The cloned RNA polymerase II transcription factor IID selects RNA polymerase III to transcribe the human U6 gene in vitro

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Although the human U2 and U6 snRNA genes are transcribed by different RNA polymerases (i.e., RNA polymerases II and III, respectively), their promoters are very similar in structure. Both contain a proximal sequence element (PSE) and an octamer motif-containing enhancer, and these elements are interchangeable between the two promoters. The RNA polymerase III specificity of the U6 promoter is conferred by a single A/T-rich element located around position −25. Mutation of the A/T-rich region converts the U6 promoter into an RNA polymerase II promoter, whereas insertion of the A/T-rich region into the U2 promoter converts that promoter into an RNA polymerase III promoter. We show that this A/T-rich element can be replaced by a number of TATA boxes derived from mRNA promoters transcribed by RNA polymerase II with little effect on RNA polymerase III transcription. Furthermore, the cloned RNA polymerase II transcription factor TFIID both binds to the U6 A/T-rich region and directs accurate RNA polymerase III transcription in vitro. Mutations in the U6 A/T-rich region that convert the U6 promoter into an RNA polymerase II promoter also abolish TFIID binding. Together, these observations suggest that in the human snRNA promoters, unlike in mRNA promoters, binding of TFIID directs the assembly of RNA polymerase III transcription complexes, whereas the lack of TFIID binding results in the assembly of RNA polymerase II snRNA transcription complexes.

[*Key Words*: RNA polymerases II and III; snRNA genes; TATA box; proximal sequence element; transcription factor TFIID]

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The vertebrate small nuclear RNA (snRNA) genes U1–U6 are members of a growing gene family that also includes the H1 and MRP/Th RNA genes. These genes share two characteristics. First, they encode short, nonpolyadenylated RNAs that are involved in the processing of other RNA molecules. The snRNAs U1, U2, and U4–U6 are involved in mRNA splicing (see Steitz et al. 1988); U3 is involved in rRNA processing (Kass et al. 1990; Savino and Gerbi 1990); H1 RNA is the RNA component of RNase P (Baer et al. 1989; Bartkiewicz et al. 1989); and MRP/Th RNA is the RNA component of RNase MRP, an endoribonuclease that cleaves the mitochondrial RNA primer for mitochondrial replication (Chang and Clayton 1987, 1989; Gold et al. 1989). Because of this common characteristic and by analogy to tRNAs and mRNAs, whose names refer to their functions, we refer to these RNAs as pRNAs, for processor RNAs, and to their genes as pRNA genes (Hernandez 1991). Second, these genes share common *cis*-acting transcriptional elements, although some, including the U1–U5 pRNA genes, are transcribed by RNA polymerase II, whereas others, including the U6, H1, and MRP/Th pRNA genes, are transcribed by RNA polymerase III (for review, see Dahlberg and Lund 1988; Hernandez 1991). As a result, RNA polymerase II and III pRNA promoter elements are more similar to each other than to either RNA polymerase II mRNA promoter elements or RNA polymerase III promoter elements such as the internal control region (ICR) of 5S genes and the A and B boxes of tRNA genes and the virus-associated (VA) genes from adenovirus 2 (Ad2).

The transcriptional elements of RNA polymerase II and III pRNA genes are exemplified by the *Xenopus* and human U2 and U6 genes, which have been used as model systems for the characterization of the *cis*-acting elements involved in the determination of RNA polymerase specificity (Mattaj et al. 1988; Lobo and Hernandez 1989; Lescure et al. 1991). The transcriptional elements of the human U2 and U6 genes are shown in Figure 1A. In RNA polymerase II pRNA genes, such as the U2 gene, an element located 3′ of the gene, the 3′ box, directs 3′ end formation of the RNA, most probably by termination of transcription (for review, see Dahlberg and Lund 1988; Hernandez 1991). The 3′ box is recognized only by

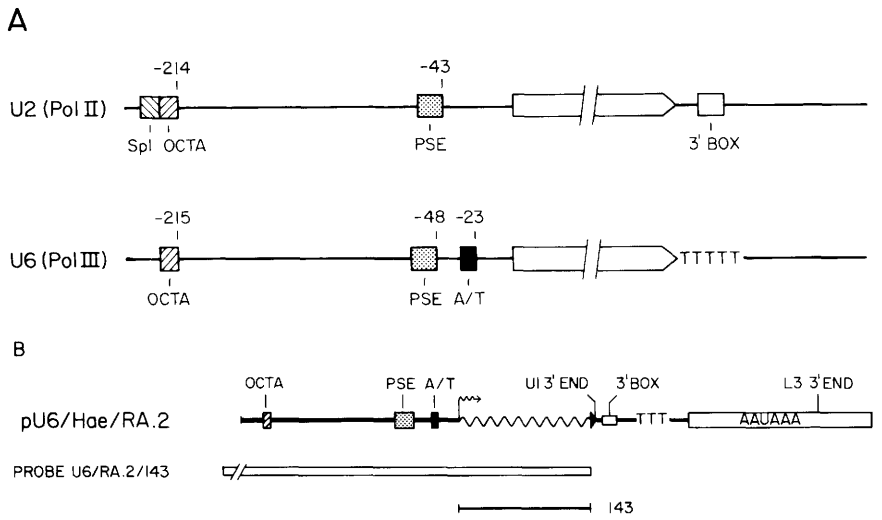


Figure 1. (*A*) Schematic representation of the promoter elements involved in RNA polymerase II transcription from the human U2 promoter and RNA polymerase III transcription from the human U6 promoter. (*B*) Structure of the hybrid U6 gene carried on the plasmid pU6/Hae/RA.2; structure of the antisense RNA probe U6/RA.2/143 and of the expected protected RNA fragment. The pU6/Hae/RA.2 insert consists of U6 5′-flanking sequences from position −241 to +1 (thick line), a fragment derived from the β-globin gene and cloned in the reverse orientation as in its natural context (wavy line), the last 6 nucleotides of the human U1 gene (solid arrowhead), 90 nucleotides of U1 3′-flanking sequences containing the U1 3′ box (open box) and a run of six Ts (labeled TTT), and the Ad2 L3 polyadenylation site. The antisense RNA probe U6/RA.2/143 is depicted by a thin box; it is protected over 143 nucleotides by RNA correctly initiated at the U6 start site of transcription.

RNA polymerase II transcription complexes derived from pRNA promoters and not by transcription complexes derived from mRNA promoters, indicating that the RNA polymerase II pRNA promoters direct the formation of transcription complexes with unique termination properties. In the RNA polymerase III pRNA genes, termination of transcription is directed by the standard RNA polymerase III termination signal, a run of T residues (Bogenhagen and Brown 1981).

The U2 and U6 promoters contain an enhancer characterized by the presence of an octamer motif. In addition, they contain, as do all pRNA promoters examined to date, a proximal sequence element (PSE), which is functionally related to the TATA box of mRNA promoters in that it is required for efficient transcription and, at least in the RNA polymerase II pRNA genes, determines the start site of transcription (for review, see Dahlberg and Lund 1988; Hernandez 1991). In both the human and *Xenopus* U2 and U6 genes, the enhancer regions and the PSEs can be interchanged with no effect on the efficiency of transcription or on the choice of RNA polymerase (Bark et al. 1987; Kunkel and Pederson 1988; Mattaj et al. 1988; Lobo and Hernandez 1989). Instead, the choice of RNA polymerase is determined by an A/T-rich sequence, similar to the TATA box of mRNA promoters, located around position −25 in the U6 promoter. In the human U6 gene, mutation of this element converts the U6 promoter into an RNA polymerase II promoter, whereas seven point mutations in the U2 promoter that create a TATA box convert the U2 promoter into a predominantly RNA polymerase III promoter (Lobo and Hernandez 1989). Thus, the A/T-rich sequence is a dominant element that defines the U6 promoter as an RNA polymerase III promoter.

Because the vertebrate U6 genes contain promoter elements that are very different from those of other RNA polymerase III genes, it is not clear whether the U6 genes use the factors involved in transcription of the 5S, tRNA, and VA genes, namely TFIIIA, TFIIIB, and TFIIIC (for review, see Geiduschek and Tocchini-Valentini 1988). These transcription factors owe their name to their elution profile from a phosphocellulose column (see Segall et al. 1980; Shastry et al. 1982). Thus, TFIIIA activity elutes in the flowthrough fraction, or A fraction; TFIIIB activity elutes in the 100–350 mM KCl fraction, or B fraction; and TFIIIC activity elutes in the 350–600 mM KCl fraction, or C fraction. Transcription of the 5S genes requires TFIIIA, which binds to the internal control region (ICR) and allows the subsequent binding of the other required factors, namely TFIIIC, TFIIIB, and RNA polymerase III (Braun et al. 1989; Gabrielsen et al. 1989; Kassavetis et al. 1989; and references therein). In contrast, transcription of the tRNA and VA genes is not dependent on TFIIIA but requires only TFIIIB; TFIIIC, which binds to the A and B box promoter elements of these genes; and RNA polymerase III (Braun et al. 1989; Gabrielsen et al. 1989; Kassavetis et al. 1989; and references therein).

The 600–1000 mM KCl fraction is not required for RNA polymerase III transcription but is required for RNA polymerase II transcription, and in a fractionation scheme for RNA polymerase II transcription factors, corresponds to the D fraction (Matsui et al. 1980; Samuels et al. 1982; Davison et al. 1983; Conaway et al. 1990). The gene for one of the active components of the D fraction, the TATA box-binding protein TFIID, has been cloned, first from *Saccharomyces cerevisiae* (Cavallini et al. 1989; Eisenmann et al. 1989; Hahn et al. 1989; Horikoshi et al. 1989; Schmidt et al. 1989) and then from a number of other species (Fikes et al. 1990; Gasch et al.

1990; Hoey et al. 1990; Hoffmann et al. 1990a,b; Kao et al. 1990; Peterson et al. 1990). TFIID binds to the TATA box of mRNA-encoding genes and directs basal transcription even in the absence of other components of the D fraction (Buratowski et al. 1988; Cavallini et al. 1988; Hoey et al. 1990; Hoffmann et al. 1990a; Pugh and Tjian 1990).

RNA polymerase III transcription of the mouse U6 gene was shown not to require TFIIIA and to require at least one factor different from those involved in 5S and tRNA gene transcription (Reddy 1988). Here, we show that RNA polymerase III transcription from the human U6 promoter can be reconstituted by a combination of the B and C or B and D fractions. Surprisingly, the C and D fractions can be replaced by human TFIID produced in *Escherichia coli*, and the resulting U6 transcription is accurate and dependent on the same promoter elements as U6 transcription in vivo or in a crude extract. TFIID binds to the wild-type U6 A/T-rich element but not to mutated versions of the A/T-rich element which, in vivo, switch the U6 promoter to an RNA polymerase II promoter. Thus, paradoxically, although in the human U6 gene, the A/T-rich element is the element responsible for RNA polymerase III selection, it seems to function by binding the key transcription factor involved in RNA polymerase II transcription from mRNA promoters, the TATA box-binding protein TFIID. In contrast to mRNA promoters, RNA polymerase II transcription from pRNA promoters can apparently occur only if the A/T-rich region is absent or mutated such that TFIID cannot bind.

Results

Figure 1B shows the structure of the parent construct, pU6/Hae/RA.2 (Lobo and Hernandez 1989), which was used in the experiments described here. It contains the U6 promoter fused to a fragment derived from the β-globin gene. Downstream of the β-globin sequences are three 3′-end formation signals in succession: the last 6 nucleotides of the U1 gene (solid arrowhead) and the U1 3′ box (open box), which together direct the formation of discrete 3′ ends on transcripts derived from RNA polymerase II pRNA promoters (Hernandez 1985); a run of T residues, which constitutes an RNA polymerase III termination signal (Bogenhagen and Brown 1981); and the Ad2 L3 polyadenylation site, which constitutes an efficient 3′-end formation signal for transcripts derived from RNA polymerase II promoters. Transcription from this construct and a number of derivatives was analyzed in transfected HeLa cells, in crude nuclear extracts, and in fractionated in vitro transcription systems. Whereas the in vivo transfection assay allows us to monitor both RNA polymerase II and III transcription from pRNA genes, the in vitro assays score only RNA polymerase III transcription because RNA polymerase II transcription from pRNA genes is not reproduced efficiently in our extracts. RNA transcripts were analyzed by primer extension, or by RNase T_1 protection of an antisense RNA probe that extends from upstream of the transcriptional start site to a position within the hybrid gene and is protected over 143 nucleotides by correctly initiated RNA (see Fig. 1B, probe U6/RA.2/143).

RNA polymerase III transcription from the U6 promoter can be directed by TATA boxes derived from mRNA-encoding genes but cannot tolerate variations in the spacing between the TATA box and the PSE

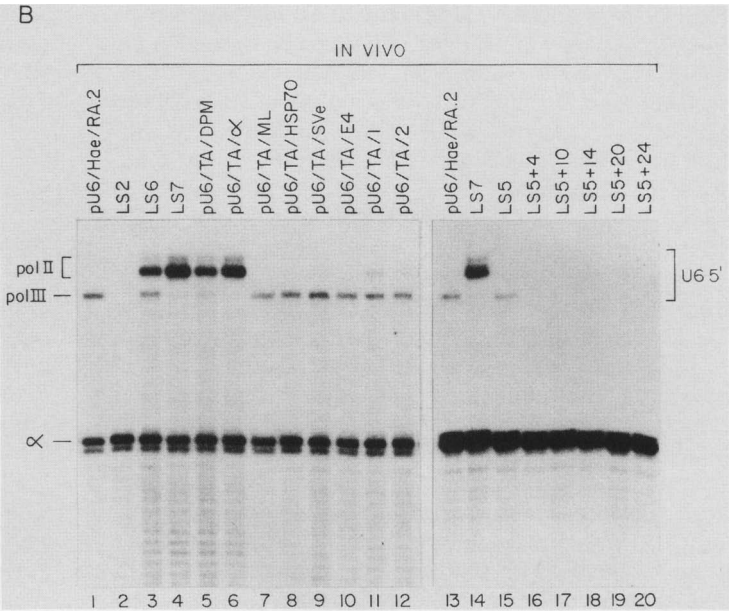
The U6 A/T-rich region is reminiscent of the TATA box of mRNA genes; therefore, we tested whether it could be replaced by TATA boxes known to be involved in RNA polymerase II transcription from mRNA-encoding genes. Figure 2A, top line, shows the sequence of the wild-type U6 A/T-rich element. It consists of a stretch of 9 A and T residues and contains an inverted perfect match (TTTATA) to the consensus TATA box sequence TATAAA. The linker scanning LS6 and LS7 mutations were tested previously and result in partial (LS6) or nearly complete (LS7) conversion of the U6 promoter into an RNA polymerase II pRNA promoter that directs initiation of transcription at sites slightly upstream of the natural RNA polymerase III U6 start site (Lobo and Hernandez 1989). We created a series of additional mutants in the A/T-rich region (Fig. 2A). pU6/TA/DPM is a double-point mutant that disrupts the TATA box by the introduction of a C and a G residue. In the other constructs, the U6 TATA box was replaced by the TATA boxes from the human α-globin promoter (pU6/TA/α), the Ad2 major late promoter (pU6/TA/ML), the human HSP70 promoter (pU6/TA/HSP70), the SV40 early region (pU6/TA/SVe), and the Ad2 E4 promoter (pU6/TA/E4). pU6/TA/1 carries a consensus TATA box, whereas pU6/TA/2 carries an inverted consensus TATA box.

These different constructs were transfected into HeLa cells along with the human α-globin gene that served as an internal reference. The resulting RNAs were analyzed by primer extension with a mixture of two primers, one complementary to RNA derived from the hybrid U6 genes and the other to RNA derived from the α-globin internal reference. As shown in Figure 2B, the wild-type U6 promoter gave rise to RNA correctly initiated at position +1 (lane 1), whereas the constructs LS6 and LS7 gave rise to a new signal corresponding to initiation by RNA polymerase II at positions −3 and −4 relative to the U6 start site (lanes 3 and 4). The construct LS2 contains a debilitated PSE and, as expected, did not produce levels of RNA detectable in this assay (lane 2). pU6/TA/DPM, with a double-point mutation in the A/T-rich region, directed transcription mainly from the upstream RNA polymerase II start site (lane 5).

Surprisingly, all of the constructs with TATA boxes from mRNA-encoding genes were efficiently transcribed by RNA polymerase III (Fig. 2B, lanes 7–10), with only one exception—the construct with the short TATA box of the α-globin gene (lane 6). The constructs pU6/TA/1 and pU6/TA/2 showed an intermediate phenotype; they directed efficient RNA polymerase III transcription and, in addition, a low level of transcription from the upstream RNA polymerase II start site. RNase T_1 protec-

A

pU6/Hae/Ra.2	TTTCTTGGC TTTATATAT CTTGTGG
LS6	..**agactcg ag**TATATAT .....
LS7	..... **TTctcgagc** .....
pU6/TA/DPM	..... **TTTcTAgAT** .....
pU6/TA/α	..... **aTaAacccT** .....
pU6/TA/ML	..... **TaTAaAagg** .....
pU6/TA/HSP70	..... **TTataAaAg** .....
pU6/TA/SVe	..... **TTTATtTAT** .....
pU6/TA/E4	..... **TaTATATAc** .....
pU6/TA/1	..... **TaTAaAcgg** .....
pU6/TA/2	..... **TTTATAcgg** .....



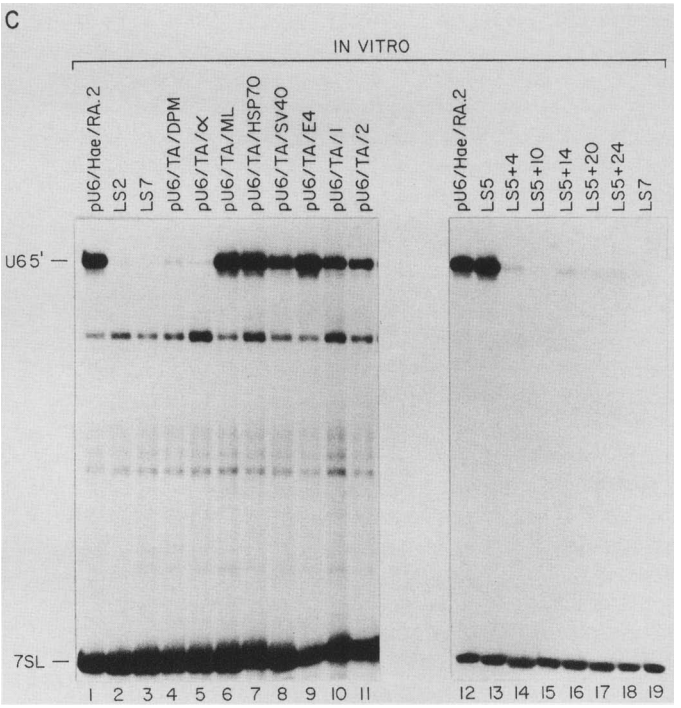


Figure 2. The U6 A/T-rich element can be replaced by several different TATA boxes derived from mRNA promoters. (*A*) Sequence of the A/T-rich element and surrounding region in the parent construct pU6/Hae/RA.2 and the indicated derivatives. (*B*) The plasmids indicated above the lanes were transfected into HeLa cells together with the internal reference plasmid pα1x72. The resulting RNAs were analyzed by primer extension with a mixture of two primers, one complementary to the β-globin portion of the hybrid RNAs derived from the U6 test plasmids and the other to α-globin RNA derived from pα1x72. The constructs LS5 + 4, LS5 + 10, LS5 + 14, LS5 + 20, and LS5 + 24 carry insertions of 4, 10, 14, 20, and 24 bp between the proximal element and the A/T-rich element. (*C*) The plasmids indicated above the lanes were incubated in a crude nuclear extract, and the resulting RNAs were analyzed by RNase T_1 protection with the U6/RA.2/143 probe. A second probe complementary to 7SL RNA endogenous to the extract was included to monitor RNA recovery. Note that the band that appears below the U6 5′ band was not reproducibly obtained and does not correspond to an RNA polymerase II or III transcription product, because it is insensitive to low and high concentrations of α-amanitin.

tion with a probe that extends from downstream of the L3 polyadenylation site to upstream of the transcription start sites (probe U6/RA.2/198 in Lobo and Hernandez 1989) indicated that the transcripts initiated at +1 ended in the run of T residues, whereas the transcripts initiated at −3, −4 ended at the U1 3′ box, as expected for transcripts derived from an RNA polymerase III and an RNA polymerase II pRNA promoter, respectively (data not shown; see Lobo and Hernandez 1989). Thus, the 9-bp U6 A/T-rich element can be replaced by a wide variety of TATA boxes with little effect on RNA polymerase III transcription as long as the stretch of uninterrupted As and Ts is at least 7 bp. Stretches of 6 A and T residues, as in the pU6/TA/1 and pU6/TA/2 mutants, promote a low level of RNA polymerase II transcription, whereas a stretch of only 5 As and Ts, as in the pU6/TA/α construct, converts the U6 promoter into an RNA polymerase II pRNA promoter.

Transcription of the same constructs was also tested in a crude nuclear HeLa cell extract. In this system, RNA polymerase II transcription from pRNA genes is undetectable, and U6 promoters with debilitated TATA boxes

therefore show a reduced level of RNA polymerase III transcription but no switch to RNA polymerase II transcription (Lobo and Hernandez 1989). As shown in Figure 2C, lanes 1–11, there is a good correlation between the levels of RNA polymerase III transcription observed in vitro and in vivo.

In all of the TATA box mutant constructs described above, the spacing between the PSE and the different TATA boxes was kept identical to the spacing in the wild-type U6 promoter. To determine whether exact spacing was required for efficient RNA polymerase III transcription, we modified a derivative of the wild-type U6 promoter (LS5; see Lobo and Hernandez 1989) by inserting 4, 10, 14, 20, and 24 bp between the PSE and the A/T-rich element. These constructs were tested in vivo and in the crude nuclear extract. As shown in Figure 2,B (lanes 16–20) and C (lanes 14–18), RNA polymerase III transcription was severely reduced by even the smallest insertion. Interestingly, these constructs did not direct RNA polymerase II transcription in vivo as determined by primer extension (Fig. 2B) and 3′-end mapping of the RNAs by RNase T_1 protection (not shown). Together, these results suggest that there is a stringent requirement for an exact spacing between the PSE and the TATA box for RNA polymerase III transcription. They also suggest that RNA polymerase II transcription from the U6 promoter is inhibited by the presence of a TATA box.

RNA polymerase III transcription from the U6 promoter is reconstituted by a combination of the phosphocellulose B and C/D fractions

As a first step to determine whether the U6 gene uses the same RNA polymerase III transcription factors as the well-characterized 5S, tRNA, and VA genes, we fractionated a crude nuclear extract over a phosphocellulose column to obtain the A, B, and C fractions involved in RNA polymerase III transcription (Segall et al. 1980) and the 600–1000 mM KCl D fraction involved in RNA polymerase II transcription (Matsui et al. 1980; Samuels et al. 1982; Davison et al. 1983; see also Materials and methods). In initial experiments we determined that transcription of the Ad2 VA gene was, as expected, reconstituted by a combination of the B and C fractions but not by a combination of the B and D fractions, whereas U6 transcription could be reconstituted to similar levels by a combination of the B and C fractions or the B and D fractions. The A fraction stimulated U6 transcription but was not essential for activity (data not shown). In subsequent fractionations we therefore eluted the phosphocellulose column with a 0.35 M KCl salt step to obtain the B fraction, and directly with a 1 M salt step to obtain a C/D fraction. The RNase T_1 protection in Figure 3A shows that the B (0.35 M) or C/D (1.00 M) fractions alone do not sustain U6 transcription (lanes 1 and 2), whereas a combination of the two results in U6 transcription (lane 3). The signal is resistant to low, but not high, levels of α-amanitin, indicating that it results from transcription by RNA polymerase III (lanes 4 and 5). In addition, the mutations LS2 and LS7, which debilitate the proximal element and the TATA box, respectively (Lobo and Hernandez 1989), reduced transcription severely (lanes 6 and 7; note that the fragments of slow mobility observed in these two lanes result from protection of the probe up to the sequence divergence at the LS2 and LS7 mutations by RNA initiated within vector sequences). Thus, transcription in the fractionated system is dependent on the same basal promoter elements as transcription in vivo. Moreover, as demonstrated by the primer extension analysis shown in Figure 3B, the site of transcription initiation is identical in vivo and in the fractionated in vitro system. Together, these results indicate that the B and C/D fractions are sufficient to reconstitute accurate U6 transcription.

The cloned general transcription factor TFIID can replace the C/D fraction for U6 transcription

Further fractionation of the C/D fraction revealed that the activity required for U6 transcription eluted in a single peak from several different chromatographic columns (not shown). This result suggested that it might

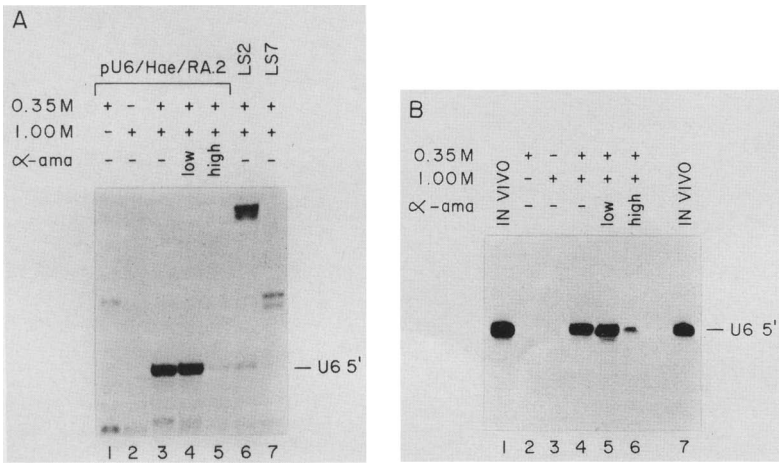


Figure 3. U6 transcription is reconstituted in a combination of the B and C/D fractions. (*A*) The plasmids indicated above the lanes were incubated in the indicated combinations of the B and C/D phosphocellulose fractions. In lanes *4* and *5*, 2.7 and 540 μg/ml of α-amanitin were included in the reactions. The resulting RNAs were analyzed by RNase T_1 protection of the U6/RA.2/143 probe. (*B*) RNAs from similar reactions were analyzed by primer extension with a primer complementary to the β-globin portion of the RNAs. In lanes *1* and *7*, primer extensions were performed with RNA from cells transfected with pU6/Hae/RA.2.

consist of a group of tightly associated polypetides or even of a single polypeptide. For this reason, and because the U6 A/T-rich region can be replaced by TATA boxes from RNA polymerase II mRNA promoters (see above), we attempted to replace the C/D fraction with human TFIID produced in *E. coli*. A human TFIID fusion protein, extended at the amino terminus by a small peptide epitope for which a monoclonal antibody (12CA5) is available (Field et al. 1988; for details, see Materials and methods), was produced in *E. coli* with the T7 expression system (Rosenberg et al. 1987; Studier et al. 1990), from the plasmid pET11cNIID (obtained from M. Tanaka). The *E. coli*-expressed TFIID was purified over an S-Sepharose column followed by a DNA affinity column, and Figure 4A shows a silver-stained gel of the resulting TFIID-containing fractions. The band labeled TFIID was highlighted in Western blots by both mAb 12CA5 and a rabbit anti-TFIID polyclonal antibody (data not shown). In Figure 4B, lanes 1–5, these fractions (fractions 6–10) were added to the HeLa cell-derived B (0.35 M) fraction and U6 transcription was monitored by RNase T_1 mapping. A U6 signal whose intensity correlated well with the relative amounts of TFIID in each added fraction was observed. This signal was not due to a contaminating *E. coli* protein, because although a fraction derived from *E. coli* transformed with the TFIID-expressing vector pET11cNIID directed U6 transcription when combined with the B (0.35 M) fraction from HeLa cells (lane 9), the matching fraction from cells transformed with the pET11c vector lacking the TFIID-coding sequences was inactive (lane 8). Figure 4C shows that the TFIID-directed U6 signal corresponds to transcription by RNA polymerase III, because it is sensitive to tagetitoxin (tagetin, lane 6), a specific inhibitor of RNA polymerase III (Steinberg et al. 1990), and to high, but not low, levels of α-amanitin (lanes 5 and 4, respectively). The integrity of the tagetitoxin and α-amanitin reagents was established in a parallel experiment, in which the sensitivity of α-globin gene transcription by RNA polymerase II in a crude extract was monitored. As expected, α-globin transcription was inhibited by low and high levels of α-amanitin but was unaffected by tagetitoxin (data not shown). Figure 4D shows that U6 transcription directed by the combination of the B fraction and TFIID is dependent on the same basal promoter elements as U6 transcription in vivo and in the crude nuclear extract, namely the PSE and the A/T-rich element (lanes 1–6). Furthermore, U6 transcription in this system tolerates replacement of the U6 A/T-rich element by the TATA box of the Ad2 major late promoter (lane 7), as it does in vivo and in crude extracts. Together, these results demonstrate that the general transcription factor TFIID can

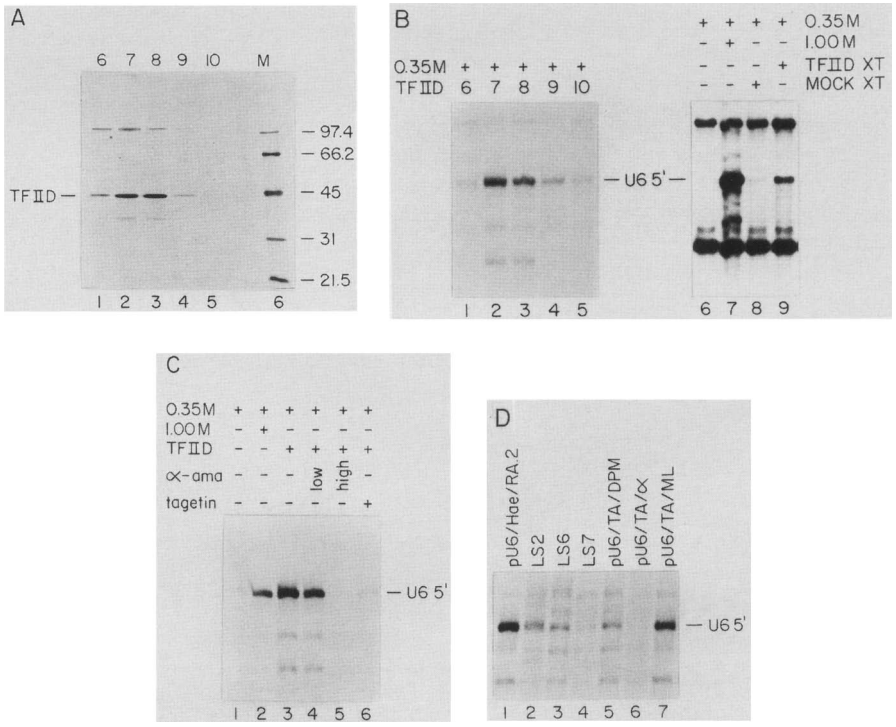


Figure 4. U6 transcription is reconstituted by a combination of the B fraction and human TFIID produced in *E. coli*. (*A*) A bacterial extract from TFIID-expressing *E. coli* was fractionated over two columns. Fractions 6, 7, and 8 (lanes *1–3*) from the 200 mM KCl elution step and fractions 9 and 10 (lanes *4, 5*) from the 300 mM KCl elution step of the second column, an oligonucleotide affinity column (for details, see Materials and methods), were fractionated by SDS-PAGE. The band corresponding to the tagged TFIID protein is indicated. (*B*) pU6/Hae/RA.2 was incubated in the B fraction supplemented with 9 μl of buffer D and 1 μl (10 ng of protein) of each of the fractions shown in *A* (lanes *1–5*), or 10 μl of buffer D (lane *6*), 10 μl of fraction C/D (lane 7), 9 μl of buffer D plus 1 μl of a control fraction from *E. coli* transformed with pET11c lacking the TFIID coding sequences (lane *8*), and 9 μl of buffer D plus 1 μl of the equivalent *E. coli* TFIID-containing fraction. The resulting RNAs were analyzed by RNase T_1 protection of the U6/RA.2/143 probe. (*C*) pU6/Hae/RA.2 was incubated in the combinations of fractions indicated above the lanes. In lanes *4* and *5*, 2.7 and 540 μg/ml of α-amanitin were included in the reactions. In lane *6*, 50 U/ml of tagetitoxin (Steinberg et al. 1990; purchased from Epicenter Technologies) was included in the reaction. Transcription directed by the combination of the B fraction and human TFIID produced in *E. coli* was accurately initiated, as determined by primer extension (not shown). (*D*) pU6/Hae/RA.2 or the constructs indicated above the lanes were incubated in a combination of the B (0.35 M) fraction and partially purified TFIID expressed in bacteria. The resulting RNAs were analyzed by RNase T_1 protection of the U6/RA.2/143 probe.

replace the C/D fraction and direct accurate RNA polymerase III transcription from the U6 promoter, and suggest that TFIID corresponds to the active component in the C/D fraction.

TFIID binds to the wild-type U6 A/T-rich region but not to mutant A/T-rich regions that switch the U6 promoter to an RNA polymerase II promoter in vivo

In TATA box-containing mRNA promoters, such as the Ad2 major late promoter, the binding of TFIID to the TATA box initiates the ordered assembly of an RNA polymerase II transcription complex (Buratowski et al. 1988, 1989; Cavallini et al. 1988; Van Dyke et al. 1988, 1989). To determine whether TFIID also binds to the U6 A/T-rich region, we performed a gel retardation assay using partially purified *E. coli*-expressed TFIID and probes containing the wild-type U6 A/T-rich region or the same region from the mutants LS7, pU6/TA/DPM, pU6/TA/α, and pU6/TA/ML. As shown in Figure 5, a complex was obtained with the wild-type and the pU6/TA/ML TATA box probes (lanes 1 and 5), but not with probes (lanes 2–4) derived from mutants that disrupt the TATA box and are not transcribed efficiently by RNA polymerase III but are transcribed efficiently by RNA polymerase II in vivo (see Figs. 2B,C). This complex contains TFIID, because it can be supershifted by mAb 12CA5 directed against the epitope tag (data not shown). Moreover, the complex is not obtained with a fraction equivalent to the TFIID-containing fraction but derived from control bacteria transformed with the pET11c vector lacking the TFIID-coding sequences (lane 6). These results strongly suggest that binding of TFIID to the U6 pRNA promoter is responsible for the RNA polymerase III specificity of this promoter, whereas absence of TFIID binding results in RNA polymerase II transcription.

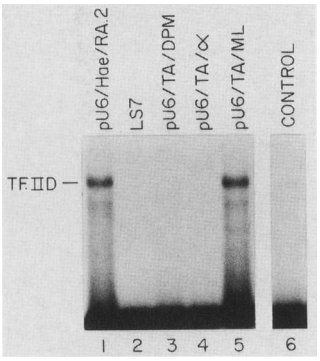


Figure 5. TFIID binds to the wild-type U6 A/T-rich element but not to mutants that, in vivo, switch the U6 promoter to an RNA polymerase II promoter. Fragments containing the A/T-rich elements present in the plasmids indicated above the lanes were incubated with partially purified TFIID expressed in *E. coli*, and the complexes were fractionated on a native gel (see Materials and methods). In lane 6, the A/T-rich element from the parent construct pU6/Hae/RA.2 was incubated with a control fraction derived from *E. coli* transformed with the pET11c vector lacking the TFIID-coding sequences.

Discussion

We have shown previously that in the human U6 promoter, the A/T-rich region constitutes a dominant element that defines the U6 promoter as an RNA polymerase III promoter (Lobo and Hernandez 1989). Mutation of this element converts the U6 promoter into an RNA polymerase II promoter, whereas introduction of an A/T-rich region into the U2 promoter switches that promoter to an RNA polymerase III promoter. Here, we show, paradoxically, that the transcription factor that binds to the U6 A/T-rich region and directs RNA polymerase III transcription from the U6 promoter is the 37-kD TFIID protein, the general transcription factor that, in mRNA promoters, directs the ordered assembly of RNA polymerase II transcription complexes (Buratowski et al. 1988, 1989; Cavallini et al. 1988; Van Dyke et al. 1988, 1989). Although our data do not prove that TFIID is part of the U6 initiation complex in vivo, they strongly suggest that this is the case, because transcription directed by the combination of the B fraction and TFIID is dependent on the same basal promoter elements (proximal element and A/T-rich region) as U6 transcription by RNA polymerase III in crude nuclear extracts and in vivo.

A *Saccharomyces cerevisiae* U6 construct lacking the U6 3′-flanking sequences and, therefore, inactive in vivo (Brow and Guthrie 1990) has recently been shown to be transcribed in vitro by a combination of a TFIIIB-containing fraction and cloned yeast TFIID (Margottin et al. 1991). The promoter elements of the U6 gene have not been as well defined as those of the vertebrate genes, but deletion analyses have shown that 5′- as well as 3′-flanking sequences are required for efficient transcription in vitro (Brow and Guthrie 1990). The 5′-flanking region contains an A/T-rich sequence around position −25 and a similarity to the 3′ portion of the PSE of the human U6 gene (Brow and Guthrie 1988). However, the functional role of these elements has not been examined, and the 5′-flanking sequences are apparently not absolutely required for transcription in vivo (Brow and Guthrie 1990). The element required for transcription in the 3′-flanking sequences is a B box similar to the B box of tRNA genes (Brow and Guthrie 1990); therefore, it is likely that transcription of the yeast U6 gene in vivo requires, in addition to TFIID, the TFIIIC factors (Brow and Guthrie 1990). Our studies with the human U6 gene show that not only can the 37-kD TFIID protein be involved in RNA polymerase III transcription, as in the case of the yeast U6 gene (Margottin et al. 1991), it can also be the factor responsible for selection of RNA polymerase III rather than RNA polymerase II by a promoter.

Models for pRNA transcription complexes

Figure 6 shows a model of hypothetical initiation complexes assembled on an RNA polymerase II mRNA promoter, on RNA polymerase II and III pRNA promoters, and on an RNA polymerase III tRNA promoter. In the mRNA promoter, the TATA box binds TFIID, and this

event initiates the ordered assembly of an RNA polymerase II transcription complex. Basal transcription is stimulated by upstream-binding factors, through direct or indirect interactions (Berger et al. 1990; Kelleher et al. 1990; Peterson et al. 1990; Pugh and Tjian 1990; Stringer et al. 1990; for review, see Ptashne and Gann 1990). In contrast, the RNA polymerase II U2 promoter does not contain a TATA box but consists of two essential *cis*-acting elements: the octamer motif and the PSE (for reviews, see Dahlberg and Lund 1988; Hernandez 1991). The octamer motif-binding protein Oct-1 is shown bound to the octamer motif, because the ubiquitous distribution of this factor correlates with the ubiquitous activity of snRNA genes (Tanaka et al. 1988 and references therein). The PSE probably binds one or several factors, possibly the Ku antigen (Knuth et al. 1990), and these factors may interact with Oct-1 either directly, as depicted in Figure 6, or indirectly. Whether direct or indirect, these interactions are probably less flexible than the interactions in mRNA promoters between enhancer-binding proteins and the basal initiation complex. Indeed, whereas enhancers from very different mRNA promoters are interchangeable, the enhancers from pRNA promoters cannot be replaced by mRNA enhancers and mRNA enhancers cannot be replaced by pRNA enhancers (Ciliberto al. 1987; Dahlberg and Schenborn 1988; Tanaka et al. 1988). Consistent with these observations, Oct-1 does not readily *trans*-activate mRNA promoters (Tanaka and Herr 1990), and the U2 promoter is not *trans*-activated by GAL4, a universal activator of mRNA promoters (Tanaka et al. 1988).

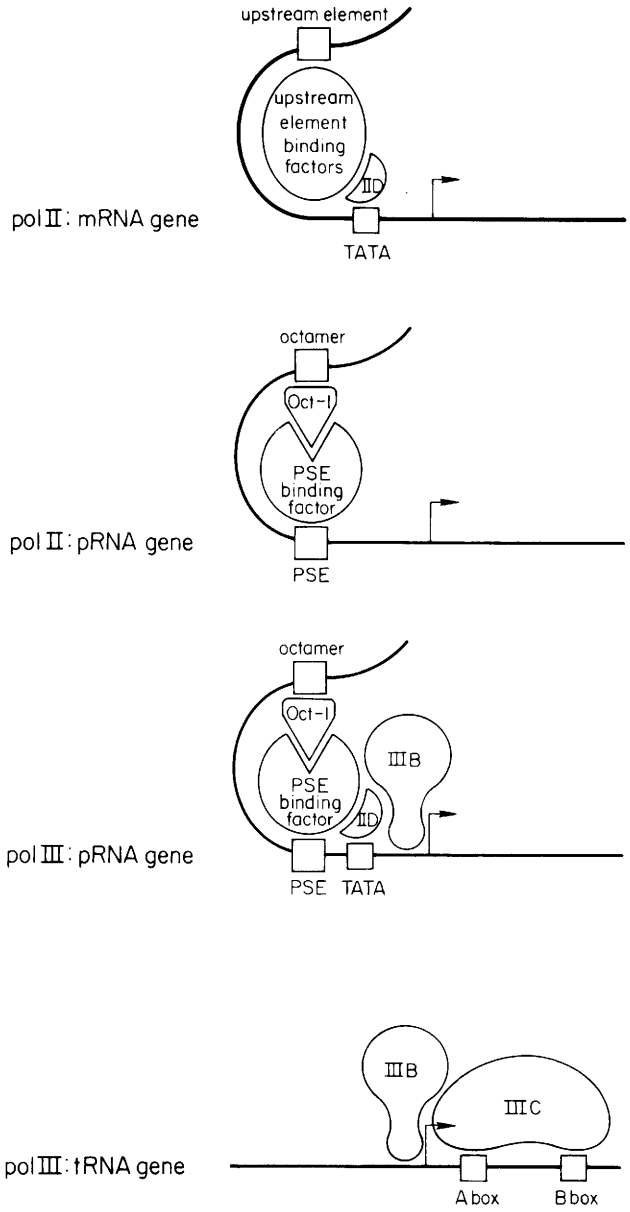


Figure 6. Hypothetical model of initiation complexes assembled on RNA polymerase II and III pRNA promoters and on a tRNA promoter.

The composition of the U2 initiation complex is undoubtedly more complicated than shown in the model. For example, the sequences immediately downstream of the PSE are involved in RNA polymerase II transcription, because in a U2 promoter with an inserted A/T-rich element that directs both RNA polymerase II and RNA polymerase III transcription, certain mutations in this region affect RNA polymerase II but not RNA polymerase III transcription (Lobo et al. 1990). Because this region is not conserved among RNA polymerase II pRNA genes, it might bind a factor with degenerate DNA sequence recognition specificity, or perhaps RNA polymerase II itself. In addition, the U2 enhancer contains an Sp1-binding site that is required for efficient transcription (Ares et al. 1985, 1987; Mangin et al. 1986; Janson et al. 1989). However, whereas six copies of the octamer motif constitute an efficient pRNA enhancer, six copies of the Sp1 motif do not efficiently stimulate U2 transcription (P. Reinagel and N. Hernandez, unpubl.). Furthermore, the Sp1-binding site is not conserved among pRNA promoters and can be replaced by the binding site for nuclear factor I with no debilitating effect on transcription (Janson et al. 1989). Together, these results show that unlike the octamer motif, the Sp1-binding site is not essential per se and can be replaced by other *cis*-acting elements; therefore, we have not included it in the model.

The U6 promoter contains an octamer motif and a proximal element in a spatial relationship similar to that in the U2 promoter. Because the U2 and U6 enhancer sequences and PSEs are interchangeable (Bark et al. 1987; Kunkel and Pederson 1988; Mattaj et al. 1988; Lobo and Hernandez 1989), we have depicted these elements binding the same *trans*-acting factors in the two promoters, although these promoters are recognized by different RNA polymerases. However, the U6 promoter contains an additional element, the A/T-rich sequence, which confers RNA polymerase III specificity. Because this element forms a functional complex with the 37-kD general RNA polymerase II transcription factor TFIID, it can be referred to as a TATA box.

How does the 37-kD TFIID protein recruit RNA poly-

merase III to the U6 promoter? A possibility is that TFIID interacts with the proximal element-binding factor and that it is the surface created by the combination of these factors that defines the U6 promoter as an RNA polymerase III promoter. An interaction between TFIID and factors bound to the PSE is suggested by several observations. First, altering the spacing between the TATA box and the PSE severely debilitates RNA polymerase III transcription both in the human U6 gene (Fig. 2B,C) and in the *Xenopus* U6 gene (Lescure et al. 1991). Second, a comparison of the promoter sequences of other cloned RNA polymerase III pRNA genes, shown in Table 1, reveals that among human genes, a distance of 16 bp between the TATA box and the proximal element is rigorously conserved. Third, in plants, both the RNA polymerase II and III pRNA promoters contain a TATA box, and the spacing between this element and an upstream sequence element (USE) determines RNA polymerase specificity. When the TATA box is close to the USE, the promoter is recognized by RNA polymerase III, but when 10 bp is inserted between the two elements, the promoter switches to RNA polymerase II (Waibel and Filipowicz 1990).

We imagine that the surface created by the combination of the 37-kD TFIID protein and the PSE-binding factors can bind and anchor TFIIIB to the DNA. Thus, such a surface would be the equivalent of that provided by TFIIIC in tRNA genes, as shown in the bottom panel of Figure 6. That TFIIIB is a part of the U6 transcription complex is suggested by the observation that the B fraction is essential for U6 transcription in vitro; the B fraction must contribute an activity other than RNA polymerase III, which is also present in the C/D fraction (Segall et al. 1980). In addition, in yeast TFIIIB has been shown to be the only essential component (besides RNA polymerase III) of initiation complexes assembled on tRNA and 5S RNA genes (Kassavetis et al. 1990). Heparin or high salt treatment of a complete initiation complex assembled on a 5S or tRNA gene will strip TFIIIA and TFIIIC from the templates, leaving TFIIIB associated with the DNA (Braun et al. 1989; Kassavetis et al. 1989, 1990). This remaining TFIIIB can sustain several rounds of transcription by RNA polymerase III, suggesting that TFIIIA and TFIIIC are assembly factors whose only role is to bring TFIIIB to the DNA, whereas TFIIIB is the transcription factor that interacts with RNA polymerase III (Kassavetis et al. 1990).

Table 1. *TATA boxes in human RNA polymerase III pRNA genes*

U6	16[a]	−34 GGC TTTATATA TCTT −20
7SK	16[a]	−35 AGG TTTATATA GCTT −21
H1	16[a]	−36 GGA ATCTTATA AGTT −22
MRP/Th	16[a]	−36 GGC TATAAAAT ACTA −22

References: (U6) Kunkel et al. (1986); (7SK) Murphy et al. (1986); (H1) Baer et al. (1989); (MRP/Th) Topper and Clayton (1990).
[a]Number of base pairs between the last base pair of the PSE and the first base pair of the TATA boxes.

Does RNA polymerase III transcription from other promoters and RNA polymerase I transcription require the TFIID polypeptide?

The observation that both the yeast and human U6 snRNA genes require TFIID for transcription by RNA polymerase III in vitro raises the question of whether other RNA polymerase III genes also use this factor. The c-*myc* gene is transcribed under certain circumstances by RNA polymerase III (Chung et al. 1987; Bentley et al. 1989), and this transcription is dependent on an intact TATA box (Bentley et al. 1989). Thus, it seems very likely that RNA polymerase III transcription from the c-*myc* promoter requires TFIID. But it is possible that other RNA polymerase III genes that contain conventional RNA polymerase III promoter elements, such as ICRs and A and B boxes, also use TFIID. Several RNA polymerase III genes contain A/T-rich sequences 20–30 nucleotides upstream of the transcription initiation site, and these sequences are often required for efficient RNA polymerase III transcription (for review, see Geiduschek and Tocchini-Valentini 1988). Furthermore, TFIID is present in the C fraction from a phosphocellulose column, as determined by Western blots (data not shown), and may well be one of the components of TFIIIC. TFIIIC can be subdivided into two fractions, referred to as TFIIIC1 and TFIIIC2, that are both required for transcription of the VA genes (Dean and Berk 1987; Yoshinaga et al. 1987). TFIIIC2 binds to the B box of the VA genes, whereas TFIIIC1 has no DNA-binding activity on its own but extends the footprint over the A box in the presence of TFIIIC2 (Yoshinaga et al. 1987, 1989). Whereas TFIIIC2 has been purified extensively to five polypeptides that may all be required for activity (Yoshinaga et al. 1989), the active components of TFIIIC1 have not been identified, and it is conceivable that they include TFIID.

Another intriguing question is whether TFIID might also be involved in transcription by RNA polymerase I. In both crude extracts and reconstituted systems containing TFIID, we occasionally observe a low amount of correctly initiated U6 transcripts even in the presence of high concentrations of α-amanitin and tagetitoxin. This low amount of transcription therefore seems to be directed by RNA polymerase I and requires TFIID. The core element of the human rRNA promoter contains several A/T-rich sequences (Haltiner et al. 1986), and one of the factors involved in RNA polymerase I transcription, SL1, has been difficult to purify to homogeneity (Learned et al. 1985). Perhaps one component of SL1 is the 37-kD TFIID protein. If this were the case, TFIID would participate in transcription initiation by all three polymerases and might have to be renamed.

Are other general RNA polymerase II transcription factors involved in the transcription of pRNA genes by RNA polymerase III?

The unexpected involvement of TFIID in transcription of the U6 gene raises the possibility that additional gen-

eral transcription factors may participate in both RNA polymerase II and III initiation complexes. For example, the general transcription factor TFIIA is known to stimulate transcription from TATA box-containing mRNA promoters (Davison et al. 1983; Fire et al. 1984) and facilitate binding of TFIID to the TATA box (Buratowski et al. 1989). These data, in conjunction with our observation that the A fraction stimulates U6 transcription in vitro (not shown), suggest that TFIIA might participate in RNA polymerase III transcription. In addition, other general RNA polymerase II and III transcription factors, which elute in similar regions of a phosphocellulose column but have never been tested for RNA polymerase II and III transcription in parallel, may turn out to be identical. Thus, perhaps as with the RNA polymerases themselves (Memet et al. 1988), the RNA polymerase II and III initiation complexes are closely related and derive from a common ancestral initiation complex.

Materials and methods

Construction of mutants

pU6/Hae/RA.2, LS7, LS5, and LS2 are described in Lobo and Hernandez (1989). The mutations in the TATA box were constructed by oligonucleotide-directed mutagenesis of pU6/Hae/RA.2 (Zoller and Smith 1982; Kunkel 1985), with the following mutagenic oligonucleotides: pU6/TA/DPM, TTCTTGGCTTTCTAGATCTTGTG; pU6/TA/α, GATTTCTTGGCATAAACCCTCTTGTGG; pU6/TA/ML, GATTTCTTGGCTATAAAAGGCTTGTGG; pU6/TA/HSP, GATTTCTTGGCTTATAAAAGCTTGTGG; pU6/TA/SVe, GATTTCTTGGCTTTATTTATCTTTGTGG; pU6/TA/E4, GATTTCTTGGCTATATATACCCTTGTGG; pU6/TA/1, GATTTCTTGGCTATAAACGGCTTGTGG; pU6/TA/2, GATTTCTTGGCTTTATACGGCTTGTGG.

The spacing mutants were constructed from LS5 by filling in the *Xho*I site at position −46 with Klenow (LS5 + 4), and inserting into the filled-in *Xho*I site one copy (LS5 + 14) or two copies (LS5 + 24) of a 10-bp double-stranded, phosphorylated *Eco*RI linker. LS5 + 10 and LS5 + 20 were constructed from LS5 + 14 by digestion with *Eco*RI, treatment with mung bean nuclease to remove the overhangs, and either religation (LS5 + 10) or insertion of a 10-bp double-stranded, phosphorylated *Eco*RI linker (LS5 + 20).

Phosphocellulose chromatography

P11 (Whatman) was prepared as described (Price et al. 1987) and equilibrated with buffer A [20 mM HEPES (pH 7.9 at room temperature), 15% glycerol, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF] containing 0.1 M KCl. Nuclear extracts from HeLa cells were prepared by the method of Dignam et al. (1983). The nuclear extract was loaded onto the P11 column (8 mg of protein/ml of packed column) at a flow rate of 0.25 column volume/hr. The column was washed with 3 column volumes of buffer A containing 100 mM KCl at a flow rate of 3 column volumes/hr and step eluted with 5 column volumes of buffer A containing 0.35 M and 1 M KCl at the same flow rate. The protein concentrations were measured by the Bradford assay, and the peak fractions were pooled to give the A, B, and C/D fractions. The B and C/D fractions were dialyzed against buffer D (Dignam et al. 1983), frozen in liquid nitrogen, and stored at −80°C.

Preparation of TFIID

A derivative of pET11c (Studier et al. 1990), pET11cNIID, containing the coding sequence for human TFIID fused at the initiation AUG codon to a fragment encoding the peptide SSYPYDVPDYASLGGPSR from the influenza virus hemagglutinin protein (Field et al. 1988), was a kind gift of M. Tanaka. TFIID was expressed as described (Studier et al. 1990) in BL21DE3–pLys S bacteria as the host. As a control, bacteria were also transformed with the pET11c vector lacking the TFIID-coding sequences. The bacteria from 500 ml of each culture were harvested by centrifugation and lysed by sonication in 50 ml of buffer A containing 0.6 M KCl and 0.1% NP-40 and then dialyzed against buffer D (Dignam et al. 1983). Each extract was loaded on a HiLoad 26/10 S-Sepharose Fast Flow column (Pharmacia) preequilibrated in buffer B [20 mM HEPES (pH 7.9) at room temperature, 5% glycerol, 0.5 mM EDTA, 5 mM $MgCl_2$, 2 mM DTT, 0.1 mM PMSF], containing 0.1 M KCl, and the column was eluted with a 10-column-volume linear salt gradient (0.1–1 M KCl). The fractions containing TFIID or the equivalent fractions from the control column were pooled, dialyzed against buffer D, adjusted to 20 mM EDTA, and loaded onto a 1-ml oligonucleotide affinity column at 4°C. The affinity column was constructed by the method of Kadonaga and Tjian (1986) with the following complementary oligonucleotides: 5′-GATTTCTTTGGCTTTATATATGACTCGAG and 5′-GCTCCTAAAGAAACCGAAATATATACTGA. The oligonucleotide affinity column was eluted with five steps of 2 ml of buffer D containing 20 mM EDTA and 0.2, 0.3, 0.4, 0.6, and 1 M KCl, respectively. TFIID eluted in the 0.2 and 0.3 M KCl elution steps.

Transcription in vitro

Transcription reactions with nuclear extracts were performed as described in Lobo and Hernandez (1989). Transcription reactions with fractionated extracts were carried out in a total volume of 50 μl and contained 10 μl of each fraction B and/or C/D, 1 μg of supercoiled template DNA, and final concentrations of 12% glycerol, 20 mM HEPES, 60 mM KCl, 5 mM $MgCl_2$, and 0.5 mM each of ATP, UTP, CTP, and GTP. Alternatively, the C/D fraction was replaced with 1–10 ng of TFIID purified from bacteria in 10 μl of buffer D, and the template concentration was lowered to 0.5 μg/ml. RNA was isolated as described (Lobo and Hernandez 1989).

Transfection into HeLa cells

Transfections were carried out by electroporation as described in Ratnasabapathy et al. (1990), except that 14.5 μg of test plasmid, 0.5 μg of the reference plasmid pα1x72 (Lobo and Hernandez 1989), and 5.5 μg of calf thymus DNA were cotransfected into 1.5×10^7 spinner HeLa cells.

RNA analyses

Primer extension and RNase T_1 protection analyses were carried out as described in Lobo and Hernandez (1989).

Mobility-shift assays

The probes for mobility-shift assays were generated by the polymerase chain reaction from an oligonucleotide complementary to the β-globin portion of the hybrid U6 constructs and the oligonucleotide used to generate the mutant construct LS5 (Lobo and Hernandez 1989). One of the primers was 5′-end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase;

hence, all probes had the same specific activity. The binding reactions were performed in a final volume of 10 μl and contained 20 mM HEPES (pH 8.4), 2.5 mM $MgCl_2$, 1 mM DTT, 1 μg of poly[d(G-C)], 12% glycerol, and 10 ng of TFIID produced in *E. coli*. In Figure 5, lane 6, the protein source was a fraction equivalent to the TFIID-containing fraction but derived from the control extract. The samples were incubated at 30°C for 30 min and loaded on a 5% polyacrylamide gel (40 : 1 acrylamide/bisacrylamide) that had been prerun for 30 min at room temperature. The running buffer was that used by Horikoshi et al. (1989b).

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References

Ares, M. Jr., M. Mangin, and A.M. Weiner. 1985. Orientation-dependent transcriptional activator upstream of a human U2 snRNA gene. *Mol. Cell. Biol.* **5:** 1560–1570.

Ares, M., Jr., J.-S. Chung, L. Giglio, and A.M. Weiner. 1987. Distinct factors with Sp1 and NF-A specificities bind to adjacent functional elements of the human U2 snRNA gene enhancer. *Genes & Dev.* **1:** 808–817.

Baer, M., T.W. Nilsen, C. Costigan, and S. Altman. 1989. Structure and transcription of a human gene for H1 RNA, the RNA component of human RNase P. *Nucleic Acids Res.* **18:** 97–103.

Bark, C., P. Weller, J. Zabielski, L. Janson, and U. Pettersson. 1987. A distant enhancer is required for polymerase III transcription of a U6 RNA gene. *Nature* **328:** 356–359.

Bartkiewicz, M., H. Gold, and S. Altman. 1989. Identification and characterization of an RNA molecule that copurifies with RNase P activity from HeLa cells. *Genes & Dev.* **3:** 488–499.

Bentley, D.L., W.L. Brown, and M. Groudine. 1989. Accurate, TATA box-dependent polymerase III transcription from promoters of the c-*myc* gene in injected *Xenopus* oocytes. *Genes & Dev.* **3:** 1179–1189.

Berger, S.L., W.D. Cress, A. Cress, S.J. Triezenberg, and L. Guarente. 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: Evidence for transcriptional adaptors. *Cell* **61:** 1199–1208.

Bogenhagen, D.F. and D. D. Brown. 1981. Nucleotide sequences in *Xenopus* 5S DNA required for transcription termination. *Cell* **24:** 261–270.

Braun, B.R., D.L. Riggs, G.A. Kassavetis, and E.P. Geiduschek. 1989. Multiple states of protein–DNA interaction in the assembly of transcription complexes on *Saccharomyces cerevisiae* 5S ribosomal RNA genes. *Proc. Natl. Acad. Sci.* **86:** 2530–2534.

Brow, D.A. and C. Guthrie. 1988. Spliceosomal RNA U6 is remarkably conserved from yeast to mammmals. *Nature* **334:** 213–218.

———. 1990. Transcription of a yeast U6 snRNA gene requires a polymerase III promoter element in a novel position. *Genes & Dev.* **4:** 1345–1356.

Buratowski, S., S. Hahn, P.A. Sharp, and L. Guarente. 1988. Function of a yeast TATA element binding protein in a mammalian transcription system. *Nature* **334:** 37–42.

Buratowski, S., S. Hahn, L. Guarente, and P.A. Sharp. 1989. Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell* **56:** 549–561.

Cavallini, B., J. Huet, J. Plassat, A. Sentenac, J. Egly, and P. Chambon. 1988. A yeast activity can substitute for the HeLa cell TATA box factor. *Nature* **334:** 77–80.

Cavallini, B., I. Faus, H. Matthes, J.M. Chipoulet, B. Winsor, J.M. Egly, and P. Chambon. 1989. Cloning of the gene encoding the yeast protein BTF1Y, which can substitute for the human TATA box binding factor. *Proc. Natl. Acad. Sci.* **86:** 9803–9807.

Chang, D.D. and D.A. Clayton. 1987. A mammalian mitochondrial RNA processing activity contains nucleus-encoded RNA. *Science* **235:** 1178–1184.

———. 1989. Mouse RNAase MRP RNA is encoded by a nuclear gene and contains a decamer sequence complementary to a conserved region of mitochondrial RNA substrate. *Cell* **56:** 131–139.

Chung, J., D.J. Sussman, R. Zeller, and P. Leder. 1987. The c-*myc* gene encodes superimposed RNA polymerase II and III promoters. *Cell* **51:** 1001–1008.

Ciliberto, G., F. Palla, G. Tebb, I.W. Mattaj, and L. Philipson. 1987. Properties of a U1 RNA enhancer-like sequence. *Nucleic Acids Res.* **15:** 2403–2417.

Conaway, J.W., D. Reines, and R.C. Conaway. 1990. Transcription initiated by RNA polymerase II and purified transcription factors from liver. *J. Biol. Chem.* **265:** 7552–7558.

Dahlberg, J.E. and E. Lund. 1988. The genes and transcription of the major small nuclear RNAs. In *Structure and function of major and minor small nuclear ribonucleoprotein particles* (ed. M.L. Birnstiel), pp. 38–70. Springer Verlag, Berlin/Heidelberg.

Dahlberg, J.E. and E.T. Schenborn. 1988. The human U1 snRNA promoter and enhancer do not direct synthesis of messenger RNA. *Nucleic Acids Res.* **13:** 5827–5841.

Davison, B.L., J.-M. Egly, E.R. Mulvihill, and P. Chambon. 1983. Formation of stable preinitiation complexes between eucaryotic class B transcription factors and promoter sequences. *Nature* **301:** 680–686.

Dean, N. and A.J. Berk. 1987. Separation of TFIIIC into two functional components by sequence specific DNA affinity chromatography. Nucleic Acids Res. **15:** 9895–9907.

Dignam, J.D., R.M. Lebovitz, and R.G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11:** 1475–1489.

Eisenmann, D.M., C. Dollard, and F. Winston. 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. *Cell* **58:** 1183–1191.

Field, J., J. Nikawa, D. Broek, B. MacDonald, L. Rodgers, I.A. Wilson, R.A. Lerner, and M. Wigler. 1988. Purification of a *RAS*-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. *Mol. Cell Biol.* **8:** 2159–2165.

Fikes, J.D., D.M. Becker, F. Winston, and L. Guarente. 1990. Striking conservation of TFIID in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. *Nature* **346:** 291–294.

Fire, A., M. Samuels, and P.A. Sharp. 1984. Interactions between RNA polymerase II, factors, and template leading to accurate transcription. *J. Biol. Chem.* **259:** 2509–2516.

Gabrielsen, O.S., N. Marzouki, A. Ruet, A. Sentenac, and P. Fromageot. 1989. Two polypeptide chains in yeast transcription factor τ interact with DNA. *J. Biol. Chem.* **264:** 7505–7511.

Gasch, A., A. Hoffmann, M. Horikoshi, R.G. Roeder, and N. Chua. 1990. *Arabidopsis thaliana* contains two genes for TFIID. *Nature* **346:** 390–394.

Geiduschek, E.P. and G.P. Tocchini-Valentini. 1988. Transcription by RNA polymerase III. *Annu. Rev. Biochem.* **57:** 873–914.

Gold, H.A, J.N. Topper, D.A. Clayton, and J. Craft. 1989. The RNA processing enzyme RNase MRP is identical to the Th RNP and related to RNase P. *Science* **245:** 1377–1380.

Hahn, S., S. Buratowski, P.A. Sharp, and L. Guarente. 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: A gene identical to the *SPT15* suppressor of Ty elements insertions. *Cell* **58:** 1173–1181.

Haltiner, M. M., S.T. Smale, and R. Tjian. 1986. Two distinct promoter elements in the human rRNA gene identified by linker scanning mutagenesis. *Mol. Cell. Biol.* **6:** 227–235.

Hernandez, N. 1985. Formation of the 3′ end of U1 snRNA is directed by a conserved sequence located downstream of the coding region. *EMBO J.* **4:** 1827–1837.

———. 1991. Transcription of vertebrate snRNA genes and related genes. In *Transcriptional regulation*. Cold Spring Harbor monograph series (In press.)

Hoey, T., B.D. Dynlacht, M.G. Peterson, B.F. Pugh, and R. Tjian. 1990. Isolation and characterization of the *Drosophila* gene encoding the TATA box binding factor, TFIID. *Cell* **61:** 1179–1186.

Hoffmann, A., M. Horikoshi, C.K. Wang, S. Schroeder, P.A. Weil, and R.G. Roeder. 1990a. Cloning of the *Schizosaccharomyces pombe* TFIID gene reveals a strong conservation of functional domains present in *Saccharomyces cerevisiae* TFIID. *Genes & Dev.* **4:** 1141–1148.

Hoffmann, A., E. Sinn, T. Yamamoto, J. Wang, A. Roy, M. Horikoshi, and R.G. Roeder. 1990b. Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). *Nature* **346:** 387–390.

Horikoshi, M., C.K. Wang, H. Fujii, J.A. Cromlish, P.A. Weil, and R.G. Roeder. 1989a. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. *Nature* **341:** 299–303.

———. 1989b. Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity. *Proc. Natl. Acad. Sci.* **86:** 4843–4847.

Janson, L., P. Weller, and U. Pettersson. 1989. Nuclear factor I can functionally replace transcription factor Sp1 in a U2 snRNA gene enhancer. *J. Mol. Biol.* **205:** 387–396.

Kadonaga, J.T. and R. Tjian. 1986. Affinity purification of sequence-specific DNA binding proteins. *Proc. Natl. Acad. Sci.* **83:** 5889–5893.

Kao, C.C., P.M. Lieberman, M.C. Schmidt, Q. Zhou, R. Pei, and A.J. Berk. 1990. Cloning of a transcriptionally active human TATA binding factor. *Science* **248:** 1646–1650.

Kass, S., K. Tyc, J.A. Steitz, and B. Sollner-Webb. 1990. The U3 small nucleolar ribonucleoprotein functions in the first step of preribosomal RNA processing. *Cell* **60:** 897–908.

Kassavetis, G.A., D.L. Riggs, R. Negri, L.H. Nguyen, and E.P. Geiduschek. 1989. Transcription factor IIIB generates extended DNA interactions in RNA polymerase III transcription complexes on tRNA genes. *Mol. Cell. Biol.* **9:** 2551–2566.

Kassavetis, G.A., B.R. Braun, L.H. Nguyen, and E.P. Geiduschek. 1990. *S. cerevisiae* TFIIIB is the transcription initiation factor proper of RNA polymerase III, while TFIIIA and TFIIIC are assembly factors. *Cell* **60:** 235–245.

Kelleher, R.J., P.M. Flanagan, and R.D. Kornberg. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* **61:** 1209–1215.

Knuth, M.W., S.I. Gunderson, N.E. Thompson, L.S. Strasheim, and R.R. Burgess. 1990. Purification and characterization of proximal sequence element-binding protein 1, a transcription activating protein related to Ku and TREF that binds the proximal sequence element of the human U1 promoter. *J. Biol. Chem.* **265:** 17911–17920.

Kunkel, G.R. and T. Pederson. 1988. Upstream elements required for efficient transcription of a human U6 RNA gene resemble those of U1 and U2 genes even though a different polymerase is used. *Genes & Dev.* **2:** 196–204.

Kunkel, G.R., R.L. Maser, J.P. Calvet, and T. Pederson. 1986. U6 small nuclear RNA is transcribed by RNA polymerase III. *Proc. Natl. Acad. Sci.* **83:** 8575–8579.

Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci.* **82:** 488–492.

Learned, R.M., S. Cordes, and R. Tjian. 1985. Purification and characterization of a transcription factor that confers promoter specificity to human RNA polymerase I. *Mol. Cell. Biol.* **5:** 1358–1369.

Lescure, A., P. Carbon, and A. Krol. 1991. The different positioning of the proximal sequence element in the *Xenopus* RNA polymerase II and III snRNA promoters is a key determinant which confers RNA polymerase III specificity. *Nucleic Acids Res.* **19:** 435–441.

Lobo, S.M. and N. Hernandez. 1989. A 7 bp mutation converts a human RNA polymerase II snRNA promoter into an RNA polymerase III promoter. *Cell* **58:** 55–67.

Lobo, S.M., S. Ifill, and N. Hernandez. 1990. *Cis*-acting elements required for RNA polymerase II and III transcription in the human U2 and U6 snRNA promoters. *Nucleic Acids Res.* **18:** 2891–2899.

Mangin, M., M. Ares, Jr., and A.M. Weiner. 1986. Human U2 small nuclear RNA genes contain an upstream enhancer. *EMBO J.* **5:** 987–995.

Margottin, F., G. Dujardin, M. Gerard, J.-M. Egly, J. Huet, and A. Sentenac. 1991. Participation of the TATA factor in transcription of the yeast U6 gene by RNA polymerase C. *Science* **251:** 424–426.

Matsui, T., J. Segall, P.A. Weil, and R. G. Roeder. 1980. Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. *J. Biol. Chem.* **255:** 11992–11996.

Mattaj, I.W., N.A. Dathan, H.W. Parry, P. Carbon, and A. Krol. 1988. Changing the RNA polymerase specificity of U snRNA gene promoters. *Cell* **55:** 435–442.

Memet, S., W. Saurin, and A. Sentenac. 1988. RNA polymerases B and C are more closely related to each other than to RNA polymerase A. *J. Biol. Chem.* **263:** 10048–10051.

Murphy, S., M. Tripodi, and M. Melli. 1986. A sequence upstream from the coding region is required for the transcription of the 7SK RNA genes. *Nucleic Acids Res.* **14:** 9243–9260.

Peterson, M.G., N. Tanese, B.F. Pugh, and R. Tjian. 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. *Science* **248:** 1625–1630.

Price, D.H., A.E. Sluder, and A.L. Greenleaf. 1987. Fractionation of transcription factors for RNA polymerase II from *Droso-*

phila K_c cell nuclear extracts. *J. Biol. Chem.* **262:** 3244–3255.
Ptashne, M. and A.A.F. Gann. 1990. Activators and targets. *Nature* **346:** 329–331.
Pugh, B.F. and R. Tjian. 1990. Mechanism of transcriptional activation by Sp1: Evidence for coactivators. *Cell* **61:** 1187–1197.
Ratnasabapathy, R., M. Sheldon, L. Johal, and N. Hernandez. 1990. The HIV-1 long terminal repeat contains an unusual element that induces the synthesis of short RNAs from various mRNA and snRNA promoters. *Genes & Dev.* **4:** 2061–2074.
Reddy, R. 1988. Transcription of a U6 small nuclear RNA gene in vitro. Transcription of a mouse U6 small nuclear RNA gene in vitro by RNA polymerase III is dependent on transcription factor(s) different from transcription factors TFIIIA, TFIIIB, and TFIIIC. *J. Biol. Chem.* **263:** 15980–15984.
Rosenberg, A. H., B. N. Lade, D.-S. Chui, S.-W. Lin, J.J. Dunn, and F.W. Studier. 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene* **56:** 125–135.
Samuels, M., A. Fire, and P.A. Sharp. 1982. Separation and characterization of factors mediating accurate transcription by RNA polymerase II. *J. Biol. Chem.* **257:** 14419–14427.
Savino, R. and S. A. Gerbi. 1990. In vivo disruption of *Xenopus* U3 snRNA affects ribosomal RNA processing. *EMBO J.* **9:** 2299–2308.
Schmidt, M.C., C.C. Kao, R. Pei, and A.J. Berk. 1989. Yeast TATA box transcription factor gene. *Proc. Natl. Acad. Sci.* **86:** 7785–7789.
Segall, J., T. Matsui, and R.G. Roeder. 1980. Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. *J. Biol. Chem.* **255:** 11986–11991.
Shastry, B.S., S.Y. Ng, and R.G. Roeder. 1982. Multiple factors involved in the transcription of class III genes in *Xenopus laevis*. *J. Biol. Chem.* **257:** 12979–12986.
Steinberg, T.H., D.E. Mathews, R.D. Durbin, and R.R. Burgess. 1990. Tagetitoxin: A new inhibitor of eucaryotic transcription by RNA polymerase III. *J. Biol. Chem.* **265:** 499–505.
Steitz, J.A., D.L. Black, V. Gerke, K.A. Parker, A. Kramer, D. Frendewey, and W. Keller. 1988. Functions of the abundant U-snRNPs. In *Structure and function of major and minor small nuclear ribonucleoprotein particles* (ed. M.L. Birnstiel), pp. 115–154. Springer-Verlag, Berlin/Heidelberg.
Stringer, K.F., C.G. Ingles, and J. Greenblatt. 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA box factor TFIID. *Nature* **345:** 783–786.
Studier, F.W., A.H. Rosenberg, J.J. Dunn, and J.W. Dubendorff. 1990. Use of T7 RNA polymerase to direct expression of cloned genes. *Methods Enzymol.* **185:** 60–89.
Tanaka, M. and W. Herr. 1990. Differential transcriptional activation by Oct-1 and Oct-2: Interdependent activation domains induce Oct-2 phosphorylation. *Cell* **60:** 375–386.
Tanaka, M., U. Grossniklaus, W. Herr, and N. Hernandez. 1988. Activation of the U2 snRNA promoter by the octamer motif defines a new class of RNA polymerase II enhancer elements. *Genes & Dev.* **2:** 1764–1778.
Topper, J.N. and D.A. Clayton. 1990. Characterization of human MRP/Th RNA and its nuclear gene: Full length MRP/Th RNA is an active endoribonuclease when assembled as an RNP. *Nucleic Acids Res.* **18:** 793–799.
Van Dyke, M.W., R.G. Roeder, and M. Sawadogo. 1988. Physical analysis of transcription preinitiation complex assembly on a class II promoter. *Science* **241:** 1335–1338.
Van Dyke, M.W., M. Sawadogo, and R.G. Roeder. 1989. Stability of transcription complexes on class II genes. *Mol. Cell. Biol.* **9:** 342–344.
Waibel, F. and W. Filipowicz. 1990. RNA-polymerase specificity of transcription of *Arabidopsis* U snRNA genes determined by promoter element spacing. *Nature* **346:** 199–202.
Yoshinaga, S.K., P.A. Boulanger, and A.J. Berk. 1987. Resolution of human transcription factor TFIIIC into two functional components. *Proc. Natl. Acad. Sci.* **84:** 3585–3589.
Yoshinaga, S.K., N.D. L'Etoile, and A.J. Berk. 1989. Purification and characterization of transcription factor IIIC2. *J. Biol. Chem.* **264:** 10726–10731.
Zoller, M. and M. Smith. 1982. Oligonucleotide-directed mutagenesis using M13-derived vectors: An efficient and general procedure for the production of point mutations in any fragment of DNA. *Nucleic Acids Res.* **10:** 6487–6500.

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