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Purification of two transcription factors required for initiation by mammalian RNA polymerase II

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ABSTRACT **We have purified two general transcription factors (FA and FE) necessary for specific transcription by mammalian RNA polymerase II to near homogeneity. Both activities are associated with peptides of ≈33 kDa. FA and FE do not replace one another and show different kinetics of action in a sarkosyl block assay. In particular, FE participated in a rapid reaction after the formation of an initial complex with the other transcription factors. Furthermore, FE can associate with purified calf thymus RNA polymerase II.**

Accurate initiation by mammalian RNA polymerase II *in vitro* is a complex reaction involving multiple factors and is a major regulatory step in many biological phenomena (1–3). Several laboratories, including ours, have identified proteins that are required for proper transcription initiation. Some of these have been partially purified and characterized (4–10). These factors can be separated functionally into two or more groups. An initial, slow reaction occurs between the DNA template, the TATA factor (10–14), and an additional factor, stimulatory factor or FF. The second group of factors, including RNA polymerase II, functions in a subsequent, more rapid reaction (10, 15, 16). One of these factors, FC, has been purified to an equimolar complex of an 80-kDa and a 30-kDa peptide and shown to associate with purified RNA polymerase II (S.K., Y. Tanaka, T.K., T. Nagaoka, S.M.W., and Y.Y. unpublished results).

Here we report the identification of two transcription factors, FA and FE, whose activity is associated with single 32- to 33-kDa peptides. Both have been purified to near homogeneity from HeLa cell nuclear extracts. FA functions in a reaction kinetically similar to that of FC, whereas FE functions in a more rapid subsequent step of transcription initiation.

MATERIALS AND METHODS

Plasmids and Templates. pAd2Δ and pβC2AT are plasmids that contain the adenovirus type 2 major late promoter and the human β-globin gene promoter from −286 to +6 ligated with a cassette that lacks guanosine residues, respectively, as described (10). pAd2MP is a plasmid that contains a minimal promoter sequence of the adenovirus major late promoter gene from −55 to +33 cloned at the *Hin*cII site of pUC19. pAd2MP was digested with *Pvu* II to create a template for a defined transcript of 232 nucleotides. pMyc6514-1 was provided by the Japanese Cancer Research Resource Bank (JCRB) and contains the human c-*myc* gene at the *Sma* I site of pUC18. pMyc6514-1 was digested with *Eco*RI, and the lengths of the transcripts from the two active promoters of the c-*myc* genes were 512 and 350 nucleotides, respectively (17).

***In Vitro* Transcription and RNA Analysis.** *In vitro* transcription activity of FE using pAd2Δ, pβC2AT, pAd2MP, and pMyc6514-1 was assayed in a 30-μl reaction mixture of 10 mM Tris·HCl (pH 7.9), 6 mM $MgCl_2$, 0.1 mM EDTA, 50 mM NaCl, 10% (vol/vol) glycerol, 600 μM CTP, 600 μM GTP, 600 μM ATP, 30 μM [α-^{32}P]UTP (5 μCi; 1 Ci = 37 GBq), and 0.5–1.5 μg of each DNA template, supplemented with purified calf thymus RNA polymerase II and other essential factors as specified in the figure legends. After incubation at 29°C for 60 min, transcripts were purified and analyzed by electrophoresis on 6% polyacrylamide/7 M urea gels as reported (10).

Purification of FE. HeLa cell nuclear extract (7530 mg) was prepared as described (18), and transcription factors FA, FB, FC, FD, FE, and FF were resolved by chromatographic procedures as reported (10). FD and FE (96 mg) were concentrated in an Amicon concentrator, dialyzed against buffer B [20 mM Tris·HCl (pH 7.9), 0.1 mM EDTA, 20% glycerol, 10 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride] containing 0.1 M NaCl, and applied onto a Sephacryl S-300 HR (Pharmacia) column (3.2 × 95 cm) equilibrated with buffer B containing 0.1 M NaCl. FD and FE were eluted at positions corresponding to apparent molecular masses of 600 kDa and 185 kDa, respectively. FE (4.3 mg) from the Sephacryl S-300 HR column was applied onto a DEAE HPLC column (7.6 mm × 100 mm, Asahipak ES-502N; Asahikasei, Kawasaki, Japan) equilibrated with buffer B containing 0.2 M NaCl. After washing with the above buffer, the column was washed at a flow rate of 4.8 ml/hr with a linear gradient of 0.2–0.4 M NaCl in buffer B. The active FE fraction (0.28 mg), which was eluted with a peak of activity at 0.3 M NaCl, was diluted to 0.2 M NaCl by using buffer B and applied again to the DEAE HPLC column equilibrated with buffer B containing 0.2 M NaCl. FE was eluted by a linear gradient of 0.22–0.32 M NaCl. FE (77 μg) was then concentrated to ≈0.4 ml in a Centricon-10 (Amicon) and dialyzed against 100 ml of 20 mM potassium phosphate (pH 6.5), 10 mM EDTA, 4 M guanidine hydrochloride at room temperature for 16 hr. After filtration through a 0.45-μM chromatodisc, FE was applied onto a TSK-250 (Bio-Rad) gel-filtration column equilibrated with the above buffer, and the column was washed at a flow rate of 1.2 ml/hr. Each fraction was renatured by dialysis against buffer B containing 0.1 M NaCl at 4°C for 8 hr. After concentration in a Centricon-10 to ≈100 μl, each fraction was kept frozen at −80°C. FE was apparently stable through several cycles of freezing and thawing.

Purification of FA. Fraction D1P2 (452 mg) was separated from HeLa cell nuclear extract (3744 mg), as described (10),

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Abbreviation: TF, transcription factor.

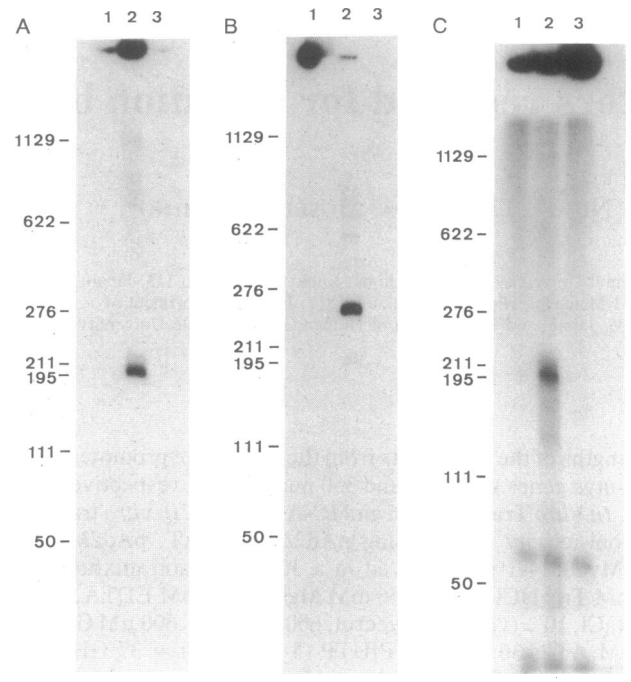

FIG. 1. Requirement of FE for transcription by RNA polymerase II. FE activity with the various DNA templates was assayed by using FE from the DEAE HPLC step as described in *Materials and Methods*. Transcripts were analyzed by electrophoresis on 6% polyacrylamide/7 M urea gels. (*A*) pAd2Δ (1.5 μg) was used as the DNA template. Lanes: 1, no FE; 2, 0.15 μg of FE; 3, 0.15 μg of FE and α-amanitin (an inhibitor of RNA polymerase II) at 0.5 μg/ml. (*B*) pAd2MP (1.8 μg) was used as the DNA template. Lanes: 1, no FE; 2, 0.15 μg of FE; 3, 0.15 μg of FE and α-amanitin at 0.5 μg/ml. (*C*) pβC2AT (2.5 μg) was used as the DNA template. Lanes: 1, no FE; 2, 0.3 μg of FE; 3, 0.3 μg of FE and α-amanitin at 0.5 μg/ml.

and was subjected to five successive chromatographic steps. D1P2 was applied to a sulfopropyl (Toso, Tokyo) column equilibrated with buffer B containing 0.1 M NaCl. After FB passed through the column, bound protein was eluted with a linear gradient of 0.12–0.27 M NaCl in buffer B. FA was detected in fractions eluted with 0.21 M NaCl. The active fractions (82.3 mg) were precipitated by the addition of $(NH_4)_2SO_4$ (0.4 g/ml) and applied to a Sephacryl S-300 HR gel-filtration column equilibrated with buffer B containing 0.1 M NaCl after the precipitate disappeared following dialysis against the equilibration buffer. FA (29.8 mg) from the gel-filtration column was dialyzed against buffer C [10 mM potassium phosphate (pH 6.6), 0.2 mM EDTA, 0.2 mM dithiothreitol, 20% glycerol] containing 0.1 M NaCl and applied to a CM-Toyo (Toso) column equilibrated with the above buffer. Bound protein was eluted with a linear gradient of 0.1–0.3 M NaCl in buffer C. The active fractions (2.0 mg) that were eluted with 0.24 M NaCl were concentrated in a Centricon-10 and applied to a TSK-250 (G3000SW) gel-filtration column (21 mm × 600 mm). The peak activity of FA (0.22 mg) was detectable at an apparent molecular mass of ≈40,000 Da and was directly applied to a CM HPLC column (7.6 mm × 100 mm, Asahipak ES502-C). The FA activity (2 μg) was detected at 0.205 M NaCl when the CM column was developed with a linear gradient of 0.13–0.25 M NaCl in buffer C.

Other Procedures. SDS/PAGE was performed as described by Laemmli (19) and stained by Ag-STAIN from Daiichi (Tokyo). Protein concentration was measured by the dye binding method (20) or absorbency at 280 nm with bovine serum albumin as standard.

Enzymes and Reagents. Endonucleases, T4 ligase, and polynucleotide kinase were purchased from New England Biolabs or Takara Shuzo (Kyoto). [α-^{32}P]UTP was from DuPont/New England Nuclear. RNA polymerase II was purified from calf thymus as described (21). Other materials were of reagent grade.

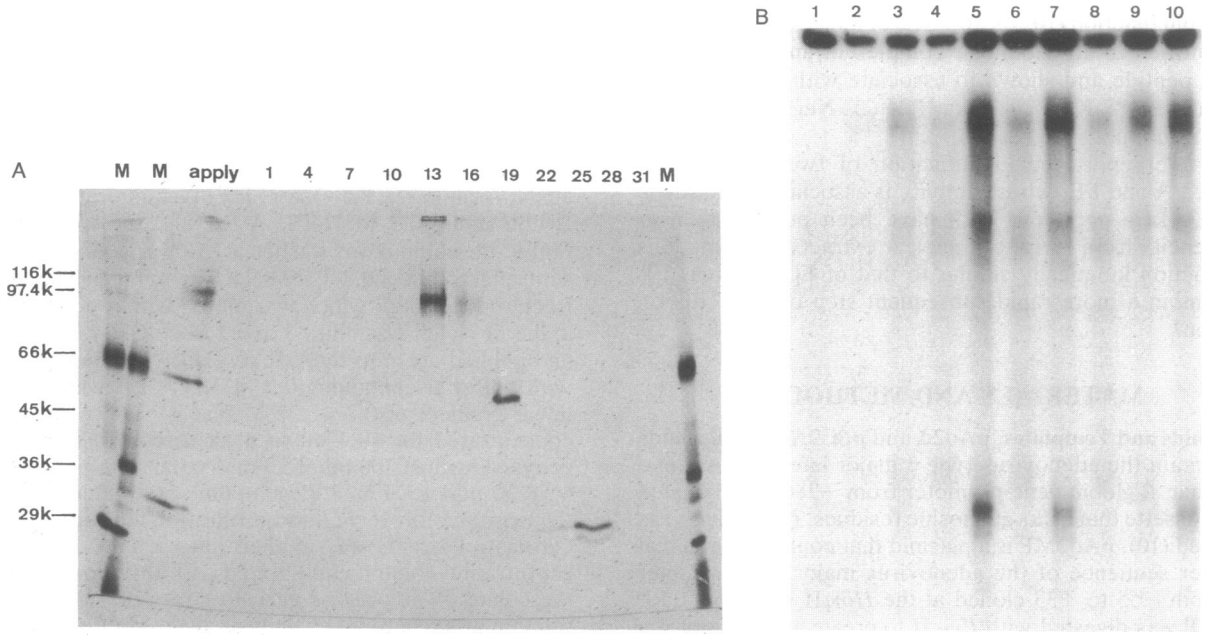

FIG. 2. TSK-250 gel-filtration chromatography of FE in the presence of 4 M guanidine hydrochloride. (*A*) FE from the DEAE HPLC step was denatured by 4 M guanidine hydrochloride and separated on a TSK-250 gel-filtration column as described in *Materials and Methods*. Twenty microliters of each fraction of elution times from 400 min to 700 min was analyzed by SDS/9% PAGE. M, molecular size markers; apply, FE (DEAE HPLC). (*B*) FE activity of the renatured fractions. Pooled fractions I (fractions 12–16), II (fractions 18–20), and III (fractions 22–26) were renatured by removal of guanidine hydrochloride, and their FE complementing activities were assayed as described in *Materials and Methods* in a reaction mixture containing 5.5 μl of I (lane 1); 5.5 μl of I and α-amanitin at 0.5 μg/ml (lane 2); 5.5 μl of II (lane 3); 5.5 μl of II and α-amanitin at 0.5 μg/ml (lane 4); 5.5 μl of III (lane 5); 5.5 μl of III and α-amanitin at 0.5 μg/ml (lane 6); 2.8 μl of II and 2.8 μl of III (lane 7); 2.8 μl of II, 2.8 μl of III, and α-amanitin at 0.5 μg/ml (lane 8); 2.8 μl of I and 2.8 μl of II (lane 9); 2.8 μl of I and 2.8 μl of III (lane 10).

RESULTS

FE Is Required for Promoter-Dependent Transcription of Several Genes. FE is essential for reconstituting *in vitro* transcription activity with the adenovirus major late promoter construct pAd2Δ as DNA template (Fig. 1*A*). To exclude specific interaction of FE with upstream (7, 22–24) or downstream (25, 26) elements of the gene and to assess the general requirement of FE, plasmid pAd2MP was constructed. The plasmid contains the major late promoter sequence from −55 to +33 relative to a cap site encompassing only the TATA sequence as the promoter element and lacking binding sites for any known specific upstream or downstream factors. FE could also act on pAd2MP, and its activity on transcription was comparable to that on the original plasmid, pAd2Δ (Fig. 1*B*). FE was also required for transcription of several other genes examined including human β-globin (Fig. 1*C*) and c-*myc* genes (data not shown). The adenovirus major late promoter gene was the strongest promoter among the genes tested and was used as a DNA template during the purification and characterization of FE.

FE Activity Is Associated with a 33-kDa Polypeptide. The FE active fraction from the Sephacryl S-300 HR gel-filtration column was further purified by two sequential chromatographic steps on a DEAE HPLC column as described in *Materials and Methods*. FE activity was eluted from the second DEAE HPLC column with a peak of protein at 0.30 M NaCl. Each fraction containing FE activity was analyzed by SDS/PAGE followed by silver staining. Comparison of transcription activity with polypeptides of each fraction suggested a possible correlation of FE activity with three major polypeptides of molecular masses of 102 kDa, 60 kDa, and 33 kDa, although the 102-kDa polypeptide apparently was eluted at a higher salt concentration than the FE activity. To separate these polypeptides, gel filtration on an HPLC column in the presence of 4 M guanidine hydrochloride, a chaotropic reagent that disrupts protein–protein interactions, was performed (Fig. 2*A*). Three pooled fractions, I (fractions 12–16), II (fractions 18–20), and III (fractions 22–26), which contained 102-kDa, 60-kDa, and 33-kDa peptides as their major components, respectively, were renatured by dialysis to remove the guanidine hydrochloride and assayed for their FE activity (Fig. 2*B*). Only fraction III was active in reconstituting FE activity. Addition of fraction II to renatured fraction III did not stimulate transcription, and fraction I was inhibitory. These results provided clear evidence that FE activity resides in a fraction whose major component is a 33-kDa polypeptide, and there is no apparent requirement of complex formation among heterologous subunits for reconstituting the *in vitro* transcription activity. The yield of FE activity through the procedure of denaturation and renaturation was about 40%, although it might have been overestimated because the guanidine column separated FE activity from the inhibitory polypeptide, fraction I.

Properties of Purified FE. Incubation of FE at 60°C for various times from 6 min to 30 min caused its activity to decrease gradually from 81% to 45% of the initial level. Thirty-nine percent of the activity was still observed after treatment at 80°C for 6 min. Treatment of the factor with 10 mM *N*-ethylmaleimide (NEM) eliminated 86% of transcription activity, whereas treatment with NEM that had been pretitrated with excess dithiothreitol (50 mM) caused a 16% decrease in activity. Inactivation by NEM suggested the existence of SH group(s) essential for transcription activity, although other groups can also react with this reagent (27). FE did not show any RNA polymerase activity in assays of the nonspecific incorporation of nucleotide with calf thymus DNA as a template. There was no stimulation of RNA synthesis by RNA polymerase II using denatured salmon sperm DNA as a template and $MnCl_2$ as a divalent cation (28), even at 3 times the amount of FE that was needed for optimal specific *in vitro* transcription. The DNA binding properties of FE were examined by both the gel mobility shift assay (29, 30) and DNase I footprinting assays (31). Neither a retarded band nor a protected region was detected under various assay conditions.

FE Is Involved in a Rapid Phase of Initiation of Transcription and Interacts with Purified RNA Polymerase II. The kinetics of action of each transcription factor has been analyzed by a sarkosyl (*N*-lauroylsarcosine) block assay whose principle depends on differential sensitivity of preinitiated and initiated complexes to the detergent sarkosyl (10, 32). The method allowed only one round of reaction of transcription to occur since 0.2% sarkosyl blocked reinitiation of reaction. The transcription activity supported by FE remained essentially constant even when FE was added only 1 min before initiation of transcription (Fig. 3*B*). The activity was totally abolished when FE was added after 0.2% sarkosyl. This indicates that its mode of action is apparently more rapid than FA (Fig. 3*A*), FB, or FC (see figure 3 in ref. 10). When FE that had been incubated with RNA polymerase II was chromatographed on a Sephacryl S-300 HR column, a peak of FE complementing activity was detected coincident with the peak of RNA polymerase II activity (Fig. 4*A*), in addition to some residual activity at fractions corresponding to free FE alone (Fig. 4 *B* and *C*). This complex was not observed with HeLa RNA polymerase I or III under the same conditions (data not shown).

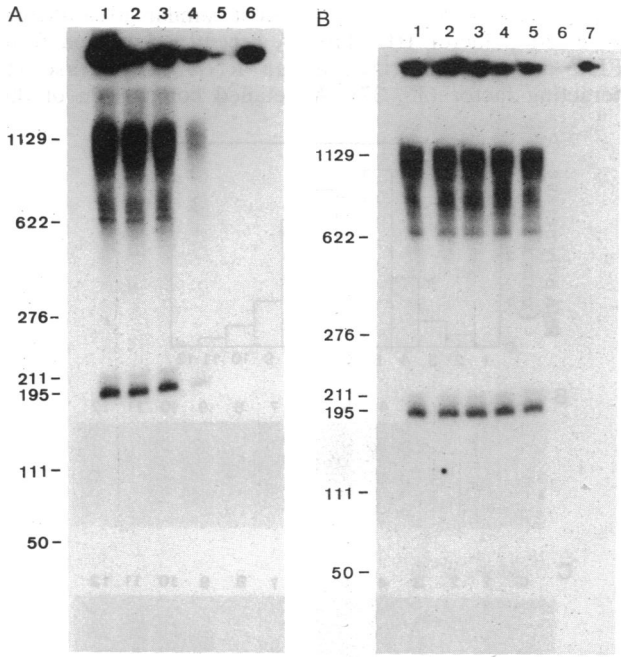

FIG. 3. Sarkosyl block assay of FA and FE. FA (0.2 μg, CM HPLC), FB (0.7 μg, sulfopropyl), FC (0.05 μg, DEAE HPLC), FE (0.1 μg, Sephacryl S-300 HR), FF (0.6 μg, DEAE-Toyo), and calf thymus RNA polymerase II (0.5 μg) were used. The mixture of factors omitting either FA or FE was first incubated with pAd2Δ (1.5 μg) in transcription buffer at 29°C. (*A*) FA (5 μl) was added at either −40 (lane 1), −20 (lane 2), −10 (lane 3), −5 (lane 4), +5 (lane 5), or +20 (lane 6) min relative to the time of addition of 0.2% sarkosyl (0 time). (*B*) FE (5 μl) was added at either −40 (lane 1), −20 (lane 2), −10 (lane 3), −5 (lane 4), −1 (lane 5), +5 (lane 6), or +20 (lane 7) min. At −1 min, 2.5 μl of a mixture of ATP, CTP, and [α-^{32}P]UTP was added to form the initiation complex; then 1.4 μl of 10 mM GTP was added at +1 min to further elongate transcripts. Incubation was continued until +40 min. FE addition at −1 min actually represented the results of adding FE just before addition of mixture of radioactive nucleoside triphosphates.

FA Activity Is Correlated with a 32-kDa Polypeptide. The FA active fraction from the CM HPLC column was applied to an analytical gel-filtration column. Each fraction was analyzed by SDS/PAGE and for its transcription activity. In each of four preparations, FA activity was correlated with a 32-kDa polypeptide (Fig. 5). The gel-filtration step appeared to reduce the amounts of the two other peptides seen in the CM HPLC preparation, but there was less protein and we cannot exclude the presence of small amounts of a 29-kDa peptide in the final preparation. Excess amounts of FA appear to be inhibitory (Fig. 5*B*, lane 12). The cause of the inhibitory effect is unknown, but when this effect was corrected for, there was a close correlation between the amount of the 32-kDa peptide and total FA activity.

DISCUSSION

Two transcription factors, FA and FE, were extensively purified by conventional chromatographic steps. To further purify FE, it was necessary to denature the protein in guanidine hydrochloride, perform molecular sieve chromatography, and renature the active protein. FE could associate with RNA polymerase II and is involved in a rapid step of transcription activation after addition of RNA polymerase II and the other transcription factors to the initiation complex. Control at such a step would permit rapid modulation of the rate of expression of specific genes.

FE does not appear to correspond to the well-characterized elongation factor IIS (33) or SII (34, 35). For example, FE showed different chromatographic properties and did not stimulate RNA synthesis on a denatured salmon sperm DNA template, as does SII. FE is most similar to a factor, transcription factor IIE (TFIIE), recently separated from TFIIF and characterized as an RNA polymerase II-interacting factor (36, 37). A detailed comparison of the chromatographic properties of FE and TFIIE supports this idea. Both bind to phosphocellulose and are eluted at 0.5 M NaCl, and both bind to a DEAE anion-exchange column under similar conditions. Both factors bind to RNA polymerase II. A final conclusion as to the correspondence of these two factors awaits further purification of TFIIE.

FA shows similar chromatographic properties to TFIIB and RNA polymerase B transcription factor 3 (BTF3) (38), although information about the peptide composition of TFIIB is not yet available and BTF3 is associated with a 27-kDa peptide. FB apparently lacks a counterpart in the literature, but it is definitely an essential factor in our assays and may be contained in one of the less pure factors used in assays by other groups. FB itself is also rather impure and may prove to be complex.

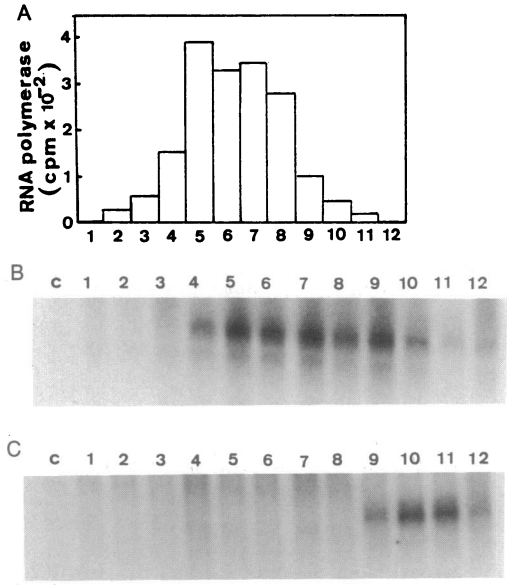

FIG. 4. Binding of FE with purified RNA polymerase II. One hundred microliters of FE (15 μg, DEAE HPLC) was incubated alone or with 75 μl of purified calf thymus RNA polymerase II (75 μg) in buffer B containing 0.075 M NaCl at 4°C for 3 hr and applied onto a Sephacryl S-300 HR column (1.4 cm × 26 cm); the column was washed at a flow rate of 3 ml/hr. Protein elution was monitored by absorbency at 280 nm. Every two fractions starting from the apparent void volume, which started reproducibly at 275 min of elution, were combined and concentrated in a Centricon-30 to 150 μl (lanes 1–12). (*A*) RNA polymerase II activity was assayed as in ref. 21. Fractions from the FE control run showed only background incorporation. FE complementing activity was assayed as described in *Materials and Methods* using 24-μl fractions of FE and RNA polymerase II (*B*) or FE alone (*C*). Lane c shows the activity observed in the absence of FE.

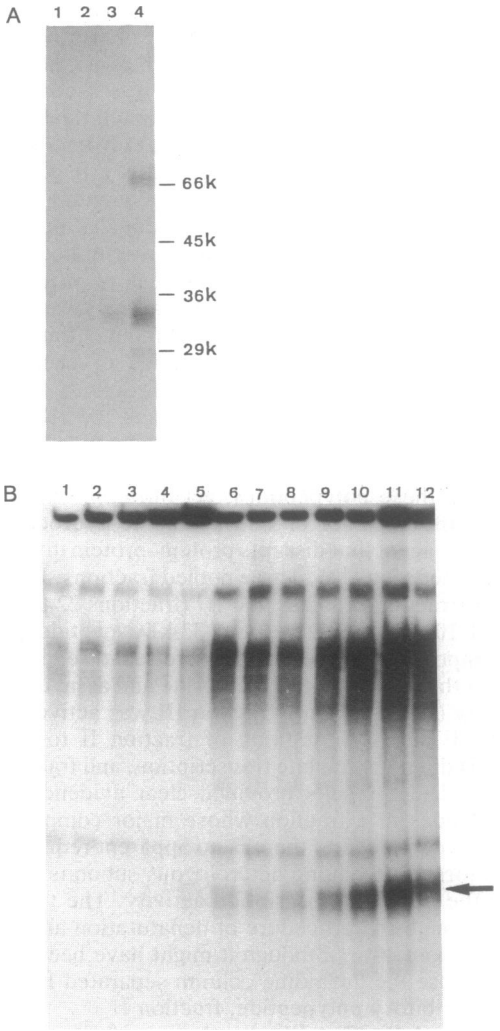

FIG. 5. SDS/PAGE and transcription assay of purified FA. The FA activity from a CM HPLC column was applied onto an analytical gel-filtration column. (*A*) SDS/PAGE without 2-mercaptoethanol. Twenty-five microliters of each fraction was analyzed on SDS/PAGE. The bands were relatively broad due to the absence of 2-mercaptoethanol. SDS/PAGE from a gel-filtration column performed in the presence of 2-mercaptoethanol showed a single sharp band with an apparent molecular mass of 32 kDa (data not shown). Gel-filtration fractions 93 and 94 (lane 1), 96 and 97 (lane 2), and 99 and 100 (lane 3), as well as CM HPLC peak fractions (lane 4) are shown. (*B*) Transcription assay. The FA activity was assayed in a reaction mixture containing 2, 4, or 8 μl of gel-filtration fractions 93 and 94 (lanes 1–3); 2, 4, or 8 μl of fractions 96 and 97 (lanes 4–6); 2, 4, or 8 μl of fractions 99 and 100 (lanes 7–9); 2, 4, or 8 μl of CM HPLC peak fractions (lanes 10–12). The specific transcript is indicated by an arrow.

In summary, three of the six general transcription factors for RNA polymerase II have been purified to near homogeneity from HeLa cell nuclear extracts. A fourth factor, the TATA factor TFIID, has recently been cloned by others (39). In spite of this recent progress, considerable work remains before the molecular components of the general RNA polymerase II transcription initiation reaction are all resolved and defined.

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1. Maniatis, T., Goodbourn, S. & Fischer, J. A. (1987) *Science* **236,** 1237–1245.
2. Kingston, R. E., Baldwin, A. S. & Sharp, P. A. (1985) *Cell* **41,** 3–5.
3. Ingram, P. W. (1988) *Nature (London)* **335,** 25–34.
4. Weil, P. A., Luse, D. S., Segall, J. & Roeder, R. G. (1979) *Cell* **18,** 469–484.
5. Matsui, T., Segall, J., Weil, P. A. & Roeder, R. G. (1980) *J. Biol. Chem.* **255,** 11992–11996.
6. Samuels, M., Fire, A. & Sharp, P. A. (1982) *J. Biol. Chem.* **257,** 14419–14427.
7. Moncollin, V., Miyamoto, N. G., Zheng, X. M. & Egly, J. M. (1986) *EMBO J.* **5,** 2577–2584.
8. Samuels, M. & Sharp, P. A. (1986) *J. Biol. Chem.* **261,** 2003–2013.
9. Burton, Z. F., Killeen, M., Sopta, M., Ortolan, L. G. & Greenblatt, J. (1988) *Mol. Cell. Biol.* **8,** 1602–1613.
10. Kitajima, S., Kawaguchi, T., Yasukochi, Y. & Weissman, S. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6106–6110.
11. Reinberg, D., Horikoshi, M. & Roeder, R. G. (1987) *J. Biol. Chem.* **262,** 3322–3330.
12. Nakajima, N., Horikoshi, M. & Roeder, R. G. (1988) *Mol. Cell. Biol.* **8,** 4028–4040.
13. Buratowski, S., Hahn, S., Guarente, L. & Sharp, P. A. (1989) *Cell* **56,** 549–561.
14. Davison, B. L., Egly, J.-M., Mulvihill, E. R. & Chambon, P. (1983) *Nature (London)* **301,** 680–686.
15. Reinberg, D. & Roeder, R. G. (1987) *J. Biol. Chem.* **262,** 3310–3321.
16. Fire, A., Samuels, M. & Sharp, P. A. (1984) *J. Biol. Chem.* **259,** 2509–2516.
17. Battey, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. & Leder, P. (1983) *Cell* **34,** 779–787.
18. Dignam, J. D., Martin, P., Shasty, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **101,** 582–598.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
21. Hodo, H. G. & Blatti, S. P. (1977) *Biochemistry* **16,** 2334–2343.
22. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
23. Carthew, R. W., Chodosh, L. A. & Sharp, P. A. (1985) *Cell* **43,** 439–448.
24. Shi, X.-P., Lee, R. & Weinmann, R. (1986) *Nucleic Acids Res.* **14,** 3729–3744.
25. Mansour, S., Grodzicker, T. & Tjian, R. (1986) *Mol. Cell. Biol.* **6,** 2684–2694.
26. Cohen, R. B., Yang, L., Thompson, J. A. & Safer, B. (1988) *J. Biol. Chem.* **263,** 10377–10385.
27. Smyth, D. G., Nagamatsu, A. & Fruton, J. S. (1960) *J. Am. Chem. Soc.* **82,** 4600–4604.
28. Weil, P. A. & Blatti, S. P. (1975) *Biochemistry* **14,** 1636–1642.
29. Garner, M. M. & Revzin, A. (1981) *Nucleic Acids Res.* **9,** 3047–3060.
30. Hennighausen, L. & Lubon, H. (1987) *Methods Enzymol.* **152,** 721–735.
31. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5,** 3157–3170.
32. Hawley, D. K. & Roeder, R. G. (1985) *J. Biol. Chem.* **260,** 8163–8172.
33. Reinberg, D. & Roeder, R. G. (1987) *J. Biol. Chem.* **262,** 3331–3337.
34. Sekimizu, K., Nakanishi, Y., Mizuno, D. & Natori, S. (1979) *Biochemistry* **18,** 1582–1588.
35. Rappaport, J., Reinberg, D., Zandomeni, R. & Weinmann, R. (1987) *J. Biol. Chem.* **262,** 5227–5232.
36. Flores, O., Maldonado, E., Burton, Z., Greenblatt, J. & Reinberg, D. (1988) *J. Biol. Chem.* **263,** 10812–10816.
37. Flores, O., Maldonado, E. & Reinberg, D. (1989) *J. Biol. Chem.* **264,** 8913–8921.
38. Zheng, X.-M., Moncollin, V., Egly, J.-M. & Chambon, P. (1987) *Cell* **50,** 361–368.
39. Horikoshi, M., Wang, C. K., Fujii, H., Cromlish, J. A., Weil, P. A. & Roeder, R. G. (1989) *Nature (London)* **341,** 299–303.