A heteromeric transcription factor required for mammalian RNA polymerase II

Shigetaka Kitajima, Yujiro Tanaka, Tatsuo Kawaguchi, Takayuki Nagaoka¹, Sherman M. Weissman² and Yukio Yasukochi

Department of Human Genetics, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, ¹Department of Clinical Genetics, School of Health Sciences, Kyorin University, 476 Miyashitacho, Hachioji, Tokyo 192, Japan and ²Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510-8005, USA

Received April 13, 1990; Revised and Accepted July 18, 1990

ABSTRACT

A general transcription factor, FC, essential for specific initiation of in vitro transcription by mammalian RNA polymerase II was identified and a procedure developed to purify it to near homogeneity from HeLa cell nuclei. Purified FC is composed of two polypeptides of apparent molecular masses 80 kDa and 30 kDa, on SDS-PAGE, and has a native size of 280 kDa estimated by gel filtration column. Both polypeptides were shown to be essential for reconstituting in vitro transcription activity. Biochemical analysis showed that the 80 kDa and 30 kDa components were present in a 1:1 molar ratio. FC was also demonstrated to interact directly or indirectly with purified RNA polymerase II. Similarities between FC and transcription factors reported by others from human, rat or *Drosophila* cells are discussed.

INTRODUCTION

A soluble cell-free system that allows accurate initiation of transcription by mammalian RNA polymerase II has been developed from cultured HeLa cell nuclear extracts (1,2,3,4). This led to identification of several accessory protein factors which are required for in vitro transcription from a DNA template containing a minimal promoter sequence (5,6,7,8,9). Among these factors, TFIID/IIA (10,11,12,13), BTF1 (14,15), or FD/FF (9) specifically associate with the promoter region of template DNA to form a DNA protein complex as a first reaction in transcription initiation. Subsequently, another set of factors [TFIIB/IIIE (16), FA/B/C/E (9) or their equivalents (8)] including RNA polymerase II interact with the above DNA protein complex to form and stabilize the initiation complex (8,9,11). The latter step may involve both catalytic action and protein-protein interaction among these factors.

Many basic questions regarding the molecular mechanism of initiation of transcription remain to be resolved. We have previously reported a fractionation scheme that resolved six components and some properties of the factors prepared from HeLa cell nuclear extract (9). As part of an effort to obtain cDNA clones of each factor, the transcription factor, FC, which is a heteromeric complex of 80 kDa and 30 kDa polypeptides has

been purified to near homogeneity. We also show that FC can associate with RNA polymerase II.

MATERIALS AND METHODS

Assay of Transcription Factor FC

In vitro transcription activity of FC using pAd2Δ and pβC2AT was assayed as reported before (9) and RNA transcripts were analyzed on 6% polyacrylamide -7 M urea gel. The pAd2Δ and pβC2AT plasmids used as DNA templates were as described (9) and the size of accurate transcripts from these promoters are 186 and 202 nucleotides, respectively. The reaction was performed in a mixture of 30 μl containing 0.2 μg FA (CM), 0.7 μg FB (Sp), 3.0 μg FD and FE (DEAE), 0.6 μg FF (DEAE), 0.5 μg of calf thymus RNA polymerase II and various amounts of fractionated FC in addition to 1.5–2.5 μg of DNA template. In some assays 0.2% Sarkosyl was added to the reaction mixture. This appeared to eliminate non-specific transcripts sometimes seen in the absence of Sarkosyl. The letter after each factor refers to the last column step (Fig. 1) used in purification of the factor for these assays.

Purification of FC

HeLa cell nuclear extract (7530 mg, 1350 ml) was prepared as described (17). Transcription factors, FA, B, C, D, E, and F were resolved as reported (9) and shown in Fig. 1. The procedures for FC purification were modified from those reported (9) and were performed at 4°C. In particular, use of a phosphocellulose column resulted in a significant purification. FC (16.8 mg, 50 ml) which had been eluted at 0.16–0.19 M NaCl during DEAE-Toyo 650 column chromatography of the D2P2 fraction (695 mg, 250 ml) was directly applied onto a phosphocellulose column (2.4 cm×10 cm) equilibrated with buffer B (20 mM Tris-HCl pH 7.9, 0.1 mM EDTA, 20% Glycerol, 10 mM β-mercaptoethanol, and 0.5 mM PMSF) containing 0.1 M NaCl. After washing the column by 80 ml of buffer B/0.3 M NaCl, FC was eluted stepwise by 60 ml of buffer B/0.5 M NaCl. FC activity was detected in the latter fractions of a large peak of eluted protein with some tailing. Fractions (0.36 mg, 22.5 ml) which gave the highest purification were concentrated to 4 ml in an Amicon concentrator with a YM30

Figure 1. A schematic representation of the chromatographic fractionation of transcription factors, FA, FB, FC, FD, FE, and FF. Columns used were DEAE Sepharose, phosphocellulose (P11), DEAE-Toyopearl 650 (DEAE-Toyo), Sephacryl S-300HR (S-300), Sulfopropyl Toyopearl 650 (Sp), TSK G-3000, CM-HPLC (Asahipak, ES-502C) and DEAE-HPLC (Asahipak, ES-502N). Details of the fractionation including buffers are described before (9).

A

B

Figure 2. FC requirement for *in vitro* transcription of pAd2Δ and pβC2AT: FC activity was assayed for reconstituting the *in vitro* transcription of pAd2Δ or pβC2AT in a total volume of 30 μl supplemented with other transcription factors, 0.2% Sarkosyl and RNA polymerase II as described in Methods. A. pAd2Δ (1.5 μg) was transcribed in a reaction mixture containing the following amounts of FC of DEAE-HPLC level. No addition (lane 1), 30 ng (lane 2), 90 ng (lane 3), 90 ng plus 0.5 μg/ml α-amanitin (lane 4). B. pβC2AT (2.5 μg) was transcribed as in Methods using FC of Sephacryl S-300HR level. 2 μg of FC (lane 1), no addition (lane 2). Specific transcripts are indicated by arrows.

Table 1. Purification of transcription factor FC from 7530 mg of HeLa cell nuclear extracts

Fraction	Protein (mg)	Activity (U) [§]	Sp a (u/mg)	Recovery (%)
D2P2	695	—	—	—
DEAE-Toyo 650	16.8	530	31.5	100
Phosphocellulose	0.36	270	750	51
Sephacryl S-300HR	0.103	216	2097	40.8
DEAE-HPLC	0.003	38	12667	27.2

[§] Units were defined arbitrarily by densitometric scanning of specific transcripts.

membrane, dialyzed against two changes of 2 l of buffer B containing 0.4 M NaCl and 0.01% Nonidet P-40 for 8 hr, and applied onto a Sephacryl S-300HR column (2.8 cm × 66 cm) equilibrated with the above buffer. FC eluted with an apparent molecular size of 280 kDa with complete recovery of activity. This fraction (0.103 mg, 15 ml) was diluted 4 times with buffer B, centrifuged at 30,000 rpm for 60 min, and applied onto a DEAE-HPLC column (7.6 mm × 100 mm, Asahipak ES-502N). A linear gradient of NaCl was developed from 0.15 M to 0.35 M in a total volume of 80 ml in buffer B and FC was detected at 0.26 M NaCl. Fractions containing transcriptional activity were concentrated by a Centricon 30 to 1 ml of buffer B/0.1 M NaCl and kept frozen at -80°C in small aliquots.

Separation of 80 kDa and 30 kDa components of FC

The purified FC (1.5 μg, 0.45 ml) was mixed with 1.5 ml of 20 mM potassium phosphate buffer pH 6.5, 10 mM EDTA, 10 mM β-mercaptoethanol, 4 M guanidine hydrochloride and dialyzed against 3 changes of 500 ml of the buffer at 4°C for 16 hr. After filtration through a 0.45 μm chromatodisc, denatured FC (0.25 ml) was applied onto a TSK 250 gel filtration column (7.5 mm × 600 mm) equilibrated with the above buffer, and eluted at a flow rate of 0.02 ml/min. 80 kDa and 30 kDa polypeptides eluted at around 610 min and 750 min, respectively. Fractions containing each polypeptide were renatured by dialysis against buffer B/0.1 M NaCl and concentrated by a Centricon 10 to approximately 100 μl.

Electroblotting and elution of proteins

The electroblotting procedure was modified from the method of Matsudaira (18). 80 kDa and 30 kDa polypeptides were separated on 10% SDS-PAGE. After electrophoresis, the gel was immersed in the electroblotting buffer (25 mM Tris-HCl pH 8.3, 192 mM Glycine, 15% Methanol) for a few seconds and sandwiched between polyvinylidene difluoride (PVDF) membranes and several sheets of Whatman 3MM papers. Polypeptides were electroblotted at a constant voltage (30 volts) in the electroblotting buffer. After the PVDF membrane was washed in H₂O for 3 min, each polypeptide was visualized by staining with 0.1% Coomassie blue in 50% methanol for 3 min and destained in 50% methanol for 3 min. Appropriate bands from the PVDF membrane were treated with acid to hydrolyze the proteins and subjected to quantitative amino acid analyses on a Beckman Model amino acid analyzer.

To determine peptide sequences, the PVDF membrane containing the protein was cut into approximately 1 mm wide pieces. After washing with cold acetone, the PVDF pieces were suspended in 100 μl of 70% formic acid containing 0.66 mM CNBr per 1 μg of blotted protein at room temperature for 24

Figure 3. DEAE-HPLC column chromatography of FC: the FC fraction from a Sephacryl S-300HR column was applied onto a DEAE-HPLC column and eluted with a linear gradient of NaCl as in Methods. A. Elution profile of protein was monitored by absorbency at 280 nm. B. 10 μ l of each fraction was assayed for FC activity using pAd2 Δ as DNA template as described in Methods. C. 15 μ l of each fraction was analyzed on 10% SDS-PAGE and visualized by silver staining.

hr. The supernatant was saved and the pieces were dried. The PVDF pieces were suspended in 100 μ l of 40% acetonitrile at 37°C for 3 hr. and 100 μ l of 40% acetonitrile, 0.05% trifluoroacetic acid at 50°C for 20 min. The supernatants were combined with the CNBr eluate and dried. The eluate was dissolved in 60 μ l of H₂O and dried again. The dried sample was digested with trypsin (1:25 weight/weight ratio to protein) and applied onto a Vydac C18 reverse phase HPLC column (2.1 mm $\times$ 250 mm). Appropriate peptides were sequenced on an Applied Biosystems Model sequencer equipped with an on-line HPLC.

Other procedures

SDS-PAGE was performed as in Laemmli (19) and stained by Ag-STAIN or 2D-SILVER STAIN II from Daiichi. Protein concentration was measured by the dye binding method (20) or absorbency at 280 nm using bovine serum albumin as a standard.

Enzymes and reagents

Endonucleases, T4 ligase, and polynucleotide kinase were purchased from New England BioLabs or Takara. [α -³²P]UTP was from Du Pont-New England Nuclear. RNA polymerase II

was purified from calf thymus as described in (21). Other materials are reagents grade.

RESULTS

Purification of transcription factor FC

We assayed FC activity by its ability to synthesize a runoff transcript from the adenovirus major late (ML) gene promoter (pAd2 Δ) in a reconstituted assay system which contained other factors resolved as in Fig. 1 and purified RNA polymerase II (Fig. 2A). Specific factors which interact with upstream (22,23,24,25,26) or downstream (27,28) elements of the adenovirus ML gene and stimulate *in vitro* transcription, have been reported in HeLa cell nuclear extracts. However, FC was also shown to be required for transcription from the minimal promoter of the human β -globin gene (Fig. 2B). FC is therefore a general transcription factor which is essential for transcription from various gene promoters. Using this assay, transcription factor FC was purified to near homogeneity by the procedures described in Methods and summarized in Table 1.

Approximately 3 μ g of protein was obtained from 7530 mg of HeLa cell nuclear extract with 7.2% recovery of activity from

the DEAE-Toyo 650 column fraction at which FC was resolved from FD and FE (Fig. 1). FC was purified 400 times from the DEAE-Toyo fraction and constituted 0.001% of protein in a

Figure 4. SDS-PAGE analysis of FC at several purification stages. From each purification step, an aliquot of FC which showed equivalent transcriptional activity was analyzed on 10% SDS-PAGE and visualized by silver staining. 1. 10 μ l of DEAE-Toyo 650 fraction. 2. 12 μ l of phosphocellulose fraction. 3. 10 μ l of Sephacryl S-300HR fraction. 4. 7 μ l of DEAE-HPLC fraction. Bands of two polypeptides, 80 kDa and 30 kDa, are indicated by arrows.

nuclear extract assuming that all of FC in the nuclear extract was partitioned into the DEAE fraction.

Fig. 3A shows the profile of elution of FC from a DEAE-HPLC column. FC eluted at 0.26–0.27 M NaCl concentration with a small symmetric peak of absorbency at 280 nm. Proteins in each fraction were analyzed on SDS-PAGE followed by silver staining (Fig. 3C). The FC activity appeared to correlate with two major polypeptides with apparent molecular masses of 80 kDa and 30 kDa although the peak of the polypeptides in each fraction are slightly displaced by one or two fractions from their transcriptional activities (Fig. 3B and 3C). This may be due to interfering activities present in the fraction or other factors supplemented with FC for assay. Such examples have been previously observed with other transcription factors (29). The 30 kDa polypeptide in each fraction appears to be present in a higher molar ratio to the 80 kDa polypeptide in a silver-stained SDS-PAGE (Fig. 3C). When the amount of each polypeptide was estimated by densitometric scanning, the results showed the ratio of 80 kDa and 30 kDa polypeptide of the same fraction differed from 0.37 : 1 to 0.78 : 1 depending on the methods of silver staining of different commercial reagent kits (compare Fig. 3C and Fig. 4 or Fig. 6B). It is well known that some polypeptides show metachromaticity and their intensities of silver staining are significantly affected by their contents of specific residue which react with the oxidizing agent of the staining solution (30, 31). Therefore it is concluded that silver staining methods are not appropriate for determination of the stoichiometric ratio of 80 kDa and 30 kDa polypeptides. FC activity was also observed to co-chromatograph with the two polypeptides in other chromatographic procedures including phosphocellulose and gel filtration columns (data not shown). Furthermore, these two components were visualized with comparable intensities of silver staining at several steps of the purification when equal amounts of FC activity from these fractions were analyzed on SDS-PAGE (Fig. 4). The data

Figure 5. Resolution and reconstitution of 80 kDa/30 kDa complex. FC from a DEAE-HPLC column (approximately 1.5 μ g) was denatured and the components were separated by a TSK 250 gel filtration HPLC column as in Methods. A. Elution profile of protein measured by absorbency at 280 nm. Arrows show the position of the elution of the 80 kDa and 30 kDa peptide that were at around 610 min. and 750 min. respectively. B. Each polypeptide was assayed for its FC complementing activity using pAd2 Δ as in Methods in a reaction mixture containing following amounts of renatured components. Total volume of reaction was kept at 30 μ l. The arrow shows the position of the specific transcript. 1. no addition of FC, 2. 8 μ l of 80 kDa polypeptide, 3. 8 μ l of 30 kDa polypeptide, 4. a mixture of 8 μ l each of 80 kDa and 30 kDa polypeptides. C. FC activity was assayed using pAd2 Δ as described in Methods in a reaction mixture of 30 μ l containing various amounts of 80 kDa and a fixed amount of 30 kDa polypeptide (6 μ l). Specific transcripts were quantitated by densitometric scanning and their relative units of activity were plotted against amounts of the 80 kDa polypeptide.

strongly suggested that FC activity is tightly associated with two polypeptides of apparent molecular weight 80 kDa and 30 kDa.

Both 80 kDa and 30 kDa polypeptides are components of transcriptional activity of FC

In order to assess the requirement of 80 kDa and 30 kDa components for transcriptional activity of FC, the polypeptides were separated through an HPLC gel filtration column in the presence of 4 M guanidine hydrochloride, renatured, and assayed for transcriptional activity (Fig. 5). As shown in Fig. 5B, renatured polypeptides were active only when both were present. The 80 kDa or 30 kDa component alone could not reconstitute transcriptional activity. The efficiency of renaturation of transcriptional activity through the procedure was low. Approximately 0.5–1% of original activity was recovered while recovery of protein could not be estimated accurately. We conclude that FC is composed of two heterologous subunits, an

80 kDa and a 30 kDa polypeptide. Since there was no difference in the size of the peptides in an SDS-PAGE of a non-reduced sample, it is unlikely that covalent linkage via disulfide bond exists between the two polypeptides. Transcription activity was also measured when the amounts of added 80 kDa polypeptide were titrated against a fixed amount of 30 kDa polypeptide. The result (Fig. 5C) showed reconstituted activity approached an apparent plateau at the point where equal aliquots of the final preparation of the two peptides were added.

Stoichiometry and native size of the heteromer of 80 kDa and 30 kDa polypeptides

To assess the stoichiometry of the interaction between the 80 kDa and 30 kDa components of FC, method other than silver staining of each polypeptide of the gel was used. The area of peak UV absorbency at 280 nm of each polypeptide separated by the HPLC gel filtration column (Fig. 5A) was integrated and quantitated.

Figure 6. Native size determination of purified FC on a Sephacryl S-300 HR column. 200 μ l of FC (0.8 μ g, DEAE-HPLC level) was applied onto a Sephacryl S-300 HR column (1 $\times$ 48 cm) equilibrated with buffer B/0.1 M NaCl and run at the flow rate of 3 ml/hr and elution of protein was monitored at 280 nm. Fraction size was 8 min. FC eluted with a peak at around 430 min. In a separate run, elution peaks of blue dextran, thyroglobulin (670 kDa), IgG (160 kDa), ovalbumin (44 kDa), and myoglobin (17 kDa) were determined and the native size of FC was calculated as in (A). Every five fractions of the experimental run starting from the apparent void volume was combined and concentrated. 20 μ l each of the fractions (1–8) was analyzed on 10% SDS-PAGE and visualized by silver staining (B).

Figure 7. Co-chromatography of FC with purified RNA polymerase II. 150 μ l of FC (10 μ g, P11) was dialyzed alone or with 75 μ l of purified calf thymus RNA polymerase II (75 μ g) against buffer B/0.1 M NaCl at 4°C for 5 hr, and applied onto a Sephacryl S-300HR column (1.4 cm $\times$ 26 cm). Protein elution was fractionated at intervals of 8 min and monitored by UV absorbency. Every three fractions which started at apparent void volume were combined and concentrated by a Centricon 30 to 100 μ l (1–8). RNA polymerase activity (A) was assayed as described in (21). No fraction of FC alone contained any RNA polymerase activity. FC complementing activity was assayed using pAd2 Δ in fractions from chromatography of FC and RNA polymerase II (B) or FC alone (C). Lane C shows the activity with buffer alone.

The molar ratio was calculated to be 0.91:1 (80 kDa:30 kDa) under the assumption that the molar absorption at 280 nm of the two peptides is proportional to their molecular weight. A_{280} will vary depending on the amino acid composition of the protein. We, therefore, transferred the FC peptides resolved on SDS-PAGE to a PVDF membrane and estimated the amount of protein by quantitative amino acid analysis. This resulted in an estimated yield of 54.0 pmoles of the 80 kDa peptide and 55.5 pmoles of the 30 kDa peptide, strongly suggesting that the molar ratio of the two peptides in extensively purified fractions was essentially 1:1. Further, the lower tyrosine content in the 80 kDa peptide (1.9%) as compared to the 30 kDa peptide (3.9%) might help explain the slightly low values for the ratio of 80 kDa to 30 kDa peptides estimated from A_{280} measurements. From the above data the 80 kDa and 30 kDa components are deduced to be associated non-covalently in a 1:1 molar ratio.

FC has a native molecular weight of 280 kDa in both crude and purified form, as estimated by gel filtration chromatography on a Sephacryl S-300HR column (Fig. 6A) and the purified FC contains only the 30 kDa and 80 kDa peptides (Fig. 6B). Since FC eluted as a 280 kDa even in the presence of 0.8 M NaCl, this suggests that the complex of two heterologous subunits, 80 kDa and 30 kDa, further aggregates.

Interaction of FC with RNA polymerase II

Since it was previously demonstrated that FC is involved in a late phase of initiation of transcription and the time course of its action resembles that of RNA polymerase II (9), the ability of FC to bind to purified RNA polymerase II was examined. When FC was incubated with RNA polymerase II and applied onto a Sephacryl S-300HR column, FC complementing activity eluted at fractions of larger molecular mass than those of FC alone (Fig. 7B and 7C). Furthermore, the peak of FC complementing activity clearly coincided with the elution peak of RNA polymerase II activity (Fig. 7A). Analysis of the proteins of these fractions showed that all of the 80 kDa and 30 kDa shifted their position of elution and co-chromatographed with RNA polymerase II activity (data not shown). The data strongly indicated that FC could associate with RNA polymerase II and both subunits of FC were involved in the formation of a ternary complex. This binding was not observed under higher concentrations of salt (buffer B/0.5 M NaCl).

Other biochemical properties

The purified form of FC contained no RNA polymerase activity and did not stimulate RNA polymerase II as assayed by a random incorporation of ribonucleotides into denatured salmon sperm DNA using $MnCl_2$ as a divalent cation. It was also free of any DNA binding activity measured by gel retardation assay or DNase I footprinting assay (9). The activity of FC was inhibited by 50% by incubation at 60°C for 5 min and was totally inactivated by incubation at 80°C for 10 min.

DISCUSSION

Our previous fractionation and attempt to purify a general transcription factor, FC, suggested that it might be a complex of heterologous polypeptides (9). Further purification described here has resulted in an apparently homogeneous form of FC. The conclusion that FC is a large molecule composed of heteromeric polypeptides of apparent molecular masses of 80 kDa and 30 kDa is supported by the following observations. (i) FC activity co-

chromatographed with the 80 kDa and 30 kDa polypeptides in various columns, including anion exchange, phosphocellulose and gel filtration columns. (ii) FC exhibited a native molecular size corresponding to a globular protein of 280 kDa on gel filtration. (iii) Quantitation by UV absorbency of each subunit separated in denaturing condition, and quantitative amino acid analysis of the separated subunits strongly indicated that the 80 kDa and 30 kDa polypeptides are present in an approximately equimolar ratio. (iv) Transcription activity of FC could be reconstituted only by a mixture of the two components. Either polypeptide alone was not active.

FC has been previously shown to function with other factors, FA, FB, and FE including RNA polymerase II in a rapid reaction occurring after initial complex formation between FD/FF and the promoter region of template DNA (9). No formation of a full-length transcript or shortened transcripts was observed in the absence of FC, suggesting that FC acted at the initiation step of specific transcription. Here it is further shown that FC interacts with purified RNA polymerase II. FC is a distinct entity from the well characterized elongation factor IIS (or SII) which also binds to RNA polymerase II (32). IIS (or SII) has been reported to be a monomeric polypeptide of 35 to 38 kDa and flow through an anion exchange column in 0.1 KCl (33,34,35,36). Moreover, it is not essential for initiation of specific transcription but stimulates the rate of elongation by allowing RNA polymerase II to pass efficiently through strong pause sites (34,35). The possibility, however, cannot be ruled out that FC might act in a very early phase of elongation of transcription along with RNA polymerase II by a different mechanism from that of IIS, although such evidence was not obtained in the assay of specific transcription. The nature of FC interaction with RNA polymerase II and the functional significance of the resulting large ternary complex are currently unknown and under investigation. It is also unknown at this moment whether FC interacts with other factor(s) or not.

Our preparation of FC resembled RAP30/74 (37,38) from HeLa cells in the following aspects. Both are composed of heteromeric subunits of similar molecular masses, although our larger subunit is somewhat bigger than its counterpart, RAP74. RAP74 is reportedly heavily phosphorylated (39) and this might affect its migration of SDS-PAGE. Second, in both cases, two components are essential for reconstituting *in vitro* transcription activity. Third, FC could bind to RNA polymerase II while RAP30/74 was originally purified by an immobilized RNA polymerase II column. However, some minor differences were observed. As estimated by gel exclusion chromatography, our preparation of FC has a native size of 280 kDa indicating large aggregate formation while that of RAP30/74 is reported to be 73 kDa (39). Furthermore, the stoichiometry of RAP30/74 retained by an RNA polymerase II column was reported to be different from 1:1, i.e. the amount of RAP74 was lower than that of RAP30 (38). Our results indicate that 80 kDa and 30 kDa polypeptides exist as a complex of 1:1 stoichiometry. This might reflect differences of purification methods. Greenblatt et al. (38,39) suspected that RAP74 has a weaker affinity for RNA polymerase II or instead interacts via RAP30, thereby resulting in lower recovery of RAP74 from their column compared with RAP30.

Our binding study showed both 80 kDa and 30 kDa were associated with purified RNA polymerase II, possibly in equimolar amounts. Recently RAP30/74 was reported to have helicase activity and the structure of a cDNA clone for the 30

kDa component of RAP30/74 (40). Within experimental error, the predicted amino acid composition of RAP30 agrees with that which we determined for the 30 kDa component of FC. We have determined the amino acid sequence of a tryptic peptide from the 30 kDa component. The amino acid sequence is as follows: X-Glu-Val-Ser-Phe-Thr-Leu-Asn-Glu-Asp-Leu-Ala-Asn-Ile-His-Asp-Ile-Gly-Gly-Lys-Pro-Ala-X-Val-. This sequence is identical to the internal amino acid sequence of RAP30 (residues 52–75). However, we were unable to detect DNA helicase activity in our most pure, transcriptionally active, FC preparation. Reinberg et al. (41,42) reported identification of their factor TFIIF from TFIIE which interacts with RNA polymerase II. While the present report was in review, a paper appeared reporting the biochemical purification of TFIIF (43). The authors achieved a substantial degree of purification and also failed to detect helicase activity in their purest preparations. Their evidence from immunologic studies concurs with our conclusions that the 30 kDa subunit is the same as that recently cloned and that the 30 kDa and 80 kDa subunits associate in an apparently higher molecular weight, perhaps multimeric complex.

FC also resembles the transcription factor $\beta\gamma$ purified from rat liver nuclei (44) or factor 5 from *Drosophila* Kc cells (45) on the basis of their biochemical characteristics, ability to associate with RNA polymerase II, and the similarity of their chromatographic properties.

Finally, our purification of FC in a homogeneous form through relatively mild methods has opened the way to more detailed study of enzymology of *in vitro* transcription reaction and regulation of gene expression *in vivo*.

ACKNOWLEDGEMENTS

We are grateful to Ms. Naoko Takemura for her HeLa cell nuclear extract preparation and expert manuscript preparation. We also thank Barbara Gramenos and Leif Madsen for manuscript preparation. We thank Dr. Kenneth Williams and Kathryn Stone for advice on electrophoresis and quantitative amino acid analysis.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture and Research Grant for Specific Diseases from the Ministry of Health and Welfare of Japan and by National Institutes of Health Grant R35CA42556.

REFERENCES

- Weil, P.A., Luse, D.S., Segall, J. and Roeder, R.G. (1979) *Cell* 18, 469–484.
- Manley, J.L., Fire, A., Cano, A., Sharp, P.A. and Geftter, M.L. (1980) *Proc. Natl. Acad. Sci. USA* 77, 3855–3859.
- Luse, D.S. and Roeder, R.G. (1980) *Cell* 20, 691–699.
- Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) *Nucleic Acids Res.* 11, 1475–1489.
- Matsui, T., Segall, J., Weil, P.A. and Roeder, R.G. (1980) *J. Biol. Chem.* 255, 11992–11996.
- Tsai, S.-Y., Tsai, M.-J., Kops, L.E., Minghetti, P.P. and O'Malley, B.W. (1981) *J. Biol. Chem.* 256, 13055–13059.
- Samuels, M., Fire, A. and Sharp, P.A. (1982) *J. Biol. Chem.* 257, 14419–14427.
- Fire, A., Samuels, M. and Sharp, P.A. (1984) *J. Biol. Chem.* 259, 2509–2516.
- Kitajima, S., Kawaguchi, T., Yasukochi, Y. and Weissman, S.M. (1989) *Proc. Natl. Acad. Sci. USA* 86, 6106–6110.
- Samuels, M. and Sharp, P.A. (1986) *J. Biol. Chem.* 261, 2003–2013.
- Reinberg, D., Horikoshi, M. and Roeder, R.G. (1987) *J. Biol. Chem.* 262, 3322–3330.
- Nakajima, N., Horikoshi, M. and Roeder, R.G. (1988) *Mol. Cell. Biol.* 8, 4028–4040.
- Buratoski, S., Hahn, S., Guarente, L. and Sharp, P. A. (1989) *Cell* 56, 549–561.
- Wasylyk, B. and Chambon, P. (1981) *Nucleic Acids Res.* 9, 1813–1824.
- Davison, B.L., Egly, J.-M., Mulvihill, E. R. and Chambon, P. (1983) *Nature* 301, 680–686.
- Reinberg, D. and Roeder, R. G. (1987) *J. Biol. Chem.* 262, 3310–3321.
- Dignam, J.D., Martin, P., Shasty, B.S. and Roeder, R.G. (1983) *Methods Enzymol.* 101, 582–598.
- Matsudaira, P. (1987) *J. Biol. Chem.* 262, 10035–10038.
- Laemmli, U. K. (1970) *Nature* 227, 680–685.
- Bradford, M.M. (1976) *Anal. Biochem.* 72, 248–254.
- Hodo, H. G. and Blatti, S. P. (1977) *Biochemistry* 16, 2334–2343.
- Miyamoto, N. G., Moncollin, V., Wintzerith, M., Hen, R., Egly, J.M. and Chambon, P. (1984) *Nucleic Acids Res.* 12, 8779–8799.
- Sawadogo, M. and Roeder, R.G. (1985) *Cell* 43, 165–175.
- Carthew, R.W., Chodosh, L.A., and Sharp, P.A. (1985) *Cell* 43, 439–448.
- Shi, X.-P., Lee, R. and Weinmann, R. (1986) *Nucleic Acids Res.* 14, 3729–3744.
- Moncollin, V., Miyamoto, N. G., Zheng, X. M. and Egly, J. M. (1986) *EMBO J.* 5, 2577–2584.
- Mansour, S., Grodzicker, T. and Tjian, R. (1986) *Mol. Cell. Biol.* 6, 2684–2694.
- Cohen, R.B., Yang, L., Thompson, J.A., and Safer, B. (1988) *J. Biol. Chem.* 263, 10377–10385.
- Dynan, W.S. and Tjian, R. (1983) *Cell* 38, 79–87.
- Switzer, R.C., Merril, C.R., and Shifrin, S. (1979) *Anal. Biochem.* 98, 231–237.
- Poehling, H.M. and Neuhoff, V. (1981) *Electrophoresis* 2, 141–147.
- Horikoshi, M., Sekimizu, K. and Natori, S. (1984) *J. Biol. Chem.* 259, 608–611.
- Sekimizu, K., Kobayashi, N., Mizuno, D. and Natori, S. (1976) *Biochemistry* 15, 5064–5070.
- Reinberg, D. and Roeder, R.G. (1987) *J. Biol. Chem.* 262, 3331–3337.
- Rappaport, J., Reinberg, D., Zandomeni, R. and Weinmann, R. (1987) *J. Biol. Chem.* 262, 5227–5232.
- Hirashima, S., Hirai, H., Nakanishi, Y. and Natori, S. (1988) *J. Biol. Chem.* 263, 3858–3863.
- Burton, Z.F., Ortolan, L.G., and Greenblatt, J. (1986) *EMBO J.* 5, 2923–2930.
- Burton, Z.F., Killeen, M., Sopta, M., Ortolan, L.G. and Greenblatt, J. (1988) *Mol. Cell. Biol.* 8, 1602–1613.
- Sopta, M., Carthew, R.W., and Greenblatt, J. (1985) *J. Biol. Chem.* 260, 10353–10360.
- Sopta, M., Burton, F. Z. and Greenblatt, J. (1989) *Nature* 341, 410–414.
- Flores, O., Maldonado, E., Burton, Z., Greenblatt, J. and Reinberg, D. (1988) *J. Biol. Chem.* 263, 10812–10816.
- Flores, O., Maldonado, E. and Reinberg, D. (1989) *J. Biol. Chem.* 264, 8913–8921.
- Flores, O., Ha, I. and Reinberg, D. (1990) *J. Biol. Chem.* 265, 5629–5634.
- Conaway, J.W. and Conaway, R.C. (1989) *J. Biol. Chem.* 264, 2357–2362.
- Price, D. H., Sluder, A. E. and Greenleaf, A. L. (1989) *Mol. Cell. Biol.* 9, 1465–1475.