THE 24,000 DALTON SUBUNIT AND THE ACTIVITY OF YEAST RNA POLYMERASES

Pablo Valenzuela,[1] Graeme I. Bell and William J. Rutter

Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

Received April 27, 1976

SUMMARY: The subunits of RNA polymerase I are partially resolved during density gradient centrifugation. An analysis of the relative subunit composition with respect to specific catalytic activity shows that the molar ratio of the 24,000 dalton subunit directly correlates with polymerase activity. Since this polypeptide is found also in polymerases II and III, it may be required for activity of all yeast nuclear RNA polymerases.

INTRODUCTION

Yeast RNA polymerases I, II and III can be purified as complex structures composed of 10-11 different subunits (1-4). Knowledge of the function of the subunits is necessary to assess the role of RNA polymerases in specific transcription. It is of interest to know which of these polypeptides are strictly required for activity, which of them are regulatory proteins which modulate the activity of a "core" enzyme, and which of these are perhaps chromosomal or other proteins which copurify with the polymerase and may have ancillary functions.

We have previously reported that some of the putative subunits are uniquely associated with individual enzymes; on the other hand, the 28,000, 24,000 and 14,500 dalton subunits seem to be common to the three yeast polymerases (4). The availability of large amounts of pure yeast polymerase I (1) has allowed its use as a paradigm to test the function of its polypeptide components. Previous

[1]Recipient of a NIH International Fellowship; on leave from the Departamento de Biologia Celular, Universidad Catolica, Casilla 114-D, Santiago, Chile.

studies have shown that the isolated polymerase I complex is composed of polypeptides with approximate molecular weights 185,000, 137,000, 48,000, 44,000, 41,000, 36,000, 28,000, 24,000, 20,000, 14,500 and 12,000 (1,2). Some of these polypeptides are more loosely associated with the complex and can be removed during electrophoresis or chromatography in ion-exchange resins. Polypeptides of 48,000 and 36,000 daltons can be removed by acrylamide gel electrophoresis under non-dissociating conditions (1,2). Sentenac and collaborators first showed that an enzyme which lacks these two subunits is less active with native calf thymus DNA but is equally active with poly d(AT) template compared to the complete enzyme (2). Hager *et al.* (4) have purified RNA polymerase I to homogeneity without the 48,000 and 44,000 dalton polypeptides and have shown that the specific activity of this enzyme on native calf thymus template is half that of the complete enzyme. This report presents evidence suggesting that the 24,000 dalton subunit is requisite for yeast RNA polymerase I activity. Since this subunit is also present in polymerases II and III, it may be required for the activity of these enzymes as well.

MATERIALS AND METHODS

Yeast (*Saccharomyces cerevisiae*) RNA polymerase was purified to fraction 4 as previously described (1). Sucrose gradient centrifugation was performed as follows: 2 mg of polymerase I dissolved in 1 ml of buffer (0.05 M Tris-HCl, pH 8.0, 12.5% glycerol, 0.0001 M EDTA, 0.5 M KCl and 0.007 M 2-mercaptoethanol) was layered on a linear 5% to 20% (w/v) sucrose gradient made in the above buffer. The gradients were centrifuged for 28 hr at 40,000 rpm in a Beckman SW-41 rotor. Fractions were collected and assayed for protein concentration (5) and RNA polymerase activity on native and denatured calf thymus DNA and and poly d(AT) templates as previously described (1). Subunit analysis of gradient fractions was carried out in 0.1% SDS-12% acrylamide slab gels using the procedure of Laemmli (6) as described elsewhere (1).

RESULTS AND DISCUSSION

When yeast RNA polymerase I was subjected to sucrose gradient centrifugation under the conditions described above, the specific activity of the purified enzyme was not constant across the enzyme-protein-containing gradient fractions (Figure 1a). The same activity profile was observed using native

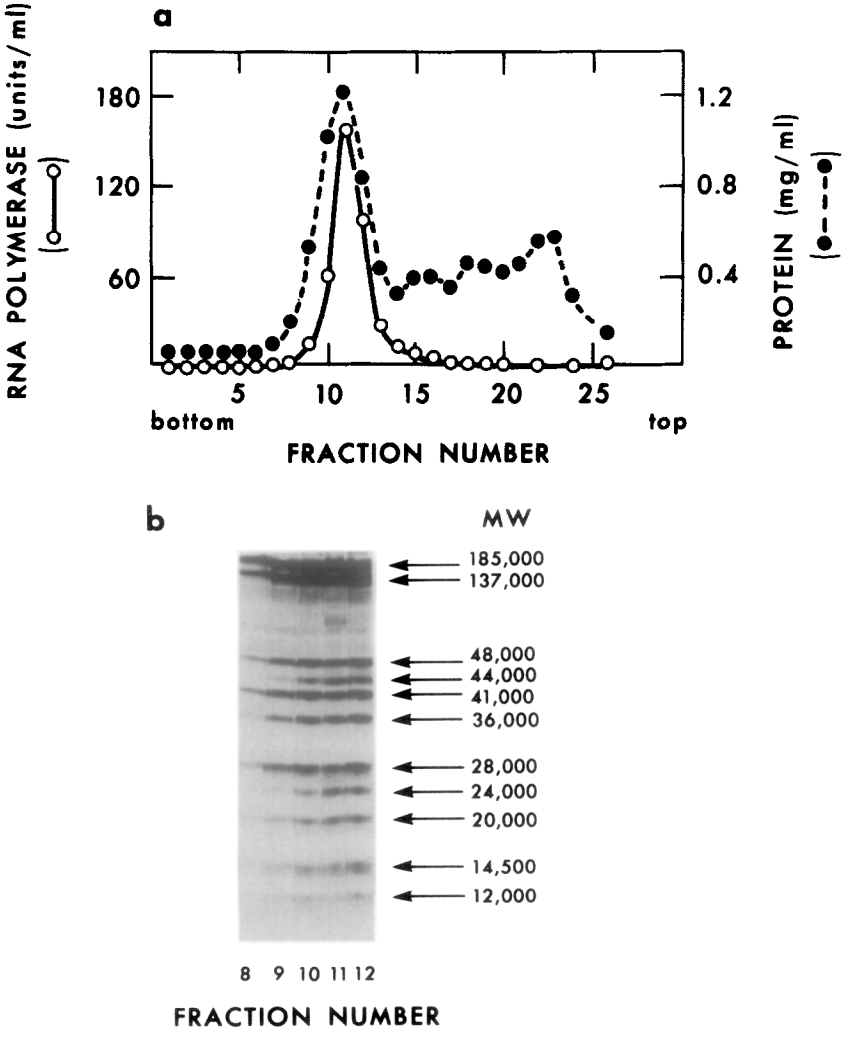

Figure 1. The subunit composition of yeast RNA polymerase I fractions from sucrose gradient centrifugation. (a) Fractions were collected and assayed for protein concentration (5) and enzyme activity on native calf thymus DNA. (b) Subunit analysis of the fractions.

and denatured calf thymus DNA and poly d(AT) templates. The gradient fractions were assayed by SDS gel electrophoresis. As shown in Figure 1b, the 44,000 and 24,000 dalton polypeptides are absent in the first fraction (fraction 8) and increase in relative amounts in the other fractions (9, 10, 11 and 12). The relative molar ratios of the polypeptides of all these fractions were determined by a densitometric analysis of the stained SDS gels. The results are shown in Figure 2. A chart correlating the molar ratio of each putative

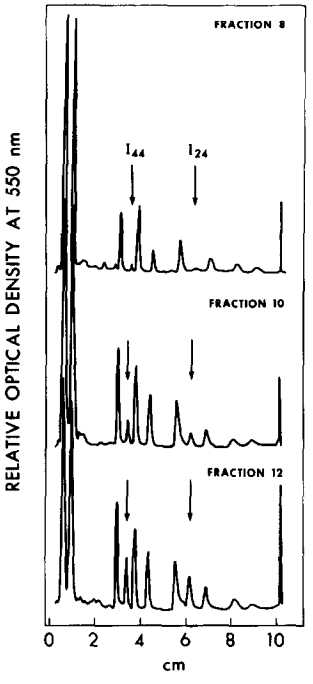

Figure 2. Densitometric tracing of yeast RNA polymerase I. Fractions correspond to those of the sucrose gradient described in Figure 1. Gel strips were scanned at 550 nm with a linear transport device attached to a Gilford spectrophotometer.

subunit (relative to the 137,000 dalton subunit) and the relative specific activity of each fraction of the gradient is shown in Figure 3. This analysis shows that the relative molar ratios of the 44,000 and 24,000 dalton polypep-

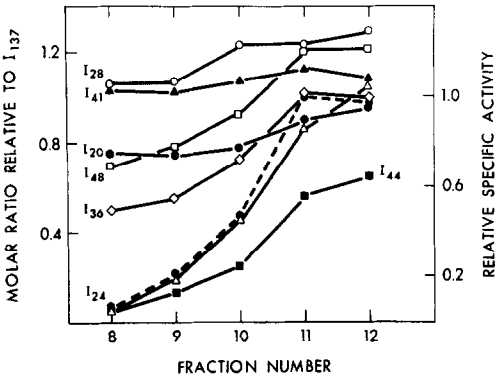

Figure 3. Correlation between the relative molar ratios of the subunits and the relative specific activity of yeast RNA polymerase I. The fractions correspond to those of the sucrose gradient described in Figure 1. Relative specific activity is designated by the dashed line.

tides increase sharply through the gradient fractions corresponding to the observed increase in specific activity of these polymerase fractions (dashed line). This relationship is essentially congruent in the case of the 24,000 dalton subunit. The results also show that the first fractions of the enzyme peak are deficient in the 48,000, 36,000 and 20,000 dalton polypeptides but their relative content does not correlate with the relative specific activity of the enzyme. The 41,000 and 28,000 dalton subunits are present at approximately constant levels in the various fractions.

Since it has been reported that the 48,000, 44,000 and 36,000 dalton polypeptides can be removed without appreciable changes in the activity of enzyme with a poly d(AT) template (2,4), our results suggest that the first fractions are inactive or less active because of the absence of the 24,000 dalton subunit. Therefore this polypeptide may be a basic component of the enzyme structure that is required for catalytic activity. A subunit of the same molecular weight is also present in yeast polymerase II and III (4) and it may also play a crucial role in the activity of these enzymes as well. Of course these experiments do not define the specific role of this polypeptide. It could be directly involved in catalysis, or in some ancillary

obligatory function of the enzyme. We have recently shown that the 24,000 dalton subunits of yeast polymerase I, II and III are phosphorylated by a yeast protein kinase (7) which suggests a possible role of phosphorylation of this polypeptide in the regulation of transcription in yeast. Reconstitution experiments may help to elucidate the function of this polypeptide.

ACKNOWLEDGEMENTS

This research was supported by grant GM21830 from the National Institutes of Health and BMS72-02222 from the National Science Foundation.

REFERENCES

1. Valenzuela, P., Weinberg, F., Bell, G. and Rutter, W.J. (1976). J. Biol. Chem. 251, 1464-1470.
2. Huet, J., Buhler, J.M., Sentenac, A. and Fromageot, P. (1975). Proc. Nat. Acad. Sci. USA 72, 3034-3038.
3. Valenzuela, P., Hager, G., Weinberg, F. and Rutter, W.J. (1976). Proc. Nat. Acad. Sci. USA 73, in press.
4. Hager, G., Holland, M., Valenzuela, P., Weinberg, F. and Rutter, W.J. (1976). in "RNA Polymerase", edited by M. Chamberlin and R. Losick, Cold Spring Harbor Laboratory, in press.
5. Laemmli, U.K. (1970). Nature 227, 680-685.
6. Lowry, O.H., Rosebrough, A.L., Farr, A.C. and Randall, R.J. (1951). J. Biol. Chem. 193, 265-275.
7. Bell, G., Valenzuela, P. and Rutter, W.J. (1976). Nature, in press.