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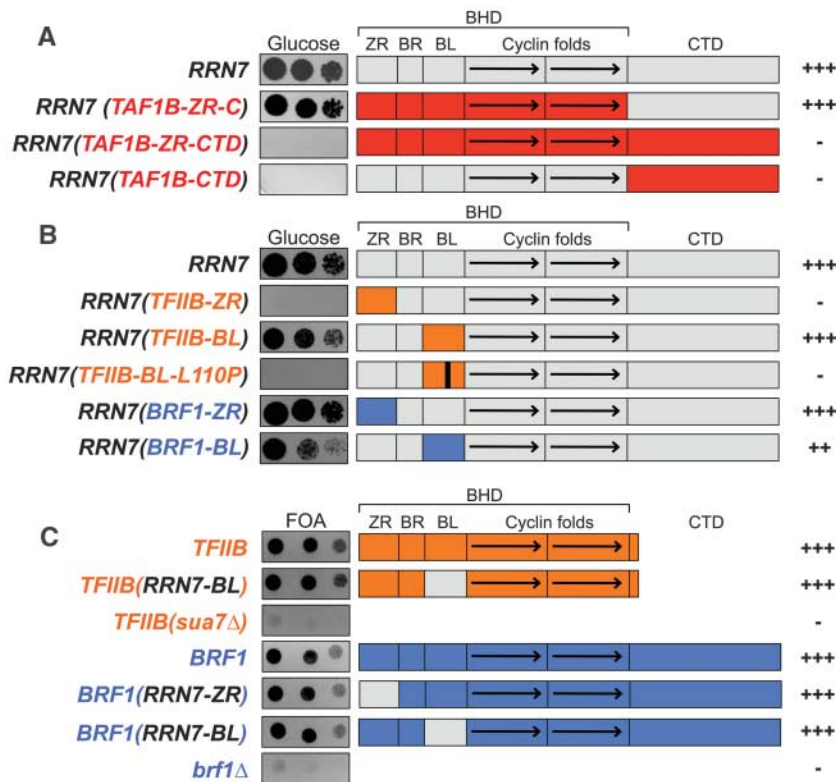


Fig. 3. Interchangeable TFIIIB family domains. *RRN7* yeast complementation assay with chimeric proteins containing (A) Rrn7 (gray) and TAF1B (red), (B) TFIIIB (orange), or BRF1 (blue). (C) *TFIIIB* and *BRF1* yeast complementation assay with indicated Rrn7 domains (gray) in place of TFIIIB or BRF1 domains. +++, WT growth; –, no growth. FOA, 5-fluoroorotic acid.

complex formation, and initiation. Although there is no sequence or notable structural similarity, TFIIIB and $\sigma 70$ show pronounced topological similarities in the way they bind to their respective Pols in near identical locations (2, 3). Combined with previous findings, our results point to the

conservation of initiation mechanisms among all multisubunit Pols.

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Supporting Online Material

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TAF1B Is a TFIIIB-Like Component of the Basal Transcription Machinery for RNA Polymerase I

Srivatsava Naidu,* J. Karsten Friedrich,* Jackie Russell, Joost C. B. M. Zomerdijk†

Transcription by eukaryotic RNA polymerases (Pols) II and III and archaeal Pol requires structurally related general transcription factors TFIIIB, BRF1, and TFB, respectively, which are essential for polymerase recruitment and initiation events. A TFIIIB-like protein was not evident in the Pol I basal transcription machinery. We report that TAF1B, a subunit of human Pol I basal transcription factor SL1, is structurally related to TFIIIB/TFIIIB-like proteins, through predicted amino-terminal zinc ribbon and cyclin-like fold domains. SL1, essential for Pol I recruitment to the ribosomal RNA gene promoter, also has an essential postpolymerase recruitment role, operating through TAF1B. Therefore, a TFIIIB-related protein is implicated in preinitiation complex assembly and postpolymerase recruitment events in Pol I transcription, underscoring the parallels between eukaryotic Pol I, II, and III and archaeal transcription machineries.

and the small nuclear RNAs and tRNAs (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 TFIIIB (2–5), BRF1 (6, 7), and TFB (8, 9), respectively. In bacteria, sigma factor performs some TFIIIB functions and shows topological similarities in association with its Pol (4, 5). TFIIIB/TFIIIB-like proteins interact with TATA-box binding protein (TBP), polymerase, and DNA in the PIC and are pivotal in early transcription events, including polymerase recruitment to promoters and postrecruitment events leading to transcription start-site selection, initiation, and promoter escape. Given the evolutionary conservation of TFIIIB-like proteins and their essential roles in transcription by eukaryotic Pols

Wellcome Trust Centre for Gene Regulation and Expression, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

*These authors contributed equally to this work.
 †To whom correspondence should be addressed. E-mail: j.zomerdijk@dundee.ac.uk

Transcription by the cellular RNA polymerases (Pols) requires general transcription factors (GTFs) for promoter recognition, preinitiation complex (PIC) formation, and initiation. Distinct Pols synthesize the major ribosomal RNAs (rRNAs) (Pol I), the mRNAs (Pol II),

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 ribosomal DNA (rDNA) promoter (13–15). Using HHpred, a server for protein remote homolog detection and structure prediction (16), we discovered that the TAF1B (TBP-associated factor

1B/TAF₆₃) 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, and tables S1 and S2). The structural similarity, conserved throughout TAF1B orthologs 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 and table S1). TAF1B orthologs contain a C-terminal domain (fig. S3) with no counterpart in TFIIB (Fig. 1 and fig. S1), 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 orthologs (fig. S3), suggesting coevolution 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 is 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 coimmunoprecipitated 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 wild-type SL1 and nucleoside triphosphates. 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).

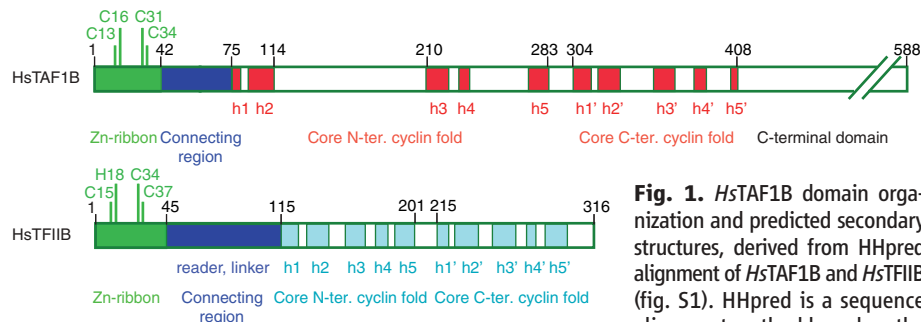


Fig. 1. *HsTAF1B* domain organization and predicted secondary structures, derived from HHpred alignment of *HsTAF1B* and *HsTFIIB* (fig. S1). HHpred is a sequence alignment method based on the pairwise comparison of profile hidden Markov models (HMMs), which facilitates protein remote homolog detection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

pairwise comparison of profile hidden Markov models (HMMs), which facilitates protein remote homolog detection and structure prediction. h denotes an α helix.

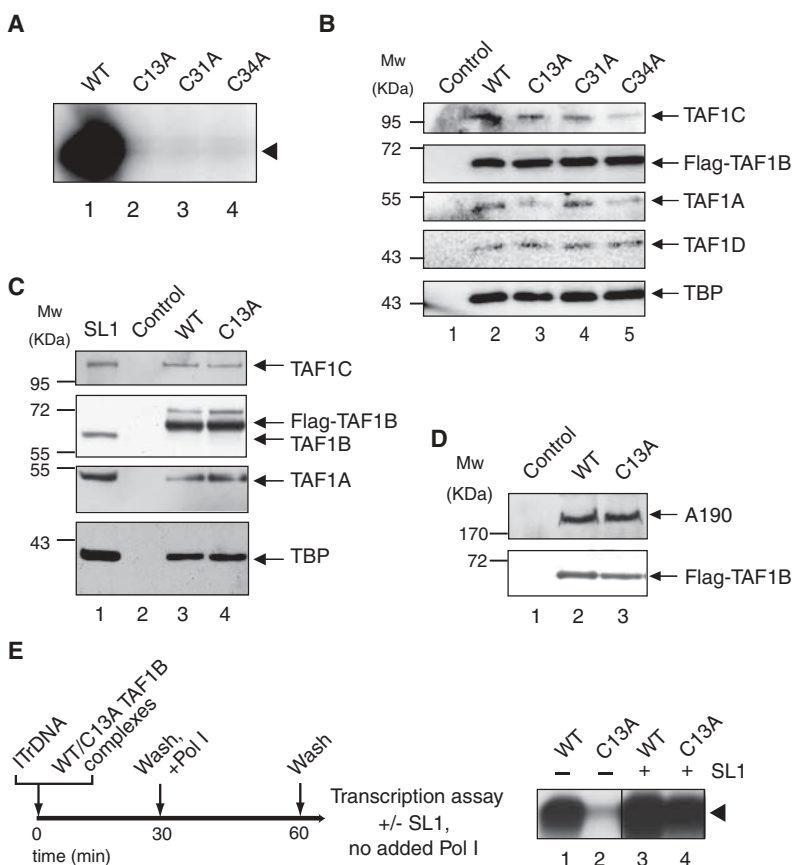


Fig. 2. The N-terminal Zn ribbon of TAF1B has a postrecruitment role in transcription. **(A)** SL1 activity of in vivo-assembled, tag-immuno-affinity-purified complexes from cells exogenously expressing tagged TAF1B wild type (WT) (lane 1) or Zn ribbon mutants C13A (lane 2), C31A (lane 3), or C34A (lane 4); in vitro transcription reactions included an immobilized human rDNA promoter template (IT-rDNA), Pol I, and upstream binding factor (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), or 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).

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 postrecruitment 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 postpolymerase recruitment role(s) for TAF1B. Such a postrecruitment 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 postrecruitment 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.

Our data suggest that the TAF1B subunit of the Pol I-specific TBP-TAF complex SL1 and its

orthologs 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) after 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 archaeal Pol (table S3).

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Role for the Membrane Receptor Guanylyl Cyclase-C in Attention Deficiency and Hyperactive Behavior

Rong Gong,^{1,2*} Cheng Ding,^{2,3*} Ji Hu,^{2*} Yao Lu,² Fei Liu,⁴ Elizabeth Mann,⁵ Fuqiang Xu,⁴ Mitchell B. Cohen,⁵ Minmin Luo^{2,6†}

Midbrain dopamine neurons regulate many important behavioral processes, and their dysfunctions are associated with several human neuropsychiatric disorders such as attention deficit hyperactivity disorder (ADHD) and schizophrenia. Here, we report that these neurons in mice selectively express guanylyl cyclase-C (GC-C), a membrane receptor previously thought to be expressed mainly in the intestine. GC-C activation potentiates the excitatory responses mediated by glutamate and acetylcholine receptors via the activity of guanosine 3',5'-monophosphate-dependent protein kinase (PKG). Mice in which GC-C has been knocked out exhibit hyperactivity and attention deficits. Moreover, their behavioral phenotypes are reversed by ADHD therapeutics and a PKG activator. These results indicate important behavioral and physiological functions for the GC-C/PKG signaling pathway within the brain and suggest new therapeutic targets for neuropsychiatric disorders related to the malfunctions of midbrain dopamine neurons.

Dopamine neurons in the midbrain ventral tegmental area and substantia nigra compacta (VTA/SNc) project their axons extensively to the forebrain and release dopamine to regulate diverse behavioral processes, such as

motor activity, cognition, motivation, and learning (1, 2). Numerous dopamine receptor agonists, antagonists, and reuptake inhibitors have been used to treat the symptoms of Parkinson's disease, schizophrenia, and attention deficit hyper-

activity disorder (ADHD) (3). Studying how the activity of midbrain dopamine neurons is selectively regulated not only can contribute to our understanding of the neurobiological mechanisms of behavioral control but also may provide insight into developing more effective treatments of psychiatric disorders.

While studying the functions of membrane guanylyl cyclases in the nervous system, we observed that guanylyl cyclase-C (GC-C)—thought to be expressed principally on intestinal mucosal cells (4, 5)—is strongly and selectively expressed throughout the VTA/SNc in mice (6, 7). GC-C mRNA colocalized with tyrosine hydroxylase (TH) (Fig. 1, A to C, and figs. S1 and S2), an enzyme critical for dopamine synthesis (1). Dual-

¹Graduate Program in Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China. ²National Institute of Biological Sciences (NIBS), Beijing 102206, China. ³College of Life Sciences, Beijing Normal University, Beijing 100875, China. ⁴Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China. ⁵Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA. ⁶School of Life Sciences, Tsinghua University, Beijing 100084, China.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: luominmin@nibs.ac.cn