Structural Studies on Yeast RNA Polymerases

EXISTENCE OF COMMON SUBUNITS IN RNA POLYMERASES A(I) AND B(I)

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The subunits of yeast RNA polymerases A(I) and B(I) were characterized using several techniques. The present studies demonstrate that the A and B enzymes possess three subunits, which are indistinguishable on the basis of molecular weight, isoelectric point, and fingerprint pattern. The three common subunits belong to the small molecular weight components of the enzymes. By polyacrylamide gel electrophoresis with sodium dodecyl sulfate they migrate with apparent molecular weights of 27,000, 23,000, and 14,500, respectively. A two-dimensional subunit mapping technique on polyacrylamide gel was used to separate the subunits according to isoelectric point and molecular weight. The common polypeptides co-migrated on three spots corresponding to isoelectric points of 9.2 (27,000), 4.5 (23,000), and 4.6 (14,500). The fingerprints of the 35S-labeled tryptic peptides of the presumptive common subunits were found to be essentially identical. Finally, the presence of common subunits was supported by the fact that antibodies against pure RNA polymerase A cross-react with and inhibit RNA polymerase B.

Except for the common subunits, it is likely that RNA polymerases A and B are primarily made of distinct gene products for the following reasons. A total of 13 polypeptide chains are present in enzyme A, whereas 10 polypeptides are found in enzyme B. The molecular weight, isoelectric point, and sulfur content of the majority of these polypeptide chains are different in the two enzymes. No similarity was found in the 35S-peptide fingerprint from a number of A and B subunits of slightly different molecular weight. Finally, antibodies against the largest subunit from RNA polymerase A do not cross react with or inhibit RNA polymerase B. The data are discussed in terms of structural organization of eukaryotic RNA polymerases.

In all eukaryotic cells RNA synthesis is catalyzed by three classes of DNA-directed RNA polymerases which can be differentiated on the basis of their α-amanitin sensitivity (1-3) and subunit structure (4). All the nuclear enzymes purified so far from mammalian or lower eukaryotic cells present a striking structural similarity. They are isolated as large multi-protein complexes containing two high molecular weight subunits and several smaller polypeptide chains (5-10). The possibility of a structural relationship between RNA polymerases A and B was first postulated by Chesterton and Butterworth (11) when almost no data existed on the structure of these enzymes. A close comparison of the subunits molecular weight later indicated that the three classes of enzyme, from the same organism, were probably made of genetically distinct proteins. However, the possible existence of a pool of small subunits common to the different classes of nuclear enzymes was suggested by the finding of polypeptide chains having identical molecular weight in mammalian enzymes (4, 6) as well as in yeast RNA polymerase A and B (10). Immunological studies also revealed that at least A and B enzymes shared common antigenic determinants (12, 13).

To better understand the involvement of the different classes of RNA polymerase in specific gene transcription it was therefore of great interest to investigate in more detail the structural relationship between these enzymes. The purpose of this paper is to demonstrate, using several criteria, that yeast RNA polymerases A and B possess three common polypeptide chains of 27,000, 23,000, and 14,500 daltons. A preliminary account of this work has been previously presented (14).

EXPERIMENTAL PROCEDURE

Materials

Enzymes—Yeast RNA polymerases A and B were prepared according to Buhler et al. (10) and DezGe and Sentenac (15). Both enzymes are homogeneous proteins when subjected to polyacrylamide gel electrophoresis under non-denaturing conditions (10, 15). 35S-labeled yeast RNA polymerases A and B were obtained by a small scale adaptation of the previously described purification procedures (10, 15). The specific radioactivity of the enzymes was 50 to 100 cpm/ng of protein. Homogeneous Escherichia coli RNA polymerase was prepared as previously described (16). TPCK-trypsin1 was purchased from Worthington.

1The abbreviation used is: TPCK-trypsin, trypsin treated with L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.
**Common Subunits to Yeast RNA Polymerases A(I) and B(II)**

**Methods**

Polyacrylamide Gel Electrophoresis with Sodium Dodecyl Sulfate—Simultaneous electrophoresis of RNA polymerases A and B was carried out in slab gel (190 x 140 x 1.2 mm) using the buffer system developed by Laemmli (17). Acrylamide concentration was 5% in the upper gel and 13% in the lower gel. Electrophoresis was carried out at 20 mA, constant current, for 4 hours at room temperature. The gel was stained with Coomassie brilliant blue 0.1% (w/v) in methanol/acetic acid/water (15/10/3/12). The radioactive spots were located by autoradiography on a Kodirex x-ray film (Kodak).

Fingerprint Maps—A mixture of 15 µg of unlabeled RNA polymerase B and 1 µg of 35S-labeled RNA polymerase A (50,000 cpm/µg) was dissolved in 6 M urea and subjected to isoelectric focusing in polyacrylamide gel slab containing 6 M urea. The protein bands were subjected to electrophoresis in the second dimension in 13% polyacrylamide gel with sodium dodecyl sulfate (18). The gel was stained with Coomassie brilliant blue to detect enzyme B subunits, whereas the subunit map of enzyme A was revealed by autoradiography. The isoelectric point of the various bands was determined after isoelectric focusing by comparing their migration with the pH gradient. This gradient was obtained by cutting a gel strip next to the focused sample, leaving small gel pieces in water for 12 hours, and measuring the pH of the solutions.

**Subunit Maps**—A mixture of 15 µg of unlabeled RNA polymerase B and 1 µg of 35S-labeled RNA polymerase A (50,000 cpm/µg) was dissolved in 6 M urea and subjected to isoelectric focusing in polyacrylamide gel slab containing 6 M urea. The protein bands were subjected to electrophoresis in the second dimension in 13% polyacrylamide gel with sodium dodecyl sulfate (18). The gel was stained with Coomassie brilliant blue to detect enzyme B subunits, whereas the subunit map of enzyme A was revealed by autoradiography. The isoelectric point of the various bands was determined after isoelectric focusing by comparing their migration with the pH gradient. This gradient was obtained by cutting a gel strip next to the focused sample, leaving small gel pieces in water for 12 hours, and measuring the pH of the solutions.

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**Results**

**Compared Subunit Structure of Yeast RNA Polymerases A and B**—A precise comparison of the molecular structure of RNA polymerases A and B was obtained by simultaneous electrophoresis of the two enzymes on a polyacrylamide gel slab in the presence of sodium dodecyl sulfate (Fig. 1). Due to the high acrylamide concentration and the use of a very long gel, excellent resolution of the small molecular weight components was achieved. As previously reported (19), RNA polymerase A contains two large subunits and several smaller polypeptides. Evidence that all these polypeptide chains are normally associated to the RNA polymerase molecule has already been presented (20). The RNA polymerase B subunit pattern is analogous to that of enzyme A (7, 21), however, the molecular structure of the two enzymes is different. Besides the large polypeptides which are clearly distinguishable, the

Fig. 1. Compared subunit structure of yeast RNA polymerases A (right) and B (left), samples of enzymes were subjected to simultaneous electrophoresis in 12.5% polyacrylamide gel slab in the presence of sodium dodecyl sulfate, as described under "Methods." The subunit molecular weights were determined and are shown in Table 1. Arrows indicate subunits of identical molecular mass (≈ 106).

Second dimension was carried out in butanol/pyridine/acetate acid/water (15/10/3/12). The radioactive spots were located by autoradiography on a Kodirex x-ray film (Kodak).

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Fingerprint Maps—A mixture of 15 µg of unlabeled RNA polymerase B and 1 µg of 35S-labeled RNA polymerase A (50,000 cpm/µg) was dissolved in 6 M urea and subjected to isoelectric focusing in polyacrylamide gel slab containing 6 M urea. The protein bands were subjected to electrophoresis in the second dimension in 13% polyacrylamide gel with sodium dodecyl sulfate (18). The gel was stained with Coomassie brilliant blue to detect enzyme B subunits, whereas the subunit map of enzyme A was revealed by autoradiography. The isoelectric point of the various bands was determined after isoelectric focusing by comparing their migration with the pH gradient. This gradient was obtained by cutting a gel strip next to the focused sample, leaving small gel pieces in water for 12 hours, and measuring the pH of the solutions.

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**Compared Subunit Structure of Yeast RNA Polymerases A and B**—A precise comparison of the molecular structure of RNA polymerases A and B was obtained by simultaneous electrophoresis of the two enzymes on a polyacrylamide gel slab in the presence of sodium dodecyl sulfate (Fig. 1). Due to the high acrylamide concentration and the use of a very long gel, excellent resolution of the small molecular weight components was achieved. As previously reported (19), RNA polymerase A contains two large subunits and several smaller polypeptides. Evidence that all these polypeptide chains are normally associated to the RNA polymerase molecule has already been presented (20). The RNA polymerase B subunit pattern is analogous to that of enzyme A (7, 21), however, the molecular structure of the two enzymes is different. Besides the large polypeptides which are clearly distinguishable, the...
number and identity of smaller components is not the same. A total of 13 polypeptide chains are present in enzyme A (Fig. 1), whereas 10 polypeptides are found in enzyme B. The molecular weight of these components was estimated by simultaneous electrophoresis with protein markers on polyacrylamide gel slab. The results are summarized in the first column of Table I. It is striking that in both RNA polymerase A and B preparations, three small polypeptides have exactly the same migration rate, corresponding to apparent molecular weights of 27,000, 23,000, and 14,500 daltons. This observation suggested the possible existence of a pool of small subunits common to yeast RNA polymerases A and B.

**Fingerprint Maps of Low Molecular Weight Subunits**—To test for the identity of subunits from A and B enzymes, the fingerprints of tryptic peptides of the presumptive common subunits were compared. Because the amount of RNA polymerase available was low, and also because the small subunits represent only 15\% (w/w) of the enzyme, we used 35S-labeled RNA polymerases (20). The radioactive subunits were separated by electrophoresis with sodium dodecyl sulfate, eluted from the gel, and digested with trypsin. 35S-labeled tryptic peptides were separated on thin layer cellulose plates and located by autoradiography (Fig. 2). The fingerprint of peptides from subunits A_{47} and B_{47} are essentially identical, although the relative intensities of the different spots differ from one experiment to another. In the case of the A_{47} and B_{47} subunits, the peptide patterns also show extensive similarities except that additional labeled peptides were found in the B_{47} subunit. These spots probably arise from contaminating polypeptides of similar molecular weight which are found in some enzyme preparations (see below). In addition to the well-defined peptide spots, in all the peptide maps one can observe a radioactive material which migrates rapidly during the chromatographic step. The fraction of radioactivity in this area varied from one experiment to another and might be related to incomplete removal of sodium dodecyl sulfate.

The use of 9 M urea and other slight modifications in the subunit mapping technique have since allowed the recovery of all subunits from RNA polymerases A and B (F. Iborra, unpublished results).

### Table I

| Molecular mass (daltons) | Isoelectric points | 35S content (\% of enzyme) |
|-------------------------|--------------------|---------------------------|
| A                       | B                  | A          | B          | A          | B          |
| 190                     |                    | *          | *          | *          | *          |
| 135                     | 150                | *          | *          | *          | *          |
| 49                      | 44.5               | 9*         | 4.45       | 41         | 167        |
| 43                      | 5.1*               | 74         |          |            |            |
| 40                      | 5.5                | 50         |          |            |            |
| 34.5                    |                    | 61         |          |            |            |
| 27                      | 27                 | 9.15       | 9.15      | 100        | 100        |
| 23                      | 23                 | 4.5        | 4.5       | 58         | 41         |
| 19                      |                    | 4.45       |           | 196        |            |
| 14.5                    | 14.5               | 7.6*       | 4.6       | 117        | 96         |
| 14                      |                    | 5.2        | 5.3       | 240        | 105        |
| 12.2                    |                    | 6.2        |           |            |            |
| 10                      |                    | 10         | *         |            |            |

Fig. 2. Fingerprints of three small subunits from RNA polymerases A and B. 35S-labeled subunits from the two enzymes (27,000, 23,000, and 14,500 daltons) were isolated and submitted to trypsin digestion, and the peptides produced were separated in two dimensions by electrophoresis and chromatography (see under Methods). The autoradiograms of the peptide maps (1-6) shown in the figure correspond to subunits A_{47}, B_{47}, A_{47}, B_{47} and A_{47}, B_{47}, respectively. Directions of electrophoresis and chromatography were as indicated. The origin corresponds to the white dot by the figure number.
Other subunits from enzymes A and B were compared by the same technique and were found to be unrelated. For instance, subunits A, and B, yielded distinct peptide patterns, as did subunits A, and B, (results not shown). This result indicates that the small difference in the molecular weight of these subunits did not arise from limited proteolysis of otherwise identical polypeptide chains. Also, no similarities were found in the fingerprints of subunits A, A, A, and A, B, A, and A, A. The recovery of polypeptides B, A, and A, was too small to allow a comparative study.

Subunit Mapping of RNA Polymerases—The peptide maps presented above concerned only 35S-labeled peptides containing cystinyl or methionyl residues. As these peptides probably represent a small fraction of the total protein, a difference between the postulated common subunits could have remained unnoticed. Alterations of bacterial RNA polymerase subunits by adenylation or phosphorylation have been reported (22-25).

To check for this possibility, a two-dimensional separation technique was devised to compare the isoelectric point of the small subunits (18). The subunits were first separated by electrophoresis on polyacrylamide gel in the presence of 6 M urea. Then, the protein bands were identified, according to molecular weight, by electrophoresis in the second dimension with sodium dodecyl sulfate (Fig. 3). The subunit maps obtained in this way have a remarkable degree of resolution. For instance, several polypeptides of about 14,500 daltons were resolved in both enzymes. Such heterogeneity, however, was not always observed, and only one of these proteins, called A, or B, in Fig. 3, was a constant component of RNA polymerases. Some subunits were not recovered. The large molecular weight subunits did not enter the gel, and the polypeptides of about 10,000 daltons migrated with the front and could not be located with certainty.

The isoelectric points of the different polypeptide chains are listed in Table I. It is clear that subunits of 27,000, 23,000, and 14,500 daltons from both enzymes have the same isoelectric point. Interestingly, the majority of the subunits are acid proteins with the exception of the subunit of 27,000 daltons, which is extremely basic (isolectric point, 9.2).

To firmly establish the identity of the common subunits, a simultaneous subunit map of RNA polymerases A and B was performed, using a mixture of 35S-labeled RNA polymerase A and an excess of unlabeled RNA polymerase B. Therefore, on the same map, RNA polymerase B subunits were located by staining with Coomassie brilliant blue, whereas enzyme A subunits were identified by autoradiography (Fig. 3). Any slight difference in migration could be detected by superposing the two maps. It was found that subunits A, and B, migrated exactly at the same place. The amount of this subunit, which is low in the present experiment, is usually much higher (18). Subunits A, and B, also migrated on the same spot. Finally, as expected from the fingerprint patterns, the 14,500-dalton subunit was identical in the two enzymes.

Immunological Relationship between RNA Polymerases A and B—Yeast nuclear RNA polymerases A(I) and B(II) were found by Hildebrandt et al. (12) to be immunologically related. As these authors did not observe the presence of the small molecular weight subunits in their enzyme preparations (26), it was of interest to confirm their results with well defined RNA polymerase preparations. The effect of antibodies against RNA polymerase A on the activity of yeast RNA polymerases A and B and Escherichia coli RNA polymerase is shown in Fig. 4A. RNA polymerases were preincubated with increasing concentrations of purified γ-globulins and assayed for activity. RNA polymerase A was drastically inhibited by A antibody. RNA polymerase B was also inhibited by the A antibody preparation, although to a lesser degree than enzyme A. In contrast, the activity of bacterial RNA polymerase was unaffected by the A antibody preparation. These results confirm the previous observation of Hildebrandt et al. (12). It is likely that the immunological relationship between the two RNA polymerases stems only from the presence of the common small subunits. Still, the possibility remained that other subunits might share common antigenic determinants as well. This is unlikely, because at least the large subunits of the two enzymes are unrelated, as shown by the following experiment. The largest subunit of RNA polymerase A was purified by gel electrophoresis and used to prepare rabbit antibodies. This antibody preparation selectively inhibited the activity of RNA polymerase A, while the activity of enzyme B and of the bacterial enzyme was not affected (Fig. 4B). By the double immunodiffusion technique, a precipitation line was obtained in the presence of native RNA polymerase A or with the
Common Subunits to Yeast RNA Polymerases A(I) and B(I)

The present studies demonstrate that yeast RNA polymerases A and B possess common subunits on the basis of the following criteria: (a) molecular weight; (b) isoelectric point; (c) 35S-peptide patterns; (d) immunological properties. The three common subunits, of 27,000, 23,000, and 14,500 daltons, belong to the small molecular mass components of the enzymes. They represent about 15% by weight of the RNA polymerase molecule. Interestingly, the 27,000 dalton subunit is very probably represented twice per molecule of RNA polymerase (10). In addition, almost all of the subunits are acid proteins, with isoelectric points around pH 5, this polypeptide is extremely basic with an isoelectric point of 9.2.

The possibility that additional subunits of A and B enzymes are partly identical is not completely excluded. For a number of reasons however, it is more likely that the two enzymes are distinct gene products. Except for the common subunits, the molecular weight, isoelectric point, and sulfur content of the majority of the polypeptide chains are different (Table I). No similarity was found in the 35S-peptide fingerprints from other small subunits of the two enzymes. Finally, antibodies against the largest subunit of enzyme A do not cross-react with or inhibit enzyme B. Therefore, the possibility that RNA polymerases A and B share large common enzymatic building blocks (12), as well as the hypothesis of an interconversion between the various RNA polymerase forms (11), is rather unlikely.

Several questions are raised by the presence of common subunits in yeast RNA polymerases A and B. They are mainly related to the structural organization of eukaryotic RNA polymerases, the function of the different polypeptide chains, and the regulation of biosynthesis of the multiple form of enzymes. From the structural point of view, it is interesting that the common subunits are part of two multiprotein complexes containing different polypeptide chains. The polypeptides, which in the two enzymes, interact with the common subunits have possibly retained the same type of subunit binding site. The results also suggest that, in spite of substantial differences in the subunits, the overall molecular organization of yeast nuclear RNA polymerases is probably the same. A similarity between A and B enzymes was also supported by the finding that RNA polymerase A* , which lacks two subunits, shows a significant α-amanitin sensitivity (20). Each common subunit probably performs the same basic function during the polymerization process by nuclear RNA polymerases. On more general grounds, a functional correspondence is likely to exist between the essential subunits of yeast enzymes. This notion is strongly supported by the elegant reconstitution of intergeneric RNA polymerase hybrids from prokaryotic organisms (27).

One additional question raised by the presence of the same subunits in yeast RNA polymerases A and B concerns the regulation of the biosynthesis of these enzymes. Sebastian et al. found that the activity of the two polymerases vary independently during the cell cycle (28) and as a function of the cell growth rate (29). If these results, which were confirmed by Carter and Dawes (30), reflect a different timing in the biosynthesis of these enzymes, it would be interesting to know how the biosynthesis of different classes of enzymes having common subunits is regulated.

The molecular structure of the yeast enzymes is very much like that of mammalian RNA polymerases, which also appear to have identical subunits, at least on the basis of molecular weight. A and B enzymes from calf thymus contain two subunits of identical charge and molecular mass (25,000 and 16,500 daltons) (6). Indeed, subunits of 25,000 daltons from calf thymus RNA polymerases A and B are indistinguishable by our subunit mapping technique. Class I, II, and III RNA polymerases from murine plasmacytoma cells contain two subunits of identical molecular mass (29,000 and 19,000 daltons), which are also found in class III RNA polymerases from Xenopus laevis (4). It is worth noting that in each case, the molecular weights of the presumptive common subunits from mammalian enzymes lie in the same range as that of the common subunits from yeast RNA polymerases. It is therefore tempting to postulate that all eukaryotic RNA polymerases share a pool of small polypeptide components which probably perform a common and basic function in the polymerization reaction. The nature of this function remains to be elucidated.

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Figure 4. Effect of anti-RNA polymerase A and anti-A150 sera on RNA polymerases A and B activity. A (left), an identical amount (3 µg) of Escherichia coli RNA polymerase or yeast RNA polymerases A or B was preincubated with varying amounts of anti-RNA polymerase A γ-globulins, in 0.1 ml of a buffer solution containing 0.02 M Tris-HCl, pH 8.0, 0.02 M NaCl, 0.1 mM EDTA/5% glycerol (v/v). After 40 min of incubation at 30°, residual activity of RNA polymerase A (0-0) and of E. coli enzyme (A—-A) was assayed using d(A-T)₄ as template (10). The activity of RNA polymerase B (●—●) was assayed with denatured calf thymus DNA (16). B (right), the same experiment was performed using antibodies against A₁₅₀ subunit (the largest subunit of RNA polymerase A). It was checked, in each case, that enzyme activity remained unaffected by preincubation with γ-globulins from a control rabbit and that inhibition was independent of the nature of the template.
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