Reconstitution of Transcription from the Human U6 Small Nuclear RNA Promoter with Eight Recombinant Polypeptides and a Partially Purified RNA Polymerase III Complex*

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The human U6 small nuclear (sn) RNA core promoter consists of a proximal sequence element, which recruits the multisubunit factor SNAPc, and a TATA box, which recruits the TATA box-binding protein, TBP. In addition to SNAPc and TBP, transcription from the human U6 promoter requires two well-defined factors. The first is hB0, a human homologue of the B′ subunit of yeast TFIIIB generally required for transcription of RNA polymerase III genes, and the second is hBRFU, one of two human homologues of the yeast TFIIIB subunit BRF specifically required for transcription of U6-type RNA polymerase III promoters. Here, we have partially purified and characterized a RNA polymerase III complex that can direct transcription from the human U6 promoter when combined with recombinant SNAPc, recombinant TBP, recombinant hB0, and recombinant hBRFU. These results open the way to reconstitution of U6 transcription from entirely defined components.

The nuclear eucaryotic RNA polymerases cannot recognize their target promoters without the help of transcription factors. In the case of RNA polymerase II, basal transcription from a TATA-containing mRNA promoter can be reconstituted in vitro with a set of recombinant or entirely defined factors, both in Saccharomyces cerevisiae and in mammalian systems (1–4). In the case of RNA polymerase III, however, this has been achieved only in the yeast system, for transcription of the U6 snRNA gene (5).

The yeast U6 snRNA promoter consists of a TATA box located upstream of the transcription start site and A and B boxes located downstream of the transcription start site (6, 7). The A and B boxes recruit TFIIIC which, together with the TATA box, then recruit TFIIIB (8). In vitro, however, the yeast U6 snRNA promoter can be transcribed in the absence of the A and B boxes and TFIIIC because the TATA box is sufficient to recruit TFIIIB90/TFC5/TFC7 (5, 15, 16). All three components have been cloned, and TFIIIB has been reconstituted from recombinant subunits (5). Thus, in the case of the yeast U6 promoter, basal RNA polymerase III transcription can be reconstituted in vitro with recombinant TFIIIB and highly purified RNA polymerase III (5).

The human U6 snRNA promoter is, unlike the yeast U6 snRNA promoter, entirely located upstream of the transcription start site (see Ref. 17 and references therein). The core promoter consists of a proximal sequence element and a TATA box, and both elements are required for efficient transcription in vitro. The proximal sequence element recruits a multisubunit complex known as SNAPc (18) or PTF (19), which has been reconstituted from recombinant subunits (20). The TATA box recruits TBP (21, 22). Until recently, however, little was known about which other TFIIIB components were required for human U6 transcription because mammalian TFIIIB was only partially characterized. We recently isolated cDNAs encoding hB0, a human homologue of yeast B′, and showed that this factor is required for RNA polymerase III transcription from the U6 promoter as well as from gene-internal promoters (23). We also isolated cDNAs encoding hBRFU, a novel homologue of yeast BRF. Unlike hBRF, a previously characterized homologue of yeast BRF that is functional for transcription from gene-internal promoter but not from the U6 promoter, hBRFU is specifically required for U6 transcription (23). Thus, in an extract depleted of both BRF and BRFU, addition of recombinant BRF and recombinant TBP specifically reconstituted transcription from the adenovirus 2 VAI gene-internal promoter, but not from the human U6 promoter (17, 24). Reciprocally, addition of recombinant hBRFU reconstituted transcription from the human U6 promoter, but not from the VAI promoter (23).

A protein identical to hBRFU (called TFIIIB50) was very recently isolated by Teichmann et al. (25) as part of a complex containing several other polypeptides. In the transcription system used by these authors, addition of recombinant hBRFU to a depleted fraction did not reconstitute U6 transcription, but addition of a BRFU-containing complex isolated from HeLa cells expressing a tagged BRFU did, suggesting that the factors associated with BRFU were absolutely required for U6 transcription. In addition, a protein called BRF2 and corresponding to a splice variant of hBRF was also reported to be involved in U6 transcription (26). In this case also, addition of recombinant BRF2 could not reconstitute U6 transcription to a depleted extract, but addition of a BRF2-containing complex immunoprecipitated from human culture cells expressing tagged BRF2 could.

Here we show that we can purify an RNA polymerase III complex that, together with recombinant SNAPc, TBP, hB0, and hBRFU, can reconstitute transcription from the human U6 promoter. These results confirm the essential role of hB0, and
Experimental Procedures

Sources of Proteins—Whole cell extract was prepared from HeLa suspension cells as described (27) and dialyzed against buffer Dm (50 mM HEPES, pH 7.9, 0.2 mM EDTA, 20% glycerol, 0.1% Tween 20, 50 mM KCl, 3 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride). Glutathione S-transferase-TBP was expressed in Escherichia coli BL21(DE3) cells with the T7 expression system, as previously described (28). The protein was then bound to glutathione-agarose beads and TBP was released from the beads by cleavage with thrombin, which cleaved just after the glutathione S-transferase moiety of the fusion protein. Mono-Q SNAPc was purified as described in Ref. 29, and recombinant SNAPc was produced in insect cells and purified as described in Ref. 20. Recombinant hTBP and hBRFu were produced in E. coli and purified as described (23).

For immunoprecipitation of an RNA polymerase III complex, rabbit polyclonal anti-BN51 antibodies (CS682) directed against the last 14 amino acids of BN51 (see Fig. 1), either crude or affinity purified, were cross-linked to protein A-agarose beads (Roche Molecular Biochemicals) with the dimethyl pimelimidate method as described in Ref. 30. The beads were washed with whole cell extract at a 1:1 ratio, incubated at room temperature for 2 h with agitation, and collected by centrifugation. The beads were then washed 4 times with 120 bead volumes of buffer D100. The washed beads were then tested directly in transcription assays. Alternatively, and with similar results, material bound to the beads was eluted by incubation with 1 bead volume of buffer D100 containing 100 μg/ml of the synthetic peptide against which the antibody was raised, and the eluate was tested in transcription assays.

The RNA polymerase III complex active in U6 transcription was purified as follows. 500 ml of HeLa whole cell extract (5785 mg of protein) was first fractionated by an 18–40% ammonium sulfate precipitation. The precipitate was resuspended in 100 ml of HEDPG (25 mM HEPES, pH 7.9, 15% glycerol, 0.1% Tween 20, 3 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride). The fraction was then fractionated by an 18–40% HEDPG sucrose gradient. One ml of the sample was equivalent to that of a 200 mM KCl solution. The sample (1650 mg of protein) was then loaded onto a 240-ml P11 phosphocellulose column (Whatman). The column was washed with 3 column volumes of HEDPG200 (HEDPG with 200 mM KCl) and bound proteins were eluted with a 5-column volume HEDPG gradient extending from 200 to 1000 mM KCl. The P11 fractions containing U6 transcription activity (eluted between 550 and 850 mM KCl) were pooled (126 mg of protein), diluted with 1400 ml of buffer D100 containing 100 μg/ml of the synthetic peptide against which the antibody was raised, and the eluate was tested in transcription assays.

The RNA polymerase III complex active in U6 transcription was purified as described (31) in a total volume of 20 ml with 100 ng of pU6/Hae/RA.2 DNA template. The reactions contained 8 μl of the RNA polymerase III fractions, 10 ng of recombinant TBP, and 4–6 μl of Mono-Q SNAPc. In Fig. 7, 4 lanes were then subjected to ultracentrifugation for 11 h at 4 °C, 49,000 rpm, in a SW55 Ti rotor. 100-μl fractions were withdrawn from the top of the gradient, tested for U6 transcription, and fractionated on 5–20% SDS-polyacrylamide gels. The resolved polypeptides were visualized by staining with silver or Coomassie Brilliant Blue R-250, or characterized by immunoblotting with antibodies against known proteins.

Transcription Assay—The U6 transcription assays were performed as described (31) in a total volume of 20 μl with 100 ng of pU6/Hae/RA.2 DNA template. The reactions contained 8 μl of the RNA polymerase III fractions, 10 ng of recombinant TBP, and 4–6 μl of Mono-Q SNAPc. In Fig. 7, the reconstitutions were performed as indicated in the figure legend.

Results

An Anti-RNA Polymerase III Immunoprecipitate Can Direct U6 Transcription When Combined with Mono-Q SNAPc and Recombinant TBP—We have described before the isolation of a cDNA clone encoding the largest subunit of RNA polymerase III and the generation of an antibody directed against the very C terminus of this subunit (32). To generate a second antibody directed against RNA polymerase III, we took advantage of the published sequence of another RNA polymerase III subunit (33), the BN51 or RPC53 subunit, to generate another anti-peptide antibody. This small subunit which is unique to RNA polymerase III was originally cloned as the gene that complemented a mutation in a temperature-sensitive cell line that arrests in G1, at the non-permissive temperature (33). Since in addition to generating antibodies, we were interested in expressing recombinant BN51 to serve as a marker in immunoblots, we generated a construct for in vitro translation of full-length BN51 from a partial cDNA clone (34) and from polymerase chain reaction fragments obtained from HeLa cell RNA. The resulting open reading frame (GenBank™ AF346574) differs from the open reading frame predicted by the BN51 sequence deposited in GenBank™ (accession number M17754) by 10 insertions, one deletion, and three nucleotide changes. As a result, the protein sequence predicted by our clone, which we refer to as hRPC53, differs from the original BN51 protein sequence (accession number AAA51838) at several positions within the N-terminal region, as depicted in Fig. 1A. The hRPC53 amino acid sequence is, however, identical to that predicted by a recent entry in GenBank™ (AK026588).

We raised rabbit polyclonal antibodies against a peptide corresponding to the last 14 amino acids of the hRPC53 sequence and tested whether this antibody could co-immunoprecipitate hRPC155, the largest subunit of RNA polymerase III. As shown in Fig. 1B, hRPC155 was detected in HeLa whole cell extract, the starting material for the immunoprecipitation, and in material immunoprecipitated by anti-hRPC53 antibodies, but not in material immunoprecipitated by preimmune antibodies (compare lanes 1 and 3 to lane 2). Thus, the anti-hRPC53 antibodies co-immunoprecipitated the largest subunit of RNA polymerase III, suggesting that they did not disrupt the multisubunit enzyme.

Wang and colleagues (35) reported the isolation of an RNA polymerase III holoenzyme capable of directing transcription from RNA polymerase III genes with gene internal promoters. We tested the ability of the RNA polymerase III complex immunoprecipitated with the anti-hRPC53 antibody to direct transcription from the gene external human U6 snRNA promoter, either on its own or combined with recombinant TBP (rTBP), a partially purified SNAPc fraction (Mono-Q SNAPc, 29), or both. As shown in Fig. 2A, neither rTBP alone, Mono-Q SNAPc alone, nor rTBP together with Mono-Q SNAPc, could direct U6 transcription (lanes 1–3). Similarly, immunoprecipitates obtained with preimmune antibodies, or mock reactions performed with just protein A beads, showed no or little activity, with or without added rTBP and Mono-Q SNAPc, (lanes 8–15). The anti-hRPC53 immunoprecipitate alone, or complemented with Mono-Q SNAPc, or TBP only, showed little or no activity (lanes 4–6). In contrast, the anti-hRPC53 immunoprecipitate complemented with both rTBP and Mono-Q SNAPc resulted in high levels of U6 transcription activity (lane 7).

To exclude the possibility that factors required for U6 transcription co-immunoprecipitated with the hRPC53 subunit of RNA polymerase III because of indirect interactions with hRPC53 through bridging DNA rather than because of protein-protein interactions, we performed immunoprecipitations in the presence of the DNA intercalating agent ethidium bromide.
This agent can eliminate interactions occurring through bridging DNA (36). As shown in Fig. 2

B.

The anti-hRPC53 antibodies immunoprecipitated a complex active in U6 transcription when complemented with Mono-Q SNAPc and rTBP regardless of whether the immunoprecipitation was performed in the absence (lane 3) or presence (lane 5) of ethidium bromide. Together, these results suggest that the anti-hRPC53 antibodies can immunoprecipitate an RNA polymerase III complex that, together with rTBP and biochemically purified Mono-Q SNAPc, is capable of directing accurate and efficient U6 transcription.

A Partially Purified RNA Polymerase III Can Reconstitute U6 Transcription When Combined with Mono-Q SNAPc and rTBP—

The results above suggest that an immunoprecipitated RNA polymerase III complex contains all factors required for U6 transcription except for SNAPc, factors other than SNAPc that might be contained in the Mono-Q SNAPc fraction, and TBP (in a form that is functional for U6 transcription). To determine whether we might be able to purify such a complex by another method than immunoprecipitation, we fractionated a HeLa whole cell extract as illustrated in Fig. 3. We tested the fractions for their ability to direct U6 transcription activity when combined with Mono-Q SNAPc and rTBP, and for the presence of the largest subunit of RNA polymerase III. As shown in Fig. 4, the fractions containing the peak of U6 transcription activity (Fig. 4A) also contained the peak of RNA polymerase III as measured by the presence of the hRPC155 subunit (Fig. 4B).

The proteins present in the phosphocellulose peak of activity were further fractionated by successive chromatography on Mono-S and Mono-Q columns, followed by fractionation on a sucrose gradient, as described under “Experimental Procedures.” Fig. 5 shows the activity profile of the sucrose gradient fractions. U6 transcription activity was recovered after the 669-kDa size marker in a broad peak with maximum activity in fraction 14. This activity profile coincided with the elution profile of the largest subunit of RNA polymerase III (Fig. 6A), panel hRPC155) as well as with that of the hRPC53 subunit of RNA polymerase III (Fig. 6A), suggesting co-purification with the bulk of RNA polymerase III. Furthermore, the recovery of U6 transcription activity in a single peak after sucrose gradient centrifugation suggested that the activity is contained within a complex, consistent with the observation that it can be immunoprecipitated with antibodies directed

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against an RNA polymerase III subunit (Fig. 2, above).

We tested the sucrose gradient fractions, as well as the Mono-Q SNAPc fraction used for U6 transcription, for the presence of RNA polymerase III factors, in particular factors that constitute the human TFIIIB activity. The results are shown in Fig. 6. We could detect traces of both TBP and BRF1 peaking with RNA polymerase III subunits, suggesting association of at least some of this complex with RNA polymerase III. In contrast, hB0, the human homologue of the B’ subunit of S. cerevisiae TFIIIB (23), peaked before the activity, and BRFU, the TFIIIB-related factor that functions in U6 transcription, was undetectable in all the sucrose gradient fractions (not shown). It is noteworthy that unlike BRF, both hB0 and hBRFU were present at substantial levels in the Mono-Q SNAPc fraction (Fig. 6, B, lane 12, and C, lane 2), which could thus provide these activities in the transcription reactions in Fig. 5. Both the La protein, which has been implicated in RNA polymerase III transcription termination and in recycling of RNA polymerase III (37–43), and TFIIA, which has been reported to be required or stimulate RNA polymerase III transcription in a mammalian system (44, 45), were detectable in the 18–40% ammonium sulfate precipitate but not in the sucrose fractions or the Mono-Q SNAPc fraction (note that the faint band present in all the lanes of the anti-La panel does not co-migrate with the La signal in the 18–40% ammonium sulfate precipitate). Finally, Oct-1, which binds to the octamer sequence in the DSE of RNA polymerase II and RNA polymerase III snRNA promoters and activates snRNA gene transcription, was not detected in the sucrose gradient fractions. Together, these results suggest that U6 transcription activity co-elutes with an RNA polymerase III complex containing RNA polymerase III and a subset of TFIIIB polypeptides, specifically the TBP-BRF complex. We refer to this complex as “sucrose gradient RNA polymerase III” (SG pol III).

U6 Transcription Can be Reconstituted by a Combination of SG Pol III, rTBP, rSNAPc, hB0, and hBRFU—The SG pol III complex was active when complemented with rTBP and biochemically purified Mono-Q SNAPc. Since we can obtain functionally active recombinant SNAPc (20), we asked whether we could replace Mono-Q SNAPc with recombinant SNAPc. We combined a peak sucrose gradient fraction as judged from hRPC155 immunoblots with either rTBP and Mono-Q SNAPc, or rTBP and SNAPc. Only the combination containing Mono-Q SNAPc resulted in U6 transcription, even though both Mono-Q SNAPc and SNAPc were active for U6 transcription when tested by complementation of a SNAPc-depleted extract (not shown). We then tested whether we might be able to recover U6 transcription by addition of hB0, hBRFU, or both factors combined. The results are shown in Fig. 7.

When we combined SG pol III with rTBP and increasing amounts of Mono-Q SNAPc, we observed U6 transcription (lanes 1 and 2), but a combination of just TBP and Mono-Q SNAPc (lane 3), or the SG pol III fraction on its own (lane 4), were inactive, as expected. When we complemented SG pol III, rSNAPc, and TBP with increasing amounts of rhB0 (lanes 5–7), or increasing amounts of rhBRFU (lanes 8–10), we obtained very low or undetectable amounts of U6 transcription. In sharp contrast, when we complemented SG pol III, rSNAPc, and rTBP with increasing amounts of hBRFU together with two fixed amounts of hB0, we could in each case detect efficient U6 transcription (lanes 11–13 and 14–16). The efficiency of U6 transcription was not greatly affected by increasing the amounts of either hBRFU or hB0, suggesting that the lowest amounts of either factors were already saturating relative to the amounts of the other factors added in the reconstitution. Together, these results allow us to reach several important conclusions. First, they reveal that the Mono-Q SNAPc fraction contains three factors functional for U6 transcription: SNAPc, hB0, and hBRFU. This is consistent with the presence of hB0 and hBRFU in this fraction as determined by immunoblot (see Fig. 6 above). Second, they confirm our previous depletion results (23) that indicated an absolute hB0 and hBRFU requirement for U6 transcription. And third, they show that all factors

FIG. 2. An anti-hRPC53 immunoprecipitate is active for U6 transcription when complemented with recombinant TBP and Mono-Q SNAPc. A, material immunoprecipitated by protein A-agarose beads cross-linked to either anti-hRPC53 (lanes 4–7) or preimmune (lanes 8–11) antibodies, or by protein A-agarose beads alone (lanes 12–15) was complemented with just buffer, rTBP alone, Mono-Q SNAPc alone, or both rTBP and Mono-Q SNAPc, as indicated above the lanes, and tested for U6 transcription. The transcription activities of rTBP alone (lane 1), Mono-Q SNAPc alone (lane 2), or rTBP together with Mono-Q SNAPc (lane 3), as well as that of whole cell extract complemented with rTBP and Mono-Q SNAPc (lane 16) are shown as controls. B, immunoprecipitations performed with anti-hRPC53 antibodies (lanes 3 and 5) or preimmune antibodies (lanes 4 and 6) either in the absence (lanes 3 and 4) or presence (lanes 5 and 6) of 75 μg/ml of ethidium bromide were tested for U6 transcription in the presence of added rTBP and Mono-Q SNAPc. The transcription activities of rTBP and Mono-Q SNAPc (lane 1) and of whole cell extract (lane 2) are also shown as controls.

FIG. 3. Purification scheme of the RNA polymerase III complex active for U6 transcription.
required for basal U6 transcription besides SNAPc, TBP, hBRFU, and hB0 are contained within the SG pol III fraction.

**DISCUSSION**

We have reconstituted U6 transcription from a combination of four recombinant factors, TBP, SNAPc, B0, and BRFU, and a purified RNA polymerase III fraction. Our data suggest that the activity in the RNA polymerase III fraction is constituted by RNA polymerase III and factors associated with it, rather than by a number of spuriously co-purifying factors. Indeed, the activity forms a single peak in a sucrose gradient (Fig. 5), and U6 transcription could also be reconstituted by an anti-RNA polymerase III immunoprecipitate (combined with Mono-Q SNAPc and rTBP) (Fig. 2). Both of these observations suggest that the activity corresponds to an RNA polymerase III-containing complex. This complex is probably different from the "holoenzyme" described by Wang et al. (35), because unlike the holoenzyme, it was not disrupted by chromatography on phosphocellulose or KCl concentrations above 300 mM.

The RNA polymerase III-containing complex contains all factors besides TBP, SNAPc, B0, and BRFU absolutely required for basal transcription from the U6 promoter. Its composition is, therefore, of high interest. Our immunoblot results suggest that the complex is not enriched in the La protein nor the transcription factor IIA. We cannot exclude, of course, that traces of these factors are present and are sufficient for transcription. Nevertheless, the data do not lend support to the idea that these factors are absolutely required for transcription in *vitro*. In the case of TFIIA, we showed before that depletion of an extract with anti-TFIIA antibodies debilitated U6 transcription, but efficient transcription was restored by addition of rTBP (47). Thus, the anti-TFIIA depletion debilitated U6 transcription because it lowered the amounts of TBP, probably because TBP was associated with TFIIA. This suggested that TFIIA is not required for U6 transcription in *vitro*, but it also suggested that the TBP that is functional for U6 transcription is associated with TFIIA (47). One possible interpretation of these results is that TFIIA may play an anti-repressor role for basal U6 transcription, for example, by displacing from TBP a repressor such as Mot1, which can repress transcription from the human U6 promoter in *vitro* (48). Such an anti-repressor function would not be revealed in an assay lacking the repressor, such as, most likely, the reconstitution assay used here. Similarly, it is entirely possible that the La protein is involved in RNA polymerase III transcription in *vivo* in a function that is not tested in the reconstitution assay.

hBRFU/TFIIIB50 can be purified as part of a multisubunit complex and functional data suggest that subunits other than BRFU in the complex are required for U6 transcription (25). Yet, we could obtain U6 transcription by combining the SG pol III fraction with recombinant SNAPc, TBP, B0, and just BRFU (Fig. 7), consistent with our previous results in which we could restore U6 transcription in a BRFU-depleted extract by addition of just recombinant BRFU (23). Addition of BRFU was required to observe U6 transcription, indicating that this factor...
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Fig. 6. A, the sucrose gradient fractions shown in Fig. 5 were analyzed by immunoblotting with antibodies directed against hRPC155, hRPC53, TBP, and Oct-1, as indicated on the left. Lane 11 (labeled C) shows 15 μl of Mono-Q peak fraction (the load for the sucrose gradient) except in the Oct-1 panel, where it shows 15 μl of the 18–40% ammonium sulfate fraction. Lane 12 shows 15 μl of the Mono-Q SNAPc fraction. The dots above the lanes indicate the peak of U6 transcription activity. B, the sucrose gradient fractions indicated on top were analyzed by immunoblotting with antibodies directed against hRPC155, hBRF, hB0, La, and TFIIA, as indicated on the left. Lane 11 (labeled C) shows 15 μl of the 18–40% ammonium sulfate fraction. Lane 12 shows 15 μl of the Mono-Q SNAPc fraction. The dots above the lanes indicate the peak of U6 transcription activity. C, the Mono-Q SNAPc, fraction (lane 2) and whole cell extract (lane 3) were analyzed for the presence of BRFU by immunoblotting with antibodies directed against BRFU. Lane 1 shows recombinant BRFU.

was not present in the SG pol III fraction, an observation confirmed by immunoblot. Together, these results suggest that in this assay, BRFU-associated factors are either not absolutely required for U6 transcription, or are present, dissociated from BRFU, in the SG pol III fraction. In any case, the absolute requirement for BRFU in the reconstitution assay confirms the essential role of this protein in U6 transcription.

Visualization of the proteins present in the SG pol III fraction by silver staining reveals some 40 to 50 polypeptides, and sequencing by mass spectrometry identified some of them as RNA polymerase III subunits (data not shown). However, the complex will need to be purified further to facilitate the identification of the polypeptides relevant to transcriptional activity. Nevertheless, one can speculate as to what these factors may be. We expect that the complex will contain all of the RNA polymerase III subunits. S. cerevisiae RNA polymerase III consists of 17 subunits, which are listed in Table I. For all subunits except C128, C37, and C25, human homologues have been characterized, as indicated in Table I, and putative homologues of C37, C25, and parts of C128 can be found in the human ESTs and nonredundant nucleotide databases (not shown).

Although it is formally possible that RNA polymerase III itself constitutes the sole activity in the SG pol III fraction absolutely required for U6 transcription, this seems unlikely because several activities besides SNAPc and the TFIIIB activity (TBP, hB0, and hBRFU) have been reported to be required for U6 transcription. Thus, human TFIIIC activity can be separated chromatographically into two activities, TFIIIC1 and TFIIIC2 (49–52). TFIIIC2, whose subunits have been cloned (53–57), corresponds to yeast TFIIIC and is not be required for U6 transcription (58, 59). In contrast, TFIIIC1, which is not well characterized, is required for transcription of all classes of RNA polymerase III promoters including U6-type promoters (58, 59).

TFIIIC1 has been shown to strengthen the weak TFIIIC2 footprint on the B box of gene internal promoters and to extend it over the upstream A box and the downstream run of T residues that constitutes the RNA polymerase III transcription terminator (60). Moreover, some components of the TFIIIC1 fraction can bind independently to the termination region (60). These components may correspond to the TFIIIC0 fraction described by Oettel et al. (59, 61), which itself contains two activities, an activity binding to the terminator region of genes with internal promoters (called TBA) and an activity required specifically for U6 transcription (TFIIICU) (61). Recently, one of the components binding to the VAI terminator region has been identified as NF1, and shown to stimulate multiple round transcription (62). Furthermore, an immunopurified TFIIIC “holocomplex,” containing both TFIIIC1 and TFIIIC2, also contains the RNA polymerase II transcription coactivators DNA topoisomerase I and PC4 (63). Both factors strongly stimulate RNA polymerase III transcription from gene-internal promoters. Thus, some or all of these activities may be required for U6 transcription and may be present in the SG pol III complex.

The RNA polymerase III-containing complex was purified on the basis of U6 transcription activity. However, we find that this complex contains the great majority, if not all, of RNA polymerase III, indicating that it does not correspond to a U6-specific RNA polymerase III complex. This is further suggested by the observation that the complex contains some BRF, which is not involved in U6 transcription (23, 24). Thus, the
fraction may contain several RNA polymerase III-containing complexes differing by the absence or presence of just a few factors, each of which with a different specificity for different types of RNA polymerase III promoters. Alternatively, perhaps less likely, it may contain a homogenous RNA polymerase III-containing complex competent for transcription of all types of RNA polymerase III promoters. The characterization of the SG-pol III fraction should lead to the identification of all factors absolutely required for basal U6 transcription.

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