito et al. (1982). After deprotection, the oligomers were purified by preparative ion-exchange high-pressure liquid chromatography as previously described (Miller et al., 1980). The oligomers were stored as 1 mM solutions in autoclaved water at −20 °C.

Oligodeoxyribonucleotides as Primers for Reverse Transcriptase. The reaction mixture contained 0.05 M Tris-HCl (pH 8.3), 0.05 M potassium chloride, 0.005 M magnesium chloride, 0.01 M DTT, 50 μM each of the four deoxyribonucleoside triphosphates, 10–15 μCi of thymidine [α-^{32}P]triphosphate, 0.2 μg of rabbit globin mRNA, 10 μM oligodeoxyribonucleotide, and 50 units of reverse transcriptase in a total volume of 10 μL. The reactions were incubated at 37 °C for 60 min and stopped by addition of 1 μL of 0.5 M EDTA. An aliquot of the reaction mixture was electrophoresed on a 10% polyacrylamide slab gel containing 7 M urea at 800 V for 45 min. The gel buffer and running buffer contained 0.089 M Tris, 0.089 M boric acid, and 0.2 mM EDTA (pH 8.0). The gel was dried and autoradiographed at −80 °C overnight using Kodak XAR-5 film and Dupont Cronex intensifying screens. The chain length of the transcript was determined by comparing its mobility with those of oligomers of known chain length (Rickwood & Hames, 1982).

Transcripts for sequencing studies were prepared in the same manner except 2 μg of mRNA was used and the thymidine [α-^{32}P]triphosphate was omitted. The unlabeled transcript was electrophoresed as described above. An aliquot of ^{32}P-labeled transcript was run in an adjacent lane to serve as a marker. After electrophoresis, the wet gel was autoradiographed at −80 °C overnight. The region of the gel containing the transcript was excised and extracted with 1 M aqueous triethylammonium bicarbonate. The transcript was desalted on a SEP-PAK C_{18} reversed-phase column (Waters Associates) as described by Lo et al. (1984). The transcripts were sequenced according to the method of Maxam & Gilbert (1980).

Translation of Rabbit Globin mRNA in Cell-Free Extracts. The commercial rabbit reticulocyte lysate, which had been pretreated with micrococcal nuclease (Pelham & Jackson, 1976), contained the following additional components: 3.5 mM magnesium chloride, 0.05 mM EDTA, 25 mM potassium chloride, 5 mM DTT, 25 μM hemin, 50 μg/mL creatine kinase, 1 mM calcium chloride, 2 mM EGTA, and 70 mM sodium chloride. Reactions were run in sterile, silanized glass tubes and were initiated by addition of the lysate. Each reaction mixture contained 25 mM HEPES (pH 7.2), 40 mM potassium chloride, 10 mM creatine phosphate, a 50 μM sample of each amino acid except methionine, 80 mM potassium acetate, 4.2 μCi of [^{35}S]methionine, 0–200 μM oligodeoxyribonucleotide, 0.3 μg of rabbit globin mRNA, and 8.3 μL of reticulocyte lysate in a total volume of 25 μL. The reactions were incubated at 25 or 37 °C and analyzed as described below. Under these conditions, 0.4 μCi (400 fmol) of methionine was incorporated at 25 or 37 °C after 60 min of incubation in the absence of oligonucleotide as determined by Cl_3CCOOH precipitation. The kinetics of incorporation were linear over the 60-min reaction period.

The commercial wheat germ extract, 80 A_{260} units/mL, which had been pretreated with micrococcal nuclease, contained the following additional components: 20 mM HEPES (pH 7.5), 5 mM magnesium acetate, 100 mM potassium chloride, 5 mM β-mercaptoethanol, 1 mM calcium chloride, and 2 mM EGTA. Reactions were initiated by addition of wheat germ extract and contained 20 mM HEPES (pH 7.5), 97.3 mM potassium acetate, 2.4 mM magnesium acetate, 1.2 mM adenosine triphosphate, 0.1 mM guanosine triphosphate, 5.5 mM creatine phosphate, 0.2 mg/mL creatine kinase, 80 μM spermidine phosphate, a 50 μM aliquot of each amino acid except methionine, 0–200 μM oligodeoxyribonucleotide, 0.3 μg of rabbit globin mRNA, and 8.3 μL of wheat germ extract in a total volume of 25 μL. Reactions were incubated at 25 °C and assayed as follows. Under these conditions, 0.55 μCi (550 fmol) of methionine was incorporated in a linear manner after 60 min of incubation in the absence of oligonucleotide as determined by Cl_3CCOOH precipitation.

Aliquots of the reaction mixture (3–5 μL) were removed at 30 and 60 min and immediately added to 10 volumes of loading buffer A (reticulocyte system) which contains 7.5 mM sodium phosphate (pH 7.0), 0.75% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 10% glycerol or to 10 volumes of loading buffer B (wheat germ system) which contains loading buffer A plus 0.4 mg/mL hemoglobin. The samples were incubated in a boiling water bath for 2 min, and 10-μL aliquots were electrophoresed on 12.5% acrylamide–0.34% methylenebis(acrylamide) slab gels prepared and run as described by Weber & Osborn (1969) and Sampson (1972). The gels were stained with a 0.1% solution of Coomassie Blue R-250 in 10% acetic acid/50% methanol overnight to locate the endogenous (reticulocyte) or added (wheat germ) α- and β-globins. Excess stain was removed by diffusion in 10% acetic acid/50% methanol. The gels were soaked in Enlightening (New England Nuclear, Inc.) for 15–30 min, dried, and fluorographed at −80 °C overnight with Kodak XAR-5 film using Dupont Cronex intensifying screens. Bands comigrating with the stained α- and β-globins were excised and counted in 10 mL of Betafluor. Typically, between 2000 and 10 000

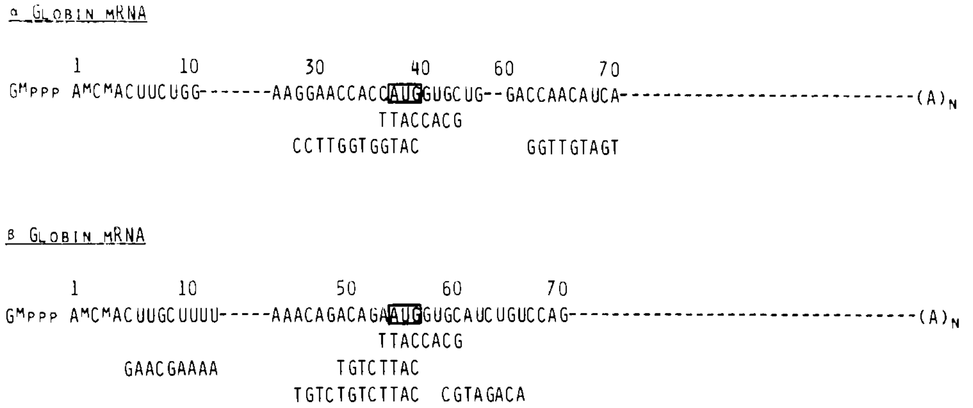

FIGURE 1: Partial nucleotide sequences of rabbit α-globin mRNA (Heindell et al., 1978) and rabbit β-globin mRNA (Efstratiadis et al., 1977). The sequences of the complementary oligodeoxyribonucleotides are shown below each mRNA sequence.

Table I: Specificity of Duplex Formation between Oligodeoxyribonucleotides and Rabbit Globin mRNA

		formation of duplexes with globin mRNA having five or more contiguous base pairs[a]		size of cDNA transcripts[b]	
oligodeoxyribonucleotide	binding site	α-globin	β-globin	expected	observed
d-AAAAGCAAG	β 4–12	0	1 (9) 1 (5)	12	nd
d-GCACCATT	α 37–43 β 53–60	1 (7)	1 (8)	43 α 60 β	41 59
d-CATTCTGT	β 49–56	0	1 (8) 4 (5)	56	55
d-CATGGTGGTTCC	α 28–39	1 (12) 1 (8) 1 (7)	0	39	41
d-CATTCTGTCTGT	β 45–56	0	1 (12) 3 (5)	56	55
d-ACAGATGC	β 59–66	0	1 (8) 1 (7)	66	65 69 98
d-TGATGTTGG	α 62–70	1 (9) 2 (5)	1 (6)	70	71

[a] Number of duplexes having five or more contiguous base pairs as found by a computer search which matches the sequence of the oligonucleotide with sequences on the mRNA. The numbers in parentheses show the number of contiguous base pairs between the oligomer and mRNA. [b] Size in nucleotide units of cDNA obtained when the oligomer is used as a primer for reverse transcriptase catalyzed copying of globin mRNA. nd = no data.

cpm were observed in each protein band.

Translation of Vesicular Stomatitis Virus mRNA in a Rabbit Reticulocyte Lysate. Vesicular stomatitis virus M, N, and NS protein mRNAs were isolated from VSV-infected mouse L cells according to the procedures of Maniatis et al. (1982). The mRNA preparation was translated in the rabbit reticulocyte lysate system at 30 °C under the conditions described above in the presence or absence of 25 μM d-CATGGTGGTTCC. Under these conditions, 0.5 μCi (526 fmol) of methionine was incorporated after 60 min of incubation as determined by Cl_3CCOOH precipitation. The reaction mixtures were analyzed by polyacrylamide gel electrophoresis on a 10% gel according to the conditions described by Laemmli (1970). The M, N, and NS proteins were located by fluorography, excised from the gel, and counted. The three proteins had mobilities identical with those isolated from intact virions. Between 3000 and 20 000 cpm were observed in each protein band.

RESULTS

Oligodeoxyribonucleotides Complementary to Globin mRNA. Figure 1 shows the sequences of the oligodeoxyribonucleotides and their complementary binding sites on α- and β-globin mRNA. The oligomers are complementary to three distinct regions of mRNA: the 5′-end sequence adjacent to the cap structure of β-globin mRNA, the initiation codon regions including the -AUG- initiation codon of α- or β-globin mRNA, and sequences in the coding region near the -AUG- codon of α- or β-globin mRNA. Potential mRNA binding sites for the oligomers were determined by a computer program which matches the oligomer sequence to corresponding complementary sequences within the mRNA. The program was used to search for potential duplex formation between the mRNA and oligomers in which the duplexes have five or more contiguous base pairs. Thus, as shown in Table I, d-AAAAGCAAG can form one duplex with nine base pairs and one duplex with five base pairs with β-globin mRNA, but no duplexes with five contiguous base pairs with α-globin mRNA.

The mRNA binding sites of the oligomers were characterized by using the oligomers as primers for reverse transcriptase. The products of the reverse transcriptase reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions as shown for d-CATGGTGGTTCC and d-CATTCTGTCTGT in Figure 2. The chain lengths and sequences of the transcripts derived from d-CATGGTGGTTCC and d-CATTCTGTCTGT were confirmed by chemical sequencing. The nucleotide sequence of the d-CATGGTGGTTCC-primed transcript was the exact complement of nucleotides 1–39 of α-globin mRNA while the sequence of the d-CATTCTGTCTGT-primed transcript was the exact complement of nucleotides 1–56 of β-globin mRNA. The chain lengths of the transcripts resulting from priming

Table II: Inhibition of Translation of Rabbit Globin mRNA by Oligodeoxyribonucleotides in a Rabbit Reticulocyte Lysate and a Wheat Germ Extract

			% inhibition[a]			
			reticulocyte[b]		wheat germ[c]	
oligomer	binding site	concn (μM)	α	β	α	β
5′ end						
d-AAAAGCAAG	β 4–12	25	−56	−37	−49	−7
		50	−75	−56	−89	−10
		100	88	87	87	91
initiation codon region						
d-GCACCATT	α 37–43	25	16	−4	16	47
	β 53–60	100	25	18	28	43
d-CATTCTGT	β 49–56	25	20	0	−27	23
		100	45	30	15	49
d-CATGGTGGTTCC	α 28–39	5	15	14	75	14
		25	47	42	77	26
		100	97	96	100	100
d-CATTCTGTCTGT	β 45–56	5	24	10	0	87
		25	20	23	13	80
		100	61	29	0	78
		184			79	93
coding region						
d-TGATGTTGG	α 62–70	25	−5	3	66	25
		100	8	4	68	39
d-ACAGATGC	β 59–66	25	−29	−2	39	32
		100	8	20	70	66
3′-noncoding region						
d-GCCACTCACTCA	α 537–548	5	2	0		
		100	−12	−6		

[a] The minus sign indicates stimulation of translation. [b] Translation carried out at 37 °C. [c] Translation carried out at 25 °C.

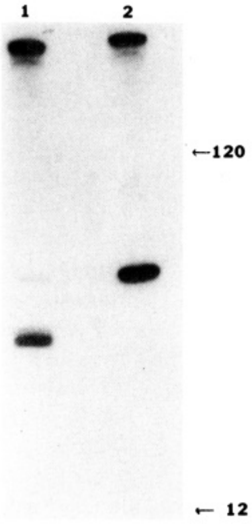

FIGURE 2: Reverse transcriptase catalyzed copying of rabbit globin mRNA using d-CATTGGTGGTTCC (lane 1) or d-CATTCTGTCTGT (lane 2) as primers. The reactions were carried out at 37 °C as described under Experimental Procedures and electrophoresed on a 10% polyacrylamide gel containing 7 M urea. The mobilities of oligonucleotides of known chain lengths are shown by arrows at the side of the autoradiogram. The material at the top of the gel is also observed in the absence of primer.

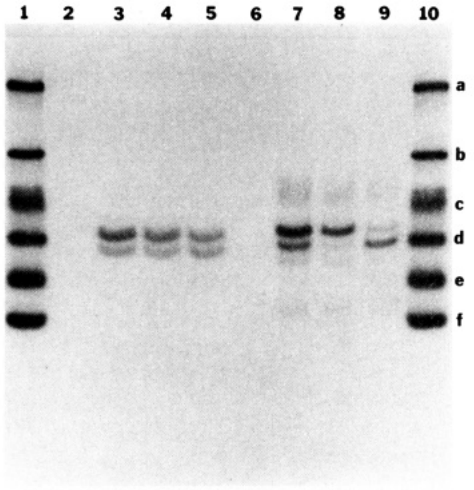

FIGURE 3: Effects of oligodeoxyribonucleotides on cell-free translation of globin mRNA in a rabbit reticulocyte lysate (lanes 2–5) and a wheat germ extract (lanes 6–9). Translations in the reticulocyte lysate were performed at 37 °C with (lane 2) no mRNA, (lane 3) globin mRNA, (lane 4) globin mRNA + 25 μM d-CATGGTGGTTCC, and (lane 5) globin mRNA + 25 μM d-CATTCTGTCTGT. Translations in the wheat germ extract were performed at 25 °C with (lane 6) no mRNA, (lane 7) globin mRNA, (lane 8) globin mRNA + 25 μM d-CATGGTGGTTCC, and (lane 9) globin mRNA + 25 μM d-CATTCTGTCTGT. Lanes 1 and 10 have the following molecular weight markers: (a) ovalbumin (43 000 daltons); (b) α-chymotrypsin (25 700 daltons); (c) β-lactoglobulin (18 400 daltons); (d) lysozyme (14 300 daltons) and cytochrome c (12 300 daltons); (e) bovine trypsin inhibitor (6200 daltons); (f) insulin (3000 daltons). The α-globin (faster migrating band) and β-globin (slower migrating band) in lanes 2–9 comigrated with authentic proteins which were located by staining the gel with Coomassie Blue as described under Experimental Procedures.

by the other oligomers were estimated from their mobilities on polyacrylamide gels and are summarized in Table I.

Effects of Oligodeoxyribonucleotides on Cell-Free Translation of Globin mRNA. Table II shows the effects of the oligodeoxyribonucleotides on translation of globin mRNA in a rabbit reticulocyte lysate and a wheat germ extract. The translation products were analyzed on polyacrylamide gels, as shown in Figure 3. The amounts of α-globin and β-globin were determined by counting regions of the dried gel corresponding to the proteins.

In the reticulocyte lysate, oligomers complementary to the initiation codon regions of either α- or β-globin mRNA inhibit translation of both mRNAs. On the other hand, oligomers complementary to the coding regions or the 3′-noncoding regions have little or no effect. Nonamer d-AAAAGCAAG, which is complementary to the 5′ end of β-globin mRNA, stimulates translation of both mRNAs at low concentrations but at high concentrations almost completely inhibits translation.

The effect of d-CATGGTGGTTCC on translation of vesicular stomatitis virus M, N, and NS protein mRNAs was tested in the reticulocyte system. This oligomer is not complementary to these mRNAs. At 30 °C, 25 μM oligomer had no effect on M or NS protein synthesis and only a slight inhibitory effect, 11%, on N protein synthesis. This same oligomer inhibits α- and β-globin synthesis 47% and 42%, respectively, at 37 °C.

Different results are obtained in the wheat germ system. Oligomers complementary to the initiation codon regions inhibit globin synthesis in a selective manner. For example, d-CATGGTGGTTCC, which is complementary to α-globin mRNA, inhibits α-globin synthesis by 77% and β-globin synthesis by 26% at a concentration of 25 μM. In contrast, d-CATTCTGTCTGT, which is complementary to β-globin mRNA, inhibits β-globin synthesis by 80% and α-globin synthesis by 13% at a concentration of 25 μM. Oligomers complementary to the coding regions also inhibit translation. At low concentrations, d-AAAAGCAAG, which is complementary to the 5′ end of β-globin mRNA, stimulates translation, although the extent of α-globin synthesis is between 7 and 9 times greater than the extent of β-globin synthesis. At 100 μM, d-AAAAGCAAG inhibits translation of both messages.

Discussion

In order to interpret the effects of sequence-specific oligodeoxyribonucleotides on mRNA translation, it is necessary to understand how these oligomers interact with mRNA. A search for complementarity between the oligomers and globin mRNA shows that each oligomer in Figure 1 can form only one completely base-paired duplex with its target mRNA (Table I). The possibility of forming partial duplexes in which the oligomer could form five or more contiguous base pairs with the mRNA was also considered. Partial duplexes of this type could be stable enough to exist under the conditions of the translation experiments (Yoon et al., 1976; Freier et al., 1983). Partial duplexes with clusters of two or three base pairs separated by nonpaired base regions or with G·T base pairs would have very low stabilities (Romaniuk et al., 1979; Alkema et al., 1982) and were therefore not considered. With the exception of d-TGATGTTGG which could form a partial duplex with nucleotides 529–534 in the 3′-noncoding region of β-globin mRNA and d-GCACCATT, which is targeted to both α- and β-globin mRNA, all the oligomers listed in Table I can form partial duplexes only with their target mRNAs. Thus, the data in Table I indicate that the oligomers used in this study should interact specifically with their target mRNAs.

The oligomer binding sites on globin mRNA were confirmed by using the oligomers as primers for reverse transcriptase. The mRNA and oligomer concentrations in these reactions were similar to those used in the translation experiments. The chain lengths of the transcripts, as determined from their mobilities on denaturing polyacrylamide gels, were consistent with the expected chain length (Table I). The sequences of the transcripts derived from d-CATGGTGGTTCC- and d-CATTCTGTCTGT-primed reactions were found to be consistent with the expected binding sites of the oligomers. Octamer d-GCACCATT gives two transcripts which are 41 and 59 nucleotides in length. The longer, major transcript results from transcription of β-globin mRNA (Baralle, 1977). The shorter transcript most likely comes from transcription of α-globin mRNA. Although there is a C·T mismatch between the 3′-terminal nucleotide of d-GCACCATT and cytidine 37 of α-globin mRNA (Figure 1), this apparently does not affect initiation of polymerization. It is also possible that the penultimate thymidine residue of the oligomer loops out, resulting in the formation of a duplex in which the 3′-terminal thymidine is base paired with adenosine 38 of α-globin mRNA.

In addition to the expected transcript, octamer d-ACAGATGC gives a minor transcript of 65 nucleotides and a major transcript of 98 nucleotides. The latter transcript may arise as a result of partial duplex formation between d-ACAGATGC and nucleotide sequences of β-globin mRNA between positions 93 and 100. Examination of the nucleotide sequences in this region shows the two 3′-terminal nucleotides and the first four nucleotides of the oligomer are complementary to nucleotides 93–94 and 96–100 of β-globin mRNA. Although the stability of such an interrupted duplex should be weak, the reverse transcriptase may help stabilize the duplex.

The effects of the oligomers on in vitro translation determined in a rabbit reticulocyte lysate at 37 °C and a wheat germ extract at 25 °C are shown in Table II. The wheat germ reactions are carried out at 25 °C in order to prevent degradation of newly synthesized proteins by endogenous protease activity present in the extract.

The effects of the oligomers in the reticulocyte lysate depend upon their mRNA binding site. Oligomers which are complementary to the initiation codon regions inhibit translation of both α- and β-globin mRNA; the extent of inhibition generally increases with increasing oligomer concentration and oligomer chain length. Thus, octamers d-GCACCATT and d-CATTCTGT are more inhibitory at 100 μM than at 25 μM. The dodecamers d-CATGGTGGTTCC and d-CATTCTGTCTGT inhibit even at a concentration of 5 μM. This behavior is consistent with oligonucleotide–mRNA binding. On the basis of the scanning model of eukaryote mRNA translation (Kozak, 1978, 1980a, 1981a,b, 1983), oligomers which bind to the initiation codon region would be expected to affect the initiation step of translation.

Oligomers which bind to the coding regions would be expected to interfere with the elongation step of translation. Both oligomers in this class, d-TGATGTTGG and d-ACAGATGG, failed to significantly inhibit translation even at high concentrations. This observation suggests that while oligomers may be quite effective in blocking initiation of translation, once translation begins, the reticulocyte ribosomes can successfully compete with the oligomer for binding to mRNA or the ribosomes can displace the oligomer from the mRNA as translation proceeds.

The above results are in agreement with the recent findings of Liebhaber et al. (1984). They found that cDNAs to human globin mRNA which cover the initiation codon or extend into the 5′-noncoding region are able to completely inhibit translation in a rabbit reticulocyte lysate. In contrast, cDNAs which cover only the coding region exclusive of the initiation codon are not effective at blocking translation. These authors postulate that a helix-destabilizing activity associated with the reticulocyte ribosomes is able to disrupt secondary structure during the elongation step but not the initiation step of translation. Thus, cDNAs or oligonucleotides bound to the coding region of mRNA would be expected to be unable to prevent translation in the reticulocyte system.

Stimulation of protein synthesis by low concentrations of d-AAAAGCAAG, which is complementary to the 5′ end of β-globin mRNA, was not expected. Since one of the first steps of translation involves interaction of the ribosome with the 5′ cap of the message (Kozak, 1978), this oligomer would be expected to interfere with the initiation step. Therefore, it is unlikely that the observed stimulation of synthesis is due to complex formation between the oligomer and mRNA. Possibly, interaction of the oligomer with some other component of the translation system results in stimulation of protein synthesis.

In contrast to their effects in the reticulocyte system, d-CATTCTGT, d-CATGGTGGTTCC, and d-CATTCTGTCTGT inhibit translation of their target mRNAs in a selective manner in the wheat germ extract (Table II, Figure 3). For example, β-globin synthesis is inhibited 6 times more than α-globin synthesis by the β-mRNA-specific oligomer d-CATTCTGTCTGT (25 μM) in the wheat germ system whereas both α- and β-globin syntheses are inhibited equally in the reticulocyte lysate. Specific inhibition of translation is not observed, however, when the dodecamers are used at higher concentrations (100 μM or greater).

Translation in the wheat germ system is also inhibited by oligomers which bind to the coding regions of globin mRNA. α-Globin synthesis is inhibited in a specific manner by d-TGATGTTGG while d-ACAGATGC inhibited both α- and β-globin synthesis to the same extent. The ability of these oligomers to inhibit translation in the wheat germ system but not the reticulocyte system could reflect stronger binding between the oligomers and the mRNA at 25 °C. However, the observation that d-TGATGTTGG is only weakly inhibitory in the reticulocyte lysate at 25 °C does not support this suggestion. It appears the different behavior of the oligomers in the two systems reflects some difference in the mechanism of translation in the wheat germ extract vs. the reticulocyte lysate. Possibly, the wheat germ ribosomes, unlike the reticulocyte ribosomes, have a reduced ability to disrupt oligomer–mRNA complexes during the elongation step of translation at 25 °C.

As in the reticulocyte lysate, d-AAAAGCAAG stimulates mRNA translation at low concentrations and completely inhibits translation at 100 μM in the wheat germ extract. However, stimulation of β-globin synthesis is 7–9 times less than that of α-globin synthesis. This suggests that although the oligomer may stimulate overall protein synthesis by a mechanism unrelated to its binding to mRNA, the oligomer inhibits β-globin synthesis more extensively than α-globin synthesis due to its specific interaction with β-globin mRNA.

Experiments in the reticulocyte lysate show that oligomers which bind to the initiation codon region are the most effective inhibitors of mRNA translation. In the wheat germ extract, the most effective inhibitors are those which bind to the initiation codon region, although oligomers which bind to the coding region near -AUG- and possibly the 5′ end are also inhibitory. In the wheat germ system, nonspecific inhibition is observed with high concentrations of d-AAAAGCAAG, d-CATGGTGGTTCC, and d-CATTCTGTCTGT. This nonspecific inhibition may be due to additional interactions of these oligomers with other components of the translation system. In general, such nonspecific interactions would be less serious at lower oligomer concentrations and higher reaction temperatures.

At low concentrations, d-CATTCTGT, d-CATGGTGGTTCC, and d-CATTCTGTCTGT specifically inhibit translation of their target mRNAs in the wheat germ system but not in the reticulocyte lysate. Since these oligomers were shown to bind specifically to their target mRNAs, it seems unlikely that nonspecific inhibition of translation in the reticulocyte lysate is due to mispairing with nontarget mRNA. This possibility seems even less likely since mispairing should be more favorable at lower temperatures, and yet specific inhibition occurs in the wheat germ system at 25 °C. Furthermore, d-GCCACTCACTCA, which is complementary to the 3′-noncoding region of α-globin mRNA, does not inhibit globin mRNA translation, and d-CATGGTGGTTCC does not inhibit translation of nontarget VSV mRNAs. Thus, it appears that some intrinsic property of globin mRNA translation in the reticulocyte system and not oligomer mispairing is responsible for the nonspecific inhibition. Experiments by Golini et al. (1976), by Kabat & Chappell (1977), and by Ray and co-workers (Ray et al., 1983) suggest that protein factors may regulate the rates at which the translation of different mRNAs is initiated. For example, it appears that an initiation factor required for translation of both α- and β-globin mRNA selectively directs translation of β-globin mRNA in reticulocyte lysates (Kabat & Chappell, 1977). A discrimination factor of this type could provide a mechanism for coordinating the translation of α- and β-globin mRNA in reticulocyte lysates. Selective inhibition of translation of one message by sequence-specific oligonucleotides could then lead to inhibition of translation of the other message.

Our studies indicate that oligodeoxyribonucleotides which bind to the initiation codon region of mRNA are very effective inhibitors of translation and, when used in the appropriate system, act as selective inhibitors of translation. There are two ways the oligomers could inhibit translation as a result of binding to mRNA. The oligomers could physically block the ribosome binding site and thus prevent attachment or movement of the ribosome on the mRNA. In this case, the oligomer and the ribosome are in direct competition for binding to the mRNA. The high oligomer concentrations required to obtain inhibition are consistent with this possibility. Alternatively, oligomer binding could cause a change in the conformation of the mRNA. Such conformational changes could be sufficient to prevent proper or productive binding of the ribosome with the message. This possibility receives support from recent studies which show that the efficiency of ribosome binding increases when the secondary structure of the 5′ end and initiation codon regions of mRNA decreases (Kozak, 1980b; Morgan & Shatkin, 1980; Lee et al., 1983).

Oligomer binding and hence the ability of the oligomer to inhibit translation could be influenced by mRNA secondary structure at the oligomer binding site. Pavlakis and co-workers (Pavlakis et al., 1980) have proposed secondary structure models for the 5′ ends of rabbit α- and β-globin mRNA. These models show that parts of the binding sites for d-CATTCTGT and d-CATTCTGTCTGT occur in hydrogen-bonded stem structures. Since these oligomers are good inhibitors of translation in both the reticulocyte and wheat germ systems, it appears that the oligomers are able to effectively compete with the mRNA secondary structure in these regions.

The results of this study show that sequence-specific oligodeoxyribonucleotides can be used to arrest translation of specific mRNAs in a selective manner in cell-free systems. Such oligomers are readily prepared by modern solid-support techniques. The observation that globin mRNA translation is coordinated in the reticulocyte lysate suggests that similar control mechanisms may also occur in the translation of other cellular or viral mRNAs. Thus, oligonucleotides may prove to be valuable probes for studying such control mechanisms in cellular extracts.

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Registry No. d-AAAAGCAAG, 96945-24-3; d-GCACCATT, 98014-39-2; d-CATTCTGT, 98014-40-5; d-CATGGTGGTTCC, 98049-94-6; d-CATTCTGTCTGT, 98014-41-6; d-ACAGATGC, 98014-42-7; d-TGATGTTGG, 98014-43-8; d-GCCACTCACTCA,

98014-44-9; reverse transcriptase, 9068-38-6.

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