The general transcription factor IIA (TFIIA) binds to the TATA binding protein (TBP) and mediates transcriptional activation by distinct classes of activators. To elucidate the function of TFIIA in transcriptional activation, point mutants were created in the human TFIIA-γ subunit at positions conserved with the yeast homologue. We have identified a class of TFIIA mutants that stimulate TBP-DNA binding (T-A complex) but fail to support transcriptional activation by several different activators, suggesting that these mutants are defective in their ability to facilitate an activation step subsequent to TBP promoter binding. Point mutations of the hydrophobic core of conserved residues from 65 to 74 resulted in various activation-defective phenotypes. These residues were found to be important for TFIIA γ-γ interactions, suggesting that γ-γ interactions are critical for TFIIA function as a coactivator. A subset of these TFIIA-γ mutations disrupted transcriptional activation by all activators tested, except for the Epstein-Barr virus-encoded Zta protein. The γY65F, γW72A, and γW72F mutants mediate Zta activation, but not GAL4-AH, AP-1, GAL4-CTF, or GAL4-VP16 activation. The γW72A mutant failed to stimulate TFIIID-DNA binding (D-A complex) but was able to form a complex with TFIID and DNA in the presence of Zta (Z-D-A complex). Thus, the ability of Zta to activate transcription with γW72A appears to result from a unique ability to form the stable Z-D-A complex with this mutant. Our results show that different activators utilize the general factor TFIIA in unique ways and that TFIIA contributes transcription activation functions in addition to the facilitation of TBP-DNA binding.

Eukaryotic transcriptional activators stimulate the assembly of general transcription factors into a stable preinitiation complex at the start site of class II promoters (1, 2). The binding of the general transcription factor IID (TFIID) to the TATA box nucleates the formation of the preinitiation complex (3, 4). TFIID consists of TATA binding protein (TBP) and TBP-associated factors (TAFs), which are essential for the reconstitution of activated transcription in vitro (5–8). Both TBP and TAFs have been shown to interact directly with different classes of transcriptional activation domains (9–16). Several experimental approaches indicate that binding of TBP to the promoter is rate-limiting in vivo and that activators that interact with TBP stimulate this step in preinitiation complex formation (17–22).

TBP binding to the TATA box can be stimulated and stabilized by the general transcription factor IID (TFIID) (3, 23, 24). TFIIA has only a modest effect on basal transcription reconstituted with TBP and a minimal set of general transcription factors (25). In contrast, activator-regulated transcription reconstituted with TFIIID (TBP plus TAFs) is strongly dependent on TFIIA (23, 26–29), suggesting that a functional interaction exists between TFIIA, activators, and the TAFs. The assembly of a TFIIA-TFIIID promoter complex has been shown to be rate-limiting in in vitro transcription reactions, and the acidic activator GAL4-AH was able to stimulate this rate-limiting step (30). The Epstein-Barr virus-encoded lytic activator, Zta, has also been shown to stimulate the formation of a TFIIA-TFIIID promoter complex that is dependent on the TAFs and the activation domain of Zta (31, 32). Thus, it is likely that some activators can stimulate TBP binding by enhancing an interaction between TFIIID and TFIIA.

How TFIIA mediates activator function is not entirely clear. TFIIA may function by directly stimulating TBP binding to the TATA box (3, 24, 23). In addition, TFIIA has been shown to compete with repressors for binding to TBP. Thus, TFIIA has been implicated as a corepressor that prevents nonproductive preinitiation complex formation (23, 34–39). TFIIA induces a conformational change in TBP (33) and interacts with the repeat of lysine residues on helix H2 of yeast TBP (30). This same basic region has been implicated in the TBP interaction with the adenovirus E1A transactivator (10). Mutations in this region of TBP were also defective for transcriptional activation by acidic activators and were reduced for their ability to bind TAF1 (5–8). Both the human and Drosophila TFIIA subunits share sequence similarity to the amino and carboxyl-terminal ends of TOA1 (αβ), with molecular weights of 35, 19, and 12 kDa, respectively (45–47). Both the human and Drosophila 35-kDa (α) and 19-kDa (β) TFIIA subunits share sequence similarity to the nonconserved spacer domain of TOA1 (αβ) is dispensable for yeast cell viability (48). Yeast deleted of TFIIA have normal RNA polymerase (pol) I and III activity, yet have reduced pol II transcription in vivo (48), indicating that TFIIA is primarily a...
pol II transcription factor.

Isolation of the cDNA encoding the human and Drosophila 12-kDa (γ) TFIIA subunit, a protein 58% homologous to TOA2 (γ), allowed the TFIIA activities to be reconstituted from recombinant components (27–29, 49, 50). Using TFIIA-depleted extracts, both basal and activated transcription in vitro required the addition of recombinant TFIIA (rTFIIA) (27, 50). Transfection of both human TFIIA cDNAs in HeLa cells stimulates activator-dependent transcription 4-fold (28). TFIIA is also required to mediate activator-dependent transcription in highly purified transcription systems reconstituted with immunopurified or holo-TFIIA (hTFIIA) (27, 28). Yeast TFIIA can substitute for human TFIIA in this system, indicating that TFIIA function is conserved in eukaryotes (27). TFIIA highly purified transcription systems reconstituted with immunopurified or holo-TFIIA (hTFIIA) (27, 28). Yeast TFIIA can substitute for human TFIIA in this system, indicating that TFIIA function is conserved in eukaryotes (27). TFIIA highly purified transcription systems reconstituted with immunopurified or holo-TFIIA (hTFIIA) (27, 28). Yeast TFIIA can substitute for human TFIIA in this system, indicating that TFIIA function is conserved in eukaryotes (27). TFIIA highly purified transcription systems reconstituted with immunopurified or holo-TFIIA (hTFIIA) (27, 28). Yeast TFIIA can substitute for human TFIIA in this system, indicating that TFIIA function is conserved in eukaryotes (27).

These mutants for TFIIA functions in transcription activation were formed as described previously (27), except for the γ73A and γ76A mutants that had only (5′) sense, and the γK101A and γK102A mutants that had only (3′) sense (51). All TFIIA mutations were confirmed by DNA sequencing in both orientations using an ABI automated 373A DNA sequencer. The resulting prSET A-IIA-γ WT or mutant constructs were expressed either in E. coli or in vitro in rabbit reticulocyte lysates (Promega) as indicated. Wild type TFIIA-γ expressed from either pQE-9 or prSET A showed no functional differences despite having different amino-terminal tags (data not shown). To create pGST-IIA-α, the TFIIA-γ fragment (codons 1–251 from the human αb gene) was derived from pQE-IIA-α (27). pQE-IIA-α was digested initially with HindIII, with a subsequent Klenow fill reaction, and followed by digestion with BamHI to isolate the α subunit fragment. The GST vector, pGEX-2T (Pharmacia Biotech Inc.), was digested initially with EcoRI, with a subsequent Klenow fill reaction, and followed by digestion with BamHI. The TFIIA-α fragment was then directionally cloned into the digested pGEX-2T vector to create pGST-IIA-α. The pGST-Zta, pGST-IIA-γ, and pGST-TBP constructs were described (27).

Protein Preparations—The pQE-18/αβ and prSET A-IIA-γ WT or mutant constructs were expressed in M15 and BL21 E. coli strains, respectively. Expressed proteins were purified under denaturing conditions on Ni-NTA agarose columns (Qiagen). The TFIIA-α protein was isolated by column fractionation with elution denaturant (8 mM urea, 0.1 M NaHPO4, 0.01 M Tris, pH 8.0, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) of decreasing pH. The purified γ mutants were renatured with equal molar amounts of WT human αβ subunit. Dialysis of the γ mutants into D100 buffer (20 mM Hepes, pH 7.9 (KOH), 20% glycerol, 0.2 mM EDTA Na2, 100 mM KCI, 7 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride) was performed as described previously (27), except for the γE109A mutant, which required a gradual multistep reduction of urea and salt concentration to allow proper renaturation. Generally, the stepwise dialysis protocol yielded 50% higher soluble concentrations of the indicated protein. TFIIA compared with Ref. 27 but did not improve the insolubility of the γD141A and γK42A mutants. TFIIA polypeptides were used at a final concentration of 0.2 μM for transcription reactions. TBP and Zta protein was prepared as described (32). GAL4 fusion proteins were purified as described (54, 55).

DNA Binding Reactions—Polyacrylamide EMSA conditions for T-A complex formation were similar to those described (56). Identical polyacrylamide EMSA results were obtained for T-A complex formation at either 22 or 30 °C for 30 min. 34 nm of human recombinant TBP was incubated with 16 nm of either WT αβ γ or WT αβ γ γ mutation to form the T-A complex. For M-g-agarose EMSA analysis of Zta-H11D-IIA complex formation, 0.2 units of TBP, 100 pmol of WT or mutant TFIIA (data not shown). Quantitation of the transcription signal was performed on a PhosphorImager 445 SI (Molecular Dynamics) and visualized by autoradiography on X-Omat-AR film (Kodak).
Distinct Activation Functions of Human TFIIA

Subsequently, equal molar amounts of WT αβ and a given γ mutant were renatured together. Soluble protein concentrations were determined by the Bradford assay and Coomassie staining of SDS-polyacrylamide gels. Purified and renatured TFIIA proteins were estimated to be approximately 70% pure. The concentration of soluble TFIIA-γ subunits was further verified by Western blot analysis of linearly titrated preparations. Fig. 1B confirms that equal amounts of TFIIA-γ subunits were present in the normalized panel of TFIIA mutants. The mutants listed in Fig. 1A had similar solubility, with the exception of γD41A and γK42A (data not shown). These two mutants were later revealed to fail to interact with αβ (see Fig. 2B), consistent with the observation that γ does not solublize in the absence of the αβ subunit (data not shown, Ref. 49).

The TFIIA-γ mutants were assayed for their ability to stimulate human recombinant TBP binding to a TATA box containing oligonucleotide in polyacrylamide gel assay EMSA (Fig. 2A). In the absence of TFIIA, TBP binds weakly to the TATA box probe (T complex; Fig. 2A, lane 1). The addition of WT rTFIIA to TBP resulted in the formation of a stable TBP-TFIIA DNA complex (T-A complex; Fig. 2A, lane 2). With the E1B TATA oligonucleotide probe, we found that human T-A complex had a faster migration than the T complex, suggesting that TFIIA may alter the conformation of the TBP-DNA complex (33). The addition of the various TFIIA-γ mutants to TBP produced varying amounts of T-A complex formation. The TFIIA-γY65A mutant did not form the T-A complex at all, while the γY3A, γD20A, γT64A, γW72A, and γD91A mutants formed weak T-A complexes (less than 35% of WT) (Fig. 2A). The γW72A mutant was unique in its ability to form an altered mobility T-A complex in polyacrylamide-EMSA gels, which was only evident on longer exposures (see below, Fig. 5A).

Mutations of the γ Subunit Affect Protein-Protein Interactions—GST fusion proteins have been previously used to demonstrate that the γ subunit homodimerized, heterodimerized with the αβ subunit, and bound Zta in an activation domain-specific manner (27). It was also reported that the αβ subunit interaction with γ was localized to the α subunit (28), which was confirmed in this study using the GST assay (Fig. 2B, top panel). 35S-labeled γ point mutants were assayed for their ability to bind to GST, GST-γ, or GST-α using similar conditions. The summary of several independent binding assays is presented at the bottom of Fig. 2B. Interactions that were significantly reduced (at least 25% of WT for α, and at least 50% of WT for γ) are indicated by a minus symbol (Fig. 2B).

Interestingly, a pattern formed from these GST experiments, indicating that certain conserved domains of γ are critical for specific protein interactions. Interactions with GST-α were most sensitive to mutations in γ residues Asp41 and Lys42, which bound at less than 10% of WT levels. The failure of γD41A and γK42A to bind α probably explains the poor solubility of these mutants upon renaturation with recombinant αβ. Homotypic interactions with GST-γ were most severely affected by mutations of a pocket of hydrophobic amino acids at positions Tyr65, Phe67, Cys68, Thr72, and Phe74 (Fig. 2B). Interestingly, we did not find a single point mutation in γ that significantly reduced Zta binding (data not shown). Thus, Zta may interact with multiple domains of γ.

We have previously demonstrated a direct protein-protein interaction between 35S-labeled γ and a GST-TBP fusion protein (27) (Fig. 2C, right panel). Since the TFIIA-γD20A, γT64A, γY65A, γW72A, and γD91A mutants all formed weak T-A complexes, these 35S-labeled γ mutants were tested for their ability to bind directly to GST-TBP. Both the γD20A and γY65A mutants were reduced in GST-TBP binding, 61 and 42% of WT levels, respectively (Fig. 2C, left panel). However, the γT64A,
TFIIA Mutants Show Activator-specific Defects—TFIIA-depleted HeLa cell extracts require rTFIIA to mediate transcriptional activation for all activation domains tested (27). The TFIIA-γ mutants were assayed for the ability to mediate transcription stimulated by several distinct types of activation domains using TFIIA-depleted HeLa extracts in an in vitro transcription assay. The GAL4 DNA binding domain was fused to distinct classes of activation domains: the synthetic acidic-rich activation domain (GAL4-AH), the herpes simplex virus-encoded VP16 activation domain (GAL4-VP16), or the proline-rich activation domain of the CCAAT-binding factor (GAL4-CTF). These GAL4-activator fusions were examined for their ability to stimulate transcription from the G5E1BTCAT promoter (five GAL4 binding sites upstream of the adenovirus E1B TATA element). The Epstein-Barr virus-encoded Zta activator was examined for its ability to stimulate transcription from the Z7E4TCAT promoter (seven ZRE binding sites upstream of the adenovirus E4 TATA element). Endogenous levels of AP-1 dependent transcription activation were tested by examining the activity of the WT human collagenase promoter. The G5E1BTCAT, Z7E4TCAT, and collagenase basal promoters have barely detectable basal levels of transcription in our in vitro transcription system in the absence of added activator or activator binding site (27).

For these activators, transcription was reduced to less than 5% in extracts depleted of endogenous TFIIA (27). Activated transcription could be restored to undepleted levels by the addition of 0.2 μM WT rTFIIA (27). To determine if the TFIIA-γ-containing mutants (WT αβ + γ mutant) coactivated transcription maximally at 0.2 μM as did WT rTFIIA in our depleted system, TFIIA-γ mutants were titrated using the Zta and GAL4-AH activators. We found that, for the panel of TFIIA-γ mutants, 0.2 μM also yielded maximal transcriptional activation (data not shown). For each activator tested in Fig. 3, the addition of 0.2 μM WT rTFIIA to TFIIA-depleted extracts was considered a 100% level of transcription, while TFIIA-depleted extracts were considered to be a 0% level of transcription. The levels of activated transcription obtained for a particular TFIIA-γ mutant and activation domain were plotted as percentage of the WT TFIIA signal (% WT) (Fig. 3).

Only one point mutation of TFIIA-γ was completely defective for all activators tested. γY65A, which failed to form the T-A complex (Fig. 2A) and had reduced binding to GST-TBP (Fig. 2C), did not mediate transcription for any activator tested, suggesting that T-A formation is required for transcription activation (Fig. 3A). However, the γY6A and γY67A mutants formed T-A complexes at near WT levels but mediated transcription at just 25% or lower values relative to WT for all activators (Fig. 3B). This indicates that T-A formation alone is not sufficient for activator function. Most significantly, the γY72A mutant supported activation only for Zta, mediating about 18-fold higher levels of Zta activity compared with GAL4-AH (Fig. 3C). The γY3A and γY68A mutants had similar transcription phenotypes to the γY72A mutant, although not as dramatic, with at least 2-fold greater activity for Zta compared with the other activators tested (Fig. 3C). In contrast, the γY91A and γY109A mutants revealed an opposite phenotype, mediating about 2-fold and 3–4-fold, respectively, higher levels of transcription activation for all the activators compared with Zta (Fig. 3, A and C). These results show that mutations in TFIIA-γ can affect some activators more dramatically than others, implying that different activators utilize TFIIA in distinct activation pathways.

Zta Overcomes TFIIA-γ Mutations That Disable Other Activators—TFIIA-γY65A and γY72A were defective for T-A formation and reduced for transcriptional activation by all activators. While γY65A was completely defective for all activators,
Fig. 3. Mutations of TFIIA-γ show activator-specific defects in reconstituted transcription reactions. A, the levels of activated transcription mediated by the TFIIA-γ mutants. Purified activator proteins GAL4-AH, GAL4-CTF, or Zta were incubated with the indicated TFIIA mutant and TFIIA-depleted HeLa cell nuclear extracts in in vitro transcription reactions. The activators used are indicated above each panel. Primer extension reaction products were quantitated by PhosphorImager analysis and graphed relative to the WT TFIIA signal (100%) and the TFIIA-depleted extract signal (0%). Wild type or mutant TFIIA was added to a final concentration of 0.2 μM. The mean and positive standard deviation are graphed for the TFIIA-γ mutants. At least three independent experiments were performed for each mutant and activator shown. B,
Conservative substitutions of γY65 and γW72 uncouple T-A formation from transcription activation. A, an immunoblot of equal amounts (200 ng) of TFIIA-WT, γY65F, and γW72F proteins was probed with rabbit TFIIA-γ Abs. B, T-A complex formation of γY65F and γW72F proteins. Human TBP was incubated either alone (lane 2) or with WT TFIIA (lane 3), TFIIA-γY65A (lane 4), TFIIA-γY65F (lane 5), TFIIA-γW72A (lane 6), or TFIIA-γW72F (lane 7). C, in vitro transcription in TFIIA-depleted nuclear extracts with the γY65F or γW72F mutants. For the in vitro transcription reactions, Zta (lanes 1-4), GAL4-AH (lanes 5-8), GAL4-CTF (lanes 9-12), or GAL4-VP16 (lanes 13-18) activator was added to TFIIA-depleted extracts. 0.2 μM WT TFIIA (lanes 1, 5, 9, and 13), no TFIIA (-) (lanes 2, 6, 10, and 14), TFIIA-γW72F (lanes 3, 7, 11, and 16), TFIIA-γY65F (lanes 4, 8, 12, and 18), TFIIA-γW72A (lane 15), or TFIIA-γY65A (lane 17) mutants were added to TFIIA-depleted reactions. Primer extension reaction products were quantitated by PhosphorImager analysis and are shown below each lane as a percentage of the WT TFIIA signal.

γW72A mediated just Zta activation but only to 30% of WT. To further investigate the mechanistic basis of these defects, two additional TFIIA-γ mutants were engineered to more conservative phenylalanine substitutions at the γY65 and γW72 residues. The γY65F and γW72F mutants were purified and solubilized as described for the other mutants. The γY65F and γW72F mutants solubilized as well as WT TFIIA (Fig. 4A) and stimulated formation of the T-A complex as well as WT TFIIA (Fig. 4B). However, when the γY65F and γW72F mutants were assayed in transcription using TFIIA-depleted extracts, only Zta was significantly coactivated by both of these mutants (Fig. 4C). The γY65F mutant coactivated Zta at 70% of WT levels, 6.5-fold better than GAL4-AH and 14-fold better than GAL4-CTF (Fig. 4C). Additionally, the γW72F mutant only coactivated Zta and not the other activation domains, at levels similar to γW72A (Fig. 4C). Since Zta is an Epstein-Barr virus-encoded viral activator, we decided to test whether another commonly studied viral activation domain, herpes simplex VP16, would show a similar phenotype to Zta with these mutants. Interestingly, like Zta, GAL4-VP16 can also form a stable magnesium-agarose EMSA complex with TFIIID and TFIIA (60). However, GAL4-VP16 failed to stimulate transcription with any of the γY65 and γW72 mutants, thus behaving markedly different from Zta (Fig. 4C). GAL4-VP16 did stimulate transcription with γY6A and γF74A to levels similar to that observed with GAL4-AH (data not shown). These results demonstrate that the transcriptional defect of the γY65F, γW72A, and γW72F mutants is not a result of the failure to form the T-A complex and further support our earlier observation that Zta can uniquely overcome TFIIA deficiencies.

Z-D-A Formation Compensates for the γW72A Defect—To investigate the mechanistic basis for the Zta-specific activation
process. Complexes were formed at either 22°C for 15 min. The structural basis of this shift in acrylamide gel EMSA (Fig. 5B, lanes 1–4) or 30°C for 60 min (bottom panel). The shifted D-A or Z-D-A complexes were quantitated by PhosphorImager analysis and are graphed below each lane as % of the WT signal.

Fig. 5. Zta overcomes the γW72A defect by stimulating a TFIIA-TFIIID promoter complex. A, γW72A forms an aberrant T-A shift in acrylamide gel EMSA. Longer exposures of EMSA shown in Fig. 2A revealed that γW72A forms a unique, slower mobility complex indicated by an asterisk. B, wild type TFIIA and γW72A were compared under increasingly favorable conditions for D-A and Z-D-A complex formation. Wild type TFIIA or γW72A was added at either 1 × (50 nM) (lanes 5–8) or 2 × (100 nM) (lanes 9–12) concentrations to stimulate hIID (0.2 units) (lanes 3–12) and Zta (16 nM) (lanes 2, 4, 6, 8, 10, and 12) complex formation. Complexes were formed at either 22°C for 15 min (top panel) or 30°C for 60 min (bottom panel). The shifted D-A or Z-D-A complexes were quantitated by PhosphorImager analysis and are graphed below each lane as % of the WT signal.

Discussion

Previous reports identified TFIIA as a critical component in the rate-limiting steps of activated transcription (30–32, 61). To better elucidate the function of TFIIA in this process, we sought to isolate TFIIA mutants that distinguish the transcription function of several different activation domains. We hypothesized that different TFIIA surfaces would be required to mediate the activation of different activators. Since a previous report determined that nearly all in frame deletions of yeast TFIIA-γ were nonviable (48), it appeared that subtle alterations of TFIIA would be more likely to yield activator-specific transcriptional defects. To avoid gross alterations of TFIIA structure, alanines were substituted in single conserved residues of TFIIA-γ in the first panel of mutants. Analysis of these TFIIA mutant proteins in DNA binding and in vitro transcription assays revealed that TFIIA interacts with different activators in distinct ways and mediates at least two mechanically distinct activator functions.

TFIIA Mutants Reveal Differences Among Transcriptional Activator Mechanisms—Our series of TFIIA mutations reveal differences in the requirements for TFIIA by distinct transcriptional activation domains. TFIIA-γ mutants Y3A, C68A, W72A, and F74A were all significantly reduced in mediating transcription for all activators compared with Zta (Fig. 3C). In contrast, E109A was reduced for Zta relative to all other activators (Fig. 3C). These results suggest that Zta interacts with TFIIA-γ in a manner distinct from the other activators tested. Zta has been shown to stimulate the TFIIA-TFIIID promoter complex, while other activators GAL-4-AH and AP-1 (Jun/Fos) fail to stimulate this complex. GAL-4-VP16 does stimulate D-A formation, but to a lesser extent than Zta (60). In contrast to Zta, GAL-4-VP16 did not activate transcription with γW72A, γW72F, or γY65F (Fig. 4C), suggesting that Zta and VP16 interact with TFIIA in mechanistically distinct ways. Zta binds directly to both γ (27) and φ8 (52), which may explain how Zta uniquely compensates for the γW72A transcriptional defect.

Transcriptional analysis of the γY6A and γF74A mutants also suggests that the activation mechanism of GAL4-CTF may
be distinct from the other activators tested. While γY6A and γF74A were reduced for most activators relative to WT (the exception being that γF74A mediates Zta activation), the effects on GAL4-CTF activity were the most severe (Fig. 3C). γY6A stimulates T-A formation at close to WT levels but is substantially reduced for the formation of Z-D-A complex (data not shown). One likely explanation for these observations is that γY6A, and possibly γF74A, fail to interact with a subset of TAFs important for D-A formation and that these TAFs are specifically important for GAL4-CTF-mediated transcription.

Other reports have found mutations of yeast TBP that specifically disrupt activated but not basal transcription in vitro (62). At least one of these mutations was likely to interfere with TFIIA binding. A more recent transfection analysis of human TBP mutations revealed that different activators had different sensitivity to TBP mutations, suggesting they interact with TBP in distinct ways (41). While several activation domains responded differently to various TBP mutants, most of the transcriptional defects could be correlated with the loss of binding to TAF250 (41). Similarly, mutagenesis of TFIIB identified a region of TFIIB that disrupted activated transcription for two activators but not basal transcription (63). In contrast, a mutagenesis analysis of the large subunit of TFIIIE did not distinguish basal from activated transcription nor differences between two types of activation domains (64). Thus, the extent to which mutations of general transcription factors affect different activators may reflect important differences in activator mechanisms and general transcription factor functions.

TFIIA Functions in Two Mechanistically Distinct Activation Steps—TFIIA stimulates the binding of TBP to DNA, and this has been considered a primary function of TFIIA in transcription activation. However, we have identified mutations of TFIIA-γ that uncouple the stimulation of TBP-DNA binding from the ability to mediate activator function for several distinct activators (Figs. 2 and 3). The γY6A, γF67A, γC68A, and γW72A mutants all stimulate T-A complex formation significantly better than they mediate transcription activity for GAL4-4AH and AP-1, relative to WT TFIIA (Figs. 2 and 3). The lack of correlation between T-A formation and transcriptional activation was most remarkable for the more conservative substitution mutants γY65F and γW72F (Fig. 4). Both of these mutants stimulate T-A formation as well as WT but failed to support transcriptional activation by GAL4-4AH, GAL4-VP16, and GAL4-CTF (Fig. 4C). The failure of these mutants to mediate transcriptional activation indicates that TFIIA contributes activation functions distinct from the stimulation of TBP-DNA binding.

Several additional activities have been ascribed to TFIIA besides stimulation of TBP-DNA binding. TFIIA induces a conformational change in TBP (33), and it is conceivable that γY65F and γW72F fail to induce a TBP conformational change necessary for transcriptional activation by GAL4-4AH, CTF, and VP16. The Drosophila αβ subunit has been shown to bind to dTAF250 (41), and mutations in TFIIA-γ may disrupt these interactions, which are critical for transcriptional activation. The requirement of TFIIA and TAFs in promoter selectivity also supports the model that TFIIA functionally interacts with TAFs (65). Alternatively, coactivators, like PC4 and HMG2, have been shown to interact with D-A complex formation (61, 66), and TFIIA-γ/W72F and γY65F may fail to interact with these coactivators in the preinitiation complex. TFIIA can also disrupt TBP-specific repressors, like DR1 (36), and although γW72F and γY65F form the T-A complex, they may fail to disrupt specific repressor-TBP interactions. TFIIA copurifies with a repressor activity specific for TBP and consensus TATA elements, and our mutations may affect the specificity of this repressor activity (67). Additionally, TFIIA makes direct contact with at least two transcriptional activators (27, 52), and it is possible that some of these TFIIA mutations have lost the ability to directly contact specific activators or coactivators necessary for transcription function. While we have not determined which of these possible TFIIA interactions have been disrupted by these mutations, our data strongly suggest that TFIIA interactions subsequent to T-A formation are essential for transcription activation.

The γ Subunit Interacts with the α and γ Subunits in Distinct Domains—Analysis of GST interaction assays, DNA binding studies and in vitro transcription reactions allow different features of TFIIA to be assigned to distinct subdomains of γ. Based upon the GST experiments in Fig. 2, the γ subunit Phe40, Asp41, and Lys12 conserved pocket was shown to be absolutely critical for α subunit interactions. These same residues were important for solubility of γ, indicating that solubility is largely dependent on the formation of α-γ heterodimers. The γ residues Tyr65, Phe67, Cys68, Trp72, Thr73, and Phe74 appear to be important for homotypic interactions. While the significance of γ-γ interactions is not clear, we (data not shown) and others (43) have found that TFIIA has a native molecular weight consistent with TFIIA being a dimer of both subunits. Thus, γ-γ interactions are likely to be important for the oligomerization state of TFIIA. The same amino acid residues important for γ-γ interactions are also important for transcriptional activation function and Z-D-A formation. We have analyzed TFIIA-γY65F and γW72A by gel filtration and found that their molecular mass was indistinguishable from that of WT TFIIA (data not shown). Thus, loss of oligomerization cannot account for the transcriptional defects of TFIIA-γY65F and γW72A. Nevertheless, we speculate that γ-γ interactions are critical for the TFIIA conformation that is necessary for activation functions.

A general model has emerged that suggests that TBPP binding to DNA is a rate-limiting step affected by several classes of transcriptional activators (19–22). TFIIA can stimulate the binding of TBP to DNA, and activators that stimulate TFIIA binding are predicted to enhance transcription (24, 32, 51, 60). The analysis of TFIIA-γ mutations presented in this study suggests that TFIIA not only enhances TBP-DNA binding but qualitatively changes the preinitiation complex. Our data suggest that TFIIA affects the recruitment of TAFs and/or coactivators into a transcriptionally active conformation. Our analysis also indicates that activators function by distinct mechanisms and that TFIIA plays a central role in distinguishing the mechanism of different activators.

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