CALF THYMUS RNA POLYMERASES EXHIBIT TEMPLATE SPECIFICITY

M. Gniazdowski, J.L. Mandel Jr., F. Gissinger, C. Kedinger and P. Chambon

Centre de Neurochimie du CNRS and Groupe de Recherche de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France

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SUMMARY. - Calf thymus RNA polymerases A and B are unable to recognize most of the initiation signals which are present in native T_4-DNA. The basis for this specificity lies in the native double-stranded structure of DNA. Addition of the E. coli RNA polymerase σ factor to the calf thymus enzymes does not stimulate transcription of native T_4-DNA.

We have recently solubilized and partially purified two DNA-dependent RNA polymerase activities A and B from calf thymus (CT) (1). Both these enzymes differed from E. coli RNA polymerase in that the inhibitors of the bacterial enzyme, rifampicin (1) and streptolydigin (unpublished results), did not inhibit the calf thymus enzymes. Moreover, the two calf thymus activities differed in that enzyme B was inhibited by α-amanitin, while this compound had no effect upon the activity of enzyme A (1). Since at least part of the transcription specificity seems to be related to the structure of RNA polymerase (2), the question arose as to whether animal RNA polymerases exhibit template specificity. We now present results showing that transcription catalyzed by calf thymus RNA polymerases is severely restricted on T_4 and λ phage DNAs.

MATERIAL AND METHODS

CT-RNA polymerases A and B were the same fractions as those previously described (1). T_4 and λ phage DNAs were purified according to Kayser and Hogness (3). σ factor and PC enzyme (core enzyme) of E. coli RNA polymerase were obtained according to

Burgess et al. (4). Incubation conditions and processing of the samples were as previously described (1) except that in the case of enzyme A Mn^{++} was 3 mM in the incubation medium. Pancreatic RNase-free DNase was purchased from Worthington.

RESULTS

Figures 1 and 2 show that at saturating level native calf thymus DNA (NCT-DNA) was the best template for both enzyme A and enzyme B. With respect to denatured calf thymus DNA (DCT-DNA) enzyme B behaved like bacterial RNA polymerase (5) while enzyme A transcribed it very poorly. This result was not due to the presence of specific DNase for denatured DNA nor to the existence of RNase specific for DNA-RNA hybrid (6) in enzyme preparation A. Na-

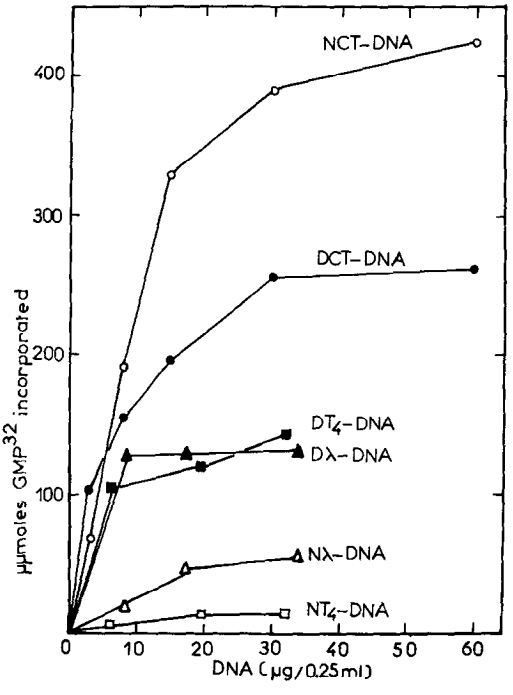

Figure 1. - Synthesis of RNA catalyzed by CT enzyme B on various templates. DNA was denatured by heating (5 min at 100°C) followed by rapid cooling in ice. 64 µg of enzyme B were added per incubation.

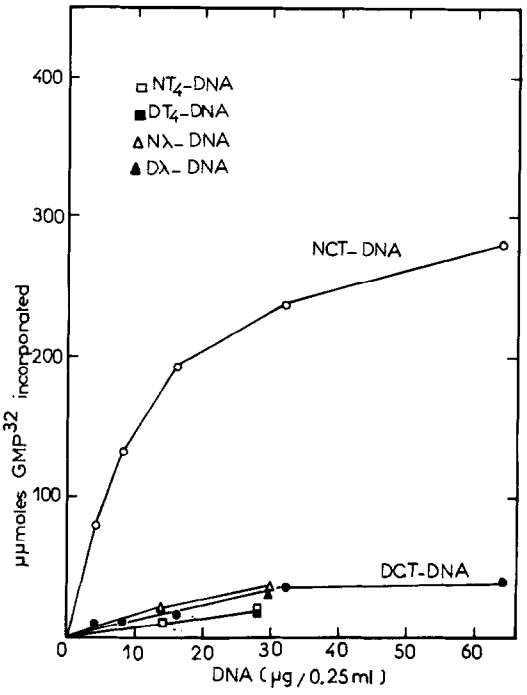

Figure 2. - Synthesis of RNA catalyzed by CT enzyme A on various templates. 75 µg of enzyme A were added per incubation.

tive T_4 or λ DNAs (NT'_4-DNA, Nλ-DNA) were poor templates for enzymes A and B while denatured T_4 or λ DNAs (DT'_4-DNA, Dλ-DNA) were nearly as effective as DCT-DNA. These results clearly indicate that the basis for restriction of transcription on phage DNA is related to native double-stranded structure.

The inability of enzymes A and B to transcribe phage DNAs could be due either to their inability to bind to phage DNAs or to initiate transcription. We investigated these points in the case of T_4 DNA. The affinity of enzymes A and B for NT'_4-DNA must be much lower than for NCT-DNA since RNA synthesis on CT-DNA was not decreased when NCT-DNA and NT'_4-DNA were added at the same time (table 1). On the other hand inhibition of RNA synthesis on NCT-DNA when enzymes A and B were preincubated with NT'_4-DNA (ta-

Table 1. - Effect of preincubation of CT enzymes with NT_4-DNA on subsequent transcription of NCT-DNA.

Preincubation conditions (2 min at 0°C)	Addition at 2 min	GMP^{32} incorporated (10 min, 37°C) µµmoles
Enzyme A	NTP, NCT-DNA	161
Enzyme A, NT_4-DNA	NTP, NCT-DNA	44
Enzyme A, NT_4-DNA NCT-DNA	NTP	166
Enzyme B	NTP, NCT-DNA	446
Enzyme B, NT_4-DNA	NTP, NCT-DNA	100
Enzyme B, NT_4-DNA NCT-DNA	NTP	425

NTP = the four nucleoside triphosphates.

Composition of preincubation mixture was identical to that of the incubation medium except for components listed in the table. 42 µg of NT_4-DNA, 32 µg of NCT-DNA, 55 µg of CT enzyme A and 64 µg of CT enzyme B were added as indicated.

ble 1) indicates that enzymes A and B can bind to NT_4-DNA even in 2 min at 0°. Inhibition was not increased when the preincubation time was 4 min. Since RNA synthesis was depressed when NCT-DNA was added after the preincubation period one must assume either that enzyme molecules bound to NT_4-DNA cannot initiate RNA synthesis, or that chain elongation was abortive or proceeded at a very low rate on NT_4-DNA.

Results presented in figure 3 suggest that initiation was restricted on NT_4-DNA since preincubation of NT_4-DNA with pancreatic DNase resulted in a several fold increase in RNA synthesis. Under the same conditions, template efficiency of NCT-DNA was not greatly modified. Direct measurements of chain initiation by incorporation of γ-^{32}P-GTP (results not shown) confirmed that ini-

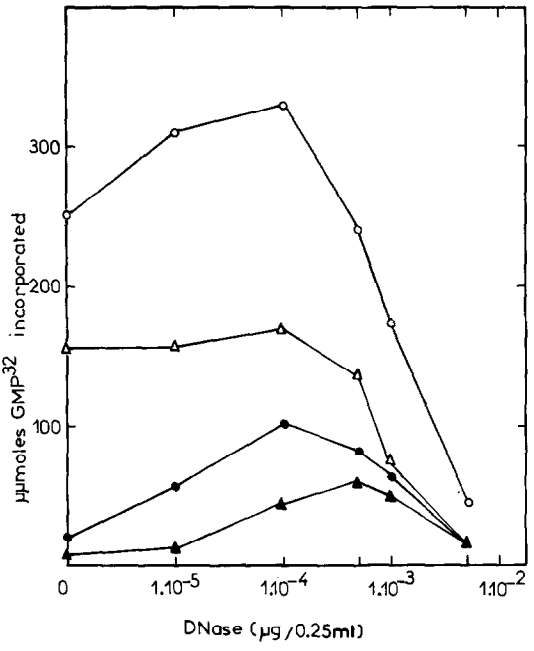

Figure 3. - Effect of pre-treatment of DNA by DNase on RNA synthesis catalyzed by CT enzymes A and B. Incubation mixtures were as described in Material and Methods except that $MgCl_2$ 5 mM was present during preincubation (20 min at 37°C). Nucleoside triphosphates and CT enzymes were added at the end of the preincubation period.
Enzyme A (75 µg) : NCT-DNA - Δ - ; NT_4-DNA - ▲ -
Enzyme B (64 µg) : NCT-DNA - O - ; NT_4-DNA - ● -

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tiation was restricted on NT_4-DNA, since it was not possible in the presence of this template to demonstrate any significative incorporation of label using either the acid precipitation method or the direct filtration technique (7).

Since initiation of transcription on NT_4-DNA by E. coli PC enzyme requires the addition of σ factor (8), we added σ factor to CT RNA polymerases. Table 2 shows that σ factor did not stimulate NT_4-DNA transcription by RNA polymerases A and B neither under conditions which were optimal for stimulating effect on PC enzyme (medium I) nor under conditions which were optimal for CT enzyme activity (medium II). Intermediate incubation conditions did not

Table 2. - Effect of E. coli σ factor on transcription of NT_4-DNA by CT enzymes A and B.

Enzyme or factor	DNA	GMP^{32} incorporated (10 min, 37°C) μμmoles	
		Incubation medium I	Incubation medium II
PC enzyme	NT_4	23	76
σ	NT_4	2	0
PC enzyme + σ	NT_4	210	114
Enzyme A	NT_4	2	17
Enzyme A + σ	NT_4	0	12
Enzyme A	NCT	70	162
Enzyme B	NT_4	3	34
Enzyme B + σ	NT_4	0	28
Enzyme B	NCT	70	437

Medium I was as described by Burgess et al. (4) and Medium II as described in Material and Methods. 28 μg of NT_4-DNA, 35 μg of NCT-DNA, 0.8 μg of PC enzyme, 2 μg of σ factor, 55 μg of enzyme A and 64 μg of enzyme B were added as indicated.

result in further stimulation. It is incidentally interesting to note that PC enzyme was quite efficient in NT_4-DNA transcription in a medium of relatively low ionic strength containing Mn^{++} and that the stimulating effect of σ factor was observed only in the presence of Mg^{++} at higher ionic strength.

DISCUSSION

Our results suggest very strongly that most of the signals encoded in NT_4-DNA and specifying start of transcription are not recognized by CT RNA polymerases A and B and that the basis for this specificity lies in the native double-stranded structure. Therefore it is not unlikely that template specificity of animal

RNA polymerases could play a fundamental role in regulation of genetic expression *in vivo*.

Enzymes A and B can bind to NT_4-DNA although their affinity (which is at least in part related to the number of binding sites per unit of DNA) for NCT-DNA is much higher. However, initiation of RNA synthesis catalyzed by enzymes A and B is severely restricted on NT_4-DNA. The correlation between induced lesions on NT_4-DNA and level of RNA synthesis suggests that, as for bacterial "core enzyme" (9), a single-strand nick or single-stranded regions in double-stranded DNA may be able to act as "artificial" initiation signal for transcription. Since even NCT-DNA contains single strand nicks, it is possible that in part its high template efficiency is related to the occurrence of single-strand nicks. It is, however, unlikely that initiation on NCT-DNA was only related to random single-strand nicks or single-stranded regions, since RNA synthesis was always much higher on CT-DNA than on DNase-treated T_4 DNA.

Besides being an intrinsic property of enzymes A and B the residual synthesis of RNA on phage DNAs could be either related to the presence of traces of DNase in our enzyme preparations or to single-strand nicks in our phage DNA preparations. We were unable to find any DNase activity in enzymes A and B looking at formation of single-strand nicks in labelled Nλ-DNA. Few single-strand nicks were present in our phage DNA preparations and could account for some of the residual RNA synthesis. Characterization of RNA synthetized on NT_4-DNA and Nλ-DNA is presently underway and could help to elucidate the meaning of this residual RNA synthesis on phage DNAs.

In many respects, CT RNA polymerases A and B behave like bacterial "core enzyme" which can also bind to T_4 phage DNA (10), but

does not initiate RNA synthesis unless σ factor is added (8). The fact that σ factor cannot stimulate NT_4-DNA transcription by enzymes A and B, could be due either to structural and functional incompatibilities between σ factor and enzymes A and B or to the preexistence in these enzymes of an animal σ-like factor which could prevent bacterial σ to bind. Further purification of animal enzymes and structural studies are in progress to elucidate this point.

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