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SIMILAR BINDING SITE FOR P_{37} FACTOR ON YEAST RNA POLYMERASES A AND B.

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SUMMARY : Yeast P_{37} protein, which stimulates transcription of native DNA by binding to RNA polymerase B, was assayed with RNA polymerases from different origins. The factor is effective on the activity of the homologous RNA polymerases A and B. Antibodies raised against the isolated subunits of RNA polymerase A were used as a probe to investigate which of the subunits are involved in the interaction with the factor. The results indicate that P_{37} interacts with both RNA polymerase A and RNA polymerase B, at the level of the 23,000 daltons subunit, which is one of the three subunits common to both enzymes.

INTRODUCTION

We have previously described a yeast protein, called P_{37}, which binds to the homologous RNA polymerase B and promotes the efficient transcription of double-stranded DNA (1). The purpose of this work is to investigate, in the first place, the effect of P_{37} on other yeast and eucaryotic transcription systems, as an approach to its physiological significance. P_{37} was found to stimulate specifically RNA polymerases A and B from yeast cells. To explain this dual effect of P_{37}, antibodies raised against each of the individual subunits of RNA polymerase A were used to investigate which of the enzyme subunits are involved in the interaction with the factor.

MATERIAL AND METHODS

RNA polymerases : Yeast RNA polymerase A was prepared as previously described (2). After the glycerol gradient centrifugation step, the enzyme was submitted to an additional chromatography on QAE-Sephadex. The enzyme (4mg) was applied to a QAE-Sephadex A25 column (0.9x10 cm) previously equilibrated with a buffer containing 0.02 M Tris-HCl pH8, 0.5 mM EDTA, 0.01 M 2-mercaptoethanol, 25 mM ammonium sulfate and 10% glycerol. Elution was performed with a 50 ml linear gradient of 25 to 500 mM ammonium sulfate. RNA polymerase A, which eluted as a single peak of constant specific activity between 90 and 160 mM salt, was recovered with 90% yield and was entirely free from contaminating nuclease activity. RNA polymerase A was assayed at 30°C in 0.1 ml mixtures containing 70 mM Tris-HCl pH8, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 1 mM dithio-

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threitol, 1 mM each of ATP, GTP and CTP, 0,5 mM (^{3}H) UTP (20 cpm/pmol), and 50 µg/ml of native calf-thymus DNA or 25 µg/ml of denatured DNA.

Yeast RNA polymerase B (1) and E. coli RNA polymerase (3) were purified and assayed as previously described.

Stimulating factor : P_{37} was purified as previously described (1), except that the last chromatographic step on phosphocellulose column was omitted. This omission had no noticeable consequence on the properties of the stimulating factor. At that stage, P_{37} was estimated to be 30% pure.

Effect of specific antibodies on RNA polymerase activity : The specific antibodies directed against the isolated subunits of yeast RNA polymerase A have been described elsewhere (4). The purified IgG were added, at the indicated concentration, to 1 µg of RNA polymerase A or 1.5 µg of RNA polymerase B in 50 µl of a buffer containing 50 mM Tris-HCl pH 7.5, 2 mM NaCl and 0.1 mM EDTA. The final IgG concentration was kept constant using control IgG from a normal rabbit. The mixtures were preincubated for 30 min at 30° C, then the residual enzyme activity was measured, in a final volume of 100 µl, by a further 20 min incubation at 30° C under the standard assay conditions (4). When measuring the effect of P_{37} on inhibition by the antibodies, the mixture of RNA polymerase and factor (0.6 µg of P_{37}) was first allowed to stand for 5 min at 30° C before addition of the antibodies.

RESULTS

Figure 1 shows the effect of P_{37} on the transcription of native calf-thymus DNA by different RNA polymerases. Yeast RNA polymerases A and B were both stimulated by the factor. In contrast, the factor did not modify in any way the transcription by E. coli RNA polymerase, holoenzyme or core enzyme. The activity of wheat-germ RNA polymerase B was not affected either, and remained very low. A negligible stimulation was observed with calf-thymus RNA polymerase B.

These results demonstrated that P_{37} specifically stimulated homologous RNA polymerases. Since it was previously shown that the factor formed a complex with RNA polymerases B, it remained to explain how it could also stimulate enzyme A. First we considered the possibility that the stimulating factor was heterogeneous, but a number of observations argued against this hypothesis. The stimulating activity displayed the same chromatographic properties when assayed with both enzymes A and B. Furthermore, when a mixture of RNA polymerase B and P_{37} factor was submitted to a glycerol gradient centrifugation, the factor cosedimented with the enzyme (1), and concomitantly the fractions on top of the gradient lost their stimulating properties on

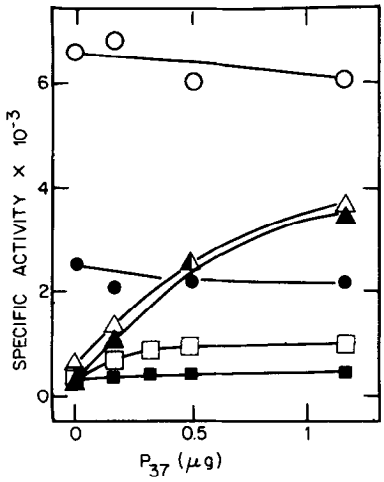

Figure 1 : Effect of P_{37} on the activity of RNA polymerases from different origins.
Increasing amounts of P_{37} (assumed to be 30% pure) were added to the reaction mixtures (100 µl) containing in each case 0.5 to 0.6 µg of RNA polymerase and 5 µg of endonuclease S_1 treated calf-thymus DNA. RNA synthesis was measured after 15 min incubation, and specific activities expressed in nanomoles of UMP incorporated into RNA in one hour per mg of RNA polymerase. Yeast RNA polymerase A (Δ-Δ) and B (▲-▲), and E. coli RNA polymerase, core enzyme (●-●) or holoenzyme (O-O), were incubated under standard conditions described under Material and Methods. For calf-thymus RNA polymerase B (□-□) and wheat-germ RNA polymerase B (■-■), the conditions were those used for yeast RNA polymerase B, except for the incubation temperature which was respectively 37° C and 25° C.

enzymes A and B (results not shown). Therefore P_{37} appears as a cofactor of RNA polymerases A and B. Kinetic studies indicated that the dissociation constant of P_{37} for RNA polymerase A was 2 to 5 times greater than the 5.10^{-8} M value calculated for RNA polymerase B (1). This weaker affinity did not allow us to isolate the RNA polymerase A - P_{37} complex as in the case of RNA polymerase B.

If one makes the assumption that P_{37} acts on RNA polymerases A and B in a similar way, this implies that the two enzymes have a similar binding site for the factor. We attempted therefore to identify the enzyme site involved in the interaction with the factor. The idea was that a class of antigenic determinants would be masked in the enzyme-factor complex. Using antibodies directed against isolated subunits, one should be able to identify the one(s) interacting with the factor. Therefore, RNA polymerase and factor

were mixed, then preincubated with the specific antibodies for a limited period of time. The residual enzyme activity was then measured under the standard assay conditions, the template being chosen so that the extent of transcription was independent of the presence of P_{37}. Under these conditions, any difference in the residual enzyme activity would reflect only the effect of P_{37} on the antibody binding. Table 1 summarizes the results obtained with the antibodies having a strong inhibitory effect on RNA polymerase A activity (4). The presence of P_{37} had no effect on the inhibition, except with the antibodies raised against the A_{23} subunit and, to a lesser extent, those directed against the A_{135} subunit. Hence, with anti-A_{23} antibodies, enzyme inhibition decreased, in the presence of P_{37}, from 31% to 9% in one case, and from 79% to 50% in the other (Table 1). As shown in figure 2, the protection by P_{37} decreased when the preincubation time with the anti-A_{23} antibodies

Table 1 : Effect of antibodies against isolated subunits of RNA polymerase A on the activity of the RNA polymerase A in the presence and absence of P_{37}.

Purified IgG	(µg)	Residual RNA polymerase activity (per cent)	
		$-P_{37}$	$+P_{37}$
Anti-A_{190}	24 120	80 21	76 23
Anti-A_{135}	110 242	48 33	58 41
Anti-A_{40}	25 62	80 59	85 62
Anti-A_{23}	55 275	69 21	91 50
Anti-A_{19}	88 220	69 53	66 54

The experimental procedure is described under Material and Methods. The template used to estimate the residual RNA polymerase A activity was denatured calf-thymus DNA. The controls, performed in the presence of IgG from a normal rabbit under the same conditions, incorporated into RNA 0.1 and 0.15 nmoles of UMP respectively in the absence and presence of P_{37}.

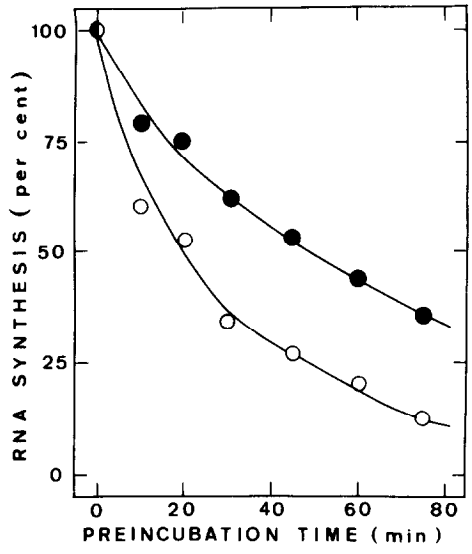

Figure 2 : Effect of P_{37} on the kinetics of RNA polymerase A inhibition by the anti-A_{23} antibodies.
A mixture of RNA polymerase A (5 µg) and purified IgG directed against A_{23} subunit (825 µg) was preincubated at 30° C in a total volume of 300 µl. Aliquots of 40 µl were withdrawn at different times to measure the residual enzyme activity as described in Material and Methods (O-O). The same experiment was performed in the presence of P_{37} (3 µg) (●-●).

increased. This probably reflected a displacement of the bound P_{37} by the antibodies which have a higher affinity for the RNA polymerase.

Interestingly, the 23,000 daltons subunit is one of the three subunits which are common to yeast RNA polymerases A and B (5). Therefore we investigated whether P_{37} also protected enzyme B against the inhibition by anti-A_{23} antibodies. Figure 3 shows that this was indeed the case. The extent of protection was of the same order of magnitude as with RNA polymerase A. The protection effect disappeared when the preincubation was performed at high ionic strength, under conditions where the RNA polymerase B - P_{37} complex is dissociated (1). The binding of P_{37} to the RNA polymerase is thus required to protect the enzyme from antibody action.

DISCUSSION

The yeast P_{37} protein stimulates the homologous RNA polymerases A and B. In both cases, P_{37} delays the inhibitory effect of antibodies directed against the common 23,000 daltons subunit. It is therefore likely that the factor

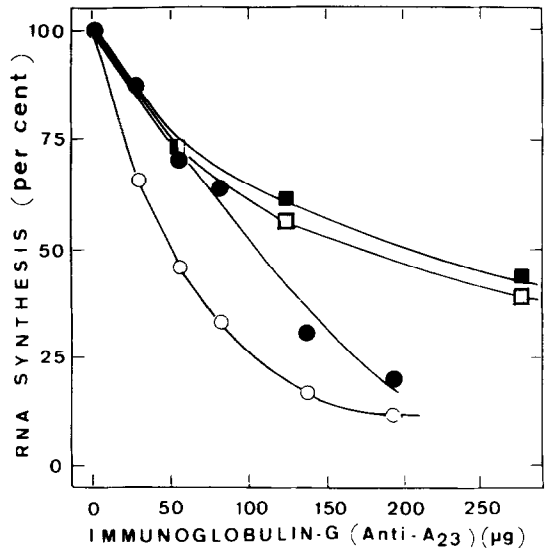

Figure 3 : Effect of P_{37} on the inhibition of RNA polymerase B by the anti-A_{23} antibodies at high and low salt concentration.
The inhibitory effect of increasing amounts of IgG directed against the A_{23} subunit was measured on the RNA polymerase B (O-O) or RNA polymerase B-P_{37} complex (●-●) as described under Material and Methods. $(rC)_n$ was used as template to estimate the residual enzyme activity, because P_{37} does not stimulate the transcription of this ribopolymer. In parallel experiments, the preincubation was performed at high ionic strength (300 mM ammonium sulfate), in the presence (■-■) or absence (□-□) of P_{37}. In this case, the final ammonium sulfate concentration during the transcription assay was 150 mM. The results, which are mean values of two duplicate experiments, are given in percent of control GMP incorporation (using control IgG) which was 11 nmoles and 3 nmoles at low and high salt respectively.

interacts, with both enzymes, at least partly at the level of this subunit. The slight protection afforded by P_{37} against the anti-A_{135} antibodies might well indicate that the subunits A_{23} and A_{135} are located near to each other in RNA polymerase A.

Stimulating factors, which appear to interact with the homologous RNA polymerases B, have also been described in calf-thymus (6), lamb-thymus (7), Ehrlich Ascites Tumor Cells (8) and Physarum polycephalum (9). These factors share with P_{37} similar properties such as molecular weight, basicity and mode of action (the mechanism by which P_{37} stimulates transcription, at the level of chain elongation, will be the subject of another report). Yet, the mammalian factors do not stimulate the homologous RNA polymerase A, which could imply that they belong to a different class of proteins. However, another possibility is that, in mammalian cells, two different factors exist, regu-

lating independently the activity of RNA polymerases A and B; these two proteins could result from the evolution of a single factor existing in lower unicellular eucaryotes like yeast and Physarum polycephalum.

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