

REVIEW

Recruitment of RNA polymerase III to its target promoters

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A key step in retrieving the information stored in the complex genomes of eukaryotes involves the identification of transcription units and, more specifically, the recognition of promoter sequences by RNA polymerase. In eukaryotes, the task of recognizing nuclear gene promoters and then transcribing the genes is divided among three highly related enzymes, RNA polymerases I, II, and III. Each of these RNA polymerases is dedicated to the transcription of specific sets of genes, and each depends on accessory factors, the so-called transcription factors, to recognize its cognate promoter sequences.

RNA polymerase I is unique among the nuclear RNA polymerases in transcribing only one set of genes, the large, tandemly repeated, ribosomal RNA genes, and thus in having to recognize a single promoter structure. RNA polymerase II transcribes the protein-coding genes (mRNA genes) as well as some small nuclear RNA (snRNA) genes. The RNA polymerase II promoters can be divided into a core region, defined as the minimal region capable of directing transcription *in vitro*, and a regulatory region. The regulatory regions are highly varied in structure, reflecting the highly varied synthesis patterns of cellular proteins and the need for exquisite and complex regulation of these patterns. The core promoters themselves come in different types that, in mRNA-encoding genes, can contain a TATA box, an initiator, a downstream promoter element, or various combinations thereof. The assembly of a functional RNA polymerase II transcription complex on a promoter consisting of just a TATA box has been extensively studied. All the factors involved in the process have been identified, and much is known about how these factors interact with DNA and with each other to recruit, eventually, RNA polymerase II (for reviews, see Orphanides et al. 1996; Woychik and Hampsey 2002). How RNA polymerase II transcription complexes assemble on TATA-less promoters is, however, not as well understood.

RNA polymerase III is dedicated to the transcription of an eclectic collection of genes whose main common features are that they encode structural or catalytic RNAs and that they are, as a rule, shorter than 400 base pairs

(bp). This length limit is consistent with the elongation properties of RNA polymerase III, which recognizes a simple run of T residues as a termination signal. The genes transcribed by RNA polymerase III encode RNA molecules involved in fundamental metabolic processes, specifically components of the protein synthesis apparatus and components of the splicing and tRNA processing apparatus, as well as RNAs of unknown function. The RNA polymerase III promoters are more varied in structure than the uniform RNA polymerase I promoters, and yet not as diverse as the RNA polymerase II promoters. They have been divided into three main types, two of which are gene-internal and generally TATA-less, and one of which is gene-external and contains a TATA box. Remarkably, we have a good, and in some cases a very detailed, understanding of how RNA polymerase III is recruited to each of these types of promoters. This provides a paradigm of how the same enzyme can be recruited to different promoter structures through different combinations of protein-DNA and protein-protein interactions. Here we summarize our present understanding of the various pathways leading to recruitment of RNA polymerase III. Other recent reviews on RNA polymerase III transcription include those by Geiduschek and Kassavetis (2001) and Huang and Marais (2001). Reviews on the regulation of RNA polymerase III transcription, which is not covered here, include those by Ghavidel et al. (1999) and Brown et al. (2000).

Structure of RNA polymerase III promoters

The three types of RNA polymerase III promoters are called types 1–3. The first RNA polymerase III promoters characterized were those of the *Xenopus laevis* 5S RNA gene (Bogenhagen et al. 1980; Sakonju et al. 1980), which encodes the small ribosomal RNA, the Adenovirus 2 (Ad2) VAI gene (Fowlkes and Shenk 1980), and various tRNA genes from *X. laevis* and *Drosophila melanogaster* (Galli et al. 1981; Hofstetter et al. 1981; Sharp et al. 1981). The 5S promoter is the only example of a type 1 RNA polymerase III promoter, and the Ad2 VAI and tRNA promoters are typical type 2 promoters. As shown in Figure 1, these promoters are intragenic. The *X. laevis* 5S gene promoter consists of an A box, an intermediate element (IE), and a C box that is conserved in the 5S promoters of different species. Together, these elements

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Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1018902>.

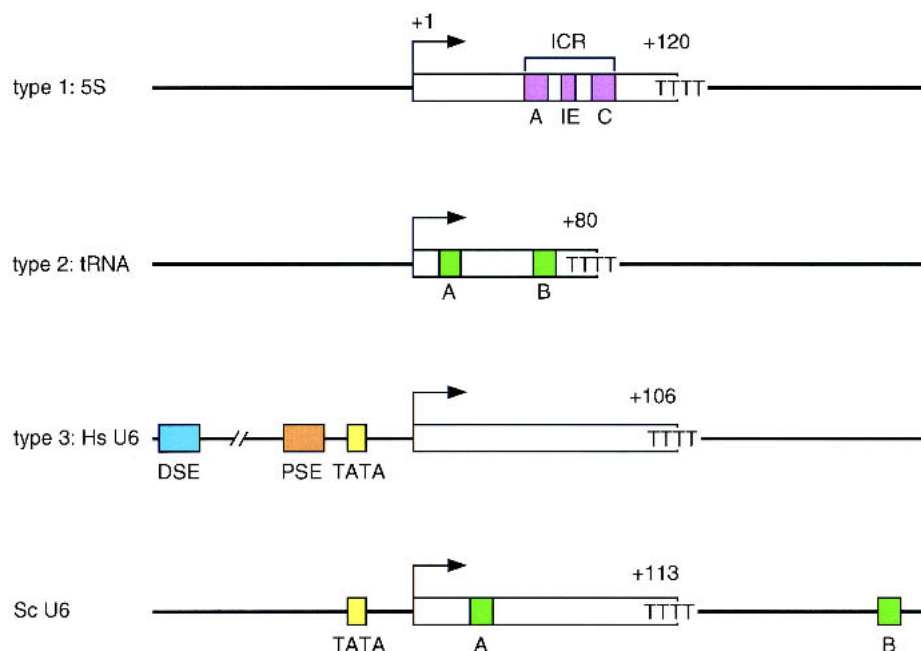


Figure 1. Different types of RNA polymerase III promoters. The type 1 promoter of the *Xenopus laevis* 5S RNA gene consists of an internal control region (ICR), which can be subdivided into A box (+50 to +60), intermediate element (IE, +67 to +72), and C box (+80 to +90). The type 2 promoter of the *X. laevis* tRNA^{Leu} gene consists of an A box (+8 to +19) and a B box (+52 to +62). The type 3 promoter of the *Homo sapiens* U6 snRNA gene consists of a distal sequence element (DSE, -215 to -240) that enhances transcription and a core promoter composed of a proximal sequence element (PSE, -65 to -48) and a TATA box (-32 to -25). The *Saccharomyces cerevisiae* promoter is a hybrid promoter consisting of a TATA box (-30 to -23), an A box (+21 to +31), and a B box located downstream of the U6 coding region (from +234 to +244 relative to the start site of transcription).

constitute the internal control region (ICR; Bogenhagen 1985; Pieler et al. 1985a,b, 1987). In the *Saccharomyces cerevisiae* 5S genes, only the C box is required for transcription (Challice and Segall 1989).

The Ad2 VAI and most tRNA promoters consist of an A box and a B box (Galli et al. 1981; Hofstetter et al. 1981; Sharp et al. 1981; Allison et al. 1983). These are well conserved in tRNA genes from various species, probably in part because they encode the tRNA D- and T-loops, which are required for tRNA function. The spacing between the A- and B-boxes varies greatly, however, in part to accommodate introns. The A-boxes of type 1 and 2 promoters are structurally related and are interchangeable in *X. laevis* (Ciliberto et al. 1983). However, this apparently reflects a similarity in sequence rather than a conserved function, because, as detailed below, the A-boxes of 5S and tRNA genes bind different transcription factors (Braun et al. 1992a).

The type 3 core promoters were identified originally in mammalian U6 snRNA genes, which encode the U6 snRNA component of the spliceosome (Krol et al. 1987; Das et al. 1988; Kunkel and Pederson 1988), and in the human 7SK gene (Murphy et al. 1986), whose RNA product has been recently implicated in the regulation of the CDK9/cyclin T complex (Nguyen et al. 2001; Yang et al. 2001). They are also found in, for example, the H1 RNA gene, which encodes the RNA component of human RNase P (Baer et al. 1989), and the gene encoding the RNA component of human RNase MRP (Topper and

Clayton 1990), as well as in genes encoding RNAs of unknown function. Their discovery came as a surprise because, unlike the then-characterized type 1 and 2 promoters, the type 3 core promoters turned out to be gene-external. As illustrated in Figure 1, they are located in the 5'-flanking region of the gene and consist of a proximal sequence element (PSE), which also constitutes, on its own, the core of RNA polymerase II snRNA promoters, and a TATA box located at a fixed distance downstream of the PSE (Hernandez and Lucito 1988; Mattaj et al. 1988; Kunkel and Pederson 1989; Lobo and Hernandez 1989). Strikingly, in the vertebrate snRNA promoters, RNA polymerase specificity can be switched from RNA polymerase III to RNA polymerase II and vice versa by abrogation or generation of the TATA box (Lobo and Hernandez 1989). Upstream of the PSE is an element referred to as the distal sequence element (DSE), which activates transcription from the core promoter.

Although the presence of a TATA box is the hallmark of type 3, gene-external, promoters, it is also found in the 5'-flanking regions of some genes with gene-internal promoter elements. Figure 1 shows an example of such a hybrid promoter, namely the *S. cerevisiae* U6 snRNA promoter. It consists of an A box located at an unusual position 120 bp downstream of the RNA coding region, and a TATA box located upstream of the transcription start site. All three of these promoter elements are required for efficient transcription in vivo (Brow and Guthrie 1990; Eschenlauer et al. 1993). Other examples

include some A- and B-box-containing tRNA genes in plants (Yukawa et al. 2000), yeast (Dieci et al. 2000), and silkworm (Ouyang et al. 2000), in which TATA boxes present in the 5'-flanking region greatly contribute to transcription efficiency. More recently, an analysis in *Schizosaccharomyces pombe* has revealed that in this organism nearly all tRNA and 5S genes contain a TATA box upstream of the transcription start site that is required for transcription (Hamada et al. 2001). Strikingly, in vitro, artificial promoters consisting of just a TATA box can direct RNA polymerase III transcription, indicating that under these circumstances, the TATA box contains all necessary information to assemble an RNA polymerase III transcription initiation complex (Mitchell et al. 1992; Roberts et al. 1995; Wang and Stumph 1995; Whitehall et al. 1995; Huang et al. 1996).

The assembly pathways directed by the different types of RNA polymerase III promoters converge on recruitment of TFIIIB and RNA polymerase III

The characterization of RNA polymerase III transcription factors started with the fractionation of a HeLa cell extract over a phosphocellulose column into three fractions known as fractions A (the phosphocellulose 100 mM KCl flowthrough), B (a 100 mM–350 mM KCl step elution), and C (a 350 mM–600 mM KCl step elution), and the observation that transcription from type 2 promoters required fractions B and C, whereas transcription from type 1 promoters required the three fractions (Segall et al. 1980). After the type 3 promoters were discov-

ered, they were shown to require the B and C fractions, or the B fraction and a D fraction eluted between 600 mM and 1000 mM KCl from the phosphocellulose column (Lobo et al. 1991). Most of the activities in these fractions required for RNA polymerase III transcription have now been characterized, both from yeast and human cells. Figure 2 shows, in a highly simplified manner, how these factors can assemble in an ordered fashion to recruit RNA polymerase III. The green arrows symbolize interactions of DNA-binding proteins with promoter elements, the blue arrows protein–protein contacts among various transcription factors, and the purple arrows protein–protein contacts between RNA polymerase III and transcription factors.

In type 2 promoters, the A and B boxes are recognized by a multisubunit complex in the C fraction called TFIIIC or TFIIIC2 (Lassar et al. 1983). This initial DNA–protein interaction then allows the recruitment of an activity in the B fraction called TFIIIB (Bieker et al. 1985; Setzer and Brown 1985). TFIIIB is composed of three polypeptides, one of which is the TATA-box-binding protein TBP. The binding of TFIIIB to the promoter in turn allows the recruitment of RNA polymerase III, mainly through protein–protein interactions with TFIIIB, although contacts with TFIIIC may also contribute (Fig. 2A). In type 1 promoters, the ICR is recognized by the activity present in the A fraction, a zinc finger protein referred to as TFIIIA (Engelke et al. 1980; Sakonju et al. 1981). Formation of the TFIIIA–DNA complex then allows for the binding of TFIIIC (Lassar et al. 1983). Thus, TFIIIA can be viewed as a specificity factor that

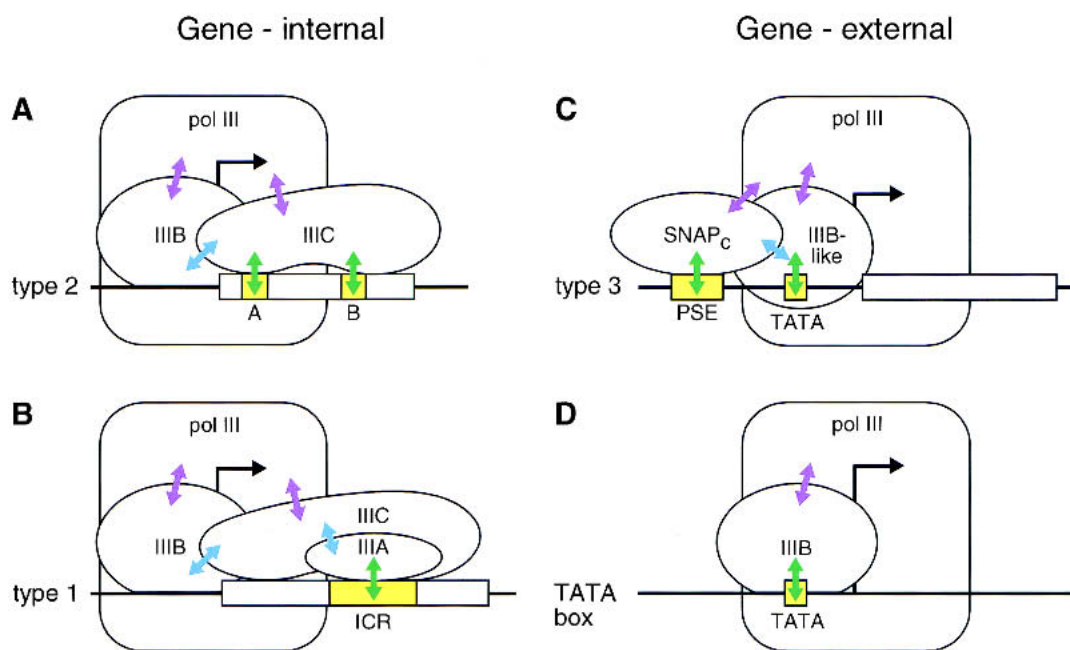


Figure 2. Different pathways for recruitment of TFIIIB and RNA polymerase III. The initiation complexes formed on type 2, 1, and 3 promoters, as well as on an artificial promoter consisting of just a TATA box, are shown. The green arrows symbolize interactions of DNA-binding proteins with promoter elements, the blue arrows protein–protein contacts among various transcription factors, and the purple arrows protein–protein contacts between RNA polymerase III and transcription factors.

alters the promoter-recognition properties of TFIIC and targets it to the 5S promoter. After the binding of TFIIC, the pathway to recruitment of the polymerase is similar to that in type 2 promoters, with the recruitment of TFIIB and RNA polymerase III (Fig. 2B). In type 3 promoters, the PSE is recognized by a multisubunit complex variously called the PSE-binding protein (PBP), the PSE transcription factor (PTF), or the snRNA activating protein complex (SNAP_c), and the TATA box is recognized by the TBP component of a specialized TFIIB-like activity (Waldschmidt et al. 1991; Murphy et al. 1992; Sadowski et al. 1993; Yoon et al. 1995; Schramm et al. 2000; Teichmann et al. 2000). These DNA-protein interactions are reinforced by protein-protein interactions between SNAP_c and TBP (Mittal and Hernandez 1997; Ma and Hernandez 2002). The binding of SNAP_c and the TFIIB-like activity then lead to recruitment of RNA polymerase III (Sepehri Chong et al. 2001), probably through protein-protein contacts with the two DNA-bound factors, SNAP_c and the TFIIB-like activity, although this has not yet been demonstrated (Fig. 2C).

Figure 2 also shows a recruitment pathway in which TFIIB is directly recruited to a TATA box without the help of protein-protein contacts with either TFIIC or SNAP_c (Fig. 2D). This pathway can be observed in vitro with *S. cerevisiae* TFIIB, and, although it is not observed in vivo, it reveals a profound aspect of RNA polymerase III transcription, namely, that TFIIB is sufficient for RNA polymerase recruitment. TFIIB was first identified as the key RNA polymerase III transcription factor by a series of experiments in which *S. cerevisiae* TFIIB was first recruited to either a 5S promoter through prior binding of TFIIA and TFIIC, or a tRNA promoter through prior binding of TFIIC (Kassavetis et al. 1990). TFIIA and/or TFIIC were then stripped from the DNA by treatment with heparin or high concentrations of salt. Under these conditions, functional TFIIA and TFIIC were released from the templates, but remarkably, TFIIB remained bound to the DNA, generating the same footprint upstream of the transcription start site as it did in the presence of TFIIA and/or TFIIC (Kassavetis et al. 1989, 1990). These stripped templates were able to support several rounds of properly initiated RNA polymerase III transcription. This suggested that, at least in yeast, TFIIB was sufficient to recruit RNA polymerase III and direct several rounds of transcription, and therefore that the main function of TFIIA and TFIIC was to recruit TFIIB to the DNA (Kassavetis et al. 1990). With the observation that just a TATA box could direct RNA polymerase III transcription in vitro and with the availability of recombinant TFIIB, it then became possible to confirm that a TATA box could direct several rounds of RNA polymerase III transcription with just recombinant TFIIB and highly purified RNA polymerase III (Kassavetis et al. 1995; R  th et al. 1996). Thus, TFIIA, TFIIC, and SNAP_c can be viewed as recruitment factors whose main function is to recruit TFIIB to promoters of various structures, which then allows the recruitment of RNA polymerase III.

The different RNA polymerase III promoters offer a

unique system in which we know how different accessory factors combine to recruit, ultimately, a TFIIB activity and RNA polymerase III. Below, we first describe briefly the subunit composition of RNA polymerase III. For a discussion of the likely three-dimensional structure of RNA polymerase III, see Geiduschek and Kassavetis (2001). We then describe the characterization of its key transcription factor, TFIIB, both yeast and human, and we summarize our present understanding of how this factor bridges DNA and RNA polymerase III. We then summarize what is known about the various factors that, in vivo, mediate the recruitment of TFIIB on most, if not all, promoters, namely, TFIIA, TFIIC, and SNAP_c. We end with a description of some factors that have been implicated in termination and recycling of RNA polymerase III.

RNA polymerase III

RNA polymerase III is well defined in *S. cerevisiae*, consisting of 17 subunits, as shown in Table 1. All the corresponding genes except for *RPC37* have been disrupted and shown to be essential (for review, see Chedin et al. 1998). Of the 17 subunits, 10 are unique to RNA polymerase III and are designated the C subunits, two are common to RNA polymerases I and III and are designated AC subunits, and five are common to the three RNA polymerases and are designated ABC subunits. The common subunits have different names in RNA polymerases I and II, as indicated in Table 1 for RNA polymerase II. C160, C128, AC40, AC19, and ABC23 are evolutionarily related to the core subunits of *Escherichia coli* RNA polymerase, as indicated in parentheses in the table. Of the C subunits, five, indicated in bold in Table 1, are specific to RNA polymerase III.

Human RNA polymerase III has been purified both by conventional chromatography (Wang and Roeder 1996) and from cell lines expressing tagged *Homo sapiens* (Hs) RPC4/RPC53/BN51 (Wang and Roeder 1997), but until recently, only five of its subunits had been characterized: HsRPC4/RPC53 (Ittmann et al. 1993; Jackson et al. 1995), HsRPC1/RPC155 (Sepehri and Hernandez 1997), HsRPC3/RPC62, HsRPC6/RPC39, and HsRPC7/RPC32 (Wang and Roeder 1997). Human RNA polymerase III has now been purified from a stable cell line expressing a doubly tagged HsRPC4 subunit, and its subunits have been identified by mass spectrometry (Hu et al. 2002). This analysis has resulted in the identification of orthologs of all of the yeast RNA polymerase III subunits except for ABC10 α , which was not detected probably because of its small size (7 kD). The newly described human subunits were named according to the guide shown in the fourth column of Table 1, in which the yeast C, AC, and ABC subunits were numbered separately in order of decreasing apparent molecular weight. Such a nomenclature would provide the same name for orthologs from different species, as shown in the fourth and sixth column in Table 1.

The characterization of human RPC8 and RPC9 brought an unexpected result. BLAST searches revealed

Table 1. Subunits of *Saccharomyces cerevisiae* and *Homo sapiens* RNA polymerase III

<i>S. cerevisiae</i> RNA Pol III subunits	MW (kD)	Accession number	Guide for nomenclature	Corresponding <i>S. cerevisiae</i> RNA Pol II subunits	<i>H. sapiens</i> RNA RNA Pol III subunits	MW (kD)	Accession number	% amino acid identities between <i>H. sapiens</i> and <i>S. cerevisiae</i> Pol III subunits
C160 (β' -like)	162.1	P04051	ScRPC1	RPB1	HsRPC1/RPC155	155.6	AAB86536	50% (1356/1391) ^a
C128 (β -like)	129.3	AAB59324	ScRPC2	RPB2	HsRPC2	127.6	AY092084	63% (1115/1133) ^a
C82	73.6	CAA45072	ScRPC3		<u>HsRPC3/RPC62</u>	60.5	NP_006459/XP_034604	22% (163/534) ^a
C53	46.6	P25441	ScRPC4		<u>HsRPC4/RPC53</u>	44.4	AY092086	28% (134/398) ^a
C37	32.1	NP_012950	ScRPC5		<u>HsRPC5</u>	79.8	AY092085	26% (160/708) ^a
C34	36.1	P32910	ScRPC6		<u>HsRPC6/RPC39</u>	35.6	NP_006457/XP_009639	26% (216/316) ^a
C31	27.7	P17890	ScRPC7		<u>HsRPC7/RPC32</u>	25.9	AAB63676/XP_036456	35% (44/223) ^a
C25	24.3	P35718	ScRPC8	RPB7	HsRPC8	22.9	AY092087	42% (201/204) ^a
C17	18.6	P47076	ScRPC9	RPB4	HsRPC9/CGRP-RC	16.8	AAC25992	30% (122/148) ^a
C11	12.5	AAD12060	ScRPC10	RPB9	HsRPC10/RPC11	12.3	NP_057394	52% (108/108) ^a
AC40 (α -like)	37.6	P07703	ScRPAC1	RPB3	HsRPAC1/RPA5,RPA39	38.6	NP_004866	47% (287/342) ^a
AC19 (α -like)	16.1	P28000	ScRPAC2	RPB11	HsRPAC2/RPA9,RPA16	15.2	NP_057056	45% (119/133) ^a
ABC27	25.1	P20434	ScRPABC1	RPB5	HsRPABC1/RPB5,RPB25	24.6	P19388	42% (207/210) ^a
ABC23 (ω -like)	17.9	AAA34989	ScRPABC2	RPB6	HsRPABC2/RPB6,RPB14.4	14.5	P41584	72% (83/127) ^a
ABC14.5	16.5	CAA37383	ScRPABC3	RPB8	HsRPABC3/RPB8,RPB17	17.1	P52434	35% (147/150) ^a
ABC10 α	7.7	AAA64417	ScRPABC4	RPB12	HsRPABC4/RPB7.0	7.0	P53803	52% (42/58) ^a
ABC10 β	8.2	P22139	ScRPABC5	RPB10	HsRPABC5/RPB10,RPB7.6	7.6	P52436	73% (67/67) ^a

Subunits in bold do not have paralogues in RNA polymerases I and II; those in bold and underlined form a complex separable from the rest of the enzyme. Subunits corresponding to the *E. coli* β' , β , α , and ω subunits are indicated. ABC27 and RPB5, ABC23 and RPB6, ABC14.5 and RPB8, ABC10 α and RPB12, ABC10 β and RPB10 designate in each case the same protein. For HsRPC62, HsRPC39, and HsRPC32, the sequence under the first accession number (Wang and Roeder 1997) differs in several positions from both the sequences deposited by NCBI (second accession number) and genomic sequences.

^aThe first number in the parentheses indicates the length of the region of similarity; the second number indicates the total length of the human protein. Reprinted from Hu et al. (2002).

that HsRPC8 is related to the RNA polymerase II subunit RPB7, as noted earlier for the *S. cerevisiae* HsRPC8 ortholog C25 (Sadhale and Woychik 1994). In addition, however, HsRPC9 is related to RPB4, and like RPB4 and RPB7, which associate with each other and form a dimer detachable from the rest of RNA polymerase II (Edwards et al. 1991; Khazak et al. 1998), HsRPC8 and HsRPC9 associate with each other (Hu et al. 2002). This strongly suggests that HsRPC8 and HsRPC9 are paralogs of RPB7 and RPB4, as indicated in Table 1, and that the corresponding *S. cerevisiae* RNA polymerase III subunits C25 and C17 can similarly associate with each other.

RPB7, but not RPB4, is essential for yeast cell viability (Woychik and Young 1989; McKune et al. 1993). RPB4 is, however, essential for cellular responses to stress (Choder and Young 1993) and thus in vivo, the requirement for the RPB4 subunit may be promoter-specific. The RPB4/RPB7 complex is thought to stabilize the open promoter complex and perhaps the early transcribing complex prior to promoter escape by binding to nascent RNA or to single-stranded DNA in the transcription bubble (Orlicky et al. 2001; Todone et al. 2001). The *S. cerevisiae* RNA polymerase III paralogs of RPB7 and RPB4, C25 and C17, are both essential for viability in yeast (Sadhale and Woychik 1994; Ferri et al. 2000). Two-hybrid and coimmunoprecipitation experiments indicate that C17 interacts with the transcription initiation factor Brf1 and with the RNA polymerase III C31 subunit (Ferri et al. 2000), which, as described below, is itself required for transcription initiation (Werner et al. 1992, 1993; Wang and Roeder 1997). Thus, the RNA polymerase III paralogs of RPB4 and RPB7 may also be involved

in transcription initiation, but in this case both subunits are essential for yeast cell viability, perhaps because most RNA polymerase III genes encode components essential for cell metabolism.

The human RNA polymerase III subunits are in general quite similar to their yeast counterparts with the notable exception of the subunits with no paralogs in RNA polymerase II (Jackson et al. 1995; Wang and Roeder 1997; Hu et al. 2002). For example, the human ortholog of yeast C37 is an 80-kD protein, HsRPC5, whose similarity to the yeast protein is confined to its N-terminal fourth, which shows 26% identity with C37 (Hu et al. 2002). Nevertheless, like yeast C37, which associates with the yeast C53 subunit (Flores et al. 1999), HsRPC5 associates with HsRPC4/RPC53, the human ortholog of yeast C53, and this association is through the HsRPC5 and HsRPC4/RPC53 domains conserved in their yeast counterparts (Hu et al. 2002). Interestingly, at least some of the subunits with no paralogs in RNA polymerase II seem to be involved in promoter recognition. The C82, C34, and C31 subunits (bold and underlined in Table 1) dissociate from a yeast enzyme carrying a mutation within the zinc finger domain of the largest subunit, and each associates with the two others in a yeast two-hybrid assay, suggesting that these three subunits form a subcomplex detachable from the rest of the enzyme (Werner et al. 1992, 1993). In the human enzyme, such a subcomplex could be demonstrated directly by sucrose gradient centrifugation under partially denaturing conditions and by reconstitution of the subcomplex from recombinant subunits (Wang and Roeder 1997). The subunits in the subcomplex are not required for ef-

efficient elongation and termination, but are required for specific initiation (Thuillier et al. 1995; Brun et al. 1997; Wang and Roeder 1997). Consistent with this observation and as detailed further below, the C34 subunit and its human counterpart interact directly with TFIIB subunits (Werner et al. 1993; Khoo et al. 1994; Wang and Roeder 1997).

Composition of TFIIB

TBP is required for transcription by RNA polymerase III both in S. cerevisiae and human cells

Although the presence of an RNA polymerase III transcription activity in the phosphocellulose B fraction was recognized in the early 1980s, the composition of this activity remained a mystery for the next 10 years. By the late 1980s, however, the concept that the TATA-box-binding protein TBP was a factor uniquely dedicated to transcription by RNA polymerase II began to change with the finding that an essential element of the U6 promoter was an A/T-rich region, that is, a potential binding site for TBP. Biochemical fractionation and reconstitution experiments then identified TBP as a factor required for transcription of both the yeast and human U6 snRNA genes (Lobo et al. 1991; Margottin et al. 1991; Simmen et al. 1991), whose binding to wild-type and mutant U6 TATA boxes correlated with transcription activity (Lobo et al. 1991). These findings established that TATA boxes are part of at least some RNA polymerase III promoters, and that they act by recruiting TBP. They also raised the possibility that TBP might be required for RNA polymerase III transcription in general. Indeed, in vitro competition experiments with TATA-containing oligonucleotides then indicated that a TATA-box-binding factor was required for transcription of the VAI and tRNA genes (White et al. 1992), and inactivation of TBP in yeast was shown to lead to defects in transcription by all three RNA polymerases (Cormack and Struhl 1992; Schultz et al. 1992). The remaining question was how to place TBP in what was then known about RNA polymerase III transcription factors.

Identification of S. cerevisiae Brf1 and Bdp1

Yeast TFIIB had been shown to consist of two chromatographically separable activities, named B' and B'', which contained polypeptides of 70 and 90 kD, respectively, that could be cross-linked to the DNA (Bartholomew et al. 1991; Kassavetis et al. 1991). A major step in the complete characterization of TFIIB came with the cloning of the gene encoding the 70-kD polypeptide, now referred to as Brf1 (TFIIB-related factor 1; for a description of a universal nomenclature of TFIIB components, see Willis 2002). The gene was cloned as a suppressor of a tRNA gene A-box mutation and called *PCF4* (López-De-León et al. 1992). It was also cloned, however, as an allele-specific high-copy suppressor of certain mutations in TBP and called *BRF1* (Colbert and Hahn 1992) or *TDS4* (Bu-

ratowski and Zhou 1992). This ability to suppress mutations in TBP suggested that Brf1 might be associated with TBP, and thus that TBP might be part of the TFIIB activity. Indeed, TBP was shown to be part of both the yeast and mammalian TFIIB activity by biochemical methods (Margottin et al. 1991; Huet and Sentenac 1992; Kassavetis et al. 1992; Lobo et al. 1992; Taggart et al. 1992; White and Jackson 1992; Chiang et al. 1993; Meyers and Sharp 1993), and to constitute a previously unrecognized, Brf1-associated, component of the B' activity (Kassavetis et al. 1992). The cloning of the gene encoding yeast B'' (B'', Kassavetis et al. 1995; TFIIB90, Roberts et al. 1996; TFC7p, Rùth et al. 1996), now referred to as *Bdp1* (B double prime 1, Willis 2002), then completed the characterization of *S. cerevisiae* TFIIB.

Identification of human Brf1 and Brf2

In *S. cerevisiae*, all RNA polymerase III promoters recruit the same TFIIB factor (Joazeiro et al. 1994). In higher eukaryotes, however, the situation is more complex, consistent with the need to transcribe much more complex genomes. Thus, the initial characterization of mammalian TFIIB not only indicated that TBP was part of the activity (Lobo et al. 1992; Taggart et al. 1992; White and Jackson 1992), but also that type 1 and 2 promoters used different components in the TFIIB fraction than type 3 promoters. Type 1 and 2 promoters were shown to require a TBP-containing complex (Lobo et al. 1992; Teichmann and Seifart 1995) consisting of TBP and a homolog of yeast Brf1 (Wang and Roeder 1995; Mital et al. 1996) referred to as HsBrf1 (*Homo sapiens* Brf1). Depletion of extracts with antibodies directed against the C-terminal half of HsBrf1 debilitated transcription from the type 2 VAI promoter, as expected, but had no effect on transcription from the type 3 human U6 snRNA promoter (Mital et al. 1996; Henry et al. 1998a). On the other hand, depletion of extracts with antibodies raised against full-length HsBrf1 or against a peptide derived from the N-terminal portion of the protein inhibited transcription from all types of RNA polymerase III promoters, although only transcription from type 1 and 2 promoters could be reconstituted by addition of recombinant HsBrf1 (Wang and Roeder 1995; Schramm et al. 2000). These observations suggested that type 3 promoters use a protein related to Brf1 in its N-terminal but not its C-terminal region, and led to the characterization of a new protein, originally called BRFU (Schramm et al. 2000) or TFIIB50 (Teichmann et al. 2000), and now referred to as HsBrf2 (Willis 2002). Thus, *S. cerevisiae* Brf1 has at least two homologs in human cells, HsBrf1 and HsBrf2.

Figure 3 shows the structure of TFIIB and various Brf proteins. *H. sapiens* and *S. cerevisiae* (Sc) Brf1 as well as HsBrf2 contain, like TFIIB, an N-terminal zinc-binding domain (green box) and a "core domain" consisting of two imperfect repeats (blue box). In addition, the Brf1 and Brf2 proteins contain C-terminal domains absent in TFIIB. Within the C-terminal segment of Brf1, three regions, designated regions I, II, and III, are conserved in

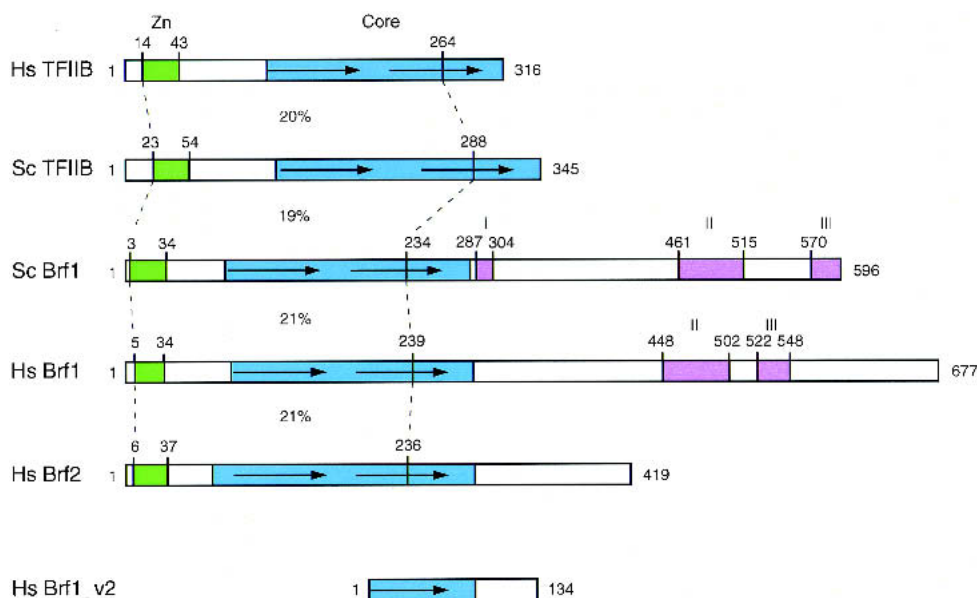


Figure 3. TFIIB, Brf1, and Brf2 form a family of related transcription factors. The location of the structured zinc ribbon as modeled in ScTFIIB and ScBrf1 (Hahn and Roberts 2000) based on the NMR structure of PfTFIIB (Zhu et al. 1996) and that of the corresponding region in HsBrf1 and HsBrf2 is indicated in green. The location of the structured core domain of TFIIB (Bagby et al. 1995; Nikolov et al. 1995) and that of the corresponding regions in the other proteins is indicated in blue. The percentages below the sequences indicate percent identities between HsBrf2 and HsTFIIB, ScTFIIB, ScBrf1, and HsBrf1 within the region of highest conservation (bracketed by the stippled lines) in pairwise alignments performed with BLAST. The purple boxes in the C-terminal regions of ScBrf1 and HsBrf1 indicate conserved regions I, II, and III (Mital et al. 1996). In HsBrf1_v2, the blue region is identical to the corresponding HsBrf1 region.

the yeasts *Candida albicans*, *Kluyveromyces lactis*, *S. pombe*, and *S. cerevisiae* (Khoo et al. 1994). Regions II and III are also conserved in the human Brf1 protein (Mital et al. 1996; Andrau et al. 1999). Consistent with the antibody depletion data, the C-terminal domain of HsBrf2 shows very little, if any, homology with Brf1.

HsBrf2 was isolated through a database search for proteins related to TFIIB and to the TFIIB-related segment of Brf1 (Schramm et al. 2000), as well as through biochemical purification of a complex, consisting of HsBrf2 and four associated proteins, required for transcription from type 3 promoters (Teichmann et al. 2000). It is clear that HsBrf2 itself is specifically required for transcription from type 3, but not types 1 and 2, promoters, but the exact role of the HsBrf2-associated factors remains to be determined. Although in one case, U6 transcription in HsBrf2-depleted extracts could be restored only by addition of the HsBrf2-containing complex immunopurified from HeLa cells expressing tagged HsBrf2 (Teichmann et al. 2000), in another case it could be restored by addition of just HsBrf2 synthesized in *E. coli* (Schramm et al. 2000). This last observation suggests that the HsBrf2-associated polypeptides may not be absolutely required for U6 transcription but may contribute to the efficiency of the reaction.

Figure 3 also illustrates the structure of HsBrf1_v2 (originally named BRF2), a factor encoded by one of at least four alternatively spliced *BRF1* pre-mRNAs (McCulloch et al. 2000). HsBrf1_v2 lacks the zinc finger domain and the first repeat that are present in Brf1 and conserved in the other proteins of the TFIIB family, as

well as the C-terminal region present in Brf1. Although HsBrf1 is not involved in human U6 transcription, HsBrf1_v2 has been implicated in U6 transcription because when antibodies recognizing all HsBrf1 variants were used to deplete extracts, U6 transcription was lost and could be specifically restored by addition of material immunopurified from cells expressing tagged HsBrf1_v2 (McCulloch et al. 2000). It will be necessary to define the composition of this immunopurified fraction to confirm the role of HsBrf1_v2 in U6 transcription.

Identification of human Bdp1

Figure 4 shows the structure of *S. cerevisiae* Bdp1. It contains a domain related to a Myb repeat, identified in the SWI-SNF and ADA complexes, the transcriptional corepressor N-Cor, and yeast TFIIB Bdp1, and therefore referred to as the SANT domain (Aasland et al. 1996). The SANT domain is absolutely required for TFIIC-dependent (but not TFIIC-independent, see below) RNA polymerase III transcription (Kumar et al. 1997). In addition, a region upstream of the SANT domain (indicated in orange in Fig. 4) is required for transcription from linear, but not supercoiled, templates (Kassavetis et al. 1998a).

Human Bdp1 cDNAs were isolated through a combination of database searches for sequences similar to the yeast Bdp1 SANT domain and library screening (Schramm et al. 2000). The structure of the protein encoded by one of these cDNAs (HsBdp1) is shown in Figure 4. It is highly related to the yeast protein within the

Schramm and Hernandez

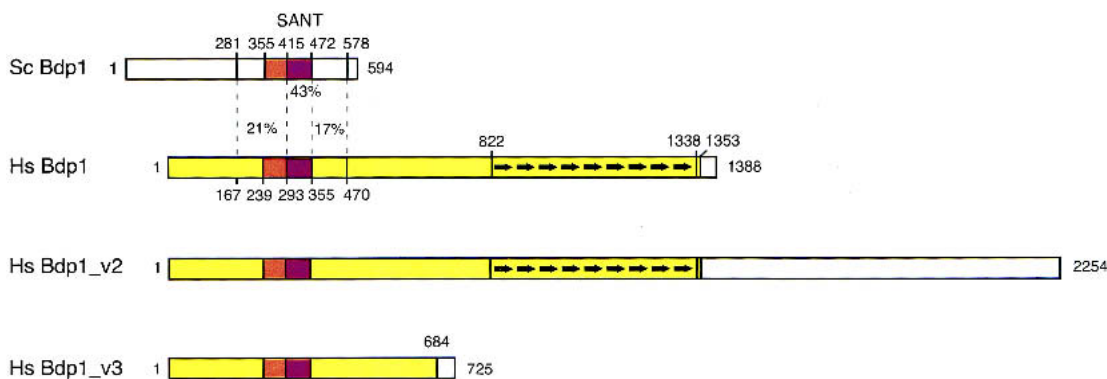


Figure 4. Comparison of the ScBdp1 and HsBdp1 polypeptides. The proteins contain a conserved SANT domain (brown box). The regions upstream and downstream of the SANT domain are also quite conserved, especially a segment upstream of the SANT domain (indicated in orange) that is required for transcription from linear, but not supercoiled, templates. The percentages indicate amino acid identities between ScBdp1 and HsBdp1 in the regions bracketed by dotted lines. HsBdp1, HsBdp1_v2, and HsBdp1_v3 are identical in the colored regions. The repeats extend from amino acids 822 to 1338. HsBdp1 and HsBdp1_v2 diverge after amino acid 1353. HsBdp1 and HsBdp1_v3 diverge after amino acid 684.

SANT domain (43% identity) as well as both immediately upstream, in a region that encompasses the segment required for transcription from linear DNA templates (21% identity), and downstream (17% identity). Outside of these regions, the two proteins are not conserved, and the human protein differs from the yeast protein by a striking C-terminal extension containing a number of repeats with potential phosphorylation sites. A number of alternatively spliced *BDP1* cDNAs have been isolated (Kelter et al. 2000; Schramm et al. 2000). Two of these encode strikingly different proteins, which are also shown in Figure 4. The longest protein (labeled HsBdp1_v2 in the figure) is identical to Bdp1 except that the last few amino acids are replaced by a 901-amino-acid extension, giving a protein of 2254 amino acids. Another cDNA encodes a 725-amino-acid protein (Bdp1_v3), which contains Bdp1 sequences up to amino acid 684, followed by a divergent 47-amino-acid extension (Kelter et al. 2000).

Which of the alternatively spliced forms of human Bdp1 are involved in RNA polymerase III transcription in vivo is not clear at present. Depletions of extracts with antibodies directed against regions both upstream and downstream of the SANT domain within the N-terminal half of human Bdp1 (Schramm et al. 2000), as well as against the repeat region (L. Schramm and N. Hernandez, unpubl.), debilitate transcription from both type 2 and 3 promoters in vitro, and transcription can be restored by addition of recombinant human Bdp1, either full-length or truncated downstream of the SANT domain. This suggests that HsBdp1 is generally required for RNA polymerase III transcription, and that the C-terminal repeats are not required for basal in vitro transcription from naked DNA templates. However, the functional protein present in HeLa cell extracts probably contains the repeat region, because it can be depleted by antibodies directed against this region. Perhaps the repeat region performs a regulatory role not scored in the in vitro transcription assay.

The characterization of human TFIIB has revealed that unlike in *S. cerevisiae*, where type 3 promoters apparently do not exist and one form of TFIIB serves all RNA polymerase III promoters (Joazeiro et al. 1994), there are at least two forms of TFIIB in human cells. As shown in Figure 5, one of them consists of HsTBP, HsBrf1, and HsBdp1 and is used by type 2 (and probably type 1) promoters. The other consists of HsTBP, HsBrf2, and HsBdp1, and is used by type 3 promoters. Future work may reveal that different spliced variants of Bdp1 are recruited to different RNA polymerase III promoters in vivo. Furthermore, in *D. melanogaster* cells, the TBP in TFIIB is replaced by a TBP-related factor called TRF1 (Takada et al. 2000). Thus, there may be a wide range of TFIIB activities in different species containing variants of each of the three TFIIB components.

In *S. cerevisiae*, *H. sapiens*, and *D. melanogaster*, Brf1 is tightly associated with TBP or TRF1 in solution, as symbolized by red bars in Figure 5. On the other hand, Bdp1 is weakly associated with the TBP–Brf1 complex in *S. cerevisiae*, and very weakly, if at all, in human cells (Kassavetis et al. 1991; Wang and Roeder 1995; Mital et al. 1996; Schramm et al. 2000). Indeed, an association between HsBdp1 and HsTBP can only be detected in GST pull-downs (blue bars in Fig. 5; Cabart and Murphy 2002). Similarly, although HsBrf2 can be shown to associate with HsTBP in GST pull-downs (Cabart and Murphy 2001, 2002), it is not strongly associated with TBP in HeLa cell extracts (Schramm et al. 2000). Thus, the TFIIB components do not always form a stable complex off the DNA.

Functions of TFIIB

In RNA polymerase II transcription, the opening of the transcription bubble that occurs after recruitment of the polymerase is dependent on TFIIE and an ATP-dependent helicase activity of TFIIF (Holstege et al. 1996; Tirode et al. 1999). In contrast, in RNA polymerase III

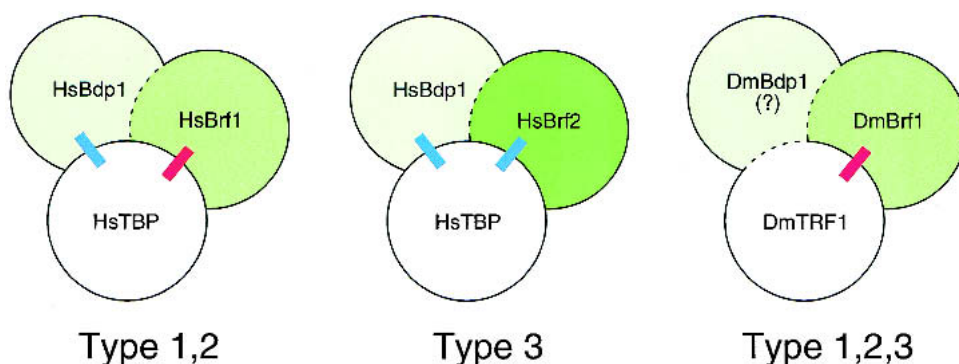


Figure 5. Promoter-selective TFIIB activities. The TFIIB components required by different classes of promoters in *Homo sapiens* and *Drosophila melanogaster* are depicted. Strong (red bars) and weak (blue bars) direct protein–protein associations in solution are indicated. Stippled lines indicate that a direct protein–protein contact has not been demonstrated. DmBdp1 has not been characterized, but a candidate gene has been identified (Schramm et al. 2000).

transcription, the opening of the transcription bubble occurs in an ATP-independent manner after recruitment of RNA polymerase III by TFIIB (Kassavetis et al. 1990, 1992). The availability, in yeast, of both recombinant TFIIB and a transcription system independent of TFIIC, that is, a system in which a TATA box can recruit TFIIB directly, has allowed detailed analyses of the functions of the TFIIB subunits. These studies have given a detailed picture of how TFIIB recognizes the TATA box and how it recruits RNA polymerase III. They have also revealed that, remarkably, TFIIB not only functions to recruit RNA polymerase III but also participates in opening of the transcription bubble.

Binding of TFIIB to the TATA box

In promoters consisting of just a TATA box, *S. cerevisiae* TFIIB binds to the DNA through recognition of the TATA box by its TBP subunit. Indeed, a mutation in the TATA box that debilitates RNA polymerase III transcription can be compensated by a mutation in TBP that alters the DNA-binding specificity of the protein and

allows it to bind to the mutated TATA box (Strubin and Struhl 1992; Whitehall et al. 1995). The TBP–TATA-box complex is then recognized by Brf1 or, in the case of the human U6 promoter, by Brf2. The similarity of both Brf1 and Brf2 to TFIIB is very striking, and immediately suggests that the conserved domains of these proteins may perform equivalent functions during assembly of RNA polymerase II and III transcription initiation complexes. The reality, however, is more complex. In TFIIB, the core domain is sufficient for association with the TATA-box–TBP complex. However, recruitment of RNA polymerase II and TFIIF to the TATA-box–TBP–TFIIB complex requires the TFIIB zinc-binding domain (Barberis et al. 1993; Ha et al. 1993; Hisatake et al. 1993; Yamashita et al. 1993; Pardee et al. 1998).

HsBrf2 resembles TFIIB in that it recognizes the TATA-box/TBP complex through its TFIIB-related core domain (Cabart and Murphy 2001). In contrast, for *S. cerevisiae* Brf1, the task of recognizing the TBP–TATA-box complex is performed by two regions of the protein, the TFIIB-related N-terminal half as well as the Brf1-specific C-terminal half, with the latter playing the major role. Thus, as summarized in Figure 6, a truncated

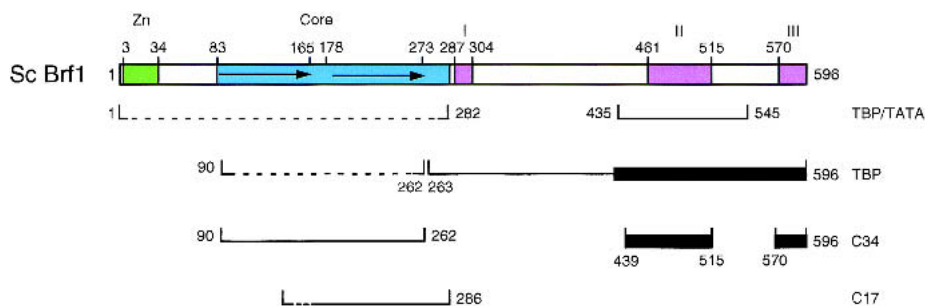


Figure 6. Functional domains of *Saccharomyces cerevisiae* Brf1. *S. cerevisiae* Brf1 is depicted, with the locations of the zinc domain, direct repeats in the core, and conserved regions I, II, and III. The brackets below indicate regions of the proteins sufficient for association with the TBP–TATA-box complex (Kassavetis et al. 1998b), TBP alone (Khoo et al. 1994), and the C34 (Khoo et al. 1994; Andrau et al. 1999) subunits of RNA polymerase III. The black boxes indicate regions where mutations or deletions have a strong negative effect on the associations. The stippled line indicates an association detected only by UV cross-linking. The upstream boundary of the Brf1 region sufficient for interaction with C17 is not precisely defined.

ScBrf1 protein retaining just the zinc-binding domain and the core associates only very weakly with a TATA-box–TBP complex. Indeed, the association is so weak that it is only detected by methods such as photochemical cross-linking (Kassavetis et al. 1997, 1998b; Colbert et al. 1998). This weak association appears to involve a TBP surface that overlaps or lies near the TFIIB-interacting surface in the “stirrup” of the second TBP repeat, because a triple-amino-acid change in TBP that disrupts the TFIIB interaction suppresses cross-linking of the N-terminal half of ScBrf1 to DNA and thus probably association with the TBP–TATA-box complex (Kassavetis et al. 1998b). On the other hand, a 110-amino-acid region encompassing conserved region II within the C-terminal half of the protein is sufficient for stable association with a TATA-box–TBP complex as well as for recruitment of ScBdp1. Moreover, the hydroxyl radical footprint observed with just the C-terminal domain of ScBrf1 is identical to that observed with the full-length protein (Colbert et al. 1998). Thus, despite the strong conservation of the core domains in TFIIB and Brf1, it appears that in ScBrf1, the function of recognizing the TBP–TATA-box complex has been largely transferred to the C-terminal half of the protein and in particular to conserved region II. This region of ScBrf1 binds the opposite face of the TBP–TATA-box complex from TFIIB and recognizes a TBP surface that overlaps that recognized by TFIIA (Colbert et al. 1998; Kassavetis et al. 1998b; Shen et al. 1998; for models of the structure of the TBP–DNA–ScBrf1 complex, see Colbert et al. 1998; Geiduschek and Kassavetis 2001). It will be important to contrast HsBrf1 and HsBrf2 with ScBrf1 to determine how these TBP-association activities have been conserved among the human Brf1 and Brf2 proteins.

ScBdp1 can associate with a preassembled TBP–ScBrf1–TATA-box complex but not with a complex lacking ScBrf1, and this confers on the yeast TFIIB–DNA complex its striking resistance to salt and heparin. ScBdp1 contacts not only ScBrf1 but also TBP, because at least one mutation in yeast TBP prevents association of ScBdp1 without affecting association of ScBrf1 (Colbert et al. 1998). ScBdp1 also contacts DNA because its assembly onto the TATA-box–TBP–ScBrf1 complex both requires DNA, and extends the DNA footprint, upstream of the TATA box (Colbert et al. 1998; Shah et al. 1999). Moreover, ScBdp1 can be cross-linked to the DNA at sites upstream of the TATA box (Shah et al. 1999). The binding of ScBdp1 to the TBP–ScBrf1–TATA-box complex induces a bend in the DNA between the TATA box and the transcription start site, which is in phase with the bend imposed by TBP on the TATA box (Leveillard et al. 1991; Braun et al. 1992b; Grove et al. 1999). This bending of the DNA has been postulated to contribute to the ScBdp1-dependent stabilization of the TFIIB–DNA complex by helping impede sliding of the DNA out of the complex (Grove et al. 1999), a hypothesis consistent with thermodynamic and kinetic data indicating ScBdp1-dependent kinetic trapping of the DNA (Cloutier et al. 2001). In the human system, HsBdp1 has been shown to assemble, albeit inefficiently, on a preformed

TATA-box–TBP–HsBrf2 complex (Cabart and Murphy 2002).

RNA polymerase recruitment by TFIIB

Figure 7 shows the known protein–protein contacts between TFIIB and RNA polymerase III subunits with arrows for contacts identified with human (solid) or yeast (hatched) subunits, respectively. Eight RNA polymerase III subunits can be cross-linked to DNA in a transcription initiation complex (Bartholomew et al. 1993). Of these, C34, which is part of the three-subunit subcomplex that is required for transcription initiation (Werner et al. 1993; Wang and Roeder 1997), maps the furthest upstream and can be localized between positions –17 and +6 relative to the transcription start site, in close proximity to TFIIB (Bartholomew et al. 1993). ScBrf1 interacts *in vivo* and *in vitro* with C34, and human Brf1 associates with the human homolog of C34, HsRPC39, *in vitro* (Werner et al. 1993; Khoo et al. 1994; Wang and Roeder 1997). As shown in Figure 6, ScBrf1 appears to contact C34 through three regions: regions II and III within the Brf1-specific C-terminal domain (Andrau et al. 1999), and another region, identified by GST pull-down assays, located within the core region in the TFIIB-related N-terminal half of the protein (Khoo et al. 1994). ScBrf1 also contacts the recently identified RNA polymerase III subunit C17 through the C-terminal half of its core region (Ferri et al. 2000). Notably, unlike the zinc-binding domain of TFIIB, the zinc-binding domain of ScBrf1 is not required for RNA polymerase recruitment (Kassavetis et al. 1997; Hahn and Roberts 2000). How HsBrf2 contacts RNA polymerase III is not known. It will be highly interesting to determine further which parts of the protein are required for assembly with TBP and SNAP_c onto the human U6 promoter and for recruitment of RNA polymerase III. Contacts between Bdp1 and RNA polymerase III subunits have not been described, but as shown in Figure 7, human TBP associates

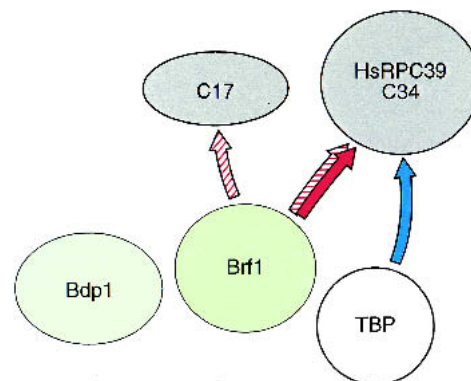


Figure 7. Protein–protein contacts between TFIIB components and RNA polymerase III subunits. The solid arrows represent contacts identified with human subunits, the stippled arrows depict contacts identified with *Saccharomyces cerevisiae* subunits.

with the HsRPC39 RNA polymerase III subunit in vitro (Wang and Roeder 1997).

Post-RNA polymerase III recruitment roles for Brf1 and Bdp1

In in vitro transcription assays with supercoiled templates, the activities of *S. cerevisiae* Brf1 and Bdp1 are surprisingly resistant to deletions. The N-terminal half of ScBrf1 forms an unstable TFIIB complex but is nevertheless capable of directing TFIIC-independent transcription from a TATA box if high amounts of ScBdp1 are supplied (Kassavetis et al. 1997). The C-terminal half on its own shows little or no transcription activity, but when the N-terminal half of ScBrf1 is added in *trans*, peptides encompassing region II mediate high levels of transcription. Perhaps most surprising, an ScBrf1 protein lacking the first 164 amino acids including the zinc-binding domain and the first TFIIB-related repeat retains up to 25% of the activity of full-length ScBrf1 for TFIIC-independent transcription from supercoiled templates in vitro (Kassavetis et al. 1997). Importantly, however, none of these ScBrf1 truncations function in vivo or for TFIIC-dependent transcription in vitro. Moreover, they do not function for TFIIC-independent transcription in vitro from a linear template, suggesting that they are somehow defective in promoter opening. Indeed, with the ScBrf1 protein lacking the first 164 amino acids, RNA polymerase III is recruited on a linear template, but the transcription bubble does not form (Kassavetis et al. 1998a). This is probably caused at least in part by the absence of the ScBrf1 zinc ribbon, because point mutations within the zinc domain show defects in promoter opening as determined by sensitivity to potassium permanganate (Hahn and Roberts 2000). Therefore, in ScBrf1, the zinc ribbon, which is not required for polymerase recruitment, plays a role at a later stage, during promoter opening.

The ScBdp1 TFIIB subunit also plays a post-RNA polymerase III recruitment role. Thus, ScBdp1 molecules lacking the conserved region upstream of the SANT domain (see Fig. 4) can direct somewhat reduced levels of transcription from supercoiled templates but are inactive with linear templates and fail to generate permanganate sensitivity around the transcription start site (Kassavetis et al. 1998a). Moreover, ScBdp1 is dispensable for transcription altogether under conditions in which promoter opening is not required (Kassavetis et al. 1999). Upon recruitment of RNA polymerase III, the *SUP4* tRNA gene promoter opens in two segments, one surrounding the transcription start site and the other located ~7 bp upstream (Kassavetis et al. 1992). With templates containing preformed bubbles extending from -9 to -5, TBP and ScBrf1 alone are sufficient to recruit RNA polymerase III and direct multiple rounds of transcription, although the efficiency is only 5% to 10% of that observed with the complete TFIIB complex. Thus, ScBdp1 plays an essential role in promoter opening.

Recruitment factors: TFIIA

In an in vitro system in which TFIIB can be recruited directly to a TATA box, TFIIB on its own is sufficient to recruit RNA polymerase III. In natural RNA polymerase III promoters, however, TFIIB is recruited to the DNA in large part through protein-protein contacts with promoter-bound recruitment factors, specifically TFIIC or SNAP_c. The type 1 5S promoters and type 2 promoters both use TFIIC, but on the 5S promoters TFIIC is recruited through the specificity factor TFIIA. TFIIA is the founding member of the C₂H₂ zinc finger family of DNA-binding proteins (Miller et al. 1985) and contains nine C₂H₂ zinc fingers. In *S. cerevisiae*, the only essential role of TFIIA is in the transcription of the 5S RNA genes, because strains engineered to express the 5S rRNA from a tRNA-type promoter and lacking TFIIA are viable (Camier et al. 1995). This may explain in part the rapid evolution of TFIIA: TFIIA sequences from various organisms are poorly conserved, even among vertebrates. As an example, human and *X. laevis* TFIAs share 61% identity over a 264-amino-acid region—of 423 and 344 amino acids for the human (Arakawa et al. 1995) and *X. laevis* (Ginsberg et al. 1984) proteins, respectively—whereas the RNA polymerase II transcription factor TFIIB is 94% identical in the two species over its entire length. TFIIA binds directly to the ICR of type 1 promoters. TFIIA also binds to 5S RNA to form the 7S storage ribonucleoprotein particle (Pelham and Brown 1980). It is present in massive amounts in immature *X. laevis* oocytes, because they accumulate 5S RNA for later use during oogenesis and the first rounds of embryonic cell division, which occur at a rapid pace in the absence of transcription. This allowed early on the purification of TFIIA to near homogeneity; indeed, *X. laevis* TFIIA was the first eukaryotic transcription factor to be purified (Engelke et al. 1980) and the first for which a corresponding cDNA was isolated (Ginsberg et al. 1984).

Upon binding of *X. laevis* TFIIA to the 5S gene, the TFIIA zinc fingers are aligned over the length of the ICR with the C-terminal zinc finger in proximity of the 5' end, and the N-terminal finger in proximity of the 3' end, of the ICR (for references, see Paule and White 2000). Zinc fingers 1–3, which contact the C box, have been reported to contribute most of the binding energy of the entire protein (Clemens et al. 1992; Liao et al. 1992). Interestingly, however, like TFIIA fragments containing fingers 1–3, fragments containing fingers 4–9 bind, in this case to the A box and intermediate element, with affinities approaching that of the full-length protein (Liao et al. 1992; Kehres et al. 1997). This observation, as well as the binding behavior of full-length proteins with zinc fingers mutated either singly or in pairs, suggest that simultaneous binding by all nine TFIIA zinc fingers to DNA requires energetically unfavorable distortions, either in the DNA, the protein, or both. Thus, there is negative cooperativity between certain zinc fingers such that loss of binding by a subset of zinc fingers has only a small negative effect on the overall stability of the complex (Kehres et al. 1997). Although TFIIA on its own is

displaced from DNA upon passage of RNA polymerase III, the unusual TFIIIA binding properties may contribute to the resilience of the complete 5S transcription complex to repeated passage of the RNA polymerase (Bogenghagen et al. 1982; Setzer and Brown 1985; Wolffe et al. 1986; Darby et al. 1988; Kehres et al. 1997).

Surprisingly little is known about how TFIIIA recruits TFIIIC to the DNA. *X. laevis* TFIIIA contains a 14-amino-acid domain located C-terminal of the ninth zinc finger, and thus located toward the 5' end of the ICR in the TFIIIA/5S gene complex, that is dispensable for DNA binding but essential for transcription (Mao and Darby 1993). In *S. cerevisiae* TFIIIA, a hydrophobic segment within an 84-amino-acid region located between zinc fingers 8 and 9 is similarly required for cell viability and transcription but not for DNA binding (Rowland and Segall 1998). These protein domains may play a role in the recruitment of TFIIIC.

Recruitment factors: TFIIIC

The transcription factor TFIIIC is capable of recognizing the TFIIIA-ICR complex on type 1 5S RNA promoters and the variously spaced A and B boxes on type 2 tRNA promoters. The structure of the factor is uniquely adapted to perform these tasks. Proteolysis studies indicate that *S. cerevisiae* TFIIIC consists of two domains separated by a flexible linker, one of which, designated τ_B , binds strongly to the B box and the other, designated τ_A , binds weakly to the A box (Marzouki et al. 1986). Depending on the distance separating the A and B boxes, the factor is visualized by scanning electron microscopy as either two tightly packed or two clearly separated globular domains of roughly similar sizes (Schultz et al.

1989). These results suggest a factor consisting of two DNA-binding modules separated by a flexible linker that can accommodate variously spaced A and B boxes.

Yeast TFIIIC

S. cerevisiae TFIIIC consists of six subunits, Tfc3/ τ_{138} , Tfc4/ τ_{131} /PCF1, Tfc1/ τ_{95} , Tfc6/ τ_{91} , Tfc8/ τ_{60} , and Tfc7/ τ_{55} , all of which have been cloned and shown to be essential for cell viability (Willis et al. 1989; Swanson et al. 1991; Lefebvre et al. 1992; Marck et al. 1993; Arrebola et al. 1998; Manaud et al. 1998; Deprez et al. 1999). Five *S. pombe* proteins have recently been identified by BLAST searches with *S. cerevisiae* TFIIIC subunits as the query, and four of them confirmed as TFIIIC subunits by immunoaffinity purification of a functional TFIIIC complex from cells expressing the tagged polypeptides (Huang et al. 2000). These four subunits, referred to as Sfc1, Sfc3, Sfc4, and Sfc6, are orthologs of *S. cerevisiae* Tfc1, Tfc3, Tfc4, and Tfc6, respectively, as shown in Table 2. The fifth one, referred to as Sfc9, shares sequence homology with the *S. cerevisiae* Tfc8 subunit within a short C-terminal segment and may, therefore, correspond to the *S. pombe* ortholog of Tfc8. The identification of these *S. pombe* subunits is very interesting because, as detailed below, it clarifies in some cases the relationship between *S. cerevisiae* and human TFIIIC subunits (Huang et al. 2000).

Human TFIIIC

The human TFIIIC fraction contains several activities, some of which are not yet completely defined. These are

Table 2. *Saccharomyces cerevisiae* TFIIIC components and orthologues in *Schizosaccharomyces pombe* and *Homo sapiens*

<i>S. cerevisiae</i> TFIIIC	<i>S. pombe</i> TFIIIC	<i>H. sapiens</i> TFIIIC2a	Comments
Tfc3/ τ_{138}	Sfc3	TFIIIC220/TFIIIC α	Tfc3 and Sfc3 are related, but neither shows sequence similarity to TFIIIC220. Tfc3 cooperates with Tfc6 for binding to DNA. Fragments of TFIIIC220 and TFIIIC110 form a subcomplex capable of binding to the B-box.
Tfc4/ τ_{131} /PCF1	Sfc4	TFIIIC102/TFIIIC γ	Tfc4 protrudes upstream of the start site. TPRs. Tfc4 contacts ScBrf1, ScBdp1, and ABC10 α . Most conserved of the TFIIIC subunits. TFIIIC102 associates with HsBrf1, HsTBP, TFIIIC63.
Tfc1/ τ_{95}	Sfc1	TFIIIC63/TFIIIC ϵ	tRNA A-box binding. Tfc1 and Tfc7 associate and can form a distinct complex. TFIIIC63 associates with TFIIIC102, HsBrf1, HsTBP, and HsRPC62.
Tfc6 (τ_{91})	Sfc6	TFIIIC110/TFIIIC β	Binds terminator. HMG-I and HMG-Y motifs, WD-40 repeats. Similarity between Tfc6 and TFIIIC110 apparent only through Sfc6. Tfc6 cooperates with Tfc3 for binding to DNA. TFIIIC110 and TFIIIC220 form a subcomplex capable of binding to the B-box. Full-length TFIIIC110 absent in TFIIIC2b. TFIIIC110 possesses HAT activity.
Tfc8 (τ_{60})	Sfc9	TFIIIC90/TFIIIC δ	Tfc8 bridges τ_B and τ_A domains as well as TFIIIB. Associates with ScTBP. Similarity of Tfc8 and Sfc9 limited to short C-terminal segment. No similarity between TFIIIC90 and the yeast proteins, but TFIIIC90 binds to TFIIIC220, 110, 63, HsBrf1, HsRPC62, and HsRPC39, and thus may be a functional homolog of the yeast proteins. TFIIIC90 displays HAT activity for histone H3 Lys 14.
Tfc7 (τ_{55})	none	none	tRNA A box binding. Tfc7 and Tfc1 associate and can form distinct complex.

summarized in Figure 8. The TFIIC fraction was originally separated into two fractions, TFIIC1 and TFIIC2, that were both required for transcription from the Ad2 VAI gene (Yoshinaga et al. 1987). The TFIIC1 fraction is only partially defined and is discussed further below. The activity in the TFIIC2 fraction seems to correspond in part to that of the yeast TFIIC complex and resides in a complex of five polypeptides referred to as TFIIC2 or TFIIC2a (Yoshinaga et al. 1989; Kovelman and Roeder 1992). Human TFIIC2a has, however, some added activities compared with *S. cerevisiae* TFIIC (see Table 2). Thus, in cell lines expressing a tagged TFIIC2a subunit, TFIIC2a can be purified by immunoprecipitation as part of a larger complex called holo-TFIIC (see Fig. 8), which is described together with the TFIIC1 fraction further below (Wang and Roeder 1998; Wang et al. 2000). Holo-TFIIC can bind to chromatin templates, relieves the chromatin-mediated repression of RNA polymerase III transcription from a tRNA gene, and displays histone acetyltransferase (HAT) activity (Kundu et al. 1999), which can be attributed to the intrinsic HAT activity of at least two of the TFIIC2a subunits (Hsieh et al. 1999a; Kundu et al. 1999).

Besides the active TFIIC2a complex, the TFIIC2 fraction contains a transcriptionally inactive TFIIC2 complex designated TFIIC2b (Fig. 8; Kovelman and Roeder 1992). TFIIC2a consists of five subunits referred to as TFIIC220/TFIIC α , TFIIC102/TFIIC γ , TFIIC63/TFIIC ϵ ,

TFIIC110/TFIIC β , and TFIIC90/TFIIC δ (see Table 2), whereas TFIIC2b, which represents 10%–20% of total TFIIC2 in actively dividing HeLa cells, lacks the TFIIC110 subunit and appears to contain a 77-kD subunit absent in TFIIC2a (see Fig. 8; Yoshinaga et al. 1989; Kovelman and Roeder 1992; Sinn et al. 1995). The nature of the 77-kD subunit is not clear; it is not recognized by antibodies generated against the last 595 amino acids of TFIIC110, suggesting that it either corresponds to an unrelated protein or to a TFIIC110 fragment devoid of epitopes recognized by the antibody.

The TFIIC220 subunit functionally corresponds to the *S. cerevisiae* Tfc3 subunit because, like Tfc3, it recognizes the B box (see below; Table 2). Very strikingly, however, it shares no sequence similarity with either *S. cerevisiae* Tfc3 or *S. pombe* Sfc3p (L'Etoile et al. 1994; Lagna et al. 1994; Huang et al. 2000). Similarly, the human TFIIC90 protein (Hsieh et al. 1999a) has no obvious sequence homolog in *S. cerevisiae*, but the set of TFIIC and TFIICB subunits it interacts with suggest that it is the functional equivalent of Tfc8 (see below). TFIIC90 has, however, the added property of an intrinsic HAT activity for both free and nucleosomal histone H3, and preferentially acetylates histone H3 Lys 14 (Hsieh et al. 1999a). The TFIIC110 subunit corresponds to the yeast Tfc6p protein, although the similarity between the two proteins is apparent only when compared with the *S. pombe* ortholog (Huang et al. 2000). Like TFIIC90, TFIIC110

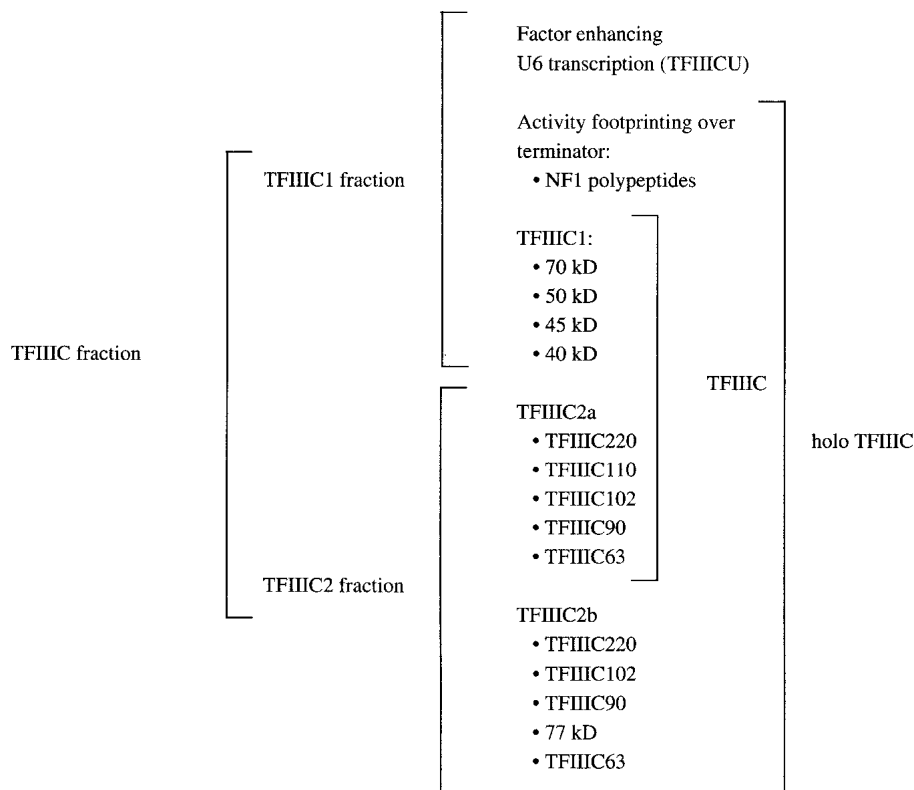


Figure 8. Schematic representing the components identified in the human TFIIC fraction. Holo-TFIIC was purified by immunoprecipitation from a cell line expressing tagged TFIIC220 (Wang and Roeder 1998). It is likely, therefore, that it contains TFIIC2b as well as TFIIC2a, although this has not been directly demonstrated. See text for references.

has intrinsic HAT activity, and acetylates free and nucleosomal histones H3 and H4 as well as nucleosomal histone H2B (Kundu et al. 1999). In contrast to the human TFIIIC subunits just described, the TFIIIC102 and TFIIIC63 subunits are very clearly related to their yeast counterparts, Tfc4 and Tfc1, respectively (Hsieh et al. 1999b). Thus, human TFIIIC2a is surprisingly divergent from *S. cerevisiae* TFIIIC in all but two of its subunits. As detailed further below, these two conserved subunits are located close to the transcription start site and interact directly with TFIIIB subunits.

Assembly of a stable initiation complex on type 1 and 2 promoters

TFIIIC performs at least three functions: It recognizes promoter elements, either directly in the case of type 2 promoters or with the help of TFIIIA in the case of type 1 promoters; it recruits TFIIIB; and it contributes to the recruitment of RNA polymerase III. For all of these functions, we have a good idea of which of the TFIIIC subunits are involved.

Binding of TFIIIC to type 2 and type 1 promoters

A recombinant complex has not yet been reconstituted either from yeast or human cells, but from both photo-

cross-linking (Gabrielsen et al. 1989; Yoshinaga et al. 1989; Bartholomew et al. 1990, 1991; Braun et al. 1992a; Kovelman and Roeder 1992) and protein–protein association experiments (Shen et al. 1996; Manaud et al. 1998; Hsieh et al. 1999a,b), TFIIIC models such as those shown in Figure 9 can be drawn. In Figure 9, the main sites of cross-linking between yeast subunits and DNA are indicated with dots. Known protein–protein contacts among TFIIIC subunits are indicated by dashes according to whether they were demonstrated with human (black) or yeast (gray) subunits.

On the *SUP4* tRNA gene, the *S. cerevisiae* Tfc3 subunit cross-links primarily just upstream of the B box and Tfc6 cross-links at the end of the gene (Bartholomew et al. 1990, 1991). The two proteins probably cooperate to bind to DNA because a mutation in Tfc6 can alleviate the binding defect of a Tfc3 mutant (Arrebola et al. 1998). Consistent with Tfc3 and Tfc6 corresponding to human TFIIIC220 and TFIIIC110, respectively, human TFIIIC220 can be cross-linked to the B box (Yoshinaga et al. 1989; Kovelman and Roeder 1992). Moreover, TFIIIC220 does not bind DNA on its own, but it is part of TFIIIC subassemblies generated by proteinase C cleavage during poliovirus infection that are still capable of binding to DNA (Clark et al. 1991). Some of these sub-complexes consist of just the N-terminal 83 kD of TFIIIC220 associated with the TFIIIC110 subunit or a

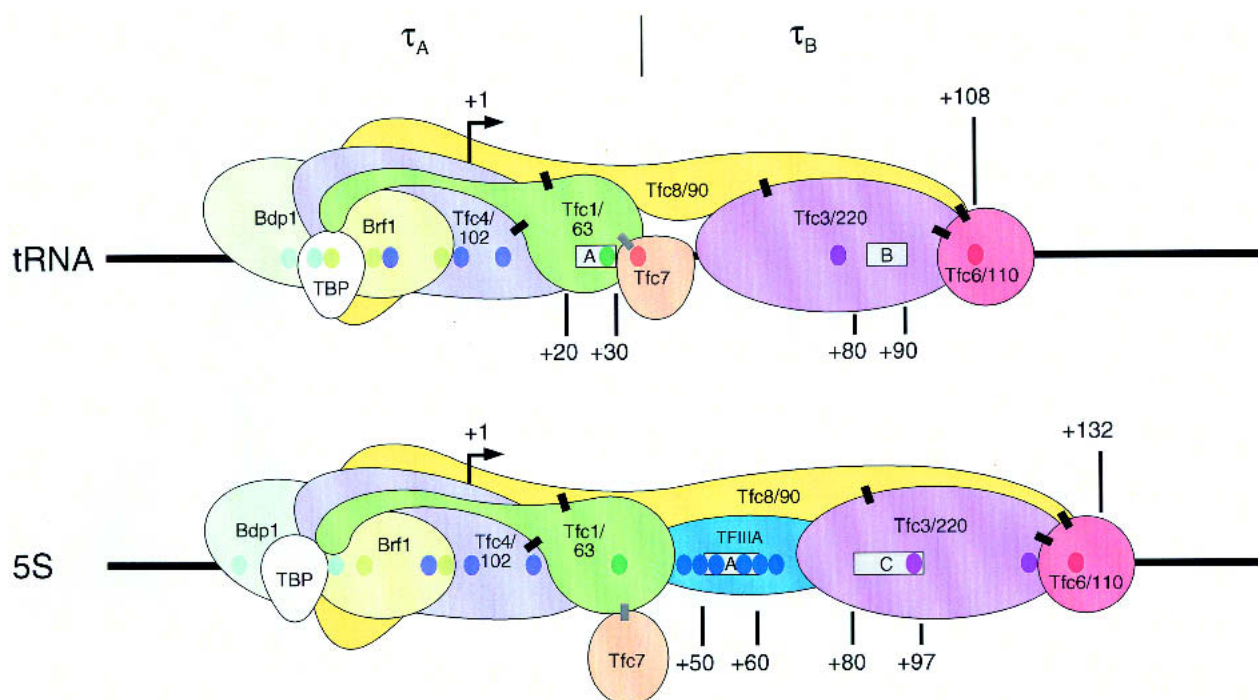


Figure 9. The position of the various *Sc* TFIIIC and TFIIIB subunits on an *SUP4* tRNA and 5S gene as determined by cross-linking and protein–protein association experiments (see text) are indicated. For the TFIIIC subunits, the names of both the *Saccharomyces cerevisiae* and the human subunits are indicated. The positions of the start site, end of the gene, and A, B, and C boxes are indicated. A bend in the DNA at the TFIIIB binding site is not illustrated in the figure. The colored dots indicate major cross-linking sites of the subunit of matching color to the DNA. The Tfc8 subunit is shown as an elongated shape extending over most of the TFIIIC complex because it appears to bridge the TFIIIC τ_A and τ_B domains as well as TFIIIB. The black and gray rectangles illustrate protein–protein contacts identified with human and *S. cerevisiae* TFIIIC subunits, respectively. Protein–protein contacts between TFIIIC and TFIIIB subunits are summarized in Figure 10A.

fragment thereof, indicating that the DNA-binding domain of TFIIC220 lies within the N-terminal region of the protein and that TFIIC220 and TFIIC110 are sufficient to generate the DNA-binding surface of the TFIIC2a complex (Shen et al. 1996). Note that an essential role for the TFIIC110 subunit in DNA binding implies that the TFIIC2b complex, which can bind DNA specifically, contains either an unrecognized TFIIC110 fragment or another protein (e.g., the 77-kD protein) providing the TFIIC110 DNA-binding function.

The yeast Tfc1 and Tfc7 subunits have strong cross-links within and near the 3' end of the A box, respectively (Bartholomew et al. 1990, 1991). Tfc7 interacts directly with Tfc1 through its C-terminal half, and the two proteins are not only part of TFIIC but also form a separate complex in yeast cells (Manaud et al. 1998). Tfc8 does not cross-link to DNA. When TFIIC is treated with protease, Tfc8 is found in the τ_B domain as determined by antibody supershift experiments. In addition, however, the temperature sensitivity observed with strains expressing an epitope tagged version of Tfc8 is specifically suppressed by overexpression of Tfc1, which resides in the τ_A domain. It is also suppressed by overexpression of TBP and ScBdp1, and Tfc8 associates with TBP in vitro (Deprez et al. 1999). Human TFIIC90 is thought to be the functional homolog of Tfc8 because it interacts with TFIIC220, TFIIC110, and TFIIC63, as well as with the TFIIB subunit HsBrf1 (Hsieh et al. 1999a). Thus Tfc8/TFIIC90 appears to bridge the TFIIC τ_B and τ_A domains as well as TFIIB, and is represented, therefore, as extending over the entire gene in Figure 9. The Tfc4 subunit cross-links to sites around and upstream of the transcription start site (Bartholomew et al. 1990, 1991) and directly contacts both the ScBrf1 and ScBdp1 subunits of TFIIB (Khoo et al. 1994; Chaussivert et al. 1995; R uth et al. 1996; Dumay-Odelot et al. 2002). Its location on the DNA is consistent with the human ortholog, TFIIC102, interacting with TFIIC63, TBP, and HsBrf1 (Hsieh et al. 1999b). Tfc1/TFIIC63 is shown extending upstream of the transcription start site because the human subunit, like TFIIC102, contacts HsBrf1 and TBP (Hsieh et al. 1999b).

On the 5S RNA genes, *S. cerevisiae* TFIIA cross-links strongly to the A box and much more weakly to positions as far upstream as +20 and as far downstream as +127, and thus extends over a large portion of the gene (only the cross-links on the A box are shown in Fig. 9; Braun et al. 1992a). TF3C appears shifted downstream as compared with its position in the tRNA gene, with a main cross-link at the 3' end of the C box and another one further downstream. As in the tRNA gene, the Tfc6 subunit cross-links at the end of the gene. There is no indication that the Tfc7 subunit contacts DNA in the 5S gene, but the Tfc1 subunit cross-links strongly upstream of the A box. Thus, the 5S A box is clearly not the functional equivalent of the tRNA A box. Rather, the functional equivalent of the tRNA A box in the 5S gene is the site of Tfc1 cross-linking, which in both genes occurs about 30 nt downstream of the transcription start site. As in the *SUP4* tRNA gene, the Tfc4 subunit cross-links

to sites around and upstream of the start site of transcription (Braun et al. 1992a).

TFIIC recruitment of TFIIB

The role of TFIIC in the recruitment of TFIIB has been intensively studied in *S. cerevisiae*. Experiments in which various TFIIB components were added sequentially to a TFIIC–DNA complex suggest that the TFIIC–DNA complex interacts initially with the Brf1 component of TFIIB (Kassavetis et al. 1992), most probably through the Tfc4 subunit, which protrudes upstream of the transcription start site (see Fig. 9). Like the interaction of TFIIC with its bipartite binding site on DNA, the interaction of TFIIC with TFIIB is highly flexible. Thus, when TFIIB is recruited by TFIIC on *SUP4* tRNA^{Tyr} gene constructs with TATA boxes inserted at various positions upstream of the A box and TFIIC is subsequently stripped from the DNA by heparin treatment, the upstream and downstream borders of the footprint occasioned by the remaining TFIIB vary according to the position of the TATA box. On the same constructs, the footprint of TFIIC alone is invariant, but the footprint attributable to TFIIC (heparin-sensitive) in the presence of TFIIB extends to cover the interval between the borders of the variant TFIIB footprint and that of the invariant TFIIC footprint observed with TFIIC alone. Thus, the placement of TFIIB is codirected by the TATA box (and thus probably the TBP subunit of TFIIB) and TFIIC. Furthermore, because the interval protected is the region to which the Tfc4 subunit of TFIIC cross-links, these results suggest that Tfc4 can extend to accommodate various spacings between TFIIB and TFIIC (Joazeiro et al. 1996).

Tfc4 contains 11 copies of the tetratricopeptide repeat (TPR) in four blocks of 5, 4, 1, and 1 repeats (Marck et al. 1993; Rameau et al. 1994; Dumay-Odelot et al. 2002). TPRs are found in a large number of proteins including subunits of the anaphase-promoting complex and the transcription repressor Ssn6. The crystal structures of several TPR domains indicate that each TPR consists of a pair of antiparallel α -helices, and that adjacent TPRs are packed in regular series of antiparallel α -helices (Das et al. 1998; Scheufler et al. 2000). Truncated Tfc4 proteins encompassing the N-terminal, middle, and C-terminal third of the protein including the first (repeats 1–5), second (repeats 6–9), and last (repeats 10 and 11) blocks of TPRs, respectively, all bind to ScBrf1 in a GST pull-down assay (Khoo et al. 1994). Quantitative in vitro equilibrium binding assays with various truncated forms of Tfc4 also detect several fragments capable of binding to ScBrf1, of which the two with the highest affinities extend from the N terminus of the protein to the end of repeat 5, and from repeat 6 to repeat 9 (Moir et al. 2002a). Interestingly, both fragments have higher affinities for ScBrf1 than a larger fragment extending from the N terminus to repeat 9 (Moir et al. 2002a), consistent with the observation that in a yeast two-hybrid assay, a similar long Tfc4 fragment shows weaker association than frag-

ments shorter at the C terminus (Chaussivert et al. 1995). These results suggest that there is autoinhibition for ScBrf1 binding within Tfc4, which is probably relieved by conformational changes during binding.

Conformational changes within Tfc4 are also suggested by an analysis of two dominant Tfc4 mutations, *PCF-1* and *PCF-2*, that map within repeats 1–3. These mutations increase transcription both in vivo and in vitro by increasing the recruitment of TFIIB, specifically the binding of Tfc4 to ScBrf1 (Rameau et al. 1994; Moir et al. 1997, 2002b). For *PCF-1*, biochemical studies indicate that this effect is achieved via a conformational change in Tfc4 that overcomes autoinhibition in the ScBrf1 binding reaction (Moir et al. 2000, 2002a,b). Thus, the TPRs appear to be involved in conformational changes that promote association with ScBrf1 and accommodate the variable placement of TFIIB. The positive effect of the mutations indicates that the recruitment of TFIIB is a limiting step in vivo, and that the conformational change may therefore serve a regulatory role.

Other associations have been observed between isolated TFIIC and TFIIB subunits. These are symbolized in Figure 10A with arrows, according to whether the association was demonstrated with human (solid) or yeast (hatched) TFIIC subunits. As just described, Tfc4 associates with ScBrf1, and it also associates with ScBdp1 (Khoo et al. 1994; Chaussivert et al. 1995; R uth et al. 1996; Dumay-Odelot et al. 2002). The human ortholog TFIIC102 associates with both HsBrf1 and HsTBP

(Hsieh et al. 1999b). Tfc8 associates with ScBdp1 and ScTBP (Deprez et al. 1999), and the human ortholog TFIIC90 with HsBrf1 (Hsieh et al. 1999a). TFIIC63 associates with both HsBrf1 and HsTBP (Hsieh et al. 1999b). Thus, there is a network of protein–protein contacts between TFIIC and TFIIB, all of which may participate in the recruitment of TFIIB during transcription initiation.

TFIIC contacts RNA polymerase III

Several of the TFIIC subunits have been shown to interact directly with RNA polymerase III subunits, and these interactions are depicted in Figure 10B with solid arrows for associations demonstrated with human subunits and hatched arrows for associations demonstrated with *S. cerevisiae* subunits. The Tfc4 TFIIC subunit interacts with the RNA polymerase III C53 subunit in a two-hybrid assay (Flores et al. 1999), and with the conserved C-terminal domain of the universal RNA polymerase subunit ABC10 α in both a yeast two-hybrid assay and in vitro. This latter interaction is likely to be functionally significant because a thermosensitive mutation within the C-terminal domain of ABC10 α that weakens the interaction can be rescued in an allele-specific manner by overexpression of a variant form of Tfc4 that strengthens the interaction (Dumay et al. 1999). Among the human TFIIC subunits, the TFIIC90 protein interacts with HsRPC62 and HsRPC39, which are both part of the subcomplex of RNA polymerase III subunits re-

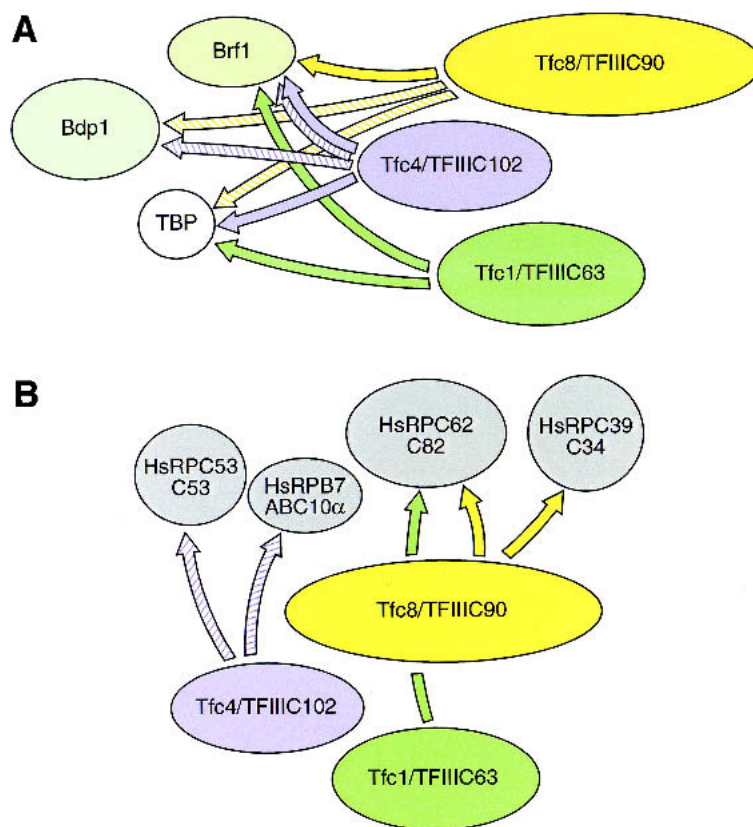


Figure 10. Protein–protein contacts between TFIIC and TFIIB subunits, and TFIIC and RNA polymerase III subunits. Contacts as determined either by in vitro or yeast two-hybrid assays are depicted. Genetic interactions have not been included. Solid arrows depict interactions shown with human proteins, stippled arrows depict interactions shown with *Saccharomyces cerevisiae* proteins. See text for references. (A) Protein–protein contacts between TFIIC and TFIIB subunits. (B) Protein–protein contacts between TFIIC and RNA polymerase III subunits.

quired specifically for transcription initiation, and TFIIC63 associates with HsRPC62 in vitro (Hsieh et al. 1999a). These TFIIC–RNA polymerase III interactions emphasize that TFIIC not only recruits TFIIB but may also participate in the recruitment of RNA polymerase III. Thus, although TFIIB is sufficient to recruit RNA polymerase III, in the cell both TFIIB and TFIIC are likely to work in concert to recruit the RNA polymerase.

Recruitment factors: SNAP_c

Type 3 promoters do not contain binding sites for TFIIA and/or TFIIC2, and, indeed, they do not require TFIIC2 for assembly of a transcription initiation complex. Thus, depletion of a transcription extract with antibodies directed against the DNA-binding subunit of TFIIC2, TFIIC220, affects transcription from the 5S, tRNA, and VAI genes but not from the 7SK and U6 snRNA genes (Lagna et al. 1994). Instead, the gene-external PSE of type 3 promoters recruits the multisubunit complex SNAP_c. SNAP_c is a complex containing five different subunits, SNAP190 (Wong et al. 1998), SNAP50/PTFβ (Bai et al. 1996; Henry et al. 1996), SNAP45/PTFδ (Sadowski et al. 1996; Yoon and Roeder 1996), SNAP43/PTFγ (Henry et al. 1995; Yoon and Roeder 1996), and SNAP19 (Henry et al. 1998b). In the human system, SNAP_c is involved in transcription of snRNA genes by both RNA polymerases II and III, as depletion of endogenous SNAP_c from transcription extracts debilitates U1 and U6 transcription and transcription can be restored in both cases by addition of highly purified recombinant SNAP_c (Henry et al. 1998b).

The subunit–subunit contacts within SNAP_c have been determined by reconstitution of partial complexes and coimmunoprecipitations of various subsets of in vitro translated full-length or truncated SNAP_c subunits (Henry et al. 1996, 1998a,b; Wong et al. 1998; Mittal et al. 1999; Ma and Hernandez 2000, 2002). Figure 11A shows the general architecture of the complex. SNAP19

and SNAP45 can each associate independently with SNAP190, and SNAP43 can associate independently with SNAP50. SNAP43 can also associate with SNAP190, but in coimmunoprecipitations of in vitro translated proteins, this association is not detectable unless SNAP19 is present. This suggests that SNAP43 has weak contacts with both SNAP190 and SNAP19, only the sum of which is measurable by the stringent coimmunoprecipitation assay (Henry et al. 1998b). However, partial complexes containing SNAP190 or a truncated SNAP190, SNAP43, and SNAP50 can be assembled both in insect cells infected with recombinant baculoviruses (Mittal et al. 1999) and from subunits overexpressed in *E. coli* (Ma and Hernandez 2000, 2002), indicating that in these situations SNAP19 is dispensable for complex assembly.

The domains involved in protein–protein contacts are summarized in Figure 11B. Within SNAP190, amino acids 84–133 are sufficient for association with SNAP19 alone, and with SNAP43 together with SNAP19. This SNAP190 region, and the N-terminal part of SNAP19, are likely to form α-helices and may be involved in a coiled-coil type of interaction with each other. In SNAP43, amino acids 164–268 are sufficient for association with SNAP190 together with SNAP19, and amino acids 1–163 are sufficient for association with SNAP50. Thus, these two association domains in SNAP43 are completely separable. The SNAP190 region required for interaction with SNAP45 lies between amino acids 1281 and 1393. The SNAP190 regions required for association with SNAP19/SNAP43 and with SNAP45 thus lie at opposite ends of the linear molecule (Ma and Hernandez 2000).

SNAP_c binds specifically to the PSE. This binding is mediated in part by an unusual Myb domain extending from amino acids 263 to 503 within SNAP190, with four and one-half Myb repeats designated the Rh (R_{half}), Ra, Rb, Rc, and Rd repeats (see Fig. 11B; Wong et al. 1998). Indeed, a SNAP190 segment consisting of just the Myb domain can bind to the PSE (Wong et al. 1998), and in the

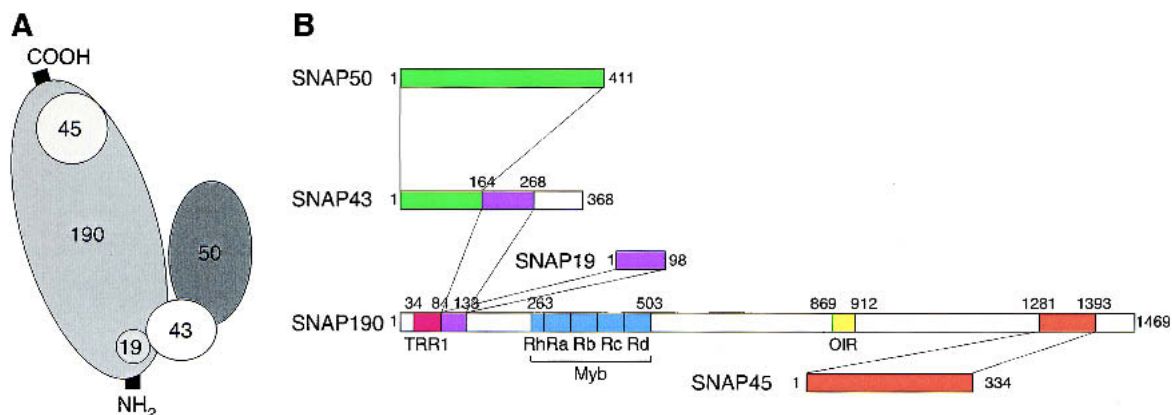


Figure 11. Subunit–subunit interactions within SNAP_c. (A) For simplicity, the complex is shown as containing one copy of each subunit, but the stoichiometry of the SNAP_c subunits has not been determined. (B) The domains of the various subunits sufficient for association with other subunits in a coimmunoprecipitation assay. The TBP recruitment region 1 (TRR1), the Myb domain with the half repeat (Rh) followed by four repeats (Ra, Rb, Rc, and Rd), and the Oct-1 interacting region (OIR) are shown.

context of the full complex, the last two Myb repeats (Rc and Rd), but not the first two and one-half (Rh, Ra, and Rb), are required for binding to the PSE (Mittal et al. 1999). However, even though the Myb domain is required, it is not sufficient. Thus, although a SNAP190 segment consisting of just the Myb domain binds DNA, larger SNAP190 segments do not. The smallest subassembly of SNAP_c subunits characterized that binds specifically to DNA consists of SNAP190 amino acids 84–505, SNAP43 amino acids 1–268, and SNAP50 (complex #8; Ma and Hernandez 2000, 2002). Consistent with the requirement for parts of SNAP190 and SNAP50 for DNA binding, UV cross-linking experiments suggest that both SNAP190 (Yoon et al. 1995) and SNAP50 (Henry et al. 1996) are in close contact with DNA.

Assembly of a stable transcription complex on the type 3 U6 snRNA promoter

As noted above, the type 3 promoters such as the U6 promoter contain, in addition to core sequences, a DSE that serves to enhance transcription from the core promoter (see Fig. 1). The DSE can contain a number of protein-binding sites, but two of them are almost invariably an SPH (also called NONOCT) element, which recruits the transcription factor STAF (also called SPH-binding factor or SBF), and an octamer sequence, which recruits the transcription factor Oct-1 (for reviews, see Hernandez 1992, 2001).

STAF is a zinc finger protein, and Oct-1 is a founding member of the POU-domain protein family. Both proteins contain two types of activation domains, one type that specifically activates mRNA-type RNA polymerase II promoters and another that specifically activates both type 3 RNA polymerase III promoters and the very similar RNA polymerase II snRNA promoters (Tanaka et al. 1992; Das et al. 1995; Schuster et al. 1998). The presence of the latter type of activation domains in STAF and Oct-1 supports the idea that these proteins activate type 3 promoters (as well as RNA polymerase II snRNA promoters) in the cell. Indeed, both proteins have been localized to snRNA promoter sequences *in vivo* by chromatin immunoprecipitation experiments (Zhao et al. 2001; Mach et al. 2002). Thus, as for the core promoter sequences of type 3 promoters, the key factors recruited by the DSE are well characterized and are available in recombinant form.

The identification of the factors binding directly to type 3 promoter elements has allowed the characterization of their assembly onto a human U6 promoter. Our present understanding of this process is summarized in Figure 12. SNAP_c binds poorly to DNA on its own. Interestingly, however, a partial complex lacking the C-terminal two-thirds of the largest subunit of SNAP_c as well as SNAP45, referred to as mini-SNAP_c, binds more efficiently to the DNA (Mittal et al. 1999). This implies that the C-terminal two-thirds of SNAP190 and/or SNAP45, which associates with the C-terminal portion of SNAP190, somehow down-regulate binding of the complex to DNA. On the U6 promoter, however, SNAP_c

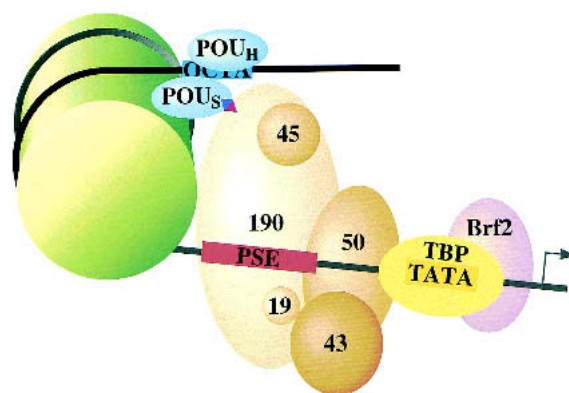


Figure 12. Human U6 transcription initiation complex. TBP (yellow) and SNAP_c (orange) bind cooperatively to the DNA, presumably through a direct protein–protein contact that involves a 50-amino-acid segment within the N-terminal region of SNAP190. TBP also binds cooperatively with Brf2. SNAP_c and the Oct-1 POU domain (blue) bind cooperatively to DNA, through a direct protein–protein contact involving a glutamic acid at position 7 within the POU_S domain (blue triangle) and a lysine at position 900 within SNAP190 (red triangle). This direct protein–protein interaction is mediated by a positioned nucleosome (green) that brings into close proximity the octamer sequence and the PSE. Adapted from Zhao et al. (2001). See text for references.

is efficiently recruited to the DNA through at least two cooperative binding interactions. SNAP_c binds cooperatively with TBP, which like SNAP_c binds poorly to DNA on its own, and with the Oct-1 POU domain (Murphy et al. 1992; Mittal et al. 1996; Mittal and Hernandez 1997).

In cooperative binding of SNAP_c and TBP, both factors recruit each other to the DNA. Mini-SNAP_c is also able to bind cooperatively with TBP, although in this case, cooperative binding results mainly in the recruitment of TBP, because mini-SNAP_c binds efficiently to DNA on its own (Mittal et al. 1999). Within the context of mini-SNAP_c, a 50-amino-acid segment within the N-terminal region of SNAP190, called TRR1 (TBP recruitment region 1; see Fig. 11) is required for cooperative binding with TBP. Interestingly, mini-SNAP_c complexes lacking these 50 amino acids are active for transcription, suggesting that during assembly of the U6 transcription initiation complex, there are redundant mechanisms to recruit TBP to the DNA. Indeed, TBP is efficiently recruited to the DNA by cooperative binding with HsBrf2 to form a complex containing mini-SNAP_c, TBP, and HsBrf2 (Ma and Hernandez 2002), and TBP is therefore shown contacting both SNAP_c and HsBrf2 in Figure 12. It remains possible that in the absence of the DSE, cooperative binding of TBP and SNAP_c is essential to recruit the full SNAP_c to the DNA, which, unlike mini-SNAP_c, binds poorly to the PSE. In the presence of the DSE, however, the full SNAP_c can be recruited to the DNA by the Oct-1 POU domain.

The Oct-1 POU domain is a bipartite DNA-binding domain consisting of two helix–turn–helix-containing DNA-binding modules: an N-terminal POU-specific

(POU_S) domain and a C-terminal POU-homeo domain (POU_H), joined by a flexible linker (Herr and Cleary 1995). The POU domains of Oct-1 and the pituitary transcription factor Pit-1 are ~50% identical, but only the Oct-1 POU domain is capable of recruiting SNAP_c to the PSE. This difference in activity is due to only one of the many amino acid differences between the two proteins, a glutamic acid at position 7 in the Oct-1 POU_S domain changed to an arginine at the corresponding position in the Pit-1 POU_S domain. Thus, an E7R mutation in Oct-1 does not affect Oct-1 binding to the octamer sequence but inhibits cooperative binding with SNAP_c, and an R7E mutation in Pit-1 imparts to this protein the ability to bind cooperatively with SNAP_c (Mittal et al. 1996).

Oct-1 associates with SNAP190, the largest subunit of SNAP_c, both in a yeast one-hybrid assay and in an electrophoretic mobility shift assay (Ford et al. 1998; Wong et al. 1998). This suggested that cooperative binding of the Oct-1 POU domain and SNAP_c may result from a protein-protein contact between the POU domain and SNAP190. Indeed, a small domain of SNAP190 extending from amino acids 869 to 912 and labeled OIR (Oct-1-interacting region) in Figure 11 above is sufficient for association with the Oct-1 POU domain in an electrophoretic mobility shift assay. Strikingly, the OIR of SNAP190 displays sequence similarity with the Oct-1 POU-domain-interacting region present in OBF-1/OCA-B (Ford et al. 1998). OBF-1 is a B-cell-specific coactivator of Oct-1, which recognizes Oct-1 bound to an octamer sequence and provides a strong activation domain of mRNA gene transcription. As with SNAP190, the association of OBF-1 with the Oct-1 POU domain is sensitive to the E7R mutation in Oct-1 (Babb et al. 1997), and, indeed, the Oct-1 POU E7 is involved in a hydrogen bond with a lysine in OCA-B as shown in the crystal structure of an OCA-B peptide associated with an Oct-1 POU/octamer sequence complex (Chasman et al. 1999). Thus, two proteins with different tissue distribution, function, and primary structure nevertheless share a common Oct-1 binding domain, and probably contact overlapping surfaces in the Oct-1 POU domain (Ford et al. 1998).

Within the SNAP190 OIR, mutation of Lys 900 to a glutamic acid (K900E mutation) inhibits cooperative binding of SNAP_c with the Oct-1 POU domain. Very significantly, however, if SNAP_c carrying the K900E mutation within SNAP190 is combined with the Oct-1 POU domain carrying the E7R mutation, significant cooperative binding is restored (Ford et al. 1998). The isolation of these paired altered specificity mutants indicates that cooperative binding requires a direct protein-protein contact, and suggests that this contact involves K900 in SNAP190 and E7 in the Oct-1 POU domain. K900 (Fig. 12, red triangle) and E7 (Fig. 12, blue triangle) are, therefore, shown contacting each other in Figure 12. In SNAP_c, then, the same region of the complex that is required for down-regulation of DNA binding is involved in a protein-protein interaction that mediates cooperative binding such that, in effect, cooperative binding inactivates a SNAP_c built-in damper of DNA binding.

The recruitment of SNAP_c by the Oct-1 POU domain contributes to transcription activation *in vitro*. Therefore, Oct-1 is likely to activate snRNA gene transcription *in vivo* not only through its transcription activation domains but also through its DNA-binding domain. This activation can be compared to activation in prokaryotes by the λ cI repressor, which activates transcription from the λ P_{RM} promoter by favoring open complex formation through direct protein-protein contacts between its DNA-binding domain and RNA polymerase (for references, see Ford et al. 1998). Strikingly, the DNA-binding domain of the λ cI repressor is structurally similar to the Oct-1 POU_S domain, and the RNA polymerase subunit contacted by the cI DNA-binding domain is the σ subunit, which is, like SNAP_c, a core promoter binding factor. This illustrates how *E. coli* and human cells can use similar protein-protein contacts to activate transcription.

The Oct-1-SNAP_c interaction was originally characterized with U6 promoter probes in which the octamer sequence had been placed close to the PSE. The exact distance separating the octamer and the PSE was not important, but not surprisingly, cooperative binding was not observed on the natural promoter, where the two sites are separated by ~150 bp. This suggested that in the natural U6 promoter the two sites may somehow be brought into close proximity for cooperative binding. Indeed, as shown in Figure 12, the human U6 promoter harbors a positioned nucleosome between the octamer sequence and the PSE, both *in vivo* as determined by micrococcal nuclease sensitivity of the chromatin and *in vitro* upon assembly of a U6 template into nucleosomes (Stunkel et al. 1997; Zhao et al. 2001). The positioned nucleosome promotes activation by the Oct-1 POU domain from its natural binding site as well as cooperative binding of Oct-1 and SNAP_c. This cooperative binding is affected by the Oct-1 E7R and the SNAP190 K900E mutations, but is restored to significant levels when the two mutant factors are combined (Zhao et al. 2001). Thus, the positioned nucleosome mediates a direct protein-protein contact between the Oct-1 POU domain and SNAP_c, and this contact is the same as the one characterized on probes containing closely spaced octamer and PSE. This provides a striking example of a nucleosome, the structural unit of chromatin, activating transcription by serving an architectural role and thus allowing cooperative interactions of factors binding to distant sites.

The assembly of the U6 transcription complex illustrates how a network of cooperative interactions ultimately increases both the affinity and the DNA-binding specificity of factors such as SNAP_c and TBP which, on their own, display slow on and off rates and relatively low DNA-binding specificity. Both SNAP_c and TBP contain built-in dampers of DNA binding. In the case of SNAP_c, the damper is deactivated by cooperative interactions with Oct-1, which dramatically enhance the SNAP_c on rate (Mittal et al. 1996). In the case of TBP, the damper is deactivated by cooperative interactions with SNAP_c (Mittal and Hernandez 1997). Because cooperative interactions between Oct-1 and SNAP_c, and SNAP_c

and TBP, only take place when the corresponding DNA-binding sites are all present, such interactions limit, in effect, the binding of these factors to promoter sequences.

Stepwise assembly versus holoenzyme

Our understanding of how RNA polymerase III is recruited to specific promoters stems from *in vitro* studies in which transcription initiation complexes are assembled by stepwise addition of various components. Such studies have brought invaluable information about the network of protein–DNA and protein–protein interactions that culminates in the specific recruitment of RNA polymerase III. *In vivo*, however, it is possible that many of the factors that mediate promoter recognition by RNA polymerase III are recruited together with the polymerase as part of a holoenzyme. Indeed, anti-epitope tag immunoprecipitates from a human cell line stably synthesizing a tagged subunit of RNA polymerase III can direct transcription from type 2 promoters on their own and from type 1 promoters when combined with TFIIB (Wang et al. 1997). Consistent with this observation, these immunoprecipitates contain the TFIIB subunits TBP and HsBrf1 as well as the TFIIC2 subunits TFIIC220 and TFIIC110 as determined by immunoblot. Tandem immunoprecipitations as well as gel filtration analyses indicate that about 10% of the RNA polymerase molecules in the immunoprecipitate is associated with TFIIB and/or TFIIC components. However, these associations are reduced by an increase in the KCl concentration from 100 mM to 150 mM, and are lost at 300 mM KCl. Nevertheless, these results raise the possibility that *in vivo*, RNA polymerase III is recruited together with some of its accessory factors, in particular TFIIB and TFIIC2 (Wang et al. 1997).

Human TFIIC1

The human TFIIC fraction contains the B-box-binding TFIIC2 complex described above as well as the TFIIC1 activity, which displays no strong DNA-binding activity on its own but extends the TFIIC2 footprint both upstream on the A box and downstream to the end of the gene (Yoshinaga et al. 1987). TFIIC1 is apparently required not only for transcription from type 1 and 2 promoters, but also for transcription from the type 3 gene-external promoters (Yoon et al. 1995). As summarized in Figure 8, this fraction contains a number of factors that have been implicated in RNA polymerase III transcription, many of which are still poorly characterized. Most of the TFIIC1 activities can be purified as part of a holo-TFIIC complex by immunopurification of TFIIC2 from a cell line expressing tagged TFIIC220 (Wang and Roeder 1998). One of them, called TFIIC1 (as opposed to the TFIIC1 fraction), remains associated with TFIIC2 after further purification of the immunoprecipitated complex on a sucrose gradient and consists of at least four polypeptides with apparent molecular masses of 70,

50, 45, and 40 kD (Wang and Roeder 1998), whose identities have not yet been reported. This relatively stable TFIIC1/TFIIC2 complex is referred to as human TFIIC (see Fig. 8). A TFIIC1 activity purified by conventional chromatography, which may correspond to the four polypeptides, binds cooperatively with both TFIIC2 and a TFIIC2/TFIIB complex, and the footprint displayed by TFIIC2 and the TFIIC1 activity on a tRNA gene extends over the A and B boxes (Oettel et al. 1997).

In addition to the TFIIC1 factor, the TFIIC1 fraction contains an uncharacterized factor that specifically enhances human U6 transcription and has been called TFIICU (Oettel et al. 1998) as well as an activity that footprints, on its own, over the terminator region of the VAI gene and a tRNA gene (see Fig. 8; Wang and Roeder 1996; Oettel et al. 1997, 1998). The latter activity is also present in holo-TFIIC but can be dissociated from the TFIIC factor with 300 mM KCl (Wang et al. 2000). It has been purified by a combination of conventional and DNA affinity chromatography to near homogeneity, and corresponds to four groups of NF1 isoforms (Wang et al. 2000). NF1 was initially identified as a cellular factor required for efficient initiation of Ad2 replication (Nagata et al. 1982), and was later found to be involved in the expression of many cellular and viral genes. The purified activity gives rise to a footprint over the two VAI terminator regions that is identical to that observed with holo-TFIIC, consistent with the idea that holo-TFIIC contains, at a minimum, TFIIC1, TFIIC2, and NF1 polypeptides (Fig. 8). The role of NF1 in RNA polymerase III transcription is further addressed below.

Termination and recycling: does the end help the beginning?

RNA polymerase III is unique among the eukaryotic RNA polymerases in recognizing a simple run of T residues as a termination signal. In a linear template, mutation or deletion of the termination signal results in the production of run-off transcripts. However, aberrant termination is not the only consequence of debilitating the terminator. Mutation of the run of T residues in the VAI gene diminished the efficiency of single- and multiple-round transcription in a HeLa cell extract (Wang and Roeder 1996; Wang et al. 2000). Similarly, in an *S. cerevisiae* system in which RNA polymerase III was limiting, deletion of the terminator affected multiple-round transcription, although in this case single-round transcription was not affected (Dieci and Sentenac 1996). These results suggest that the run of T residues can contribute to the efficiency of initiation and reinitiation, and therefore that it may play a role in RNA polymerase recycling. In higher eukaryotes, a number of factors have been implicated in efficient termination and RNA polymerase III recycling, including the La protein, NF1 polypeptides, DNA topoisomerase I, and PC4.

La protein

The first factor implicated in transcription termination and recycling was the La antigen (Gottlieb and Steitz

1989a,b). La binds to the poly-U tail at the end of RNA polymerase III transcripts (Stefano 1984), and could be demonstrated to stimulate transcription in a system in which transcription complexes were (1) preassembled on immobilized templates; (2) allowed to undergo a single round of transcription in the presence of heparin and sarcosyl, which resulted in stripping of RNA polymerase III; (3) washed; and (4) incubated with either fresh RNA polymerase alone or RNA polymerase together with La. The effect could be attributed to improved transcript release and transcription reinitiation (Maraia 1996), and was abolished by phosphorylation of Ser 366 in La (Fan et al. 1997). On the other hand, however, depletion of La from crude *X. laevis* extracts did not affect RNA polymerase III transcription (Lin-Marq and Clarkson 1998). Thus, although La can clearly affect RNA polymerase III transcription in certain in vitro systems, it is not clear that the protein plays such a role in the cell.

NF1 polypeptides

Other factors implicated in transcription termination and efficiency are the NF1 polypeptides in the TFIIC1 fraction, which footprint over the VAI terminator region (Wang et al. 2000). NF1 polypeptides form a family of proteins with multiple functions encoded by a large number of alternatively spliced RNAs derived from four different genes. Individual NF1 proteins can dimerize with themselves and with other NF1 variants, but all variants have the same DNA-binding specificity and recognize the consensus sequence 5'-YTGGCANNNTGC CAR-3'. Sequences that closely match this consensus are found in the two VAI terminators, immediately downstream of the run of T residues, and mutations in these NF1-binding sites resulted in the appearance of read-through transcripts, although the effect was not as severe as with mutation of the run of T residues. Furthermore, addition of NF1 together with the TFIIC factor improved the ratio of correctly terminated transcripts over read-through transcripts derived from a dC-tailed template, in which RNA polymerase III initiates transcription at the dC tail in the absence of transcription initiation factors (Wang et al. 2000). Together, these results suggest an involvement of NF1 polypeptides in transcription termination, although the interpretation of the dC-tailed template experiment is complicated by the fact that initiation at tailed templates tends to result in the production of RNA-DNA hybrids, which are known to inhibit transcription termination by RNA polymerase III (Campbell and Setzer 1992).

In addition to a qualitative effect on transcription termination, NF1 polypeptides can affect transcription efficiency. Thus, depletion of NF1 polypeptides from an extract severely reduced VAI transcription, and efficient transcription could be restored by addition of either purified NF1 activity or recombinant CTF1, the largest NF1 variant derived from the *NF1-C* gene (Santoro et al. 1988). However, in a highly purified transcription system, the stimulatory effect of NF1 was reportedly not observed, suggesting that NF1 serves to counteract nega-

tive factors not present in the purified system (Wang et al. 2000). Thus, NF1 can increase VAI transcription efficiency in a crude extract, perhaps by facilitating transcription termination.

Depletion of NF1 reportedly also reduced transcription from the 5S RNA, a tRNA, and the human U6 snRNA genes, suggesting that NF1 polypeptides are generally required for efficient RNA polymerase III transcription in crude extracts (Wang et al. 2000). It is not clear, however, how NF1 is recruited to other RNA polymerase III terminators with no obvious NF1-binding sites close to the run of T residues. Perhaps in these cases, protein-protein interactions with TFIIC2 (or, for genes such as the human U6 snRNA gene that do not recruit TFIIC2, some other transcription factor) mediate NF1 recruitment.

DNA topoisomerase I and PC4

Like NF1, both DNA topoisomerase I and PC4 can suppress read-through transcripts in the dC-tailed template assay (Wang and Roeder 1998; Wang et al. 2000). However, these factors seem to differ from NF1 in that even though they enhance TFIIC interactions in the downstream region of a tRNA gene and the VAI gene, they do not generate a footprint on their own, and the footprint observed in the presence of TFIIC does not appear to extend over the termination region (Wang and Roeder 1998; Wang et al. 2000). DNA topoisomerase I and PC4 are present in holo-TFIIC, but unlike NF1, they are present only in trace amounts. Thus, DNA topoisomerase I and PC4 seem less likely than NF1 to participate in RNA polymerase III transcription in vivo.

Conclusion

The study of RNA polymerase III promoters has provided perhaps the best illustration of how promoters with different structures rely differently on DNA-protein and protein-protein contacts to recruit, ultimately, the same RNA polymerase. Thus, TFIIB can be recruited to a TATA box through direct binding to DNA, or to many DNA sequences through binding to TFIIC. TFIIC itself can bind directly to the B box of type 2 promoters or can use the accessory factor TFIIA to bind to type 1 promoters. And in type 3 promoters, TFIIB is recruited through a combination of DNA contacts with the TATA box and protein contacts with SNAP_c. These promoters also illustrate how a transcription machinery has diversified and expanded from *S. cerevisiae* with its relatively simple genome to higher eukaryotes with their much more complex genomes to include new factors, such as SNAP_c, or multiple variations of a factor unique in yeast, such as HsBrf1 and HsBrf2.

RNA polymerase III transcription is tightly regulated; it is controlled by growth conditions, during the cell cycle, and by a number of viruses (for review, see Brown et al. 2000). These controls involve RNA polymerase III transcription factors, in particular TFIIC2 and TFIIB, as well as, perhaps, RNA polymerase III itself. For example,

the ratio of active TFIIC2a versus inactive TFIIC2b is altered by the Ad2 E1A protein and the SV40 large T antigen (Brown et al. 2000). TFIIB activity is regulated by a number of factors including P53 and retinoblastoma protein (Brown et al. 2000), as well as CK2, both in yeast and human cells (Ghavidel and Schultz 2001; Johnston et al. 2002). And in yeast, RNA polymerase III can be found associated with Maf1, a negative regulator of RNA polymerase III transcription (Pluta et al. 2001). So far, reconstitution of RNA polymerase III transcription with completely defined (recombinant) transcription factors has been achieved only in the *S. cerevisiae* system with a promoter that can recruit TFIIB directly through a TATA box. An urgent task, which will allow a detailed understanding of the mechanisms by which RNA polymerase III transcription is regulated, is a description of the complete set of transcription factors sufficient for RNA polymerase III transcription from more complex promoters, both in naked DNA and in chromatin templates.

Acknowledgments

We thank Winship Herr, Ping Hu, David R. Setzer, Ian Willis, and the reviewers for comments that greatly improved the manuscript; and J. Duffy and P. Renna for artwork and photography. This work was funded in part by NIH grant GM38810. N.H. and L.S. are supported by the Howard Hughes Medical Institute.

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Schramm and Hernandez

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Schramm and Hernandez

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Genes Dev. 2002, **16**:

Access the most recent version at doi:[10.1101/gad.1018902](https://doi.org/10.1101/gad.1018902)

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