Transcription Factor (TF)-like Nuclear Regulator, the 250-kDa Form of Homo sapiens TFIIIB", Is an Essential Component of Human TFIIIC1 Activity*

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The general human RNA polymerase III transcription factor (TF) IIIC1 has hitherto been ill defined with respect to the polypeptides required for reconstitution of its activity. Here we identify Homo sapiens TFIIIB (HsBdp1) as an essential component of hTFIIIC1 and hTFIIIC1-like activities. Several forms of HsBdp1 are described. The 250-kDa form of HsBdp1, also designated the “transcription factor-like nuclear regulator,” strictly co-eluted with TFIIIC1 activity over multiple chromatographic purification steps as revealed by Western blot with anti-HsBdp1 antibodies and by MALDI-TOF analysis. In addition, TFIIIC1 activity could be depleted from partially purified fractions with anti-HsBdp1 antibodies but not with control antibodies. Moreover, highly purified recombinant HsBdp1 could replace TFIIIC1 activity in reconstituted transcription of the VAI gene in vitro. Furthermore, smaller proteins of ~90–150 kDa that were recognized by anti-HsBdp1 antibodies co-eluted with TFIIIC1-like activity. Finally, cytoplasmic extracts from differentiated mouse F9 fibroblast cells that lacked TFIIIC1 activity could be made competent for transcription of the VAI gene by the addition of TFIIIC1, TFIIIC1-like, or recombinant HsBdp1. These results suggest that HsBdp1 proteins represent essential components of TFIIIC1 and TFIIIC1-like activities.

RNA polymerase III transcribes genes encoding small, untranslated RNAs including tRNA, 5 S rRNA, and U6 small nuclear RNA genes (reviewed in Ref. 1). In the yeast Saccharomyces cerevisiae, genes transcribed by RNA polymerase III are governed by promoter elements comprised of A and C boxes (type 1) or A and B boxes (type 2) that are located downstream of the transcription initiation site. Two transcription factors (TF), TFIIIB and TFIIIC, are necessary and sufficient for the transcription of type 2 genes (tRNA), whereas transcription of the type 1 S RNA gene requires, in addition, TFIIIA. S. cerevisiae (Sc) TFIIIC is a stable complex of six polypeptides, whereas ScTFIIIB consists of a less stable association of three components, namely the ScTBP, ScBrf1, and ScBdp1 proteins (reviewed in Refs. 1–3; for the new nomenclature for TFIIIB components, see Ref. 4).

In human cells, three distinct promoter types have been described. In addition to the intragenic type 1 and type 2 promoters similar to those found in yeast, there exist promoters (type 3, found in U6 and 5S RNA genes) that are located exclusively within the transcription initiation site. Primary promoter recognition of type 3 promoters is achieved by PBP/PTF/SNAPc, whereas type 2 or type 1 promoters are primarily recognized by TFIIIC2 alone or by TFIIIC2 and TFIIIA, respectively (for review, see Refs. 1–3, 5). Human TFIIIC fractions can be chromatographically separated into two distinct activities, TFIIIC1 and TFIIIC2 (6, 7), that are both required for transcription of type 1 and type 2 genes. TFIIIC2 is a stable complex of five subunits and seems to represent the functional homologue of yeast TFIIIC1. Human TFIIIC1 is less well defined. It is essential only for the transcription of type 1 and type 2 genes but also for that of type 3 genes and, thus, for all known human genes transcribed by RNA polymerase III (6, 8, 9). TFIIIC1 is not an assembly factor for TFIIIBβ (defined below) but is required for the recruitment and initiation of RNA polymerase III (10, 11). Also identified are two other activities, TFIIIC1-like and TFIIIC1′, that are functionally similar to TFIIIC1 but are chromatographically distinct (9, 12–14).

Human TFIIIB has also been chromatographically separated into two functionally distinct activities. HsTFIIIBα is active in the transcription of type 3 genes, whereas HsTFIIIBβ is specific for the transcription of type 1 and 2 genes (15). Like ScTFIIIB, HsTFIIIBβ is minimally comprised of a stable HsTBP/HsBrf1 complex that associates reversibly with a human homologue (HsBdp1) of ScBdp1 (16, 17). HsTFIIIBα activity does not reflect a highly stable complex of proteins but rather a loose association of HsTBP, HsBrf2, and HsBdp1 that can be assembled on a U6 promoter in a stepwise fashion in vitro (15, 17–21). Two forms of HsBdp1 were shown to be active in the transcription of U6/7SK and VAI genes in vitro. HsBdp1(1–846) is comprised of the N-terminal 846 amino acids (TFIIIB150) (17) (NCBI accession number AA09268), whereas HsBdp1(1–1388) consists of the N-terminal 1388 amino acids (Hb)19 of full-length HsBdp1. A third form of HsBdp1, the “transcription factor-like nuclear regulator,” which represents full-length HsBdp1(1–2254), has been described (22), but this protein was not analyzed for HsBdp1
activity. In this paper and for reasons of clarity and simplicity, we denote the three reported forms of HsBdp1 according to the number of amino acids (indicated within parentheses) contained within each form, as shown above. All three proteins are translation products of the same gene, and both HsBdp1-(1–846) and HsBdp1-(1–1388) likely represent splicing variants of full-length HsBdp1-(1–2254). Interestingly, Northern blot analyses have revealed several HsBdp1 transcripts, ranging in size from 1.6 to 9.5 kb and showing tissue-specific expression levels (22), that might arise from differential splicing.

According to amino acid (aa) sequence features, three parts of the HsBdp1 protein can be distinguished. The N-terminal part (aa 1–822) contains a region that shows sequence homology with SeBdp1. The central part (aa 823–1327) contains nine repeats of a 55-aa motif. The C terminus (aa 1328–2254) displays sequence motifs that also are found in topoisomerase II and elongation factor 1α.

Here we report that HsBdp1-(1–2254) is an integral component of human TFIIIC1 activity. We demonstrate that a 250-kDa protein, recognized in Western blots by anti-HsBdp1 antibodies, co-eluted with TFIIIC1 activity over several purification steps. This protein was identified by MALDI-TOF as HsBdp1-(1–2254). In addition and importantly, we show that anti-HsBdp1 antibodies depleted TFIIIC1 activity from a partially purified fraction. Furthermore, we demonstrate the ability of HsBdp1-(1–846), expressed in a baculovirus-based system and purified to homogeneity, to functionally replace TFIIIC1 in reconstituted in vitro transcription assays with partially purified components. Finally, we show that recombinant HsBdp1-(1–846) was also able to reconstitute transcription by RNA polymerase III in cytoplasmic extracts that were prepared from differentiated F9 mouse fibroblast cells and lack TFIIIC1 activity.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmid pVKU contains a single copy of the adenoviral VAI gene inserted into pUC18 (23).

Recombinant HsBdp1-(1–846)—Recombinant HsBdp1-(1–846) was expressed in a baculovirus system as described for Brfl (16). For affinity purification of Bdp1-(1–846), whole cell extracts prepared from Sf21 cells were dialyzed against Buffer B containing 300 mM KCl and 0.1% Triton X-100, whole cell extracts prepared from Sf21 cells were dialyzed against Buffer B containing 300 mM KCl and 0.1% Triton X-100, whole cell extracts prepared from Sf21 cells were dialyzed against Buffer B containing 300 mM KCl and 0.1% Triton X-100, whole cell extracts prepared from Sf21 cells were dialyzed against Buffer B containing 300 mM KCl and 0.1% Triton X-100, whole cell extracts prepared from Sf21 cells were dialyzed against Buffer B containing 300 mM KCl and 0.1% Triton X-100.

Buffers—Buffer A contained 20 mM HEPES (pH 7.9), 10% (v/v) glycerol, 3 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride. Buffer B contained 20 mM Tris-HCl (pH 7.9), 10% (v/v) glycerol, 3 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride. All protein fractions, except that containing RNA polymerase III, were subsequently subjected to chromatography over phosphocellulose (Whatman P11), generating fractions PCA, PCB, PCC, and PCD (25).

Preparation of Cytoplasmic Extract (HEK S100) and Purification of Transcription Factors—Cytoplasmic extract from human embryonic kidney cells (HEK S100) was prepared as described (24). The S100 extract was dialyzed against Buffer A containing 100 mM KCl and subsequently subjected to chromatography over phosphocellulose (Whatman P11), generating fractions PCA, PCB, PCC, and PCD (25). The PCB, PCC, and PCD fractions were subsequently dialyzed against Buffer B (60 mM KCl). All further purification steps were conducted in Buffer B.

PCB Fraction—The TFIIIB contained in fraction PCB was separated by chromatography on EMD DEAE Fractogel (Merck) into fractions containing TFIIIBα, TFIIIBβ, and RNA polymerase III as described previously (15). TFIIIBβ was further purified by chromatography over MonoQ (11), which also separated TFIIIBβ (elution from 280 to 300 mM KCl) from TFIIIC1 (elution from 200 to 250 mM KCl). TFIIIBβ activity co-eluted with HsTBP and HsBrf1 on MonoQ but was separated from any protein showing cross-reactivity with anti-HsBdp1 antibodies (data not shown). The TFIIIBβ-containing MonoQ fractions typically contained 0.4 mg/ml protein.

The EMD DEAE Fractogel fraction containing RNA polymerase III activity was further purified by chromatography over single-stranded DNA cellulose as described (18) and diluted with glycerol to a final concentration of 50% (v/v) glycerol. The protein concentration was 0.1 mg/ml.

PCC Fraction—TFIIIC1-like (TFIIHC0), TFIIHC1, and TFIIHC2 were purified by chromatography over phosphocellulose and MonoQ as described (9). A typical protein concentration of TFIIHC2 activity-containing fractions eluting from MonoQ was 0.2 mg/ml. For further purification of TFIIHC1, 4 mg of the MonoQ fraction (0.4 mg/ml) was loaded onto a 1-ml MonoS column (Amersham Biosciences) and eluted with a 30-ml linear salt gradient from 200 to 600 mM KCl. MonoS fractions containing TFIIHC1αko or TFIIHC1αα were diluted with Buffer B to a final concentration of 100 mM KCl and loaded onto a 0.7 ml MiniQ column (Amersham Biosciences). Bound proteins were eluted with a 20-ml linear gradient from 100 to 300 mM KCl. Respective peak fractions were then loaded onto a 150-ml High Prep Superdex 200 XK 10/70 column (Amersham Biosciences) and developed with Buffer B containing 200 mM KCl.

Alternatively, after chromatography on MonoS and before chromatography on MiniQ, an additional purification step on MiniS (Amersham Biosciences) was included. In this case, MonoS fractions with TFIIHC1αko or TFIIHC1αα activity were diluted to 300 mM KCl with Buffer B and applied to MiniS. Bound proteins were eluted with a 30-ml linear salt gradient from 300 to 600 mM KCl.

TFIIHC1-like was similarly purified from MonoS and Superdex 200 under identical conditions. The TFIIHC1-like fraction used in Fig. 6 was derived from MonoQ and further purified by EMD-So.3 Fractogel (Invitrogen) as described (14).

F9 Cells—F9 cell culture, extract preparation, and chromatography were performed as described (14).

In Vitro Transcription—In vitro transcription assays were reconstituted either with fractions containing 2 μg of TFIIIBβ, 50 μg of RNA polymerase III, and 1 μg of TFIIHC2 or with 30 μg of F9 parietal endomer (PE) cytoplasmic extract.

Transcription reactions (60–90 μl) contained 0.5 μg of plasmid DNA, 600 μM each ATP, CTP, and UTP, 30 μM GTP, 3 μCi of [α-32P]GTP (Hartmann), and 15 units of RNase block ribonuclease inhibitor (Eppendorf) in Buffer B containing 60 mM KCl. After incubation at 30 °C for 90 min, RNA was purified and subjected to electrophoresis on denaturing 7 m urea and 6% polyacrylamide gels. The gels were analyzed by autoradiography and a Fuji FLA-3000 Bio Imaging analyzer.

SDS-Gel Electrophoresis and Preparation for MALDI-TOF Analysis—Purified fractions containing TFIIHC1 activity were subjected to SDS-PAGE. TFIIHC1-containing fractions eluting from Superdex 200 were concentrated on Strataclean resin beads (Stratagene) as described (26). All other fractions were loaded directly onto gels. Staining of the gels was performed using Simply Blue™ Safe Stain (Invitrogen). MALDI-TOF analysis was performed at the Zentrum für Molekularbiologie (ZMBH), Zentrale Einheit Biomolekulochemie in Heidelberg.

Antibodies—Antibodies were purified as described (26). Anti-HsBdp1 antibody was raised against aa 211–357 of HsBdp1, expressed in Esherichia coli and purified under nickel-nitritolactric acid-agarose. Anti-HsTBP (15) and anti-HsBrf1 antibodies (16) were as described. 125I-labeled secondary anti-rabbit or anti-mouse antibodies were obtained from Amersham Biosciences.

Western Blot—SDS-PAGE and blotting on polyvinylidene difluoride membrane were carried out as described (15) with the following modification, i.e. the secondary anti-rabbit or anti-mouse antibody (Amersham Biosciences) was 125I-labeled. Immunocomplexes were detected by autoradiography or with a Fuji FLA-3000 Bio Imaging analyzer. Antibody solutions were preblocked by preincubation of the HsBdp1 antibody for 2 h at room temperature with a 5–10-fold excess of recombinant HsBdp1-(1–846).

Immunodepletion—Immunodepletions were performed using 1 ml HiTrap Protein G columns (Amersham Biosciences). Columns were loaded with 0.7 mg of either preimmune serum (mock) or anti-HsBdp1 antiserum. Coupling with dimethylpimelimidate was as described (18). 0.7 ml (140 μg) of a MonoQ TFIIHC1 fraction was loaded onto each column and incubated for 30 min at 4 °C. Proteins eluting with the flow-through from each column were collected and subsequently tested for their TFIIHC1 activity in reconstituted transcription in vitro.

PESTfind analysis—The analysis of possible PEST sequences was performed using the PESTfind program at the EMBNet Austria (27, 28).
RESULTS

Purification of TFIIIC1 for MALDI-TOF Analysis—The polypeptide composition of human transcription factor TFIIIC1 has not yet been resolved. In an attempt to identify the proteins required for the reconstitution of TFIIIC1 activity, we established a purification scheme (Fig. 1A) that allows the preparation of highly purified protein samples with TFIIIC1 activity. After phosphocellulose and MonoQ chromatography (6), we subjected TFIIIC1-containing fractions to chromatography over MonoS. Bound proteins were eluted with a linear salt gradient from 200 to 600 mM KCl. Interestingly, TFIIIC1 activity was split into two distinct peaks upon chromatography over MonoS (Fig. 1B). The first peak eluted at 340 mM KCl (fraction 11), and the second peak at eluted 400 mM KCl (fractions 16 and 17). This separation of both activities was reproduced with more than five independent TFIIIC1 preparations. We therefore designated the activity eluting with 340 mM KCl as TFIIIC1low (low ionic strength) and that eluting with 400 mM KCl as TFIIIC1high (high ionic strength). Neither activity could be converted into the other upon the rechromatography of each activity on MonoS or MiniQ columns, suggesting that they represent unique and different forms of human TFIIIC1 activity (data not shown).

Western blot analysis of MonoS fractions with anti-HsBdp1 antibodies revealed co-elution of TFIIIC1low and TFIIIC1high activities with a doublet of ~220–250 kDa proteins that probably correspond to full-length HsBdp1-(1–2544) (Fig. 1C). Proteins that migrated with the approximate sizes of 210, 110, 85, and 65 kDa were recognized by anti-HsBdp1 antibodies but did not co-elute with TFIIIC1 activity from MonoS (Fig. 1C). The Western blot in Fig. 1D shows the specificity of our anti-HsBdp1 antiserum (lanes 1 and 4) as compared with the commercially available anti-(Hs)TFIIIB T-20 antiserum (lanes 2 and 5) from Santa Cruz Biotechnology Inc. Both antisera detect the baculovirally expressed HsBdp1-(1–846) protein (lanes 1 and 2) and the 220–250-kDa forms of HsBdp1 (lanes 4 and 5), whereas the controls without a specific first antibody were negative (lanes 3 and 6). Furthermore, reactivity with the 220–250-kDa bands was prevented by preblocking the anti-HsBdp1 antiserum with increasing amounts of affinity-purified recombinant HsBdp1-(1–846) (lanes 10–12).

We further purified each activity, TFIIIC1low and TFIIIC1high, over two additional resins, MiniQ and Superdex 200 (Fig. 1A). Interestingly, both forms of TFIIIC1 eluted at 220 mKCl from MiniQ (Fig. 2A). As evidenced by Western blots with anti-HsBdp1 antibodies, both peaks of TFIIIC1 activity showed strict co-elution with the same 220–250 kDa protein doublet (Fig. 2B) that co-eluted with these activities after MonoS chromatography (Fig. 1C). Likewise, both TFIIIC1low and TFIIIC1high activities showed a similar elution profile close to the void volume on Superdex 200 columns, indicating that both reside in large protein complexes (see "Discussion"). In this case, TFIIIC1low and...
TFIIIC1\textsubscript{high}, active fractions again showed precise co-elution with the 220–250-kDa polypeptide. As shown in Fig. 3B, TFIIIC1 activity in a reconstituted transcription assay with the VAI gene was reconstituted with TFIIIC1. TFIIIC1\textsubscript{high} active fractions were then concentrated (see “Experimental Procedures”) and loaded onto an 8% SDS-polyacrylamide gel. The Superdex 200 fraction that contained TFIIIC1\textsubscript{low} activity showed a large number of contaminating polypeptides, thus excluding an informative MALDI-TOF analysis of TFIIIC1\textsubscript{low}. In contrast, the Sephadex 200 fraction with TFIIIC1\textsubscript{high} activity exhibited a higher degree of purity, thus allowing identification of an ~250-kDa protein that appeared to correspond to one of the anti-HsBdp1 reactive proteins that co-eluted with TFIIIC1 activity (Figs. 1C and 2B). Subsequent MALDI-TOF analysis revealed probable identity (score 110; protein scores 61 are significant; \( p < 0.05 \)) of this protein with the 250-kDa form of HsBdp1 (transcription factor-like nuclear regulator) (22).

HsBdp1 Is an Essential Part of TFIIIC1 Activity—To further determine whether HsBdp1-(1–2254) plays an essential role in reconstituting TFIIIC1 activity, we performed an immunodepletion assay with anti-HsBdp1 antibodies. TFIIIC1-containing MonoQ fractions were subjected to chromatography over HiTrap protein G columns to which either anti-HsBdp1 antibodies or preimmune antibodies (mock) had been coupled.

The flow-through fractions of these columns were tested for TFIIIC1 activity in a reconstituted transcription assay with the VAI gene. As shown in Fig. 3A, the flow-through of the column with coupled preimmune antibodies contained a level of TFIIIC1 activity comparable with that observed in the input fraction (compare lane 2 with lanes 3–9). In contrast, the flow-through from the column containing coupled anti-HsBdp1 antibodies showed almost no activity (Fig. 3A, lanes 10–16). A Western blot of the corresponding input and flow-through fractions revealed that the 220–250-kDa HsBdp1-(1–2254) doublet was nearly completely depleted by the anti-HsBdp1 column (Fig. 3B, lane 3) but left at the input level by the preimmune column (lane 2). These results suggest that HsBdp1-(1–2254) is an integral and essential component of TFIIIC1 activity. TFIIIC1, TFIIIC1-like, and Recombinant HsBdp1-(1–846) Are Functionally Exchangeable—As shown previously (9, 12) and in Fig. 4A, MonoQ chromatography of a PCC (see “Experimental Procedures”) fraction resulted in the separation of a TFIIIC1-like activity from the conventional TFIIIC1 activity. Because of their functional exchangeability in transcriptional assays, the TFIIIC1 and TFIIIC1-like fractions were compared by Western blot (Fig. 4B) with respect to the presence of HsBdp1-related polypeptides. As expected, HsBdp1 (220–250 kDa) co-eluted with TFIIIC1 activity (fraction 20). Faster migrating proteins that were detected by anti-HsBdp1 antibodies in fractions with TFIIIC1 activity (fractions 19–22) were dispensable for TFIIIC1 activity because they did not co-elute with TFIIIC1 from MonoS (Fig. 1C). In contrast, fractions with TFIIIC1-like activity (fractions 10–14) contained no detectable immunoreactive proteins of 220–250 kDa. However, many immunoreactive proteins of uncertain specificity were observed in the 70–150 kDa size range. Because this did not allow an unambiguous determination of the active form of HsBdp1, proteins containing TFIIIC1-like activity were subjected to further purification on MonoS. Surprisingly, development of the column with a linear salt gradient (200–600 mM KCl) split the activity into two distinct peaks, as was seen previously for TFIIIC1 (Fig. 5A).

TFIIIC1\textsubscript{like\textsubscript{low}} activity was recovered in fractions 11–14 (~350 mM KCl), whereas TFIIIC1\textsubscript{like\textsubscript{high}} activity was found in fractions 19 and 20 (~450 mM KCl). A Western blot indicated several immunoreactive proteins whose distributions correlated well with the two TFIIIC1-like activities. They included proteins with apparent sizes of ~150, 100, and 90 kDa, with the 150-kDa protein possibly showing the best correlation and the 90-kDa protein being the most abundant (Fig. 5B). It is thus not clear whether one or two or all of these proteins contribute to TFIIIC1-like activity.

Because we observed a strict co-elution of the 220–250-kDa forms of HsBdp1 (corresponding to HsBdp1-(1–2254)) with TFIIIC1 activities and a concordant elution of the smaller forms of HsBdp1 (probably corresponding to HsBdp1-(1–846) or HsBdp1-(1–1388)) with TFIIIC1-like activity, and because we could deplete TFIIIC1 activity with anti-HsBdp1 antibodies from a partially purified fraction, we determined whether recombinant human HsBdp1-(1–846), purified to homogeneity, was able to replace TFIIIC1 or TFIIIC1-like in the reconstituted transcription assays with the VAI gene (Fig. 6A). Whereas no transcription could be observed in a reconstituted system lacking TFIIIC1 activity (lane 1), TFIIIC1 (lane 2), HsBdp1-(1–846) (lane 3), or TFIIIC1-like (lane 4) were all able, to different degrees, to functionally complement this TFIIIC1-deficient transcription system.

Recently, we reported that TFIIIC1 plays a central role in the regulation of transcription by RNA polymerase III during differentiation of F9 fibroblasts (14). Because rHsBdp1-(1–846) could replace TFIIIC1 in the transcription assay reconstituted with purified fraction, we asked whether it could reconstitute RNA polymerase III transcription activity in inactive cytoplasmic extracts from differentiated F9 PE cells. As shown in Fig. 6B and consistent with published results, cytoplasmic...
PE cell extracts were transcriptionally active when complemented with TFIIIC1 (lane 4) but not when assayed alone (lane 1) or when complemented with a HsTBP-HsBrf1 (TFIIIB/H9252) complex (lane 2). Importantly and consistent with the results presented above, rHsBdp1-(1–846) also was able to reconstitute transcription by RNA polymerase III in these extracts.

To determine whether the lack of TFIIIC1 activity in PE cell extracts reflected the absence of Bdp1 proteins, derived chromatographic fractions were analyzed by Western blot with anti-HsBdp1 antibodies (Fig. 6C). TFIIIC1 activity-containing fractions from F9 embryonal carcinoma cell extracts that had been purified on phosphocellulose (lane 1) and MonoQ (lanes 3–9) were compared with the corresponding purified fractions from F9 PE cell extracts (lane 2 and lanes 10–16) that did not show any TFIIIC1 activity (14). Although 220–250 kDa Bdp1 polypeptides could readily be detected in the PCC (lane 1) and TFIIIC1 activity-containing MonoQ fractions from embryonal carcinoma cells (lanes 5–7), no Bdp1 could be detected in the corresponding fractions from a PE cytoplasmic extract (lane 2 and lanes 12–14). These results suggest that loss of TFIIIC1 activity during the differentiation process may reflect either reduced transcription and translation of the HsBdp1 gene or enhanced degradation of Bdp1 or both. The identification of 12 PEST consensus sites throughout the HsBdp1 protein (see “Experimental Procedures”) is consistent with a possible degradation involving the proteasome complex (27, 28).

DISCUSSION

220–250-kDa Forms of HsBdp1 Co-elute with TFIIIC1 Activity—TFIIIC1 activity was first described more than 15 years ago by Berk and co-workers (6). Since then, the molecular basis of the TFIIIC1 activity has remained obscure, and the apparent lack of a yeast homologue has heightened the mystery. TFIIIC1 activity was reported to be essential for the transcription of all genes by human RNA polymerase III (6, 8, 9). It was shown to enhance the binding of TFIIIA, TFIIIC2, and PBP/PTF/SNAPc (9, 13) to promoter sequences (reviewed in Ref. 2), and its activity was shown to be regulated during the differentiation of F9 mouse embryonic carcinoma cells into parietal endoderm cells (14). Furthermore, the description of functional counterparts of TFIIIC1 further complicated an understanding of TFIIIC1 structure and function. Thus, TFIIIC1-like and TFIIIC1/H11032 differed from TFIIIC1 in chromatographic behavior but were similar in functional properties (9, 12, 13). A central question posed by these observations is related to the molecular composition of TFIIIC1 and its related activities. Here, we were able to show that TFIIIC1 activity resides within high molecular mass forms (~220 and 250 kDa) of HsBdp1. The relevance of HsBdp1-(1–2254) to TFIIIC1 activity is supported by MALDI-TOF identification of the protein by a purified active preparation and by the loss of activity upon immunodepletion from a TFIIIC1 fraction. Surprisingly, we were able to resolve two chromatographically distinct but functionally equivalent forms of TFIIIC1 (low and high). Because HsBdp1 proteins were common to both activities, they appear to be closely related to each other. Elution of both TFIIIC1 activities close to the void volume of Superdex 200 further indicated that they might be present in large “complexes” of >600 kDa each.
This suggests that the HsBdp1 components of individual TFIIIC1 activities could be associated with a yet unknown number of interacting proteins. Variations in the complements of associating proteins might determine the distinct (340 versus 400 mM KCl) elution profiles of individual TFIIIC1 activities on MonoS chromatography.
Alternate explanations for the apparently large size of TFIIIC1 based on gel filtration are the oligomerization of HsBdp1 molecules and/or molecule shapes that differ from those of the molecular mass standards used for gel filtration chromatography. In such a situation, the chromatographically distinct forms of TFIIIC1 on MonoS chromatography could reflect different posttranslational modifications of HsBdp1. Both possibilities, i.e. association of HsBdp1 with other proteins and posttranslational modification, are not mutually exclusive, and future experiments will address these questions.

**Smaller Forms of HsBdp1 Co-elute with TFIIIC1-like Activity**—TFIIIC1-like activity was separated from TFIIIC1 activity upon chromatography on MonoQ (9) and found to co-elute with smaller HsBdp1-related polypeptides with apparent masses of 150, 100, and 90 kDa based on SDS-PAGE. Nevertheless and surprisingly, TFIIIC1-like activity could also be resolved into two distinct fractions upon subsequent chromatography on MonoS. Interestingly, it had been reported (22) that HsBdp1 RNA might be subject to alternative splicing, ultimately resulting in the translation of three different HsBdp1 proteins of 2254, 2187, and 796 amino acids. This result suggests that smaller variants of HsBdp1 might result from natural RNA splicing and translation events instead of, or in addition to, either natural or artifactual (in vitro) proteolytic degradation events. Furthermore, the presence of a variety of mRNAs encoding HsBdp1 was demonstrated by Northern blot analysis (22). Some of these RNAs show tissue-specific distributions, possibly allowing a tissue-specific synthesis of individual subforms of HsBdp1. Therefore, the smaller versions of HsBdp1 that appear to contribute to TFIIIC1-like activities could possibly fulfill cell type-specific functions in vivo.

Western blots with anti-HsBdp1 antibodies also revealed a variety of other proteins that did not perfectly co-elute with TFIIIC1 or TFIIIC1-like activities. It presently is not clear whether these proteins represent other, possibly inactive forms of HsBdp1 or whether they are unrelated in sequence to Bdp1 but share a common epitope(s).

TFIIIC1 and TFIIIC1-like activities differ in their apparent molecular mass on the basis of Superdex 200 size exclusion chromatography (> 600 kDa for TFIIIC1 in contrast to 150–250 kDa for TFIIIC1-like; data not shown). These results indicate that TFIIIC1-like might reflect HsBdp1 alone or HsBdp1 in association with only a few polypeptides, whereas TFIIIC1 might represent a complex with a number of other proteins (as discussed above).

**TFIIIC1, TFIIIC1-like, and Recombinant Human HsBdp1 (1–846) Are Functionally Exchangeable in the Reconstitution of Transcription by RNA Polymerase III**—The co-elution of HsBdp1 proteins with TFIIIC1 and TFIIIC1-like activities, as well as the depletion of TFIIIC1 activity with anti-HsBdp1 antibodies, provided good indications for a functional contribution of HsBdp1 proteins to the various TFIIIC1 activities. However, they did not formally prove that these proteins constitute TFIIIC1 or TFIIIC1-like activity. In contrast, the complementation assays with the TFIIIC1-deficient in vitro transcription system revealed that recombinant HsBdp1(1–846), with TFIIIC1 and TFIIIC1-like activities, was able to reconstitute transcription by RNA polymerase III and, thus, that an HsBdp1-derived polypeptide must contribute to the TFIIIC1 and TFIIIC1-like activities.

TFIIIC1 and rHsBdp1(1–846) were also shown to reconstitute transcription in inactive extracts from differentiated F9 PE cells. In contrast to what was observed in transcription assays reconstituted with purified factors, HsBdp1(1–846) exhibited higher activity as compared with TFIIIC1 in this assay. This variable ability of TFIIIC1 or rHsBdp1(1–846) to complement distinct reconstitution systems might indeed indicate that additional factors, variably present in individual reconstitution systems, might modulate HsBdp1 activity. However, the replacement of TFIIIC1 activity with recombinant baculovirus-expressed HsBdp1(1–846) in two completely different reconstitution systems unambiguously proves that the N-terminal 846 amino acids of HsBdp1 are sufficient to exert all essential TFIIIC1 or TFIIIC1-like functions for VAI transcription by...
RNA polymerase III in vitro. Therefore, whereas factors possibly associated with HsBdp1 might possess important functions for regulating TFIIIC1 activity or RNA polymerase III transcription activity in general, these factors are not able to replace the essential function of HsBdp1 for TFIIIC1 activity.

Complementation of transcriptionally inactive extracts from differentiated F9 cells by recombinant human HsBdp1(1–846) also shed light on another, hitherto unresolved question. Meisnner et al. (14) reported that the loss of RNA polymerase III transcription ability during F9 cell differentiation is accompanied by the loss of TFIIIC1 activity, whereas Alzubari and White (29) found that TFIIIB activity was diminished. With the finding of a functional equivalence between TFIIIC1 and HsBdp1 and the knowledge that complete HsTFIIIB activities are regulated during the differentiation of F9 cells. 

Identity of TFIIIC1 and HsBdp1 Simplifies the Picture of the Human RNA Polymerase III Transcription Apparatus—A long standing question was answered by our resolution of at least one molecular entity of TFIIIC1. The demonstration that HsBdp1 can functionally replace TFIIIC1, at least in the transcription of genes with internal promoter elements, simplifies the view of the human RNA polymerase III transcription system and its comparison to yeast. The results presented here for VAI RNA gene transcription suggest that the transcription of tRNA genes in human cells requires a DNA-binding HsTFIIIC2 complex, HsTFIIIBβ (composed of HsTBP, HsBrf1, and HsBdp1), and RNA polymerase III itself and is thus largely comparable with transcription factor requirements in the yeast S. cerevisiae. In the end, TFIIIBβ in humans has turned out to be composed of a stable HsTBP-HsBrf1 complex that associates reversibly with HsBdp1, just as for the related components of ScTFIIIB (11) (reviewed in Ref. 2). An important difference between human and yeast is the obvious existence of various human Bdp1 splicing variants and the evolutionary acquisition of the central repeats and the C-terminal extension of HsBdp1.

Diversification of the RNA polymerase III transcription apparatus during evolution is observed in the case of the U6 gene. Transcription of the U6 gene in yeast largely depends on promoter elements downstream of the transcription initiation site, whereas U6 gene promoter elements are located entirely 5′ of the transcription initiation site in humans. Evolution of two completely different U6 promoters has been accomplished by the emergence of a novel DNA-binding protein complex (PBP/PTF/SNAPc) and a novel form of human TFIIIB (HsTFIIIBα, HsTFIIIBβ) (17) containing HsBdp1 proteins, it is reasonable to assume that both studies utilized different protein fractions containing HsBdp1 (TFIIIC1 or TFIIIB) for the reconstitution of transcription. In addition, the functional interchangeability of TFIIIC1 and rHsBdp1(1–846) in reconstituting transcription by RNA polymerase III in extracts from differentiated cells also suggests that neither TBP nor Brf1 activities are regulated during the differentiation of F9 cells.

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Transcription Factor (TF)-like Nuclear Regulator, the 250-kDa Form of Homo sapiens TFIIB”, Is an Essential Component of Human TFIIC1 Activity
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