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TAF1B is a TFIIB-like component of the basal transcription machinery for RNA polymerase I

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Abstract

Transcription by eukaryotic RNA polymerases (Pols) II and III and archaeal Pol requires structurally-related general transcription factors TFIIB, Brf1 and TFB, respectively, which are essential for polymerase recruitment and initiation events. A TFIIB-like protein was not evident in the Pol I basal transcription machinery. We report that TAF1B, a subunit of Pol I basal transcription factor SL1, is structurally related to TFIIB/TFIIB-like proteins, through predicted N-terminal Zn-ribbon and cyclin-like fold domains. SL1, essential for Pol I recruitment to the ribosomal RNA gene promoter, also has an essential post-polymerase recruitment role, operating through TAF1B. Therefore, a TFIIB-related protein is implicated in pre-initiation complex assembly and post-polymerase recruitment events in Pol I transcription, underscoring the parallels between eukaryotic Pol I, II and III and archaeal transcription machineries.

Transcription by the cellular RNA polymerases (Pols) requires general transcription factors (GTFs) for promoter recognition, pre-initiation complex (PIC) formation and initiation. Distinct Pols synthesize the major ribosomal RNAs (Pol I), the messenger RNAs (Pol II) and the small nuclear and transfer RNAs (Pol III) in eukaryotes, whereas a single Pol directs transcription in archaea(1). Basal transcription by Pols II and III and archaeal Pol requires the structurally-related GTFs TFIIB(2-5), Brf1(6, 7) and TFB(8, 9), respectively. In bacteria, sigma factor performs some TFIIB functions and shows topological similarities in association with its Pol(4, 5). TFIIB/TFIIB-like proteins interact with TBP, polymerase and DNA in the PIC and are pivotal in early transcription events, including polymerase recruitment to promoters and post-recruitment events leading to transcription start-site selection, initiation and promoter escape. Given the evolutionary conservation of TFIIB-like proteins and their essential roles in transcription by eukaryotic Pols II and III and archaeal Pol, the Pol I basal transcription machinery is predicted to include a TFIIB-like component.

Pol I basal transcription factor SL1 is a complex of TBP and TBP-associated factors(10-12), essential for recruitment of Pol I to the human rDNA promoter(13-15). Using HHpred, a server for protein remote homologue detection and structure prediction(16), we discovered that the TAF1B (TBP-associated factor 1B/TAF₁63) subunit of human SL1 is structurally similar to TFIIB, having the signature N-terminal Zn-ribbon and core domain with two potential cyclin-like folds (Fig. 1, Fig. S1, Tables S1, S2). The structural similarity, conserved throughout TAF1B orthologues from human TAF1B to yeast Core Factor subunit Rrn7(17), extends to Brf1, Brf2 and TFB and to plant TFIIB-like proteins such as pBrp, which is important for Pol I transcription(18) (Fig. S2, Table S1). TAF1B orthologues contain a C-terminal domain (Fig. S3) with no counterpart in TFIIB (Fig. 1, Fig. S1),

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distinct from the C-terminal domains of Brf1 and Brf2 (Fig. S2). In TFIIB, a flexible connecting region incorporates the B-reader(4), encompassing the B-finger region(5, 19), which is important for transcription start site positioning(4), transcription initiation(20) and processes during promoter escape(21, 22), and the B-linker, which is implicated in promoter opening and/or transcription bubble stabilization(4). A region involved in promoter opening or open complex stabilization in Pol III transcription lies in the connecting region of Brf1(23, 24). The connecting region of TAF1B contains residues essential for Pol I transcription (Fig S4), but lacks the highly conserved reader and linker residues of TFIIB and TFB (4) (Fig. S2), indicating not necessarily the absence of functional equivalents but divergence of this region with the specialization of eukaryotic RNA polymerase into classes I, II and III. There is little homology between the connecting regions of the higher eukaryotic TAF1B proteins and the yeast Rrn7 orthologues (Fig. S3), suggesting co-evolution with species-specific elements of the Pol I transcription machineries and the rDNA(25).

The TFIIB Zn-ribbon is essential for recruitment of Pol II to the promoter(19, 26, 27). The Brf1 Zn-ribbon is also involved in polymerase recruitment, though not essential for this(24), and in promoter opening(27). TAF1B interacts with RRN3 and is, therefore, implicated in recruitment of initiation-competent Pol I(14, 28). To investigate potential functional similarities to TFIIB or TFIIB-like proteins further, we tested *in vivo*-assembled SL1 complexes containing TAF1B Zn-ribbon mutants for activity in *in vitro* transcription. Mutations C13A, C31A and C34A abolished Pol I transcription (Fig. 2A and Fig. S4B). All SL1 subunits co-immunoprecipitated with the TAF1B mutants C13A, C31A and C34A (Fig. 2B), suggesting no defect in SL1 assembly in cells. TAF1B Zn-ribbon mutation did not affect the ability of SL1 to bind the rDNA promoter template (Fig. 2C) or to bind Pol I (Fig. 2D). rDNA promoter templates bound with TAF1B Zn-ribbon mutant-containing SL1 and Pol I were washed, then assayed for transcription activity following the addition of WT SL1 and NTPs. Promoter-specific Pol I transcription activity was detectable, suggesting that mutation of the TAF1B Zn-ribbon does not affect the ability of SL1 to recruit initiation-competent Pol I(14) to the rDNA promoter template (Fig. 2E).

Therefore, mutation of the TAF1B Zn-ribbon abrogates SL1 activity in Pol I transcription and this is not the consequence of a defect in SL1 assembly, SL1 binding to rDNA or recruitment of initiation-competent Pol I. Pre-opening of the rDNA promoter from -1 to +2 does not bypass the requirement for an intact Zn-ribbon (Fig. S5). Our data suggest that the Zn-ribbon domain of TAF1B has an essential post-recruitment role in Pol I transcription at a step preceding synthesis of the first 40 nucleotides (Fig 2). Analyses of mutations in the connecting region and the N-terminal cyclin-like fold domain (Fig. S4) also indicate a post-polymerase recruitment role(s) for TAF1B. Such post-recruitment role(s) could be intrinsic to the Zn-ribbon, the connecting region and the N-terminal cyclin-like fold domains of TAF1B. Alternatively, one or more of these domains could affect the positioning within Pol I of TAF1B domains essential for post-recruitment event(s), by analogy to TFIIB/TFIIB-like proteins (4, 5, 27). TAF1B has a role in Pol I recruitment as a component of the SL1 complex and, perhaps, directly through its interaction with RRN3, but our data imply that the Zn-ribbon of TAF1B is not essential for recruitment. This finding is reminiscent of Brf1(24) and likely to reflect functional redundancy in Pol I recruitment through other TAF1B domains or other SL1 TAFs, such as TAF1C (TAF₁₁₀), which also interacts with RRN3 in Pol I(14, 28). Collectively, these findings indicate that the roles of Pol I basal transcription factor SL1 extend beyond PIC formation.

To conclude, our data suggest that the TAF1B subunit of the Pol I-specific TBP-TAF complex SL1 and its orthologues are structurally related to TFIIB, TFB, Brf1 and Brf2. TAF1B is functionally related to TFIIB, TFB and the Brf proteins, in that it interacts with

TBP(10, 11), binds promoter DNA sequences(29), is implicated in polymerase recruitment(14, 28) and has an additional role(s) post-recruitment of polymerase (Fig. 2 and Fig. S4), in events leading to initiation and/or promoter escape. Therefore, Pol I transcription factor SL1 appears to integrate essential functions of Pol II GTFs TFIID and TFIIB. These findings extend and underscore parallels between the transcription machineries of eukaryotic Pals I, II and III and archael Pal (Table S3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig 1. *HsTAF1B* domain organization and predicted secondary structures, derived from HHpred alignment of *HsTAF1B* and *HsTFIIB* (Fig. S1). HHpred is a sequence alignment method based on the pairwise comparison of profile Hidden Markov Models (HMMs) which facilitates protein remote homologue detection and structure prediction. (h denotes an α -helix).

Fig 2.

The N-terminal Zn-ribbon of TAF1B has a post-recruitment role in transcription. **(A)** SL1 activity of *in vivo*-assembled, tag-immuno-affinity purified complexes from cells exogenously-expressing tagged TAF1B WT (lane 1), Zn-ribbon mutants C13A (lane 2), C31A (lane 3) or C34A (lane 4); *in vitro* transcription reactions included an immobilised human rDNA promoter template (IT-rDNA), Pol I and UBF. **(B)** Composition of *in vivo*-assembled SL1 complexes (immunodetection). **(C)** Binding of the rDNA promoter template by SL1 complexes, isolated as in **A**, containing WT TAF1B (lane 3), C13A (lane 4), purified endogenous SL1 (lane 1) (immunodetection). Control, no TAF1B expressed (lane 2). **(D)** Binding of Pol I to SL1 complexes containing WT TAF1B (lane 2) or C13A (lane 3) (immunodetection of Pol I subunit A190). Control (lane 1) as in **C**. **(E)** Initiation-competency of Pol I recruited to the rDNA promoter by SL1 complexes containing WT TAF1B (lanes 1 and 3) or C13A (lanes 2 and 4) (transcription assay +/- SL1, no added Pol I). IT-rDNA is defined in **A**.