Functional Differences between Yeast and Human TFIID Are Localized to the Highly Conserved Region

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Summary

TFIID, the general transcription factor that binds TATA promoter elements, is highly conserved throughout the eukaryotic kingdom. TFIID is highly conserved throughout the eukaryotic kingdom. TFIID isolated from yeast cells can functionally substitute for human TFIID in transcription reactions reconstituted in vitro with basal initiation factors prepared from human cells (Buratowski et al., 1988; Cavallini et al., 1989). Conversely, TFIID isolated from human cells can functionally replace yeast TFIID in reconstituted yeast nuclear extracts (Fianaqan et al., 1990). Yeast and human TFIID have nearly identical DNA sequence requirements for TATA-dependent transcription in vitro (Wobbe and Struhl, 1990), and TFIID from an evolutionarily distant fission yeast, Schizosaccharomyces pombe, can carry out the essential functions necessary for cell viability in S. cerevisiae (Fikés et al., 1990). These functional similarities among eukaryotic TFIID's represent another of the accumulating pieces of evidence for a common molecular mechanism of transcriptional initiation by RNA polymerase II.

Introduction

Most eukaryotic promoters contain a TATA element that is required for accurate and efficient transcription by RNA polymerase II. TATA elements, consensus sequence TATAAA, are specifically recognized by the general transcription factor TFIID (Davison et al., 1983; Parkor and Topol, 1984; Nakajima et al., 1988). Binding of TFIID to the TATA element is the first step in assembling the active transcription complex that is composed of multiple initiation factors in addition to RNA polymerase II (Fire et al., 1984; Reinberg et al., 1987; Van Dyke et al., 1988; Buratowski et al., 1989). Detailed mutational analysis of TFIID element function indicates that the level of TFIID-dependent transcription in vitro strongly, but not absolutely, correlates with the level of transcriptional activation in vivo (Wobbe and Struhl, 1990). Genetic (Struhl, 1986; Homa et al., 1988; Simon et al., 1988) and biochemical (Sawadogo and Roeder, 1985; Abmayr et al., 1988; Hai et al., 1988; Hori-koshi et al., 1988a, 1988b) experiments suggest that TFIID functionality interacts with upstream activator proteins that stimulate TATA element-dependent transcription, and TFIID can directly and selectively associate with the acidic transcriptional activation region of VP16 in vitro (Stringer et al., 1990). However, the question of whether upstream activator proteins stimulate transcription by directly contacting TFIID or by interacting with a distinct protein that serves as an adaptor to the basic transcription machinery (Berger et al., 1990; Kambadur et al., 1990; Kelleher et al., 1990; Meisterernst et al., 1990; Pugh and Tjian, 1990) remains unanswered and controversial. As expected for a general transcription factor, TFIID is required for the viability of the yeast Saccharomyces cerevisiae, and mutant TFIID proteins can result in strains with altered transcriptional initiation patterns (Eisenmann et al., 1989).

TFIID is highly conserved throughout the eukaryotic kingdom. TFIID isolated from yeast cells can functionally substitute for human TFIID in transcription reactions reconstituted in vitro with basal initiation factors prepared from human cells (Buratowski et al., 1988; Cavallini et al., 1989). Conversely, TFIID isolated from human cells can functionally replace yeast TFIID in reconstituted yeast nuclear extracts (Fianaqan et al., 1990). Yeast and human TFIID have nearly identical DNA sequence requirements for TATA-dependent transcription in vitro (Wobbe and Struhl, 1990), and TFIID from an evolutionarily distant fission yeast, Schizosaccharomyces pombe, can carry out the essential functions necessary for cell viability in S. cerevisiae (Fikés et al., 1990). These functional similarities among eukaryotic TFIID's represent another of the accumulating pieces of evidence for a common molecular mechanism of transcriptional initiation by RNA polymerase II.

The deduced amino acid sequences of TFIID's from baker's yeast (Cavallini et al., 1989; Hahn et al., 1988; Hori-koshi et al., 1989; Schmidt et al., 1989), fission yeast (Fikés et al., 1990; Hoffmann et al., 1990a), fly (Hoey et al., 1990; Muhich et al., 1990), plant (Gasch et al., 1990), and human (Hoffmann et al., 1990b; Kao et al., 1990; Pette-son et al., 1990) are remarkably similar, with the C-terminal 180 amino acids being at least 80% identical in sequence. This conserved "core" domain contains an interrupted direct repeat, a short basic repeat, and a region rich in glutamine residues.

Despite the striking structural and functional similarities among eukaryotic TFIID's, recent observations suggest the possibility that these proteins may differ significantly in their ability to respond to upstream activator proteins. Competition experiments involving purified TFIID proteins suggest that human and fly TFIID can respond to the Sp1
activator protein, whereas yeast TFIID cannot (Pugh and Tjian, 1990). Moreover, the human N-terminal region appeared to be required for transcriptional stimulation by Sp1 or GAL4-VP16 under conditions in which endogenous TFIID in the extract was significantly inactivated by heat treatment (Peterson et al., 1990). From these rather indirect biochemical experiments, it has been proposed that species-specific behavior of TFIID reflects the differential interaction of putative adaptor proteins with the divergent N-terminal regions.

In this paper, we demonstrate that human TFIID cannot replace yeast TFIID for cell growth. Surprisingly, the nonconserved N-terminal region is not responsible for this functional distinction between yeast and human TFIID, and in fact is relatively unimportant for yeast TFIID function in vivo. Analysis of yeast–human hybrid TFIID s indicates that several regions within the conserved core are responsible for the phenotypic difference, with some regions being more important than others. Thus, subtle structural differences between these proteins have a profound effect on some essential function(s) of TFIID in yeast cells.

Results

The Complementation Assay for Determining TFIID Function In Vivo
We used the plasmid shuffle complementation assay (Boeke et al., 1987) to determine the function of the various TFIID proteins in yeast cells (Figure 1). In brief, we constructed a parental yeast strain carrying a deleted version of the endogenous TFIID gene. Because TFIID is essential for cell growth (Eisenmann et al., 1989), this parental strain also contains a centromeric plasmid carrying a wild-type copy of the yeast TFIID gene and the URA3 selectable marker. The TFIID constructs to be tested are introduced on a centromeric plasmid containing the TRP1 marker. The resulting strains contain two plasmid-borne copies of TFIID, each of which can be lost at a frequency of about 1% per generation. These strains are plated on medium containing 5-fluoroorotic acid, a uracil analog that prevents the growth of cells containing the URA3 gene, thereby selecting against cells carrying the wild-type yeast TFIID gene present on the URA3 plasmid. Thus, the ability to grow under these conditions directly reflects the functionality of the TFIID protein encoded by the TRP1 plasmid. This is a stringent test for TFIID activity because the derivative in question must carry out all essential functions to permit cell growth. However, this complementation assay does not address potential nonessential functions of TFIID.

Human TFIID Does Not Substitute for Yeast TFIID In Vivo
To determine if human TFIID could substitute in vivo for yeast TFIID, we wanted to express the human protein in as similar a context as possible to that of yeast TFIID. We made a precise fusion of the yeast TFIID promoter and 5′-untranslated region to the beginning of the human TFIID protein-coding region. In addition, we precisely fused the end of the human coding region to the 3′-untranslated and transcriptional termination regions of the yeast gene. This artificial gene, therefore, is identical to the yeast TFIID gene used in this study in all respects except for the coding region. Moreover, by using single-copy plasmids containing the native yeast transcriptional initiation and termination signals to express human TFIID, the protein levels should be comparable with those in normal yeast cells.

As assayed by the plasmid shuffle technique outlined above, yeast cells containing human TFIID as the sole source of the TATA-binding protein are unable to grow (Figure 2). As expected, the control molecule expressing yeast TFIID supports cell growth. This indicates that at normal protein levels, human TFIID cannot functionally substitute for yeast TFIID in vivo. Even when multiple copies of the human TFIID gene are supplied by using a 2μ vector, cell growth is not observed after normal incubation times such as those used in Figure 2. However, slow growing colonies are eventually observed after prolonged incubation. This weak complementation presumably results from the overexpression of human TFIID due to increased gene dosage. Thus, human TFIID is extremely inefficient.
TFIID Function Is Species Specific

A

B

Figure 2. The Conserved Regions of Yeast and Human TFIID Are Nonequivalent

The sequence of the TFIID is presented schematically with sequences derived from yeast (solid) and human (open) indicated. The limit of the N-terminal and C-terminal regions are indicated as are the positions of the hybrid junctions (defined according to residues in yeast TFIID). Growth of the strains containing these TFIID s is indicated to the right.

but not completely incompetent, at carrying out the essential functions of yeast TFIID.

Yeast and Human TFIID Functionally Differ in Their Conserved C-Terminal Domains

The 180 C-terminal residues of yeast and human TFIID are 80% identical, whereas the N-terminal regions differ considerably in length and sequence. To determine whether the functional distinction between yeast and human TFIID is a result of differences in the divergent N-terminal regions or in the conserved C-terminal cores, we generated reciprocal TFIID hybrid proteins by exchanging the yeast and human N-termini (Figure 2).

Surprisingly, the yeast N-terminal region can be functionally replaced by the human N-terminal region, suggesting that this nonconserved region is not required for viability in yeast (strains 3 and 4). Consistent with this notion, strain 5, which contains a deleted version of yeast TFIID that lacks the N-terminal region (residues 4-63), grows at least as well, and perhaps slightly better, than the strain containing full-length yeast TFIID. In this regard, it is worth noting that the isolated C-terminal core domain binds more efficiently to TATA elements than the intact protein (Horikoshi et al., 1990; Lieberman et al., 1991).

On the other hand, a hybrid protein consisting of the yeast N-terminus and the human core domain fails to complement and in fact behaves indistinguishably from the full-length human protein (strain 4). Thus, the divergent N-terminal regions are neither necessary nor contributory to cell viability, and they cannot explain the failure of human TFIID to substitute for yeast TFIID. Instead, differences between the conserved core domains account for the phenotypic distinctions between yeast and human TFIID.

Figure 3. Analysis of Small Human TFIID Regions That Poison Yeast TFIID

The portions of the hybrid TFIID derived from yeast (solid) and human (open) are indicated along with the positions of the hybrid junctions (defined according to residues in yeast TFIID). Growth of strains containing these TFIID s is indicated to the right.

Regions of the Human TFIID Core Domain That Poison the Yeast Protein

To further delimit the portion of the conserved region that is functionally different between yeast and human, we analyzed additional hybrid TFIID s that contain subregions of human TFIID embedded within an otherwise entirely yeast protein (Figure 3). Portions of yeast TFIID that cannot be functionally replaced by the homologous region of human TFIID should contain residues critical for function in yeast that are not conserved between the two proteins. Region C1 (corresponding to yeast residues 67-170) contains differences that contribute to yeast function because strain 7 grows detectably less well than strain 1. More significantly, the fact that molecule 6 almost completely fails to complement the TFIID deletion suggests that differences in 69 C-terminal residues (region C2, corresponding to yeast residues 172-240) are extremely important. Molecules 8, 9, and 10 are swaps designed to localize the crucial differences within region C2. However, the resulting strains grow as well as wild type (strain 1), indicating that noncomplementation by molecule 6 results from a cumulative effect of at least two subregions within region C2. We conclude that the failure of human TFIID to complement in yeast cells reflects amino acid differences in more than one region of the highly conserved core domain, but that the most important differences lie within region C2.
Regions of the Yeast TFIID Core Domain That Rescue an Otherwise Human TFIID

The experiments described in Figure 3 define regions of yeast TFIID core that cannot be replaced by the homologous region of human TFIID to generate a fully functional protein. To determine if the same regions of yeast TFIID could supply the essential functions to an otherwise human protein, we generated and analyzed a reciprocal set of hybrid TFIID (Figure 4). Some functionally important differences map to region C1 because the strain containing molecule 11 shows modest growth. Strain 12, however, grows almost as well as strain 2, which contains the wild-type TFIID, suggesting that the most important differences map to region C2. These results are in complete accord with those obtained with the reciprocal hybrid TFIID (strains 6 and 7 of Figure 3).

In an attempt to subdivide region C2, we first analyzed molecules in which only the small subregions C2a, C2b, and C2c are derived from yeast TFIID, with the rest of the protein being entirely human (Figure 4). Molecule 13 shows a small amount of complementing activity, whereas molecules 14 and 15 show none. This suggests that important differences in region C2 are spread over at least two subregions, a conclusion consistent with the reciprocal hybrid proteins (molecules 8, 9, and 10). Molecules 16, 17, and 18 are derivatives of human TFIID in which pairwise combinations of subregions C2a, C2b, and C2c have been replaced by the corresponding yeast regions. Although strains 16 and 17 grow as poorly as strains 13 and 15, strain 18 clearly grows better than either. We conclude that while the effects in region C2 seem to be cumulative, subregions C2a and C2c seem to contain most of the functionally important differences.

Functional Yeast-Human Hybrid TFIIDS Support Transcriptional Activation by GAL4, GCN4, and ACE1

Because yeast cells contain approximately 1000 essential genes (Goebel and Petes, 1986), most of whose promoters contain TATA elements, our cell viability assay for TFIID function requires that the protein efficiently activate the transcription of many genes. To examine the hybrid TFIID for their ability to respond to specific upstream activator proteins, the strains described in Figures 2–4 were plated on appropriate selective media. Strains containing the various functional TFIID proteins grow in the presence of 40 mM aminotriazole (requires GCN4 activation) or 100 μM copper sulfate (requires ACE1 activation), and they are able to utilize galactose as a sole source of carbon (requires GAL4 activation). Their relative growth rates under these more stressful conditions are in good accord with the relative growth rates in standard glucose minimal medium. Thus, yeast–human hybrid TFIID that support cell viability also respond to the GCN4, GAL4, and ACE1 transcriptional activator proteins.

Comparable In Vivo Protein Levels of Yeast, Human, and Hybrid TFIID

We determined the intracellular TFIID levels in several yeast strains to eliminate the trivial possibility that the observed phenotypes reflect differences in the amounts, rather than in the activity, of the various proteins. To enable consistent immunodetection of the various TFIID, we fused an 11 amino acid sequence encoding the HA-1 epitope from flu virus (Wilson et al., 1984; Field et al., 1988) to the N-termini of yeast, human, and several hybrid TFIID proteins. The resulting TRP7 plasmids were introduced into a strain containing the wild-type yeast TFIID gene on a centromeric plasmid. As expected, the proteins migrate in accord with their predicted molecular weight, and an increased amount of epitope-tagged human TFIID is observed when the gene is encoded on a multicity plasmid. Since the flu-tagged TFIID show the same growth properties as their nontagged parents (data not shown), it is clear that the functional difference between yeast and human TFIID results from amino acid sequence dissimilarities and not protein levels.

Discussion

Yeast and Human TFIID Are Functionally Distinct In Vivo

Despite the structural and biochemical similarities among eukaryotic TATA-binding proteins, human TFIID does not effectively carry out the essential function(s) of yeast TFIID. This clearly reflects an inherent functional distinction between the two proteins because human TFIID does not
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proteins (in kd) are indicated at the left. The strong de& promoter (Hope and Struhl, 1966) thus accounting for its the other TFIID proteins, lacT-TFIID was expressed from the relatively a band that represents an unrelated protein in the yeast extract. Unlike band representing TFIID from strain 12 is due to its comigration with shorter (due to its yeast C-terminus). The increased intensity of the yeast TFIID. Hybrid 12 contains the human N-terminus, but migrates from strain 12 is due to its comigration with a band that represents an unrelated protein in the yeast extract. Unlike the other TFIID proteins, lacT-TFIID was expressed from the relatively strong de& promoter (Hope and Struhl, 1988), thus accounting for its increased band intensity. The mobility of molecular weight marker proteins (in kd) are indicated at the left.

not support cell growth when expressed at levels comparable with those of yeast TFIID in wild-type cells. However, human TFIID is not completely inactive in yeast cells, because its overproduction does permit extremely weak cell growth. Thus, at some detectable level, human TFIID can productively interact with the other components of the basic yeast transcription machinery.

The cell viability assay for TFIID function is very stringent because it requires efficient transcription of many genes. While human TFIID could fail to support cell growth by seriously compromising the transcription of one or several genes, it is more likely to generally reduce the transcription of most genes. Even in this latter case, it is entirely possible that these strains could show promoter-specific effects, such as those observed with several mutant yeast TFIIDs (Eisenmann et al., 1989).

The N-Terminal Region of Yeast TFIID Is Dispensable for Transcriptional Activity In Vivo

From in vitro transcription experiments, it has been suggested that the divergent N-terminal regions are responsible for species-specific differences among TFIIDs regarding their ability to respond to upstream activator proteins (Peterson et al., 1990; Pugh and Tjian, 1990). We were surprised, therefore, to discover that the N-terminal region of yeast TFIID could be replaced by the human N-terminal region or indeed simply be deleted without a detrimental effect on cell viability. Thus, the yeast TFIID core region is sufficient for all the essential functions of a TATA-binding protein as well as for the ability to respond to the three upstream activator proteins tested (GCN4, GAL4, and ACE1). However, we cannot eliminate the possibility that the N-terminal region could play a specialized role that either positively or negatively affects the transcription of certain nonessential genes.

In vitro, the N-terminal domains of yeast, fly, and human TFIID are not required for binding to TATA elements or for initiating transcription in reactions reconstituted with mammalian factors and RNA polymerase II (Horikoshi et al., 1990; Peterson et al., 1990; Pugh and Tjian, 1990). In the plant Arabidopsis thaliana, the N-terminal region of TFIID is only 18 amino acids (Gasch et al., 1990), suggesting that it may be functionally unimportant. In contrast, it has been reported that the N-terminal regions of fly and human TFIID are noooooory for transcriptional activation by Sp1 (Peterson et al., 1990; Pugh and Tjian, 1990). One explanation for this apparent discrepancy is that the N-terminal domains play fundamentally different roles in the various species. Alternatively, the fly and human domains might be required for activation by a specific class of transcription factors that do not exist in yeast. In this regard, Sp1 does not contain acidic activation regions that function in essentially all eukaryotic species (Courrey and Tjian, 1988), and it does not stimulate transcription in yeast cells (G. Gill, E. Pascal, and R. Tjian, unpublished data cited in Pugh and Tjian, 1990; A. S. P. and K. S., unpublished data). However, this hypothesis does not easily explain the apparent requirement of the human N-terminal domain for activation mediated by the acidic domain of GAL4-VP16 (Peterson et al., 1990).

Multiple Regions in the Core Domain Contribute to the Functional Difference between Yeast and Human TFIID

The failure of human TFIID to substitute for yeast TFIID in vivo is clearly due to differences between the highly conserved C-terminal domains. To map the determinants for this functional distinction, we examined the phenotypes conferred by yeast/human and human/yeast hybrid proteins. Most importantly, each of the seven pairs of reciprocal hybrid TFIIDs yields complementary phenotypes. That is, the degree to which a given region of yeast TFIID increases the function of human TFIID is in excellent accord with the degree to which that same region of human TFIID decreases the function of yeast TFIID. This reciprocality strongly argues against artifacts such as differential synthesis, stability, and folding of the proteins. Instead, it strongly suggests that the phenotypic distinctions among the hybrid proteins reflect the contributions of the swapped regions.

The basic conclusion from the analysis of hybrid TFIIDs is that no single region of the conserved domain is responsible for the functional difference between the two proteins. Both region C1 (116 amino acids with 10 differences) between yeast and human) and the nonoverlapping region C2 (68 amino acids with 15 differences and one extra residue in the human) contribute to the phenotypic distinction, with region C2 playing a more significant role. Within region C2, subregions C2a (25 residues with five differences)
and C2c (15 residues with five differences and one extra residue in human) are responsible for most of the important functional differences. Indeed, a derivative of human TFII D that contains only ten yeast-specific residues supports efficient cell growth (strain 18).

With respect to the sequence motifs within the conserved domain, part of the α homology region lies within subregion C2a, which contributes determinants for the difference between yeast and human TFII D. The basic repeat lies entirely within region C1 as does the first of the direct repeats. The second of the direct repeats lies almost entirely in region C2a and C2b. Although these observations may be suggestive, it is important to note that a functional role for these sequence motifs has never been established. In addition, our experimental approach does not map functionally important regions per se, but rather regions that contribute to the distinction between yeast and human TFII D.

Mechanistic Implications

Our results strongly suggest that subtle differences throughout the entire conserved region cumulatively account for the failure of human TFII D to function efficiently in yeast cells. The scattered nature of these determinants might indicate that the entire core domain constitutes a single structural unit that carries out the various functions of TATA-binding proteins. In support of this idea, short deletions throughout the entire conserved region of yeast TFII D invariably abolish the ability to bind DNA and to support transcription in vitro (Horikoshi et al., 1990). However, it is possible that the important differences between yeast and human TFII D, though dispersed throughout the primary sequence, may form part of a single surface in the correctly folded molecule. TFII D must perform several distinct functions during the transcriptional initiation process such as specific binding to TATA promoter elements and interactions with one or more of the components of the basic transcription machinery. In addition, TFII D might also interact with upstream activator and/or adaptor proteins, and it might be required for melting of DNA near the initiation region. Given this functional complexity of TFII D, there are several mechanisms to account for the observed difference between the yeast and human proteins.

One possibility is that the proteins might differ slightly in their DNA-binding properties. For example, human TFII D might have a slightly lower affinity for TATA elements such that it is insufficiently bound to yeast promoters when present at normal intracellular concentrations for yeast TFII D. Alternatively, the DNA-binding specificities of the proteins might not be identical, such that human TFII D alters the mRNA levels of the numerous yeast genes. In this regard, we reported subtle differences in the DNA sequence requirements for purified yeast TFII D and partially purified human TFII D (Wobbe and Struhl, 1990). However, more recent experiments indicate that this apparent difference in sequence specificity is due to other components in the impure human TFII D preparation, because it is not observed when yeast and human TFII Ds are prepared from Escherichia coli cells expressing the cloned genes (C. R. Wobbe and K. S., unpublished data). Thus, the available evidence argues against an intrinsic difference between yeast and human TFII Ds in terms of DNA binding.

Another, perhaps more intriguing, possibility is that the functional distinction reflects a species-specific interaction with a transcription factor(s) that itself is functionally divergent between yeast and human. In this view, subtle differences between the critical surfaces would affect the strength of the protein–protein interaction. It seems very likely that the basic transcription machineries of yeast and human are not interchangeable because mammalian TFII B, TFII E, and RNA polymerase II cannot be substituted with activities from yeast nuclear extracts. Moreover, yeast differ from most other eukaryotic organisms in the requirements for spacing between the TATA element and mRNA initiation site (Chen and Struhl, 1985; Hahn et al., 1985; McNeil and Smith, 1986; Nagawa and Fink, 1985).

The specific models suggested here are not mutually exclusive, and other explanations are certainly possible. More detailed biochemical and genetic analyses of the hybrid proteins should provide further insight into the molecular differences between eukaryotic TATA binding proteins.

Experimental Procedures

Construction of DNA Molecules

The 2.4 kb EcoRI–BamHI fragment containing the yeast TFII D gene was cloned from strain KY30 (Chen and Struhl, 1988) by screening a yeast library with an oligonucleotide based on the sequence of the gene provided by Arnie Berk (Chen et al., 1989). This fragment contains 1 kb upstream and 500 bp downstream of the coding region, and hence should contain the entire promoter and transcriptional initiation region. The sequenced region corresponds entirely to that contained in the original yeast genomic clone (Wobbe and Struhl, 1990). Thus, the available evidence argues against an intrinsic difference between yeast and human TFII Ds in terms of DNA binding.

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TFIID Function Is Species Specific

strains were grown overnight in appropriate glucose minimal medium prior to or during the short incubation in YPD medium. However, the number of colonies in a spot is variable, with more colonies being observed with derivatives that encode functional TFIID tagged TFIIDs, and the resulting strains were grown to mid-log phase for 15 min at 4°C. The Bradford assay was used to determine protein concentrations using bovine serum albumin as a standard.

Phenotypic Analysis

Yeast strain BYA is a derivative of KY320 (relevant genotype ura3-52 trpl1-5 a-h3-200) (Chen and Struhl, 1988), in which the chromosomal TFIID locus was replaced by a version deleted between the Xbal and Hindlll sites in the coding region (removes amino acids 172 to 225 and creates a frameshift); in addition, the chromosomal HIS3 locus has been restored to wild type. The strain also carries the URA3 centromeric plasmid YCP6 (Hope and Struhl, 1986) with the yeast TFIID gene cloned as a 2.4-kb EcoRI-BamHI fragment. Yeast strain BYA22 is identical to BYA2 except that the TFIID chromosomal locus is deleted between the SpeI and XbaI sites, effectively removing the entire gene. For both strains, the structure of the deleted TFIID locus was verified by genomic blotting. The behavior of each TFIID derivative tested was identical in BYA2 and BYA22.

DNA molecules encoding the various TFIID proteins were introduced into BYA2 by selection for Tdr . The resulting strains were grown overnight in appropriate glucose minimal medium lacking uracil and tryptophan, transformed for about three generations in YPD medium to reach an OD of 1, and washed in H2O. Approximately 106 cells (in 10 µl) were spotted on glucose minimal medium containing 1 mg/ml 5-fluoroorotic acid, 50 µg/ml uracil, 25 µg/ml adenine, and 0.6% agarose, and incubated at 30°C for 5-6 days. Given the frequency of plasmid loss, each spot should contain about 100 cells capable of surviving the 5-fluoroorotic acid selection and hence providing a functional assay for the TFIID derivative encoded on the TRP7 plasmid. However, the number of colonies in a spot is variable, with more colonies being observed with derivatives that encode functional TFIIDs. The probable reason for this variability is that cells losing the URA3 plasmid prior to or during the short incubation in YPD medium can grow only if they encode a functional TFIID on the TRP1 plasmid.

Measurement of Intracellular TFIID Levels

BYA2 was transformed with plasmids encoding various flu epitope-tagged TFIID s, and the resulting strains were grown to mid-log phase in glucose minimal medium containing casamino acids but lacking uracil (tryptophan was added to allow growth of the BYA2 control strain). Cells were harvested and washed twice at 0°C in 25 mM Tris-phosphate (pH 6.7), 2 mM PMSF. Cell pellets (approximately 0.25 ml packed volume) were frozen in liquid nitrogen, stored at -70°C for 2 hr, and then thawed on ice. The pellets were resuspended at 0°C in 0.5 ml of the same buffer, and acid-washed glass beads were added to the meniscus. Celldisruptingorganismsbyvigorousvortexing,usingten 15 s bursts with cooling on ice between each cycle. The extract was transferred to a new tube and clarified by centrifugation at 12,000 x g for 15 min at 4°C. The Bradford (Bio-Rad) assay was used to determine protein concentrations using bovine serum albumin as a standard. Total TFIID was precipitated from an extract (50 µg) without prior centrifugation, fitted with ammonium acetate (1.25A250, ascites fluid, 14 mg protein obtained from the Gorilla Institute) was used at a 1:1,000 dilution followed by a 1:7,500 dilution of goat anti-mouse IgG-specific antibody conjugated to alkaline phosphatase (PromoImmun system, Promega).

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