Drosophila 230-kD TFIID subunit, a functional homolog of the human cell cycle gene product, negatively regulates DNA binding of the TATA box-binding subunit of TFIID

Tetsuro Kokubo,¹ Da-Wei Gong,¹ Shinya Yamashita,^{1,2} Masami Horikoshi,^{3,4} Robert G. Roeder,³ and Yoshihiro Nakatani^{1,5}

¹National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892 USA;

³Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, New York 10021 USA;

⁴Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

A *Drosophila* cDNA encoding the largest TFIID subunit (p230) was isolated using a degenerate oligodeoxynucleotide probe based on an amino acid sequence of the purified protein. The entire cDNA sequence contains an open reading frame encoding a polypeptide of 2068 amino acids, corresponding to a calculated molecular mass of 232 kD. The deduced amino acid sequence showed a strong sequence similarity with the protein encoded by a human gene (CCG1) implicated in cell cycle progression through G₁, suggesting that p230 may be a target for cell cycle regulatory factors. The recombinant protein expressed in Sf9 cells via a baculovirus vector interacts directly with the TATA box-binding subunit of TFIID (TFIID τ or TBP) from *Drosophila*, human, and yeast. Surprisingly, recombinant p230 inhibits the TATA box-binding activity and function of TFIID τ , suggesting that p230 interactions with TFIID τ , and possible modulations thereof by other factors may play an important role in TFIID function.

[Key Words: *Drosophila*; TFIID subunit; cell cycle gene product; TATA box-binding activity]

Received March 4, 1993; revised version accepted April 13, 1993.

Transcription factor TFIID is a multisubunit protein complex in both *Drosophila* (Dynlacht et al. 1991; Kokubo et al. 1993) and human (Tanese et al. 1991; Takada et al. 1992; Zhou et al. 1992) and plays a central role in transcription by RNA polymerase II (for review, see Roeder 1991; Gill and Tjian 1992; Pugh and Tjian 1992). The TATA box-binding subunit (TFIID τ) of TFIID, together with other general factors, is active in basal transcription but differs from native TFIID in several functional properties, as described below.

One way in which native TFIID and TFIID τ differ is the manner in which each binds to promoters. TFIID τ binds only to the TATA box region in all of the promoters tested by footprint analysis, whereas native TFIID displays a variety of interactions in a promoter-dependent manner. Native TFIID footprints extend from the TATA box through the downstream region in the adenovirus major late (Nakajima et al. 1988) or human gfa (Nakatani et al. 1990b) promoter, whereas they are re-

stricted to the TATA box in the human *hsp70* and adenovirus E4 promoters (Nakajima et al. 1988). Functionally, downstream binding is thought to stabilize TFIID-TATA box interactions, resulting in stimulation of basal transcription from the gfa promoter (Nakatani et al. 1990b). Moreover, it has been demonstrated that sequence-specific activators can affect downstream interactions, presumably via conformational changes in native TFIID, which are correlated with enhanced functional interactions of other general factors (Horikoshi et al. 1988a,b). More recently, it has been found that TFIID τ cannot mediate activator-induced transcription (e.g., see Hoey et al. 1990; Hoffmann et al. 1990; Peterson et al. 1990), in contrast to the earlier observations with native TFIID (Sawadogo and Roeder 1985a; Horikoshi et al. 1988a,b). Such data have suggested that one or more TFIID subunits interacting with the downstream regions might be essential for transcriptional activation.

Another functional difference between native TFIID and TFIID τ is that native TFIID mediates basal transcription from TATA-less promoters, whereas TFIID τ does not (Smale and Baltimore 1989; Nakatani et al. 1990b; Pugh and Tjian 1991; Zhou et al. 1992). This suggests

²Present address: Central Research Laboratory, Nippon Suisan Kaisha Ltd., Hachioji, Tokyo 192, Japan.

⁵Corresponding author.

that components of native TFIID other than TFIID τ are required for interaction with TATA-less promoters and that these components may interact with initiator-binding factors such as TFII-I (Roy et al. 1991) and YY1 (Seto et al. 1991).

Previously, we purified native TFIID from *Drosophila* embryos by immunoaffinity chromatography using antibodies against TFIID τ and identified nine tightly associated polypeptides (230, 110, 85, 62, 58, 42, 28, 22, 21 kD) as presumptive TFIID subunits in addition to TFIID τ (Kokubo et al. 1993). We have now isolated and characterized a cDNA encoding the 230-kD subunit (p230), which has been shown to interact directly with TFIID τ . The deduced amino acid sequence of p230 shows a significant sequence similarity with a human cell cycle gene (CCG1) (Sekiguchi et al. 1988, 1991). The fact that CCG1 can complement a temperature-sensitive mutant cell blocked in progression through G₁ suggests a role for a part of CCG1 in cell cycle regulation. A more direct role for p230 in the regulation of initiation is indicated by the ability of p230 to inhibit TATA box binding of TFIID τ .

Results

Molecular cloning of p230

Previously, we demonstrated that TFIID τ interacts directly with only the largest subunit (p230) of TFIID (Kokubo et al. 1993), suggesting a central role for p230 in assembly of the TFIID protein complex. To study the functional role of p230 in TFIID more precisely, we have used a probe based on a partial amino acid sequence to isolate the corresponding cDNA. To determine the amino acid sequence, the subunits of affinity-purified TFIID were separated by SDS-PAGE, transferred onto a PVDF membrane, and identified with Ponceau S staining. The protein band corresponding to the largest subunit of TFIID was excised and digested with lysyl-endopeptidase on the membrane. The digested peptides were separated by reverse-phase HPLC and subjected to amino acid sequence analysis. One of the resulting protein sequences (N'-GLDSMLLEVIDLK-C') was used to design a degenerate oligodeoxynucleotide probe (see Materials and methods). We first screened a genomic library rather than a cDNA library for the following reasons: (1) the cDNA encoding the p230 might be rare in the library; (2) the *Drosophila* genome is relatively small (1.7×10^5 kb; Ashburner 1989); and (3) having an exact DNA sequence encoding a peptide facilitates screening of a cDNA library. Three positive clones were isolated from a screen of 10^5 plaques using the oligodeoxynucleotide probe. To select a relatively small fragment encoding the peptide sequence from the >15-kb insert DNA, one of the genomic clones (pG1A) was digested with various restriction endonucleases and hybridized with the screening probe. A 700-bp *AluI* fragment that hybridized to the probe was sequenced and found to encode the predicted peptide sequence (data not shown). The resulting genomic sequence was then used to screen a cDNA library.

A cDNA clone, p21, encoding part of p230 (position 45–1135; Fig. 1) was isolated from a random-primed *Drosophila* embryo cDNA library. To isolate cDNAs encoding flanking amino- and carboxy-terminal regions, both random and oligo(dT)-primed libraries were screened using appropriate probes (Fig. 1B). Seven overlapping clones (Fig. 1B) were sequenced and yielded the composite sequence shown in Figure 1A. All overlapping sequences were identical except that (1) clone p19 contained dT instead of dC at the nucleotide position of 1800, which caused a P $\rightarrow$ S change at the amino acid position of 575; and (2) clone pL17 contained a poly(A) tail after position 6337, which probably had been produced by different polyadenylation. The entire cDNA contains an open reading frame encoding a polypeptide of 2068 amino acids, corresponding to a calculated molecular mass of 232 kD. Southern blotting analysis showed that p230 is encoded by a single gene (Fig. 2). Comparison of the deduced protein sequence with the protein sequence data library (SwissProt) showed a strong sequence similarity to the protein encoded by a human cell cycle gene, CCG1 (Fig. 3). The structural domains and intriguing protein motifs (Fig. 8a, below) based on the sequence similarity are discussed below.

The p230 cDNA product copurifies with TFIID τ

A critical point to conclusively prove that the isolated cDNA encodes the p230 subunit is to determine whether the endogenous protein corresponding to the isolated cDNA copurifies with TFIID τ . To test this, a polyclonal antibody against a bacterially expressed amino-terminal portion (amino acids 1–352; Fig. 1A) was prepared. The elution profiles of the two proteins were then compared by Western blotting of fractions from each purification step. There was good correlation in elution profiles between the two proteins on gel filtration (data not shown) and heparin–5PW HPLC (Fig. 4A,B). In addition, the elution profiles of these two proteins in heparin–5PW HPLC were the same as that of TFIID activity (Fig. 4C). Moreover, p230 in the highly purified TFIID fraction isolated by anti-TFIID τ immunoaffinity chromatography also reacted with antibody against the isolated cDNA product (Fig. 5). We conclude that the isolated cDNA encodes the *Drosophila* TFIID subunit p230.

Species-nonspecific interaction between p230 and TFIID τ

Among TFIID subunits, only p230 strongly interacts with TFIID τ (Kokubo et al. 1993). To confirm that the isolated cDNA product binds to TFIID τ , the entire open reading frame was assembled from five clones (p21, p19, p23, p5A3, and pL17; Fig. 1B) and then expressed in Sf9 cells using the baculovirus system. The 230-kD protein was specifically detected in a cell extract harboring the recombinant plasmid by Coomassie brilliant blue staining (Fig. 6A) and Western blot analysis against anti-recombinant p230 antibody (Fig. 6B). This recombinant protein migrated at the same position as the native p230

Figure 1. (See following page for B and legend.)

Figure 2. *Drosophila* p230 is encoded by a single copy gene. *Drosophila* embryo genomic DNA was digested with *Hind*III (lane 1), *Eco*RI (lane 2), and *Bam*HI (lane 3) and hybridized with a random-primed probe made from a fragment spanning nucleotide positions 45–1135 (Fig. 1A).

by both Western and far Western blots (data not shown). Far Western blotting showed that the 230-kD protein specifically interacted with *Drosophila* TFIID τ (Fig. 6C). These results further confirm the identity of the cloned cDNA to be that encoding TFIID subunit p230.

Recently, we and others have demonstrated that both *Drosophila* and human TFIID have a multimeric structure consisting of TFIID τ and several associated subunits, and that the associated subunits are essential for the full range of TFIID activities (Dynlacht et al. 1991; Tanese et al. 1991; Takada et al. 1992; Zhou et al. 1992; Kokubo et al. 1993). The multimeric structure of TFIID is in striking contrast to its counterpart in yeast, which is purified as a single subunit (e.g., see Horikoshi et al. 1989a). Consistent with this and despite the strong evolutionary conservation of the carboxy-terminal core of TFIID τ , the TFIID τ species from yeast to man differ in some aspects of function (Cormack et al. 1991; Gill and

Tjian 1991; Poon et al. 1991; Zhou et al. 1991; Berkenstam et al. 1992). To test whether the capability for interaction with p230 was conserved, *Drosophila* p230 was probed with both human (Fig. 6D) and yeast (Fig. 6E) TFIID τ . These latter species of TFIID τ interacted as efficiently as did *Drosophila* TFIID τ (Fig. 6C), indicating that the p230–TFIID τ interaction is not species specific and raising the admittedly speculative possibility that yeast may contain a p230 counterpart.

p230 negatively regulates the TATA box-binding of TFIID τ

p230 was expressed in Sf9 cells as a histidine fusion protein and purified by Ni-agarose affinity chromatography to determine its biochemical characteristics. The 230-kD protein, which cross-reacts with anti-recombinant p230 antibody (data not shown), was purified to >70% purity, whereas no band was observed at the corresponding position in the mock preparation from uninfected Sf9 cells (Fig. 7A).

Native TFIID gives footprints on the adenovirus major late promoter that extend from 9 bp upstream of the TATA box to 35 bp downstream from the transcription initiation site with characteristic hypersensitive sites (Nakajima et al. 1988; Zhou et al. 1992). To determine whether p230 contributes to the downstream interaction, DNase I footprinting was carried out (Fig. 7B). Surprisingly, p230 inhibited the TATA box binding by TFIID τ (lanes 3–8), whereas the mock preparation did not (lane 18). On the other hand, p230 by itself did not interact with the promoter region (lanes 10–15). These results were confirmed by gel retardation analysis (Fig. 7C). The TFIID τ –promoter complex decreased as the amount of p230 increased (lanes 3–8). In agreement with these binding studies, p230 also inhibited basal transcription activity in a reconstituted system dependent on TFIID τ (Fig. 7D). To test whether the inhibitory effect of p230 is specific to TFIID τ , three other DNA-binding proteins (AP-1, USF, and Oct-1) were tested. In each case, p230 effected a slight increase in DNA binding, indicating that the inhibitory activity of p230 is specific to TFIID τ binding (data not shown). From these observations we conclude that p230 can negatively regulate the TATA box-binding activity of TFIID τ .

Figure 1. Nucleotide and deduced amino acid sequence of *Drosophila* cDNA encoding p230. (A) The open reading frame is defined by translation start and stop codons (boxed in the nucleotide sequence). The positions corresponding to the determined protein sequences are underlined. (B) Positions of overlapping cDNA clones that have been sequenced are shown by the nucleotide numbers. Thick lines represent restriction fragments that were used to construct an expressible cDNA containing an entire open reading frame. Note that (1) clone p19 contains dT instead of dC at nucleotide position 1800, which causes a P $\rightarrow$ S change at amino acid position 575 (underlined); and (2) clone pL17 contains a poly(A) tail after position 6334.

Figure 3. Comparison of the deduced amino acid sequence of *Drosophila* p230 and human CCG1 (human p250). The positions of structural domains I-IV and potential structural motifs, including HMG box, nuclear localization signal (NLS), and bromodomains, are indicated. Direct repeats are represented by arrows.

Figure 4. The cDNA product coelutes with TFIIID τ . TFIIID was fractionated by heparin–5PW HPLC. Each fraction was separated by SDS-PAGE and transferred onto a nitrocellulose membrane, and the blot was probed with either anti-recombinant p230 antibody (A) or anti-TFIIID τ antibody (B). TFIIID activity in each fraction was measured in a TFIIID-dependent reconstituted transcription system in vitro (C). The expected positions for p230 (A), TFIIID τ (B), and transcription product (C) are indicated by arrows. The numbers indicate fraction numbers.

Discussion

p230 is a Drosophila CCG1 homolog

The amino acid sequence encoded by the *Drosophila* p230 cDNA shows extensive sequence similarity (50% identity, 62% similarity) to human CCG1 (Sekiguchi et al. 1991). In addition, we also determined a partial protein sequence for human p250 (Takada et al. 1992) and found it to be identical to that of CCG1 (K. Hisatake, S. Hasegawa, R. Takada, Y. Nakatani, M. Horikoshi, and R.G. Roeder, unpubl.). Hence, we conclude that *Drosophila* p230 is a CCG1 homolog.

Structural domains of p230

Overall structure. On the basis of its amino acid sequence and comparison to CCG1 (human p250), p230 can be divided into four subdomains, as shown in Figure 8A. The small amino-terminal domain (I) (amino acids 1–235) is a highly acidic region (26%) that is moderately conserved between *Drosophila* and human (25% identity, 35% similarity; Fig. 3). This domain is also rich in glycine and serine residues (Gly-Ser, 20%), suggesting

that it might form a fairly flexible and/or closely packaged structure as a result of the small side chains of these amino acids. In contrast, the large domain II (amino acids 236–1706) is well conserved (55% identity, 67% similarity; Fig. 3), especially in its central region (amino acids 610–1178) (72% identity, 83% similarity). Domain II contains three intriguing structural motifs (Sekiguchi et al. 1991): an HMG box (amino acids 1246–1360) (Jantzen et al. 1990); a potential nuclear localization signal (amino acids 1445–1452) (Chelsky et al. 1989); and 120-amino-acid direct repeats that contain the bromodomain (Haynes et al. 1992; Tamkun et al. 1992) in each repeat (amino acids 1457–1577 and 1578–1699). Both the high mobility group (HMG) box and the bromodomain will be discussed later.

Domain III (1707–1993 amino acids) is barely conserved in primary sequence (15% identity, 26% similarity) between *Drosophila* and human, but both species contain a remarkable number of acidic residues (*Drosophila*, 24%; human, 31%). This acidic domain has two consecutive negative charges, both of which are consensus target sites for casein kinase II (CKII) (Kuenzel et al. 1987), which is known to control the activity of several transcription factors (Berberich and Cole 1992; Lüscher et al. 1990; Lin et al. 1992; Marais et al. 1992; Voit et al. 1992). CCG1 has similar multiple target sites for CKII in

Figure 5. Anti-cDNA product antibody cross-reacts with purified TFIIID. TFIIID was purified by protein A–Sepharose with (lanes 1,3) or without (lanes 2,4) anti-TFIIID τ antibody. Purified protein was fractionated by SDS-PAGE and visualized either by silver staining (left) or by Western blotting (right). For Western blotting, the blot was probed with either anti-recombinant p230 antibody (top) or anti-TFIIID τ antibody (bottom). Migration positions of molecular-size standards are indicated at left. Dots indicate specifically detected bands on antibody lanes that are considered to be TFIIID subunits; the estimated molecular mass of each polypeptide is shown between the panels. Although the 85-kD polypeptide is parenthesized because it is fairly faint on this gel, it was detected more clearly in other experiments.

Figure 6. Recombinant p230 interacts directly with various species of TFIID. Sf9 cells were infected with a recombinant baculovirus containing the entire protein-coding sequence of p230 (lanes 1), wild-type baculovirus (lanes 2), and no virus (lanes 3). Cell extracts were fractionated with SDS-PAGE and stained with Coomassie brilliant blue (A) or transferred onto a nitrocellulose membrane. Nitrocellulose blots were reacted with anti-p230 antibody (B) or with [35 S] methionine-labeled probe prepared by in vitro translation in rabbit reticulocyte lysates programmed by RNA encoding either *Drosophila* TFIID (C), human TFIID (D), or yeast TFIID (E). The positions corresponding to p230 are indicated by arrows.

this domain and has been shown to be phosphorylated in vitro by CKII (Sekiguchi et al. 1991). Recently, Zhou et al. (1992) showed that the human 250-kD TFIID subunit [probably equivalent to human p250 (Takada et al. 1992)] is metabolically labeled with 32 P-labeled phosphate in vivo. It is therefore of interest to localize the precise sites of such phosphorylation and to determine whether they regulate the TFIID activity.

The most carboxy-terminal domain (IV) (amino acids 1994–2068) is a glutamine-rich tail (30%) present in the *Drosophila*, but not in the human, sequence. It has been shown for some proteins that the glutamine-rich region is important for transcriptional activation (Courey and Tjian 1988; Gerster et al. 1990) and dimerization (Courey and Tjian 1988), suggesting that these sites might also be interfaces for intermolecular protein–protein interactions. Thus, the *Drosophila*-specific domain IV might interact with some other factors in a species-specific manner.

HMG box. The HMG box is a DNA-binding motif that was first recognized in HMG proteins (Jantzen et al. 1990). Figure 8B shows the amino acid sequence alignment of the HMG box of p230 with other members of the family. Remarkably, only the HMG box of p230 is interrupted by the insertion of a 35-amino acid sequence. In addition, both p230 and CCG1 differ from the others by the absence of four amino acids. It has been proposed that DNA binding by the HMG box can induce a bend in the DNA helix (Giese et al. 1992). DNA bending is supposed to facilitate assembly of a higher-order multiprotein complex by juxtaposing widely separated target sites to be aligned spatially. Thus, p230 might facilitate assembly of a multiprotein complex that involves transcriptional activators and basal transcriptional machinery via DNA bending in response to transcriptional ac-

tivators. It will be important to ascertain whether the HMG box of p230 is active in DNA binding and bending and to further clarify the functional role of the 35-amino-acid insertion.

The bromodomain. The other characteristic structure in domain II is the direct repeat of a 120-amino-acid unit that contains a protein motif called the bromodomain. An alignment of the bromodomains from several proteins is shown in Figure 8C. The bromodomain was proposed as a feature common to several global transcriptional activators (Georgakopoulos and Thireos 1992; Haynes et al. 1992; Tamkun et al. 1992) that help gene-specific activators to function effectively (Jiang and Stillman 1992; Laurent and Carlson 1992; Peterson and Herskowitz 1992). Although the way in which the members of this family cooperate with gene-specific activators is not known, some global activators, such as SNF2 (Davis et al. 1992; Laurent et al. 1992), STH1 (Laurent et al. 1992), and Brahma (Tamkun et al. 1992), contain a helix motif that is believed to enhance transcription via a conformational change in chromosome structure (Hirshhorn et al. 1992; Travers 1992). However, other factors can still function as global transcriptional activators even though they are devoid of helicase domains, suggesting that the bromodomain itself might represent a more important structural motif for their function. It is significant that p230 also contains the bromodomain, as TFIID must cooperate with various gene-specific transcriptional activators to regulate transcription in a manner that may be similar to the action of some global transcriptional activators.

How does the CCG1 mutant cause G₁ arrest?

The human gene encoding CCG1 was isolated as a sup-

pressor of temperature-sensitive G_1 phase mutants of the hamster BHK21/13 cell line (Sekiguchi et al. 1988). The other example of G_1 arrest caused by a mutation in the general transcriptional machinery is an RNA polymerase II mutant (Burstin et al. 1974; Waechter et al. 1984).

Therefore, a variety of defects in the transcriptional machinery driven by RNA polymerase II may produce G_1 arrest. However, the CCG1 mutant has one notable feature: Only particular genes are affected by its mutation (Hirschhorn et al. 1984; Liu et al. 1985) in contrast to the

Figure 7. (See facing page for legend.)

broader effect of an RNA polymerase II mutant. Thus, the mutated CCG1 might have defects in a domain that is especially important for proper expression of certain cell cycle regulatory factors.

The mutated CCG1 may fail to activate genes because of a defect in phosphorylation. It is well known that the cdc/cdk kinase family strictly controls the cell cycle in eukaryotes by associating with cyclins, which are regulatory subunits of this kinase (for review, see Hunter and Pines 1991; Reed 1991). The particular domain of CCG1 might be regulated by phosphorylation in a cell cycle-specific manner. To evaluate this possibility, it will be important to determine whether CCG1 is actually phosphorylated in vivo and whether certain events correlate with different phases of the cell cycle.

p230 is a negative regulator of the TATA box-binding activity of TFIID τ

TFIID is thought to be a candidate as a target for transcriptional activators. Certain sequence-specific activators are known to cause a conformational change of native TFIID (Horikoshi et al. 1988a,b); thus, although native TFIID protects only the TATA box region in the adenovirus E4 promoter, protection is extended farther downstream in response to the addition of transcription activators. In addition, direct interactions between TFIID τ and various transcriptional activators have been shown (Stringer et al. 1990; Horikoshi et al. 1991; Lee et al. 1991; Liberman and Berk 1991; Seto et al. 1992; Truant et al. 1993). Although these findings indicate that TFIID is a potential target for activators, the molecular mechanism for transcriptional activation is unclear.

Here, we demonstrated that baculovirus-expressed p230 inhibits both the TATA box-binding activity of TFIID τ and basal transcription activity in a reconstituted system dependent on TFIID τ . To investigate the specificity of the inhibitory effect more thoroughly, truncated versions of p230 were expressed in and purified (>95% purity) from another organism (*Escherichia coli*). The truncated protein containing the amino-terminal portion (amino acids 1–352) of p230 also inhibits TFIID τ binding to the TATA box, whereas identically purified carboxy-

terminal portions do not (T. Kokubo, unpubl.). These results strongly support the conclusion that the inhibitory effect shown in this paper is the result of the inherent character of p230 rather than to contaminants.

How might the inhibitory activity of p230 be involved in transcriptional regulation? First, it must be emphasized that the present analysis reveals primarily the potential inhibitory effect of p230 on TFIID τ function in the absence of any other TFIID subunits, whereas it is clear that native TFIID can interact functionally with at least some promoters (Sawadogo and Roeder 1985a; Nakajima et al. 1988; Zhou et al. 1992) in the absence of activators. Hence, the negative regulatory potential of p230 must be controlled (counteracted) by other subunits in native TFIID and could be modulated further by the interactions of other regulatory factors (activators or repressors) with TFIID.

Materials and methods

Purification and amino acid sequencing of p230

For purification of p230, 720 ml of nuclear extract from 0- to 12-hr embryos (~3.5-kg embryo) was loaded onto a Sephacryl S300 gel filtration column that had been pre-equilibrated in 0.1 M KCl–buffer C [25 mM HEPES (pH 7.6), 0.1 mM EDTA (pH 8.0), 12.5 mM MgCl₂, 10% glycerol, 0.1% NP-40, 1 mM DTT]. Transcriptionally active TFIID fractions were pooled and clarified by centrifugation at 190,000 g for 60 min. The supernatant was subjected to chromatography on heparin–5PW HPLC. Active TFIID fractions were purified further on an immunoaffinity column on which anti-dTFIID τ antibodies had been covalently immobilized. Bound TFIID subunits were eluted with EGTC buffer [0.1 M glycine (pH 2.5), 50% ethyleneglycol, 10% Tween 20].

To obtain peptide sequences of p230, eluted proteins from the immunoaffinity column were precipitated with TCA and separated on a 4–20% gradient SDS–polyacrylamide gel. After blotting onto a PVDF membrane and staining with Ponceau S, the p230 band was excised from the filter, washed extensively with water (LeGendre and Matsudaira 1989), and digested with lysyl–endopeptidase. The cleaved peptides were resolved by reverse-phase HPLC column, and peak fractions were subjected to automated Edman degradation to determine amino acid sequences.

Figure 7. p230 negatively regulates TATA box-binding activity of TFIID τ . (A) Protein was purified from Sf9 cells infected with a recombinant baculovirus containing the sequence encoding the p230 (lane 1), and no virus (lane 2), as described in Materials and methods. Purified protein was fractionated with SDS-PAGE and stained with Coomassie brilliant blue. The arrow indicates the position of p230; numbers at right show the positions of standard molecular mass markers. (B) DNase I footprint analysis. A probe containing the adenovirus major late promoter sequence was incubated with no protein (lanes 1, 9, 16), with 6 ng of purified recombinant *Drosophila* TFIID τ alone (lanes 2, 17), with *Drosophila* TFIID τ and p230 (lanes 3–8), with p230 alone (lanes 10–15), with *Drosophila* TFIID τ and 2 μ l of mock preparation from uninfected Sf9 cells (lane 18), or with 2 μ l of mock preparation (lane 19). The amount of p230 is 0.01 μ l (lanes 3, 10), 0.1 μ l (lanes 4, 11), 0.3 μ l (lanes 5, 12), 1 μ l (lanes 6, 13), 1.5 μ l (lanes 7, 14), and 2 μ l (lanes 8, 15). Protein concentrations of p230 and mock preparation are 0.27 and <0.01 mg/ml, respectively. The A+G-specific reaction was used as a molecular mass marker. The positions from the transcription initiation site and of the TATA box are indicated. (C) Gel retardation analysis. Experiments were carried out as described for B, except that materials were analyzed on an acrylamide gel containing TGMg buffer (see Materials and methods). The position of the *Drosophila* TFIID τ –promoter complex is shown by an arrow. Although there was a slight increase in TFIID τ –promoter complex formation upon the addition of low concentrations of p230, we have not found this slight effect to be reproducible. (D) p230 inhibits basal transcription. In vitro transcription in a TFIID τ -dependent assay was performed either without (lane 1) or with (lane 2) p230. Reactions contained 18 ng of TFIID τ and 3 μ l of p230, when present. The correctly initiated RNA products are indicated by an arrow.

Figure 8. Structure of p230. (A) Schematic overall structure of p230. The lower line indicates amino acid sequence positions. Four domains within p230 are depicted by brackets: the acidic and Gly/Ser-rich domain I; the highly conserved domain II containing the HMG box; a potential nuclear localization signal (NLS), and the 120-residue direct repeats, which each include a bromodomain (Br), the acidic domain III, and the glutamine-rich domain IV. (B) Amino acid sequence alignment of the HMG boxes of p230 with other members of the family. The amino acid positions are indicated at left. Dashes indicate gaps introduced to maintain optimal alignment. Shading indicates residues identical to or conserved with the sequence of p230. Conserved amino acid substitution groups are (E, D), (K, R, H), (N, Q), (A, I, L, M, V), (F, W, Y), (S, T), (C), (G), (P). Abbreviations for the proteins aligned are as follows: (CCG1) Human cell cycle gene 1 (Sekiguchi et al. 1991); (HMG-1) human high mobility group protein 1 (Wen et al. 1989); (LG-1) chromosome-associated protein from *Tetrahymena thermophila* (Roth et al. 1987); (LEF-1) murine lymphoid enhancer-binding factor 1 (Travis et al. 1991); (TCF-1) human T-cell factor 1 (van de Wetering et al. 1991); (Mc) mating-type protein of *S. pombe* (Kelly et al. 1988); (UBF) human RNA polymerase I upstream binding factor (Jantzen et al. 1990); (mt a1) mating-type protein of *Neurospora crassa* (Staben and Yanofsky 1990); (SRY) murine testis-determining gene product (Gubbay et al. 1990). (C) Sequence alignments of bromodomain proteins. Shading indicates residues identical to or conserved with either the first or second bromodomain of p230. Amino acid positions and conserved amino acid substitution groups are represented as in B. The number 1 or 2 at the end of the abbreviations indicates the first or second bromodomain localized in the same molecule. Abbreviations for the proteins aligned are as follows: (CCG1) The same as in B; (GCN5) yeast GCN5 gene product (Georgakopoulos and Thireos 1992); (RING3) human homolog of FSH1 (Beck et al. 1992); (FSH1) *Drosophila* female sterile homeotic gene product (Haynes et al. 1989); (SPT7) yeast SPT7 gene product; (BRM) *Drosophila brahma* gene product (Tamkun et al. 1992); (SNF2) yeast SNF2 gene product (Laurent et al. 1991); (STH1) yeast STH1 gene product (Laurent et al. 1991).

Isolation of genomic and cDNA clones encoding p230

A *Drosophila* genomic library (Promega) was screened using ³²P end-labeled oligodeoxynucleotide-probe mixtures of 5'-GGI (C/T)TIGA(C/T)TCIATG(C/T)TI(C/T)TIGA(G/A)GTIATIGA-3' and 5'-GGI(C/T)TIGA(C/T)AG(C/T)ATG(C/T)TI(C/T)TIGA-(G/A)GTIATIGA-3'. Hybridization was performed for 12 hr at 42°C in 50 mM phosphate buffer (pH 7.4) containing 10% formamide, 0.1% SDS, 1 M NaCl, 5× Denhardt's solution, and 50 µg/ml of single-stranded DNA; filters were washed in 4× SSC several times at room temperature. The positive clone pG1A was digested with *AluI*, and the 700-bp DNA fragment, which was hybridized with a screening probe, was sequenced. The

DNA sequence corresponding to the peptide sequence (amino acid position 63–78; Fig. 1A) was used to screen a random-primed *Drosophila* embryo cDNA library. The overlapping cDNA clones, which cover the entire protein-coding region, were isolated from random (B. Hovemann, unpubl.) and oligo(dT) (Poole et al. 1985; Zinn et al. 1988)-primed libraries, using appropriate probes labeled by random primers (Fig. 1B).

Southern blot analysis

Genomic DNA isolated from 0- to 12-hr *Drosophila* embryos was digested with *HindIII*, *EcoRI*, and *BamHI*, fractionated on a

0.8% agarose gel, and transferred onto Hybond-N (Amersham). Hybridization was carried out at 55°C in a solution containing 5× SSC, 0.5% SDS, 5× Denhardt's solution, 100 µg/ml of salmon sperm DNA, and 1× 10⁶ cpm/ml of random-primed probe containing a p21 cDNA fragment (Fig. 1B). The filter was washed with a solution containing 0.1× SSC and 0.1% SDS at 65°C for 30 min.

Antibodies and Western blotting

The amino-terminal portion of p230 (amino acids 1–352; Fig. 1A) was expressed in *E. coli* as a histidine–fusion protein and purified by affinity chromatography using Ni-agarose (Qiagen) as described (Yamashita et al. 1992). The purified protein was then injected into New Zealand white rabbits to prepare specific antiserum. The antisera for TFIID τ was produced by immunizing the synthetic peptide corresponding to amino acids position 30–47 (Hoey et al. 1990; Muhich et al. 1990) and affinity-purified as described (Kokubo et al. 1993).

For Western blotting, proteins were separated on 4–20% gradient SDS–polyacrylamide gel, transferred to nitrocellulose membranes, and probed with either anti-TFIID τ or anti-p230 antibody (Harlow and Lane 1988). Detection of the immune signal was done with the alkaline phosphatase system (Promega).

In vitro transcription analysis

The TFIID-dependent transcription system contained recombinant *Drosophila* TFIIB, human TFIIE/F/H fraction, and partially purified human RNA polymerase II as described previously (Yamashita et al. 1992). To determine the TFIID elution profile on the chromatogram, each fraction was incubated in the transcription system with a *Drosophila hsp70* promoter (Clos et al. 1990), and activity was measured by primer extension analysis as described previously (Nakatani et al. 1990a). To determine the effect of p230 on basal transcription, activity was measured by the G-less cassette method (Sawadogo and Roeder 1985b) with G6–HIV template (Kato et al. 1991) as described previously (Yamashita et al. 1992).

Expression of p230 in Sf9 cells

The entire open reading frame was assembled from five clones (p21, p19, p23, p5A3, and pL17) using appropriate restriction enzymes (Fig. 1B) and subcloned into expression plasmid pVL1392 (Pharmingen). The recombinant virus was isolated using a BaculoGold system, according to the manufacturer's instructions (Pharmingen), and was infected into Sf9 cells to express p230.

Far Western blotting

Whole-cell extracts (35 µg protein) from Sf9 cells infected with p230 recombinant baculovirus, wild-type baculovirus, and no virus were separated on 4–20% gradient SDS–polyacrylamide gel and transferred onto a nitrocellulose membrane. Renaturing of the protein blot was performed as described (Takada et al. 1992). Briefly, the blot was denatured with 6 M guanidine hydrochloride in buffer A [20 mM Tris-HCl (pH 7.9), 0.25 M KCl, 0.2 mM EDTA, 10 mM 2-mercaptoethanol, 0.5 mM PMSF, 10% (vol/vol) glycerol], followed by successive treatments with 3.0, 1.5, 0.75, and 0.375 M guanidine hydrochloride in buffer A. After blocking, the renatured blot was incubated with 500 µl of buffer A containing 1% skim milk mixed with 50 µl of rabbit reticulocyte lysate containing ³⁵S-labeled TFIID τ for 12 hr at 4°C.

RNA synthesis and ³⁵S-labeled protein synthesis from *Drosophila* (Hoey et al. 1990; Muhich et al. 1990), human (Hoffmann et al. 1990), and yeast (Horikoshi et al. 1989b) TFIID τ cDNAs with rabbit reticulocyte lysate were performed according to a standard protocol (Promega). The blot was then washed with buffer A, dried, and exposed at room temperature.

Purification of recombinant p230

A histidine tag was introduced at the carboxy-terminal end of p230 by in vitro mutagenesis (Kunkel et al. 1987), using the mutagenic oligodeoxynucleotide 5'-TATGCCTGGACTTTTCACCA-CCACCACCACCACTAGAATTCTTAGAAAATAAAACG-3'. The histidine–fusion protein was expressed as described above and purified as follows: The cell pellet was suspended in 1/10 culture volume of buffer B (20 mM Tris-HCl at pH 7.9, 0.2 mM EDTA, 10% glycerol, 2 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride) containing 6 M guanidine-HCl and 500 mM KCl. The suspension was passed (>10 times) through a syringe fitted with a 21-gauge needle to reduce the viscosity. After centrifugation (8000 g for 20 min), the supernatant was applied onto a Ni-agarose column. After washing with buffer B containing 6 M guanidinium-HCl, 500 mM KCl, and 10 mM imidazole, protein was eluted with the same buffer containing 100 mM imidazole. The eluate was then dialyzed against buffer B containing 1 M guanidine-HCl and 500 mM KCl overnight at 4°C, and then dialyzed against buffer B containing 500 mM KCl for 10 hr at 4°C.

DNA-binding analyses

To prepare the probe, the downstream end of the adenovirus major late promoter (–119 to +61 of the transcription initiation site) was ³²P-labeled with polynucleotide kinase. Each complete system contained the following: 5 × 10⁴ dpm of probe (~10 fmoles), 2.4 µg/ml of dGdC (Pharmacia), 35 mM HEPES–KOH buffer (pH 8.0), 7.5 mM MgCl₂, 6% (vol/vol) glycerol, 60 mM KCl, 6 mM dithiothreitol, 60 µM EDTA, and appropriate transcription factors (as indicated in the legend to Fig. 7), in a total volume of 25 µl. Binding reaction was carried out at 30°C for 40 min. For DNase I footprinting analysis, products were analyzed on a sequencing gel after DNase I digestion, phenol–chloroform extraction, and ethanol precipitation. For gel retardation analysis, products were analyzed on a 4% polyacrylamide gel (59 : 1) containing TGM buffer (25 mM Tris, 192 mM glycine, and 2 mM MgCl₂) and 5% (vol/vol) glycerol at 100 V using TGM as a running buffer.

Acknowledgments

Protein sequence analysis was provided by The Rockefeller University Protein Sequencing Facility, which is supported in part by the National Institutes of Health (NIH) Biomedical shared instrumentation grants and funds provided by the U.S. Army and Navy for purchase of equipment. We are grateful to C. Wu for advice on fly techniques, M. Brenner for AP-1 and critical reading of the manuscript, R. Takada for help on protein sequence analysis, Y. Luo for Oct-1, P. Pognonec for USF, T. Kornberg, K. Zinn, and B. Hovemann for the *Drosophila* cDNA libraries, C. Parker and R. Tjian for the *Drosophila* TFIID τ cDNA clone, and D. Schoenberg for editing the manuscript. S.Y. is supported by Nippon Suisan Kaisha Ltd. M.H. was an Alexandrine and Alexander Sinsheimer Scholar. A portion of this study was supported by NIH grants CA42567 and AI27397 (to R.G.R.) and GM45258 (to M.H.), general support from the Pew

Trusts to The Rockefeller University, and grants from Uehara Memorial Foundation, Kato Memorial Bioscience Foundation, and for Special Project Research from the Ministry of Education, Science, and Culture of Japan (to M.H.).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

Note added in proof

The reference K. Hisatake et al. cited in the Discussion has been published in *Nature* **362**: 179–181.

References

- Ashburner, M. 1989. *Drosophila: A laboratory handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Beck, S., I. Hanson, A. Kelly, D.J. Pappin, and J. Trowsdale. 1992. A homologue of the *Drosophila* female sterile homeotic (fsh) gene in the class II region of the human MHC. *DNA Sequencing* **2**: 203–210.
- Berberich, S.J. and M.D. Cole. 1992. Casein kinase II inhibits the DNA-binding activity of MAX homodimers, but not Myc/Max heterodimers. *Genes & Dev.* **6**: 166–176.
- Berkenstam, A., M.d.M. Vivanco Ruiz, D. Baretino, M. Horikoshi, and H.G. Stunnenberg. 1992. Cooperative in transactivation between retinoic acid receptor and TFIID requires an activity analogous to E1A. *Cell* **69**: 401–412.
- Burstein, S.J., H.K. Meiss, and C. Basilico. 1974. A temperature-sensitive cell cycle mutant of the BHK cell line. *J. Cell. Physiol.* **84**: 397–408.
- Chelsky, D., R. Ralph, and G. Jonak. 1989. Sequence requirement for synthetic peptide mediated translocation to the nucleus. *Mol. Cell. Biol.* **9**: 2487–2492.
- Clos, J., J.T. Westwood, P.B. Becker, S. Wilson, K. Lambert, and C. Wu. 1990. Molecular cloning and expression of a hexameric *Drosophila* heat shock factor subject to negative regulation. *Cell* **63**: 1085–1097.
- Cormack, B.P., M. Strubin, A.S. Ponticelli, and K. Struhl. 1991. Functional differences between yeast and human TFIID are localized to the highly conserved region. *Cell* **65**: 341–348.
- Courey, A.J. and R. Tjian. 1988. Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. *Cell* **55**: 887–898.
- Davis, J.L., R. Kunisawa, and J. Thorne. 1992. A presumptive helicase (MOT1 gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**: 1879–1892.
- Dynlacht, B.D., T. Hoey, and R. Tjian. 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. *Cell* **66**: 563–576.
- Georgakopoulos, T. and G. Thireos. 1992. Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* **11**: 4145–4152.
- Gerster, T., C.G. Balmaceda, and R.G. Roeder. 1990. The cell type-specific octamer transcription factor OTF-2 has two domains required for the activation of transcription. *EMBO J.* **9**: 1635–1643.
- Giese, K., J. Cox, and R. Grosschedl. 1992. The HMG domain of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Cell* **69**: 185–195.
- Gill, G. and R. Tjian. 1991. A highly conserved domain of TFIID displays species specificity in vivo. *Cell* **65**: 333–340.
- . 1992. Eukaryotic coactivators associated with the TATA box binding protein. *Curr. Opin. Genet. Dev.* **2**: 236–242.
- Gubbay, J., J. Collignon, P. Koopman, B. Capel, A. Economou, A. Munsterberg, N. Vivian, P. Goodfellow, and B.R. Lovell. 1990. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature* **346**: 245–250.
- Harlow, E. and D. Lane. 1988. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Haynes, S.R., B.A. Mozer, D.N. Bhatia, and I.B. Dawid. 1989. The *Drosophila* fsh locus, a maternal effect homeotic gene, encodes apparent membrane proteins. *Dev. Biol.* **134**: 246–257.
- Haynes, S.R., C. Dollard, F. Winston, S. Beck, J. Trowsdale, and I.B. Dawid. 1992. The bromodomain: A conserved sequence found in human, *Drosophila* and yeast proteins. *Nucleic Acids Res.* **20**: 2603.
- Hirschhorn, R.R., P. Aller, Z.A. Yuan, C.W. Gibson, and R. Baserga. 1984. Cell-cycle-specific cDNAs from mammalian cells temperature sensitive for growth. *Proc. Natl. Acad. Sci.* **81**: 6004–6008.
- Hirschhorn, J.N., S.A. Brown, C.D. Clark, and F. Winston. 1992. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes & Dev.* **6**: 2288–2298.
- Hoey, T., B.D. Dynlacht, M.G. Peterson, B.F. Pugh, and R. Tjian. 1990. Isolation and characterization of the *Drosophila* gene encoding the TATA box binding protein, TFIID. *Cell* **61**: 1179–1186.
- Hoffmann, A., E. Sinn, T. Yamamoto, J. Wang, A. Roy, M. Horikoshi, and R.G. Roeder. 1990. Highly conserved core domain and unique N terminus with presumptive regulatory motifs in a human TATA factor (TFIID). *Nature* **346**: 387–390.
- Horikoshi, M., M.F. Carey, H. Kakidani, and R.G. Roeder. 1988a. Mechanism of action of a yeast activator: direct effect of GAL4 derivatives on mammalian TFIID-promoter interactions. *Cell* **54**: 665–669.
- Horikoshi, M., T. Hai, Y.S. Lin, M.R. Green, and R.G. Roeder. 1988b. Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell* **54**: 1033–1042.
- Horikoshi, M., C.K. Wang, H. Fujii, J.A. Cromlish, P.A. Weil, and R.G. Roeder. 1989a. Purification of a yeast TATA box-binding protein that exhibits human transcription factor IID activity. *Proc. Natl. Acad. Sci.* **86**: 4843–4847.
- . 1989b. Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box. *Nature* **341**: 299–303.
- Horikoshi, N., K. Maguire, A. Kralli, E. Maldonado, D. Reinberg, and R. Weinman. 1991. Direct interaction between adenovirus E1A protein and the TATA box binding transcription factor IID. *Proc. Natl. Acad. Sci.* **88**: 5124–5128.
- Hunter, T. and J. Pines. 1991. Cyclins and cancer. *Cell* **66**: 1071–1074.
- Jantzen, H.M., A. Admon, S.P. Bell, and R. Tjian. 1990. Nuclear transcription factor hUBF contains a DNA-binding motif with homology to HMG proteins. *Nature* **344**: 830–836.
- Jiang, Y.W. and D.J. Stillman. 1992. Involvement of the SIN4 global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**: 4503–4514.
- Kato, H., M. Horikoshi, and R.G. Roeder. 1991. Repression of HIV-1 transcription by a cellular protein. *Science* **251**:

- 1476–1479.
- Kelly, M., J. Burke, M. Smith, A. Klar, and D. Beach. 1988. Four mating-type genes control sexual differentiation in the fission yeast. *EMBO J.* 7: 1537–1547.
- Kokubo, T., R. Takada, S. Yamashita, D.-W. Gong, R.G. Roeder, M. Horikoshi, and Y. Nakatani. Identification of TFIID components required for transcriptional activation by USF. *J. Biol. Chem.* (in press).
- Kuenzel, E.A., J.A. Mulligan, J. Sommercorn, and E.G. Krebs. 1987. Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides. *J. Biol. Chem.* 262: 9136–9140.
- Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154: 367–382.
- Laurent, B.C. and M. Carlson. 1992. Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. *Genes & Dev.* 6: 1707–1715.
- Laurent, B.C., M.A. Treitel, and M. Carlson. 1991. Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation. *Proc. Natl. Acad. Sci.* 88: 2687–2691.
- Laurent, B.C., X. Yang, and M. Carlson. 1992. An essential *Saccharomyces cerevisiae* gene homologous to SNF2 encodes a helicase-related protein in a new family. *Mol. Cell. Biol.* 12: 1893–1902.
- Lee, W.S., C.C. Kao, G.O. Bryant, X. Liu, and A.J. Berk. 1991. Adenovirus E1A binds the TATA-box transcription factor TFIID. *Cell* 67: 365–376.
- LeGendre, N. and P. Matsudaira. 1989. Purification of proteins and peptides by SDS-PAGE. Academic Press, San Diego, CA.
- Liberman, P.M. and A.J. Berk. 1991. The Zta *trans*-activator protein stabilizes TFIID association with promoter DNA by direct protein–protein interaction. *Genes & Dev.* 5: 2441–2454.
- Lin, A., J. Frost, T. Deng, T. Smeal, N. Al-Alawi, U. Kikkawa, T. Hunter, D. Brenner, and M. Karin. 1992. Casein kinase II is a negative regulator of c-Jun DNA binding and AP-1 activity. *Cell* 70: 777–789.
- Liu, H.T., C.W. Gibson, R.R. Hirschhorn, S. Rittling, R. Baserga, and W.E. Mercer. 1985. Expression of thymidine kinase and dihydrofolate reductase genes in mammalian ts mutants of the cell cycle. *J. Biol. Chem.* 260: 3269–3274.
- Lüscher, B., E. Christenson, D.W. Litchfield, E.G. Krebs, and R.N. Eisenman. 1990. Myb DNA binding inhibited by phosphorylation at a site deleted during oncogenic activation. *Nature* 344: 517–522.
- Marais, R.M., J.J. Hsuan, C. McGuigan, J. Wynne, and R. Treisman. 1992. Casein kinase II phosphorylation increases the rate of serum response factor-binding site exchange. *EMBO J.* 11: 97–105.
- Muhich, M.L., C.T. Iida, M. Horikoshi, R.G. Roeder, and C.S. Parker. 1990. cDNA clone encoding *Drosophila* transcription factor TFIID. *Proc. Natl. Acad. Sci.* 87: 9148–9152.
- Nakajima, N., M. Horikoshi, and R.G. Roeder. 1988. Factors involved in specific transcription by mammalian RNA polymerase II: Purification, genetic specificity, and TATA box-promoter interactions of TFIID. *Mol. Cell. Biol.* 8: 4028–4040.
- Nakatani, Y., M. Brenner, and E. Freese. 1990a. An RNA polymerase II promoter containing sequences upstream and downstream from the RNA startpoint that direct initiation of transcription at the same site. *Proc. Natl. Acad. Sci.* 87: 4289–4293.
- Nakatani, Y., M. Horikoshi, M. Brenner, T. Yamamoto, F. Benard, R.G. Roeder, and E. Freese. 1990b. A downstream initiation element required for efficient TATA box binding and in vitro function of TFIID. *Nature* 348: 86–88.
- Peterson, C.L. and I. Herskowitz. 1992. Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. *Cell* 68: 573–583.
- Peterson, M.G., N. Tanese, B.F. Pugh, and R. Tjian. 1990. Functional domains and upstream activation properties of cloned human TATA binding protein. *Science* 248: 1625–1630.
- Poole, S.J., L.M. Kauvar, B. Drees, and T. Kornberg. 1985. The engrailed locus of *Drosophila*: Structural analysis of an embryonic transcript. *Cell* 40: 37–43.
- Poon, D., S. Schroeder, C.K. Wang, T. Yamamoto, M. Horikoshi, R.G. Roeder, and P.A. Weil. 1991. The conserved carboxy-terminal domain of *Saccharomyces cerevisiae* TFIID is sufficient to support normal cell growth. *Mol. Cell. Biol.* 11: 4809–4821.
- Pugh, B.F. and R. Tjian. 1991. Transcription from a TATA-less promoter requires a multisubunit TFIID complex. *Genes & Dev.* 5: 1935–1945.
- . 1992. Diverse transcriptional functions of the multisubunit eukaryotic TFIID complex. *J. Biol. Chem.* 267: 679–682.
- Reed, S.I. 1991. G1-specific cyclins: in search of an S-phase-promoting factor. *Trends Genet.* 7: 95–99.
- Roeder, R.G. 1991. The complexities of eukaryotic transcription initiation: Regulation of preinitiation complex assembly. *Trends Biochem. Sci.* 16: 402–408.
- Roth, S.Y., I.G. Schulman, R.G. Cook, and C.D. Allis. 1987. The complete amino acid sequence of an HMG-like protein isolated from the macronucleus of *Tetrahymena*. *Nucleic Acids Res.* 15: 8112.
- Roy, A.L., M. Meisterernst, P. Pognonec, and R.G. Roeder. 1991. Cooperative interaction of an initiator-binding transcription initiation factor and the helix-loop-helix activator USF. *Nature* 354: 245–248.
- Savado, M. and R.G. Roeder. 1985a. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. *Cell* 43: 165–175.
- . 1985b. Factors involved in specific transcription by human RNA polymerase II: Analysis by a rapid and quantitative in vitro assay. *Proc. Natl. Acad. Sci.* 82: 4394–4398.
- Sekiguchi, T., T. Miyata, and T. Nishimoto. 1988. Molecular cloning of the cDNA of human X chromosome gene (CCG1) which complements the temperature-sensitive G1 mutants, tsBN462 and ts13, of the BHK cell line. *EMBO J.* 7: 1683–1687.
- Sekiguchi, T., Y. Nohiro, Y. Nakamura, N. Hisamoto, and T. Nishimoto. 1991. The human CCG1 gene, essential for progression of the G1 phase, encodes a 210-kilodalton nuclear DNA-binding protein. *Mol. Cell. Biol.* 11: 3317–3325.
- Seto, E., Y. Shi, and T. Shenk. 1991. YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. *Nature* 354: 241–245.
- Seto, E., A. Usheva, G.P. Zambetti, J. Momand, N. Horikoshi, R. Weinmann, A.J. Levine, and T. Shenk. 1992. Wild-type p53 binds to the TATA-binding protein and represses transcription. *Proc. Natl. Acad. Sci.* 89: 12028–12032.
- Smale, S.T. and D. Baltimore. 1989. The “initiator” as a transcription control element. *Cell* 57: 103–113.
- Staben, C. and C. Yanofsky. 1990. *Neurospora crassa* a mating-type region. *Proc. Natl. Acad. Sci.* 87: 4917–4921.
- Stringer, K.F., C.J. Ingles, and J. Greenblatt. 1990. Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. *Nature* 345: 783–786.

- Takada, R., Y. Nakatani, A. Hoffmann, T. Kokubo, S. Hasegawa, R.G. Roeder, and M. Horikoshi. 1992. Identification of human TFIID components and direct interaction between a 250 kDa polypeptide and the TATA box-binding protein (TFIID γ). *Proc. Natl. Acad. Sci.* **89**: 11809–11813.
- Tamkun, J.W., R. Deuring, M.P. Scott, M. Kissinger, A.M. Patatucci, T.C. Kaufman, and J.A. Kennison. 1992. *brahma*: A regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**: 561–572.
- Tanese, N., B.F. Pugh, and R. Tjian. 1991. Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. *Genes & Dev.* **5**: 2212–2224.
- Travers, A.A. 1992. The programming of transcriptional competence. *Cell* **69**: 573–575.
- Travis, A., A. Amsterdam, C. Belanger, and R. Grosschedl. 1991. LEF-1, a gene encoding a lymphoid-specific protein with an HMG domain, regulates T-cell receptor α enhancer function. *Genes & Dev.* **5**: 880–894.
- Truant, R., H. Xiao, C.J. Ingles, and J. Greenblatt. 1993. Direct interaction between the transcriptional activation domain of human p53 and the TATA box-binding protein. *J. Biol. Chem.* **268**: 2284–2287.
- van de Wetering, M., M. Oosterwegel, D. Dooijes, and H. Clevers. 1991. Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J.* **10**: 123–132.
- Voit, R., A. Schnapp, A. Kuhn, H. Rosenbauer, P. Hirschmann, H.G. Stunnenberg, and I. Grummt. 1992. The nucleolar transcription factor mUBF is phosphorylated by casein kinase II in the C-terminal hyperacidic tail which is essential for transactivation. *EMBO J.* **11**: 2211–2218.
- Waechter, E., C. Avignolo, E. Freund, C. Riggenschach, W. Mercer, P.M. McGuire, and R. Baserga. 1984. Microinjection of RNA polymerase II corrects the temperature-sensitive defect of tsAF8 cells. *Mol. Cell. Biochem.* **60**: 77–82.
- Wen, L., J.K. Huang, B.H. Johnson, and G.R. Reeck. 1989. A human placental cDNA clone that encodes nonhistone chromosomal protein HMG-1. *Nucleic Acids Res.* **17**: 1197–1214.
- Yamashita, S., K. Wada, M. Horikoshi, D.-W. Gong, T. Kokubo, K. Hisatake, N. Yokotani, S. Malik, R.G. Roeder, and Y. Nakatani. 1992. Isolation and characterization of a cDNA encoding *Drosophila* transcription factor TFIIB. *Proc. Natl. Acad. Sci.* **89**: 2839–2843.
- Zhou, Q.A., M.C. Schmidt, and A.J. Berk. 1991. Requirement for acidic amino acid residues immediately N-terminal to the conserved domain of *Saccharomyces cerevisiae* TFIID. *EMBO J.* **10**: 1843–1852.
- Zhou, Q., P.M. Lieberman, T.G. Boyer, and A.J. Berk. 1992. Holo-TFIID supports transcriptional stimulation by diverse activators and from a TATA-less promoter. *Genes & Dev.* **6**: 1964–1974.
- Zinn, K., L. McAllister, and C.S. Goodman. 1988. Sequence analysis and neuronal expression of fasciclin I in grasshopper and *Drosophila*. *Cell* **53**: 577–587.

Drosophila 230-kD TFIIID subunit, a functional homolog of the human cell cycle gene product, negatively regulates DNA binding of the TATA box-binding subunit of TFIIID.

T Kokubo, D W Gong, S Yamashita, et al.

Genes Dev. 1993, **7**:

Access the most recent version at doi:[10.1101/gad.7.6.1033](https://doi.org/10.1101/gad.7.6.1033)

References

This article cites 80 articles, 32 of which can be accessed free at:
<http://genesdev.cshlp.org/content/7/6/1033.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).