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A bipartite DNA binding domain composed of direct repeats in the TATA box binding factor TFIID

(transcription factor/transcription initiation/RNA polymerase II/*in vitro* transcription/structural motifs)

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ABSTRACT **Point mutations in residues comprising the interrupted direct repeats of TFIID eliminated DNA binding in an electrophoretic mobility shift assay. In contrast, mutations in nonconserved residues within the direct repeat regions or in lysine residues comprising the intervening basic repeat had no effect on DNA binding. However, small spacing changes (addition or deletion of one to three residues) in the basic repeat eliminated DNA binding. These results argue for a bipartite DNA binding domain composed of direct repeats with a strict spacing and orientation. Surprisingly, some direct repeat mutations that inhibited DNA binding failed to show a corresponding inhibition of basal transcription, indicating compensating interactions of TFIID with other general factors. The implications of these and other recent results for TFIID structure, promoter recognition, and interactions with other factors are discussed.**

Transcription initiation by RNA polymerase II is effected by a number of general initiation factors that interact through core (minimal) promoter elements (reviewed in ref. 1) and further regulated by gene-specific factors that interact at distal control elements (reviewed in ref. 2). The most common core promoter element is the TATA box, and the direct recognition of this element by TFIID (3, 4), in a manner that may be facilitated by TFIIA (5, 6), nucleates the assembly of the remaining factors, beginning with TFIIB, into a functional preinitiation complex (5–7). The earlier suggestions that TFIID might be a target for various activators (3, 8–11) have received further support by the demonstration of direct interactions between TFIID and different activators (12–14). Thus, TFIID interacts with DNA, with at least two general factors, and with some activators.

Yeast TFIID and the corresponding TATA-binding subunit (TFIIDτ) of natural TFIID from higher organisms contain a highly conserved 180-residue carboxyl-terminal domain (reviewed in refs. 15 and 16) that is necessary and sufficient for DNA binding and for core promoter function (basal transcription) (17). TFIID has somewhat unusual binding properties, compared to most site-specific DNA binding proteins, in that it binds as a monomer (rather than a dimer) (17), requires elevated temperatures (4, 8, 17, 18), and exhibits very slow on and off rates (3, 4, 8, 17–19). The absence in TFIID of any of the motifs (leucine zipper, zinc finger, helix–turn–helix, etc.) common to many site-specific DNA-binding proteins (20) also suggests the presence of an unusual DNA binding domain. Among the structural features noted from sequence analysis are (*i*) two interrupted direct repeats (21), which could impart a partial similarity to the folded TFIID and which contain portions of *myc*-related helix–loop–helix (HLH) domains (HLH/*myc* homology) implicated in protein–protein interactions (22); (*ii*) a central basic core with a potentially helical lysine repeat region (23); and (*iii*) a region with sequence similarity to bacterial σ factors (23). It has been speculated (21, 23) that these motifs might be variously involved in intramolecular protein–protein interactions and DNA binding (direct repeats), intermolecular protein–protein interactions (HLH/*myc* homology), specific TATA recognition (σ homology), and either DNA binding or interactions with acidic activators (lysine repeat).

In light of the central role of TFIID in the regulation of preinitiation complex assembly and function, and its unusual DNA binding properties, it was important to map domains and specific residues important for these parameters. An earlier analysis (17) of internal deletions revealed that the N-terminal 62 residues of yeast TFIID were dispensible for binding and basal transcription, whereas all internal deletions between residues 62 and 240 (C terminus) destroyed both activities concommitantly. To extend these studies, and to define residues important for DNA binding versus basal or regulated transcription, we have constructed and analyzed a set of point mutants in the various motifs. This analysis has provided evidence for the involvement of the direct repeats, but not the central basic core, in DNA binding and a strict spacing requirement of the direct repeats. These findings confirm and extend the conclusions of a study (24) published since this work was completed.

MATERIALS AND METHODS

The creation of TFIID mutants employed oligonucleotide-directed mutagenesis as described (17). The correct introduction of the various mutations was verified by DNA sequencing. Expression and functional analysis are as described in the figure legends.

RESULTS

Involvement of Direct Repeats in DNA Binding. To map more precisely the TFIID DNA binding and functional domains, and to test their possible separability, a series of site-directed mutants in the conserved C-terminal core of yeast TFIID was constructed. Since lysine and leucine residues contribute extensively to the direct repeats and the central basic core, one series of mutations involved lysine to leucine and leucine to lysine changes. The mutant cDNA-encoded proteins were obtained by translation of the corresponding cDNA-derived RNAs in a reticulocyte lysate. Analysis of the ^{35}S-labeled polypeptides (Fig. 1) revealed that equal amounts of wt and mutant forms of TFIID were expressed. Curiously, the forms of TFIID with mutations in the HLH-related regions of the direct

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Abbreviations: wt, wild type; AdML, adenovirus major late; HLH, helix–loop–helix.

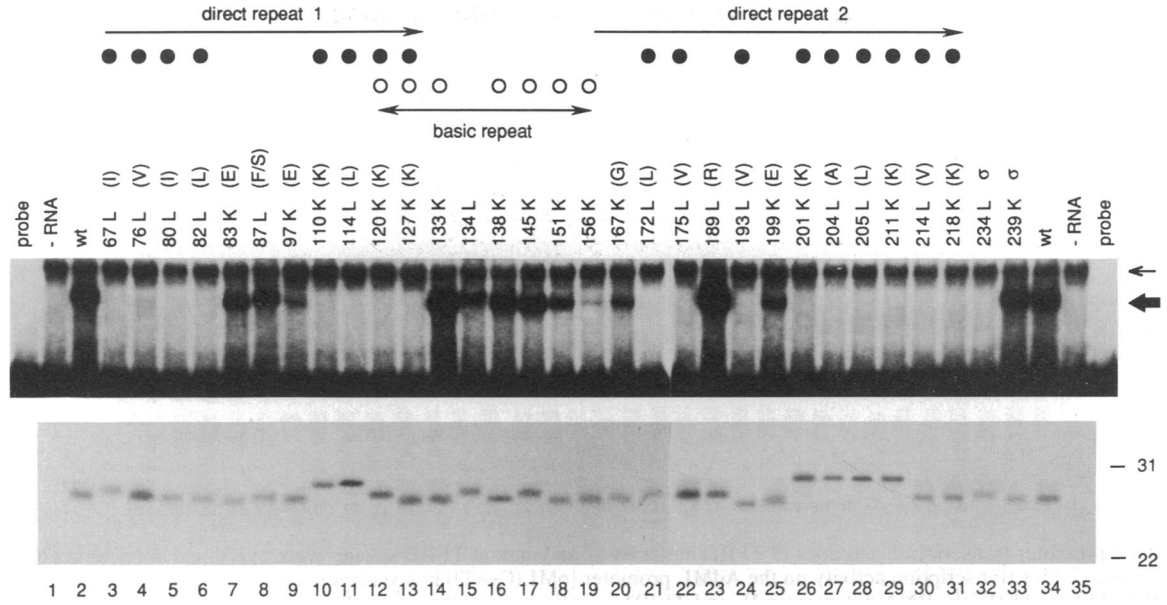

FIG. 1. Specific TATA box binding activities of TFIID mutants. cDNA-derived RNAs were translated in reticulocyte lysates, and the derived proteins were analyzed by electrophoretic mobility shift assay with a TATA-containing fragment (−138 to +46) of the adenovirus major late (AdML) promoter (25). (*Top*) Graphic representation of structural motifs in TFIID. For the analyzed TFIID mutations (at the residues indicated above each of the lanes in *Middle*), the open and closed circles indicate, respectively, lysine residues in the basic repeat region and lysine or leucine residues in the direct repeats that show identity or conservative changes between the direct repeats; the letters in parentheses indicate the amino acid at the corresponding position in the other direct repeat region. (*Middle*) Mobility shift analysis with TFIID proteins. The thick and thin arrows indicate, respectively, specific and nonspecific DNA–protein complexes. Lanes 2–34, analyses of the TFIID species mutated (K to L or L to K) at the positions indicated at the top of each lane. Lanes 1 and 35, negative controls with no TFIID RNA additions to the lysates. wt, Wild type. (*Bottom*) SDS/PAGE analysis of TFIID proteins labeled with [^{35}S]methionine. Sizes of molecular weight markers are indicated ($M_r \times 10^{-3}$).

repeats (at residues 110K, 114L, 201K, 204L, 205L, and 211K) showed drastic changes in mobility, indicating some unusual structural features in these regions.

The TATA binding activity was measured by mobility shift assay (23) with a consensus TATA-containing AdML promoter fragment (Fig. 1). This assay revealed a TFIID–DNA complex (thick arrow) shown by competition experiments with wt versus mutant TATA oligonucleotides to reflect specific binding to the TATA box (data not shown; see ref. 23). When the lysine residues present in the central basic core (residues 127–156) were analyzed, mutations in those residues (133K, 138K, 145K, 151K, and 156K) lying between the direct repeats had no effect on specific DNA binding, whereas mutations in those residues (120K and 127K) within and comprising part of the first direct repeat eliminated DNA binding. Thus, the latter lysine residues should be considered part of the conserved amino acids in the direct repeats. In a more extended analysis of residues within the direct repeats (Fig. 1, summarized in Fig. 3*b*), all mutations in residues that were identical or reflected conservative changes between the two direct repeats destroyed TATA binding activity (67L, 76L, 80L, 82L, 110K, 114L, 120K, and 127K in the first repeat and 172L, 175L, 193L, 201K, 204L, 205L, 211K, 214L, and 218K in the second repeat). Significantly, in all cases in which mutations at analogous positions in the direct repeats were analyzed (82L/172L, 110K/201K, 120K/211K, and 127K/218K), each mutation showed the same effect on TATA box binding activity. In contrast, mutations in nonconserved residues within the regions containing the two repeats had, in all cases, no effect on TFIID binding (residues 83K, 87L, and 97K in the first repeat and 189L and 199K in the second repeat). Although the reciprocal K to L and L to K mutations are rather drastic, it was only when these changes occurred in the residues comprising the direct repeats that binding was altered. Thus, the K to L and L to K mutations in nonconserved positions serve as important negative controls. Taken together, these results indicate that individual residues comprising the direct repeats, but not those comprising the restricted basic (lysine) repeat, are important for DNA binding.

Lack of Distinction Between Domains Required for TATA Box Binding and Basal Transcription Activities. To test for residues that might be important for basal transcription but not for DNA binding, the various mutant proteins were analyzed in a system reconstituted with the AdML promoter and other general factors from human cells. In this case, the wt and mutant TFIID proteins were obtained by expression of the corresponding cDNAs in bacteria. As shown in Fig. 2, the mutant proteins were expressed at levels comparable to the wt protein and showed relative mobilities similar to those observed for the proteins expressed in reticulocyte lysates. In transcription assays the mutant proteins showed either undetectable activities or activities comparable to that displayed by the wt protein (Fig. 2). A comparison of the effects of mutations on specific DNA binding versus basal transcription (summarized in Fig. 3) reveals that most of the mutations that altered TATA binding also inhibited basal transcription, whereas all of the mutants that were active in TATA binding were also active in basal transcription. Thus the analysis failed to reveal any residues that might be specifically required for basal transcription and suggested further that individual lysine residues in the basic repeat are not required absolutely for TATA binding or for basal transcription.

One surprising but interesting result of this analysis was that some mutations (at residues 110K, 114L, 120K, and 127K in direct repeat 1 and 211K and 218K in direct repeat 2) that inhibited DNA binding in the mobility shift assay nonetheless failed to show inhibitory effects on basal transcription. These results suggest that the capacity for stable TFIID binding in the absence of other factors is not necessarily essential for basal transcription and that cooperative interactions with other general factors may stabilize TFIID binding and compensate for the loss of some binding potential. Nonetheless, the apparently more stringent binding

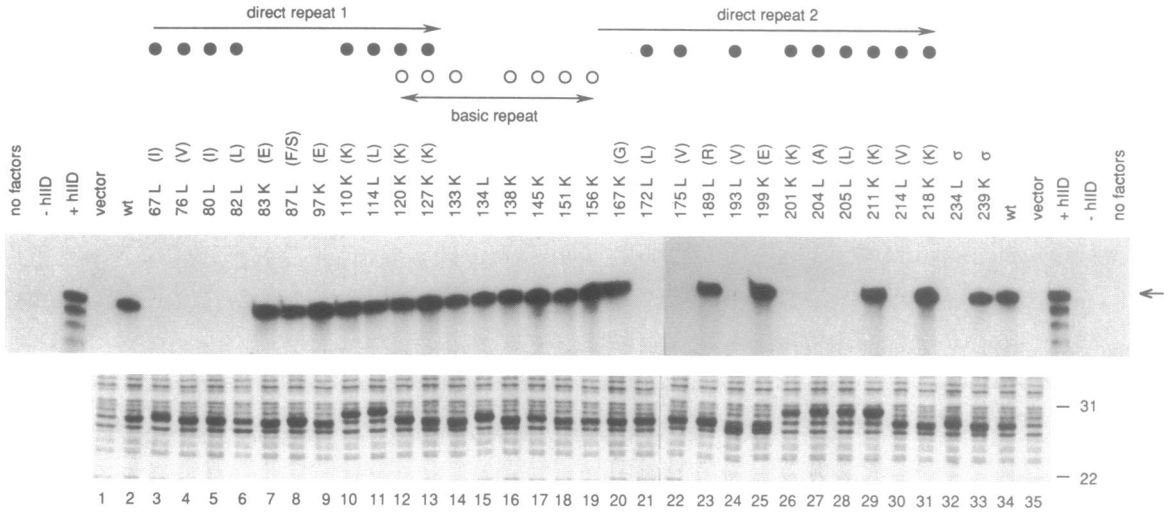

FIG. 2. Core promoter transcription activities of TFIID mutants. wt and mutant TFIID species were expressed in bacteria (26) and derived proteins were assayed for transcription activity on the AdML promoter [pML(C_2AT)] in a system reconstituted with a complete set of general factors (TFIIA, TFIIB, TFIIE/F, RNA polymerase II, and TFIID as indicated) as described (27). (*Top*) As indicated in the legend to Fig. 1. (*Middle*) Transcription activity of TFIID proteins. The arrow indicates the transcripts resulting from specific initiation. Lanes 2–34, analyses of the TFIID species mutated at the positions indicated above each lane. Lanes 1 and 35, analysis of extracts from bacteria transformed with an expression vector lacking TFIID coding sequences. Other controls with human TFIID or with no TFIID or no factors are as indicated. (*Bottom*) SDS/PAGE analysis of TFIID proteins expressed in bacteria. Sizes of molecular weight markers are indicated ($M_r \times 10^{-3}$).

conditions of the mobility shift assay have allowed identification of TFIID residues that are important for intrinsic binding strength and that might be potentially more important for transcription of other (weaker) promoters. It is also interesting to note that those mutated residues that affected binding but not basal transcription, as well as those that resulted in abnormal SDS gel mobilities (above), fall within the regions of the direct repeats that contain sequence similarities to the HLH domain.

Strict Spacing Requirements for the Direct Repeats. Given the involvement of both direct repeats in DNA binding, it was of interest to determine whether alterations in their spacing affected DNA binding and transcriptional activation. For this purpose mutants with one-, two-, or three-residue insertions (alanines) or deletions near residue 141 in the center of the basic repeat and near residue 156 (see Fig. 4) in the region between the basic repeat and direct repeat 2 were constructed, expressed, and analyzed as described above. As shown in Fig. 4, each of the 12 mutations completely destroyed DNA binding (Fig. 4*a*) and basal transcription (Fig. 4*b*) activities. These results suggest a rather inflexible TFIID structure, with a fixed spacing and orientation of the two direct repeats that is necessary for DNA binding (and activation) and determined by the structure of the intervening basic repeat.

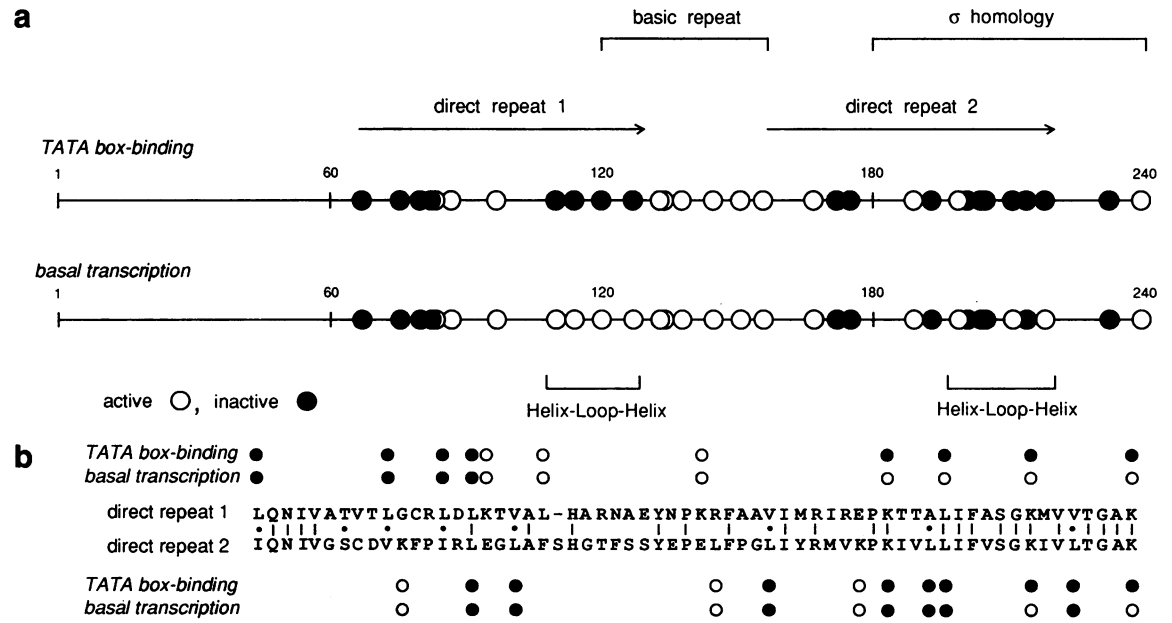

FIG. 3. Schematic of structural motifs in TFIID and summary of the effects of mutations within the regions comprising the basic repeat and the two direct repeats. (*a*) The TATA binding and transcription data for the 31 TFIID mutants analyzed in Figs. 1 and 2 are summarized. Regions encompassing residues contributing to the interrupted direct repeats (21), the basic (lysine) repeat (23), the σ homology (23), and the partial HLH regions (22) are indicated. (*b*) Detailed comparison of the effects of mutations in residues within the direct repeat regions. The direct repeat 1 and direct repeat 2 regions encompass residues 67–127 and 157–218, respectively. Open and closed circles indicate, respectively, maintenance and loss of activity. Basal transcription for 201K is listed as inactive, although a weak activity can be observed.

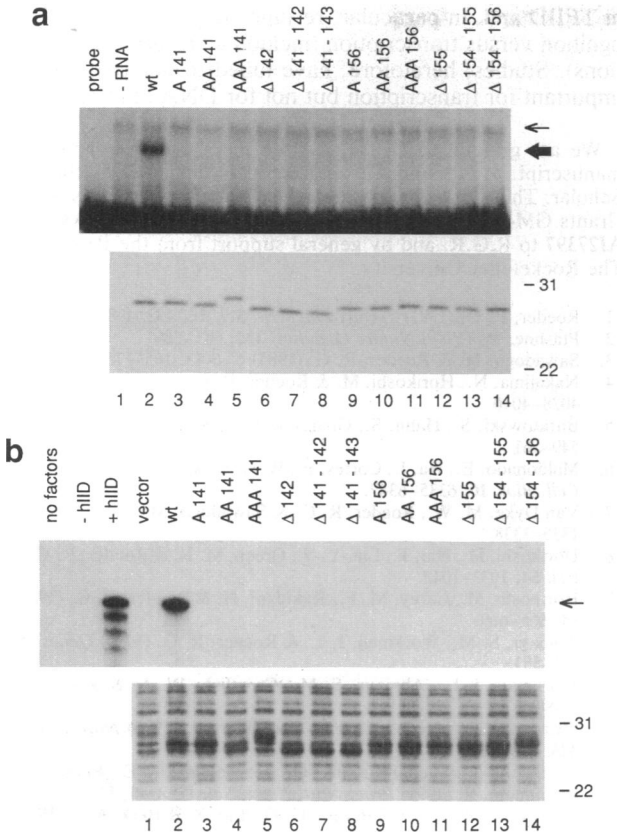

FIG. 4. TATA box binding and core promoter transcription activities of small insertion or deletion mutants in the basic repeat region. (*a*) Mobility shift (upper panel) and SDS/PAGE (lower panel) analyses of TFIID mutants expressed in reticulocyte lysates as in Fig. 1. The thick and thin arrows indicate specific (TFIID–TATA) and nonspecific DNA–protein complexes, respectively. (*b*) Transcription (upper panel) and SDS/PAGE (lower panel) analyses of TFIID mutants expressed in bacteria. The wt and mutant yeast TFIID species analyzed are indicated above each lane. For the insertion mutants, one, two, or three alanine residues were inserted at either residue 141 or 156, and for the deletion mutants, one, two, or three residues were deleted, as indicated. Sizes of molecular weight markers are indicated ($M_r \times 10^{-3}$).

DISCUSSION

As an extension of our earlier study (17) showing that the nonconserved N terminus of yeast TFIID is not essential for DNA binding, the present analysis of an extensive series of point mutants in the conserved C-terminal core demonstrates that the direct repeats, separated by the basic (lysine) repeat region, comprise a unique bipartite DNA binding domain. A schematic model in which the DNA is contacted by the symmetrically folded repeats, along with the σ homology region that overlaps the C-terminal repeat, is shown in Fig. 5. Further details of the model, including features of the various TFIID structural motifs important for DNA binding and promoter activation, as well as aspects of DNA structure, are discussed below.

A Bipartite DNA Binding Domain for TFIID. Twenty-five independent mutations in the direct repeat regions were examined and, without exception, all of those in identical or conserved residues lost DNA binding activity whereas those in nonconserved residues did not. At the same time, mutations in lysine residues comprising the basic repeat in the intervening region were without effect. These results, which included analysis of residues at equivalent positions in each of the direct repeats, clearly establish the bipartite nature of the DNA binding domain. The results of a recent study of dominant negative mutations in the direct repeats of TFIID

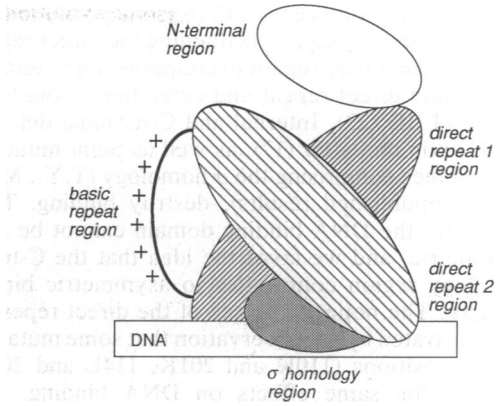

FIG. 5. Proposed model for TFIID binding to DNA. The model emphasizes (*i*) a pseudosymmetrical structure of the DNA binding domain contributed by the direct repeats, giving a structure formally equivalent to dimeric site-specific binding proteins; (*ii*) contribution of the σ homology/C terminus to the DNA binding domain with concomitant alteration of the symmetry; and (*iii*) the lack of involvement of the nonconserved N terminus and the basic repeat region (linking the direct repeats) in DNA binding. Although not shown, regions of the direct repeats are believed to interact with the minor groove of DNA within the TATA element and to bend the flanking regions toward the upper surface of the protein. Note that more detailed models involving antiparallel β-ribbon structures in the direct repeats (28) can change the orientation and relative disposition of the N-terminal, C-terminal, and basic repeat regions.

also support this view (24). That a precise orientation of the structures dictated by the direct repeat is required for DNA binding was indicated by the detrimental effect of various spacing mutations (±1–3 residues) in the basic repeat region. Our recent studies have indicated that TFIID binding to the TATA element involves backbone phosphate contacts and base contacts through the minor groove (29), with concomitant DNA bending (26). Others (30) have noted possible sequence relationships between integration host factor (IHF) and regions in the direct repeats. These and the present results have led us to suggest (29) that the DNA backbone (phosphate) and the minor groove contacts may involve specific residues symmetrically disposed in antiparallel β ribbons formed by parts of each direct repeat, analogous to the models for IHF and HU proteins (31, 32). In general, β-sheet interactions in the minor groove show low sequence specificity (28); however, stable TFIID–TATA element interactions could result from specific recognition by TFIID of changes in DNA geometry after DNA bending. Since most site-specific DNA binding proteins recognize the DNA as dimers, whereas TFIID binds to the TATA element as a monomer (17), it is relevant to note that the proposed model for the DNA binding domain of TFIID yields the formal equivalent of a dimeric protein. It has been suggested earlier (references in ref. 24) that the TFIID structure may have arisen during evolution by duplication of an ancestral gene whose product recognizes its target as a dimer.

Another question that arises concerns the basis for asymmetric transcription and the possible role therein of TFIID binding. Asymmetric promoter interactions of TFIID, apparent in natural human TFIID (3, 4, 33) and in the recombinant yeast TFIID analyzed here (19, 26, 29, 33), could be determined by asymmetric TATA elements (ref. 34, but see also ref. 35), by flanking sequence context effects (4, 19, 29, 33), or by cooperative interactions with other factors interacting at the initiation region (36). Relevant to this point, yeast TFIID contacts are skewed toward the 3′ end of the TATA element of the major late promoter and involve contacts in the major groove of the flanking (G+C)-rich region (29). Regarding TFIID itself, the asymmetry and specificity of

binding may reflect sequence variations (some evolutionarily conserved) between the regions containing the direct repeats as well as the σ homology region overlapping the C-terminal end of the second direct repeat and extending to the C-terminal end of TFIID (23). Internal and C-terminal deletions that impinge upon the latter (17), as well as point mutations in specific residues comprising the σ homology (T.Y., M.H., and R.G.R., unpublished results), destroy binding. Thus, though bipartite, the DNA binding domain cannot be completely symmetric, and we favor the idea that the C-terminal/σ homology region contributes to asymmetric binding and specificity. The nonequivalence of the direct repeats is further demonstrated by the observation that some mutations in equivalent positions (110K and 201K, 114L and 205L), though having the same effects on DNA binding, have different effects on transcription (Fig. 3*b*).

Domains for Interactions with Other Factors. The chemical characteristics (high basic charge) and potential α-helical structure of the central basic (lysine) repeat led to previous speculation that this region might be involved in direct interactions either with DNA or with acidic activators (23). The present analysis with single point mutations in the basic repeat residues suggests that this region is not directly involved in DNA interactions but leaves open the possibility of a major involvement in activator function. Related, recent studies have also implicated the basic repeat in TFIID interaction with the adenovirus E1A activator (14). On the other hand, we recently have found that TFIIA interactions with TFIID involve specific residues in the basic repeat as well (D. K. Lee, J. DeJong, S. Hashimoto, T. K. Kim, M.H., and R.G.R., unpublished results). These results are consistent with previous speculation of a functional interplay between TFIIA and transcriptional activators (37).

One intriguing result is the observation that some of the direct repeat mutations that eliminated DNA binding in the mobility shift assay were without effect on basal transcription (Fig. 3). Though seemingly paradoxical, these results are explained most easily by more stringent conditions for DNA binding in the electrophoretic mobility shift assay and the possibility of compensating cooperative interactions with other factors in the transcription assay. Interestingly, the mutations that show these divergent effects (on DNA binding versus transcription) fall within the direct repeat regions with sequence similarity to HLH proteins. HLH domains are known to potentiate homomeric and heteromeric protein–protein interactions between various regulatory factors (reviewed in ref. 38). On this basis it was suggested previously (22) that the related regions in TFIID might facilitate interactions with other proteins and/or intramolecular interactions between the direct repeats of TFIID. Similarly, the compensation for the loss of TFIID binding potential in the HLH region mutants could reflect direct interactions of these domains with related domains in other general factors such as TFIIE (39) or facilitated interactions between the direct repeats by factors (e.g., TFIIA) interacting elsewhere. Analyses of appropriate TFIID mutants in conjunction with the various general initiation factors will allow these hypotheses to be tested.

Promoters lacking TATA elements nonetheless require TFIID (refs. 40 and 41; T. Yoganathan, M.H., S.H., R.G.R., and B. H. Sells, unpublished results), whose interactions at the promoter are likely to be mediated in part by cooperative interactions with factors interacting either at other core promoter elements (see ref. 36) or at adjacent regulatory elements (3, 8, 9). It should be possible to use such promoters (and associated factors) to map promoter-dependent factor interaction domains on TFIID independently of the ability of TFIID to interact with its primary recognition site (TATA element). This may allow a finer dissection of structure–function relationships in TFIID and, in particular, residues important for DNA recognition versus transcription (including various factor interactions). Studies, heretofore, have failed to determine residues important for transcription but not for DNA binding.

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