The Human Homologue of Drosophila TRF-proximal Protein Is Associated with an RNA Polymerase II-SRB Complex*

Hua Xiaos, Yong Tao‡, and Robert G. Roeder
From the Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, New York 10021

Mammalian RNA polymerase II holoenzymes are large complexes that have been reported to contain, in addition to RNA polymerase II, homologues of several yeast SRBs, various general transcription factors, and other polypeptides. On the basis of its copurification with an SRB-containing RNA polymerase II complex by conventional chromatography procedures, we have identified a human homologue of Drosophila TRF-proximal protein, designated hTRFP, and isolated its cognate cDNA. Antibody specific for SRB7 can immunoprecipitate hTRFP and RNA polymerase II and, reciprocally, antibody specific for hTRFP can immunoprecipitate RNA polymerase II and SRB7. These data indicate that hTRFP is an integral component of an RNA polymerase II-SRB complex. Whereas the precise function of hTRFP remains to be determined, the hTRFP-containing RNA polymerase II-SRB complex supports basal level transcription and, relative to RNA polymerase II alone, enhances transcriptional activation by Gal4-VP16 in the presence of cofactor PC4. Thus, hTRFP may regulate transcription of class II genes through association with the RNA polymerase II-SRB complex.

Eukaryotic RNA polymerase II holoenzymes have been purified from yeast and mammalian cells by a variety of procedures (reviewed in Refs. 1 and 2). The eukaryotic RNA polymerase II holoenzyme (3) was first isolated from yeast extract as a multicomponent complex containing RNA polymerase II, TFIIB, TFIIF, TFIIFH, and a group of cofactors (SRBs) originally identified by genetic analysis (4). Another yeast holoenzyme lacking all general initiation factors except TFIIA alone, enhances transcriptional activation by Gal4-VP16 in the presence of cofactor PC4. Thus, hTRFP may regulate transcription of class II genes through association with the RNA polymerase II-SRB complex.

* This work was supported by National Institutes of Health Grants AI37327 and CA42567 (to R. G. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AF097725.

† These two authors contributed equally to this work.
‡ Supported in part by a fellowship from the National Institutes of Health.
¶ Present address: DuPont Agricultural Products, Stine-Haskell Research Center, Bldg. 300, Newark, DE 19714.
‡ To whom correspondence should be addressed. Tel.: 212-327-7600; Fax: 212-327-7949; E-mail: roeder@rockvax.rockefeller.edu.

The nucleotide sequence(s) reported in this paper has been submitted with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: CTD, C-terminal domain of the largest subunit of RNA polymerase II; PAGE, polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; EST, expressed sequence tag.
For large scale purification for polypeptide sequence analysis, 100 ml of nuclear extract in BC300 were loaded directly onto an affinity column (2 ml) containing immobilized anti-SRB7 antibody. The column was washed with BC300 and BC1000 and subsequently eluted with 0.1 M glycine buffer (pH 2.5). The eluates were concentrated by trichloroacetic acid precipitation, and proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. The proteins on the membrane were visualized by Ponceau S staining. The 28-kDa polypeptide was excised and digested with endoproteinase Lys-C. Peptides were isolated by high pressure liquid chromatography and subject to amino acid sequence analysis. Derived sequences were used to identify an EST clone that was sequenced by the Rockefeller University core facility.

**Antibody Preparation**—Expression plasmids pRSET-his-tTRFP, pRSET-his-SRB7, and pRSET-his-CDK8 were constructed by inserting the full-length cDNA (amplified by polymerase chain reaction) into the plasmid pREST-6his (33). This created, in each case, an NdeI site at the N terminus and a BamHI (BglII for CDK8) site at the C terminus. His-tagged tTRFP, His-tagged SRB7, and His-tagged CDK8 were used to prepare antibody as described previously (33). All antibody affinity columns were prepared with antigen-purified antibodies as described (33). Anti-RPB6 was provided by Dr. Zhengxin Wang.

**RESULTS AND DISCUSSION**

**Purification of a Human RNA Polymerase II-SRB Complex**—A HeLa nuclear extract was fractionated by conventional chromatographic steps according the scheme in Fig. 1A, with the holoenzyme being monitored by immunoblot with antibodies against RNA polymerase II and the previously described SRB7 component (17, 18) of human holoenzyme. On the phosphocellulose column most of RNA polymerase II and SRB7 were detected in the 0.5 M KCl step fraction. On the subsequent hydroxylapatite column, RNA polymerase II was detected in the 0.2, 0.3, and 1 M phosphate buffer (pH 7.9) fractions and in the 0.5 M phosphate buffer (pH 6) fraction. SRB7 and CDK8 were detected mainly in the 1 M phosphate (pH 7.9) and the 0.5 M phosphate buffer (pH 6) fractions. Because RNA polymerase II and SRB7 were present in a more highly purified state in the 0.5 M phosphate buffer (pH 6) fraction, which contained only about 5% of total input protein, this fraction was subjected to chromatography on a gel filtration column. RNA polymerase II and SRB7 appeared to copurify in fractions corresponding to a size of about 2 MDa. The peak fractions of RNA polymerase II and SRB7 were pooled, and equal portions were subjected to independent immunoprecipitations with antibody specific for SRB7 and with antibody for the RPB6 subunit of RNA polymerase II. Each antibody immunoprecipitated a common population of about 30 polypeptides (Fig. 1B), and the presence of SRB7 and RNA polymerase II in both immunoprecipitates was verified by immunoblot analysis with anti-SRB7 and anti-RPB1 antibodies (data not shown). The fact that each antibody immunoprecipitated most of the major polypeptides in the input gel filtration fraction reflects the high degree of purification (about 400-fold) of SRB7 and RNA polymerase II at this step, whereas the few input proteins that were not precipitated indicate the specificity of the immunoprecipitation. A direct comparison of the anti-SRB7 immunoprecipitate with highly purified core RNA polymerase II revealed several polypeptides specifically immunoprecipitated with anti-SRB7, with the same molecular weight as the subunits of RNA polymerase II (Fig. 1C). As a further control to show that the major group of polypeptides was specifically precipitated by anti-SRB7 antibody, control beads containing only protein A and beads containing protein A and bound anti-SRB7 antibody were used to immunoprecipitate proteins directly from HeLa nuclear extract. SRB7 and RNA polymerase II, as well as the 28-kDa protein (see below), were specifically detected in the anti-SRB7 immunoprecipitates by Western blot analysis and silver staining (data not shown).

**Identification of Human TRF-proximal Protein**—Because the antibody against SRB7 was previously reported to be able to immunoprecipitate the holoenzyme (17), an anti-SRB7 antibody affinity column was used to purify the holoenzyme on a
Polymerase II-SRB complex.

A, Colons TRFP. polypeptide (Fig. 1) was excised from the PVDF membrane. A 28-kDa larger scale. The polypeptides of the holoenzyme were resolved and digested with protease. Three polypeptide sequences, lane 1 with RNA polymerase II. HeLa nuclear extract (Fig. 2) (27). TRF-proximal protein, whose cognate cDNA was found up-

sequence similarity and 44% sequence identity with Drosophila and core RNA polymerase II preparations added to the reaction contained equivalent amounts of RNA polymerase II. Reactions were carried out in the presence or absence of activator Gal4-VP16 and in the presence of PC4 or USA as indicated. Arrows indicate the RNA transcripts from the specific DNA templates.

To further determine whether hTRFP is present in a preassembled protein complex containing RNA polymerase II and SRBs, the hydroxylapatite fraction was subjected to gel filtration on Sephacryl S-500, and derived fractions were immunoblotted with antibodies directed against hTRFP, the RPB1 subunit and SRB7. This result, together with a reciprocal experiment in which hTRFP was immunoprecipitated with anti-SRB7 antibody (as evident from Fig. 1A and from the purification protocol that generated the hTRFP employed for direct sequence analysis (see above)), indicates that hTRFP is associated with RNA polymerase II and SRBs in vivo.

Effect of the hTRFP- and SRB-containing RNA Polymerase II Complex on in Vitro Transcription—Because it was previously shown that SRB-containing yeast holoenzyme and mediator complexes are able to support transactivation (5) (reviewed in Ref. 1), we tested whether the hTRFP-containing RNA polymerase II-SRB complex could support both basal and activated transcription. Because our purified hTRFP- and SRB-containing RNA polymerase II complex appears not to contain any general transcription factors as judged by Western blot (data not shown), an in vitro system reconstituted with ectopic general transcription factors was used to assay transcription. To compare the activity of the hTRFP-containing RNA polymerase II complex with that of the 12-subunit core RNA polymerase II, the amount of the complex assayed was adjusted to contain the same amount of RNA polymerase II as the core RNA polymerase II preparation that was assayed (based on quantitative immunoblot assays with anti-RPB1 antibody; data not shown). As shown in Fig. 4, the assay system has little or no basal transcriptional activity without RNA polymerase II (lanes 1 and 2), and the hTRFP-containing RNA polymerase II-SRB complex can substitute for core RNA polymerase II in effecting basal transcription from a minimal promoter (lanes 3 and 4). This complex could not support transcriptional activation by Gal4-VP16 in the absence of the USA cofactor fraction (28) or the recombinant PC4 (29) (lanes 3 and 4). However, addition of either the USA cofactor fraction (lanes 7 and 8) or PC4 (lanes 5...
and Z. X. Wang for providing anti-RPB6 antibody and core RNA polymerase (lanes 9 and 10) but was not able to support transcriptional activation in the presence of the USA cofactor fraction (lanes 13 and 14). This latter result may reflect the presence in the partially purified USA fraction not only of other co-activators, including PC2 (30), but also substantial amounts of SRB7, CDK8, and hTRF6 (data not shown). Thus, SRB and mediator components in USA may synergize with other endogenous co-activators (including PC4 and PC2), as observed for purified PC4 and holoenzyme components, to effect high level activation in conjunction with core RNA polymerase II. As observed previously (28, 29), the overall increased level of transcription with USA relative to PC4 (lane 8 versus lane 6) reflects the effects of USA components on basal transcription (lanes 7 versus lane 3 and lane 13 versus lane 9).

The present results demonstrate that hTRF6 is a component of an RNA polymerase II-SRB complex that can synergize with coactivators derived from the USA fraction to enhance transcriptional activation. Our results are also consistent with a recent report by Kornberg and colleagues (31) of a mouse cell line derived mediator complex that contains SRBs, MED proteins, and other polypeptides. The amino acid sequence of a peptide derived from the mediator complex that contains SRBs, MED proteins, and other polypeptides. The amino acid sequence of a peptide derived from a holoenzyme complex that contains SRBs, MED proteins, and other polypeptides. The amino acid sequence of a peptide derived from a holoenzyme complex that contains SRBs, MED proteins, and other polypeptides. The amino acid sequence of a peptide derived from a holoenzyme complex that contains SRBs, MED proteins, and other polypeptides.

Acknowledgments—We are grateful to our colleagues for discussions and Z. X. Wang for providing anti-RPB6 antibody and core RNA polymerase II. Peptide sequencing was performed by the Protein Sequencing Facility of The Rockefeller University.

REFERENCES

1. Greenblatt, J. (1997) Curr. Opin. Cell Biol. 3, 310–319
2. Koleske, A. J., and Young, R. A. (1994) Nature 368, 466–469
3. Koleske, A. J., and Young, R. A. (1994) Nature 368, 466–469
4. Nonet, M. L., and Young, R. A. (1989) Genetics 123, 715–724
5. Kim, Y. J., Bjorklund, S., Li, Y., Sayre, M. H., and Kornberg, R. D. (1994) Cell 77, 599–609
6. Li, Y., Bjorklund, S., Jiang, Y. W., Kim, Y. J., Lane, W. S., Stillman, D. J., and Kornberg, R. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10684–10688
7. Gustafsson, C. M., Myers, L. C., Li, Y., Redd, M. J., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (1997) J. Biol. Chem. 272, 48–50
8. Myers, L. C., Gustafsson, C. M., Bushey, D. A., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (1998) Gene Dev. 12, 45–54
9. Song, W., Treich, I., Qian, N., Kuchin, S., and Carlson, M. (1996) Mol. Cell. Biol. 1, 115–120
10. Lee, Y. C., Min, S., Kim, B. S., and Kim, Y. J. (1997) Mol. Cell. Biol. 8, 4622–4632
11. Lee, Y. C., and Kim, Y. J. (1997) Mol. Cell. Biol. 9, 5364–5370
12. Thompson, C. M., Koleske, A. J., Chao, M. D., and Young, R. A. (1993) Cell 73, 1361–1375
13. Thompson, C. M., and Young, R. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4587–4590
14. Lee, T. I., Wyrick, J. J., Koh, S. S., Jennings, E. G., Gadbois, E. L., and Young, R. A. (1992) Cell 70, 883–894
15. Ossipow, V., Tasaan, J. P., Nigg, E. A., and Schübler, U. (1995) Cell 83, 137–146
17. Chao, D. M., Gadbois, E. L., Murray, P. J., Anderson, S. F., Sonu, M. S., Parvin, J. D., and Young, R. A. (1993) Nature 363, 82–85
18. Maldonado, E., Shiekhattar, R., Sheldon, M., Cho, H., Drapkin, R., Rickert, P., Lees, E., Anderson, C. W., Linn, S., and Reinberg, D. (1996) Nature 381, 86–89
21. Nakajima, T., Udvardi, C., Anderson, S. F., Lee, C. G., and Young, R. A. (1997) Nature 385, 557–561
22. Cho, H., Orphanides, G., Sun, X., Yang, X. J., Opyrko, V., Lees, E., Nakatani, Y., and Reinberg, D. (1998) Mol. Cell. Biol. 8, 5355–5363
23. Xiao, H., Tao, Y., Greenblatt, J., and Roeder, R. G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5605–5610
25. Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. R., Kingston, R. E., and Young, R. A. (1996) Cell 84, 235–244
26. Scully, R., Anderson, S. F., Chao, D. M., Wei, W., Ye, L., Young, R. A., Livingston, D. M., and Young, R. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5805–5810
27. Crowley, T. E., Hoey, T., Liu, J. K., Jan, Y. N., Jan, L. Y., and Tjian, R. (1993) Nature 361, 981–993
28. Ge, H., and Roeder, R. G. (1994) Cell 78, 513–523
29. Kretzschmar, M., Stelzer, G., Roeder, R. G., and Meisterernst, M. (1994) Mol. Cell. Biol. 4, 3927–3937
30. Jiang, Y. W., Vescchambera, P., Erdjument-Bromage, H., Tempst, P., Conaway, J. W., Conaway, R. C., and Kornberg, R. D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8538–8543
31. Tao, Y., Guermah, M., Martinez, T., Oeschgeschlager, T., Hasegawa, S., Takada, R., Yamamoto, T., Hirakoshi, M., and Roeder, R. G. (1997) J. Biol. Chem. 272, 6714–6721

2. Y. Tao, W. Gu, H. Xiao, and R. G. Roeder, unpublished observation.
The Human Homologue of *Drosophila* TRF-proximal Protein Is Associated with an RNA Polymerase II-SRB Complex

Hua Xiao, Yong Tao and Robert G. Roeder

*J. Biol. Chem.* 1999, 274:3937-3940.
doi: 10.1074/jbc.274.7.3937

Access the most updated version of this article at [http://www.jbc.org/content/274/7/3937](http://www.jbc.org/content/274/7/3937)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 10 of which can be accessed free at [http://www.jbc.org/content/274/7/3937.full.html#ref-list-1](http://www.jbc.org/content/274/7/3937.full.html#ref-list-1)