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Selective inhibition of *Escherichia coli* protein synthesis and growth by nonionic oligonucleotides complementary to the 3′ end of 16S rRNA*

(oligonucleoside methylphosphonates/chemical synthesis/ribosome binding/cell-free translation/cell growth)

KRISHNA JAYARAMAN, KEVIN MCPARLAND, PAUL MILLER, AND PAUL O. P. TS'O

Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

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ABSTRACT **A series of nonionic oligonucleotide analogues, the deoxyribooligonucleoside methylphosphonates, were synthesized. The base sequences of these compounds, d(A*p*G*p*G*p*), d(A*p*G*p*G*p*)$_2$, and d[(A*p*G*p*G*p*)$_2$T], are complementary to the Shine–Dalgarno sequence (-A-C-C-U-C-C-U-) found at the 3′ end of bacterial 16S rRNA. These nonionic oligonucleotide analogues were tested for their ability to inhibit the *in vitro* translation of mRNAs in cell-free systems of *Escherichia coli* and rabbit reticulocyte. In the *E. coli* system, both d(A*p*G*p*G*p*)$_2$ and d[(A*p*G*p*G*p*)$_2$T] effectively inhibited MS-2 RNA-directed protein synthesis but they had much less effect on either poly(U)- or poly(A)-directed polypeptide synthesis. In the reticulocyte system, these compounds had no significant effect on the translation of globin mRNA. The observation that d[(A*p*G*p*G*p*)$_2$[^{3}H]T)] binds to 70S ribosomes (association constant, 2.0×10^4 M^{-1}, 37°C) together with the specificity of the inhibitory action of these compounds on protein synthesis strongly suggests that inhibition of translation is a consequence of analogue binding to Shine–Dalgarno sequence of 16S rRNA. The oligonucleoside methylphosphonates inhibited both protein synthesis (without concurrent inhibition of RNA synthesis) and colony formation by *E. coli* ML 308-225 (a permeable mutant) whose cell wall contains negligible quantities of lipopolysaccharide but had no effect on wild-type *E. coli* B. Our preliminary results on the uptake of oligodeoxyribonucleoside methylphosphonates by *E. coli* B show that these cells are not permeable to oligomers longer than 4 nucleotidyl units. Although oligodeoxyribonucleoside methylphosphonates are taken up by mammalian cells in culture, this series of analogues had negligible inhibitory effects on colony formation by transformed human cells. This study indicates that this class of nonionic oligonucleotide analogues can be used to probe and regulate the function and structure of nucleic acids of defined sequence within living cells.**

Single-stranded exposed regions of cellular nucleic acids are potential target regions for base-pairing interactions with complementary oligonucleotides. Binding of oligonucleotides to these regions can be used to probe and regulate the structure–function relationship of nucleic acids in both biochemical and cellular systems. Deoxyribooligonucleotides complementary to the reiterated 3′- and 5′-terminal nucleotides of Rous sarcoma virus 35S RNA inhibited the translation of the RNA in a cell-free system as well as the virus production of chicken fibroblast tissue cultures (1, 2). Studies in our laboratory have shown that an oligonucleotide ethylphosphotriester complementary to the amino acid-accepting stem of most tRNAs had a transient but specific inhibitory effect on the growth of mammalian cells in culture (3). More recently, we have studied the effects of oligo(dA) methylphosphonate analogues (complementary to the anticodon loop of tRNA^{lys}) on bacterial and mammalian cells in culture (4). These analogues contain an isosteric 3′-5′ linked methylphosphonate group which replaces the normal phosphodiester linkage of nucleic acids.

In this paper, we focus our attention on the 3′ end of 16S rRNA because the base-complementary interaction between the 3′-terminal polypyrimidine sequence -C-C-U-C-C-U- of 16S rRNA in the ribosome and the polypurine sequence -A-G-G-A-G-G- preceding the initiator triplet of mRNA is believed to be an essential recognition step in the initiation of protein synthesis in *Escherichia coli* (5). There have been several reports in support of this hypothesis (6–8). In comparison to the prokaryotic system, the 3′-terminal sequence of eukaryotic 18S rRNA differs from that of the 16S rRNA sequence (9) and, so far, there has been no concrete experimental evidence to suggest that initiation of eukaryotic protein synthesis involves a base-pairing mechanism similar to that of the prokaryotic system. This difference in the prokaryotic and eukaryotic systems led us to explore the possibility that protein synthesis in bacteria could be selectively inhibited by oligonucleotides complementary to the 3′ end of 16S rRNA. Inhibition of protein synthesis would be manifested by a reduction of colony formation by treated cells.

In order to exploit this possibility in living cells, we have synthesized a series of deoxyribooligonucleoside methylphosphonates with base sequences complementary to the 3′ end of 16S rRNA. Nonionic oligonucleoside methylphosphonates have a number of unique physical and biochemical properties (10) including (*a*) the ability to form stable complexes with complementary polynucleotides, (*b*) the ability to penetrate the membranes of living cells, and (*c*) resistance to hydrolysis by cellular nucleases. In this paper, we report the synthesis of d[(A*p*G*p*G*p*A*p*G*p*G*p*)T] and its intermediates and the effect of these analogues on cell-free protein synthesis in *E. coli* and rabbit reticulocyte systems as well as the effects of these analogues on *E. coli* B, *E. coli* ML 308-225 (a permeable mutant), and transformed human cells in culture.

MATERIALS AND METHODS

2′-Deoxyadenosine, 2′-deoxyguanosine, and thymidine were obtained from P-L Biochemicals and were checked for purity by paper chromatography before use. Poly(U) and poly(A) were purchased from Sigma. MS-2 RNA was a product of Miles. [^{3}H]Thymidine (101 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), [^{3}H]lysine (54 Ci/mmol), [^{3}H]leucine (55 Ci/mmol), and

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Abbreviations: *p*, 3′–5′ linked methylphosphonate group. The symbols used to represent protected nucleosides and oligonucleoside methylphosphonates follow the IUPAC–IUB Commission on Biological Nomenclature Recommendations (1976).

* This is paper no. 4 of the series "Nonionic Oligonucleoside Methylphosphonates." Paper no. 3 is ref. 4.

[^{3}H]phenylalanine (35 Ci/mmol) were obtained from ICN. [^{3}H]Uridine (25 Ci/mmol), ^{3}H-labeled L-amino acid mixture, and the rabbit reticulocyte cell-free protein synthesizing systems were purchased from New England Nuclear.

Preparation, purification, and isolation of deoxyribooligonucleoside methylphosphonates were carried out as described (10). The base ratios of the products were determined by depurination with 80% acetic acid (5 hr at 60°C). The resulting bases were separated by high-performance liquid chromatography on a reverse-phase Partisil ODS-2 column (Whatmann) with a 5–20% acetonitrile gradient in water (50 ml, total). Adenine and guanine had retention times of 5.6 and 2.0 min, respectively. Under the same conditions, d(A*p*G*p*G*p*) and d(A*p*G*p*G*p*A*p*G*p*G*p*) had retention times of 12.5 and 18.0 min, respectively. The ratio of bases was determined from the area of the peaks. For d(A*p*G*p*G*p*) and d(A*p*G*p*G*p*A*p*G*p*G*p*), the ratio of adenine to guanine was 1:1.9 and 1:1.95, respectively.

Dialysis experiments (11) were performed in 30-μl plexiglass chambers separated by a dialysis membrane. The equilibration buffer contained 60 mM Tris·HCl (pH 7.5), 120 mM NH_4Cl, 6 mM $MgCl_2$, 0.6 mM dithiothreitol, 0.6 mM GTP, 200 pmol of *E. coli* B ribosomes, and 135–175 pmol of ^{3}H-labeled deoxyribooligonucleoside methylphosphonates. The chambers were equilibrated at the desired temperature for 2 days before measurement.

A cell-free protein-synthesizing system and 70S ribosomes from *E. coli* B were prepared according to the method of Nirenberg (12). Cell-free protein synthesis in a rabbit reticulocyte system was performed by using a cell-free translation system purchased from New England Nuclear (lot J1157AW). For the translation of globin mRNA, the reactions were run in 25.0 μl of buffer containing 2 μl of translation mixture, 2 μg of globin mRNA, 79 mM potassium acetate, 0.65 mM magnesium acetate, 0–100 μM oligomer, and 14 μM [^{3}H]leucine. Reactions were initiated by addition of 5 μl of reticulocyte lysate. Aliquots (4 μl) were removed at various times and added to 0.1 ml of bovine serum albumin (100 μg) solution. The protein was precipitated by heating with 1 ml of 10% trichloroacetic acid at 70°C filtered on G/F filters, and assayed for radioactivity in Betafluor.

E. coli ML 308-225 cells (a gift from Chien Ho, Carnegie–Mellon University, Pittsburgh) were grown at 37°C in minimal salt medium supplemented with 1% glucose (13). *E. coli* B cells were grown in M9 medium as described (14). Protein synthesis and RNA synthesis were carried out in cells grown to midlogarithmic phase ($\approx 5.0 \times 10^8$ cells per ml). Aliquots (50 μl) of cells were preincubated with 15 μl of medium or medium containing the compounds for 1–2 hr at 22°C. *E. coli* ML cells were transferred to a water bath maintained at 10°C. After 10 min, 3 μl of [^{3}H]uridine (100 μCi/ml) or 3 μl of [^{3}H]leucine (50 μCi/ml) was added; then 15-μl aliquots were withdrawn at 0, 5, 10, and 20 min and added to 200 μl of lysing buffer (2.0% $NaDodSO_4$/0.02 M EDTA) and heated at 70°C for 20 min. For protein synthesis experiments, bovine serum albumin (100 μg) and 20% trichloroacetic acid (1 ml) were added and the solution was heated at 70°C for 15 min. Then the solution was filtered; the filter was then washed and assayed for radioactivity. For RNA synthesis experiments, cold 5% trichloroacetic acid was added after lysis of the cells, and the solution was filtered without heating. The final concentration of oligomers in these experiments was 100 μM.

For determination of colony formation, *E. coli* ML 308-225 cells were incubated for 2 hr in 100 μl of medium containing 75–160 μM of oligonucleoside methylphosphonate. The solution was then diluted to 1.0 ml with the medium. To 0.9 ml of this solution, 2.0 ml of 0.5% bactoagar was added at 37°C and the solution was poured onto 100-mm plates containing 1.5% bactoagar. After solidification, the plates were incubated at 37°C for 36 hr, and the colonies were counted. The final concentration of the oligomers on the plate was 2.6–5.5 μM.

In vitro aminoacylation experiments were done as described by Barrett *et al.* (15).

Growth experiments were done by treating 15 μl of cells ($\approx 1 \times 10^8$ cells per ml) in 15 μl of medium (control) or medium containing 150 μM of the compounds at 37°C. Aliquots (4 μl) were withdrawn at different time intervals and appropriately diluted. The number of cells was determined by using a Hausser counting chamber and a Zeiss phase-contrast microscope.

RESULTS

Table 1 summarizes the reaction conditions and yields in the preparation of the deoxyribooligonucleoside methylphosphonates. Because the trinucleotide sequence d(A-G-G) is repeated in the heptamer, condensation of the trinucleotide blocks was considered to be more favorable than the stepwise addition of mononucleotides. The fully protected heptamer was prepared by condensing T(OAC) or [^{3}H]T(OAC) with the protected hexamer (data not shown). The low yields obtained in these preparations are attributed to the large number of dG residues present in these sequences. The trimer d(A*p*G*p*G*p*) and hexamer d(A*p*G*p*G*p*A*p*G*p*G*p*) were deblocked from d([$(MeO)_2$Tr]bzA*p*ibuG*p*ibuG*p*CNEt) and d([$(MeO)_2$Tr]-bzA*p*ibuG*p*ibuG*p*bzA*p*ibuG*p*ibuG*p*CNEt), respectively, and hence obtained with the 5′-terminal methylphosphonate group. Reactions carried out on a small scale (<0.01 mmol) were deblocked as such and the product was isolated by paper chromatography. The purity of the oligomers was examined mainly by high-performance liquid chromatography and paper chromatography. The UV spectral properties and paper chromatographic mobilities are given in Table 2.

The interaction of d(A*p*G*p*G*p*A*p*G*p*G*p*[^{3}H]T) and d(A*p*G*p*G*p*[^{3}H]T) with 70S ribosomes was studied by equilibrium dialysis. The heptamer has a high apparent association constant which decreases with increasing temperature (4.67×10^5 M^{-1} at 0°C; 1.72×10^5 M^{-1} at 22°C; 2.0×10^4 M^{-1} at 37°C). As expected, the tetramer, which has only three bases complementary to the 3′ end of 16S rRNA, has a proportionately lower association constant (1.44×10^4 M^{-1} at 22°C).

Table 1. Preparation of protected oligodeoxyribonucleoside methylphosphonates

3′-Methylphosphonate		5′-OH		MST	Product		
						Yield	
Component	mmol	Component	mmol	mmol	Name	mmol	%
d([$(MeO)_2$Tr]ibuG*p*)	2.77	d(ibuG*p*CNEt)	2.99	6.64	d(ibuG*p*ibuG*p*CNEt)	0.75	27
d([$(MeO)_2$Tr]bzA*p*)	1.1	d(ibuG*p*ibuG*p*CNEt)	0.725	2.2	d([$(MeO)_2$Tr]bzA*p*ibuG*p*ibu-G*p*CNEt)	0.15	21
d([$(MeO)_2$Tr]bzA*p*-ibuG*p*ibuG*p*)	0.033	d(bzA*p*ibuG-*p*ibuG*p*CNEt)	0.04	0.132	d([$(MeO)_2$Tr]bzA*p*ibuG*p*ibu-G*p*bzA*p*ibuG*p*ibuG*p*CNEt)	0.01	30

Table 2. UV spectra and chromatographic mobilities of oligodeoxyribonucleoside methylphosphonates

Oligomer	UV spectra* λ max., nm	λ min., nm	$\varepsilon_{260/280}$	$\varepsilon_{max.}$‡	Paper chromatography,† R_F
d(A*p*G*p*G*p*)	257	228	2.11	—	0.77
d(A*p*G*p*G*p*T)	257	229	2.02	4.19×10^4	0.88
d(A*p*G*p*G*p*A*p*G*p*G*p*)	257	229	2.11	6.6×10^4	0.27
d(A*p*G*p*G*p*A*p*G*p*G*p*T)	257	232	2.06	7.33×10^4	0.39

* In water at pH 7.0.
† Run in solvent F; R_F of pT, 0.41. Solvent F is *n*-propanol/NH_4OH/H_2O, 55:10:35 (vol/vol).
‡ Obtained by comparing the absorbance of a solution of the oligomer in water at pH 7.0 to that of the same solution at pH 1.0. The oligomer extinction coefficient was calculated from the observed hyperchromicity of the oligomer at pH 1.0 by using the following extinction coefficients: dA at pH 1.0, 14.1×10^3; dG at pH 1.0, 12.3×10^3.

The effects of the oligomers on cell-free protein synthesis in *E. coli* B system are summarized in Table 3. In general, the hexamer and heptamer exhibited inhibitory activities but the trimer and tetramer did not. Poly(U)-directed polyphenylalanine synthesis and poly(A)-directed polylysine synthesis were not inhibited appreciably by hexamer and heptamer at 37°C. The inhibition was greater at 22°C than at 37°C. At higher concentrations, the hexamer inhibited polylysine synthesis directed by poly(A) more effectively than polyphenylalanine synthesis directed by poly(U). Whereas d(A*p*G*p*G*p*) and d(A*p*G*p*G*p*T) did not cause appreciable inhibition of the translation of MS-2 RNA in the *E. coli* system, d(A*p*G*p*G*p*A*p*G*p*G*p*) and d(A*p*G*p*G*p*A*p*G*p*G*p*T) were effective inhibitors in dose-dependent manner, even at low concentrations. As a negative control for sequence specificity, d(CpCpApApGpC*p*-chlorophenylphosphate), a hexamer not complementary to the 3′ end of 16S rRNA, was used. This oligomer was found to be much less effective in inhibiting translation of MS-2 RNA in the *E. coli* system. In contrast to their effects on the *E. coli* system, both d(A*p*G*p*G*p*A*p*G*p*G*p*) and d(A*p*G*p*G*p*A*p*G*p*G*p*T), which are not complementary to the 3′ end of eukaryotic 18 S rRNA, did not have appreciable inhibitory effects on the translation of globin mRNA in a cell-free reticulocyte system (at 100 μM and 22°C, 16% and 17%, respectively).

The effects of deoxyribooligonucleoside methylphosphonates on the colony formation by *E. coli* B, *E. coli* ML 308-225, and transformed human cells (HTB 1080) as well as the effects of these analogues on cellular protein synthesis in *E. coli* B and *E. coli* ML-308-225 were investigated. Oligomers d(A*p*G*p*G*p*), d(A*p*G*p*G*p*A*p*G*p*G*p*), and d(A*p*G*p*G*p*A*p*G*p*G*p*T) inhibited colony formation by *E. coli* ML 308-225 cells effectively (Table 4).

Table 3. Effect of deoxyribooligonucleoside methylphosphonates on cell-free protein-synthesizing system from *E. coli* B

Oligomer	Conc., μM	Inhibition, % Poly(U)* 22°C	Poly(U)* 37°C	Poly(A)† 22°C	Poly(A)† 37°C	MS-2 RNA 22°C
d(A*p*G*p*G*p*)	100	8	0	0	0	5
d(A*p*G*p*G*p*T)	100	—	—	—	—	0
d(A*p*G*p*G*p*A*p*G*p*G*p*)	12.5	—	—	—	—	45
	25	0	0	0	0	75
	50	19	0	29	14	88
	100	39	18	80	27	—
d(A*p*G*p*G*p*A*p*G*p*G*p*T)	25	0	0	0	0	77
d(CpCpApApGpC*p*)‡	100	—	—	—	—	21

* At 260 μM in UMP residues.
† At 225 μM in AMP residues.
‡ p = *p*-chlorophenylphosphate.

These analogues had virtually no effect on colony formation by *E. coli* B cells and only a small inhibitory effect on colony formation by transformed human cells.

Results of the study on cellular protein synthesis by *E. coli* ML 308-225 support the observation on the colony formation by these two strains of bacteria. The rates of incorporation, by *E. coli* ML 308-225 cells, of exogenous [^{3}H]leucine into hot trichloroacetic acid-precipitable material and of [^{3}H]uridine into cold trichloroacetic acid-precipitable material were found to be quite rapid at 37°C and 22°C; however, the incorporation leveled off in 5 min at these temperatures. Hence, incorporation of [^{3}H]leucine and [^{3}H]uridine by this *E. coli* mutant were studied at a lower temperature (10°C). The incorporation was linear up to 10 min at this temperature. d(A*p*G*p*G*p*A*p*G*p*G*p*T) inhibited protein synthesis by *E. coli* ML 308-225 but not by *E. coli* B. Some variation in the extent of inhibition was observed between experiments, and the inhibition was found to be in the range of 20–45%. Under the same experimental conditions, d(A*p*G*p*G*p*A*p*G*p*G*p*T) had no effect on RNA synthesis as measured by [^{3}H]uridine incorporation. d(A*p*G*p*G*p*T) has no effect on either protein synthesis (Table 4) or RNA synthesis (data not shown).

In addition to the studies on colony formation, experiments were done on the growth of *E. coli* ML 308-225 in mass culture in the presence of the oligonucleotide analogues. There was no inhibition of growth during the first 4 hr (Fig 1). At the rapid growth period, between 4 and 12 hr, the growth of the treated culture was inhibited up to 50% in the presence of either trimer or heptamer. At the end of this growth period, 24 hr after initiation of the culture, the treated and untreated cultures had approximately the same number of cells.

DISCUSSION

The oligodeoxyribonucleoside methylphosphonates used in this study were prepared by the general procedures reported earlier

Table 4. Effect of deoxyribooligonucleoside methylphosphonates on colony formation

Oligomer (at 75 μM)	Inhibition, % *E. coli* ML 308-225*	*E. coli* B*	Human cells HTB 1080†
d(A*p*G*p*G*p*)	75–98	0	—
d(A*p*G*p*G*p*A*p*G*p*G*p*)	78–97	0	—
d(A*p*G*p*G*p*A*p*G*p*G*p*T)	67–97	0	10
d(A*p*G*p*G*p*T)	0	0	—
d(G*p*G*p*T)	5	—	—

* At either 22°C or 37°C.
† At 37°C.

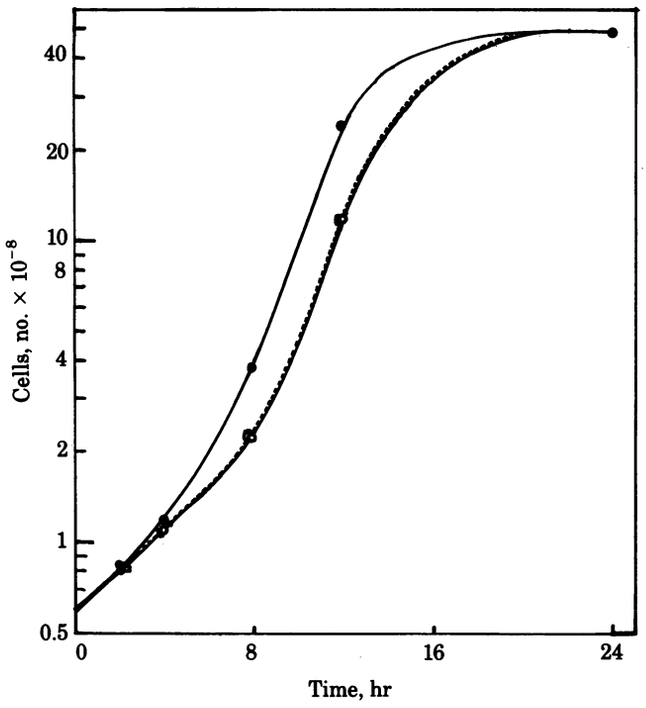

FIG. 1. Effect of oligonucleoside methylphosphonates on *E. coli* ML 308-225 cells growing in culture. ●, Control; ○, in the presence of 150 μM d(A*p*G*p*G*p*); □---□, in the presence of 150 μM d[(A*p*G*p*G*p*)$_2$T].

(10). The yields in these preparations are somewhat low, which can be attributed to the reactions involving deoxyguanosine residues. Similar difficulties have been encountered in the preparation of oligonucleotide phosphotriesters containing deoxyguanosine residues (16).

The two phosphonate analogues, d(A*p*G*p*G*p*[^{3}H]T) and d(A*p*G*p*G*p*A*p*G*p*G*p*[^{3}H]T), which are complementary to the 3′ end of 16S rRNA, exhibit high affinity for 70S ribosomes as studied by equilibrium dialysis. Earlier studies on the interaction of a pentanucleotide (G-A-dG-dG-U) with *E. coli* 30S and 70S ribosomes have shown that this pentamer specifically binds to a site on the 30S subunit (7). Hence, it is very likely that the observed binding of the tetramer and heptamer analogues with 70S ribosomes is due to their formation of complexes with the complementary regions at the 3′ end of 16S rRNA.

This conclusion is further supported by studies on the effect of these analogues on cell-free protein synthesis. Both the hexamer and heptamer effectively inhibit the translation of MS-2-RNA in the *E. coli* cell-free system while having a much lower inhibitory effect on the translation of poly(U) and poly(A). The *in vitro* aminoacylation of $tRNA_{coli}$ is not inhibited by these oligomers, suggesting that the inhibition of aminoacylation of tRNAs does not play a role in the inhibition of translation of MS-2 RNA. The inhibition observed in the *E. coli* system, therefore, is most likely at the ribosome site. Because the synthetic mRNAs, unlike natural mRNAs, lack specific initiation sites, the results also support the conclusion that the inhibition of translation of MS-2 RNA may arise from competition between the oligonucleotide analogues and the homologous sequence within the preinitiator region of MS-2 RNA. Our results are in agreement with those obtained by Taniguchi and Weissmann (6) and Eckhardt and Luhrmann (7). They observed an inhibition of formation of phage mRNA-70S ribosome initiation complex in the presence of oligonucleotides complementary to the 3′ end of 16S rRNA. In contrast, no inhibition of poly(U)-dependent $tRNA^{Phe}$ binding to 70S ribosomes was found. Additional support for the base-complementary interaction of oligonucleotide analogues with the 3′ end of 16S rRNA comes from the inability of these analogues to inhibit the translation of globin mRNA in a rabbit reticulocyte system. Although the 3′-end sequences of 18S rRNA and 16S rRNA are similar, 18S rRNA specifically lacks the -C-C-U-C-C-U- sequence found in 16S rRNA, and hence the oligonucleotide analogues cannot form stable complexes with 18S rRNA in reticulocyte ribosomes.

Although the oligomers inhibit translation of mRNAs in the *E. coli* B cell-free system, these oligomers have no effect on either protein synthesis or colony formation by the intact *E. coli* B cells; however, as shown in Table 4, these oligomers inhibit the protein synthesis and growth of an *E. coli* mutant (ML 308-225). Our experiments on the uptake of oligonucleotides by *E. coli* B cells indicate that they are permeable to d(A*p*[^{3}H]T), T*p*[^{3}H]T, and T*p*T*p*[^{3}H]T but not to (T*p*)$_4$[^{3}H]T and (T*p*)$_8$[^{3}H]T (unpublished data). Thus, oligonucleoside methylphosphonates longer than 4 nucleotide units cannot enter the cell. The cutoff size of the nonionic oligonucleotides observed here agrees with the size limit found for oligosaccharides and oligopeptides (17, 18). In contrast to *E. coli* B, *E. coli* ML 308-225 cells were permeable to d(A*p*G*p*G*p*A*p*G*p*G*p*[^{3}H]T) (unpublished data). This *E. coli* mutant has only small quantities of lipopolysaccharide in the outer membrane of the cell wall (19). The reduction in lipopolysaccharide content may increase the permeability of cell wall toward oligonucleoside methylphosphonates. Thus, the difference in the permeability of the cell walls of these two bacteria can explain why the hexamer and heptamer do not have any effect on intact *E. coli* B cells but inhibit protein synthesis, colony formation, and culture growth of *E. coli* ML 308-225.

The specific inhibitory effects of oligonucleotide analogues in the cell-free systems is also indicated by the following observations at the intact cellular level (Table 4). (*i*) The oligonucleotide analogues inhibit protein synthesis and growth of *E. coli* ML 308-225 cells but have little or no effect on human cells. (*ii*) Although d(A*p*G*p*G*p*A*p*G*p*G*p*) and d(A*p*G*p*G*p*A*p*G*p*G*p*T) inhibit colony formation by *E. coli* ML 308-225, d(A*p*G*p*G*p*T) has no effect; and (*iii*) d(A*p*G*p*G*p*A*p*G*p*G*p*T) inhibits protein synthesis without concurrent inhibition of RNA synthesis.

Because we have found that oligonucleoside methylphosphonates (T*p*)$_n$T (n = 1,4,8) are effectively taken up by Syrian hamster cells (unpublished results), the lack of inhibition of human cell colony formation is unlikely to be attributable to the inability of these oligomers to penetrate the human cells. At present, the observed inhibitory effect of d(A*p*G*p*G*p*) on colony formation by *E. coli* ML 308-225 cells requires additional investigation for an adequate explanation because the trimer has no effect on cellular protein and RNA synthesis in this mutant. Also, the trimer has no effect on protein synthesis in the *E. coli* B cell-free system.

The temporary inhibition of the growth of *E. coli* ML 308-225 cells in mass culture by the trimer and heptamer (Fig. 1) is in agreement with the expectation that the oligomers may not have caused a permanent damage to the functioning of ribosomes. The cells can overcome this inhibition by synthesizing more ribosomes, or the inhibitory effect of the oligomers will not be detectable when the capacity of the ribosomes for protein synthesis is no longer the factor limiting growth.

The results of our studies further demonstrate the feasibility of using oligonucleoside methylphosphonate analogues for probing the structure–function relationship of nucleic acids in biochemical systems as well as in living cells. The results also suggest that, by choosing an appropriate complementary sequence of oligonucleotide, one can selectively regulate either

bacterial or mammalian cellular nucleic acid function. Thus, the appropriate use of nonionic nucleic acid analogues, such as the oligonucleoside methylphosphonates, may have great significance in basic research and in practical applications.

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1. Stephenson, M. L., & Zamecnik, P. C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 285–288.
2. Zamecnik, P. C. & Stephenson, M. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 280–284.
3. Miller, P. S., Braitermann, L. T. & Ts'o, P. O. P. (1977) *Biochemistry* **16,** 1988–1996.
4. Miller, P. S., McParland, K., Jayaraman, K. & Ts'o, P. O. P. (1980) *Biochemistry*, in press.
5. Shine, J. & Dalgarno, I. (1975) *Nature (London)* **254,** 34–38.
6. Taniguchi, T. & Weissmann, C. (1978) *Nature (London)* **275,** 770–772.
7. Eckhardt, H. & Luhrmann, R. (1979) *J. Biol. Chem.* **254,** 1185–1188.
8. Steitz, J. A. & Jakes, K. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4734–4738.
9. Hagenbuchle, O., Santer, M. & Steitz, J. A. (1978) *Cell* **13,** 551–563.
10. Miller, P. S., Yano, J., Yano, E., Carroll, C., Jayaraman, K. & Ts'o, P. O. P. (1979) *Biochemistry* **18,** 5134–5143.
11. Miller, P. S., Barrett, J. C. & Ts'o, P. O. P. (1974) *Biochemistry* **13,** 4887–4896.
12. Nirenberg, M. (1963) *Methods Enzymol.* **6,** 17–23.
13. Davis, B. D. & Mingioli, E. S. (1950) *J. Bacteriol.* **60,** 17.
14. Bolle, A., Epstein, R. H., Salser, W. & Geiduschek, E. P. (1968) *J. Mol. Biol.* **33,** 339–362.
15. Barrett, J. C., Miller, P. S. & Ts'o, P. O. P. (1974) *Biochemistry* **13,** 4898–4906.
16. Miller, P. S., Cheng, D. M., Dreon, N., Jayaraman, K., Kan, L. S., Leutzinger, E. E., Pulford, S. M. & Ts'o, P. O. P. (1980) *Biochemistry* **19,** 4688–4698.
17. Decad, G. & Nikaido, H. (1976) *J. Bacteriol.* **128,** 325–336.
18. Payne, J. W. & Gilvarg, C. (1968) *J. Biol. Chem.* **243,** 6291–6299.
19. Kaback, H. R. (1971) *Methods Enzymol.* **22,** 99–120.