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Domain structure of a human general transcription initiation factor, TFIIF

Masatomo Yonaha, Teijiro Aso[1,+], Yasushi Kobayashi, Haren Vasavada[1], Yukio Yasukochi, Sherman M.Weissman[1] and Shigetaka Kitajima*

Department of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan and [1]Department of Genetics, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Ave., New Haven, CT 06536, USA

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ABSTRACT

The structural and functional domains of a general transcription initiation factor, TFIIF (RAP30/74, FC), have been investigated using various deletion mutants of each subunit, both in vivo and in vitro. An in vivo assay showed that the N-terminal sequence containing residues of 1 – 110 of RAP30 that is located close to a σ homology region interacts with a minimum sequence of residues 62 – 171 of RAP74 to form a heteromeric interaction. Reconstitution of in vitro transcription activity by deletion mutants of RAP74 clearly indicated that both N-terminal residues 73 – 205 and C-terminal residues 356 – 517 are essential for full activity, the former interacting with RAP30, thus complexing with RNA polymerase II. From these data, the functional significance of domain structure of TFIIF is discussed in terms of its σ homology sequences and complex formation with RNA polymerase II in the initiation and elongation of transcription.

INTRODUCTION

The initiation of transcription of protein-coding genes is a complex process involving multiple components (1). The development of a soluble cell-free system composed of minimal or core promoter DNA, RNA polymerase II, and protein factors in nuclear extract has led to the identification of basic transcription factors essential for specific and accurate initiation of transcription in vitro (2,3,4,5,6,7,8). So far, at least six factors have been found to be required for transcription initiation, TFIIA, IIB, IID, IIE, IIF and IIG (9,10), and additional factors probably exist (11,12).

Recently, cDNAs encoding TFIID (13,14), IIB (15), IIE (16,17,18) and IIF (19,20,21) have been successfully cloned and the primary structure of each factor reported. However, detailed analysis of structure and function of each factor is essential in order to understand the basic molecular mechanism of protein–protein interaction in the transcription initiation reaction.

TFIIF (22,23), also termed FC (24), RAP30/74 (25), βγ in rat (26), or factor 5 in Drosophila (27) is one of the general initiation factors. TFIIF can bind directly to RNA polymerase II (22,24,25) and suppresses nonspecific binding of RNA polymerase II to DNA (24,28,29). Studies on the assembly of transcription initiation complexes using the gel shift assay show that TFIIF is required for RNA polymerase II to assemble into a preinitiation complex formed by promoter DNA and the general factors TFIID, IIA and IIB (30,31). Furthermore, TFIIF also stimulates transcript elongation by RNA polymerase II (22,27,32). Thus, TFIIF is not only an initiation factor but an elongation factor. TFIIF could be a target of regulation at these two steps of mRNA synthesis.

TFIIF is a heteromer composed of a small (RAP30) and a large (RAP74) subunit (23,24,25). We have recently cloned cDNA encoding the large subunit of TFIIF that interacts with the small subunit in vivo and shown that bacterially expressed proteins of both could replace the transcription initiation activity of native TFIIF (20). In this article, the structure of TFIIF was investigated by using various deletion mutants of each subunit both in vivo and in vitro. We show that a small portion of N-terminal domain of RAP74 interacts with RAP30 while the C-terminal region is also essential for transcription activity.

MATERIALS AND METHODS

Expression of recombinant protein

Plasmids for expression of recombinant RAP30 (r30) and RAP74 (r74) were constructed by insertion of each full length open reading frame into the NcoI and BamHI sites of the T7 expression vector, pET-3d as described (20). To construct plasmids expressing C-terminal deletion mutants of RAP74, the NcoI site at the translation initiation site was first converted to an NdeI

* To whom correspondence should be addressed

+ Present address: Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, USA

site by inserting a synthetic oligonucleotide. Restriction fragments from the NdeI site to the SalI site, to each of three StuI sites, to the FspI site and to the HindIII site, encoding from the first methionine to amino acids 435, 356, 256, 136, 205 and 73, respectively, were prepared. The nucleotides corresponding to the C-terminal region of each encoded peptide were converted to a BamHI site by filling in with Klenow polymerase and ligating on BamHI linkers. These modified C-terminal fragments were subcloned into the NdeI and BamHI sites of the His-tagged expression vector, pET15b. Plasmids expressing N-terminal deletions of RAP74 were constructed by inserting restriction fragments from HindIII, FspI or StuI sites to the BamHI site that encodes polypeptides from the 73rd, 205th and 356th amino acid to the C-terminal end, respectively, into the NdeI and BamHI sites of pET15b. The sequences around both the initiation and termination regions of each clone was determined by the di-deoxy method and confirmed to be in frame with the N-terminal 6-His sequence of pET15b.

BL21(DE3) cells were transformed by each plasmid and the cells were grown at 37°C in an LB medium containing 100μg/ml ampicillin until A_{600} reached 0.6, at which time IPTG was added to a final concentration of 1.0mM and the cells were allowed to grow for an additional 3 hours. The cells were collected by centrifugation at 4,000g for 10 minutes and suspended in 1/20 of the original culture volume using lysis buffer containing 20mM Tris-HCl (pH7.9), 5mM EDTA, 25mM NaCl, 0.5mM PMSF and 14mM β-mercaptoethanol. After sonication and centrifugation at 10,000g for 15 minutes, RAP74 and its deletion mutants were solubilized in the supernatant but RAP30 was retained in the pellet.

Protein purification

The supernatant fraction containing expressed RAP74 was applied onto a P11 column equilibrated with buffer B (20mM Tris−HCl (pH7.9), 0.2mM EDTA, 20%Glycerol, 10mM β-mercaptoethanol) containing 0.1M NaCl. After washing the column by buffer B/0.5M NaCl, RAP74 or its deletion mutants were eluted stepwise by B/1.0M NaCl. C-terminal deletion proteins of r74(1−73) and r74(1−136), and N-terminal deletions of r74(205−517) and r74(356−517) were sequentially purified on a Nickel column to near homogeneity according to the pET-His protocols of Novagen. For other deleted RAP74s proteins, the fractions from the P11 column that contained approximately 500μg of the protein were subjected on SDS-polyacrylamide gel electrophoresis, and stained with 4M Na-acetate. The protein was eluted from the gel and renatured as described (33) with minor modifications. Briefly, the protein was eluted into 2ml of 20mM Tris−HCl (pH7.9), 0.1mM EDTA and 0.15M NaCl by rotary shaking overnight. The eluted protein was then aceton-precipitated, rinsed twice by 80% aceton, and dissolved into 50μl of 6M guanidine-HCl. After diluting with 250μl of buffer B containing 0.3M NaCl, the protein was renatured by dialysis against the same buffer at 4°C overnight. RAP30 protein was prepared from a pellet of cell lysate as described before (20).

Production of anti-RAP74 and anti-RAP30 anti-serum

The full-length RAP74 (~1mg) and RAP30 (~20mg) prepared as above were separated on 10% SDS-PAGE and visualized by staining with 4M Na-acetate. The band containing each protein was cut out and the gel was emulsified in Freund complete adjuvant for injection. Two female New Zealand White rabbits were each injected with approximately 100μg of RAP74 or 300μg of RAP30 a week after preimmune serum had been bled. After three timed boosting injections with 75μg of RAP74 or 200μg of RAP30, serum against each protein was obtained at days 38 and 50.

In vivo binding by CAT assay

Plasmids for expressing GAL4-RAP30 or VP16-RAP74 fusions were constructed by inserting PCR-generated fragments of RAP30 or RAP74 into the EcoRI site of plasmid pSG424 (34) or pAASVVP16 (35), respectively, as in (20). PCR fragments were generated by standard methods with primers encoding the amino acids at both ends of each fragment and containing extraneous nucleotides comprising EcoRI sites at their 5′ ends. The junctions of the resultant constructs were sequenced to ensure the fragments were in frame. The reporter plasmid, G5EC, which contains five binding sites for the GAL4 protein and the E1B TATA box in front of the CAT gene, was kindly provided by Drs. I.Sadowski, and M.Ptashne.

CV1 (African green monkey kidney) cells were grown in DMEM supplemented with 10% FCS. The reporter and effector plasmids (10μg each) were cotransfected by calcium phosphate precipitation into 10^6 CV1 cells in 10cm plates. After 6 hours, the cells were treated with 15% glycerol in complete medium for 3 minutes, washed and maintained in the same medium. After 48−72 hours, cell extracts were prepared and assayed for CAT activity (36).

Assay of *in vitro* transcription

In vitro transcription activity of recombinant TFIIF was assayed as before (20,24) with minor modifications, and RNA transcripts were analyzed on 6% polyacrylamide-7M urea gel. A mixture of r30 and r74 was first incubated at 29°C for 30 minutes, then incubated for another 30 minutes with other factors containing the D1P2 fraction (a crude fraction containing FA and FB by our nomenclature, see reference 9), 0.4μg FE and 0.5μg RNA polymerase II. The preinitiation complex was allowed to assemble by adding a mixture of 5ng recombinant TFIID and 2μg pMLC2AT. After incubating at 29°C for 30 minutes, nucleotide triphosphates were added to a final concentration of 600μM ATP, UTP and 5μM [α-^{32}P] CTP (~5μCi). After 60 minutes, the reaction was stopped and radioactive RNA was isolated and analyzed. Recombinant TFIID and the plasmid pMLC2AT were provided by Dr A.Berk (14) and Dr R.Roeder (37), respectively.

Other procedures

SDS-PAGE was performed as in Laemmli (38), and the gel were stained by 2D-SILVER STAIN II from Daiichi. Immunoblotting was performed by using alkaline phosphatase-conjugated goat anti-rabbit IgG as a second antibody as in the Western Light TM protocol provided by Boehringer. Methods for subcloning and di-deoxy sequencing were as described in (39).

RESULTS

Size and western blot of recombinant TFIIF

Bacterially expressed subunits of TFIIF, r74 and r30, were purified as described in Materials and Methods, and analyzed on 10% SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1A, r74 and r30 migrated at rates consistent with molecular weights of 76 and 29.2kDa, respectively, compared with 78 and 30kDa for native HeLa TFIIF. This suggests that both subunits are post-translationally modified in vivo. Western blot analysis

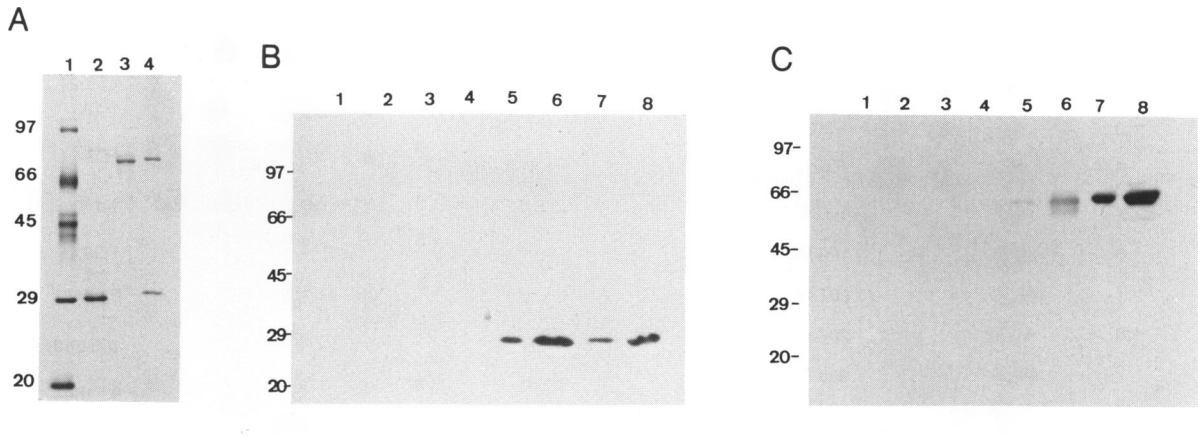

Figure 1. SDS-PAGE analysis and immunoblots of recombinant and HeLa native TFIIF. (**A**) Recombinant RAP30 (r30) and RAP74 (r74) expressed and purified as in Methods were analyzed on 10% SDS-PAGE along with HeLa native TFIIF purified as in (24) and visualized by silver staining. 1. size marker 2. r30 (20ng) 3. r74 (50ng) 4. HeLa TFIIF (60ng). (**B**) and (**C**) After two different amounts of r30 (10ng in lane 1 and 5, 30ng in lane 2 and 6 in B) or r74 (20ng in lane 1 and 5, 60ng in lane 2 and 6 in C) along with HeLa TFIIF (30ng in lane 3 and 7, 90ng in lane 4 and 8 in both B and C) were separated on 10% SDS-PAGE, proteins were electroblotted to nitrocellulose membrane and immunoreaction was performed using 1:2000 dilution of preimmune (lanes1−4) or anti-RAP30 anti-serum (lanes 5−8 in B), or preimmune (lanes 1−4) or anti-RAP74 anti-serum (lanes 5−8 in C) prepared as in Method. The second antibody was 1:10000 diluted anti-rabbit IgG. A rainbow protein marker from Amersham was used as monitoring electroblotting and also as a size marker.

using anti-serum against expressed proteins detected each corresponding subunit of HeLa TFIIF (Fig. 1B, 1C) providing immunological evidence that the recombinant proteins are derived from expression of cDNAs for each subunit of TFIIF.

Fig. 2 shows the polypeptide pattern of various mutant RAP74s prepared as in Materials and Methods. We found that the RAP74 C-terminal deletions larger than r74(1−136) and the N-terminal deletions larger than r74(205−517) were consistently contaminated with two major NH_2-terminal fragments of sizes around 20 and 30kDa. These two NH_2-terminal fragments co-chromatographed with intact RAP74 in anion-exchange or gel filtration columns and were detected by immunoblot (data not shown). These smaller N-terminal polypeptides were retained on a Nickel column even in the presence of 6M guanidine-HCl or 8M urea indicating the presence of a histidine repeat at their N-terminus. When the cell lysates were prepared in the presence of various protease inhibitors (PMSF, antipain, benzamidine and chymostatin) the sizes and quantities of these smaller fragments did not change, and other fragments corresponding to proteolytic products were not observed. It is speculated that these small fragments might result from unique terminated or inefficient translation at one or more sites between the 136th and 205th amino acid, and not from proteolysis. Therefore, we employed a gel-extraction method (33) and renatured the eluted protein as described in Materials and Methods. It is noted here that feasibility and applicability of the method, especially for the purpose of efficient renaturation of the eluted protein, seem to be dependent on the amount of protein in addition to its own renaturability. So an experiment was performed on a large scale using approximately 500μg of expressed protein. From the mobility of each polypeptide of the expressed proteins on SDS-PAGE, the M.wt. of r74s containing the C-terminal sequence down to the 435th, 356th, 256th, 205th, 136th and 73rd amino acid were calculated to be 74, 62, 32, 27, 21 and 16kDa, respectively. The M.wt. of proteins N-terminally deleted down to the 73rd, 205th and 356th residue were 69, 56 and 27kDa, respectively. The proteins were all immunologically shown to

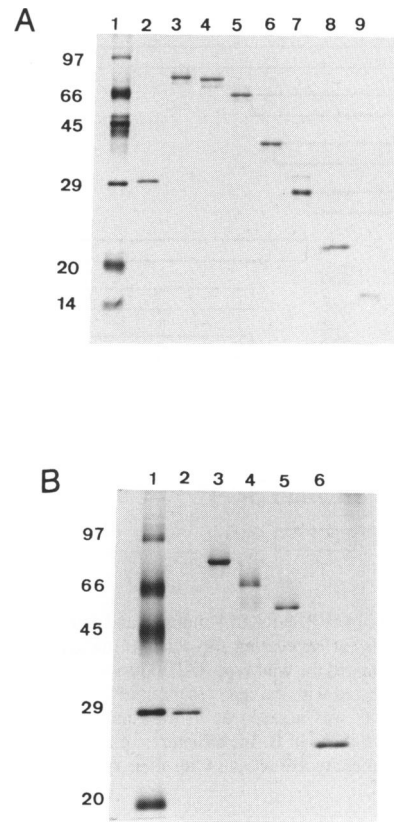

Figure 2. SDS-PAGE of various deletion mutants of RAP74. C-terminal deletions (A) and N-terminal deletions (B) prepared as in Methods were analyzed on 11% SDS-PAGE and stained by silver staining to determine both their purity and the amounts of protein that gave apparent stoichiometry to r30. (**A**) C-terminal deletions. 1. marker 2. r30 3. r74(1−517) 4. r74(1−435) 5. r74(1−356) 6. r74(1−256) 7. r74(1−205) 8. r74(1−136) 9. r74(1−73). (**B**) N-terminal deletions. 1. marker 2. r30 3. r74(1−517) 4. r74(73−517) 5. r74(205−517) 6. r74(356−517).

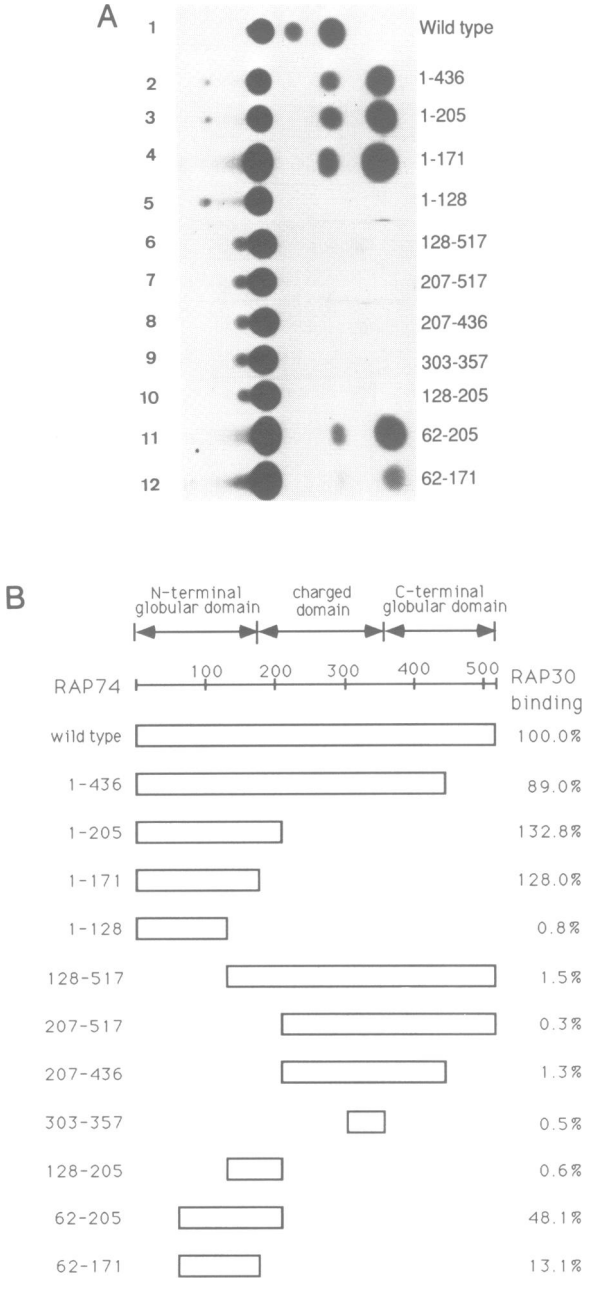

Figure 3. CAT assay by VP16-RAP74 mutants and wild-type GAL4-RAP30. Two effector plasmids for expressing the deletion mutants of RAP74 fused to VP16 activating domain and the wild-type RAP30 fusion with GAL4 DNA binding domain were cotransfected with the reporter plasmid G5EC into CV1 cells. CAT activity of cell extracts was assayed as in (36) and typical results of several experiments are shown in **A**. In **B**, the structures of a series of deletion mutants of RAP74 used in the study are shown with their relative activities to the full length protein.

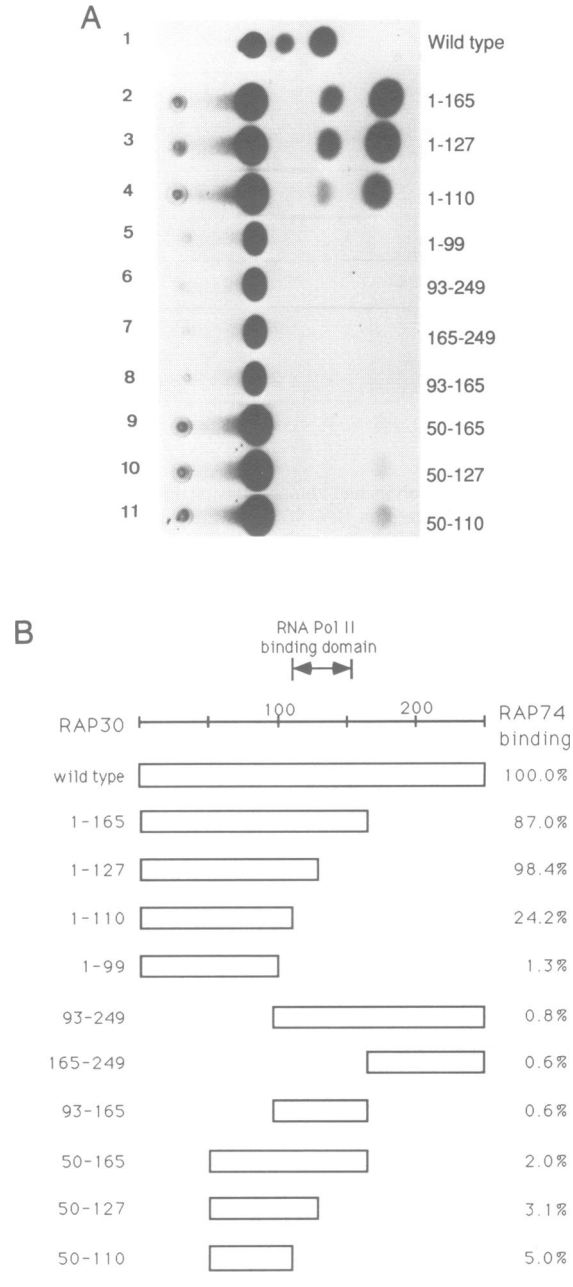

Figure 4. CAT assay by GAL4-RAP30 mutants and wild-type VP16-RAP74. Two effector plasmids for expressing the deletion mutants of RAP30 fused to the GAL4 DNA binding domain and the wild-type RAP74 fusion with the VP16 activating domain were cotransfected with the reporter plasmid G5EC into CV1 cells and CAT activity of cell extracts was assayed. In **A**, typical results of the experiments are shown. In **B**, the structures of RAP30 mutants are shown with their relative activities to that of wild clone.

be RAP74 deletions by immunoblots using anti-RAP74 antibodies (data not shown).

Interaction of RAP74 with RAP30 *in vivo*

The RAP30 subunit of TFIIF has been shown to bind to RNA polymerase II (25,29,40). Interaction of RAP74 with RAP30 is essential for transcription initiation activity (23,24,25,26). Cotransfection of GAL4-RAP30 and VP16-RAP74 has been shown to transactivate the expression of the chloramphenicol acetyl transferase (CAT) gene containing a GAL4 binding site as a cis-acting element at an upstream sequence, indicating RAP74 and RAP30 interact in vivo (20).

The above experiment was extended to determine the sequence of both subunits essential for this interaction as described in the Materials and Methods. Fig. 3 showed that the clones of RAP74 whose C-terminal sequences were deleted up to the 171th amino

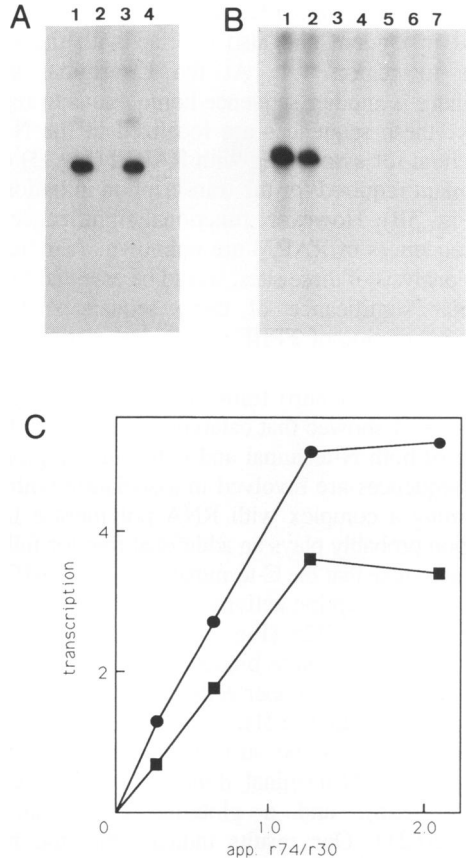

Figure 5. In vitro transcription activity of the C-terminal deletion of RAP74. Each transcriptional activity of r74s of C-terminal deletions prepared as in Methods was reconstituted with a stoichiometric amount of r30 and assayed as described in Methods. (**A**) Each amount of full length RAP74 protein, r74(1−517) and r30 shown in Fig. 1A was assayed. 1. HeLa native TFIIF 2. none 3. r74(1−517) and r30 4. as in 3. plus 0.5μg/ml α-amanitin. (**B**) Various C-terminal deletion r74s were assayed with r30 using each amount of protein shown in Fig. 2A. 1. r74(1−517) 2. r74(1−435) 3. r74(1−356) 4. r74(1−256) 5. r74(1−205) 6. r74(1−136) 7. r74(1−73). For r74s shorter than r74(1−356), different amounts of proteins were assayed, but no activity was observed (data not shown). **C**) r74(1−517) (circles) and r74(1−435) (squares) was each titrated against the fixed amount of r30 and resulting transcripts were quantitated by an image analyzer (Fuji BAS2000) and expressed as an arbitrary unit.

acid residue (lanes 2,3 and 4) stimulated the CAT activity to the same extent as the wild type clone, but further deletion of the C-terminal sequence up to the 128th residue resulted in a complete loss of the CAT activity (lane 5). By contrast, transfection with clones encoding various regions of C-terminal (lanes 6 and 7) or internal sequences (lane 8,9 and10) did not show any CAT activity. This indicates that the region of RAP74 responsible for the interaction with RAP30 in vivo is located at its N-terminal domain. The peptide consisting of amino acids 62−171 was the smallest fragment responsible for the effect although its activity was only 13% of the intact clone (lane 12). Clones 1−171 and 62−205 showed activities higher than that of the clone 62−171 suggesting that the flanking regions of 62−171 at both the N-terminus (up to 1) and C-terminus (down to 205) have positive effects on the interaction. Fig. 4 showed the CAT activities of various deletion mutant clones of RAP30. The clones of sequential deletions from the C-terminal end up to the 110th residue stimulated the CAT activity while the 1−110 clone showed only control levels of activity (lane 2,3 and 4). It was also found that C-terminal flanking sequence down to the 127th or 165th amino acid residue slightly stimulated the activity of clone 1−110. Further deletion of the C-terminal 11 amino acid residues from the clone 1−110 totally abolished the stimulation (lane 5). Other clones containing various regions of RAP30 sequence could not stimulate the CAT activity. This indicates that region 1−110 of RAP30 contains a minimum sequence essential for interacting with RAP74 in vivo.

Catalytic activity of the expressed proteins

Recombinant RAP30 (r30) and RAP74 (r74) together substitute for the transcription activity of TFIIF in vitro (20,21). As shown in Fig. 5A, r30 and r74 were indeed active in our assay. The specific activity of the bacterially expressed TFIIF was estimated to be 5−10% of the native HeLa factor, and its activity required preincubation with other transcription factors derived from HeLa nuclear extract. Under these conditions, each of the deletion mutants of RAP74 was assayed along with a stoichiometrical equivalent amount of r30 for their activity of supporting in vitro transcription. Fig. 5B showed that only two RAP74 C-terminal deletions, r74(1−517) and r74(1−435), were active. In contrast, other truncated forms of RAP74 containing a shorter C-terminal region were all inactive. Since r74(1−435) was reproducibly less active than r74(1−517), a fixed amount of r30 was mixed with varying amounts of each of these r74 proteins (Fig. 5C). The activity of the mixture augmented with increasing amounts of r74 and reached a plateau at a molar concentration of r74 which was approximately equal to that of r30 (Fig. 5C). The profile has been also observed when HeLa TFIIF activity was reconstituted by each subunit separated under denaturing conditions (24). At this plateau, the activity for r74(1−435) was 62% of that of full length r74. Thus deletion of C-terminal 82 amino acids from RAP74 still provided an active protein that supported in vitro transcription at a lower efficiency, and further deletion of the C-terminal region up to the 356th amino acid residue resulted in a complete loss of activity. Fig. 6 shows the effect of the N-terminal deletions of RAP74 on its catalytic activity. r74(73−517) was about as active as a full length protein, while two other forms, r74(205−517) and r74(356−517), were inactive. These data strongly indicated that both N-terminal 73−205 and C-terminal 356−517 regions are essential for RAP74 to function as a transcription factor.

DISCUSSION

TFIIF, a transcription initiation factor for mammalian RNA polymerase II, has been analyzed in terms of structural and catalytic function using deletion mutants of each subunit.

The N-terminal sequence 62−171 of RAP74 and N-terminal sequence 1−110 of RAP30 were shown to be minimum domains essential for interacting with each other to form a heteromer, although both N- and C-terminal flanking sequences of RAP74 and a small portion of C-terminal sequence of RAP30 stimulated the interaction (Figs. 3 and 4). These sequences form a slightly hydrophobic domain compared with other region of the proteins, as deduced by computer analysis. It is conceivable subunits of TFIIF associate with each other by hydrophobic interaction. However, it is also possible that these domains might not be directly involved in the association but that deletions of these regions might induce a conformational change of each protein, affecting the interaction.

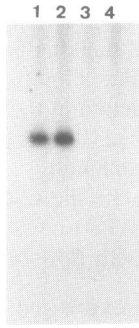

Figure 6. In vitro transcription activity of N-terminal deletion of RAP74. Each r74 of N-terminal deletions prepared as in Methods was assayed with r30 for its supporting TFIIF activity. Amounts of each protein employed are shown in Fig. 2B. 1. r74(1−517) 2. r74(73−517) 3. r74(205−517) 4. r74(356−517). Assays by using different amounts of r74(205−517) and r74(356−517) were negative (data not shown).

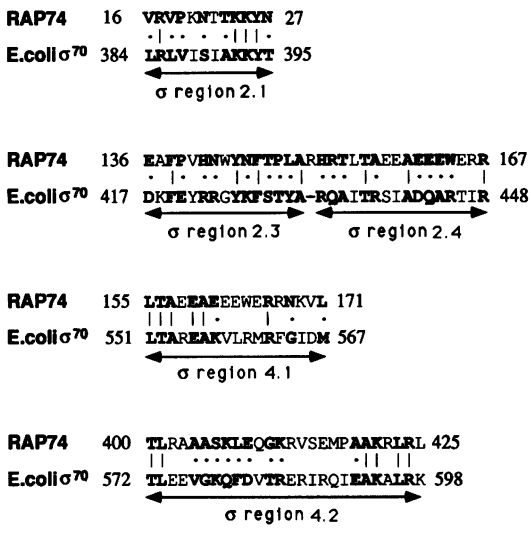

Figure 7. Alignment of RAP74 sequence with σ^{70}. The predicted RAP74 amino acid sequence was compared with that of the major σ factor of *E.coli*, σ^{70}, using the Sequence Analysis Software Package, GCG version 7.1 of the Institute of Medical Science of University of Tokyo. Bold face letters represent amino acids identical or similar between two proteins with the numbers of amino acid residues.

RAP30 has been reported to contain a sequence homologous to region 1b and 2 of σ factors of bacteria, which has been shown to be a binding site for the core component of *E.coli* RNA polymerase and possibly for mammalian RNA polymerase II (19,40). Since there are sequence similarities between the large subunits of prokaryotic and eukaryotic RNA polymerases (41,42,43,44,45), it is likely that RAP30 interacts with the largest subunit of mammalian RNA polymerase II. Thus, RAP30 forms a large complex with RAP74 and RNA polymerase II at the closely located sequences, 1−110 and 111−152, respectively, although these sequences might overlap. In contrast to hydrophobic interaction between RAP30 and RAP74, binding of RAP30 to RNA polymerase II is likely to be electrostatic, easily dissociable at a salt concentration of 0.2−0.4M (24,46).

RAP74 is also found to contain σ homology sequences as shown in Fig. 7. N-terminal homology sequences correspond to regions 2.1, 2.3 and 2.4 of *E.coli.* σ^{70}, while the last two homologies are repeated in tandem and also overlap with the sequences homologous to region 4.1. At the C-terminal sequences 400−425, there is another sequence homologous to region 4.2. Interestingly, these sequences are localized in the N-terminal domain essential for associating with RAP30 (Fig. 3) or the C-terminal domain required for the transcription initiation activity of TFIIF (Fig. 5B). However, functional significance of the σ homology sequences of RAP74 are unknown. A more detailed mutagenesis analysis of these sites, would be essential to elucidate the molecular significance of these sequences during the heteromeric interaction of TFIIF and its association with RNA polymerase II.

Reconstruction of in vitro transcription activity by deletion mutants of RAP74 showed that catalytic activity of r74 requires the presence of both N-terminal and C-terminal regions. Since N-terminal sequences are involved in associating with RAP30, thereby forming a complex with RNA polymerase II, the C-terminal region probably plays an additional role for full activity. It is intriguing to note that the C-terminal region of RAP74, which is essential for transcription activity, is homologous to a region of s between 400 and 425 (Fig. 7). It also contains regions homologous to the phosphate binding loop (P-loop) of human thymidine kinase, which is imperfectly repeated between 428 and 446, as reported by others (21).

RAP74 is proposed to be structurally separated into three regions, a globular N-terminal domain (1−179), a charged domain (180−356), and a globular C-terminal domain (357−517) (20,21). Our results indicate that the functional domains are apparently correlated with the proposed structure. A minimum sequence of amino acids 62−171 essential for binding to RAP30 is localized in the N-terminal globular domain, and there are sequences required for transcription activity at the C-terminal globular domain. The central charged domain was not sufficient for either heteromeric interaction with RAP30 or transcription activity by our assays although a small portion of the N-terminal sequence of this domain stimulated association with RAP30 (Fig. 3). We observed that the bacterially expressed RAP74 protein forms a tetrameric aggregate in solution, and this property was assigned to an internal region from 73 to 356 (data not shown). Therefore, the central domain might provide the protein with the ability to form the tetrameric self-aggregate seen with native HeLa TFIIF (24).

TFIIF is required for RNA polymerase II to associate with DAB complex (30,31), while neither the RAP30 nor RAP74 component of TFIIF are likely to interact directly with a component(s) of the DAB complex. Recently physical interaction of the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II with TATA-binding protein has been shown and it was proposed that the CTD is one of the components of the RNA polymerase II that interacts with the DAB complex during recruitment of the enzyme (47,48). TFIIF, possibly along with TFIIE, might be involved in a change of DNA conformation around the initiation site that is induced by binding of RNA polymerase II to the preformed DAB complex and represents a transition from a closed to an open complex (30).

Overall there remain many questions about molecular function of TFIIF in formation of preinitiation complex, initiation and elongation of transcription. Our present study could be an initial step toward elucidating the functional role of TFIIF in a complicated process of protein−protein interaction in transcription.

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