Transcription factor (TF) II A performs two important regulatory functions during RNA polymerase II transcription: it is required for efficient binding of TFII D to a core promoter and it mediates the effects of upstream activators, both through direct interaction with the TATA box binding protein (TBP). To begin studying how TFII A mediates these effects, we used a highly sensitive protease footprinting methodology to identify surfaces of human TFII A participating in TFII A-TBP-TATA ternary complex formation. Chymotrypsin and proteinase K cleavage patterns of TFII A bearing a [32P]-end-labeled γ subunit revealed that amino acids 59–73 were protected from cleavage both in the context of an immobilized ternary complex and in a binary complex with TBP alone. In contrast, amino acids 341–367 in the β portion of a [32P]-labeled α-β subunit were protected in the ternary but not in the binary complex, implying that those residues interact with promoter DNA. The regions of human TFII A identified by protease footprinting are homologous to and encompass the yeast TFII A residues that contact TBP and DNA in the recently solved crystal structure of the yeast ternary complex. The conservation of the regions and residues mediating complex formation implies that yeast and human TFII A employ the same mechanism to stabilize the binding of TFII D to a core promoter.

Transcription of protein-encoding genes by RNA polymerase II (pol II) \(^1\) is regulated by an intricate array of protein-protein and protein-DNA interactions \((1–4)\). Understanding how these interactions mediate formation of a transcription complex over a core promoter is a central problem in the field of gene expression. In the step-wise model, transcription complex assembly is nucleated by binding of the general initiation factors (Transcription Factor) TFII D and TFII A to the TATA box generating the “DA complex” \((5)\). The parallel between the ability of gene activators to facilitate DA complex formation and to activate transcription suggests that the complex plays a key role in regulation \((6)\).

TFII D is a multisubunit complex consisting of TATA box binding protein (TBP) and eight or more TBP-associated factors (TAFs) \((7–10)\). TBP alone, when it is substituted for TFII D, can nucleate the formation of a basal transcription complex that is fully functional but not responsive to activators. In contrast, TAFs are thought to act as co-activators because they are dispensable for basal transcription but are required to obtain activator-responsive transcription \(\textit{in vitro}\). In addition to TAFs, a fraction called upstream-factor stimulatory activity (USA), which contains both positive and negative co-activators, potentiates transcriptional activation \((11)\).

In the DA complex, TFII A functions both to stabilize the relatively weak binding between TFII D and the TATA box and is necessary for activator recruitment of TFII D \((12–14)\); its role in recruitment depends on the TAFs since activators have no effect on TBP-TFII A complex formation. In the step-wise model, the formation of the transcription complex is completed by the successive association of TFII B, RNA polymerase/TFI IF, TFII E, and TFII H \((2, 4)\). An alternative model for complex formation involves a holoenzyme \((15)\) containing RNA polymerase II and many of the other general initiation factors. The holoenzyme was discovered initially in yeast and more recently in mammalian cells. Both the yeast and some mammalian versions have been reported to lack TFII D and TFII A \((16–18)\). One possible function of the DA subcomplex is to form an activator-responsive platform for recruitment of the holoenzyme.

Human (and \textit{Drosophila}) TFII A is a multisubunit protein consisting of three subunits called α (LN), β (LC), and γ (S) \((19–25)\). α and β are synthesized as a precursor that is processed proteolytically to generate the mature subunits although the unprocessed form is functional \(\textit{in vitro}\). Yeast TFII A consists of only two subunits, TOA1 and TOA2 \((26, 27)\). TOA1 is homologous at its amino and carboxyl termini to regions of the human α and β subunits, respectively, and TOA2 is homologous to the human γ subunit \((19–22)\). Analysis of systematic internal deletion mutants of both subunits demonstrated that all of the regions conserved between yeast and human TFII A were required for yeast viability \((28)\). For TOA1 (α and β), these deletion mutants defined the amino- and carboxyl-terminal ends as essential but the nonconserved, middle region as dispensable for viability. A deletion mutant removing residues 217–240 abolishes binding to TBP. For TOA2 (γ), all of the internal deletions but one resulted in inviable yeast, making it difficult to genetically define functional domains. Alanine scanning mutants of both subunits were screened for temperature-sensitive (ts) growth phenotypes. In TOA1, all the ts mutations were in basic residues between residues 253 and 259. These mutants bound normally to TBP but could not form complexes, implying a decreased ability to bind DNA. Among the three ts mutants identified in TOA2, one double mutant, D73A/D74A, bound less tightly to TBP although complex formation was unaffected, and the other two exhibited no detectable difference
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in their ability to bind TBP or form ternary complexes. Human TFIIAγ has also been analyzed by alanine substitution mutations and Y65A (equals Y69 in yeast TOA2) caused the greatest decrease in the ability of IIA to stabilize TBP binding (29).

The crystal structure of the ternary complex containing the yeast (γ)CYC1 TATA box, γTBP, and γTFIIA was recently published (30, 31). In the crystal structure, γTBP, which encodes two 80-amino acid direct repeats, folds into a pseudosymmetric saddle-like shape containing a hydrophobic concave surface composed of a 10-strand antiparallel β-sheet, which interacts with the minor groove of the DNA. TBP binding distorts the TATA box by inserting phenylalanine residues between the first and final base pairs of the recognition site, resulting in bending of the DNA by 80° and broadening of the minor groove (32, 33). γTFIIA, on the other hand, is composed of a four-helix bundle and a 12-strand β-barrel (30, 31). The β-barrel is made of six strands each from the carboxyl terminus of TOA1 (TOA1C—human TFIIAβ) and the carboxyl terminus of TOA2 (γ). The four-helix bundle is composed of the amino terminus of TOA1 (TOA1N—human TFIIAα) and the amino terminus of TOA2. The interaction between TFIIA and TBP places the β-sheets of TOA2 and TBP in close proximity, resulting in a continuous 16-strand β-sheet. TFIIA interacts with DNA through basic residues in TOA1C that lie within a loop connecting two β-strands.

DNA-protein cross-linking studies have confirmed certain aspects of the structure in solution (34, 35). Collectively, these studies show cross-linking was observed between DNA and a α upstream of the TATA box, between β and both sides of the TATA box, and with γ at one position upstream of the TATA. In the crystal structure of the yeast complex, only a region homologous to part of the β subunit contacts DNA.

To study how TFIIA stabilizes TFIIID/TBP binding, we investigated the assembly of the ternary complex containing a mammalian TATA box, human TBP, and human TFIIA. Presently, there is no genetic analysis of the α-β subunit of human TFIIA or crystal structure of free TFIIA. To identify the interactions mediating complex formation, the differences between free TFIIA and complexed TFIIA were characterized using protease footprinting. The interactions of TFIIA with TBP and DNA were identified along with the accessible regions of TFIIA. The results of our protease footprinting studies are placed within the context of the recently solved x-ray structure of the yeast ternary complex.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—To engineer vectors containing both His and HMK tags at alternate termini, pET2a-N-HMK was constructed by cloning an oligonucleotide, 5′-TATGCCCACCCCGCAGTGTGGGATCC-3′, encoding the HMK phosphorylation site (HMK tag) between the Ndel and XhoI sites of pET2a (Novagen). pET11d-N-His-C-HMK was constructed by cloning an oligonucleotide, 5′-CTAGACCCACCAACACACCA-3′, encoding the His tag between the NheI and BamHI sites of pET11d-C-HMK (36). A BamHI fragment encoding TFIIA α-β and a BamHI-BglII fragment encoding TFIIA γ coding regions were synthesized by PCR and ligated into the BamHI site of pET11d-NheI-C-HMK and pET2a-N-HMK, respectively, using T4 ligase. pET11d-TFIIAαδ81 was made by digesting pET11d-TFIIAαδ81-His at the EcoRI site within the coding region, repairing with the Klenow fragment and deoxyxenoside triphosphates, and inserting a stop codon oligonucleotide, 5′-CTAATCTAGAGTG-3′, using T4 DNA ligase. pC-HMK-TFIIAαδ38, pC-HMK-TFIIAαδ129, and pC-HMK-TFIIAαδ254 were constructed by deleting the coding region between the NdeI and SacI (Δδ38), NdeI (Δδ129), and NdeI (Δδ254) sites within the coding regions, respectively, by digesting with these restriction enzymes, filling in with the Klenow fragment, and ligating using T4 DNA ligase. These constructs encode internal deletions between residues 3 and 38, 129 and 254, respectively.

**Protein Purification**—TFIIA and GST-TBP (The GST-TBP expression vector was a generous gift of Arnie Berk, UCLA) were purified as described (20, 37). TFIIA containing the HMK tag on the carboxyl terminus of the α-β subunit was further purified by affinity chromatography over a GST-TBP affinity resin (28). The TFIIA deletion constructs were transferred into BL21(DE3) and inoculated into 250 ml of Luria broth containing 50 μg/ml ampicillin. When the cell culture reached an A600 of 0.6, protein expression was induced for 1.5 h by the addition of isopropyl-β-D-galactopyranoside. After 0.5 h, the following steps were performed at 4 °C. The cells were collected by centrifugation in a Sorvall SA600 rotor at 5,000 rpm for 5 min, washed with Buffer A (20 mM HEPES, pH 7.9, 0.2 mM EDTA, 0.5 mM KCl), collected again by centrifugation at 5,000 rpm for 5 min, resuspended in 50 ml of Buffer A containing 0.5 mM DTT, 0.5 mM PMSF, 50 μg/ml benzonidamide, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A, and lysed by sonication. For TFIIAαδ81 and for TFIIAαδ38 and δ129, the membrane fraction was extracted with 30 ml of Buffer B (20 mM HEPES, pH 7.9, 0.2 mM EDTA, 20% glycerol) containing 0.2 mM KCl, 8 mM urea, 0.5 mM DTT, and 0.5 mM PMSF. The urea was removed by step-wise dialysis into Buffer B containing 0.3 mM KCl, 0.5 mM DTT, and 0.5 mM PMSF and by decreasing concentration of urea from 2 to 0.5 to 0 mM. For TFIIAαδ254, the lysate was precipitated by adding 0.20, 0.25, and 0.30 g/ml of (NH₄)₂SO₄, stirring for 30 min, and then collecting the precipitate by centrifugation at 10,000 rpm for 10 min. TFIIAαδ254 was in the 0.25 g/ml precipitate and was solubilized in 3 ml of Buffer D containing 0.3 mM KCl, 0.5 mM DTT, and 0.5 mM PMSE. Recombinant human TBP was a generous gift from Zong Juo and Richard Dickerson (UCLA) (38).

**Complex Formation and Proteolysis**—TFB-TFIIA-TATA box ternary complexes were formed in a 50-μl mixture containing 3 pmol of biotinylated adenovirus E4 DNA template, a synthetic bintynilated oligonucleotide (biotin-5′-GGATCCCGCTGAATATACCTCGTCG-3′) immobilized on streptavidin-conjugated M-280 magnetic beads (Dynal), with 500 ng of TBP, and 400 ng of αδ254-labeled TFIIA. After 30 min at 30 °C, the complexes were washed three times with 100 μl of binding buffer (12 mM HEPES, pH 7.9, 60 μM MgCl₂, 0.02 M NaCl, 2 mM Nonidet P-40, 0.5 mM DTT) and digested with 5–200 ng of either proteinase K (Promega) or chymotrypsin (Sigma) alongside free TFIIA or TFIIA incubated with immobilized DNA. TFIIA incubated with immobilized DNA was only washed once to maintain some of the protein in the presence of the resin for the control reaction. After 1.5 min, the cleavage reactions were terminated by addition of PMSF to 10 mM and freezing in dry ice. SDS-loading dye was added, and the cleavage products were fractionated on 15% total acrylamide-3.3% cross-linker or 16.3% total acrylamide/5.0% cross-linker tricine-SDS gels. Binary complexes were formed and footprinted as described (36). Protease sequencing ladders were generated by denaturing 120 ng of labeled TFIIA in 10 mM HEPES, pH 7.9, 10% glycerol, 50 mM KCl, 2 mM urea, 1 mM DTT, and 0.1% SDS at 5 °C and digesting at 37 °C with 3 μg of endoproteinase Lys-C (1–10 min), 1.5 μg of chymotrypsin (5–90 min), or 5 μg endoproteinase Glu-C (1–10 min) (Promega). The cleavage sites were assigned using a combination of the known amino acid sequence, prestatine molecular weight markers (Bio-Rad), and deletion markers. The positions of the digestion products in the experimental lanes were mapped using all of these markers and a semi-log plot of mobility based on these markers. The precision of the semi-log plot in the protected regions is ±2 residues. The gels were autoradiographed by exposure to XAR-5 film. The autoradiographs were scanned into Adobe Photoshop 3.0 using a ScanMaker III (Microtek), imported into Microsoft PowerPoint 4.0, and labeled and printed onto glossy paper on a Tektronix printer. For quantitation, either the autoradiographs were scanned with a laser densitometer or the gel was exposed to a Phosphorimage scanner, quantitated using ImageQuant software (Molecular Dynamics). Sample lanes in which the extent of digestion was similar, as judged by the percentage of full-length material remaining in the experimental and control lanes, were compared, and the ratio of these signals was used to normalize other signals. To determine the extent to which each band was protected, the signal ratio of a digestion product in the experimental lane to that of the control lane was calculated and then normalized using the signal ratio of full-length protein.

**RESULTS**

**Protease Footprinting Methodology**—Protease footprinting represents a potentially powerful technology for examining protein-protein and protein-DNA interactions. In its simplest form, the concept is analogous to DNase I footprinting except that, rather than employing nucleases to map a protein binding site on a 32P-end-labeled DNA fragment, proteases are used to map protein contacts on a 32P-end-labeled target protein. The
advantage of protease footprinting over deletion and point-mutation analyses is that interactions are studied in the context of a largely native wild-type protein, bypassing the potential for deleterious effects that mutants might have on the overall structure. Furthermore, because the complexed protein is compared with the free protein, conformational changes upon interaction may also be detected quite readily.

In a previous study, we employed protease footprinting to map the binding sites of the viral activator VP16 and Drosophila TAF$_{4}$ on human TFIIB (36). The VP16 binding sites correlated with those defined by mutational studies (39), whereas the TAF$_{4}$ binding site was not previously identified. The subsequent crystal structure of the TBP-TFIIB-TATA box ternary complex (40) revealed that the VP16 and the TAF$_{4}$ binding sites defined by protease footprinting formed a solvent-accessible wedge and were in close proximity, consistent with their ability to mediate complex formation (41). Our current study characterizes the interactions of human TFIIA within the TFIIA-TBP-TATA ternary complex to understand how TFIIA assists TBP and TFIID binding to DNA, a step that serves as a key target for transcriptional activators. Although our study was performed independently, comparison with the recently solved crystal structure of the yeast ternary complex validates the utility of the protease footprinting technology for studying intricate protein-protein interactions.

**Experimental Design**—Fig. 1 describes the protease footprinting procedure used in our study. The TFIIA used for these experiments contains a unique $^{32}$P-end label on one of its two subunits ($\alpha$-$\beta$ or $\gamma$) (Fig. 1A). The $^{32}$P-end-labeled TFIIA, the free molecule, or that in the form of either a binary complex with GST-TBP or a ternary complex with TBP bound to an immobilized adenosine E4 TATA box oligonucleotide is digested with a nonspecific protease to generate a nested set of digestion products (Fig. 1B). The products are fractionated on a high resolution SDS-polyacrylamide gel and autoradiographed to reveal a ladder (Fig. 1B). The limited proteolysis pattern of TFIIA in the complex is compared with that of free TFIIA to identify changes in cleavage that occur as a consequence of complex formation. The loss (or increase) of specific cleavage products is taken as an indicator of an interaction. To determine whether these footprints are due to a direct interaction with TBP or the TATA box, protease footprinting of GST-TBP-TFIIA binary complexes formed in the absence of DNA is compared with the results obtained with the ternary complexes.

$^{32}$P-end-labeled TFIIA was generated by constructing molecules of TFIIA in which one subunit had an HMK tag (Arg-Arg-Ala-Ser-Val; RRASV) engineered onto one of its termini and a His tag onto the other for purification using nickel affinity resins (see Fig. 1A). These molecules were phosphorylated on the HMK tag using [$\gamma$-$^{32}$P]ATP and heart muscle kinase; the labeling was specific as equimolar amounts of a TFIIA molecule containing untagged subunits incorporated less than 5% of the amount of $^{32}$P generated using tagged subunits (data not shown).

Fig. 1C shows an example of how the binding reactions were optimized. Increasing amounts of TFIIA, $^{32}$P-end-labeled on the $\gamma$ subunit, were first incubated with a resin containing an immobilized TATA box and subsequently washed to remove unbound protein. The bound fraction was electrophoresed on standard SDS-polyacrylamide gels and visualized by autoradiography. The bound fraction of TFIIA was electrophoresed on standard SDS-polyacrylamide gels and autoradiographed. Lane 1 is 3% of the input end-labeled TFIIA for the experiments shown in lanes 8 and 9.

FIG. 1. **Experimental design.** A, specific $^{32}$P-end labeling of TFIIA. Recombinant TFIIA subunits bearing a heart muscle kinase phosphorylation site (HMK tag) at one terminus and a His-tag at the other were overexpressed and purified from *Escherichia coli* by Ni-NTA chromatography. The tagged subunits were then joined with a partner lacking the HMK tag and labeled with [$\gamma$-$^{32}$P]ATP and heart muscle kinase to generate TFIIA $^{32}$P-end-labeled at the HMK tag of one subunit. B, schematic of protease footprinting protocol. To form ternary complexes, recombinant human TBP (the carboxyl-terminal 181 amino acids) and human $^{32}$P-end-labeled TFIIA were incubated with a biotinylated oligonucleotide containing the TATA box from the adenovirus E4 promoter immobilized on streptavidin:magnetic beads. (Binary complexes were assembled by incubating glutathione-agarose-immobilized GST-TBP with human $^{32}$P-end-labeled TFIIA, not shown.) The complexes were washed with an excess of binding buffer to remove unbound protein and then digested with limiting amounts of a broad specificity protease. The products were fractionated on high resolution SDS-polyacrylamide gels and visualized by autoradiography. C, specific ternary complex formation. Increasing amounts of TFIIA $^{32}$P-end-labeled on the $\gamma$ subunit were incubated with biotinylated TATA box oligonucleotides immobilized on M-280 streptavidin resin with (lanes 3, 5, 7, and 9) or without (lanes 2, 4, 6, and 8) TBP and then washed extensively. The bound fraction was electrophoresed on standard SDS-polyacrylamide gels and autoradiographed. Lane 1 is 3% of the input end-labeled TFIIA for the experiments shown in lanes 8 and 9.
by mapping the known amino acid sequence and prestained markers against the migration of the digestion products on a semi-log plot. Proteinase K footprinting revealed that a subset of the same residues (amino acids 59–73) was protected in the ternary complex (compare lanes 20–23 with lanes 16–19 and 24–27) with concomitant DNA-dependent enhanced cleavages (asterisks) flanking the protected region. Using laser densitometry, the three cleavage products between residues 59 and 73 in the proteinase K footprint were 79%, 93%, and 79% protected in the ternary complex (compare procedures" for normalization procedure).

Protease footprinting of the binary TBP-TFIIA complex (Fig. 2B) demonstrates that the protection described above represents a direct interaction with TBP. When compared with free TFIIA (lanes 10–13) or TFIIA incubated with GST alone (lanes 2–5), proteinase K digestion of a binary complex between TFIIA and GST-TBP immobilized on glutathione agarose (lanes 6–9) revealed a footprint. Alignment against the sequencing ladder and deletion marker (lanes 14–16) revealed that the footprint encompassed the same region (residues 59–73) of the TFIIAγ subunit as that identified in the ternary complex (Fig. 2A) with the minor difference that the DNA-dependent enhanced bands were absent. When samples digested to a comparable extent were quantitated using a PhosphorImager and compared, there was >90% inhibition of proteolysis of the lowest three cleavage products on the gel. Taken together, our results suggest that residues 59–73 of the γ subunit mediate a direct protein–protein interaction with TBP. The residues in human TFIIAγ identified by protease footprinting, 59–73, are homologous to residues 63–77 in yeast TOA2, which completely encompasses the TBP-interacting amino acid residues in the crystal structure. We will return to this point in the discussion (30, 31). The digestion pattern also reveals that the γ subunit is divided into a protease-resistant amino-terminal half, which precludes a protease footprinting analysis of this region, and a more protease-sensitive carboxyl-terminal half (see Figs. 2A and 2B).
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The TFIIAα-β Subunit Makes a Direct Contact with DNA via the β Subunit—Fig. 3A demonstrates that TFIIA makes a direct contact with DNA via the β portion of the α-β subunit. TFIIA containing α-β 32P-labeled at the carboxyl terminus was assembled into a ternary complex and footprinted using chymotrypsin. A striking feature of this digest is the very small number of accessible chymotrypsin cleavage sites, only 5 throughout the length of this subunit. Similar results were obtained using several proteases. The digestion patterns between free TFIIA (lanes 11–13) and TFIIA in the presence of the TATA box oligo alone (lanes 3–5). Lane 1 contains 0.2% of the input TFIIA used in the binding reaction, and lanes 2, 6, and 10 are mock digestions. The brackets identify the protected regions, which include sites in the basic region of the β subunit (amino acids 341–354). The pound signs denote the presence of background proteolytic products. A schematic of TFIIAα-β is on the right with the position of the basic region denoted by plus signs. The HMK tag and His tag are represented by dark and light cross-hatching, respectively. Lys-C, clostripain, and Glu-C sequencing ladders are shown in lanes 14–16, respectively. B, binary complexes. GST-TBP-IIA binary complexes were assembled and subjected to proteolysis by chymotrypsin (lanes 7–9) in parallel with free TFIIA (lanes 7–9) and TFIIA in the presence of GST (lanes 11–13) and TFIIA in the presence of GST alone as a control (lanes 3–5). Mock digestions to identify any background proteolysis are shown in lanes 2, 6, and 10. Lane 1 contains 0.2% of the input TFIIA used in the binding reactions. Asterisks on the left identify the regions protected in the ternary complex. A pound sign identifies a background proteolytic product that occurs in the presence of the GST resin. Deletion markers are in lanes 14–16, and clostripain, Lys-C, and Glu-C sequencing ladders are shown in lanes 17–19, respectively. The estimated positions of the lysine, arginine, and glutamic acid residues are shown on the right.

FIG. 3. Chymotrypsin footprinting of the α-β subunit. A, ternary complexes containing TFIIA labeled on the carboxyl terminus of the α-β subunit were assembled and subjected to proteolysis by chymotrypsin (lanes 7–9) in parallel with free TFIIA (lanes 11–13) or TFIIA in the presence of the TATA box oligo alone (lanes 3–5). Lane 1 contains 0.2% of the input TFIIA used in the binding reaction, and lanes 2, 6, and 10 are mock digestions. The brackets identify the protected regions, which include sites in the basic region of the β subunit (amino acids 341–354). The pound signs denote the presence of background proteolytic products. A schematic of TFIIAα-β is on the right with the position of the basic region denoted by plus signs. The HMK tag and His tag are represented by dark and light cross-hatching, respectively. Lys-C, clostripain, and Glu-C sequencing ladders are shown in lanes 14–16, respectively. B, binary complexes. GST-TBP-IIA binary complexes were assembled and subjected to proteolysis by chymotrypsin (lanes 7–9) in parallel with free TFIIA (lanes 7–9) and TFIIA in the presence of GST (lanes 11–13) and TFIIA in the presence of GST alone as a control (lanes 3–5). Mock digestions to identify any background proteolysis are shown in lanes 2, 6, and 10. Lane 1 contains 0.2% of the input TFIIA used in the binding reactions. Asterisks on the left identify the regions protected in the ternary complex. A pound sign identifies a background proteolytic product that occurs in the presence of the GST resin. Deletion markers are in lanes 14–16, and clostripain, Lys-C, and Glu-C sequencing ladders are shown in lanes 17–19, respectively. The estimated positions of the lysine, arginine, and glutamic acid residues are shown on the right.

DISCUSSION
We have identified, using protease footprinting, interactions made by human TFIIA upon assembly of the TFIIA-TBP-TATA box ternary complex. Each subunit makes a series of contacts with one of the other components of the complex, the TFIIAγ subunit with TBP and the IIαα-β subunit with DNA. These interactions allow TFIIA to form a bracket that stabilizes TBP binding. These results are summarized schematically in Fig. 4. Together, TBP and TFIIA function to provide specificity and stability. The preference of TBP for the TATA box provides the specificity required to bring TFIID to the promoter, but the weak affinity of TFIIA for DNA is used advantageously to
Correlations with Crystal Structure and Biochemical Data—

The contacts made by TFIIA within the human ternary complex as measured by protease footprinting are similar to those made within the yeast ternary complex as identified in the crystal structure. Fig. 5A is a ribbon diagram illustrating the recently solved crystal structure of the yeast ternary complex (30, 31) in which yTOA1 (α and β), yTOA2 (γ), TBP, and the TATA box oligonucleotide are yellow, green, blue, and white, respectively. In the structure, the two subunits of yTFIIA are highly intertwined and form a boot-shaped structure in which TOA2 (γ) interacts with TBP and TOA1 (α and β) interacts with DNA. TFIIA binds to the underside of TBP along the loop (stirrup) (42) connecting β-strand 2 and β-strand 3. A mutagenesis study, in which radical changes were introduced into surface residues of human TBP, identified an interaction between TFIIA and residues encompassing the stirrup within the first direct repeat of TBP, consistent with the crystal structure (43).

The ability of these TBP mutants to support transcriptional activation in vivo and TBP-TFIIA complex formation in vitro was greatly diminished.

In Figs. 5B, C, E, and F, our footprinting results are superimposed on space-filling models of yeast TFIIA (Fig. 5, B and E) alone and in the ternary complex (Fig. 5, C and F). Fig. 5, B and C, shows the γ-TBP interaction using the same view as in Fig. 5A. Fig. 5B illustrates yTFIIA alone in which TOA1 (i.e. hTFIIAα-β) is yellow and TOA2 (i.e. hTFIIAγ) is green. The residues identified by protease footprinting as interacting with TBP (hTFIIAγ residues 59–73 homologous to yTOA2 residues 63–77) are colored red. Of the 15 residues in the protected region, 11 of the positions are homologous between human and yeast including all those that directly contact TBP.

In Fig. 5C, TBP (blue) and the TATA box oligonucleotide (white) have been added to TFIIA. The protected region in red forms β-strands that are part of the domain that is intertwined with the β-strands of yTOA1 to form the β-barrel. A comparison of Fig. 5B with 5C illustrates that the edge of the concave β-sheet region of TBP covers the “red” region and explains why TBP protects that region from proteolysis.

Fig. 5, D–F, is a separate view of the ternary complex used to optimize presentation of the region of hTFIIAα-β protected in the ternary complex. Fig. 5, E and F, superimposes the interaction between TFIIAα-β and DNA identified by protease footprinting onto TFIIA alone (Fig. 5E) and in the ternary complex (Fig. 5F). In this case, the residues protected in hTFIIAα-β (TOA2) are labeled as red, and this region lies within the β-strands of the β-portion of hTFIIAα-β. Within the identified region, 16 of the 27 residues are homologous between yeast and human, including 6 basic ones.

The region of the human TFIIAα-β subunit protected from proteolysis in the ternary complex includes the homologous residues of yeast TOA1 shown to contact DNA directly. In Fig. 5F, when γTBP (blue) and the TATA box oligonucleotide (white) are added to yTFIIA (shown from the same view and with the same color scheme in Fig. 5E), the DNA runs across the “red” residues, which is consistent with the observed protease footprint. It appears from the figure that the protected residue at the bottom of the figure may still be accessible from underneath. However, the TATA box oligonucleotide used for our footprinting experiments is longer than the one used in the crystallography studies, which would increase the protection of that site.

The one interaction observed in the crystal structure that was not detected in our study was the insertion of the penultimate tryptophan residue of yTOA1 into a crevice created where TBP and γTOA2 interact. Because that tryptophan is the carboxyl-terminal residue in hTFIIAα-β, it is probably too close to our HMK tag for detection.

Although the protease footprinting analysis does not identify other interactions of TFIIA within the ternary complex, it is possible that a protease-resistant region forms other contacts. The lack of a proteolytic reagent that cleaves at every surface position does tend to limit the protease footprinting approach. One possible limitation is revealed by mutational studies that indicate that an interaction exists between a highly acidic region of hTFIIA α-β and the H2 helix of TBP (44–46). We have not identified such a region in our study.

Interactions of TFIIA with TBP in the absence of DNA have been studied using “GST-pulldown” experiments. Interactions of the α, β, and γ subunits (or their homologues) individually with TBP have been identified. However, in view of the crystal structure, the previous studies will have to be reevaluated because the subunits are intertwined and probably will not fold properly on their own. Our studies define an interaction between only the γ subunit and TBP.

A protein-DNA cross-linking study concluded the α subunit interacts with promoter DNA upstream of TBP centered at −45, the γ subunit is close to only −39, and the β subunit can be cross-linked to both sides of TBP (35). These data are similar to an earlier study (34). We detected only an interaction between the β portion of the α-β subunit and DNA. The absence of an α-DNA interaction may simply reflect the use of a TATA box oligonucleotide that is too short to detect this interaction. It may also be possible that some proportion of TFIIA undergoes a conformational change within the ternary complex leading to these other DNA contacts, a finding supported by at least one recent study (21).

Based on protease sensitivity, it is plausible that other proteins will interact with TFIIA via the carboxyl terminus of the γ subunit and with the α subunit. Because the γ subunit binds TBP, regulatory proteins interacting with the γ subunit could modulate the ability of TFIIA to associate with TBP. There is evidence that the Epstein-Barr virus activator ZEBRA overcomes a mutation in the γ subunit, which decreases TBP binding and complex formation (29). Because the α subunit is upstream within the ternary complex (30, 31), α is positioned to stabilize binding of TFIIID to promoters, which contain diverse sequences flanking the TATA box.
more easily interact with activators or possibly TAFs that extend toward upstream bound activators.

Our understanding of how the transcription complex is assembled requires an understanding of each step, particularly the key regulatory ones. We have characterized the formation of a promoter-bound complex containing TBP and TFIIA. We will use this study as a starting point to perform parallel studies with TFIIID and to examine the interactions of activators. It will be particularly interesting to determine how (and if) TAFs change the interaction of IIA with TBP. The ability of activators to both facilitate complex assembly and induce a conformational change in the DA complex could be studied. These future studies will benefit from using smaller proteolytic reagents, especially given the larger size of TFIIID relative to TBP. Recent experiments by the labs of Meares, Heyduk, and Berk for insightfully discussing comments on the manuscript, and Thai Nguyen for constructing some of the TFIIA deletion mutants.

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