Autoinhibition of TFIIIB70 Binding by the Tetra tricopeptide Repeat-containing Subunit of TFIIIC*

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Robyn D. Moir, Karen V. Puglia, and Ian M. Willis‡

From the Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

An important step in the assembly of RNA polymerase III transcription complexes on tRNA and 5 S genes is the interaction between the tetra tricopeptide repeat (TPR)-containing subunit of TFIIIC (TFIIIC131) and the TFIIIB-related subunit of TFIIIB (TFIIIB70/Brf1). A fragment of TFIIIC131 that contains the hydrophobic amino terminus and two TPR arrays, with five and four repeats, respectively (Nt-TPR9), is sufficient to support an interaction with TFIIIB70. Here we evaluate the contribution of each TPR array to TFIIIB70 binding. Both TPR arrays bind independently to TFIIIB70 with TPR6–9 having a 4-fold higher apparent affinity than TPR1–5. However, the TPR arrays are not sufficient for a high affinity interaction with TFIIIB70. The addition of amino-terminal sequences increases the affinity of TPR1–5 18-fold to create a high affinity TFIIIB70 binding site (Nt-TPR5, 44 ± 6 nm). Although the Nt-TPR5 and TPR6–9 fragments are contained entirely within the Nt-TPR9 fragment, the affinity of the latter is significantly lower than either of these smaller fragments. The results demonstrate that the TFIIIB70 binding sites in TFIIIC131 are subject to autoinhibition. We propose that the binding of TFIIIB70 to these sites within the TFIIIC complex may proceed in an ordered fashion.

In eukaryotic cells, the transcription of 5 S rRNA, tRNA, and related genes by RNA polymerase III (pol III)1 is dependent on transcription factor (TF) IIIC for promoter recognition and recruitment of the initiation factor TFIIIB to the DNA upstream of the transcription start site (1–3). In Saccharomyces cerevisiae, TFIIIC (also called τ) is a large (≈520 kDa) six subunit complex (Ref. 4 and references therein) whose structure in electron micrographs reveals two flexibly linked globular domains (αA and αB) that interact, respectively, with the variably spaced internal A and B block promoter elements of tRNA genes (5, 6). The recruitment of TFIIIB by promoter-bound TFIIIC is mediated initially by protein-protein interactions between the tetra tricopeptide repeat (TPR)-containing subunit of TFIIIB (TFIIIB70/Brf1) and the TFIIIB-related subunit of TFIIIB (TFIIIB70/Brf1 (7–10)). Subsequently (or simultaneously) TBP is incorporated into the complex by protein-protein interactions with TFIIIB70 and TFIIIC60 (4, 9, 11, 12). These interactions serve to drive DNA binding by TBP (7). The assembly of TFIIIB is completed with the recruitment of TFIIIB90/B* by the other TFIIIB components and TFIIIC131 (11, 13). These binding reactions are thought to proceed in a concerted manner that involves a series of conformational changes in the DNA and the proteins (14). These structural changes kinetically trap the DNA within TFIIIB (15) making the TFIIIB-DNA complex very stable and resistant to dissociation by high salt treatment and polyionics (16).

The TPR motif is a degenerate 34-amino acid motif that is often found in tandem arrays (17, 18). TPR-containing proteins are ubiquitous and participate in a diverse range of processes that are linked only by the primary function of the TPR as a protein-protein interaction domain (19–21). Individual TPRs fold into two antiparallel α-helices separated by a turn (22). Three adjacent TPR motifs stack together to form a right-handed superhelix that creates an amphipathic channel (18, 23–26). In longer TPR arrays, the superhelix may be extended (24, 25) or it may be interrupted by a single long α-helix (23). For example, in the seven tandem TPRs of PEX5, TPRs1–3 and TPRs5–7 are arranged in two antiparallel superhelices separated by a hinge region (TPR4). The TPR4 hinge forms a single long helix rather than the usual TPR fold and thus enables the two TPR superhelices to surround the ligand (23). Amino acid residues that line the TPR superhelical groove provide the ligand binding interface and confer binding specificity, as exemplified by the TPR domains of the co-chaperone Hop (26). Alternatively, the internal groove can function as a scaffold upon which a non-TPR ligand binding interface is organized, as for the p67phox subunit of NADPH oxidase (24). TPR domains may also function as ligand-dependent modifiers of protein activity: Either deletion of or lipid binding to the TPR domain of protein phosphatase 5 (PP5) activates the catalytic activity of the enzyme (27).

TFIIIC131 contains 11 TPRs organized into two tandem arrays of five and four repeats, respectively, and two single repeats (28). Dominant mutations in and around the second TPR increase pol III transcription from a tRNA A block promoter in vivo, and thus the function of this region has been implicated in an important limiting step (10, 29). Biochemical studies with TFIIIC preparations from the dominant TPR mutant strains have demonstrated that increased transcription of wild-type and mutant templates results from the facilitated recruitment of TFIIIB70 (10). However, the mechanism underlying this facilitated recruitment is complex: Biochemical data for the PCF1–2 mutation (T167I in TPR2) are consistent with a conformational change within TFIIIC131 that affects the extent to which the binding reaction can proceed (10). TPR-mediated changes in the nature and/or accessibility of the TFIIIB70 binding site may also underlie the 4-fold decrease in the two-hybrid interaction that occurs when the...
minimal TFIIIB70 interaction domain, r131–1TPR–(1–165), is extended up to the end of TPR9 (8). Changes in the configuration of TFIIIC131 during preinitiation complex assembly are also indicated by site-specific DNA-protein photocross-linking experiments (7) and have been proposed to underlie the capacity of TFIIIC to vary the placement of TFIIIB on the DNA (30). Although a role for TPRs in this conformational flexibility of TFIIIC has been suggested (3), the function of TPRs in the recruitment of TFIIIB to DNA is not well understood at the present time.

To better understand the contribution that each TPR domain makes to the recruitment of TFIIIB70, we have characterized fragments of TFIIIC131 using a quantitative equilibrium binding assay (31). We demonstrate that each TPR array (TPR1–5 and TPR6–9) binds independently to TFIIIB70 with differential affinity and that addition of the amino terminus to TPR1–5 creates a high affinity site for TFIIIB70 binding (Nt-TPR5). Finally, because the binding sites in Nt-TPR5 and TPR6–9 have higher affinity for TFIIIB70 than a larger fragment (Nt-TPR9) containing both of these sites, the results provide evidence for autoinhibition in the interaction between TFIIIC131 and TFIIIB70.

EXPERIMENTAL PROCEDURES

Plasmid Construction—A wild-type clone of the gene encoding the 131-kDa subunit of yeast TFIIIC131 (PCR1/TC4 [28, 29]) was used as a template to generate truncated genes. Pdu DNA polymerase (a high fidelity polymerase) was used for PCR amplification prior to cloning into pET2di-d as previously described (31). The truncated proteins all contained a six-histidine tag at the carboxyl terminus and included: Nt-TPR9 (formerly TFIIIC131(1–580), amino acids 1–580 (Ref. 31); Nt-TPR5, amino acids 1–303; TPR1–5, amino acids 118–303 with the substitutions Q118M and R119G; IVRTPR6–9 was expressed and purified under native conditions on Ni2+ nitrilotriacetic acid-agarose resin (Qiagen), followed by heparin-agarose purification. The proteins were detected by fluorescence analysis using PicoGreen reagent, Molecular Probes, Inc., yeast TFIIIC (10 fmol), TFIIIB70 (3000 fmol), TBP (1000 fmol), and TFIIIB90 (400 fmol) as required in various experiments and were incubated at 20 °C for 60 min before heparin treatment and electrophoresis. At this level of input TFIIIC, TFIIIB70 was limiting as a TF and TFIIIB90 were present in saturating amounts for TFIIIB-DNA complex assembly. In controls, TFIIIC proteins, these proteins were added to preformed TFIIIC-DNA complexes prior to the addition of the TFIIIB subunits.

Quantitation and Data Analysis—Digital images collected on photostorage screens were quantified using ImageQuant software. Individual lines, one lane wide, were analyzed using Peak Finder to calculate peak areas corresponding to the TFIIIB-DNA complex. These values, when paired with the corresponding TFIIIC131 fragment concentration, yielded a transition curve describing the inhibition reaction. Importantly, the TFIIIC-DNA concentration in complex assembly assays never exceeded 0.25 nm. This is more than 150-fold lower than the apparent equilibrium dissociation constant determined for the complex with the highest affinity (Table I). Thus, under the conditions employed, the difference between the total and free TFIIIC131 fragment concentration in the reactions is negligible. The upper limit of complex assembly was determined by non-linear least squares analysis using the Hill equation in Microcal Origin version 5.0 software (Microcal Software Inc.) This limiting value was used to generate a scaled inhibition isotherm in which the relative level of TFIIIB-DNA complex formation was expressed as a function of TFIIIC131 fragment concentration. Multiple scaled data sets for each TFIIIC131 fragment were then simultaneously re-fit to the Hill equation. Errors associated with the apparent equilibrium dissociation constants and the Hill co-efficients were determined during curve fitting. Inhibition by TPR1–5, unlike the other TFIIIC131 fragments, was incomplete and reached a plateau at around 9–24% of the uninhibited level in different experiments. This fragment also did not easily remain in solution at high concentrations (>400 μM). However, at concentrations of ≤0.5 μM, fragment inhibition of complex assembly was well described by the Langmuir binding polynomial (i.e. the Hill equation with the Hill co-efficient fixed at 1.0).

Sequence Data—S. cerevisiae TFIIIC131 homologs from Schizosaccharomyces pombe (AL031555.1), Homo sapiens (AF131231.1, and Kluyveromyces lactis (AF229111) were retrieved by BLASTp searches and were used as annotated. A protein query of S. cerevisiae TPR2 to unfinished nucleotide sequence data bases recovered homologs in Candida albicans and Drosophila melanogaster (gnl Stanford_5476 C.albicans_C04–2667 C. albicans unfinished fragment of complete genome, D. melanogaster AE003804 [Full Sequence] genomic scaffold 14200013386047). The respective contigs were translated in all reading frames to identify the extent of homology to S. cerevisiae TFIIIC131. Because many non-related TPR-containing proteins were recovered in this manner, TFIIIC131 homologs were required to conform in size and TPR domain organization to TFIIIC131. The putative Caenorhabditis elegans ortholog (Z70783.1) was excluded from our analysis because sequence homology was limited to only the first TPR array and the carboxyl terminus. The initial protein alignment was performed using ClustalW software, Version 1.8 (available at www.workbench.sdsc.edu), and subsequent realignment was made manually by visual inspection.

RESULTS

Multiple Fragments of TFIIIC131 Interact with TFIIIB70—In a previous study, we expressed the amino-terminal half of TFIIIC131 up to the end of TPR9 (Fig. 1A, Nt-TPR9) and characterized its structure in solution by circular dichroism and partial proteolysis (31). Additionally, using a coupled equilibrium binding assay, we showed that the interaction between Nt-TPR9 and TFIIIB70 is well described by a single-site binding model and an apparent equilibrium dissociation constant (Kd) of 333 nM. To examine the ability of different TFIIIC131 structural domains to interact with TFIIIB70, we have now expressed and analyzed several truncated derivatives of Nt-TPR9 (Fig. 1A). The individual TPR arrays were expressed, independently, with (Nt-TPR5 and IVR+TPR6–9) or without (TPR1–5 and TPR6–9) adjacent non-TPR sequences. Among these fragments, Nt-TPR5 is known to encompass the minimal TFIIIB70 interaction domain defined by two-hybrid experiments (8). In addition, a fragment containing both TPR arrays and the intervening region (IVR) was also prepared (TPR1–9). These truncated proteins were expressed...
Our previous characterization of Nt-TPR9 showed that it interacts with TFIIIB70 in solution and inhibits the formation of TFIIIB70-TFIIIC-DNA complexes as assayed by native gel electrophoresis (31). We therefore assayed each of the truncated TFIIIC131 proteins for the ability to interact similarly with TFIIIB70. At a concentration of 3 μM, all of the truncated TFIIIC131 proteins were competent for binding to TFIIIB70 (Fig. 1C). The loss of TFIIIB70-TFIIIC-DNA complexes upon addition of the TFIIIC131 fragments is readily detected by native gel electrophoresis and can be quantified directly if the gel quality is high. However, even in the best experiments, accurate quantitation is technically demanding because of the limited separation of the TFIIIC-DNA and TFIIIB70-TFIIIC-DNA complexes and requires the use of peak-fitting software. We therefore chose to quantify the interaction between the TFIIIC131 fragments and TFIIIB70 indirectly using our previously described coupled equilibrium binding assay (31). This assay monitors the inhibition of heparin-resistant TFIIIB-DNA complex formation that occurs when TFIIIC131 fragments compete with TFIIIC-DNA for binding to TFIIIB70 in the presence of excess TBP and TFIIIB90. Titrations of each TFIIIC131 fragment were performed, and the heparin-stripped TFIIIB-DNA complexes were resolved on native gels. The quantitation of these complexes as a function of TFIIIC131 fragment concentration produces an inhibition isotherm (see “Experimental Procedures”). Global (simultaneous) analysis of multiple titrations of each fragment is then performed to accurately describe the pattern of inhibition and to determine an apparent dissociation constant for the fragment

**Fig. 1. Multiple regions of TFIIIC131 bind TFIIIB70.** A, a schematic representation of the structural domains in TFIIIC131 and each of the TFIIIC131 fragments analyzed in this study. Full-length TFIIIC131 includes a hydrophilic amino-terminal region (forward hatches), 11 TPR repeats (solid), which are grouped into two TPR arrays, and two solo repeats and a helix-loop-helix region (backward hatches). B, SDS-polyacrylamide gels of the purified TFIIIC131 proteins. Protein size markers are annotated in kilodaltons. Lane 1, Nt-TPR9; lane 2, Nt-TPR5; lane 3, TPR1–9; lane 4, IVR+TPR6–9; lane 5, TPR1–5; lane 6, TPR6–9. C, a native polyacrylamide gel of TFIIIB70-TFIIIC-DNA (B70.IIIC.DNA) complexes formed in the presence or absence of the different TFIIIC131 fragments. Reactions contained either no TFIIIC131 competitor protein, lane 1; or 60 pmol of the following proteins, Nt-TPR9, lane 2; Nt-TPR5, lane 3; TPR1–9, lane 4; IVR+TPR6–9, lane 5; TPR1–5, lane 6; and TPR6–9, lane 7.

as carboxyl-terminal hexahistidine fusions in *Escherichia coli*, purified under denaturing conditions on Ni²⁺-nitrilotriacetic acid-agarose, and refolded. The Nt-TPR5 and TPR1–9 proteins were additionally purified to separate the full-length proteins from smaller species (see “Experimental Procedures”). With one exception (IVR+TPR6–9, see below), all of the protein preparations were >90% pure (Fig. 1B). For IVR+TPR6–9, the full-length protein was determined to be 50% pure. The remainder of this preparation contained two smaller TFIIIC131 fragments in approximately equimolar proportions. The larger of these fragments contained TPR5–6–9 with about 5 kDa of IVR sequence, whereas the smaller fragment had its amino terminus within the TPR array (Fig. 1B). In our previous work (31), we noted the aberrant mobility of Nt-TPR9 (apparent molecular mass of 79 kDa compared with the predicted value of 69 kDa) on SDS-polyacrylamide gels. This property is attributed to the hydrophilic amino terminus, because an uncharacteristic mobility is also seen for Nt-TPR5 (apparent molecular mass of 48 kDa compared with the predicted value of 36 kDa) but not for TPR1–9 (predicted molecular mass of 55 kDa, Fig. 1B, lanes 1–3).

**B70.C.DNA**

**C.DNA**
TPR9 and IVR–TPR6–9 may solely mediate the interaction between Nt-TPR9 and TFIIIB70.

**TPR Domains 1–5 and 6–9 Are Sufficient for TFIIIB70 Binding**—The Nt-TPR5 and IVR+TPR6–9 fragments contain different TPR arrays and interact independently with TFIIIB70. Each TPR array may therefore provide an interaction surface with TFIIIB70. Indeed, both TPR1–5 and TPR6–9 inhibit the formation of TFIIIB70-TFIIC-DNA (Fig. 1C) and heparin-resistant TFIIIB-DNA complexes (Fig. 3), and the resulting inhibition isotherms conform to the single-site binding model (Ta-
able I). However, these two TPR domains exhibit very different apparent affinities for TFIIIB70 (Table I). The TPR1–5 inhibition isotherm gave an apparent $K_D$ of $773 \pm 87$ nM, which is about 18-fold lower than the apparent $K_D$ for Nt-TPR5. These data indicate that the amino-terminal region adjacent to TPR1–5 is primarily responsible for the high affinity binding of Nt-TPR5 to TFIIIB70 and presumably contains most of the binding determinants for this interaction. Attempts to independently express and purify the amino-terminal region up to the beginning of TPR1 were unsuccessful. However, two-hybrid experiments have shown that this region alone is not sufficient for an interaction with TFIIIB70 (8).

The inhibition isotherm for TPR6–9 yielded an apparent $K_D$ for TFIIIB70 binding of $177 \pm 27$ nM, which is more than 2-fold higher than the upper limit estimated for IVR+TPR6–9 (Table I). Because TPR6–9 is clearly sufficient for binding to TFIIIB70, the lower affinity of IVR+TPR6–9 suggests that IVR sequences interfere with this interaction. It is also interesting to note that the affinity of TFIIIB70 for TPR6–9 is 4-fold higher than that for TPR1–5.

The TPR1–9 fragment contains both TPR domains and has an apparent affinity for TFIIIB70 (210 ± 20 nM) that is statistically identical to TPR6–9. Thus, the addition of TPR1–5 and the IVR appears to have had a negligible effect on the apparent $K_D$ of TPR6–9 for TFIIIB70. However, unlike the other TFIIIC131 fragments, the slope of the TPR1–9 isotherm ($-1.8 \pm 0.3$, Table I) diverges significantly from the value of unity. This result has two simple interpretations: One possibility is that the binding stoichiometry of the reaction has changed such that two molecules of TFIIIB70 are now bound for each molecule of TPR1–9. The presence of independent TFIIIB70 binding sites in TPR1–5 and TPR6–9 is consistent with this notion. However, for the apparent affinity of the TPR1–9 interaction to be equivalent to that for TPR6–9, the low affinity interaction between TPR1–5 and TFIIIB70 (Table I) must be increased in the context of the larger fragment. Although this could be achieved in principle by interactions between two bound TFIIIB70 molecules, this mechanism is unlikely to be biologically relevant (see below) and lacks experimental support. A second explanation for the high Hill coefficient is that a single molecule of TFIIIB70 binds cooperatively to TPR1–9. This model is biologically appropriate (by analogy to the pol II ternary complex (32)) and is also consistent with the presence of two independent TFIIIB70 binding domains in TPR1–9. Importantly, this mode of binding predicts that different regions of TFIIIB70 interact with the two TPR domains. This prediction is supported by two-hybrid experiments that indicate distinct regions of TFIIIB70 interact with TFIIIC131 (8).

**A Structural Model for TFIIIB70 Recognition by TPR6–9**—To gain some insight into the nature of the TFIIIB70 binding sites in the TPR1–5 and TPR6–9 fragments, we carried out a phylogenetic analysis of these regions in known or presumed TFIIIC131 orthologs. The identification of functionally important residues using this approach is especially informative, because the crystal structures of several TPR proteins have recently been solved with and without bound ligands. These structures allow attention to be focused on highly conserved amino acids other than those involved in the TPR fold. A similar phylogenetic analysis of the TPRs in PEX5 together with the TPR structure of protein phosphatase 5 successfully predicted the residues involved in binding the peroxisomal targeting signal 1 (23, 33).

An alignment of TPRs1–9 of *S. cerevisiae* TFIIIC131, *H. sapiens* TFIIIC102, and the predicted orthologs from *C. albicans*, *S. pombe*, *K. lactis*, *D. melanogaster*, and *Arabidopsis*

*thaliana* is shown in Fig. 4. TPRs are highly degenerate motifs. Consistent with this, only 24 residues out of 306 contained in the nine TPRs are identical across all seven species and only 13 of these residues map to TPR consensus positions (1–8, 1–20, 1–28, 2–27, 2–28, 3–4, 3–20, 3–27, 4–20, 4–23, 5–8, 7–20, and 8–20). Accordingly, the other 11 residues are likely to participate in functionally important ligand interactions. Seven of the non-consensus TPR residues that are identical from yeast to humans map to TPRs1–5 (positions 1–9, 1–26, 2–7, 2–15, 2–34, 4–10, and 4–15). These residues are distributed evenly between the helices (A and B) and the connecting loops of the repeat (Table II). In contrast, the four non-consensus TPR residues that are phylogenetically identical and map to TPRs6–9 (positions 6–7, 7–3, 8–10, and 9–7) are all located in the A helix of the repeat. The restricted distribution of these residues in TPR6–9 is even more striking when the analysis is relaxed to include amino acids of similar character (e.g. basic or acidic residues, etc.). For example; 14 of the 17 non-TPR consensus residues in TPRs6–9 that are identical or highly conserved through evolution map to solvent-exposed positions on the A helix (Table II). In the TPR crystal structure of protein phosphatase 5, the corresponding residues project into the cradle-shaped superhelical groove (18). Moreover, in the structures of Hop and PEX5 bound to their respective peptide ligands, a subset of these residues are involved directly in interactions with the peptides (23, 26). In particular, the Hop-peptide complexes involve interactions with five A helix residues spread over three adjacent TPRs. The affinity of these complexes and of a complex formed between Hop and a large (25 kDa) fragment of Hsp70 in 100 mM potassium acetate is 10–20 μM (26). Given the much higher apparent affinity of the TPR6–9/TFIIIB70 interaction (determined at the same monovalent ion concentration) and the large number of phylogenetically conserved residues in the A helix of TPR6–9, it seems likely that a significant number of these residues will interact directly with TFIIIB70.

As indicated in Table II, the non-TPR consensus residues in TPR1–5 that are identical or highly conserved through evolution are not concentrated in helix A but are evenly distributed through both the helices and the adjoining loops. This suggests that interactions distinct from those in the ligand binding channel of the TPR array are of considerable importance in this region. Notably, the dominant mutations in TFIIIC131 that increase TFIIIB70 recruitment map throughout TPRs1–3 across both the A and B helices (10).

**DISCUSSION**

This report extends previous studies on the TFIIIC131–TFIIIB70 interaction in several important ways. First, each of the two TPR arrays in TFIIIC131 was found to be sufficient for binding to TFIIIB70; TPR6–9 was shown to have a 4-fold higher apparent affinity for TFIIIB70 than TPR1–5, and potential sites of interaction for TFIIIB70 in the superhelical groove of TPR6–9 were identified from a TPR structure-based analysis of residues that are identical or highly conserved from yeast to humans. Second, amino-terminal sequences of TFIIIC131 (preceding TPR1) were found to increase the apparent affinity of TPR1–5 for TFIIIB70 by 18-fold. Thus, of the TFIIIC131 fragments that have been assayed biochemically, the Nt-TPR5 fragment has the highest TFIIIB70 binding affinity. Based on its high affinity, we anticipate that TFIIIB70 binding to this domain is likely to be biologically important (discussed further below). Third, a comparison of apparent binding affinities of the TFIIIC131 fragments that exhibit single-site binding behavior (Nt-TPR5 > TPR6–9 > Nt-TPR9 > IVR+TPR6–9 > TPR1–5) indicates that the interaction of TFIIIB70 with the large Nt-TPR9 fragment is inhibited by an
intramolecular mechanism. Nt-TPR9 contains two distinct TFIIIB70 binding domains; a high affinity site located in Nt-TPR5 and a lower affinity site in TPR6–9. The present experiments do not distinguish which of these sites in Nt-TPR9 interacts with TFIIIB70 (or indeed whether parts of each site may contribute to the interaction). However, autoinhibition of both TFIIIB70 binding sites is necessary to account for the lower affinity of Nt-TPR9 relative to Nt-TPR5 and TRP6–9.

Given the equivalent affinities of the TPR6–9 and TPR1–9 fragments and the fact that addition of the hydrophilic amino-terminal domain to TPR1–9 reduces the Hill co-efficient from approximately two to one, we favor the idea that the amino-
terminal domain in Nt-TPR9 is unable to present the high affinity binding site for TFIIIB70 and blocks access of TFIIIB70 to TPR1–5. Similarly, the lower affinity of Nt-TPR9 relative to TPR6–9 suggests a modest negative effect of sequences aminoterminal to TPR6 on TFIIIB70 interactions with TPR6–9. The ability of the IVR region to inhibit the interaction of TPR6–9 with TFIIIB70 suggests that it may be responsible for the lower affinity of Nt-TPR9.

The current studies have been conducted with fragments of a subunit from the six subunit TFIIIC complex. It is important, therefore, to consider whether the multiple TFIIIB70 binding sites identified in TFIIIC131 (Table 1, Refs. 9 and 34) play a role in TFIIIC function and whether autoinhibition of TFIIIB70 binding is biologically significant. Definitive answers to these issues are not yet available. However, as discussed below, numerous observations are consistent with these possibilities. Chaussivert et al. (8) have reported two-hybrid interactions between different regions of TFIIIB70 and TFIIIC131. Their results show a strong interaction between the TFIIIB-like half of TFIIIB70 and Nt-TPR5. These findings are complementary to those reported here and, together, suggest that the TFIIIB-like half of TFIIIB70 binds the high affinity site in Nt-TPR5. In the same study, a substantially weaker (4-fold) two-hybrid interaction between Nt-TPR9 and TFIIIB70 was found to be partially dependent on the carboxyl terminus of TFIIIB70. These data are consistent with autoinhibition of the high affinity TFIIIB70 binding site in vivo and suggest that the unique carboxyl-terminal region of TFIIIB70 mediates an interaction with TFIIIC131, potentially via TPR6–9.

In this work, we have shown that deletion of certain regions in TFIIIC131 can relieve autoinhibition of TFIIIB70 binding in vitro. The dominant activating mutations that map to TPRs1–3 of TFIIIB70 may function in a similar manner in vivo (10). Biochemical studies with TFIIIC purific from a PCF1–2 strain (which carries a T167I mutation in TPR2 of TFIIIC131) have suggested that the mutation facilitates a conformational change thereby enabling higher levels of TFIIIB70 binding than the wild-type factor. Based on the work described here, dominant mutations in the first TPR array may disrupt one intramolecular interaction that otherwise inhibits exposure of one of the TFIIIB70 binding sites. Experiments to explicitly examine this mechanism are in progress.

The proposition that the high affinity TFIIIB70 binding site in TFIIIC131 is occluded within Nt-TPR9 as well as within the TFIIIC complex and must therefore be uncovered at some point in the assembly of TFIIIB is readily compatible with the changes in TFIIIC131 photocross-linking to DNA that have been documented during complex assembly (7). Additional support is provided by deletions of specific TPRs in TFIIIC131, which are deleterious for interactions with TFIIIB70 (ΔTPR1) (8) but which increase interactions between TFIIIC131 and either TFIIIB90 (ΔTPR2) (13) or ABC10α (ΔTPR1, ΔTPR2, and ΔTPR3) (35). These deletions in the first TPR array suggest that the structure of this domain is inhibitory for TFIIIC131 interactions with TFIIIB90 and ABC10α. Inhibitory effects of TFIIIC131 have also been revealed in a comparison of TFIIID-dependent and TFIIIC-dependent assembly of TFIIIB using TFIIIB70 deletion mutants (36). Perhaps the high affinity interaction of TFIIIB70 with Nt-TPR5 must be relaxed or exchanged to allow further complex assembly.

One implication of the autoinhibition we have described is that the different TFIIIB70 binding sites in TFIIIC131 may be presented in a temporal sequence during preinitiation complex assembly. What advantages might the ordered presentation of these sites provide? Studies of other TPR-containing proteins suggest that binding to a TPR array might provide: (i) an interaction surface on which to facilitate structural changes in TFIIIB70 (31), perhaps to expose its latent DNA binding activity (37, analogous to the chaperone-like function supported by the TPR-like 14–3–3 proteins, reviewed in ref. 38); (ii) a ligand-dependent change in structure that would allow TFIIIC131 interactions with additional proteins, such as TFIIIB90 (35, 36) as seen for ligand-bound PEX5 recognition by its receptor (23); and/or (iii) a ligand-dependent structural change to reverse an inhibitory interaction (as seen for PPS (27)) or promote further structural changes in the TPRs themselves. This later possibility is suggested by recent structures in which two TPR helices failed to pack against one another and instead formed a single long helix (39, 40).

The structures of several TPR-ligand complexes have been solved and show that the functionally important residues for binding in a TPR groove are ligand-specific. The co-chaperone Hop contains two TPR arrays (designated 1 and 2A) that recognize a common motif. Consequently, the ligand binding residues in these arrays are identical (26). However, the specific ligand binding residues in Hop differ from those in the PEX5 TPR complex with PTS1 peptide (23) and the TPR-like 14–3–3 protein-peptide complex (41). Interestingly, the identity and/or position of the TPR residues in TFIIIC131 that are predicted to contribute to ligand binding (Table II) are different from the above examples. Thus, the TPRs of TFIIIC131 are likely to employ a mode of ligand recognition that is distinct from those described thus far. Moreover, the conserved residues in the groove of TPRs1–5 and TPRs6–9 are sufficiently different from one another that each array is likely to have a different binding specificity. Efforts to confirm the identity of the TPR binding determinants, based on the phylogenetic data, are underway.

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Robyn D. Moir, Karen V. Puglia and Ian M. Willis

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