

COMPETITION BETWEEN RNA POLYMERASE  
AND DNA POLYMERASE FOR THE DNA TEMPLATE\*

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A gap in our understanding of the mechanism of DNA-directed RNA synthesis, particularly as it occurs in vivo, results from a lack of information about how transcription of the DNA template is initiated and terminated. Are there specific "start-stop" signals, perhaps in the form of unique nucleotide sequences or structural conformations, at which the enzyme binds and from which it dissociates? And if "signals" do exist, what is their nature and how does the enzyme recognize and respond to them?

One approach to this problem is to examine the interaction between RNA polymerase and the DNA template. The earliest information was deduced from kinetic experiments which showed that natural and synthetic polyribonucleotides inhibit DNA transcription in vitro (Krakow and Ochoa, 1963; Tissieres, Bourgeois and Gros, 1963; Fox and Weiss, 1964); DNA was also shown to inhibit transcription from RNA homopolymer templates (Krakow and Ochoa, 1963; Fox, Robinson, Haselkorn and Weiss, 1964). Initially these observations, as well as the findings that single-stranded DNA at low concentrations is a more effective template (Hurwitz, Furth, Anders and Evans, 1962; Chamberlin, 1963; Fox and Weiss, 1964; Stevens and Henry, 1964) and inhibitor (Wood and Berg, 1964) than double-stranded DNA, were interpreted in the context of classical Michaelis-Menten (competitive inhibition) kinetics (Fox et al., 1964; Fox and Weiss,

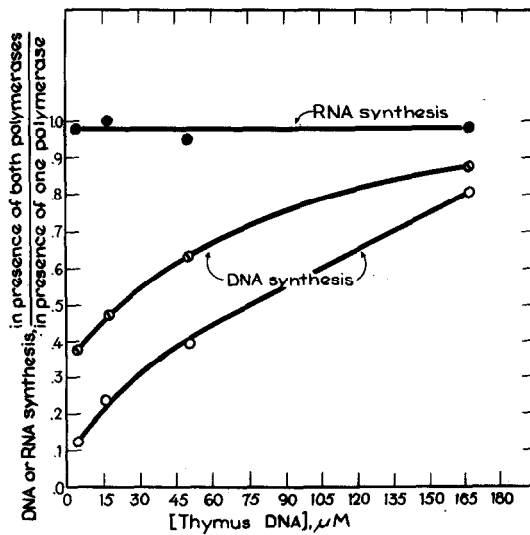
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1964; Wood and Berg, 1964). But the situation is actually more involved: instead of a readily dissociable complex between enzyme and polynucleotide, the interaction of RNA polymerase and DNA template (or RNA) produces a relatively poorly dissociating complex, so that the inhibition is actually "pseudo-competitive" (see note to Wood and Berg, 1964).

Evidence for virtually irreversible associations of RNA polymerase and DNA has been obtained from several types of experiments. Bremer and Konrad (1964) showed that once transcription of a particular DNA molecule was under way, those enzyme molecules engaged in the copying were unable to initiate transcription of another DNA. This conclusion is reinforced by the finding that DNA completely inhibits poly dAT (Berg, unpublished) or poly dC (Chamberlin, private communication) transcription when it is added several minutes before addition of template, but produces little inhibition if it is added several minutes after mixing of enzyme and template. A similar conclusion was also made from studies of the inhibitory properties of RNA (Weiss, 1964). The low dissociation of RNA polymerase from polynucleotides also explains the non-competitive ("high-efficiency") inhibition of DNA-directed polyadenylate synthesis by nucleoside polyphosphates (Chamberlin and Berg, 1964). Indeed, because of the lack of appreciable dissociation of the enzyme from template DNA and from the newly formed RNA, the transcription reaction in vitro proceeds at maximal rate for only a short time with the resulting accumulation of a ternary complex of DNA, RNA polymerase and RNA (Bremer and Konrad, 1964).

The nature of the putative enzyme-DNA complex, in particular its stoichiometry, the sites on the DNA at which enzyme binding occurs, and the interactions which produce such strong binding, is not known. In the present study we show that RNA polymerase binds strongly at or near the ends of double-stranded DNA, preventing DNA polymerase from initiating replication of the same template. Moreover, RNA polymerase, by binding to DNA, inhibits the action of DNA exonuclease I (Lehman, 1960), exonuclease II (Lehman and Richardson, 1964) and exonuclease III (Richardson, Lehman and Kornberg, 1964), but does not affect the activity of micrococcal nuclease (Cunningham, Catlin and de Garilhe, 1956) and E. coli endonuclease I (Lehman, Roussos and Pratt, 1962).



Legend to Figure 1. Inhibition of DNA Synthesis by RNA Polymerase.

The data are expressed as the ratio of initial rates of either RNA or DNA synthesis observed in the presence of the two enzymes, to that found with each enzyme by itself. ●-● RNA synthesis; ○-○ DNA synthesis in the presence of RNA polymerase; ○-○ DNA synthesis in the presence of RNA polymerase and the four ribonucleoside triphosphates.

The reaction mixtures (0.3 ml) contained 20  $\mu\text{moles}$  of Tris buffer, pH 7.4, 2  $\mu\text{moles}$  of  $\text{MgCl}_2$ , 2  $\mu\text{moles}$  of 2-mercaptoethanol, varying quantities of calf thymus DNA (Kay, Simmons and Dounce, 1952) pre-treated with DNase as described by Aposhian and Kornberg (1962). 10  $\mu\text{g}$  of RNA polymerase (Fraction IV (Chamberlin and Berg, 1962)) and 2  $\mu\text{g}$  of DNA polymerase (hydroxylapatite fraction (Richardson *et al.*, 1964)). Where indicated the reaction mixtures contained either 100  $\mu\text{moles}$  each of ATP, GTP, UTP and  $\alpha\text{-P}^{32}\text{CTP}$  ( $5$  to  $10 \times 10^6$  cpm/ $\mu\text{mole}$ ), or 10  $\mu\text{moles}$  each of dATP, dGTP, dCTP or dTTP- $\text{C}^{14}$  ( $4.5 \times 10^6$  cpm/ $\mu\text{mole}$ ) or all of the above eight nucleoside triphosphates. After 20 min. at  $37^\circ$ , 1.5 ml of cold 3.5% perchloric acid (PCA) and 0.5 mg of albumin were added and the precipitate was washed three times with 1.5-ml aliquots of PCA by centrifugation and then dissolved in 1.5 N  $\text{NH}_4\text{OH}$ . An aliquot was added to a toluene-ethanol-POP-POPOP mixture containing Cab-o-sil and the  $\text{P}^{32}$ ,  $\text{C}^{14}$ , or both isotopes were measured in a Tri-Carb scintillation spectrometer.

## RESULTS

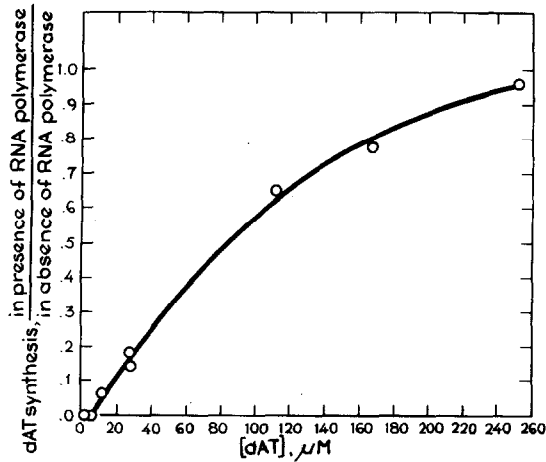
The original plan of the experiment was to determine if, at limiting template concentrations, the concomitant synthesis of RNA (by RNA polymerase (Chamberlin and Berg, 1962)) would affect DNA formation (by DNA polymerase (Richardson, Schildkraut, Aposhian and Kornberg, 1964)). Although at saturating levels of template each enzyme had no influence on the other's activity (Hurwitz *et al.*, 1962), the effect at limiting levels of template is striking.

Figure 1 shows that RNA synthesis is unaffected by the presence of DNA polymerase and the four deoxynucleoside triphosphates. By contrast, DNA synthesis is inhibited strikingly by RNA polymerase at low concentrations of DNA but is inhibited hardly at all at higher DNA levels. The presence of four ribonucleoside triphosphates is not necessary for inhibition by RNA polymerase; in fact the inhibition of DNA synthesis at low primer concentrations is even more pronounced in the absence of the ribonucleoside triphosphates.

RNA polymerase (in the absence of ribonucleoside triphosphates) inhibits the replication of the dAT copolymer by DNA polymerase. As with DNA primers, the inhibition is quite marked at low dAT concentrations but completely reversed at high dAT levels (Figure 2).

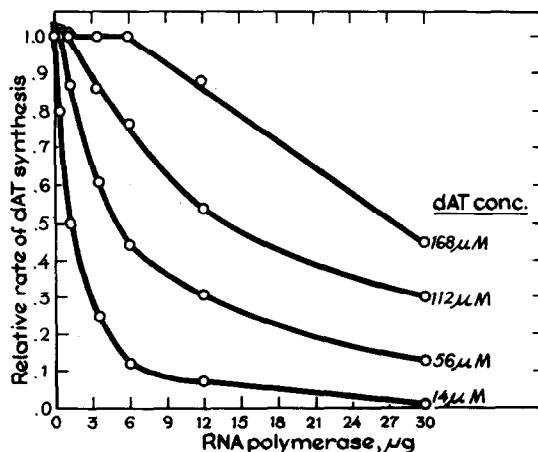
The amount of inhibition of dAT synthesis depends upon the amount of RNA polymerase added and on the dAT template concentration (Figure 3). With any given amount of dAT, increasing quantities of RNA polymerase produce greater inhibition. For example, the rate of dAT synthesis with 6  $\mu\text{g}$  of RNA polymerase and 14  $\mu\text{M}$  of dAT was not appreciably affected by up to a five-fold increase in the level of DNA polymerase.

One explanation for the inhibition of DNA synthesis by RNA polymerase is that RNA polymerase binds strongly to DNA at or near the sites where replication is initiated. As more RNA polymerase is added more template molecules (or replication sites) are blocked; addition of fresh DNA templates would then be expected to provide new sites for initiating DNA synthesis. Since DNA polymerase very likely initiates replication at the ends of helical DNA chains, it seems possible that inhibition by RNA polymerase results from its strong association at or near the ends of the DNA template.



Legend to Figure 2. Inhibition of dAT Replication by RNA Polymerase.

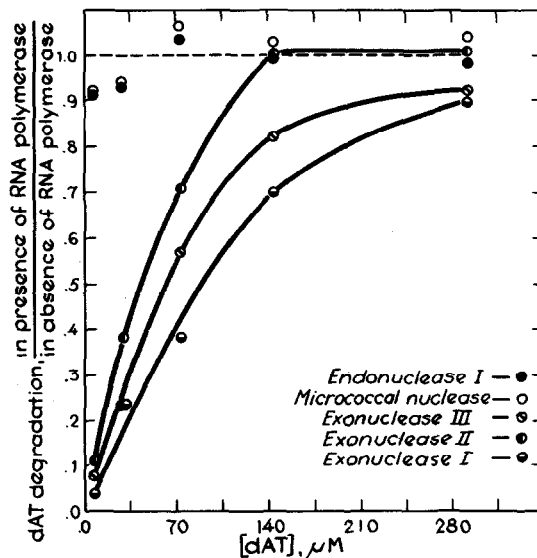
The data are expressed as the ratio of the initial rate of dAT synthesis in the presence of RNA polymerase to that observed in the absence of RNA polymerase. The reaction mixture (0.3 ml) contained 20  $\mu\text{moles}$  of Tris buffer, pH 7.4, 2  $\mu\text{moles}$  of  $\text{MgCl}_2$ , 2  $\mu\text{moles}$  of 2-mercaptoethanol, 10  $\mu\text{moles}$  each of dATP and dTTP- $\text{C}^{14}$ , dAT copolymer (Schachman, Adler, Radding, Lehman and Kornberg, 1960), 10  $\mu\text{g}$  of RNA polymerase (where indicated) and 0.5  $\mu\text{g}$  of DNA polymerase. dAT synthesis was measured as described in Fig. 1.



Legend to Figure 3. Inhibition of dAT Replication at Different Concentrations of RNA Polymerase and dAT.

The conditions of the reaction and method of assay were essentially those described in Figures 1 and 2.

According to this model, other enzymes which initiate their attack on DNA at the 3'-hydroxyl terminus, the end which has been implicated in DNA replication (Richardson, Schildkraut and Kornberg, 1963), should also be inhibited by RNA polymerase at low but not at high levels of DNA. Accordingly, the effects of RNA polymerase on the degradation of dAT by exonuclease I (Lehman, 1960), exonuclease II (Lehman and Richardson, 1964) and exonuclease III (Richardson *et al.*, 1964) and by two endonucleases (micrococcal nuclease (Cunningham *et al.*, 1956) and *E. coli* endonuclease I (Lehman *et al.*, 1962)) were examined. Figure 4 shows that the activity

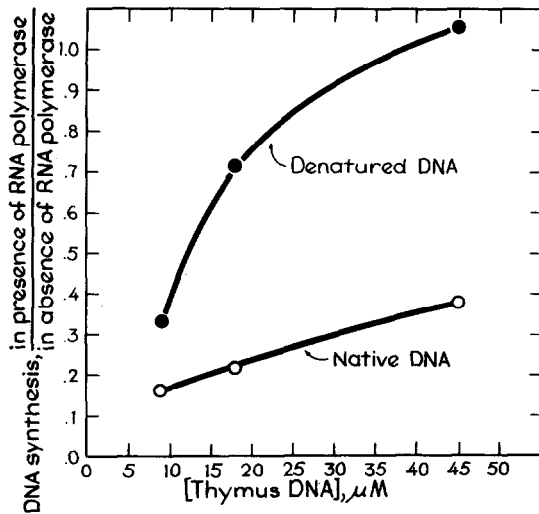


Legend to Figure 4. Effect of RNA Polymerase on Endo- and Exonuclease Action.

The data are expressed as the ratio of the initial rates of each degradative process in the presence of RNA polymerase to the rate in the absence of the polymerase. The reaction mixture (0.3 ml) contained 20  $\mu\text{moles}$  of Tris buffer, pH 7.4, 2  $\mu\text{moles}$  of  $\text{MgCl}_2$ , 2  $\mu\text{moles}$  of 2-mercaptoethanol, variable quantities of  $\text{C}^{14}$ -labeled dAT copolymer ( $4 \times 10^5$  cpm/ $\mu\text{mole}$ ) and an amount of each of the enzymes listed so that not more than 30% of the dAT was converted to an acid-soluble form in 30 min. at  $37^\circ$ . At the end of the incubation 50  $\mu\text{g}$  of calf.thymus DNA and 0.5 ml of cold 3.5% trichloroacetic acid were added. After centrifugation an aliquot of the supernatant fluid was counted in a dioxane-naphthalene-POP-POPOP solvent in the Tri-Carb scintillation spectrometer.

of micrococcal nuclease and endonuclease I is virtually unaffected by RNA polymerase at any level of dAT substrate. But, as predicted by the model, each of the exonucleases is markedly inhibited by RNA polymerase at low levels of dAT and only slightly inhibited at higher dAT concentrations.

It has been stated that RNA polymerase has a greater "affinity" for single-stranded than for double-stranded DNA (Hurwitz *et al.*, 1962; Fox and Weiss, 1964; Stevens and Henry, 1964). But since the enzyme-DNA complex is so poorly dissociable one cannot describe the interaction as an "affinity" in the Michaelis-Menten sense. It seems rather that RNA polymerase finds more combining sites per equivalent of DNA nucleotide in single-stranded than in double-stranded DNA (Wood and Berg, 1964). Support for this view comes from an experiment comparing the ability of increasing concentrations of denatured and native DNA to reverse the inhibition of DNA synthesis by RNA polymerase (Fig. 5). Per nucleotide



Legend to Figure 5. Difference in the Inhibition of DNA Synthesis by RNA Polymerase Using Denatured and Native Calf Thymus DNA as Templates.

The experimental conditions and method of assay were those described in Fig. 1 except that either native calf thymus DNA or a heated sample of calf thymus DNA (heated at a concentration of 75  $\mu\text{g}/\text{ml}$  in 0.01 M Tris, pH 7.4, for 10 min. in boiling water and cooled rapidly) was used.

residue, denatured calf thymus DNA is far more effective in overcoming the inhibition by RNA polymerase than is native calf thymus DNA.

#### DISCUSSION

The present study further supports the notion that RNA polymerase forms a poorly dissociable complex with DNA. Helical DNA to which RNA polymerase is bound is not available as a template for DNA replication or as a substrate for exonuclease action, which we interpret to mean that RNA polymerase binds strongly at or near the 3'-hydroxyl terminus.

It is interesting to note that DNA polymerase, in contrast to RNA polymerase, probably forms a readily dissociable complex. Relatively large amounts of DNA polymerase, with or without the deoxynucleoside triphosphates, do not interfere with RNA polymerase activity at any level of DNA template. Moreover, in experiments which measure inhibition of replication of dAT copolymer by calf thymus DNA (with dATP and dTTP as the only substrates), the same amount of inhibition is observed whether the calf thymus DNA is mixed with the enzyme before, after, or at the same time as the dAT copolymer (Berg, unpublished experiments). As one would predict, even after replication of DNA by DNA polymerase is under way the addition of RNA polymerase to the reaction inhibits further replication (Berg, unpublished).

The fact that there are more binding sites for RNA polymerase in single-stranded than in helical DNA also merits some comment. This conclusion is suggested by studies showing that per unit of DNA nucleotide, single-stranded DNA saturates a given amount of RNA polymerase more effectively than does double-stranded DNA, whether measurements are made of the kinetics of RNA synthesis (Hurwitz *et al.*, 1962; Fox and Weiss, 1964), by inhibition of transcription of dAT copolymer (Wood and Berg, 1963), or as in the present case by neutralization of the inhibitory activity of RNA polymerase. Moreover, the single-stranded DNA requirement for polyadenylate synthesis (Chamberlin and Berg, 1963; Stevens, 1964) and the transcription of the  $\Phi$ X174 DNA ring structure (Bassel, Hayashi and Spiegelman, 1964; Sinsheimer and Lawrence, 1964) indicate that RNA polymerase does not require 3'-hydroxyl ends to initiate complementary copying. Therefore it seems reasonable to suppose that the

enzyme can bind to any unpaired segment of the DNA. The ends of helical DNA molecules are probably "frayed" or unpaired (Zimm, personal communication) and therefore provide sites for binding RNA polymerase.

The fact that RNA polymerase readily binds to dAT or even to homopolymers (Krakow and Ochoa, 1963; Fox and Weiss, 1964; Fox et al., 1964) argues against the need for assuming the existence of a specific sequence as the unique site of enzyme attachment. Rather, we are led to wonder whether in "native" DNA there exist short regions where base-pairing between the two strands is interrupted, thereby providing sites at which RNA polymerase can bind and initiate RNA synthesis. Such interruptions might only be several nucleotides in length and be restricted to regions where methylation or other modifications of the keto-amino functions could destabilize intrastrand hydrophobic interactions between adjacent bases or interstrand hydrogen bonding between paired bases. In this regard it has been shown that d6-methyl AT copolymer is relatively unstable to thermal denaturation but can still direct dAT synthesis with DNA polymerase (Lazarus and Swartz, 1964). Quite clearly further studies are needed to assess the physiologic significance and nature of the strong binding of RNA polymerase to DNA templates.

#### SUMMARY

RNA polymerase reacts with native and denatured DNA to give relatively poorly dissociated complexes. Such complexes are unavailable as templates for replication by DNA polymerase or as substrates for exonucleases I, II, or III, although they are readily degraded by micrococcal nuclease of E. coli endonuclease I. We interpret these findings to mean that RNA polymerase binds strongly at or near the 3'-hydroxyl terminus of helical DNA chains.

#### ACKNOWLEDGMENTS

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