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Transcription factor TFIIIB and transcription by RNA polymerase III

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Abstract

pol (RNA polymerase) III is charged with the task of transcribing nuclear genes encoding diverse small structural and catalytic RNAs. We present a brief review of the current understanding of several aspects of the pol III transcription apparatus. The focus is on yeast and, more specifically, on *Saccharomyces cerevisiae*; preponderant attention is given to the TFs (transcription initiation factors) and especially to TFIIIB, which is the core pol III initiation factor by virtue of its role in recruiting pol III to the transcriptional start site and its essential roles in forming the transcription-ready open promoter complex. Certain relatively recent developments are also selected for brief comment: (i) the genome-wide analysis of occupancy of pol III-transcribed genes (and other loci) by the transcription apparatus and the location of pol III transcription in the cell; (ii) progress toward a mechanistic and molecular understanding of the regulation of transcription by pol III in yeast; and (iii) recent experiments identifying a high mobility group protein as a fidelity factor that assures selection of the precise transcriptional start site at certain pol III promoters.

The pol (RNA polymerase) III transcription apparatus is devoted to the production of small structural and catalytic RNAs. For the budding yeast *Saccharomyces cerevisiae* (*Sc*), it is fully enumerated; for the human, some gaps remain in the specification of the component parts and understanding their functions.

This brief account emphasizes the current understanding of transcription by pol III in budding yeast and only briefly comments on the divergences of other pol III systems (see also [1–5]). The Supplementary Section (http://www.biochemsoctrans.org/bst/034/bst0341082add.htm) presents colour images to accompany the primary text.

The pol III transcription apparatus and its promoters

Internal promoters (Supplementary Figure 1 at http://www.biochemsoctrans.org/bst/034/bst0341082add.htm) that serve as the DNA-binding sites of TFs (transcription initiation factors) TFIIIA (for the ~150 5 S rRNA genes) and TFIIIC (for the other pol III-transcribed genes) are cardinal features of pol III transcription units. Binding of proteins within a transcription unit implies displacement at each round of RNA synthesis. Nevertheless, pol III promoters do not have to be newly marked for every successive round of transcription because TFIIIC places the third initiation factor, TFIIIB, upstream of the transcriptional start site, and TFIIIB, in turn, is able to repetitively recruit RNA polymerase to the promoter [6]. TFIIIC also interacts directly with a subunit common to pols I, II and III (10α) [7], but it is not known whether this interaction is important for recruitment to the promoter, termination of transcription or pol III recycling [8,9], and whether it has the opportunity to intervene in each cycle of RNA synthesis.

TFIIIA, the archetype Zn finger protein, the six-subunit TFIIIC, the three-subunit TFIIIB and the 17-subunit pol III constitute the essential RNA polymerase III transcription apparatus of budding yeast (Supplementary Figure 2 at http://www.biochemsoctrans.org/bst/034/bst0341082add.htm). No additional factors for transcript elongation or termination are known and none is required *in vitro*. All ten genes encoding the component proteins of TFIIIA, TFIIIB and TFIIIC are essential, as are all 17 genes encoding the subunits of pol III. Five of these pol III subunits are shared with the two other nuclear RNA polymerases; two subunits are common to pols I and pol III (with paralogue subunits in pol II); five pol III subunits have separate pol I and II paralogues; and five are pol III-specific. Three of the last-cited subunits form a subassembly that interacts with TFIIIB in the pol III promoter complex [10]. Two subunits are in a separate subassembly that attaches to the pol III core together with subunit C11 (C refers to pol III, also known as pol C, and 11 refers to the original names of pol III subunits that were based on approximate molecular mass) [11]. C11, a partial paralogue of pol II subunit Rpb9 and elongation factor TFIIS, is associated with another specific property of pol III, the factor-independent retractive hydrolytic cleavage of the nascent transcript [12,13] that is in part responsible for the extraordinarily high fidelity of pol III transcription (N. Alic, E. Landrieux, P. Baudouin-Cornu, E. Favry, M. Riva and C. Carles, personal communication).

Key words: Bdp1, Brf1, budding yeast, RNA polymerase III, TATA-box-binding protein (TBP), transcription initiation factor.

Abbreviations used: ChIP, chromatin immunoprecipitation; ChIP-chip, ChIP-array hybridization; COC, chromosome-organizing clamps; *Hs*, *Homo sapiens*; pol, RNA polymerase; *Sc*, *Saccharomyces cerevisiae*; *Sp*, *Schizosaccharomyces pombe*; TBP, TATA-box-binding protein; TBPc, core of TBPc; TF, transcription initiation factor; TPR, tetratricopeptide.

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The remaining 11 subunits constitute a core enzyme whose structure has been modelled by combining a *de novo* structure determination of the C17–C25 heterodimer subassembly with pol II homology-based modelling [14–17].

The six subunits of the very large TFIIIC [5.2×10^5 a.m.u. (atomic mass units)] assemble into two domains (τA and τB) that attach to two internal promoter elements (boxes A and B respectively) of the 273 *Sc*tRNA genes and a handful of other genes. A flexible tether connecting τB and τA allows TFIIIC to adapt to the diverse box A–box B spacings of its transcriptome, with one exception: box B of the U6 spliceosomal snRNA (small nuclear RNA) gene (*SNR6*) is located downstream of the transcription terminator, and the approx. 220 bp box A–box B separation of *SNR6* exceeds the span of the tether connecting τA and τB. DNA compaction by chromatin proteins is required to assure TFIIIC-dependent transcription of the U6 gene and co-occupancy of its box A and box B. However, the large size of TFIIIC and the extended DNA site that it occupies also appear to be adapted to excluding repressive chromatin from all but the longest pol III-transcribed genes [18]. The recent reconstitution of active *Sc*TFIIIC from recombinant subunits (co-expressed in insect cells) opens new avenues for the dissection and analysis of TFIIIC assembly, function and interactions [19].

TFIIIB

The three subunits of TFIIIB, which have been a focus of our research, are TBP (TATA-box-binding protein), Brf1 and Bdp1. The 596-amino-acid Brf1 is a hybrid: its N-terminal half ($Brf1_N$) is a paralogue of pol II initiation factor TFIIB; the pol III-specific C-terminal half ($Brf1_C$) provides most of the affinity for binding to TBP and Bdp1, but it is $Brf1_N$ that is essential for the transcriptional activity of TFIIIB. The 594-amino-acid Bdp1 is specific to pol III, with no paralogues in other transcription systems.

*Sc*TBP and Brf1 associate sufficiently stably to co-purify, while the more weakly held Bdp1 separates during chromatographic purification. That is not the situation for TFIIIB of other organisms. The TBP and Brf1 subunits of the fission yeast *Schizosaccharomyces pombe* (*Sp*) TFIIIB do not interact stably in the absence of DNA; TBP must first associate with its TATA box before it is joined by Brf1. Indeed, a TATA box is an essential/ubiquitous promoter element of all *Sp*pol III-transcribed genes [1,20]. For the human [*Hs* (*Homo sapiens*)], Brf paralogues, Brf1 and Brf2, operate in conjunction with two distinct modes of pol III transcription [2,21] and Bdp1 separates from Brf1 and TBP at the first step of purification, instead co-purifying with TFIIIC. *Hs*Bdp1 has only recently been identified as an essential (possibly the only globally essential) component of the transcription factor fraction previously called TFIIIC1 [22].

Most pol III-transcribed genes of budding yeast do not have TATA boxes and accurate placement of TFIIIB upstream of their transcriptional start sites is secured by DNA (box A)-bound TFIIIC. The small minority of *Sc* genes with TATA boxes (*SNR6* and a handful of tRNA genes) are autonomously bound by TFIIIB *in vitro* through its TBP subunit. Transcription of these genes as DNA *in vitro* does not require TFIIIC [23].

TFIIIC brings TFIIIB to the promoter principally through interactions of its Tfc4 subunit (part of the τA subassembly) with both halves of Brf1. Tfc4 contains two clusters of TPR (tetratricopeptide) repeats within its N-proximal approx. 570 amino acids. The interaction with Brf1 is multivalent, involving three separate TPR elements, two sites in $Brf1_N$ (one in repeat I and another in repeat II), and an additional site in $Brf1_C$'s homology segment II, and presents two interesting features: (i) the TPR array–Brf1 interaction competes with an internal interaction between two segments of the TPR array; as a consequence, Brf1 binding probably reconfigures the N-terminal arm of Tfc4; (ii) the TPR-interacting site in $Brf1_C$ also binds to TBP and Bdp1 (for more about this, see below) [24]. It is probable that this small $Brf1_C$ segment cannot simultaneously accommodate all three of these interactions. In addition, the Brf1-binding sites of the TPR arrays also overlap with a Bdp1-binding site [25]. If TFIIIB is recruited stepwise to the promoter, with TBP–Brf1 placed on DNA first and Bdp1 attaching subsequently, then it is likely that assembly of the promoter complex involves a sequence of realignments (structural rearrangements) of its constituent parts. The available information is compatible with the following hypothetical sequence of steps: (i) the initial interaction of Brf1 is with the N-terminal arm of TFIIIC subunit Tfc4; (ii) Brf1-bound TBP anchors the Brf1-TFIIIC complex to DNA, in the process extending Tfc4 so that it lies close to DNA upstream of the transcriptional start site; (iii) interactions between Brf1 and Tfc4 are reconfigured to accommodate a supplanting interaction with Bdp1 ([26] interpreted in the light of [4,24]).

As already stated, each half of Brf1 interacts with TBP. The stronger interaction is with the evolutionarily conserved homology segment II of $Brf1_C$. A determination of the structure of a ternary complex shows the 70-amino-acid segment of Brf1 that constitutes most of the homology segment II interacting with TBP along a very large interface (~3200 Å^2; 1 Å = 0.1 nm) that extends over the top of the TBP saddle (the DNA-distal surface) and the lateral surface of the N-proximal half of TBP [27]. Homology segment II is also the principal binding site for Bdp1. A dense site-directed mutagenesis of Brf1 amino acids 440–508 (informed by the structure of this Brf1–TBP–DNA complex) shows that homology segment II in fact serves as a two-sided adhesive mediating the attachment of TBP, Brf1 and Bdp1 (Supplementary Figure 3 at http://www.biochemsoctrans.org/bst/034/bst0341082add.htm). The Bdp1 segment that interacts with this $Brf1_C$ site, a SANT [SWI3, ADA2, N-COR (nuclear receptor co-repressor) and TFIIIB] domain, is its most conserved part. These domains assume a compact helix–loop–helix–loop–helix fold (see e.g. [28]). The C-end of $Brf1_C$ (amino acids 498–596) is not required for Bdp1 binding but is essential for full transcriptional activity [29].

An analysis that combines site-specific TBP mutagenesis with analysis of protein-DNA photochemical cross-linking,

in vitro transcription and homology modelling shows that the weaker TBP interaction of $Brf1_N$ resembles that of its paralogue TFIIB, with the two repeat domains of $Brf1_N$ grasping the C-terminal stirrup of TBP. A mutation of a surface-exposed TBP residue that affects interaction with Bdp1, and is located close to the proposed $Brf1_N$–TBP interface, represents a repetition of an already referred to theme: significant Bdp1 interactions within TFIIIB cluster to sites that are also involved in interactions between the other two subunits.

The N-terminal 68 amino acids of *Sc*Brf1 share prominent features with the corresponding segment of TFIIB, including an N-terminal Cys_4 Zn-ribbon domain and an extended segment, the B-finger, that insert into a pol II channel, permitting close approach to the catalytic centre and to the transcribed (template) DNA strand in the vicinity of the transcriptional start site [30]. Thus this N-terminal domain of TFIIB is intimately connected with initiation of transcription [31], and also with the release of pol II from the promoter complex, since a steric clash blocking further elongation is predicted once a 5-nt transcript has been made [30]. Surprisingly, the corresponding N-terminal domain of *Sc*Brf1 is not essential for pol III recruitment to the promoter or for selection of the transcriptional start site. Additional interactions with TFIIIB that involve other pol III subunits and other sites evidently suffice for that.

TFIIIB is also a co-determinant, with pol III, of promoter opening. This is the conclusion drawn from the observation that TFIIIB assembled with certain Brf1 and Bdp1 mutants recruits pol III to a promoter that is presented as linear DNA but fails to open the promoter, and that these defects are compensated for by partially pre-opening the promoter (i.e. unpairing different 3–5 bp segments of the transcription bubble). The defect of Brf1 lacking its N-terminal domain is rescued by unpairing DNA around the transcriptional start site [32]. This is consistent with the expectation that the Brf1 N-terminal domain and the paralogous domain of TFIIB insert in the same way into their conjugate RNA polymerases [30], and that the inserted N-terminal domain of Brf1 provides a stabilizing interaction with the transcribed (template) strand that favours promoter opening.

The defect of a contiguous cluster of Bdp1 deletions is repaired by pre-opening the upstream end of the transcription bubble. It has been argued that promoter opening by pol III has to initiate at this upstream end, near bp –10 (relative to +1 as the transcriptional start site) and propagate downstream, past the transcriptional start site, because of the topological constraint that is imposed by tight TFIIIB binding to upstream DNA [33]. Thus it is initiation of promoter opening that is blocked by these Bdp1 mutations.

Occupancy of pol III-transcribed genes by the transcription apparatus and the nuclear location of pol III transcription

Recent ChIP-chip [ChIP (chromatin immunoprecipitation)-array hybridization] analyses specify the genome-wide distribution of TFIIIC, TFIIIB and pol III in the budding yeast cell [34–36]. Most members of the pol III transcriptome, the approx. 150 5 S rRNAs and 273 tRNAs, U6 spliceosomal RNA, signal recognition particle RNA and RNase P RNA genes, are already known, and the array analysis shows the expected general presence of TFIIIB, TFIIIC and pol III. The ChIP-chip analysis uncovers one 'new' pol III transcription unit (*SNO52*) encoding an RNA involved in rRNA processing. A few loci recruit TFIIIC and TFIIIB but little or no pol III; several loci recruit TFIIIC only [35], raising two interesting questions: (i) what kind of chromatin environment can prevent TFIIIC from bringing TFIIIB to the promoter? (ii) Does TFIIIC-only occupancy have any significance for chromosome architecture and dynamics? Indeed, clusters of B boxes binding TFIIIC (but recruiting no pol III) serve as COC (chromosome-organizing clamps) for the *Sp* genome. These COC tether DNA to the nuclear periphery and also serve as boundary element barriers to heterochromatin spreading [37].

Active transcription requires the periodic displacement of TFIIIC [38], and the efficiency of ChIP (that is, enrichment over background) is generally lower for TFIIIC than it is for TFIIIB. The problem for interpreting this consistently observed correlation in mechanistic terms is that there is no direct way to deduce relative gene occupancy of different proteins, for example TFIIIB and TFIIIC, from relative enrichment in ChIP. Changes of occupancy under conditions that diminish pol III transcription are free of (or at least less subject to) this indeterminacy. While pol III occupancy (ChIP enrichment) diminishes sharply in late growth phase and in stationary cells, TFIIIB occupancy diminishes less and TFIIIC remains bound. Indeed, on a short time scale, when pol III transcription diminishes (2–3-fold) in response to essential nutrient depletion, pol III occupancy diminishes more rapidly than does TFIIIB occupancy, and TFIIIC occupancy increases somewhat (~40–70% for the best-quantified cases) [36] consistent with expectations based on the simple notion that elongating pol III strips TFIIIC off the intragenic promoter. However, the reported effects are quantitatively modest and are not observed for all sites of transcription, implying the existence of additional constraints.

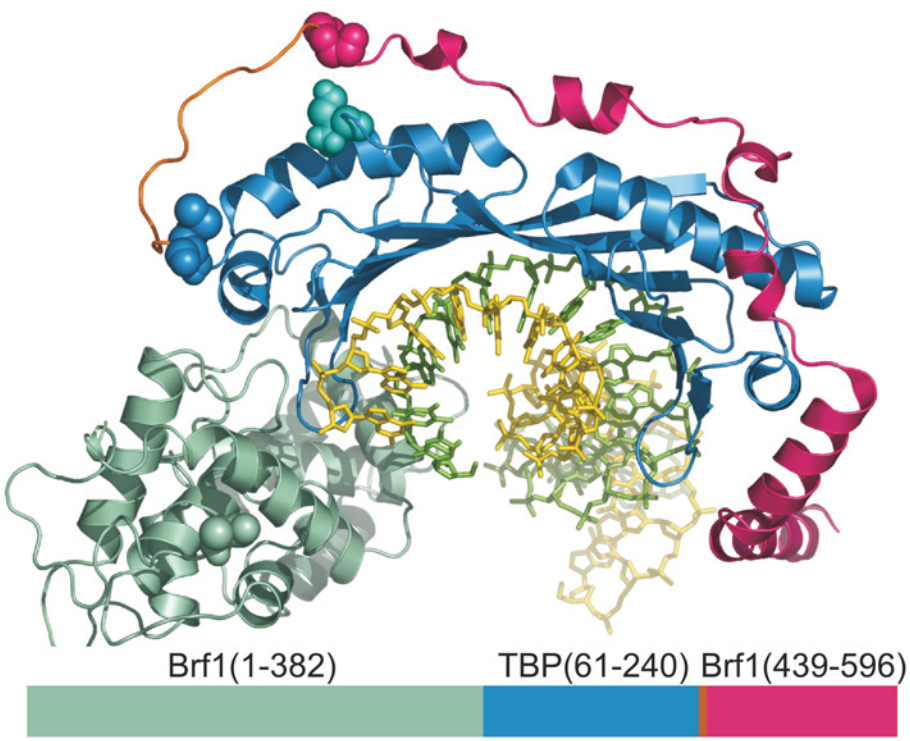
*Sc*pol III transcription is anticipated to be nucleolar, since all tRNA genes examined (in formalin-fixed cells) are nucleolar and since the repressive effect of tRNA genes on vicinal pol III-transcribed genes is dependent on this nucleolar localization [39,40]. Needless to say, placing 273 sites that are broadly distributed over all 17 yeast chromosomes in the nucleolus or at the nucleolus-nucleoplasm interface places enormous constraints on chromosome architecture.

A global repressor of pol III transcription

Transcription by pol III is highly regulated in yeast [41], as it is in metazoans [5,42,43]: repressed in response to nutrient limitations, DNA damage and interruptions of the secretory pathway that produce stress on the plasma membrane [44,45]. The common mediator for signalling this repression of the

Figure 1 | The sites of Brf1 attachment to TBP, and the design of a fusion protein that places the TBPc between N- and C-proximal domains of Brf1

The $Brf1_N$–TBPc–$Brf1_C$ triple fusion protein effectively replaces the functions of both Brf1 and TBP *in vitro*, and also replaces Brf1 function *in vivo*, where it creates a TBP paralogue that is dedicated to pol III transcription. The ribbon representation of TBP core (amino acids 61–240; blue) and Brf1 (amino acids 439–506; red) and stick representation of DNA (yellow and green) are from the solved structure of a ternary complex [27]. The N-terminal Brf1 amino acid 439 and the C-terminal TBP amino acid 240 (space-filled) are joined by a $(Gly\text{-}Ser)_6$ flexible linker (orange) for which a possible path is drawn. Brf1 (amino acids 76–273; green) is modelled into the solved structure of a TFIIB–TBP–DNA complex [53,54]. The structure and location of Brf1 amino acids 274–382 are not specified. A cartoon identifying the segments of the fusion protein is drawn below the model [but the $(Gly\text{-}Ser)_6$ linker does not show up on this scale]. Reproduced from [49] with permission. © 2005 The National Academy of Sciences.



*Sc*pol III machinery is the 45 kDa Maf1; cells lacking *Sc*Maf1 fail to respond to repressive signalling [41,45–47].

Maf1 is a phosphoprotein that responds to repressive signalling by dephosphorylation (mediated directly or indirectly by protein phosphatase 2a) and import into the nucleus. Dephosphorylation is essential for cytoplasm → nucleus transfer and for repression. The multiple forms of Maf1 that exist in the cell are probably in dynamic steady states even when transcription by pol III is active. Dephosphorylation and nuclear accumulation of Maf1 are accompanied by its increased association with repressed pol III genes (detected by ChIP-chip), and correlates with greatly decreased occupancy by pol III [46,47]. How Maf1 mediates repression of transcription remains to be determined. Targets of direct interaction include the N-terminal domain of the largest pol III subunit (C160) and Brf1 [44,46,48]. *In vitro*, a moderate excess of unmodified rMaf1 blocks TFIIIB assembly on the tRNA gene promoter as well as pol III recruitment to preassembled TFIIIB–promoter complexes [48]. These essentially stoichiometric interactions could support a repression mechanism, but it remains to be seen whether Maf1-mediated pol III repression is either direct or stoichiometric *in vivo* [48,48a].

Reconfiguring the connectivity of the TBP–Brf1 complex

The above-summarized structure determination (of the $Brf1_C$ interface with TBP) and mutation analysis (of the $Brf1_N$–TBP interface) show the highly conserved core of TBP sitting between two domains of Brf1 that retain their function when separated from each other (Figure 1). This image has motivated a redesign of the Brf1–TBP complex that joins these two subunits together by inserting the conserved TBPc (core of TBP; amino acids 61–240) between the two domains of Brf1 (Figure 1). The structure-informed TBPc–$Brf1_C$ junction is provided by a flexible $(Ser\text{-}Gly)_6$ linker; the $Brf1_N$ junction

to the N-terminus of TBPc (more a matter of guesswork) eliminates a poorly conserved segment of Brf1 (amino acids 383–438) and includes a Brf1 segment (amino acids 366–382) that is non-essential and not obviously structured as a short connector. The resulting triple fusion protein is fully functional and able to replace TBP as well as Brf1 for accurately initiating TFIIIC-dependent transcription *in vitro*. It also is able to replace Brf1 *in vivo*, while creating a TBP paralogue in yeast that is 'privatized' for pol III transcription [49]. Current genome-wide approaches to the interactions and functions of yeast proteins emphasize the prevalence of multiprotein assemblies and the participation of individual proteins in multiple assemblies with different specialized functions (e.g. [50]). Re-engineering the covalent connectivity of components of multiprotein complexes is a general way of more incisively analysing their diverse functions by re-dedicating them to specific tasks in the cell. Of course, this is what has also happened in the evolution of Brf1 as a fusion of a TFIIB paralogue with a protein that specifies the distinctive and, in budding yeast, tight TBP interaction that characterizes the pol III transcription apparatus.

A fidelity factor for transcriptional initiation

The preferred initiation sites of *Sc*pol III-transcribed genes are marked, very simply, by a pyrimidine preceding the initiating purine, i.e. by YR at –1/+1 of the non-transcribed strand. Such a minimal Initiator Element offers little selective power. Specification of unique start sites therefore must depend on precisely constrained placement of pol III by its initiation factors and correspondingly constrained promoter opening. It has been noted that the fidelity of transcriptional initiation *in vitro* at a tRNA gene promoter offering an especially rich clustering of alternative start sites diminishes as the component transcription proteins are purified. The difference of start site selectivity between relatively crude Bdp1-containing fractions and recombinant Bdp1 is especially notable when the other components are respectively highly purified (TFIIIC and pol III) and recombinant (TBP and Brf1), suggesting that a factor required for accurately initiating transcription is lost during Bdp1 purification [51]. Recent experiments identify two abundant and closely related HMGB (high mobility group B) family proteins, Nhp6a and Nhp6b, as the 'missing' fidelity factor and show that they also exert their effect on transcriptional initiation *in vivo*. Nhp6 distinctively alters the TFIIIC τA subassembly's interaction with the box A promoter element, as seen by chemical footprinting, and generates a corresponding change of footprint consistent with a more confined DNA site occupancy by TFIIIB. A coherent interpretation of these findings proposes a specific interaction of Nhp6 with TFIIIC that anchors/strengthens the τA–box A interaction, generating less 'wobble' in the placement of TFIIIB and, as a further consequence, in the placement of pol III [52]. Since it specifically modulates a TFIIIC–promoter interaction, and affects fidelity of transcription, it is appropriate to include Nhp6 as a component (albeit a minor one) of the pol III transcription apparatus.

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