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Quantitative hybridization-arrest of mRNA in *Xenopus* oocytes using single-stranded complementary DNA or oligonucleotide probes

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ABSTRACT

The expression of heterologous mRNA in Xenopus oocytes was quantitatively inhibited by coinjection of single-stranded complementary DNA or synthetic complementary oligonucleotides. The lymphokines Interleukin-2 (IL-2) and Interleukin-3 (IL-3) were used as model systems to test the effectiveness of this procedure. Messenger RNA samples were hybridized to single stranded complementary DNA or oligonucléotides, injected into oocytes and the oocyte incubation medium assayed for the presence or absence of specific translation products 48 hours later. When IL-2 mRNA was hybridized to a large excess of long (490 bases) single standed complementary DNA, the expression of IL-2 was effectively blocked (>98%). Complementary oligonucleotides (18-23 bases) were almost as effective as the polynucleotide in inhibiting IL-2 activity (>95%). Oligonucleotides derived from the 5' end, middle or 3' end of the coding sequence were all effective in arresting IL-2 mRNA translation. Oligonucleotide hybrid-arrest was effective even when no NaCl was present in the hybridization buffer, indicating that the annealing reaction could occur within the oocyte after injection. Definite proof that hybrid-arrest could occur in vivo was shown by the fact that oligonucleotides injected before or after mRNA injection, while not as effective as co-injection, still showed substantial inhibition of specific mRNA translation. The oligonucleotide hybrid-arrest method was equally effective in the case of IL-3, demonstrating its general applicability.

INTRODUCTION

Hybridization-selection and translation of specific mRNAs has been an extremely useful technique in identifying and characterizing specific DNA sequences or clones from cDNA banks (1,2,3). The usual protocol involves binding DNA from clones to nitrocellulose filters and then hybridizing mRNA to the immobilized DNA for several hours. Messenger RNA bound to the filters can then be eluted and assayed for a specific protein or activity and the unbound mRNA can be tested for removal of the sequence in question. The advantage of this procedure is that extraneous mRNA can easily be removed from the hybridized mRNA by simply washing the filters. The disadvantage is that quantitative removal of a specific sequence is not

possible by filter hybridization, so that the unbound fraction will still contain measurable levels of the mRNA being tested.

In our studies of the lymphokine IL-2 (4,5) we wished to establish conditions of quantitative hybrid-arrest while preserving the biological activity of the remainder of the mRNA. This was desired for two reasons. The first reason was that we wished to determine if the IL-2 mRNA population was composed of only one species or several functionally similar species with diverse nucleotide sequences. By blocking the expression of the known IL-2 mRNA sequence, we could more easily detect the presence of any non-homologous IL-2 mRNA species. The second reason was that we wished to determine if our mRNA preparations contained other non-IL-2 lymphokine activities such as B-cell growth factor, IL-1, etc. By eliminating entirely the IL-2 fraction of the mRNA we could avoid any complications arising from the biological activity of IL-2 in the assays. In this report we describe experiments designed to accomplish quantitative hybrid-arrest using mRNA translation in Xenopus oocytes to test for efficacy of the procedure. We found that single stranded complementary DNA was very effective in blocking the translation of IL-2 mRNA, with >98% of the activity inhibited by this method. Oligonucleotides complementary to the IL-2 coding sequence were also found to be effective, whether they were derived from the 5', middle or 3' end of the gene. Similar results were obtained using oligonucleotides specific for IL-3 mRNA.

MATERIALS AND METHODS

Messenger RNA Isolation

Total cytoplasmic mRNA was isolated from Jurkat cells, normal human peripheral blood lymphocytes (PBLs) and Wehi-3 cells by methods described previously (6,7). The Jurkat cells and PBLs were induced for IL-2 production prior to mRNA isolation (4). Wehi-3 cells, a mouse promyelomonocytic line, are constitutive producers of IL-3 (8).

Xenopus Oocyte Assay

Samples of mRNA were injected into Xenopus oocytes (9) and the incubation medium assayed for IL-2 or IL-3 40-48 hours later.

IL-2 and IL-3 Assay

IL-2 was assayed by measuring ^{3}H-thymidine uptake in the IL-2 dependent murine line HT-2 (10). A similar assay for IL-3 was done using the IL-3 dependent murine cell line FD-5 (11). Both assays are linear in the range from about 10 to 90% of the maximum signal obtained.

Table 1. Oligonucleotides Used In This Study.

1.	Beta IFN	(17mer)	5' GC AAT TTT CAG AGT CAG 3'
2.	IL-2 A	(18mer)	5' AGA ACT TGA AGT AGG TGC 3'
3.	IL-2 B	(20mer)	5' GT GGC CTT CTT GGG CAT GTA 3'
4.	IL-2 C	(23mer)	5' C TTC TAG AGA CTG CAG ATG TTT 3'
5.	IL-2 D	(21mer)	5' GT CTC GTC AGC ATA TTC ACA C 3'
6.	IL-2 E	(18mer)	5' TAG TGT TGA GAT GAT GCT 3'
7.	IL-3 A	(21mer)	5' CG GTG GGT ATC CCG GCC ACT G 3'
8.	IL-3 B	(22mer)	5' CG GTT AGG AGA GAC GGA GCC AG 3'

The sequences given here are the complement of the coding sequences. See Fig. 1 for their positions in the coding sequences.

Construction of IL-2 M13 Clones

The 490 base-pair RsaI to StuI fragment from the Jurkat cDNA clone containing the entire IL-2 coding sequence (12,13) was subcloned into the SmaI site of M13mp8 (14). Clones containing the fragment inserted in both orientations were obtained, and preparative amounts of single stranded (ss) DNA from the phage particles were isolated by standard protocols (15).

Oligonucleotide Construction

Oligonucleotides were constructed by the deoxynucleoside phosphoramidite method (16) on a Biosearch SAM-1 machine. Table 1 shows the sequence of the oligonucleotides and Figure 1 shows their positions in the IL-2 (12,13) and IL-3 (17,18) coding sequences. The sequences given in Table 1 are the complement of the coding sequences. Beta IFN, the oligonucleotide specific for beta-interferon (19), was used as a negative control in this study.

Hybridization-Arrest Protocols

Hybrid-arrest using single stranded IL-2 M13 DNA was done as follows. Ten μg of mRNA was hybridized to 5 μg of ss IL-2 M13 DNA in 50 ul of 0.4 M NaCl, 0.02 M Tris-HCl (pH 7.0), 2 mM EDTA and 0.1% SDS. Samples were heated to 70° C for 5 min, incubated at 55°C for 20 min, chilled and diluted with 50 μl TE (TE = 10 mM Tris-HCL and 1 mM EDTA, pH 7.5) and precipitated 2X from ethanol. The precipitates were dissolved in 20 μl of 50 mM NaCl and 50 nl aliquots were used for injection into oocytes.

In order to separate the mRNA-M13 DNA hybrids from unhybridized mRNA, samples were hybridized as described above and then fractionated on sucrose gradients. Forty μg of mRNA and 20 μg of ss IL-2 M13 DNA were hybridized in 50 μl, diluted with 150 μl ETS (= TE + 0.5% SDS), and layered directly on 12

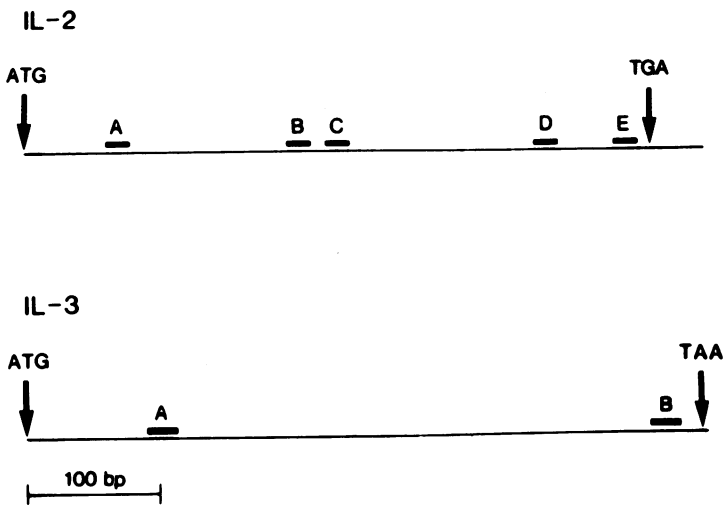

Figure 1. Position of oligonucleotides in the IL-2 and IL-3 coding sequences. See Table 1 for sequence of each oligonucleotide.

ml 5-20% w/w linear sucrose gradients made in NETS (= ETS + 100 mM NaCl). The gradients were centrifuged in a Beckman SW 40 rotor at 25,000 rpm for 17 hr at 20°C. 0.6 ml fractions were collected, extracted 1X with TE saturated phenol-chloroform (1/1, v/v), and precipitated 2X from ethanol with 10 µg wheat germ tRNA as carrier. After washing with ethanol and drying, the fractions were dissolved in 10 µl of 50 mM NaCl and 50 nl aliquots were injected into oocytes. To heat denature the hybrids, samples were heated to 90°C for 5 min. and quick chilled on ice.

For oligonucleotide hybrid-arrest, 4-5 µg of mRNA was assembled with various concentrations of oligonucleotides in 5 µl of buffer containing 10 mM Tris-HCl (pH 7.5) and 0.0 to 0.4 M NaCl. Samples were heated to 65°C for 5 min, incubated at 37°C for 30 min, 30 min at room temperature, and then chilled on ice. Samples containing high salt concentrations were adjusted down to 0.1 M NaCl with ice-cold 10 mM Tris-HCl (pH7.5) prior to injection into oocytes. Samples containing less than 0.1 M NaCl were adjusted with their own incubation buffer to keep the mRNA concentrations the same as the high salt samples. 50 nl aliquots were injected into oocytes. In one experiment the oligonucleotides and mRNA were injected sequentially. In this case, the oligonucleotides were injected in buffer containing 100 mM NaCl at a concentration of 50 pmoles per ul. The mRNA was injected in the same buffer

at a concentration of 1 mg per ml. Sequential injections were done about 1 hour apart and 20-30 nl were used for each injection.

Hybridization using the M13 DNA and oligonucleotides was carried out to a Cot value of >100X the Cot1/2 value of an mRNA with a complexity of IL-2 or IL-3 (20). In our cDNA cloning experiments (4), we estimated that the abundance of IL-2 mRNA was less than 0.1% and a similar value can be estimated for IL-3 mRNA from the data presented in the IL-3 cDNA cloning paper (17). Thus, in the experiments described here, the M13 DNA and oligonucleotides are the "driver" in the hybridization reactions, and the large amounts ensures that the annealing reaction is driven to completion.

RESULTS

Hybrid-Arrest with Single Stranded (ss) IL-2 M13 DNA

Table 2 summarizes the results of hybrid-arrest when PBL and Jurkat mRNA were annealed to ss IL-2 M13 DNA followed by injection into oocytes. Samples 1 and 2 show that when PBL and Jurkat mRNA was hybridized with the complementary or (-) strand DNA, greater than 98% of the IL-2 activity in the oocyte supernatants was lost relative to the positive controls, samples 5 and 6. Samples 3 and 4 show that hybridization of the mRNA to the (+) or coding strand has little or no effect on IL-2 activity, indicating that the loss of activity in the hybrid-arrested samples was not due to an artefactual translational block caused by M13 DNA itself.

The data presented in Table 2 showed that hybrid-arrest of IL-2 mRNA with ss complementary DNA was very effective, although there was still some low,

Table 2. Hybrid-Arrest of IL-2 mRNA With ss IL-2 M13 DNA.

	mRNA	DNA Addition	CPM in Assay	% Inhibition
1.	PBL	(-) M13 DNA	3,657	98.9
2.	Jurkat	(-) M13 DNA	3,389	98.8
3.	PBL	(+) M13 DNA	285,589	15.0
4.	Jurkat	(+) M13 DNA	287,407	0.0
5.	PBL	none	336,171	----
6.	Jurkat	none	282,474	----

The values in the table are the average of duplicate determinations with the background subtracted. The maximum signal in this assay was 400,007 cpm and the background was 1584 cpm.
(-) M13 DNA = IL-2 clone with complementary strand
(+) M13 DNA = IL-2 clone with coding strand

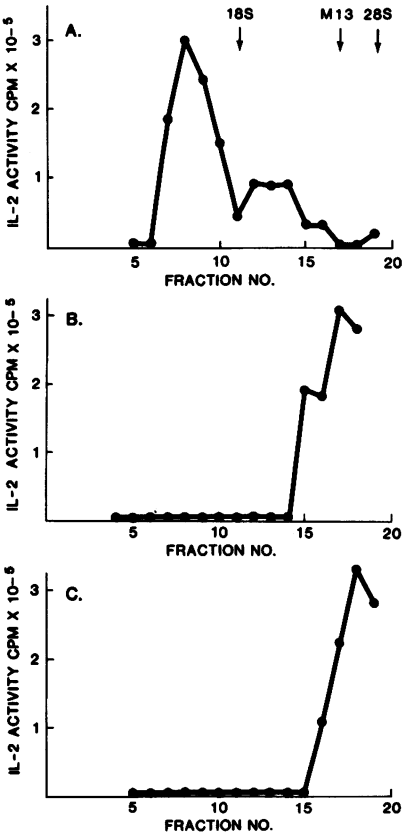

Figure 2. Sucrose gradient fractionation of IL-2 mRNA/IL-2 M13 DNA hybrids. (A) IL-2 activity profile of PBL mRNA hybridized to the coding or (+) ss IL-2 M13 DNA. (B) Activity profile of PBL mRNA hybridized to the complementary or (-) ss IL-2 M13 DNA. (C) Activity profile of Jurkat mRNA hybridized to (-) ss IL-2 M13 DNA.

but measurable activity remaining. To make the data even less ambiguous, IL-2 mRNA was removed from the bulk of the mRNA by sucrose gradient centrifugation of hybrid-arrested samples; aliquots from each fraction were then tested for IL-2 activity. Fig. 2 shows the results of this experiment. Panel A is the IL-2 activity profile obtained when PBL mRNA was hybridized with the (+) or coding strand of IL-2, and Panel B is the profile obtained with the (-) or complementary strand. The results are very clear in this case. For example, the fractions containing the peak activity in Panel A (fractions 7-10 gave 187,000 cpm, 301,000 cpm, 246,000 cpm and 154,000 cpm, respectively) contain less than 500 cpm in Panel B, and inhibition of greater than 99%. In addition, almost all the IL-2 activity in the gradient shown in Panel B could be recovered, after heat denaturation, in the fractions containing M13 DNA (fractions 15-18), demonstrating that the IL-2 mRNA was in hybrid form with the ss M13 DNA. Panel C shows the result when Jurkat mRNA was hybridized to

Table 3. IL-2 Hybrid-Arrest With Several Oligonucleotides

	Oligomer	CPM in Assay	% Inhibition
1.	IL-2 A	11,690	95.6
2.	IL-2 A w/o inc.	21,529	91.9
3.	IL-2 B	12,266	95.4
4.	IL-2 D	6,291	97.7
5.	IL-2 E	35,416	86.7
6.	Beta IFN	257,450	2.6
7.	mRNA Alone	264,403	----
8.	Buffer	431	----

The maximum signal in this assay was 265,378 cpm and the background was 3650 cpm. The background has been subtracted from the above values. Each hybridization reaction contained 50 pmoles oligonucleotide and 5 μg mRNA in 5 μl buffer containing 200 mM NaCl.

the ss (-) IL-2 M13 DNA. Essentially the same result was obtained as was with PBL mRNA; >99% of the IL-2 mRNA activity was removed from the region where IL-2 mRNA normally sediments.

From the above data we can conclude that hybridization of mRNA with ss complementary DNA is an extremely effective way to arrest a specific biological activity when assayed in Xenopus oocytes.

Hybrid-Arrest with Oligonucleotides

Frequently, oligonucleotide probes are derived from analyzing amino acid sequence data of purified proteins. Because of the degeneracy of the code, the initial probes usually contain a mixture of some or all the possible coding sequences contained within the chosen amino acid sequence. Before using the probes on a large scale (e.g. screening a large cDNA bank), it would be advantageous to know whether the deduced oligonucleotide sequences are,in fact, correct. In our study of IL-2 (4,5), several oligonucleotides were synthesized for use as probes, sequencing primers, or in site specific mutagenesis. We have used these oligomers to show that they are also very effective in the hybrid-arrest of mRNA in vivo. A similar result was obtained with oligonucleotides derived from the murine IL-3 cDNA sequence (17,18).

Table 3 gives the results of hybrid-arrest of IL-2 mRNA using several different oligomers. See Table 1 and Fig. 1 for oligomer designation and their positions in the cDNA sequence. All hybridizations were done in 200 mM NaCl with 5 μg mRNA and 50 pmoles of oligonucleotide. The data show that all the IL-2 oligomers effectively block the expression of IL-2 activity, with

Table 4. IL-2 Hybrid-Arrest: Effect of NaCl Molarity

	Oligomer &	[NaCl]	CPM in Assay	% Inhibition
1.	IL-2 C	200mM	1,822	98.8
2.	IL-2 C	100mM	2,536	98.4
3.	IL-2 C	0mM	4,030	97.4
4.	IL-2 E	200mM	11,832	92.3
5.	IL-2 E	100mM	13,892	91.0
6.	IL-2 E	0mM	24,177	84.3
7.	Beta IFN	200mM	159,035	----
8.	mRNA Alone		154,396	----
9.	Buffer		0	----

The maximum signal in this assay was 313,506 cpm and the background was 3686 cpm. Hybridization conditions were as in Table 3 except that the NaCl molarity was varied.

values ranging from 86.7% to 97.7% inhibition. The oligomer IL-2 E (sample 4) may be somewhat less effective because of its position at the 3' end of the coding sequence, ending only one triplet before the stop codon. The Beta-IFN oligomer used as a control, had no effect on the assay, showing that the inhibition by the IL-2 oligomers was not due to a general blockage of mRNA translation in the oocyte. Sample 2 (IL-2 A w/o incub.) is the same as sample 1 except that it was immediately chilled and diluted after 65°C treatment. Since the inhibitory effect was almost the same (95.6% vs 91.9%), it appears that the actual hybridization time was not too important under these conditions.

The data presented in Table 4 summarizes an experiment in which the NaCl molarity was varied in the mRNA-oligomer hybridization buffer. The conditions were the same as described in Table 3 except that the NaCl concentration ranged from 200 mM to 0 mM. Surprisingly, the molarity of salt was not too important in effecting hybrid-arrest, since the inhibition with IL-2 C or IL-2 E was almost the same whether they were hybridized in buffer with 200 mM or 0 mM NaCl. These results demonstrate that hybrid-arrest of mRNA can occur within the oocytes AFTER injection, since no prior annealing could occur in the samples incubated without NaCl. IL-2 C, which gave the greatest inhibition (98.8%), has two mismatches at positions 9 and 14 (C to G and A to C, respectively). This shows that under "physiological" conditions in the oocyte, hybrid formation of mRNA with an oligomer containing mismatches can still be very efficient.

Table 5. IL-2 Hybrid-Arrest: Effect of Injecting Oligonucleotides Before or After mRNA Injection

	Oligomer & Order of Injection		CPM in Assay	% Inhibition
1.	IL-2 A	1st	146,801	28.3
2.	IL-2 A	2nd	53,127	74.1
3.	IL-2 C	1st	70,803	65.4
4.	IL-2 C	2nd	22,835	88.8
5.	IL-3 A	1st	172,123	15.9
6.	IL-3 A	2nd	200,296	2.2
7.	mRNA Alone		204,758	---
8.	Buffer		0	---

The maximum signal in this assay was 321,953 cpm and the background was 2575 cpm. Oligonucleotides were injected before (1st) or after (2nd) mRNA was injected. The oligomer and mRNA concentrations were 50 pmoles/ul and 1 mg/ml, respectively. 20-30 nl of each were injected about 1 hour apart.

Table 5 shows the effect on hybrid-arrest of injecting oligonucleotides and mRNA sequentially. In this experiment the oligonucleotides were injected about one hour before or after the mRNA was injected. As can be seen injecting the oligonucleotides AFTER the mRNA had a strong inhibitory effect on IL-2 expression. The oligomers IL-2 A and C blocked IL-2 expression by 74.1 and 88.8%, respectively, when injected one hour after the mRNA injections. Although the level of inhibition was not as great as seen when the oligomers and mRNA were co-injected, the result, nevertheless,

Table 6. IL-2 Hybrid-Arrest: Effect of Oligonucleotide Concentration

	Oligomer & PM Used		CPM in Assay	% Inhibition
1.	IL-2 A	50 pmoles	584	99.8
2.	IL-2 A	20 pmoles	920	99.6
3.	IL-2 A	5 pmoles	11,250	95.4
4.	IL-2 B	50 pmoles	10,273	95.8
5.	IL-3 A	50 pmoles	225,050	8.1
6.	mRNA Alone		244,977	----
7.	Buffer		0	----

The maximum signal in this assay was 394,138 cpm and the background was 3495 cpm. Hybridization conditions as in Table 3 except the oligonucleotide concentration was varied and the annealing buffer contained 0.4 M NaCl.

Table 7. IL-3 Hybrid-Arrest: Effect of Oligonucleotide Concentration

	Oligomer & PM Used	CPM in Assay	% Inhibition
1.	IL-3 A 50 pmoles	2,856	97.4
2.	IL-3 A 20 pmoles	8,080	92.7
3.	IL-3 A 5 pmoles	11,901	89.2
4.	IL-3 B 50 pmoles	0	100.0
5.	IL-2 B 50 pmoles	106,437	3.8
6.	mRNA Alone	110,609	------
7.	Buffer	126	------

The maximum signal in this assay was 166,742 cpm and the background was 391 cpm. 4.2 ug mRNA was used each sample in a volume of 5 µl buffer containing 0.4 M NaCl.

demonstrates that the oligonucleotides can hybridize to mRNA and block their translation *in vivo*.

Table 6 shows the effect of decreasing the oligomer concentration on the magnitude of hybrid-arrest. In this experiment 0.4 M NaCl was used in the hybridization buffer. Only a small effect was seen over a 10 fold range from 50 pmoles to 5 pmoles (99.8% to 95.4%, respectively) using IL-2 A. Thus it is possible to use <5 pmoles of oligomer to get >90% hybrid-arrest by this protocol. This is an important fact to know if one is testing a large oligonucleotide mixture containing only one correct sequence.

The results presented in Table 7 show that the oligomer hybrid-arrest protocol is applicable to other lymphokines, in this case IL-3. The results of the oligomer dilution series with IL-3 A are very comparable with those obtained with IL-2 A in the IL-2 assay (see Table 6). Again, as little as 5 pmoles of oligomer is effective in hybrid-arrest; in this case an 89% inhibition of activity. IL-3 B appears to be particularly effective, since it completely blocked the expression of IL-3.

DISCUSSION

Single-stranded phage DNAs containing cDNA sequences have been shown to be useful in hybridization-selection and release translation studies *in vitro* (21). In this study we used the M13 cloning system to demonstrate that hybridization-arrest may be followed *in vivo* by injection of samples into *Xenopus* oocytes and subsequent assay of the medium for specific translation products. In addition we found that complementary oligonucleotides, 18 to 23 bases in length, were almost as effective as the cloned (490 bases) single-

stranded DNA for this purpose. In both cases specific mRNA activity can be almost entirely eliminated by hybridization of the probes prior to injection. The oligomers were also able to block activity substantially when injected before or after mRNA injections, proving that annealing of the mRNA to the short probes can occur within the oocyte under these conditions.

Although long ss DNA probes would be expected to be more efficient in hybrid-arrest of mRNA, the use of oligonucleotides may be more useful in general for this purpose. It is much easier to obtain a high molar concentration of a specific sequence by using oligomers than with cloned probes. For instance, if one uses M13 vectors (7.2 kb), more than 350X the mass is needed to achieve a specific molarity than with an oligomer probe of 20 bases. Thus, 50 pmoles of 20mer is about 0.33 μg of DNA, but the same molarity of M13 probe would require more than 115 μg of ss DNA. This point may be especially important if one wishes to block the activities of several mRNA species in the same RNA preparation. Another advantage of using oligomers is the fact that one does not need an actual clone to do these studies; one only requires the DNA sequence and the availability of oligonucleotide synthesis capabilities. An example is the IL-3 mRNA hybrid-arrest experiment described in this paper (see Table 7). In this case we wished to determine whether the IL-3 activity found in our mouse cell line was encoded by the same sequence as was described in two recent publications (17,18). By simply using two oligomers specific for the 5' and 3' end of the IL-3 coding sequence, we determined not only that the IL-3 mRNA in our cell line was the same, but that there is probably only one IL-3 mRNA species in these cells since the oligonucleotides inhibited the IL-3 activity to nearly 100%. This type of information is extremely useful, especially in the area of lymphokine biology, where there is still some uncertainty as to whether or not a defined activity is elicited by a single factor or several factors.

The data presented in this report have shown that complementary oligonucleotides are capable of annealing to and blocking translation of mRNA under "normal" physiological conditions in Xenopus oocytes (see Table 4 & 5). Some previous reports have shown that defined oligonucleotides can be used to study gene regulation in vitro. These reports included studies of 16S rRNA function (22-24), Rous Sarcoma virus translation (25), and tryptophan operon transcription and termination (26,27). Rous Sarcoma virus replication and cell transformation was also shown to be inhibited in vivo by incubation of infected cells in the presence of a tridecamer complementary to the 5' and 3' terminal repeats of the virus (28). Recently, studies have shown that hybrid-

arrest (or hybrid-regulation) may be a widespread naturally occurring phenomenon in the control of gene expression in vivo. For example, the insertion sequence of transposon Tn10, IS10, may negatively control its transposase protein at the translational level by annealing with a small RNA encoded by one of its own promoters (29). A similar situation exists for the ompF gene in E. coli, in which regulation at the translational level is accomplished by hybridization with a complementary RNA encoded by the related ompC gene (30). Hybridization of small nuclear RNAs to splice regions in precursor RNAs may play a role in processing of the initial RNA transcripts (31-34). It is known that the plasmid copy number of ColE1 is partly controlled by hybridization of the primer RNA with a short complementary RNA transcript (35). Directed control of gene expression has also been accomplished artificially in vivo by transformation of E.coli (36) or transfection of L-cells (37) with plasmids containing genes inserted in the wrong orientation relative to the promoter. Inhibition of expression is accomplished by hybridization of the correct transcript with "anti-sense" RNA synthesized from the plasmid. Thus, it appears that nucleic acid hybrid formation may play a significant role in vivo in the control of DNA replication, RNA transcription and mRNA translation.

Finally, although the method described in this report was shown to be very effective against heterologous mRNAs, it may also have some utility in inhibiting the translation of endogenous, maternal mRNAs, such as those containing "homeo-box" sequences (38). Injection of complementary oligonucleotides prior to or after fertilization, might block the expression of the maternal mRNAs; analysis of the results may prove helpful in identifying the genes important in the early embryogenesis of Xenopus. The feasibility of this approach has been demonstrated by two recent papers which have shown that injected antisense RNA can produce phenocopy mutants in Drosophila (39) and block translation of globin mRNA in Xenopus oocytes (40).

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