STUDY ON YEAST RNA POLYMERASE. EFFECT OF α-AMANITAN AND RIFAMPICIN

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Received 25 February 1970

1. Introduction

It has recently been found by Kedinger et al. [1], Seifart [2], Jacob et al. [3] and Novello et al. [4] that mammalian RNA polymerase is inhibited by α-amanitin, a toxic peptide isolated from *Amanita phalloides*, whereas *E. coli* RNA polymerase is insensitive to this drug. On the other hand, the reverse situation occurs in the presence of rifampicin [5, 6]. To establish further that structural differences in RNA polymerase of prokaryotic and eukaryotic cells exist, we have studied the effect of these drugs on RNA polymerase isolated from yeast. In the present work, it is shown that yeast RNA polymerase is insensitive to rifampicin, but completely inhibited by α-amanitin, although the amount of toxic peptide required is rather high.

Yeast RNA polymerase purified according to Frederick et al. [7], required denatured DNA as template and Mn^{2+} ions as reported by these authors. Its specific activity was 400 nmoles of ^{3}H-UMP incorporated per hr per mg protein. *E. coli* RNA polymerase and γ-^{32}P-GTP were prepared as previously described [8, 9].

2. Results and discussion

2.1. *Influence of rifampicin and α-amanitin on RNA synthesis by yeast and E. coli RNA polymerase*

Fig. 1 shows that yeast enzyme is unaffected by amounts of rifampicin hundred times higher than those required to inhibit to 80 percent the *E. coli* enzyme.

In contrast, as shown in fig. 2, yeast enzyme can be completely inhibited by α-amanitin, whereas the *E. coli* RNA polymerase is virtually unaffected by the highest concentration used.

Assuming that our yeast enzyme preparation is 25 percent pure as inferred from gel electrophoresis, and that its molecular weight is of the order of 500,000 daltons, it appears that complete inhibition requires a ratio of about 500 amanitin molecules per molecule of RNA polymerase, which is two orders of magnitude higher than the amount required to inhibit the mammalian enzyme [1, 2]. This crude calculation indicates that although yeast RNA polymerase behaves, on the whole, like mammalian RNA polymerase, there are definite differences between RNA polymerases from various eukaryotic cells.

2.2. *Influence of α-amanitin on chain initiation and elongation*

The mode of action of α-amanitin on mammalian RNA polymerase is still unknown. There is evidence that the toxic peptide interferes with the elongation of RNA chains [1–4]. It was of interest to investigate its effects on chain initiation and elongation by yeast RNA polymerase. This enzyme, like *E. coli* RNA polymerase, initiates with purine nucleoside triphosphates, and GTP chains are predominant using denatured calf thymus DNA as template [7]. Fig. 3 shows that, although the initiation of chains, as expressed by γ-^{32}P-GTP incorporation, is less affected than total RNA synthesis, most of the inhibition can be accounted for by a decrease in the number of chains initiated. In this experiment, α-amanitin was added before the beginning of the reaction. The kinetics in fig. 4 show that yeast RNA polymerase, in

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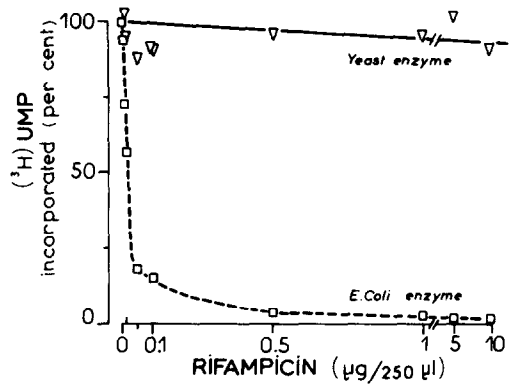

Fig. 1. Resistance of yeast RNA polymerase to rifampicin. The assay mixtures (0.25 ml) contained heat-denatured calf thymus DNA 23 µg/ml, 70 mM tris-HCl buffer pH 7.75, 13 mM β-mercaptoethanol, 4 mM $MnCl_2$, 1 mM CTP, ATP, GTP, 0.2 mM ^{3}H-UTP (1700 cpm/mmole), yeast RNA polymerase 80 µg/ml and rifampicin as indicated in the figure. Incubation was carried out for 10 min at 37° and the RNA recovered by acid precipitation with 5 percent TCA and counted. *E. coli* RNA polymerase (20 µg/ml) was assayed as already described [8] with 13 µg/ml of native calf thymus DNA and nucleoside triphosphates as in the yeast enzyme assay. The results are expressed in percent of ^{3}H-UMP incorporation in the absence of rifampicin which was 1.3 nmoles/10 min in both cases.

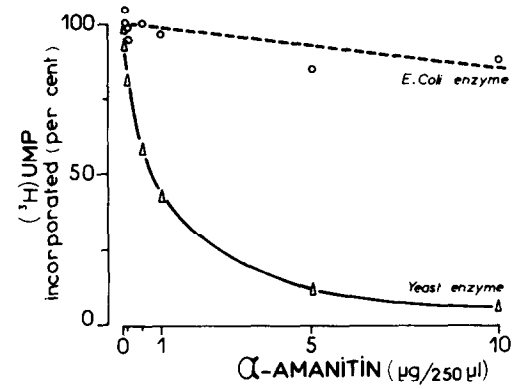

Fig. 2. Inhibition of yeast RNA polymerase by α-amanitin. RNA synthesis by yeast and *E. coli* RNA polymerase was assayed as described in the legend to fig. 1 except that α-amanitin was added in place of rifampicin.

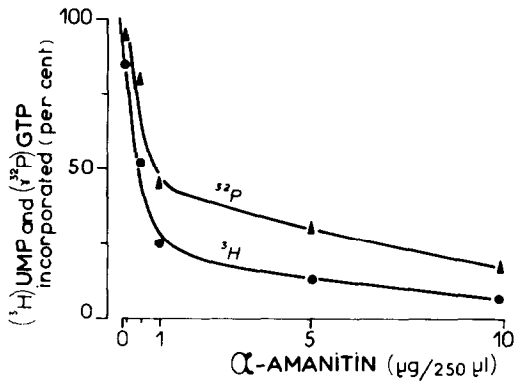

Fig. 3. Effect of α-amanitin on RNA chain initiation and elongation. RNA synthesis by yeast RNA polymerase was assayed as described in fig. 1, except that 0.13 mM γ-^{32}P-GTP (500 cpm/pmole) replaced non-radioactive GTP. RNA polymerase and α-amanitin were mixed and the reaction started by the addition of the nucleoside triphosphates and DNA. RNA was recovered by acid precipitation. Incorporation in the absence of inhibitor was 0.75 nmole ^{3}H-UMP and 5 pmoles γ-^{32}P-GTP in 30 min.

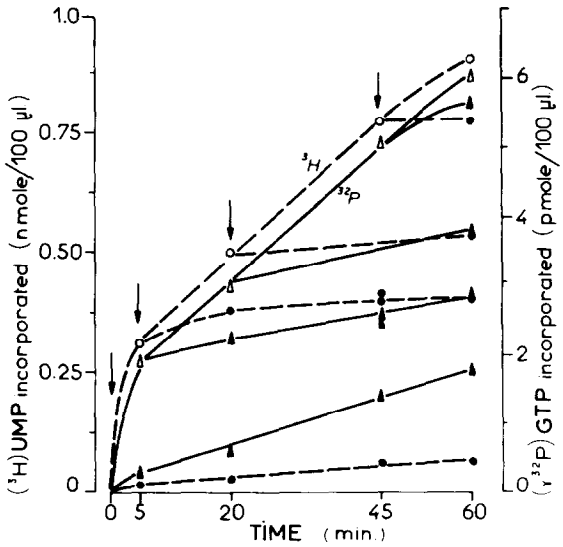

Fig. 4. Kinetics of RNA chain initiation and elongation by yeast RNA polymerase. Effect of the addition of α-amanitin at different times. Assay mixture, scaled up to 3 ml, was as described in fig. 3, except for yeast enzyme (160 µg/ml) and DNA (34 µg/ml). Aliquots were taken at the time indicated by the arrows, α-amanitin (32 µg/ml) added and further incubated. The incorporation of ^{3}H-UMP and γ-^{32}P-GTP, was measured on 100 µl aliquots at different times.

absence of α-amanitin, initiates throughout the 60 min of the incubation. In this respect the yeast enzyme also behaves like *E. coli* RNA polymerase which can repeatedly initiate on single stranded DNA. If α-amanitin is introduced while RNA synthesis is under way, ^{3}H-UMP incorporation is stopped immediately and chain reinitiation is almost completely blocked. Thus, as in the case of mammalian enzyme, α-amanitin inhibits chain propagation, possibly by interfering with the formation of the phosphodiester bond. As a consequence, when added before initiation, the peptide may prevent the formation of the first phosphodiester bonds, thus inhibiting γ-^{32}P-GTP incorporation. Up to now, we found no toxicity of α-amanitin toward growing yeast cells, but it is not known whether the drug can enter the cells.

A general feature of the mammalian RNA polymerases isolated so far seems to be their high efficiency on denatured templates [2, 6, 10]. Our preparation of yeast RNA polymerase is an extreme example of this situation as it is completely unable to use native DNA as template. It could be an incomplete or damaged enzyme, unable to carry out chain initiation on doubled stranded templates. The addition of the *E. coli* initiation factor, σ, [11, 8] does not stimulate the yeast enzyme with T_4 DNA as template. In fact yeast RNA polymerase does not synthesize RNA on native calf thymus DNA. However this DNA is a good template for the basic *E. coli* enzyme devoid of σ factor [11]. It is thus probable that the binding and initiation processes are quite different in these two *in vitro* systems.

Note added in proof

While this paper was written the work of Wintersberger and Wintersberger (FEBS Letters, 6 (1970) 58), came to our attention showing the insensitivity of RNA synthesis in yeast to rifampicin which is confirmed by the results presented in fig. 1 of this paper.

Acknowledgements

We are greatly indebted to Prof. T.Wieland, (Heidelberg), for a gift of α-amanitin, to Prof. L.Silvestri of Lepetit S.A. (Milan) for a sample of rifampicin, and to Prof. J.C. Mounolou (Paris) who performed the assays with yeast cells.

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