Transcription Factor IID Mutants Defective for Interaction with Transcription Factor IIA

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Transcription factor IID (TFIID) recognizes the TATA element of promoters transcribed by RNA polymerase II (RNAPII) and serves as the base for subsequent association by other general transcription factors and RNAPII. The carboxyl-terminal domain of TFIID is highly conserved and contains an imperfect repetition of a 60–amino acid sequence. These repeats are separated by a region rich in basic amino acids. Mutagenesis of the lysines in this region resulted in a conditional phenotype in vivo, and the mutant proteins were defective for interactions with transcription factor IIA in vitro. Binding of TFIID to DNA was unaffected. These results suggest that the basic domain of TFIID is important for protein-protein interactions.

TRANSCRIPTION FACTOR IID (TFIID) is essential for positioning the RNA polymerase II (RNAPII) initiation complex on promoter DNA (*1–4*). A multifunctional protein, TFIID binds in a sequence-specific manner to promoter DNA and interacts with the general transcription initiation factors TFIIA and TFIIB (*1–6*). Regulation of basal transcription initiation may be mediated by the interaction of other factors with TFIID (*2, 5, 7–9*). It is therefore essential to identify the domains of TFIID that carry out each of its functions. The evolutionarily conserved COOH-terminal region of TFIID consists of an imperfectly repeated 60–amino acid sequence linked by a conserved basic domain (*10, 11*). Genetic studies suggest that the repeats of TFIID participate in DNA binding (*12*). We sought here to explore the function of the highly conserved basic domain that links the repeats.

The distinguishing character of the linker is its high content of basic residues that could theoretically lie along one face of an α helix (*11*). Therefore, we targeted the lysines in this region for mutagenesis (Fig. 1A). Codons for lysines at positions 133, 138, 145, 151, and 156 were individually changed to leucine codons, and the mutant genes were introduced into yeast by plasmid-shuffling (*13*). Recipient strains carried a deletion of the chromosomal TFIID gene or genes. The mutants all grew normally at 22°, 30°, and 36°C. However, at 38°C their growth was different from that of wild type (*14*). In a haploid yeast strain, the mutant TFIID genes caused slowed growth. In a diploid strain, the K133L mutation (lysine to leucine at amino acid position 133) caused a slow growth phenotype, and the other mutants were nonviable. The different phenotypes in haploid and diploid yeast strains can be explained in terms of the amount of TFIID relative to the ploidy of the cell. The mutant phenotypes were recessive, as they could be rescued by a plasmid-borne or chromosomal copy of the wild-type TFIID (wtTFIID) gene (*14*). These results indicate that the basic region is important for TFIID function in vivo.

Because each of the single mutations produced a similar phenotype, it seemed likely that the individual lysines were contributing to the same function of TFIID. If so, mutation of two lysines might further impair TFIID function and yield a more severe phenotype. To test whether the effects of the lysine mutations were additive, we constructed double mutants by changing Lys^{133} and one of the other lysines to leucines and then tested their ability to support growth at various temperatures. Double mutants K133,151L and K133,156L exhibited phenotypes similar to K151L and K156L (*14*). However, the double mutants K133,138L and K133,145L were nonviable in haploids and diploids at 37°C (Fig. 1B). Thus, we conclude that lysine residues 133, 138, and 145 together contribute to the function of the TFIID linker domain.

In order to explore their molecular defect, the single and double mutant proteins were produced in bacteria (*15*). The single mutant proteins behaved like wtTFIID in all in vitro assays. During purification, the double mutant proteins eluted from negatively charged columns at a lower salt concentration than did the single mutant or wild-type proteins (*14*). This suggests that the mutated lysines are normally exposed on the surface of the protein and may be in a position to interact with other components of the initiation complex. It was suspected that the basic residues of this region might participate in DNA binding. However, all mutant proteins exhibited normal binding to the adenovirus major late promoter and the CYC1 promoter at 30° and 37°C (Figs. 2 and 3 and *14*). Deoxyribonuclease (DNAse) I protection experiments revealed no differences between mutant and wild-type binding (*14*). Because the mutant proteins retain a DNA binding–competent conformation at 37°C, it is unlikely that the in vivo defect is a result of destabilization of the protein.

The mutant TFIID proteins were tested for the ability to complement a reconstituted mammalian in vitro transcription reaction that lacked TFIID (*6*). No difference was observed between wild-type and mutant proteins (*14*). Analysis of initiation complexes formed by mutant or wild-type yeast TFIID and mammalian initiation factors (*1, 15*) on the adenovirus major late promoter (AdMLP) revealed no qualitative differences, although the amount of complexes formed with the mutant proteins was slightly less than that with wtTFIID (*14*). Both of these analyses were performed under conditions where transcription factor IIA (TFIIA) was not required for initiation complex formation.

We next tested whether the basic linker region was important for interaction with yeast TFIIA (yTFIIA) (*16*). The wild-type

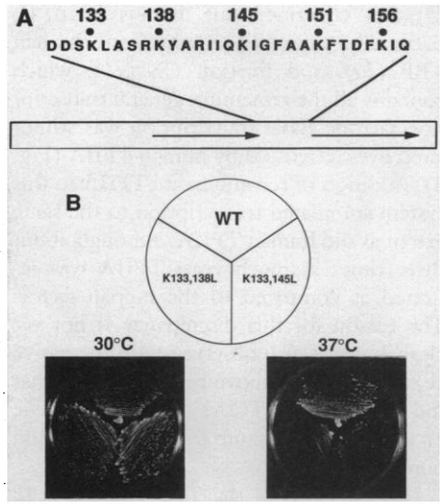

Fig. 1. Conditional phenotype of TFIID lysine mutants. (**A**) Schematic of the yeast TFIID protein. The repeats of TFIID are shown as arrows. The protein sequence of the basic region linking the repeats is shown. The mutated lysines are numbered. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (**B**) Phenotypes of double point mutants. The double mutants were derived from the single mutants by conventional cloning techniques. The amino acid changes are of the indicated lysines to leucines (K-residue numbers-L). The wild-type (WT) or the doubly mutated genes were used to replace the *spt*15-21 allele in strain FY567 (*22*). Similar results were obtained with plasmid-borne copies of the mutant genes (*13*). Strains were streaked on YPD plates and allowed to grow for 2 days at 30° or 37°C. While the wild-type gene supported growth at both temperatures, the mutants were temperature-sensitive.

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or doubly mutated TFIID proteins were incubated with AdMLP DNA and yTFIIA (*1*), and the resulting complexes were resolved by native gel electrophoresis (Fig. 2). At 30°C, mutant K133,145L and yTFIIA formed a complex with mobility similar to wtTFIID and yTFIIA (Fig. 2A, lanes 2 and 6), but a complex between K133,138L and yTFIIA migrated somewhat faster (Fig. 2A, lane 4). The bands corresponding to mutant TFIID-yTFIIA complexes were broad, indicating that some dissociation may have occurred during electrophoresis. At 37°C, the mobility of the wtTFIID-yTFIIA complex was the same as at 30°C. However, the mutant TFIID-yTFIIA complexes migrated much faster at 37°C (lanes 10 and 12). Similar

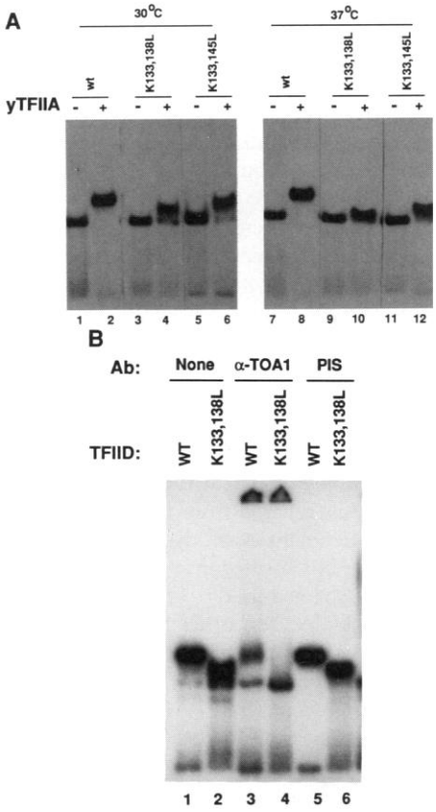

Fig. 2. Altered interactions of TFIID lysine mutants with TFIIA. (**A**) Temperature-sensitive interaction between mutant TFIID and yTFIIA. Binding reactions containing yTFIIA, probe DNA, and either wild-type (lanes 1, 2, 7, and 8) or mutant TFIID (lanes 3 to 6 and 9 to 12) were assembled on ice. The reaction mixture was divided, and one portion was incubated at 30°C (lanes 1 to 6) and the other at 37°C (lanes 7 to 12). The 30°C reaction was resolved by native gel electrophoresis at room temperature, while electrophoresis of the 37°C reaction was performed at 37°C (*1, 15*). (**B**) Disruption of complexes by antibodies to yTFIIA. Complexes were assembled as in (A) with wtTFIID (lanes 1, 3, and 5) or mutant K133,138L (lanes 2, 4, and 6). In addition, lanes 3 and 4 received immune serum (1 μl) against the large subunit of yTFIIA (α-TOA1) while lanes 5 and 6 received 1 μl of preimmune serum (PIS).

electrophoretic mobility shift patterns were observed when the yeast *CYC1* promoter was used as the probe, demonstrating that this effect is not promoter-specific (*14*).

In order to verify that the aberrant mobility complexes were composed of mutant TFIID and yTFIIA, we used polyclonal antisera to probe the complexes. The complexes were completely dependent on added TFIID and were disrupted by antibodies to TFIID (*14*). Antibodies to the large subunit of yTFIIA (*17*) disrupted both the wild-type and mutant TFIID-yTFIIA complexes (Fig. 2B, lanes 3 and 4). These results confirm the composition of the complexes. The mobility difference between mutant TFIID-yTFIIA and wt-TFIID-yTFIIA complexes suggests that a TFIIA-dependent conformation change occurs in either the proteins or DNA within the complex and that this conformational change is affected by the TFIID mutations. The fact that this conformation is more affected at higher temperatures suggests that this may be the molecular defect responsible for the temperature-sensitive phenotype in vivo.

We observed a more striking difference between mutant and wtTFIID proteins when they were incubated with human TFIIA (*18*). The mutants were completely unable to interact with human TFIIA, even at lower temperatures (Fig. 3). Thus, despite the conservation of the TFIID linker region, some species-specific contacts must exist between yTFIIA and mutant yTFIID that allow formation of the aberrant mobility complexes. The results presented here demonstrate that lysines within the basic repeat region of TFIID are essential for normal interactions with TFIIA.

Several observations suggest that these basic residues might directly contact TFIIA. The wild-type and mutant TFIID-promoter complexes migrated with identical mobility, arguing against an overall conformation difference in TFIID. The mutant proteins had reduced affinity for negatively charged columns relative to wild-type protein, suggesting that the lysines are normally exposed on the surface of the protein. Finally, TFIIA binds tightly to positively charged columns (*16–19*) and is therefore predicted to have exposed acidic regions.

Several other proteins interact with TFIID, at least in mammalian cells (*8, 9*). Some of these proteins are not required for basal transcription initiation, but are required for response to transcriptional activators. Factor NC1 may repress initiation by binding to TFIID (*8*); this interaction is incompatible with TFIID-TFIIA binding, suggesting that TFIIA and NC1 may interact with the same domain of TFIID. Another factor, TFIIG, has been found to have some functional properties in common with

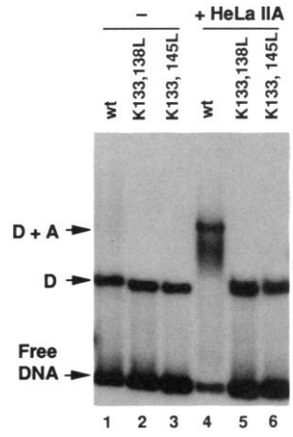

Fig. 3. Loss of interactions between mutant TFIID and human TFIIA. Binding reactions that contained wild-type (lanes 1 and 4) or mutant TFIID (lanes 2, 3, 5, and 6) were incubated at room temperature with (lanes 4 to 6) or without (lanes 1 to 3) TFIIA derived from HeLa cells (*18*), and complexes were analyzed by native polyacrylamide gel electrophoresis (*1, 15*).

TFIIA (*20*) and may also interact with the basic domain of TFIID.

Studies in vitro suggest that initiation complexes containing TFIIA are not stimulated by upstream transcriptional activators (*8*). One possible explanation for this observation is that binding of TFIIA to TFIID masks a domain of TFIID that can interact with a component of the activation machinery [for example, an acidic activation domain or an adapter molecule (*7, 9, 21*)]. It will be of interest to determine whether the basic region mutations affect response to upstream activators.

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13. An M13 phage containing the *SPT15* (TFIID) gene was used to generate single-stranded DNA. Lysine codons (AAA or AAG) at the indicated positions were mutated to leucine codons (TTG) by oligonucleotide-directed mutagenesis as described [T. A. Kunkel, J. D. Roberts, R. A. Zakour, *Methods Enzymol.* **154**, 367 (1987)]. The mutated genes were cloned into the polylinker of plasmid pUN45, which contains the *TRP1* gene as well as *ARS* and *CEN* sequences [S. J. Elledge and R. W. Davis, *Gene* **70**, 303 (1988)]. The plasmids were introduced into yeast by plasmid-shuffling as described [J. Boeke, J. Truehart, B. Natsoulis, G. R. Fink, *Methods Enzymol.* **154**, 164 (1987)]. The haploid strain was YDE11 (*MATa, ura3-52, trp1Δ1, leu2Δ1, spt15Δ::LEU2*, {pDE32-7}), and the diploid strain was YDE13 (*MATa/MATα, ura3-52/ura3-52, trp1Δ1/trp1Δ1, leu2Δ1/leu2Δ1, spt15Δ::LEU2/spt15 Δ::LEU2*, {pDE32-7}). pDE32-7 carries the wild-type *SPT15* (TFIID) gene, a 2-μm replication origin, and the *URA3* gene. After loss of the pDE32-7, the strains were transferred as patches onto YPD (1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, and 2% Bacto-agar) plates and then replica plated. Incubation was on YPD plates for 2 days at the appropriate temperatures.
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22. Strain FY567 contains the *spt15-21* allele, which suppresses the His⁻ and Lys⁺ phenotypes caused by *his4-917δ* and *LYS2-173r2* insertion mutants [D. M. Eisenmann, C. Dollard, F. Winston, *Cell* **58**, 1193 (1989)]. Therefore, the strain is His⁺ and Lys⁻. Fragments that contained the double mutant TFIID genes, which do not suppress the insertions (*14*), were used to transform strain FY567. Cells in which the spt15-21 allele had been replaced were isolated by selecting for a His⁻ and Lys⁺ phenotype. Replacement by the double mutant TFIID allele was confirmed by testing for the temperature-sensitive phenotype.
23. We thank F. Winston, D. Eisenmann, K. Arndt, and other members of the Winston lab for their generosity with yeast strains, materials, and advice; S. Hahn for the generous gift of antiserum to TFIIA; J. Bittner for help in purification of proteins; and R. Buratowski, G. Fink, A. Koleske, D. Pellman, A. Sachs, and B. Tidor for critical reading of the manuscript. Supported by NIH grant R29-GM46498 and grants to the Whitehead Fellows program from the Lucille P. Markey Charitable Trust and the W. M. Keck Foundation.

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A Human Gene Responsible for Zellweger Syndrome That Affects Peroxisome Assembly

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The primary defect arising from Zellweger syndrome appears to be linked to impaired assembly of peroxisomes. A human complementary DNA has been cloned that complements the disease's symptoms (including defective peroxisome assembly) in fibroblasts from a patient with Zellweger syndrome. The cause of the syndrome in this patient was a point mutation that resulted in the premature termination of peroxisome assembly factor–1. The homozygous patient apparently inherited the mutation from her parents, each of whom was heterozygous for that mutation.

ZELLWEGER SYNDROME (ZS) [McKusick 214100 (*1*)] is a fatal autosomal recessive disease, with clinical evidence of severe neurologic abnormalities, dysmorphic features, hepatomegaly, and multiple renal cysts (*2*). This syndrome is characterized by the absence of catalase-containing particles (peroxisomes) (*3*), resulting in multiple metabolic disturbances such as defects in peroxisomal β-oxidation and plasmalogen biosynthesis (*4, 5*). Earlier studies suggested that ineffective assembly of peroxisomes was likely to be the syndrome's primary effect (*6, 7*). We previously showed that a cloned rat cDNA encoding the peroxisome assembly factor–1 (PAF-1) (*8*), a 35-kD peroxisomal integral membrane protein, restores the assembly of peroxisomes in one peroxisome-deficient Chinese hamster ovary (CHO) cell mutant (Z65) (*9*).

There are five to eight complementation groups that affect generalized peroxisomal disorders such as ZS, neonatal adrenoleukodystrophy, infantile Refsum's disease, and hyperpipecolic acidemia, implying that several genes are required for peroxisome assembly (*10–12*). To find clues to the etiology of generalized peroxisomal disorders, we searched for patients affected in the same complementation group as the CHO mutant Z65. We used fibroblasts from nine American and Japanese patients with generalized peroxisomal disorders from eight complementation groups. Fusion of the fibroblasts from all patients with Z65 resulted in the appearance of peroxisomes (Fig. 1A), thereby implying that all the patients' pathogenic genes differed from that of Z65. But one female infant, MM, diagnosed with ZS, could not be classified into any of the previously characterized complementation groups. Unlike the other hybrids, the cell hybrids of MM's skin fibroblasts with Z65 cells lacked peroxisomes (Fig. 1, B and C).

We transfected rat PAF-1 cDNA into

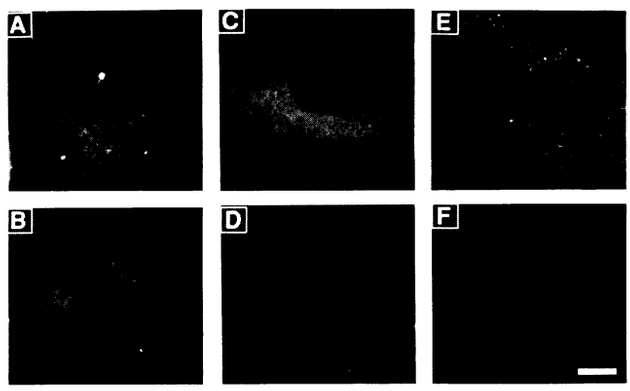

Fig. 1. Immunofluorescent staining of peroxisomes with the use of an antibody to catalase. (**A**) Hybrids (*14*) of Z65 cells and human fibroblasts GM228 (Human Genetic Mutant Cell Repository, Camden, New Jersey) from a patient with ZS [group E or group 1 (*12*)]. (**B**) Hybrids of Z65 with MM's fibroblasts (*15*). (**C**) MM's fibroblasts used for fusion in (B). (**D**) MM's fibroblasts transfected with the rat PAF-1 cDNA (*8, 16*). (**E**) MM's fibroblasts transfected with human PAF-1 cDNA. (**F**) MM's fibroblasts transfected with a PAF-1 cDNA isolated from MM's own fibroblasts. Antibodies to rat catalase [(A) and (B)] and human catalase [(C) through (F)] were used. Numerous catalase-positive particles, peroxisomes, were present in the cytoplasm of cells in (A), (D), and (E). Bar, 15 μm.

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