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The *Drosophila* 110-kDa transcription factor TFIID subunit directly interacts with the N-terminal region of the 230-kDa subunit

(TATA box-binding factor/subunit structure/RNA polymerase II/transcriptional activation/transcriptional initiation)

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ABSTRACT **Transcription initiation factor TFIID is a multimeric protein complex that plays a central role in transcriptional regulation by facilitating promoter responses to various activators. cDNAs encoding the 110-kDa subunit of *Drosophila* TFIID (p110) were isolated with 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 921-amino acid polypeptide with a calculated molecular mass of 99,337 Da. The recombinant protein expressed in Sf9 cells via a baculovirus vector interacts directly with the 230-kDa subunit of TFIID (p230). Together with the previous observation that the TATA box-binding subunit of TFIID (TFIIDτ or TBP) interacts directly with only p230 among the TFIID subunits, this result suggests that p110 forms a complex with TFIIDτ via p230. A binding study using various p230 mutants indicated that both p110 and TFIIDτ interact with the N-terminal 352-amino acid portion of p230, suggesting a functional communication between p110 and TFIIDτ via p230 interactions.**

Transcription of protein-encoding genes in eukaryotes is regulated by various gene-specific transcriptional factors that bind to distinct DNA elements (for review, see refs. 1–3). In general, sequence-specific activators contain both DNA-binding and activation domains. Various activation domains rich in acidic, glutamine, or proline residues have been characterized. Although these domains are thought to activate transcription by interacting with the RNA polymerase II transcription machinery (3–5), their mechanism of action is poorly understood.

In addition to RNA polymerase II, accurate transcription initiation *in vitro* requires at least six general transcription initiation factors: TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (for review, see refs. 3 and 6). The first step in preinitiation complex formation involves TFIID binding to the TATA box in the promoter region. Subsequently, TFIIB binds to the TFIID–promoter complex and acts as a "bridging" factor to recruit the preformed RNA polymerase II–TFIIF complex to the promoter. The subsequent binding of TFIIE and TFIIH completes formation of the preinitiation complex. Consistent with the possibility that they may stimulate transcription by enhancing the rate of preinitiation complex formation or function, various activators have been shown to interact with the TATA box-binding subunit of TFIID (TFIIDτ or TBP) (7–13) and TFIIB (14). Although such interactions may be important for activator-dependent transcription, it is clear that they are insufficient because systems reconstituted with TFIIDτ in place of TFIID effect only basal promoter functions (15–17).

In contrast to TFIIDτ, the intact TFIID complex mediates responses to various activators *in vitro* (4, 5, 18–22). These findings strongly suggest that an additional TFIID subunit(s) plays a critical role in activator-dependent transcription. One possible role would be to serve as an additional direct target for activators, along with TFIIDτ (7–13) or TFIIB (14), and to transduce signals from these interactions to other components of the preinitiation complex to facilitate either assembly or function.

Recently, we purified a native TFIID complex from *Drosophila* embryo extracts by immunoaffinity chromatography with anti-TFIIDτ antibody and identified nine tightly associated polypeptides (230, 110, 85, 62, 58, 42, 28, 22, and 21 kDa) that are candidates for TFIID subunits (22). To further understand the role of the 110-kDa TFIIDτ-associated polypeptide (p110), we have cloned and expressed the corresponding cDNA and report structural motifs and functional properties of p110. Tjian and colleagues have reported the structural characterization of *Drosophila* TFIID (23) and, recently, have described both the cloning of p110 and somewhat distinct functional properties (24).

MATERIALS AND METHODS

Isolation of cDNA Clones Encoding p110. Purification and amino acid sequencing of p110 were done as described (25). *Drosophila* cDNA libraries (26, 27) were screened by using a ^{32}P end-labeled oligodeoxynucleotide probe of 5′-CARGCIC-ARATIMGICCIATIGGICC-3′. Hybridization was done for 12 hr at 42°C in 50 mM phosphate buffer (pH 7.4)/10% (vol/vol) formamide/0.1% SDS/1 M NaCl/5× Denhardt's solution/single-stranded DNA at 50 μg/ml; filters were washed several times in 4× standard saline citrate at room temperature.

Antibodies and Immunoblotting. Two portions of p110 (residues 201–458 and 457–693) were expressed in *Escherichia coli* as histidine fusion proteins and purified by affinity chromatography using Ni-agarose (Qiagen, Studio City, CA) as described (25). The purified proteins were mixed and injected into New Zealand White rabbits to prepare polyclonal antiserum. Immunoblotting was done as described (25).

***In vitro* Transcription Analysis.** *In vitro* transcription was done as described (22) on a template DNA containing the *Drosophila* hsp70 promoter.

Expression of Recombinant Proteins in Sf9 Cells. The entire open reading frame was assembled from clones KZ4 and AR19, using appropriate restriction enzymes (Fig. 1*B*). To remove excess untranslated region, a *Bgl* II site was introduced at position 240 by *in vitro* mutagenesis (28). For Ni-agarose affinity purification, six histidine residues were inserted at the C-terminal end (28). The 3.0-kb *Bgl* II–*Dra* I (Fig. 1*B*) fragment was subcloned into the *Bgl* II–*Sma* I-digested

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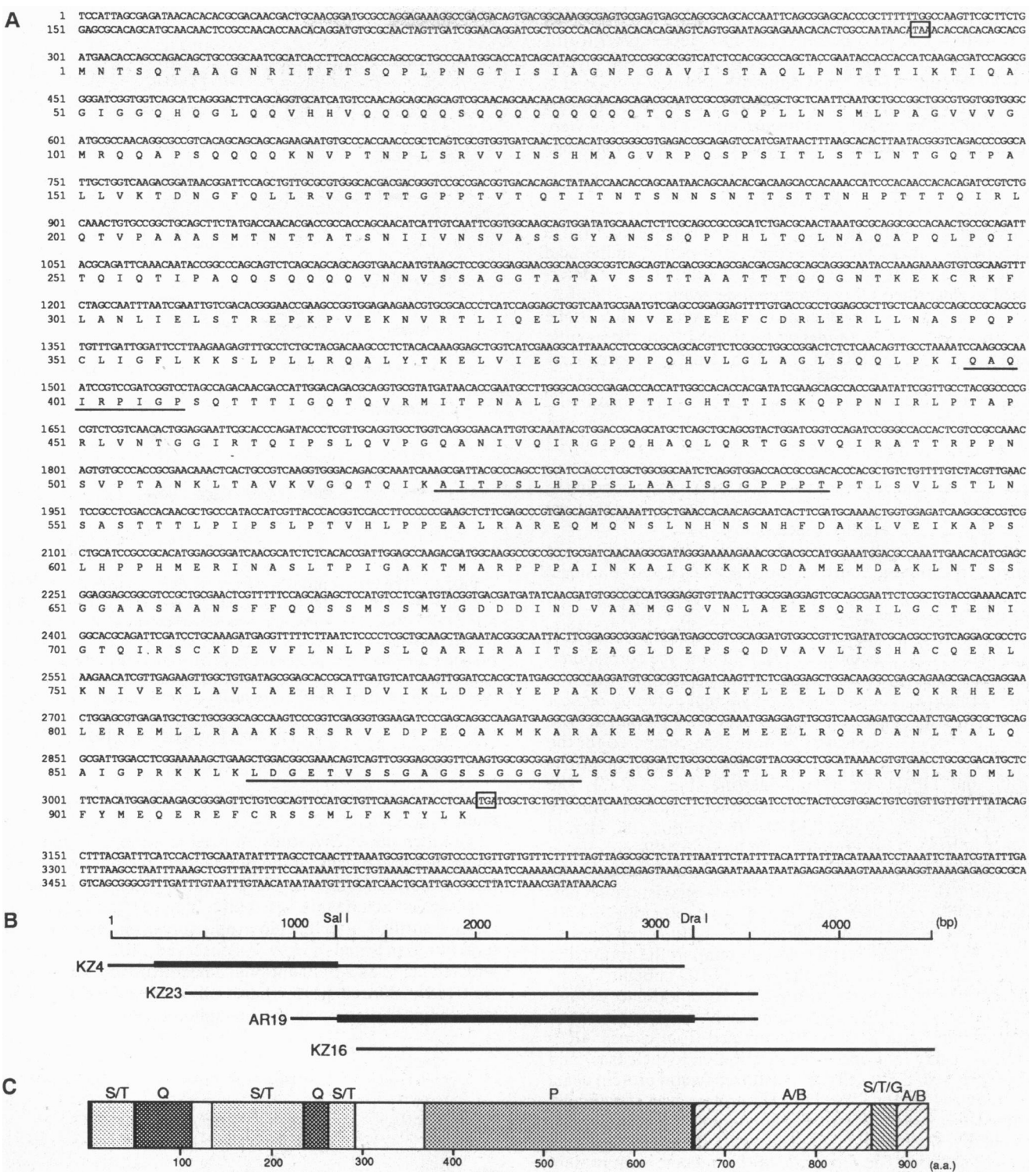

FIG. 1. Nucleotide and deduced amino acid sequence of *Drosophila* cDNA encoding p110. (*A*) The open reading frame is defined by translation start and stop codons (boxed in the nucleotide sequence). The positions corresponding to the determined protein sequences are underlined. Note that several nucleotides (positions 1563, 1585, and 2334) within the protein-coding region differ from the sequence reported by Hoey *et al.* (24). (*B*) Positions of overlapping cDNA clones that have been sequenced are shown by the nucleotide numbers. Thick lines represent restriction fragments used to construct an expressible cDNA containing an entire open reading frame. (*C*) Schematic overall structure of *Drosophila* 110-kDa subunit. The lower numbers indicate positions of the amino acid sequence. Q, glutamine-rich domain; S/T, serine/threonine-rich domain; A/B, acidic/basic domain; P, proline-rich domain; S/T/G, serine/threonine/glycine-rich domain.

plasmid pVL1392 (PharMingen, San Diego). For the truncated p230 mutants, six histidine residues and a stop codon were introduced at the indicated points (see Fig. 5). The histidine-fusion protein was expressed and purified as described (25).

Immunoprecipitation. For *in vivo* interaction, Sf9 cells were coinfected with recombinant baculovirus containing p110 and p230 cDNA sequences. Whole-cell lysates were prepared as described (25). For *in vitro* interaction, purified recombinant proteins were mixed, as described in the figure legends, and incubated for 1 hr at 30°C.

For immunoprecipitation, samples were incubated with protein A-Sepharose with or without p110 antibody for 1 hr

with gentle rocking at 4°C. The beads were washed three times with 0.1 M KCl/buffer C [25 mM Hepes (pH 7.6)/0.1 mM EDTA (pH 8.0)/12.5 mM $MgCl_2$/10% (vol/vol) glycerol/0.1% Nonidet P-40/1 mM dithiothreitol] and resuspended in EGTG buffer [0.1 M glycine (pH 2.5)/50% ethylene glycol/10% Tween 20] to elute proteins. The eluates were precipitated with acetone and detected by immunoblotting.

RESULTS

Molecular Cloning of p110. TFIID was purified from a *Drosophila* embryo nuclear extract, and partial amino acid sequences of p110, the second largest subunit, were determined as described (25). Three peptide sequences (Fig. 1*A*) were obtained, and a degenerate oligodeoxynucleotide probe (see *Materials and Methods*) was designed from the peptide sequence N′-QAQIRPIGP-C′, with the least sequence diversity among the back-translated sequences of the peptides. More than 10 cDNA clones were isolated from a *Drosophila* embryo cDNA library; four clones, KZ4, KZ13, KZ15, and AR19 (Fig. 1*B*), were sequenced. Sequence analysis showed that the entire cDNA contains an open reading frame encoding a 921-amino acid polypeptide (Fig. 1*A*), corresponding to a calculated molecular mass of 99,337 Da. The structural features (Fig. 1*C*) are discussed below.

RNA blotting analysis yielded a single 5-kb band (data not shown). In addition, Southern blotting analysis gave a single band (data not shown). These results suggest that p110 is encoded by a single gene.

The cDNA Product Encodes the 110-kDa TFIID Subunit. If the isolated cDNA encodes a TFIID subunit, the corresponding endogenous protein should cochromatograph with the TFIID complex. To test this, a polyclonal antibody against bacterially expressed cDNA products was prepared. The elution profiles on heparin-5PW were then compared for the encoded endogenous protein, the 230-kDa subunit of TFIID (p230), and TFIIDτ by immunoblotting (Fig. 2 *A–C*). The elution profile of the cDNA-encoded product closely matched that of p230 and TFIIDτ. In addition, the elution profiles of these three proteins from heparin-5PW correlated with TFIID activity (Fig. 2*D*). In a further test of the association of p110 with TFIID, antibody against the recombinant cDNA product was found to coprecipitate both TFIIDτ and p230 subunits, along with p110, from nuclear extract (Fig. 3). These results strongly support the notion that the isolated cDNA encodes the p110 TFIID subunit.

p110 Directly Interacts with p230. To determine subunit interactions of TFIID using recombinant factors, the entire coding sequence of p110 was assembled from clones AR19 and KZ4, using appropriate restriction endonucleases, and then expressed in Sf9 cells as a histidine-fusion protein using a baculovirus vector. The 110-kDa protein was specifically detected in extracts from cells containing the recombinant plasmid by immunoblot analysis with anti-p110 antibody (data not shown). The migration of this recombinant protein was nearly identical to that of the endogenous p110 in the *Drosophila* embryo extract-derived TFIID fraction (Fig. 4*A*).

Previously, we found that only the largest subunit of TFIID (p230) interacts directly with TFIIDτ (22), suggesting that p230 might be a central unit in the TFIID protein complex and that other subunits might interact with TFIIDτ via p230. Accordingly, we tested for an interaction between p110 and p230. To test for this interaction *in vivo*, Sf9 cells were coinfected with both p110- and p230-encoding recombinant baculoviruses, and derived extracts were subjected to immunoprecipitation with anti-p110 antibody followed by immunoblot analysis. As predicted, anti-p110 antibody coimmunoprecipitated p230 with p110 from doubly infected cells, whereas neither p230 nor p110 was immunoprecipitated in extracts from control cells infected with p230-baculovirus alone (Fig. 4*B*).

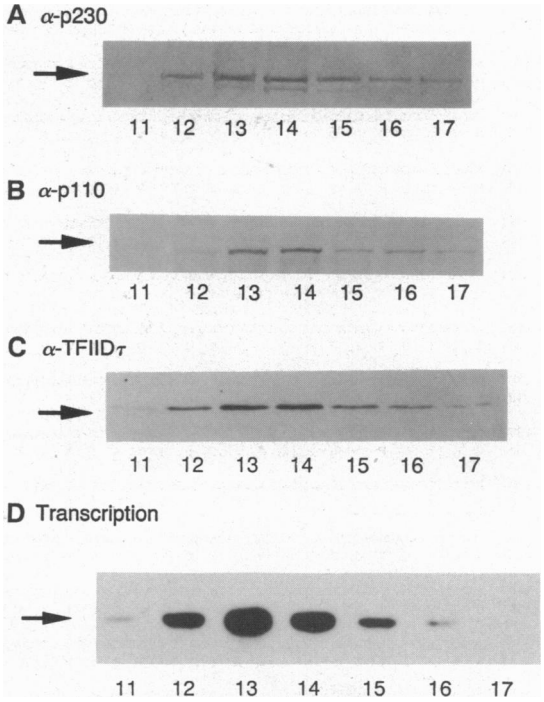

FIG. 2. The cDNA product cochromatographs with other TFIID subunits and with TFIID activity. TFIID was fractionated by heparin-5PW HPLC. Each fraction was separated by SDS/PAGE and transferred onto a nitrocellulose membrane. The resulting blot was probed with either anti-p230 antibody (*A*), anti-p110 antibody (*B*), or anti-TFIIDτ antibody (*C*). Functional TFIID activity in each fraction was measured in a TFIID-dependent reconstituted transcription system (*D*). The expected positions for p230 (*A*), p110 (*B*), TFIIDτ (*C*), and the specifically initiated transcription product (*D*) are indicated by arrows. The numbers indicate the fraction numbers.

Although the *in vivo* study showed that the p230 and p110 interacted specifically, it did not exclude the possibility that one or more endogenous Sf9 protein(s) mediated the interactions. To exclude this possibility, both p110 and p230 were affinity-purified after baculovirus expression, and their interaction was tested *in vitro*. As observed with extracts from infected cells, anti-p110 antibody specifically coimmunoprecipitated p230 and p110 when both were mixed together, whereas p230 alone was not precipitated (Fig. 4*C*).

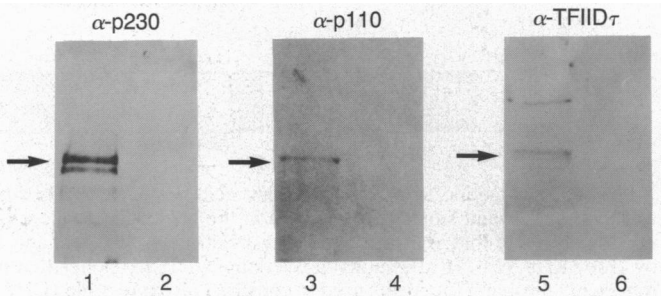

FIG. 3. Antibody against the cDNA-encoded protein coimmunoprecipitates other TFIID subunits with p110. Protein was immunoprecipitated from *Drosophila* embryo nuclear extract by protein A-Sepharose with (lanes 1, 3, and 5) or without (lanes 2, 4, and 6) anti-p110 antibody. Precipitated proteins were analyzed by immunoblot by using either anti-p230 antibody (lanes 1 and 2), anti-p110 antibody (lanes 3 and 4), or anti-TFIIDτ antibody (lanes 5 and 6). The expected positions for p230 (*Left*), p110 (*Middle*), and TFIIDτ (*Right*) are indicated by arrows.

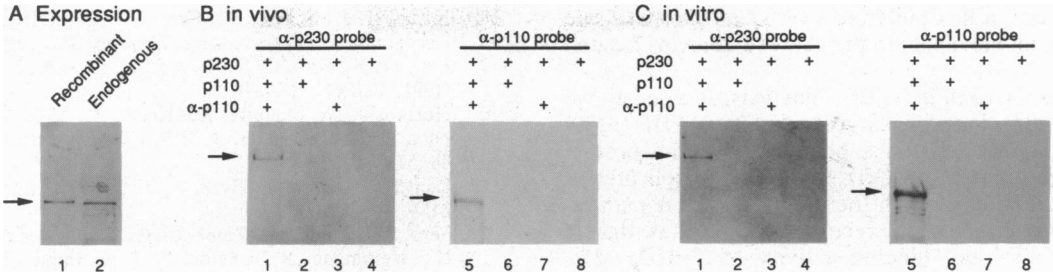

FIG. 4. p110 interacts with p230 both *in vivo* and *in vitro*. (*A*) Expression of p110. Recombinant p110 expressed in Sf9 cells using the baculovirus system (lane 1) and endogenous p110 (lane 2) were detected by immunoblot. The expected position for p110 is indicated by an arrow. (*B*) *In vivo* interaction of p110 and p230. Sf9 cells were infected with either a mixture of p230- and p110-encoding baculovirus (lanes 1, 2, 5, and 6) or only with p230-encoding baculovirus (lanes 3, 4, 7, and 8). Cell extracts were incubated with protein A-Sepharose with (lanes 1, 3, 5, and 7) or without (lanes 2, 4, 6, and 8) anti-p110 antibody. Precipitated proteins were analyzed by immunoblot using either anti-p230 antibody (lanes 1–4) or anti-p110 antibody (lanes 5–8). The expected positions for p230 (*Left*) and p110 (*Right*) are indicated by arrows. (*C*) *In vitro* interaction of p110 and p230. p230 was incubated with (lanes 1, 2, 5, and 6) and without p110 (lanes 3, 4, 7, and 8) *in vitro*. Immunoprecipitation was done as described in *B*.

p110 Interacts with the N-Terminal Region of p230. Previously, we isolated a *Drosophila* cDNA encoding the largest TFIID subunit (p230). The entire cDNA sequence contains an open reading frame encoding a polypeptide of 2068 amino acids, and the derived baculovirus-expressed protein was found to interact with TFIIDτ (25). Recently, a mutational analysis indicated that TFIIDτ interacts with the N-terminal region (residues 1–342) of p230 (T.K., unpublished observation). To determine which portion of p230 interacts with p110, an *in vitro* interaction study was done. Truncated versions of p230 were incubated with p110 *in vitro*, precipitated with anti-p110 antibody, and analyzed by immunoblot analysis. All truncated proteins were coimmunoprecipitated with p110, whereas a control analysis with p230 derivatives alone gave no precipitation with anti-p110 antibody (Fig. 5). Similar results were also obtained by *in vivo* interaction experiments using Sf9 cells (data not shown). Thus, we conclude that p110, as well as TFIIDτ, interacts with the N-terminal region (residues 1–352) of p230. These results suggest that p110 may communicate with TFIIDτ via p230 binding.

DISCUSSION

p110 Is a TFIID Subunit. In our previous study, we purified native TFIID from *Drosophila* embryos by immunoaffinity chromatography using anti-TFIIDτ antibody and identified nine tightly associated polypeptides (230, 110, 85, 62, 58, 42, 28, 22, and 21 kDa) as candidates for TFIID subunits (22). Some of these polypeptides, including p110, were also detected by Dynlacht *et al.* (23) in a similar type of analysis. Here we have isolated the cDNA encoding the TFIIDτ-associated 110-kDa polypeptide (p110). An immunologic approach using an antibody against the encoded cDNA product revealed that the corresponding endogenous protein cochromatographs with other TFIID subunits (TFIIDτ and p230) and with TFIID transcription activity. In addition, the antibody coimmunoprecipitates other TFIID subunits. Similarly, our preliminary studies have shown that antibody against other recombinant-TFIID subunits (p230 and TFIIDτ) also coimmunoprecipitates p110 (data not shown). From these results, we conclude that p110 is a TFIID subunit and that it is encoded by the cDNA we have isolated.

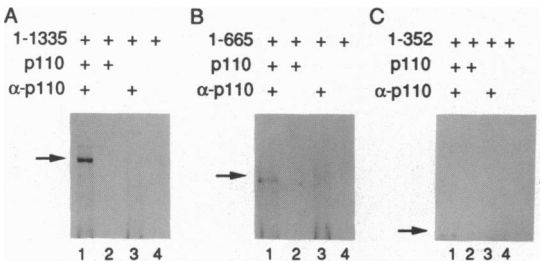

FIG. 5. p110 interacts with the N-terminal region of p230. C-terminal-truncated p230 mutants (residues 1–1335, *A*; residues 1–665, *B*; residues 1–352, *C*) were incubated with (lanes 1 and 2) and without (lanes 3 and 4) p110. Samples were incubated with protein A-Sepharose with (lanes 1 and 3) or without (lanes 2 and 4) anti-p110 antibody. Precipitated protein was detected by immunoblot by using anti-p230 antibody. The expected positions for truncated p230 mutants are indicated by arrows.

Overall Structure of p110. A search of both nucleotide (GenBank, European Molecular Biology Laboratory, EMBL-Update, and GBUpdate) and protein (SwissProt, Protein Identification Resource, GenPept, and GPUpdate) data bases failed to identify proteins related to p110, indicating that p110 is an additional type of transcription factor. While this manuscript was in preparation, Hoey *et al.* (24) also reported the cloning of a cDNA encoding the same protein and many of structural motifs described below.

On the basis of its deduced amino acid sequence, p110 can be divided into several subdomains, as shown in Fig. 1*C*. The N-terminal region contains three Ser/Thr-rich subdomains (residues 3–47, 135–232, and 270–293; 31%, 39%, and 50% Ser/Thr content, respectively) and two Gln-rich subdomains (residues 49–111 and 233–265, 43% and 39% glutamine content, respectively). These structural domains are also found in Sp1, a sequence-specific activator found in human (but not *Drosophila*). The N-terminal region of p110 (residues 3–293) shares low sequence similarity with Sp1 (24% identity and 38% similarity). The fact that this region is important not only for activation but also for multimerization (29) suggested that p110 and Sp1 might interact with each other through these regions, as recently demonstrated in Hoey *et al.* (24). Thus, p110 may be an adaptor between Sp1, or structurally related activators in *Drosophila*, and other general transcription initiation factors.

The central region of p110 (residues 370–668) is rich in proline (14%). The C-terminal domain (residues 381–499) of transcription factor CTF is also rich in proline (20%) and is needed for transcription activation (30). Although p110 has low sequence similarity with the CTF C-terminal domain (25% identity and 35% similarity), the functional relevance between the two factors is unclear.

The C-terminal region contains three subdomains: the acidic/basic domain (residues 672–863; 39% charged amino acids); the glycine/serine/threonine-rich domain (residues 866–882; 35% glycine, 47% serine/threonine); and the most C-terminal acidic/basic domain (residues 888–921; 35% charged amino acids). The glycine/serine/threonine-rich do-

main might impart a flexibility (as a result of the small side chains of these amino acids) to the charged domains located at both ends.

Possible Role for p110 in TFIID Functions. Previously we (25) and others (35) cloned the largest subunit of TFIID (p230) and determined that p230 is a homologue of the protein encoded by a human gene (CCG1; refs. 31–34) implicated in cell-cycle progression through the late G_1 stage. A particularly interesting property of recombinant p230 is that it inhibits the TATA box-binding activity of TFIIDτ (25). Because native TFIID can bind to the promoter, the negative regulation by p230 must be counteracted by another subunit(s) of TFIID. In the present study, we have determined that p110 binds to an N-terminal region (between residues 1–352) of p230. Although p230 contains various structural motifs, including a high-mobility group box and 120-residue bromodomain-containing direct repeats (25, 32, 33), the N-terminal 352-amino acid portion does not contain any obvious known motif. Importantly, TFIIDτ also interacts with this same region of p230 (T.K., unpublished observation). Thus, the binding of p110 to p230 may counteract the inhibitory interaction between p230 and TFIIDτ, and this could be a point for control by other activators or repressors. It also is possible that while p110 by itself does not interact detectably with TFIIDτ, it might do so when bound to p230.

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