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Oligonucleotides complementary to a promoter over the region −8...+2 as transcription primers for *E. coli* RNA polymerase

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ABSTRACT

Primer-dependent transcription by E.coli RNA polymerase on T7 promoter A2 has been studied. Synthetic deoxyribonucleotides complementary to the promoter over the region -8...+2 were taken as primers. A ribonucleoside residue was present at the 3'-end of some of these oligonucleotides. The octanucleotide complementary to the region -8...-1 appeared to be an active primer. Oligonucleotides having lengths from 3 to 6 nucleotide residues complementary to the promoter over the region -4...+2 also exhibited primer activity. The latter was some 5-10 times greater in the case of oligonucleotides having a ribonucleoside residue at the 3'-end. Oligonucleotides which on complementary binding do not reach the center of phosphodiester bond synthesis, as well as the decanucleotides (-8...+2) and octanucleotides (-6...+2) of both the ribo- and deoxyribo-series were inactive as primers.

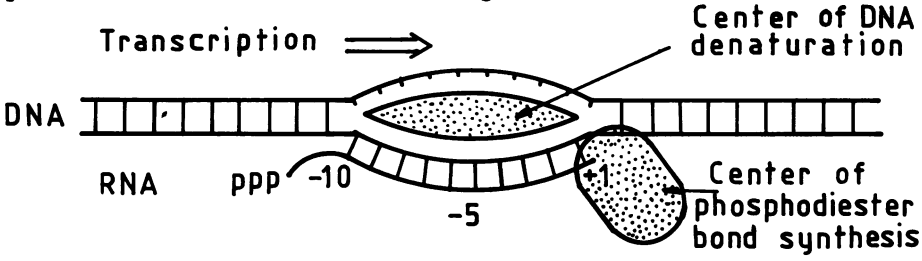
INTRODUCTION

DNA-dependent RNA polymerases, unlike other enzymes of template-dependent biosynthesis of nucleic acids, do not use any primers and are able to start synthesis of RNA de novo at definite points of double-stranded promoter-containing templates. However, it is known, that diribonucleotides of the structure NpN complementary to promoter in immediate vicinity to the startpoints of primer-independent transcription may act as primers and are incorporated into the 5'-ends of the growing RNAs (1-4). To our knowledge, the primer activity of longer oligonucleotides in promoter-directed transcription has not been studied.

Profound conformational changes of the transcription complex take place at the beginning of RNA synthesis.Johnston and McClure (5) have found that the dinucleotides of the structure pppNpN synthesized by E.coli RNA polymerase on pro-

moter templates in the presence of two NTPs do not form stable enzyme-template-product complexes and easily dissociate into solution. After the dissociation, the enzyme synthesizes a new pppNpN molecule, etc. It has been found later (6) that the ternary transcription complex becomes stable (and fully productive) only after the synthesis of some 8-10 phosphodiester bonds. Hansen and McClure (7) have presented evidence that the σ-subunit of RNA polymerase (which serves for specific binding of the enzyme to promoter) dissociates from the ternary transcription complex after the synthesis of 8-10 phosphodiester bonds. These facts are sufficient to assume that the synthesis of the first 10 phosphodiester bonds may be regarded as a descrete stage of transcription for which we propose a name "primer synthesis stage".

We have published a hypothesis (6) according to which increase in the stability of the ternary complex after the start of transcription at the "primer synthesis stage" is due to formation of a heteroduplex over the region between the positions -1 and -10 of the codogeneous strand:



Chemical modification studies (8,9) have lead to the conclusion that RNA polymerase when it forms an "open" complex prior to the start of transcription unwinds DNA over the region -8...+3. For this reason, taking into account the above mentioned hypothesis, it seemd interesting to find out if it is possible to bypass the "primer synthesis stage" by taking ready primers - synthetic oligonucleotides complementary to the region of promoter denatured by RNA polimerase.

In the present studies we investigated the primer activities of oligodeoxyribonucleotides complementary to the promoter A2 of T7 DNA over the region -8...+2. The 3'-termini of some of these oligonucleotides contained a ribonucleoside

rather than a deoxyribonucleoside residue, because it was anticipated that the ribo-moiety better fits the center of phosphodiester bond synthesis. The main conclusion of our studies is that some of such oligonucleotides do act as efficient primers.

MATERIALS AND METHODS

RNA polymerase E.coli B, isolated according to ref.(10), was a generous gift of Dr. V.M.Lipkin (Shemyakin Institute of Bioorganic Chemistry, Moskow). The preparation contained more than 90% of holoenzyme, as suggested by SDS gel-electrophoresis, and exhibited a specific activity 36,000 units/mg, when analyzed according to ref.(10).

The fragment of T7 DNA containing the promoter A2 was obtained by action of E.coRI restriction nuclease upon the previously described plasmid pSK T7A2 (11) and purified by chromatography on Sepharose 2B. The fragment obtained was 110 b.p. long; the startpoint of transcription on it resides at a distance of 29 b.p. from its left-hand end.

[γ-^{32}P]ATP and [α-^{32}P]NTPs (specific radioactivity more than 2000 Ci/mmole) were from Isotop (USSR) or Amersham (England).

p-Chlorophenyl esters of N-acyl-3'-levulinyl nucleoside-5'-phosphates, p-chlorophenyl, β-cyanoethyl-N-acylnucleoside-5'-phosphates, triisopropylsulfochloride, di-β-cyanoethyl-N-acyl-nucleoside-5'-phosphates were the products of Novosibirsk Institute of Organic Chemistry.

The synthesis of the oligonucleotides pd(TACAAATCGC), pd(CGC), pd(TACAAATCG)rC and pd(CG)rC was performed by the triester method starting with 5'-chlorophenyl esters of nucleotides (12,13). The synthesis of the decanucleotide pd(TACAAATCGC) was run in pyridine in the presence of 1-(benzenesulphonyl)-3-nitro-1,2,4-triazole as the condensing reagent. The synthesis of the decanucleotide pd(TACAAATCG)rC and of the trinucleotides pd(CGC) and pd(CG)rC was performed in chloroform in the presence of triisopropylbenzenesulfochloride and N-methylimidazole (1:2). Two cyanoethyl groups were used to protect the 5'-phosphate. The 3'-hydroxyl groups of the OH-components were blocked by levulinate. The base residues were protected

as usually (12,13). At all the stages the P- and OH-components were taken in concentration 0.2 M and at a ratio close to equimolar. A 3-fold excess of the condensing reagents over the P-components was used. The cyanoethyl groups were removed by a mixture of dry acetonitrile and triethylamine (1:1) (14). The OH-components after the removal of levulinate residue (15) were isolated by chromatography on silica gel in a gradient of methanol in chloroform. Complete removal of the protecting groups was performed by treatment with p-nitrobenzaldoxymate at 20° during 24 h (16) followed by treatment with concentrated ammonia (2 days at 20°). The structures of the oligonucleotides were confirmed by sequencing according to ref. (17).

Partial apurinization and cleavage of oligonucleotides were performed essentially as in (18). An aqueous solution of an oligonucleotide (5 mg/ml) was mixed with two volumes of formic acid, and the mixture was kept for 1h at 22°. Formic acid was removed by evaporation with methanol, and the residue was dissolved in 10% piperidine (to concentration of nucleotide material ca. 5 mg/ml). The solution was kept at 95° during 30 min and a few times evaporated with methanol.

Fractionation of cleavage products. The mixture of oligonucleotides obtained by apurinization and cleavage of decanucleotide pd(TACAAATCGC)* (10-20 A_{260} units) was separated by ion-exchange chromatography on Partisil 10SAX (10 ml column, linear gradient of KH_2PO_4 from 0.02 to 0.3 M in 30% acetonitrile, 60 ml) using the liquid chromatograph Altex (USA). Oligonucleotides which were not resolved by ion-exchange chromatography were further purified by reversed-phase chromatography on Nucleosil C-18 using microcolumn liquid chromatograph "OB-4" (19). Two A_{260} units of oligonucleotides were applied to a 190 µl column. Elution was performed by 1ml of a linear gradient of methanol (0 to 40%) in 20 mM ammonium acetate (pH 4.8) at a flow rate 100 µl/min. The separated oligonucleotide fractions were evaporated and desalted by chromatography on Lichroprep RP-18. The oligonucleotide was applied to the column (no more than 50 A_{260} units per 1 ml of the resin), the column washed with 0.05 M triethylammonium

bicarbonate (pH 7.5), and the substance recovered with 25% aqueous methanol. Methanol was removed by evaporation, and the oligonucleotide dissolved in water.

The products of degradation of the decanucleotide pd(TACAAATCG)rC were at first separated by chromatography on dihidroxyboryl cellulose (Collaborative Research,USA). For this purpose, the residue after the evaporation of cleavage products with methanol was dissolved in 1 ml of buffer A (0.2M NaCl - 10 mM $MgCl_2$ - 50 mM morpholine-HCl(pH 7.7) - 10% ethanol) and applied to a 10 ml column with dihydroxyboryl cellulose. The column was washed with buffer A to remove oligonucleotides which did not contain a 3'-terminal ribonucleoside residue. The application and washing was performed at 6-10°. After this, the column was warmed to room temperature, and the substances adsorbed eluted with 0.2 M sodium acetate (pH 5) - 1M NaCl. The two fractions obtained were desalted by chromatography on LiChroprep RP-18. After this, each of them was subjected to ion-exchange chromatography on Partisil 10SAX followed by reversed-phase chromatography on Nucleosil C-18 and desalted as described above.

Dephosphorylation of oligonucleotides and phosphorylation of their 5'-ends. In order to remove 3'-phosphate groups and/or to introduce a [5'-^{32}P]-label, oligonucleotides were at first dephosphorylated by immobilized E.coli phosphomonoesterase and then rephosphorylated by polynucleotide kinase. Dephosphorylation was performed in 10 mM Tris-HCl (pH 8.8) - 10 mM $MgCl_2$ - 10 mM mercaptoethanol by phosphomonoesterase immobilized on BrCN-sepharose (10 µl of suspension per 10 nmoles of oligonucleotide). The reaction was run during 1h at 37° with agitation. The extent of dephosphorylation was estimated by thin layer chromatography on PEI-cellulose sheets in 0.3 M LiCl - 10 mM Tris-HCl (pH 8) - 7 M urea. Phosphomonoesterase was removed by centrifugation, [γ-^{32}P]ATP (100-1000 µCi) and polynucleotide kinase were added to the supernatant and the mixture kept during 30 min at 37°. after this unlabeled ATP was added to $5 \cdot 10^{-4}$ M, and the mixture incubated 15 min more at 37°. The solution was applied to LiChroprep RP-18 (50 µl), and the column washed with 0.5 ml of 0.05 M triethylammonium

bicarbonate to remove ATP. The radioactive oligonucleotide was eluted with 25% methanol and evaporated. Rephosphorylation by non-radioactive ATP was performed similary, but the incubation with [γ-^{32}P]ATP was omitted.

Primer-dependent transcription. A reaction mixture containing E.coli RNA polymerase, T7 A2 promoter fragment, 25mM Tris-HCl (pH 7.9), 50 mM NaCl, 10 mM $MgCl_2$, 10 mM mercaptoethanol was incubated during 2 min at 37°. oligonucleotide was then added and the incubation continued for 10 min more. The reaction was started by addition of nucleoside-5'-triphosphates. After 10 min reaction at 37° an equal volume of stopping solution (20 mM EDTA - 100 µg/ml tRNA - 0.02% xylene cyanol FF - 0.02% bromphenol blue - 0.02% orange G - 7 M urea) was added. The samples were applied to 25% polyacrylamide gel (30x20x0.05cm) prepared according to ref. (20). Gel-electrophoresis was run at 1200-1500 V for 2-3 h. The products were vizualized by autoradiography. To estimate the quantities, the radioactive zones were cut out and counted by means of Mark-III (Nuclear Chicago, USA) scintillation counter.

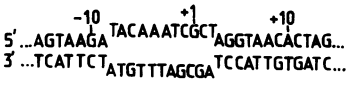
RESULTS

The preparation of oligonucleotide primers

We have synthesized two decanucleotides complementary to the codogeneous strand of T7 promoter A2 over the region -8...+2 (Fig.1).

The difference between these two decanucleotides is that one consisted of only deoxyribonucleotide units and the other

A



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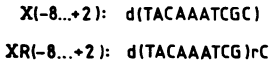

Fig.1 A. Structure of a part of the promoter A2 of the T7 phage DNA. The region denatured by RNA polymerase when an open complex is formed (deduced from (6)) is shown by separation of strands. Position +1 is the startpoint of transcription (rightward).

B. Structures of the synthetic decanucleotides.

Table 1. Structures of oligonucleotides and the methods of their preparation.

Designation	Structure	Region of promoter to which the oligonucleotide is complementary	Method of preparation
X(-8...+2)	d(pTpApCpApApApTpCpGpC)	(-8...+2)	Chemical synthesis
XR(-8...+2)	d(pTpApCpApApApTpCpGp)rC	(-8...+2)	
III(-8...-6)	d(pTpApC)	(-8...-6)	Partial apurinization of X(-8...+2), degradation by piperidine, chromatography, dephosphorylation, rephosphorylation
IV(-8...-5)	d(pTpApCpA)	(-8...-5)	
V(-8...-4)	d(pTpApCpApA)	(-8...-4)	
VIII(-8...-1)	d(pTpApCpApApApTpC)	(-8...-1)	
III(-1...+2)	d(pCpGpC)	(-1...+2)	Chemical synthesis
IIIR(-1...+2)	d(pCpGp)rC	(-1...+2)	
IV(-2...+2)	d(pTpCpGpC)	(-2...+2)	Partial apurinization of X(-8...+2) or XR(-8...+2), degradation by piperidine, chromatography
V(-3...+2)	d(pApTpCpGpC)	(-3...+2)	
VI(-4...+2)	d(pApApTpCpGpC)	(-4...+2)	
VIII(-6...+2)	d(pCpApApApTpCpGpC)	(-6...+2)	
IVR(-2...+2)	d(pTpCpGp)rC	(-2...+2)	
VR(-3...+2)	d(pApTpCpGp)rC	(-3...+2)	
VIR(-4...+2)	d(pApApTpCpGp)rC	(-4...+2)	
VIIIR(-6...+2)	d(pCpApApApTpCpGp)rC	(-6...+2)	

contained a ribocytidine residue at the 3'-end. They were named X(-8...+2) and XR(-8...+2), respectively. To name other oligonucleotides, we use the same system: the Roman numeral indicates the length of the oligonucleotide, the letter "R" means that a ribonucleoside residue is present at the 3'-end, and the Arabic numerals in brackets corresponds to the region of the promoter to which given oligonucleotide is complementary.

In order to obtain shorter primers, we either used chemical synthesis, or subjected decanucleotides X(-8...+2) and

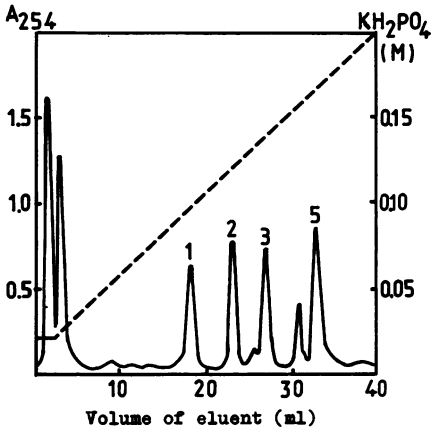

Fig.2 Ion-exchange chromatography on Partisil 10SAX of the products of degradation of XR(-8...+2) retained by dihydroxyboryl cellulose.

1 - IVR(-2...+2)
2 - VR(-3...+2)
3 - VIR(-4...+2)
4 - VIIIR(-6...+2)
5 - XR(-8...+2)

Solid line - A_{254}
Broken line - concentration of KH_2PO_4

XR(-8...+2) to limited apurinization and degradation by piperidine (Table 1). The mixtures obtained were subjected to chromatographic separations.

The products of degradation of the oligonucleotide XR(-8...+2) were at first separated by chromatography on dihydroxyboryl cellulose. This adsorbent retained oligonucleotides having a ribonucleoside residue at the 3'-end, but did not retaine other degradation productes. The two fractions obtained were subjected to ion-exchange chromatography on

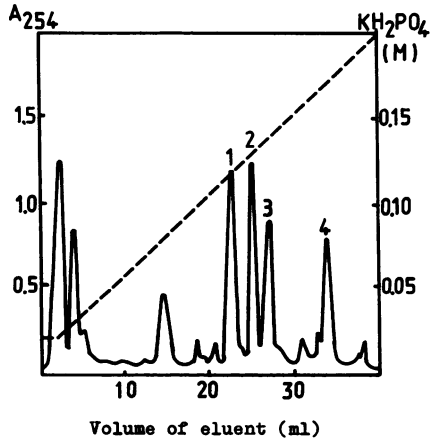

Fig.3 Ion-exchange chromatography on Partisil 10SAX of the fraction of the products of degradation of XR(-8...+2) non-retained by dihydroxyboryl cellulose. Dephosphorylation and rephosphorylation of these compounds gave the following products:

1 - III(-8...-6)
2 - IV(-8...-5)
3 - V(-8...-4)
4 - VIII(-8...-1)

Solid line - A_{254}
Broken line - concentration of KH_2PO_4

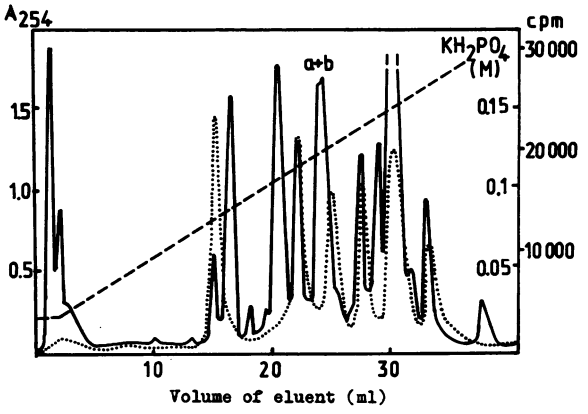

Fig.4 Chromatography of the products of degradation of X(-8...+2) on Partisil 10SAX; 20 A_{260} units were applied; ca. 1 μCi of [5'-^{32}P]X(-8...+2) was added to the unlabeled decanucleotide before the degradation. Solid line - A_{254}; broken line - concentration of KH_2PO_4; dotted line - radioactivity (cpm).

anionite Partisil 10SAX (Figs.2,3) followed by reversed-phase chromatography on Nucleosil C-18.

The products of degradation of the oligonucleotide X(-8...+2) were subjected directly to chromatography on Partisil 10SAX (Fig.4). To identify the 5'-terminal products a small amount of the same [5'-^{32}P]decanucleotide was added to the mixture prior to degradation. Oligonucleotides having the same charges (like IV(-8...-5) and VI(-4...+2) and some others) and hence non-resolved by ion-exchange chromatography on Partisil 10SAX were separated by reversed-phase chromatography on Nucleosil C-18 using the microcolumn liquid chromatograph OB-4 (19) (Fig.5).

The 5'-terminal products of the degradation of the decanucleotides possesed phosphate residues at their 3'-ends. To remove them, the oligonucleotides were dephosphorylated by E.coli phosphomonoesterase. The 5'-phosphate residues were then regenerated by rephosphorylation with T4 polynucleotide kinase. The structures of all the oligonucleotides obtained were confirmed by sequencing according to ref.(17).

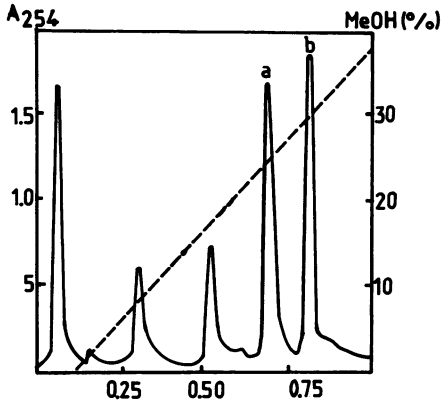

Fig.5 Rechromatography on Nucleosil C-18 of the peak (a+b) (Fig.4) non-resolved on Partisil 10SAX; 2 A_{260} units applied. Chromatography was run as described in Materials and Methods.

a - pd(TACA)p; its dephosphorylation and rephosphorylation gave IV(-4...+2)

b - VI(-4...+2)

Solid line A_{260}; broken line - concentration of methanol.

Studies of the primer activities of non-radioactive oligonucleotides in the presence of radioactive substrates.

Reaction mixture which contained RNA polymerase and the promoter fragment A2 (its length was 110 b.p.(11)) was incubated for 2 min at 37°. The oligonucleotide was then added, and the mixture was kept during 10 min at 37°. After this an [α-^{32}P]NTP was added which was complementary to the residue of template next to the 3'-end of the primer. The mixture was incubated at 37° for additional 10 min, and the products were analyzed by electrophoresis in 25% polyacrylamide gel. The lengths of the products were determined by comparison of their mobilities with those of radioactive oligonucleotides of known structures.

It is seen in Fig.6 that primer activity is exhibited by the oligonucleotides III(-1...+2), IV(-2...+2), V(-3...+2), VI(-4...+2), by similar oligonucleotides of the R-series and also by the oligonucleotide VIII(-8...-1). In their presence, radioactive products were accumulated in the reaction mixtures; the lengths of these products were one nucleotide longer than those of the starting oligonucleotides. Determination of the quantities of the products showed that under the conditions employed oligonucleotides IIIR(-1...+2), IVR(-2...+2), VR(-3...+2) and VIR(-4...+2) having a ribonucleoside residue at the 3'-end gave some 5-10 times more products (0.1-0.3

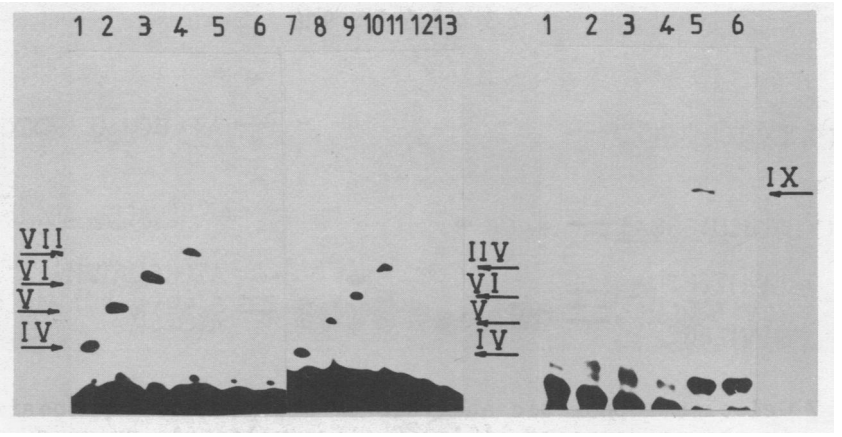

Fig.6 Electrophoretic analysis of the products synthesized by RNA polymerase in the presence of different oligonucleotides and a single [α-^{32}P]NTP. Concentrations of oligonucleotides was 10^{-5}M, of NTPs - 10^{-6}M, of RNA polymerase and A2 promoter fragment - 10^{-7} M. Roman numerals correspond to lengths of the products whose positions are indicated by arrows.

A. The reaction mixtures contained UTP and the following oligonucleotides:

1 - IIIR(-1...+2); 7 - III(-1...+2);
2 - IVR(-2...+2); 8 - IV(-2...+2);
3 - VR(-3...+2); 9 - V(-3...+2);
4 - VIR(-4...+2); 10 - VI(-4...+2);
5 - VIIIR(-6...+2); 11 - VIII(-6...+2);
6 - XR(-8...+2); 12 - X(-8...+2);
13 - no oligonucleotides.

B. The reaction mixtures contained the following NTPs and oligonucleotides:

1 - ATP alone;
2 - ATP+III(-8...-6)
3 - ATP+IV(-8...-5)
4 - ATP+V(-8...-4)
5 - GTP+VIII(-8...-1)
6 - GTP alone

moles per mole of promoter A2) than similar oligodeoxyribonucleotide primers having deoxynucleoside 3'-termini (0.02 - - 0.05 moles per mole of promoter A2). The oligonucleotide VIII(-8...-1) gave 0.02 mole of product per mole of promoter A2. All other oligonucleotides listed in Table 1 gave less than 0.002 moles of products per mole of A2 promoter. Non--complementary trinucleotides pd(CGA), pd(CGT) and pd(CGG) did not give detectable products; non-complementary NTPs were not attached to primers active with complementary ones (data not shown).

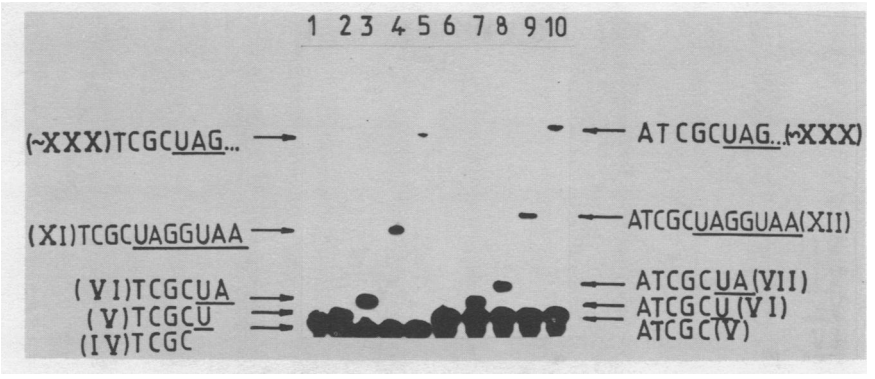

Fig.7 Gel-electrophoretic analysis of the products synthesized in the presence of [5'-^{32}P] oligonucleotide primers and different combinations of non-radioactive NTPs. The reaction mixtures contained $5 \cdot 10^{-7}$M RNA polymerase and A2 promoter fragment, $5 \cdot 10^{-6}$M oligonucleotides and 10^{-4}M of each indicated NTP.

Lanes 1-5: IVR(-2...+2) and:
1 - no NTPs
2 - UTP
3 - UTP+ATP
4 - UTP+ATP+GTP
5 - UTP+ATP+GTP+CTP

Lanes 6-10 VR(-3...+2)and:
6 - no NTPs
7 - UTP
8 - UTP+ATP
9 - UTP+ATP+GTP
10 - UTP+ATP+GTP+CTP

The Roman numerals denote the lengths of the products.

Studies of the primer activities of radioactive oligonucleotides in the presence of non-radioactive substrates.

In these experiments, the reaction mixtures contained RNA polymerase, the A2 promoter fragment, a [5'-^{32}P] oligonucleotide primer of the R-series, and different combinations of non-radioactive substrates: UTP, UTP+ATP, UTP+ATP+GTP or UTP+ATP+GTP+CTP. Fig.7 shows the result of a typical experiment with IVR(-2...+2) and VR(-3...+2). It is seen, that the lengths of the products synthesized were those following from the known primary structure of the promoter A2 and the combination of the oligonucleotide and NTPs. For example the combination of [5'-^{32}P] IVR(-2...+2) and three NTPs (UTP+ATP+GTP) led to a product which had a length of 11 nucleotide residues in accord with the structure shown in Fig.1 (see Table 2). Addition of four substrates resulted in a product which was about 30 nucleotide

Table 2. Amounts of products synthesized from [5'-^{32}P] oligonucleotide primers and different combinations of non-radioactive NTPs.

Oligonucleotide	Substrates	Length of product (found)	Structure of product (theory)*	Amount of product (moles per mole of A2)
IVR(-2...+2)	UTP	5	pTCGCU	2.6
IVR(-2...+2)	UTP+ATP	6	pTCGCUA	2.3
IVR(-2...+2)	UTP+ATP+GTP	11	pTCGCUAGGUAA	0.3
VR(-3...+2)	UTP	6	pATCGCU	1.6
VR(-3...+2)	UTP+ATP	7	pATCGCUA	1.2
VR(-3...+2)	UTP+ATP+GTP	12	pATCGCUAGGUAA	0.4
VIR(-4...+2)	UTP	7	pAATCGCU	1.3
VIR(-4...+2)	UTP+ATP	8	pAATCGCUA	0.8
VIR(-4...+2)	UTP+ATP+GTP	13	pAATCGCUAGGUAA	0.6

* Underlined is the "ribo" part of products.

residues long, i.e. the run-off product (the startpoint of transcription is located 29 nucleotides to the left from the right end of the codogenic strand of the A2 promoter fragment (11)). Hence, the above oligonucleotides ending at position +2 do occupy the predicted positions in the transcription complexes relative to template.

Similar resultes were obtained with IIIR(-1...+2) and VIR(-4...+2). However, no products at all were formed in the presence of [5'-^{32}P]oligonucleotides VIIIR(-6...+2) and XR(-8...+2) with any of the combination of substrates (data not shown).

The amounts of products synthesized with the above mentioned active primers were relatively great when the combinations of primers and substrates allowed them to be short (n<6, see Table 2). Presumably, this was because they relatively easy dissociated from the ternary complex enzyme--template-product, and abortive synthesis was the case (cf.(6)). On the other hand, combinations leading to products of n>6 resulted in smaller yields, presumably because

of the higher stability of the ternary complexes. This explanation is in accord with the model discussed in the Introduction.

DISCUSSION

The above presented evidence suggests that E.coli RNA polymerase is able to elongate oligodeoxyribonucleotide primers complementary to the codogeneous strand of a promoter. Our data confirm the conclusion following from the results of the chemical modification studies (8,9), that the region (-8...+3) is unwound in the "open complex". Moreover, they show, that this region of the codogeneous strand is able to interact with complementary oligonucleotides.

The primer activities of oligonucleotides depend on their lengths and positions relative to template. The results are summarized in Fig.8. It is seen that oligonucleotides whose 3'-end does not reach the center of phosphodiester bond synthesis (position between -1 and +1) are not active as primers. Oligonucleotides having lengths from 3 to 6 nucleotide residues whose 3'-end reaches the position +2 are active as primers, suggesting that they may shift the active center of phosphodiester bond synthesis to a position between +2 and +3. Such oligonucleotides with a 3'-terminal ribonucleoside instead of the 3'-deoxyribonucleoside residue are more active primers, presumably because ribose residue better fits the center of phosphodiester

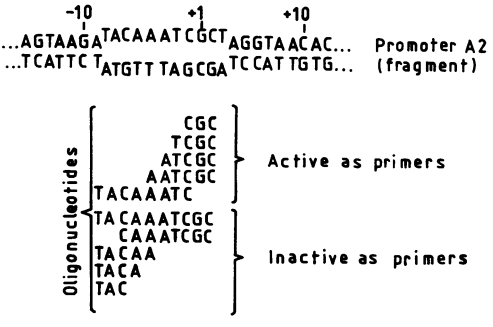

Fig.8 Results of the study of the activity of oligonucleotides as primers (summary).

bond synthesis. The octanucleotide VIII(-8...-1) whose 3'-end enters the startpoint of non-primed transcription is also an active primer.

The primer activity of oligonucleotides demonstrated by the present studies has important consequences. Firstly, it supports the hypothesis (6) that the stability of the ternary complex enzyme-template-product depends on the filling of the single-stranded region upstream the startpoint of RNA synthesis - at least, existence of such a single-stranded region capable to accept productive primers is shown unequivocally. In this connection it would be of great interest to find out whether oligonucleotide primers induce dissociation of the σ-subunit from the ternary complex.

Secondly, the primer activity of oligonucleotides opens some new possibilities of investigation of the topography of the transcription complex. For example, primers may be used to obtain a synchroneous population of complexes shifted to a given position relative to the startpoint of transcription and to study the state of DNA in these "shifted" complexes by means of chemical modification approaches, e.g. those proposed in refs. (8,9) ("footprints"). Such synchroneous populations have been practically unavailable before because of the unstability of ternary complexes at the early steps of unprimed transcription. Finally, new affinity reagents -- derivatives of active primers - may be obtained and used to study the topography of the transcription compex.

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* A minor amount of [5'-^{32}P]pd(TACAAATCGC) was added to the mixture prior to apurinization.

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