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Residues in the WD Repeats of Tup1 Required for Interaction with α2

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The yeast transcriptional repressor Tup1 contains seven WD repeats which interact with the DNA-binding protein α2. We have identified mutations in Tup1 that disrupt this interaction. The positions of the amino acids changed by these mutations are consistent with Tup1 being folded into a seven-bladed propeller like that formed by another WD repeat-containing protein, the β subunit of the heterotrimeric G protein used in signal transduction. Our results also indicate that the interaction between Tup1 and α2 resembles the interaction between Gβ and Gα, suggesting that a similar structural interface is formed by WD repeat proteins that are used in both transcriptional regulation and signal transduction.

The WD repeat is a 40-amino-acid motif found in proteins involved in a wide variety of cellular processes ranging from signal transduction to RNA processing (3, 30). Proteins containing WD repeats are often physically associated with other proteins and are believed in many cases to act as scaffolds upon which multimeric complexes are built (21). The structure of the GTP-binding protein (G protein) heterotrimer has revealed that the seven WD repeats of the β subunit (Gβ) fold into a circular, seven-bladed propeller with a water-solvated central channel and a relatively flat top and bottom formed by the turns connecting the β-strands that make up each propeller blade (17, 26, 33). The α subunit (Gα) sits asymmetrically on top of the propeller, contacting both the flat top surface and one of the sides parallel to the central channel.

Because many of the amino acids in Gβ that contribute to the integrity of the propeller are hallmarks of the WD motif, it has been proposed that all WD proteins fold into propellers in which the internal β-strands form a rigid skeleton that is fleshed out on the surface by specialized loops to which other proteins bind (22). We obtained evidence for this idea by examining the interaction between the yeast repressor Tup1, a WD protein whose biological function is unrelated to that of Gβ, and the cell type regulator α2. Tup1 represses the transcription of a large number of genes in *Saccharomyces cerevisiae* by interacting with various proteins bound to DNA sequences found upstream of target genes and interfering with transcription (4, 20, 28, 36). Tup1 is known to interact directly with at least one of these proteins, the homeodomain protein α2 (15). This interaction requires both the extreme N terminus of α2 and the WD repeats of Tup1, as a deletion of the respective region of either protein results in a loss of binding. In order to delineate which parts of the WD repeats are important for this interaction, we screened for point mutations in Tup1 that affect binding to α2 but that leave other functions of Tup1 intact.

MATERIALS AND METHODS

Plasmids. Plasmid pKK631 is a 2μm *LEU2* plasmid containing *TUP1* fused to *lexA* via *Bam*HI sites engineered at the stop codon of *TUP1* and the start codon of *lexA*; the *TUP1* sequences in pKK631 have been modified to eliminate the naturally occurring *Bam*HI site within the coding sequence of *TUP1* and to introduce a *Bam*HI site at sequences coding for amino acids 333 to 335 and a *Sal*I site at sequences coding for amino acids 436 to 438. Plasmid pKK630 is identical to pKK631 except that the *Bam*HI fragment containing sequences coding for amino acids 334 to 713 of Tup1 has been deleted. Plasmid pKK339 is three α2 operators upstream of the *URA3* gene carried on the TRP1/ARS/CEN vector pRS314 (24). Plasmid pKK602 is an *ADE2*-marked integrating version of the LexA-repressible *cycl*::*lacZ* reporter pCK30 (13).

Mutant versions of the *TUP1-lexA* plasmid (pKK631) were recovered from yeast as previously described (10) and sequenced. In cases where the plasmid contained more than one mutation, each of the single mutations was introduced into pKK598 by oligonucleotide-directed site-directed mutagenesis (16). All of the mutations were then subcloned into pKK448, a plasmid expressing Tup1 that is not fused to LexA, and the resulting plasmids were transformed into yeast strain SM1196 (9). Transformants were screened for β-galactosidase activity by filter assay. Table 1 summarizes the mutations which created alleles of *TUP1* that are able to derepress the *mfa2*::*lacZ* reporter. Plasmid pKK598 is the *Bam*HI-*Hin*dIII fragment of *TUP1* subcloned into the f1 origin-containing plasmid pUCf1 (Promega). Plasmid pKK448 contains the *TUP1* gene with a *Sal*I site engineered in at sequences coding for amino acids 436 to 438; the *LEU2* and 2μm sequences on pKK448 are derived from pΔSJ1, which is pSJ1 in which the *Sal*I-*Xho*I fragment of the polylinker has been deleted. pSJ1 is a 2μm *LEU2* plasmid (12).

All of the glutathione *S*-transferase (GST)-Tup1 expression plasmids were derived from pGST-CTERM (15), which expresses GST fused to amino acids 254 to 713 of Tup1. The mutant versions of pGST-CTERM were generated by

TABLE 1. Tup1 mutants are defective for repression of the α2-regulated reporter *mfa2*::*lacZ*[a]

Tup1 amino acid change	β-Galactosidase activity (Miller units)
Control (no Tup1)	170 ± 20
None (wild type)	20 ± 6
C348R	177 ± 20
Y445C	57 ± 3
S448P	168 ± 4
E463N	115 ± 10
Y489H	61 ± 20
Y580H	99 ± 7
L634S	86 ± 2
K650N	135 ± 10
N673S	174 ± 20
S674P	139 ± 10
I676T	41 ± 10
I676V	62 ± 6

[a] Yeast strain KKY110 (*MAT*α Δ*tup1 mfa2*::*lacZ*) was transformed with a wild-type or mutant *TUP1* plasmid and assayed for β-galactosidase activity. The level of repression conferred by the wild-type *TUP1* plasmid is incomplete relative to the level obtained with chromosomally expressed Tup1, possibly as a result of plasmid loss (31). Values are means ± standard deviations.

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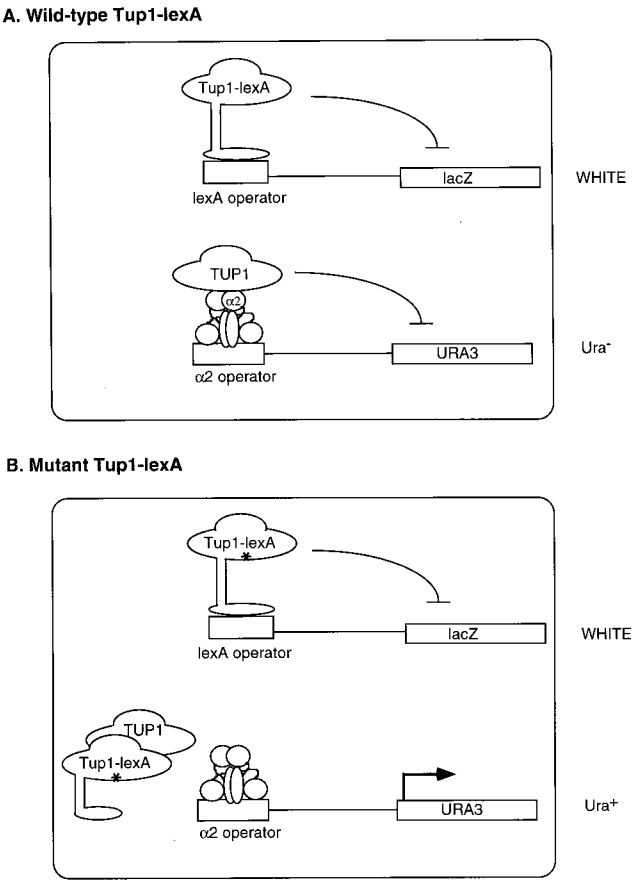

FIG. 1. Screen for mutations in Tup1-LexA that affect interaction with α2. (A) Wild-type Tup1-LexA binds to the *lexA* operator and represses the *lacZ* reporter; either Tup1 or Tup1-LexA binds to α2 and represses the *URA3* reporter. Hence, *MAT*α *TUP1* colonies expressing wild-type Tup1-LexA are white and Ura$^-$. (B) A Tup1-LexA mutant that cannot bind to α2 is able to repress from a *lexA* operator but interferes with α2-mediated repression, possibly by titrating some downstream repression component away from α2. Thus, colonies expressing a mutant fusion are white and Ura$^+$. For simplicity, we have shown the mutant Tup1-LexA binding to endogenous Tup1 and forming heteromers that are incompetent for α2 binding, but the mutant could be titrating any component of the repression machinery downstream of α2. A Tup1-LexA fusion rather than Tup1 itself was used in order to screen against mutations that merely destabilize, unfold, or truncate Tup1 or affect its ability to interact with downstream components of the repression machinery, since such mutations would presumably cause derepression of the both the *lacZ* and *URA3* reporters.

subcloning appropriate restriction fragments from the mutant pKK448 plasmids into pGST-CTERM. The $\alpha2^{\Delta2-12}$ expression plasmid was constructed by introducing the deletion mutation into pAV99 (32) by site-directed mutagenesis (16), with the oligonucleotide 5′-GGA TTT AAA CTC ATC TGT GAT TTG CAT ATG CTG TTT CCT GTG TGA AAT TGT TAT-3′, and by subsequently removing the *Bam*HI fragment downstream of the $\alpha2^{\Delta2-12}$ coding sequence.

The *anbl*::*lacZ* reporter was constructed by inserting the *Xho*I-*Bam*HI fragment from pLGΔ312S (7) into the *Xho*I and *Bam*HI sites of pKK480. pKK480 is the *Sma*I-*Sal*I fragment of pRY52 inserted into the *Sma*I and *Xho*I sites of pRY52. pRY52 was provided by Roger Yocum and is pLG669 (8) with a *Bgl*II linker inserted into the *Hae*III site. The *suc2*::*lacZ* reporter was constructed by inserting a *Bam*HI-cut PCR fragment containing the upstream regulatory region of *SUC2* into the *Bam*HI site of pLGΔSS (11). The PCR fragment was generated with the oligonucleotides 5′-GCC GGG ATC CGC TCA AAA AAG TAC GTC ATT TAG AAT TTG-3′ and 5′-CTC CGG ATC CGG TCA TCA TAT ACG TTA GTG AAA AGA AAA GC-3′ as primers and plasmid pRB58 (2, 23) as the template. The *mr2*::*lacZ* reporter is pZZ2 (35).

Yeast strains. All yeast strains are congenic to EG123 (27). KKY131″ (*MAT*α *trp1 leu2 ura3 his4 ade2*; carries pKK602 and pKK339) was constructed by transforming pKK602 and pKK339 into KKY135. KKY135 is 246.1.1 (25) in which the *ADE2* gene has been partially deleted. KKY110 (*MAT*α *trp1 leu2 ura3 his4* Δ*tup1 mfa2*::*lacZ*) and KKY103 (*MAT*α *trp1 leu2 ura3 his4* Δ*tup1*) were constructed by introducing an unmarked *TUP1* deletion into SM1196 (9) and 246.1.1, respectively, using plasmid pRT164 as described previously (15).

PCR mutagenesis and screen for Tup1-LexA mutants. Mutants were generated by amplifying a region of *TUP1* under mutagenic PCR conditions and cotransforming the PCR product into yeast with a gapped plasmid containing homology to both ends of the PCR product (19). The PCR product was made with the oligonucleotides 5′-CCA CTC TAA ACC TAT CCC-3′ and 5′-CCT CTT CCT GCA ACA GAC GAA TCC-3′ as primers and plasmid pKK631 as template DNA. Reactions were carried out in commercial 1× PCR buffer plus $MgCl_2$ (Boehringer Mannheim Biochemicals) supplemented with 1 mM concentrations of dGTP, dCTP, and dTTP; 200 μM dATP; 500 μM $MnCl_2$; 3 mM $MgCl_2$; and 2.5 U of *Taq* polymerase (Boehringer Mannheim Biochemicals). The PCR product was cotransformed with *Bam*HI-cut pKK630 into KKY135″. The transformants were grown on synthetic medium lacking tryptophan and leucine and then replica plated onto medium lacking tryptophan, leucine, and uracil. Ura$^+$ colonies were patched onto medium lacking tryptophan, leucine, and uracil and assayed for β-galactosidase activity by filter assay as previously described (15).

Liquid β-galactosidase assays. Quantitative β-galactosidase assays were performed as previously described (18), except that yeast cells were permeabilized with 0.0025% sodium dodecyl sulfate and 5% chloroform and the cell debris was removed by centrifugation before the optical density at 420 nm of the sample was read, thus eliminating the need to correct for light scatter. Activities are reported in Miller units and represent assays performed in triplicate on three independent transformants.

α2-binding assays. Bacterial extracts containing both α2 and $\alpha2^{\Delta2-12}$ were passed over glutathione-agarose columns bearing various GST-Tup1 fusions. The columns were then washed and eluted with high salt concentrations. Purification of GST-Tup1 fusions, preparation of α2-containing bacterial extracts, and affinity chromatography were performed essentially as described previously (15).

RESULTS

Isolation of dominant negative Tup1 mutants. Our screen for *TUP1* mutants specifically defective in interacting with α2 took advantage of two properties of Tup1. First, a fragment of Tup1 lacking the WD repeats cannot bind to α2 and will disrupt repression of α2-regulated genes in wild-type strains when overexpressed, presumably because the fragment of Tup1 binds to some downstream component of the repression machinery and titrates it away from α2 (14). Second, a Tup1-LexA fusion represses transcription from a *lexA* operator in the absence of α2 (14, 29). From these results we concluded that the two major functions of Tup1—interaction with α2 and interaction with the repression machinery—are separable and hence capable of being disarmed independently. Reasoning that a Tup1-LexA mutant defective in binding to α2 would interfere with repression of an α2-regulated reporter by wild-type Tup1 but would maintain the ability to repress from a *lexA* site, we set up a screen to look for such mutants (Fig. 1).

We introduced random mutations into a *TUP1-lexA* fusion

TABLE 2. Point mutations do not affect repression by plasmid-expressed Tup1-LexA[a]

Tup1 amino acid change	β-Galactosidase activity (Miller units)	
	TUP1 strain	Δ*tup1* strain
Control (no Tup1)	529 ± 80	129 ± 50
None (wild type)	17 ± 10	9 ± 3
C348R	20 ± 3	19 ± 4
S448P	32 ± 10	28 ± 20
Y489H	22 ± 6	ND[b]
Y580H	19 ± 8	29 ± 2
L634S	32 ± 13	ND
I676V	22 ± 10	ND

[a] The Tup1-LexA expression plasmids were cotransformed with pJK1621 into either 246.1.1 (*TUP1*) or KKY103 (Δ*tup1*), and the transformants were assayed for β-galactosidase activity. Reporter JK1621 is *cyc1*::*lacZ* with four *lexA* sites upstream of the upstream activation sequence (13). Values are means ± standard deviations.

[b] ND, not determined.

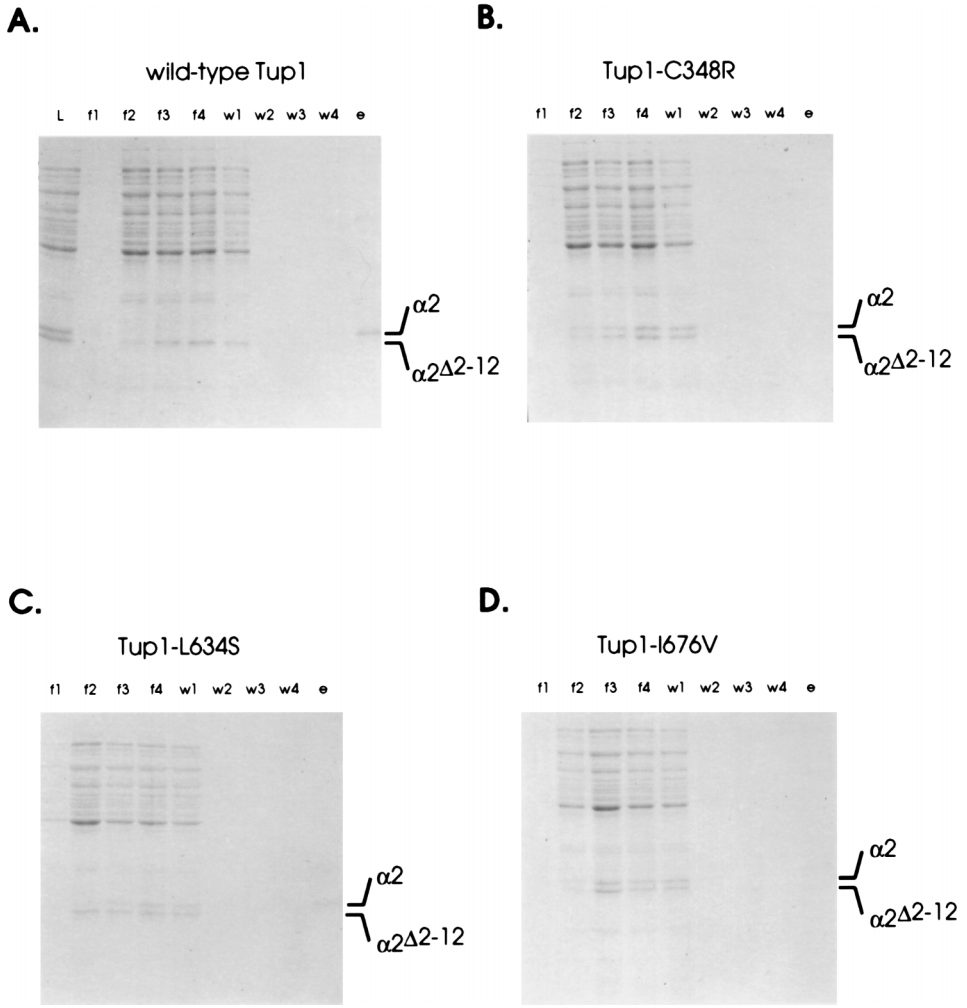

FIG. 2. Binding of wild-type and mutant GST-Tup1 fusions to α2. Shown are Coomassie-stained gels of fractions of a bacterial extract containing α2 that has been passed over a column containing glutathione-agarose beads bearing GST-Tup1 (wild type), GST-Tup1 (C348R), GST-Tup1 (L634S), or GST-Tup1 (I676V). The load is the same for all of the experiments and is shown only in panel A (lane L). The other lanes show flowthrough fractions (f1 through f4), wash fractions (w1 through w4), and the eluate (e). The depletion of α2 from the flowthrough fractions indicates binding to the column. Recovery of α2 in the high-salt eluate is usually incomplete, making comparisons of the eluate fractions from different experiments difficult. The truncated form of α2 does not bind well to Tup1 and is included as a negative control to show that the α2-Tup1 interaction is specific.

borne on a high-copy-number plasmid under modified PCR conditions and transformed the DNA into a *MAT*α *TUP1* strain carrying an α2-repressed *URA3* gene and a Tup1-LexA-repressible *lacZ* reporter. We selected for transformants able to grow in the absence of uracil and screened the resulting Ura^+ colonies for β-galactosidase activity by filter assay. Of the 30,000 transformants examined, 150 were Ura^+, and 75 of these were white by filter assay. Plasmid DNA was isolated from 24 of the white Ura^+ colonies and sequenced; 12 unique mutations in *TUP1* were identified. Because the other 12 plasmids that we sequenced all contained one of these 12 mutations, the remaining 51 white Ura^+ colonies were not examined further.

Tup1 mutants are defective for α2-mediated repression. In order to quantitate the ability of the *TUP1* mutants to complement for *TUP1* function and to confirm that any defect in repression observed is not an artifact of the LexA fusion, we introduced the mutations into a plasmid that expresses Tup1 that is not fused to LexA and transformed the resulting plasmids into a *MAT*α Δ*tup1* strain carrying the α2-repressible

TABLE 3. Effects of Tup1 point mutations on repression of other Tup1-regulated reporters[a]

Tup1 mutation	β-Galactosidase activity (Miller units)			
	anb1::*lacZ*	*suc2*::*lacZ*	*rnr2*::*lacZ*	*mfa2*::*lacZ*
Control (no Tup1)	350 ± 60	130 ± 22	10.5 ± 2	170 ± 20
None (wild type)	18 ± 2	8 ± 1	2.6 ± 0.3	20 ± 6
C348R	107 ± 40	19 ± 2	6.0 ± 1	177 ± 20
S448P	49 ± 8	18 ± 3	8.3 ± 2	168 ± 4
Y489H	32 ± 5	14 ± 3	2.8 ± 0.2	61 ± 20
Y580H	54 ± 16	22 ± 6	4.5 ± 1	99 ± 7
L634S	66 ± 17	18 ± 3	4.6 ± 0.7	86 ± 2
I676V	46 ± 12	11 ± 3	4.8 ± 0.6	41 ± 10
Δ255–713	74 ± 12	22 ± 5	8.2 ± 1	176 ± 10

[a] KKY103 (*MAT*α Δ*tup1*) carrying an *anb1*::*lacZ*, *suc2*::*lacZ*, or *rnr2*::*lacZ* reporter was transformed with a wild-type or mutant *TUP1* plasmid and assayed for β-galactosidase activity. Relevant numbers from Table 1 are reproduced in the rightmost column for ease of comparison. Values are means ± standard deviations.

A. TUP1

D strand — A strand — B strand — C strand

330 PREIDVELHKSLDH..TSVV**C**CVKFSNDGEYLATGC.NKTTQVYR

429 KDVENLNTSSSPSS..DL**Y**IR**S**VCFSPDGKFLATGAEDRLIRIWD

472 IENRKIVMILQGH..EQDI**Y**SLDYFPSGDKLVSGSGDRTVRIWD

516 TGQCSLTLSIEDGV..TTVAVSPG...DGKYIAAGSLDRAVRVWD

572 VERLDSENESGTGH..KDSV**Y**SVVFTRDGQSVVSGSLDRSVKLWN

616 TPNSGTCEVTYTGH..KDFV**L**SVATTQNDEYILSGS**K**DRGVLFWD

658 KKSGNPLLMLQGH..R**NS**V**I**SVAVANG*NVFATGSGDCKARIWK

D-A loop — B-C loop

B. Gβ

D strand — A strand — B strand — C strand

45 MRTRRTLRGH..LA**K**IYAMHWGTDSRLLLSAS**Q**DGKLIIWD

84 SYTTNKVHAIPLR..SS**W**VMTCAYAPSGNYVACGG**L**DNICSIYN

126 LKTREGNVRVSRELSGH..**T**G**Y**LSCCRFL.DDNQIVTSSG**D**TTCALWD

171 IETGQQTTTFTGH..TG**D**V**M**SLSLAPDTRLFVSGA**C**DASAKLWD

213 VREGMCRQTFTGH..ES**D**INAICFFPNGNAFATGS**D**DATCRLFD

255 LRADQELMTYSHDNIICGITSVSFSKSGRLLLAGYDDFNCNVWD

299 ALKADRAGVLAGH..DNRVSCLGVTDDGMAVATGS**W**DSFLKIWN

D-A loop — B-C loop

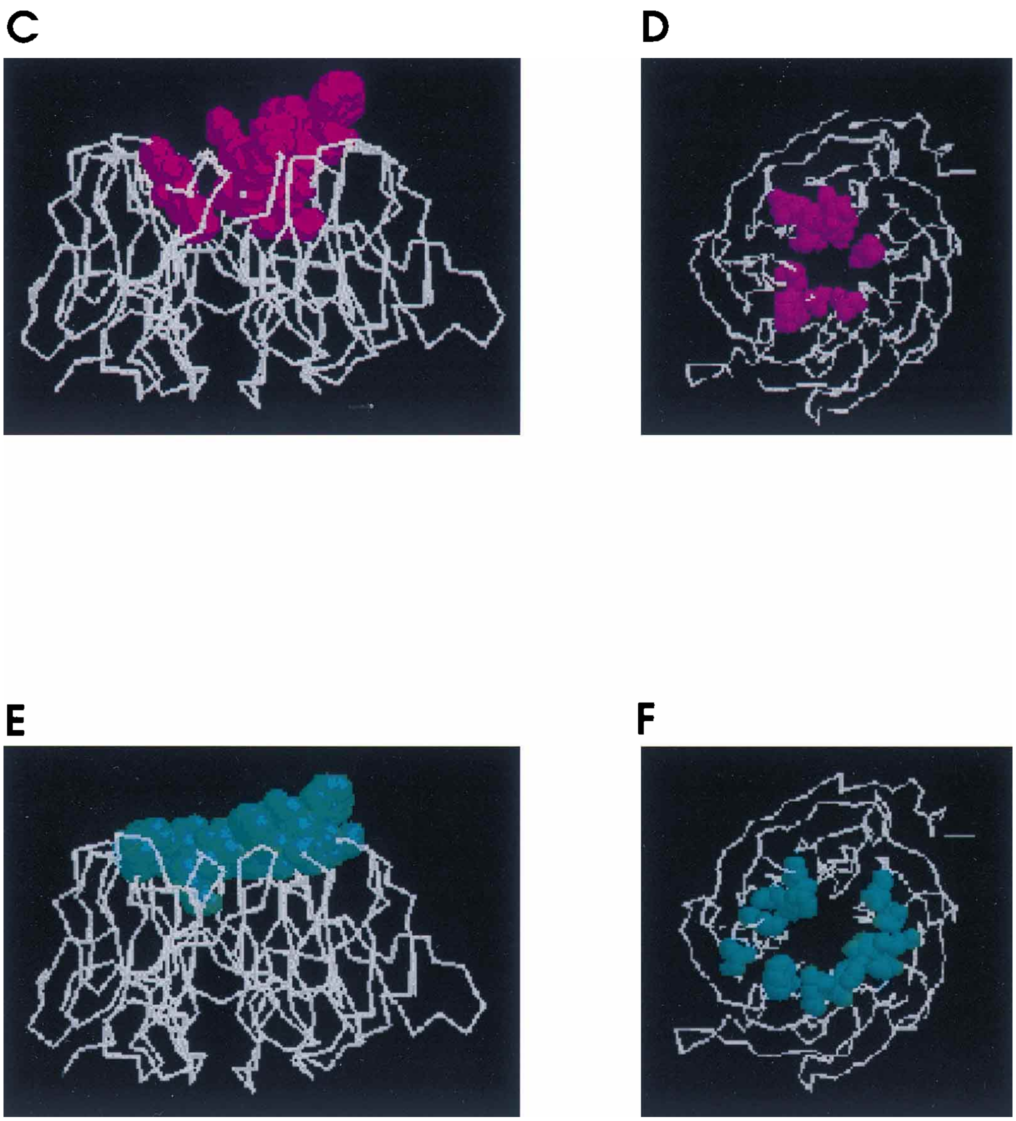

FIG. 3. Similarity between the region of Tup1 that binds α2 and the region of Gβ that binds Gα. (A) Sequence of the Tup1 WD repeats, with the amino acids that are changed in mutants defective for α2 binding in bold type. The asterisk represents a stretch of seven amino acids that disrupts the spacing of WD7 and presumably forms a loop on the bottom surface of Tup1. (B) Sequence of the Gβ WD repeats, with the amino acids that contact Gα in bold type (17, 26, 33). The seven rows in panels A and B are preceded by the positions of the amino-terminal residues of each repeat. The conserved WD (or WD-like) sequence at the end of each repeat is underlined. The solid lines above the repeats indicate the amino acids that form the four β-strands (strands A through D) which make up each propeller blade in Gβ. The dotted lines below the repeats indicate the interstrand loops that form the upper surface of Gβ. (C and D) Model for the structure of the WD repeats of Tup1, as viewed from the side and top, respectively. The backbone is drawn in white from the coordinates for the structure of Gβ; the amino acids of Gβ that are in the same position as the amino acids of Tup1 involved in α2 binding are highlighted in purple. (E and F) The structure of Gβ, as viewed from the side and top, respectively. The backbone is drawn in white, and the amino acids that contact Gα are highlighted in green (17, 26, 33). Structures were drawn with Rasmol with coordinates provided by Stephen Sprang.

reporter *mfa2*::*lacZ*. As expected, the mutants failed to repress the reporter gene to the same extent as does wild-type Tup1 (Table 1). In contrast, the wild-type and mutant versions of Tup1-LexA repressed from a *lexA* site to approximately the same degree (Table 2), suggesting that the mutations do not debilitate the interaction between Tup1 and downstream components of the repression machinery and that the defect in carrying out α2-mediated repression is due to an inability of the mutant Tup1 proteins to bind α2.

Tup1 mutants are defective for α2 binding. We next examined the ability of the mutant Tup1 proteins to bind to α2 in vitro by affinity chromatography. Each of the mutants was expressed as a GST fusion in *Escherichia coli*, purified, and immobilized on glutathione-agarose beads. Bacterial extracts containing α2 and $\alpha2^{\Delta2-12}$, a version of α2 that has a deletion and does not bind to Tup1, were passed over the beads, which were subsequently washed and eluted with high salt concentrations. As shown in Fig. 2A, α2 bound to the wild-type-Tup1 column and was absent from the flowthrough and wash fractions, whereas $\alpha2^{\Delta2-12}$ did not bind to the column and was present in the flowthrough and wash fractions. In contrast, the flowthrough and wash fractions of three of the mutant-Tup1 columns contained both α2 and $\alpha2^{\Delta2-12}$, indicating that the mutant-Tup1 columns retained α2 less efficiently than did the wild-type-Tup1 column (Fig. 2B to D). The remaining nine Tup1 mutants also showed a decrease in α2 binding by this assay (data not shown). Each of the column experiments was repeated two to six times with individually prepared columns, and similar results were obtained each time. Hence, we believe that the difference between the wild-type- and mutant-Tup1 columns is unlikely to be due to slight variations in column volume or protein concentration on the beads and that it reflects instead a decrease in the ability of the mutant proteins to bind α2.

Tup1 mutants do not fully repress other Tup1-regulated reporters. Although the WD repeats of Tup1 are absolutely required for α2-mediated repression, deletion of all seven repeats causes only partial derepression of other Tup1-repressed genes (29), indicating that the DNA-binding proteins found upstream of these genes probably recruit Tup1 via a domain outside of the WD repeats. Nevertheless, the repeats are required for full repression of all genes that have been tested and could serve as an additional site of interaction between Tup1 and these other DNA-binding proteins. We were therefore interested in determining whether mutations which debilitate the Tup1-α2 interaction also affect repression of other Tup1-regulated genes.

We examined the ability of six of the Tup1 point mutants to repress three other Tup1-regulated reporters: a glucose-repressed reporter (*suc2*::*lacZ*), a hypoxic reporter (*anbl*::*lacZ*), and a DNA damage-inducible reporter (*rnr2*::*lacZ*). A Δ*tup1* strain was cotransformed with a reporter and a wild-type or mutant *TUP1* plasmid and assayed for β-galactosidase activity. As a control, the reporter-carrying strains were also transformed with the deletion mutant Δ255–713, which lacks all seven WD repeats and partially represses *ANB1* and *SUC2* (29). In almost all cases, repression by the mutants was weaker than wild-type repression but stronger than that achieved by the Δ255–713 mutant (Table 3). Furthermore, the Tup1 mutants which were capable of only weak α2-mediated repression also showed weak repression of *anb1*::*lacZ*, *suc2*::*lacZ*, and *rnr2*::*lacZ*. There is, however, no strict hierarchy for strength of repression that applies to all four reporters tested. For example, Tup1 with a Y580H mutation is the weakest mutant with respect to *suc2*::*lacZ* repression but has an intermediate phenotype with respect to the other three reporters.

DISCUSSION

In summary, we have genetically identified residues in the WD repeats of Tup1 that are required for the Tup1-α2 interaction. The simplest explanation for the defect in binding is that the mutations change amino acids in Tup1 that contact α2. Hence, the mutants provide a test for the prediction that Tup1 is folded into a β-propeller, since the affected amino acids are predicted to lie close to one another on the surface of Tup1. When we used the coordinates for the Gβ structure and the homology between Tup1 and Gβ to construct a model for the structure of Tup1, we found that all of the amino acids that are changed in the α2-binding mutants reside on one face of the predicted Tup1 propeller (Fig. 3). This surface of Tup1 is analogous to the surface of Gβ which interacts with a β-strand–β-strand–α-helix cluster in Gα.

Our results, then, provide evidence for several of the generalizations regarding WD proteins that have emerged from the study of the structure of Gβ. First, our data support the prediction that the Tup1 WD repeats form a β-propeller and are consistent with recent proteolysis experiments showing that much of Tup1 is folded into a compact, trypsin-resistant structure (5). Although the trypsin-resistant fragment of Tup1 is the size of six WD repeats rather than seven, the long linker between WD1 and WD2 contains several trypsin sites and is likely to be exposed as an extended loop on the upper surface of Tup1. Second, the mutations that we have identified affect amino acids that are completely conserved among Tup1 homologs from other yeasts but not among WD proteins in general (1, 34), lending credence to the notion that surface amino acids that are evolutionarily conserved within a functional family of WD proteins are likely to be involved in interacting with specific proteins. Finally, the similarity between the Tup1-α2 interaction and the major Gβ-Gα interaction suggests that the flat surfaces of the propeller might be used by WD proteins in general as a protein-binding surface.

Although the structure of the G protein heterotrimer clearly demonstrates that the sides of the propeller are capable of making protein-protein contacts, the flat upper surface is particularly interesting because it binds not only Gα but also some of the numerous downstream effectors that Gβγ consorts with after abandoning Gα (6). Likewise, the flat upper surface of Tup1 is probably utilized to contact not only α2 but also the assorted DNA-binding proteins found upstream of other Tup1-regulated genes, since other genes that we have tested are partially derepressed by the *TUP1* mutations isolated in this work. Given that one of the distinguishing features of WD proteins is their ability to engage a number of different partners, it is tempting to speculate that the flat surfaces composed of flexible loops from each WD repeat are designed to provide binding sites for many proteins within a relatively small area.

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