YEAST DNA DEPENDENT RNA POLYMERASES I, II AND III. THE EXISTENCE OF SUBUNITS COMMON TO THE THREE ENZYMES

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Received June 30,1976

SUMMARY: The subunits of purified yeast RNA polymerases I, II and III have been analyzed by two-dimensional polyacrylamide gel electrophoretic subunit mapping techniques. The results suggest that polymerases I and III have two subunits in common, the 41,000 and 20,000 dalton peptides, which are not present in polymerase III. The 14,500 dalton peptide by all criteria is identical in polymerases I, II and III. The 28,000 and 24,000 subunits appear identical in polymerases I and II but have different charge properties in polymerase III.

INTRODUCTION

RNA polymerases I, II and III from eucaryotes are distinct enzymatic entities (1,2). Like the procaryotic enzyme each protein is characterized by two large unique subunits, but the eucaryotic counterparts have a much more complex array of smaller subunits (1,2). For example, yeast RNA polymerases I, II and III, as isolated, contain 8 to 13 polypeptides each (2,3). The differences in the molecular size of the various peptides suggest that the three polymerases are composed largely of products of different genes and hence are not interconvertible assemblies. However, the possibility that some of the peptides are very similar or identical has not been ruled out. Three polypeptides (28,000, 24,000, 14,500 daltons) have identical molecular weights in each of the three enzymes (2) and immunological cross-reaction between enzymes I and II has been reported (4). Furthermore, Thonart *et al.* have recently isolated a series of temperature-sensitive yeast mutants which are impaired in RNA synthesis (5). Mutations in any

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of three complementation groups affects the *in vitro* and *in vivo* activity of all three nuclear RNA polymerases. These results suggest the presence of three common subunits required for activity. Buhler *et al.* have already presented evidence suggesting the existence of three common subunits in polymerases I and II (6). We present here complementary studies of all three polymerases. The results suggest that polymerases I and III have two subunits in common which are not present in polymerase II (the 41,000 and 20,000 dalton peptides). In addition, the 14,500 dalton peptide by all criteria is identical in polymerases I, II and III. The 28,000 and 24,000 subunits appear identical in polymerases I and II but have different charge properties in polymerase III. Our results thus support the contention that some subunits are uniquely associated with each enzyme. Others are common between polymerases I and III, and I and II, still others are common between all three polymerases.

MATERIALS AND METHODS

Materials: Reagents, resins and other materials used are described in previous reports (2,3). Polymin P was obtained as a 50% solution in water from Gallard-Schlesinger, New York. A 5% stock solution was prepared as described by Burgess and Jendrisak (7).

Enzyme purification: Yeast (*Saccharomyces cerevisiae*) RNA polymerase I was purified as described previously (3). After removal of polymerase I by phosphocellulose (3), the extract was precipitated with Polymin P and polymerases II and III extracted with a buffer containing ammonium sulfate. The purification of polymerase II was continued by chromatography in DEAE-cellulose, phosphocellulose and sucrose gradient centrifugation. Polymerase III was purified by successive chromatographic steps in DEAE-cellulose, DEAE-Sephadex and DNA-cellulose. Enzymes I, II and III have specific activities (3) of 250, 450 and 150 units per mg of protein respectively. The detailed procedure for the purification of yeast polymerases II and III will be reported elsewhere (Valenzuela, P., Bell, G., Weinberg, F. & Rutter, W.J., manuscript in preparation). We thank G. Hager for a sample of polymerase II used in the initial phase of this research.

Gel electrophoresis: Sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis in one dimension was carried out by the method of Laemmli (8) as described previously (3). Two-dimensional subunit maps were performed in two systems. System a: 20 µg of each polymerase or a mixture of them were dissolved in 60 µl of a solution containing 8 M urea, 1 M 2-mercaptoethanol and 0.9 N acetic acid, heated in boiling water for 3 min and subjected to electrophoresis in 2.5 M urea - 10% acrylamide slabs as described by Panyim and Chalkley (9). For the second dimension, the corresponding strip of gel was equilibrated by stirring for 2 hr at room temperature in a solution containing 1% SDS, 2% 2-mercaptoethanol and 0.05 M Tris-HCl, pH 6.8 and layered on top of a polymerized 12% acrylamide gel slab containing 0.1% SDS (8). System b: electrophoresis in the first dimension was performed in 8 M urea, pH 8.6,

5% acrylamide gel slabs (11). For the second dimension, the corresponding strip of gel was equilibrated and electrophoresed as described in system a. After electrophoresis in the second dimension the gels were fixed, stained and destained as described previously (3). The position of each subunit spot in the map was determined by comparison with a standard sample of enzyme run parallel to the map in the second dimension.

Preparation of antiserum: Pure polymerase I (1 mg) was mixed with one volume of Freund's complete adjuvant and then injected into the hind toe pads of a rabbit. Two further injections of 1 mg in Freund's incomplete adjuvant were given 4 and 8 weeks later. Blood was collected by heart puncture 14 days after the third injection. The serum was separated from the clotted cells and stored at -20°C.

TABLE 1: Subunit Composition of Yeast RNA Polymerases I, II and III

Polymerase I MW $x10^{-3}$	Polymerase II MW $x10^{-3}$	Polymerase III MW $x10^{-3}$
185	170	160
137	145	128
		82
48		
44		
41		41
36		34
	33.5	
28	28	28
24	24	24
20		20
	18	
14.5	14.5	14.5
12.3	12.5	11

Enzymes were purified as described under Materials and Methods. Molecular weights were calculated from the electrophoretic mobilities ( ) as described previously (3). Polypeptides of 53,000, 40,500 and 37,000 daltons previously reported in polymerase III (2) do not occur in our present preparations and therefore presumably represent contaminants. The molar ratios of each subunit are reported elsewhere (2,3).

RESULTS AND DISCUSSION

The purified nuclear RNA polymerases from yeast have a complex subunit structure (2) (Table 1) and the majority of the subunits have a unique molecular weight. However, as indicated, each enzyme contains certain subunits of identical mass which suggests the existence of common subunits. Hildebrandt et al. (4) and Buhler et al. (6) have reported that yeast nuclear polymerases

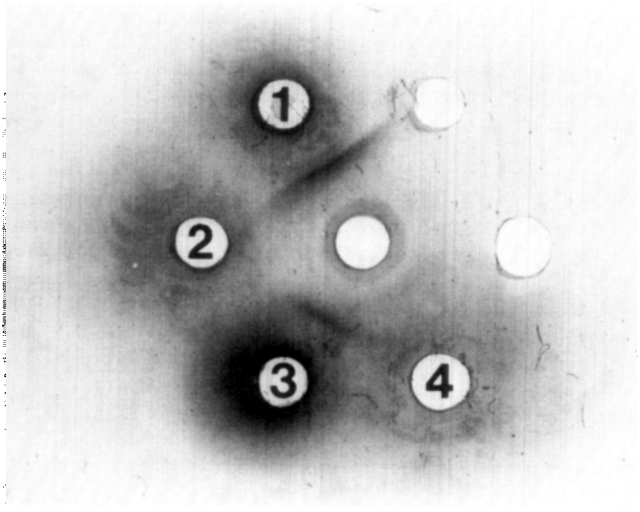

Figure 1. Double diffusion in agar. Immunodiffusion against rabbit antiserum was done at 4°C in 0.6% agar in 0.05 M sodium barbital, pH 8.2 and 0.2% sodium azide. The center well contained 5 µl of anti-polymerase I antiserum. Antigen wells contained: 1.5 µg of polymerase I (1); 2.5 µg of polymerase II (2); 3.5 µg of polymerase III (3); 4.2 µg of E. coli RNA polymerase (4). After formation of precipitin lines, the immunodiffusion plates were washed with 0.2% sodium bicarbonate overnight. The washed plates were then stained with 0.1% Coomassie brilliant blue in methanol:water:acetic acid (5:5:1) and destained in 10% acetic acid.

I and II share common antigenic determinants. Buhler et al. have presented evidence that the antigenic similarity between polymerase I and II is probably due to the presence of common small subunits since antibody prepared against subunit I_{185} did not cross-react with polymerase II (6). We have prepared antibodies to purified polymerase I. This antibody does not cross-react with polymerase II but does cross-react with polymerase III (Fig. 1) which suggests that polymerases I and III share common antigenic determinants which are lacking in polymerase II, in addition to those determinants shared between I and II. This observation suggests that there may be subunits common to polymerases I and III as well as between I and II.

We have also examined the question of common subunits by two-dimensional gel electrophoresis using systems similar to those that have been successfully used to resolve ribosomal proteins (10,12). Except for the two largest sub-

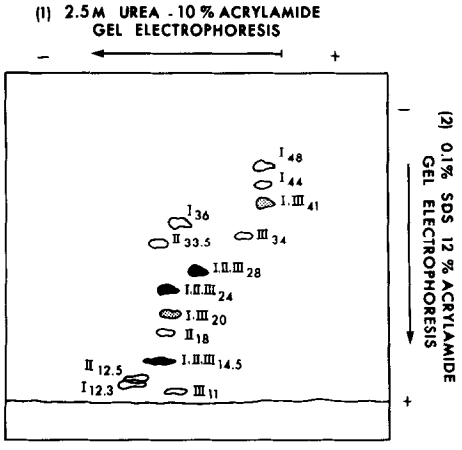

Figure 2. Schematic representation of an acid urea-SDS two-dimensional electrophoretogram of subunits of yeast RNA polymerases I, II and III. Solid spots represent co-migration of subunits of three enzymes; dashed spots represent co-migration of subunits of two enzymes; clear spots represent migration of subunits of an individual enzyme. Details in Materials and Methods.

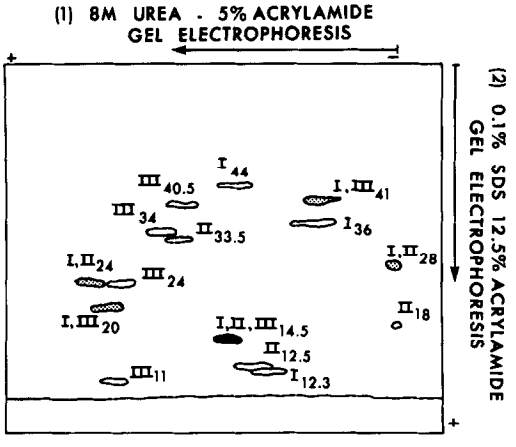

Figure 3. Schematic representation of basic urea-SDS two-dimensional electrophoretogram of subunits of yeast RNA polymerases I, II and III. Meaning of the intensity of the spots as indicated in Figure 2.

units, these systems effectively resolve the subunits of the enzymes. Figure 2 shows a schematic representation of the results obtained for polymerases I, II and III in the acid urea-SDS system. The first dimension, in 2.5 M urea and 0.9 N acetic acid, separates the polypeptides on the basis of the positive

charge density and the second dimension on the basis of molecular weight. The following subunits migrate together in this system: I_{41} and III_{41}; I_{28}, II_{28} and III_{28}; I_{24}, II_{24} and III_{24}; I_{20} and III_{20}; $I_{14.5}$, $II_{14.5}$ and $III_{14.5}$.

The electrophoretic mobility of the subunits was also examined in a second system designed to maximize the separation of the subunits by charge differences in the first dimension. The polypeptides were first separated by 5% acrylamide gel electrophoresis in the presence of 8 M urea, pH 8.8 and then subjected to 12.5% acrylamide gel electrophoresis in the presence of SDS. The results obtained are shown in Figure 3. The following subunits migrate together in this system: I_{41} and III_{41}; I_{28} and II_{28}; I_{24} and II_{24}; I_{20} and III_{20}; $I_{14.5}$, $II_{14.5}$ and $III_{14.5}$. Subunit III_{28} was not detected by this technique, presumably it is slightly more basic than I_{28} and II_{28} and migrates to the cathode in the first dimension. Subunit III_{24} was found slightly less acidic than I_{24} and II_{24}.

The data presented here do not allow a clear identification of the three polymerase genes described by Thonart *et al.* (5). However, there is a very likely possibility that these three genes correspond to subunits 28,000, 24,000 and 14,500 daltons. We have found that the 24,000 dalton peptide is susceptible to phosphorylation by yeast protein kinase (13), so that the difference in charge of III_{24} with respect to I_{24} and II_{24} may be due to a different degree of phosphorylation of an identical protein. Chemical modification (like acetylation or methylation) may also explain the different mobility of III_{28}. The report of Thonart *et al.* (5) also indicates that these three common subunits are required for catalytic activity in all three enzymes. The observation of Valenzuela *et al.* (14) who have isolated yeast polymerase I which lacks the 24,000 dalton subunit and is inactive on native and denatured DNA as well as poly (dA-dT) templates supports the idea of a catalytic role for this subunit in all three polymerases. Bell *et al.* (13) have demonstrated the *in vivo* and *in vitro* phosphorylation of the 24,000 dalton subunit of yeast polymerases I, II and III (also the 20,000 dalton subunit of polymerases I and III).

Their observations also support the hypothesis that the 24,000 dalton subunit is common to the three enzymes (and the 20,000 dalton subunit common to polymerases I and III). The phosphorylation of these common subunits also suggests that the polymerases may be regulated in the same manner under some circumstances.

Interestingly, mammalian RNA polymerases also have subunits of the same molecular weight: those of 25,000 and 16,500 daltons in calf thymus polymerases I and II and those of 29,000 and 19,000 daltons in polymerases I, II and III of murine plasmacytoma cells (1). All eucaryotic RNA polymerases may have a set of identical subunits which perform functions common to the three polymerases.

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