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Genetics

Mutations in RNA polymerase II enhance or suppress mutations in *GAL4*

(*RPO21*/heptapeptide repeats/transcription factors/*Saccharomyces cerevisiae*)

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ABSTRACT **The activation domains of eukaryotic DNA-binding transcription factors, such as GAL4, may regulate transcription by contacting RNA polymerase II. One potential site on RNA polymerase II for such interactions is the C-terminal tandemly repeated heptapeptide domain in the largest subunit (RPO21). We have changed the number of heptapeptide repeats in this yeast RPO21 C-terminal domain and have expressed these mutant RNA polymerase II polypeptides in yeast cells containing either wild-type or defective GAL4 proteins. Although the number of RPO21 heptapeptide repeats had no effect on the activity of wild-type GAL4, changing the length of the C-terminal domain modified the ability of mutant GAL4 proteins to activate transcription. Shorter or longer RPO21 C-terminal domains enhanced or partially suppressed, respectively, the effects of deletions in the transcriptional-activation domains of GAL4. The same RPO21 mutations also affected transcriptional activation by a GAL4-GCN4 chimera. These data suggest that the activation domains of DNA-binding transcription factors could interact, either directly or indirectly, with the heptapeptide repeats of RNA polymerase II.**

The primary structure of RNA polymerase subunits has been exceptionally well conserved during evolution. In the yeast, *Saccharomyces cerevisiae*, three closely related genes, *RPO11*, *RPO21*, and *RPO31*, encode the largest subunit polypeptide of RNA polymerases I, II, and III, respectively (1–3). These three polypeptides share extensive homology amongst themselves and also with the largest subunit, β', of the *Escherichia coli* RNA polymerase (3, 4). However, the polymerase II subunit, RPO21, differs significantly from the analogous polymerase I and III subunits and from β' by the presence of a unique C-terminal domain (4). This domain, also conserved in the RPO21 polypeptide of higher eukaryotes (5, 6), consists of a tandemly repeated heptapeptide sequence with the consensus amino acid sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The *S. cerevisiae* polypeptide has 26 or 27 of these heptapeptide repeats (4, 7), whereas the RPO21 polypeptides of *Drosophila* and mammals have 44 and 52 repeats, respectively (5, 6). The importance of this novel domain of eukaryotic RNA polymerase II for enzyme function has been demonstrated by genetic experiments in yeast, *Drosophila*, and mammalian cells. Deletions in RPO21 that leave <10 of the 26 yeast repeats are lethal in yeast (6, 7); a mutation deleting 22 of the 44 *Drosophila* repeats is lethal (8); and a mouse polymerase II polypeptide with only 25 of the 52 mammalian repeats is nonfunctional in rodent cells (9).

A role for this heptapeptide repeat domain of RNA polymerase II in the initiation of transcription has been suggested by some indirect experiments. Purification of RNA polymerase II usually yields two forms of the enzyme (II_A and II_B), which differ only in the size of their largest subunit (10). We and others have shown that proteolysis of the largest subunit of RNA polymerase II_A removes the C-terminal heptapeptide repeat domain and generates RNA polymerase II_B (4, 5). Earlier experiments had demonstrated that a monoclonal antibody recognizing the II_A enzyme but not the II_B form prevented accurate initiation of transcription by RNA polymerase II at a variety of promoters *in vitro* but had no effect on nonspecific transcription (11). Our laboratory has shown since that a monoclonal antibody raised against a synthetic peptide containing five of the heptapeptide repeats inhibits the formation of stable initiation-competent complexes at the adenovirus major late promoter *in vitro* (unpublished observations). The role of the repeats in initiation is, however, ambiguous since the *Drosophila* RNA polymerase II, proteolytically treated to remove the RPO21 C-terminal domain, can still initiate transcription accurately *in vitro* (8). If these heptapeptide repeats do act at a step of transcription initiation, protein(s) that interact with this part of RNA polymerase II have yet to be identified. Such proteins may include transcription factors that activate transcription when bound to DNA.

One of the best characterized DNA-binding transcription factors is the protein GAL4 (for a review, see ref. 13). This yeast polypeptide comprises two functionally distinct regions—namely, an N-terminal DNA-binding domain (14) and a C-terminal domain necessary for transcriptional activation (15). Within the C-terminal activating domain, there are two regions, rich in acidic amino acids, that themselves are transcriptional activators when fused to the N-terminal DNA-binding domain (16). Current models of transcriptional regulation propose the interaction, direct or indirect, of these acidic domains with the transcription machinery (17, 18). Since we and others (6, 7, 9, 19, 20) have suggested that the activation domains of transcription factors may contact the repeating heptapeptides present at the C terminus of the largest subunit of RNA polymerase II, we have examined the effects of alterations in this part of RPO21 on transcriptional activation by wild-type and mutant forms of GAL4. We made a series of directed alterations in the C-terminal domain of RPO21 and expressed in yeast cells RPO21 polypeptides with either fewer or more heptapeptide repeats than in the wild-type polypeptide. Such shortening or lengthening of the RPO21 C-terminal domain had the effect of enhancing or partially suppressing, respectively, the deleterious effects of deletions in the transcriptional-activation domains of GAL4. These experiments provide some support for the hypothesis that GAL4 may contact the RPO21 heptapeptide repeats.

MATERIALS AND METHODS

Yeast Strains, Growth, and Manipulation. The *S. cerevisiae* strain used to construct new strains for these experiments was LP112 (**a**/α *can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 ade2-1/ade2-1*), constructed from the isogenic haploid strains W3031A and W3031B (21) and obtained from J. Segall. The

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wild-type *RPO21* strain referred to in this study is the haploid W3031B. Yeast cells were grown either in YEPD medium (1% yeast extract/2% peptone/2% glucose) or minimal medium (0.67% yeast nitrogen base without amino acids and containing either 2% glucose or 2% each raffinose and galactose) supplemented with the appropriate amino acids. Yeast were transformed integratively by using the spheroplast technique of Orr-Weaver *et al.* (22). Plasmids containing 2-μm sequences were transferred into yeast by the LiCl transformation technique (23).

Construction of Mutant Strains. We have described (6) the use of pRPO21:CEE, containing part of the *RPO21* locus and the entire *URA3* gene, to construct *rpo21* deletion mutations, one of which was *rpo21-Δ88* (6). Integration of pRPO21:CEE results in a partial duplication of the *RPO21* locus. To generate stable transformants with no *RPO21* duplication, diploid cells carrying the integrated *rpo21-Δ88* cassette were plated on medium containing 1 mg of 5-fluoroorotic acid per ml, and 5-fluoroorotic acid-resistant colonies were selected (24). These diploids were then sporulated, and tetrads were dissected to obtain a haploid *rpo21-Δ88* allele-containing strain.

The *rpo21-ylt* allele-containing strain was constructed as follows. A *Hpa* II–*Hin*dIII fragment of the yeast *RPO21* gene (nucleotides 4911–5723), encoding 26 heptapeptide repeats, was subcloned between the *Acc* I and *Hin*dIII polylinker sites of a pEMBL18$^+$ derivative, in which the polylinker *Xba* I site had been blunted with mung-bean nuclease. From this intermediate construct, a *Bam*HI–*Hin*dIII fragment containing the repeat-encoding DNA was subcloned into pRPO21-Bl (6) between its *Bam*HI and *Hin*dIII sites, replacing the DNA encoding the distal 14 repeats of RPO21 with DNA encoding 26 repeats. Nucleotide sequence analysis confirmed that the fusion was in-frame (see Fig. 1). After subcloning this modified *RPO21* DNA into pRPO21:CEE, this DNA was used in turn to integratively transform LP112 cells. Sporulation and tetrad analysis of Ura$^+$ transformants confirmed that this *rpo21* mutation was not lethal in haploid yeast. Stable Ura$^-$ haploid strains were selected on 5-fluoroorotic acid-containing media. All steps in the construction of *rpo21* mutant strains were verified by Southern analysis of yeast genomic DNA by using appropriate restriction enzyme digestions.

The three haploid yeast strains containing alleles *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt* were transformed integratively with a reporter plasmid pRY171 containing a *GAL1-lacZ* fusion gene and the *URA3* gene for selection (25). pRY171 was linearized with *Apa* I to target the integration event to the *URA3* locus. Single-copy integrants were detected by Southern analysis. In each of the three resulting *URA3:RY171*, *rpo21* strains, the endogenous *GAL4* gene was rendered nonfunctional by a single-step gene-replacement technique (26). Starting with a pEMBL18$^+$ vector into which had been subcloned a *Pst* I–*Eco*RI fragment from the 1.45-kilobase (kb) *Eco*RI *TRP1 ARS1* DNA (27), a 12-base-pair *Eco*RI linker was cloned at the *Pst* I site. The resulting construct was cut with *Eco*RI, and the 0.83-kb fragment containing *TRP1* DNA was then subcloned into the *Eco*RI site of pMA235 (16), disrupting the plasmid *GAL4* gene with *TRP1* coding sequences. An *Sph* I–*Sal* I fragment of *GAL4 TRP1* DNA, subcloned into pEMBL19$^+$, was then used to transform the three Ura$^+$ yeast strains. Ura$^+$, Trp$^+$ transformants were checked by Southern analysis to identify those with the expected DNA rearrangements. The three *URA3:RY171*, *gal4:TRP1* strains were further transformed with each of seven plasmids expressing one of the following: wild-type GAL4 (pMA210), no GAL4 (pMA200), mutant GAL4 proteins (pMA237, CD14XT, SD15, and CD19XX) or a GAL4-GCN4 chimeric polypeptide. All of these GAL4 polypeptides assessed for activity in these experiments were expressed

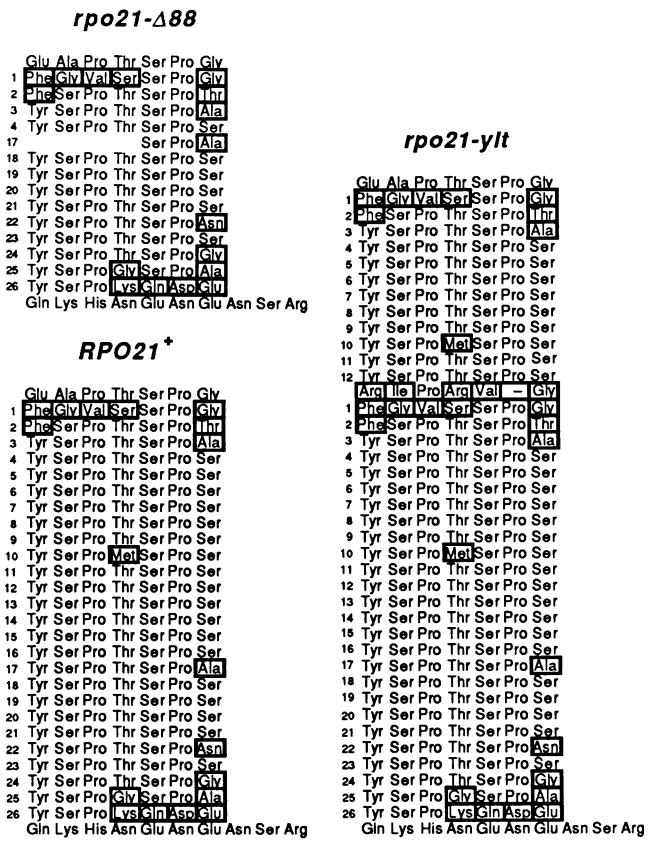

FIG. 1. Amino acid sequences of the C-terminal domains of the three alleles of *RPO21* used in these studies: *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt*. The boxed residues represent amino acids within the heptapeptide repeats that differ from the consensus sequence. The heptapeptide repeats are numbered according to their position in the RPO21$^+$ polypeptide.

from the *ADH1* promoter on a 2-μm *HIS3* vector as described by Ma and Ptashne (16). The chimeric GAL4-GCN4 transcription factor was constructed by introducing a 12-base-pair *Eco*RI linker at the *Bam*HI site at nucleotide position 937 in *GCN4* DNA. A resulting *Eco*RI fragment (≈2.0 kb) encoding amino acids 54–281 of GCN4 was then inserted into the 2-μm *HIS3* yeast vector pMA235. pMA235, a derivative of pMA210, contains a 12-base-pair *Eco*RI linker at the *Cla* I site in the *GAL4* gene (16).

Immunological Detection of RPO21 Polypeptides. Protein extracts were prepared by the method of Ohashi *et al.* (28) from 20 ml of yeast cells grown to midlogarithmic phase (OD_{600} ≈ 1.5). One-tenth of each protein preparation was fractionated on a 5% polyacrylamide/sodium dodecyl sulfate gel and electrophoretically transferred onto nitrocellulose by using a Bio-Rad Transblot apparatus following the manufacturer's directions. A mouse monoclonal antibody (JEL352) directed against a synthetic peptide of five consensus heptapeptide repeats (unpublished work) was used to detect RPO21 polypeptides. An alkaline phosphatase-coupled secondary antibody (Bio-Rad) was used for visualization of the primary antibody.

β-Galactosidase Assays. For β-galactosidase assays of each *rpo21* strain transformed with one of the seven *HIS3* plasmids described above, three independent *URA3:RY171*, *gal4:TRP1*, *HIS3* transformants were restreaked onto glucose minimal medium plates; from each restreak, three isolated colonies were assayed in duplicate for β-galactosidase activity. The colonies were grown to an OD_{600} of ≈1.5 in raffinose/galactose minimal medium, and 1-ml aliquots were assayed essentially as described by Guarente (29), except that the pelleted cells were resuspended in 0.15 ml of Z buffer,

and 0.7 ml of *o*-nitrophenyl β-galactoside (2 mg/ml) was added to each assay tube. The β-galactosidase activities, expressed in units, were normalized to the OD_{600} of the cultures and to the assay time (29). We note that the β-galactosidase values reported for the GAL4 expression plasmids in our experiments were lower than those reported by Ma and Ptashne (16) for the same GAL4 plasmids. This is most likely a consequence of the different yeast strains used.

RESULTS

A model of transcriptional regulation, which specifies interactions between GAL4 and the RPO21 heptapeptide repeats (6), predicts that the ability of GAL4 to act as a transcriptional activator might be modified by changing the number of RPO21 repeats available for such interactions. To test this prediction, we constructed yeast strains in which the wild-type *RPO21* gene had been modified at its 3′ coding region so that the mutant strains expressed an RPO21 polypeptide with either less or more repeats than are present in the wild-type RPO21 C-terminal domain. We then compared the transcriptional activating abilities of both wild-type and functionally defective GAL4 proteins in each of these mutant *rpo21* strains to their activities in a strain with a wild-type RPO21 polypeptide. The mutant *gal4* genes we chose to examine were selected from a larger panel of *gal4* deletions described by Ma and Ptashne (16) and encoded GAL4 proteins with a broad range of transcriptional activating abilities. These mutant *gal4* genes with activating-region deletions were introduced into each strain on 2-μm vectors, and GAL4-dependent transcription was assayed by using a *GAL1-lacZ* fusion reporter gene.

Expression of RPO21 Polypeptides with Varying Numbers of Heptapeptide Repeats. For the experiments reported in this paper we used an *RPO21* deletion mutation, *rpo21-Δ88*, which encodes an RPO21 polypeptide containing 13 2/7 heptapeptide repeats (6). The C-terminal domain of this mutant peptide therefore contains only half as many repeats as the wild-type RPO21 polypeptide (see Fig. 1). We also constructed a mutant *rpo21* gene that encodes a longer C-terminal domain containing 38 heptapeptide repeats. This mutant allele, *rpo21-ylt*, contains DNA encoding the first 12 heptapeptide repeats, followed by a sequence encoding the entire yeast C-terminal domain of 26 repeats (Fig. 1). Both of these mutant alleles were integrated stably into the genome of haploid yeast, replacing the wild-type *RPO21* locus. While the *rpo21-ylt* strain had no detectable growth phenotype, the *rpo21-Δ88* strain, as noted previously (6), grew more slowly than the wild-type strain on both rich and minimal media.

To establish that the mutant *rpo21* yeast strains synthesized RPO21 polypeptides of the predicted molecular weight and to ensure that these mutant proteins were not being modified unexpectedly by posttranslational proteolytic events, we analyzed the RPO21 polypeptides being expressed by the mutant strains. RPO21 in protein extracts of the three haploid yeast strains, *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt*, was detected immunologically with a monoclonal antibody raised against a synthetic peptide consisting of five consensus RPO21 C-terminal repeats. This immunoblot (Western) analysis showed that each mutant strain expressed an RNA polymerase II largest subunit protein of a size predicted by its respective *RPO21* allele and that these polypeptides appeared to be stably maintained in the cells (Fig. 2).

The rpo21-Δ88 polypeptide is present in these extracts at a level comparable to that of wild-type and rpo21-ylt polypeptides. Its apparent underrepresentation in this Western blot is due to the specificity of the detecting antibody for the repeating heptapeptides. The amount of RPO21 polypeptide detected was proportional to the number of C-terminal repeats encoded by the *RPO21* allele of each strain; 13 in *rpo21-Δ88*, 26 in *RPO21*$^+$, and 38 in *rpo21-ylt*. We have repeated the immunodetection, using an antibody raised against an N-terminal portion of RPO21, which is common to each of the RPO21 polypeptides used in these experiments, and we have confirmed that the level of RPO21 expression does not differ significantly in these three strains (data not shown). The diffuse bands migrating more slowly than the major RPO21 polypeptide in Fig. 2 likely represent phosphorylated forms of the RPO21 protein (30). The significance of this phosphorylation of the yeast RPO21 polypeptide has not been addressed in these studies.

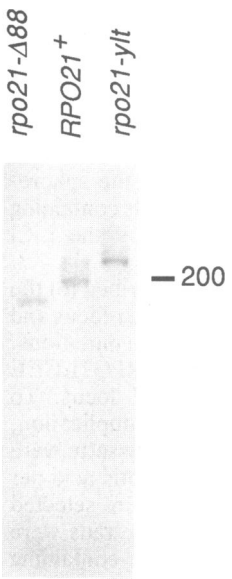

FIG. 2. RPO21 polypeptides present in cell extracts from *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt* strains. Total cell proteins from each strain were fractionated on a 5% polyacrylamide/sodium dodecyl sulfate gel, transferred to nitrocellulose, and blotted with a monoclonal antibody preparation raised against a 37-amino acid peptide containing five of the RPO21 consensus heptapeptide repeats. Size is indicated in kDa.

The Length of the RPO21 C-Terminal Domain Does Not Affect Wild-Type GAL4 Activity. We transformed each of the three yeast strains, *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt*, with a 2-μm vector containing the wild-type *GAL4* gene. GAL4-dependent transcription in each strain was measured by determining the level of expression of β-galactosidase from the single-copy *GAL1-lacZ* reporter gene present in these cells. Fig. 3*A* shows the results of assays of β-galactosidase activity in extracts of the three strains. The amount of β-galactosidase activity was ≈600 units regardless of the length of the RPO21 C-terminal domain. Therefore, the wild-type GAL4 polypeptide activates transcription to the same extent in the wild-type and mutant *RPO21* strains examined here. Strains transformed with the plasmid pMA200, which lacks *GAL4* DNA, expressed 0.2 unit of β-galactosidase (data not shown).

The Activity of Mutant GAL4 Proteins Depends upon the Number of Repeats Present in the RPO21 C-Terminal Domain. We next examined the ability of four different mutated GAL4 proteins, with activation-domain deletions (diagrammed in Fig. 3), to activate transcription in strains containing the *rpo21-Δ88*, *RPO21*$^+$, and *rpo21-ylt* alleles. The activity of the mutant GAL4 proteins in the *RPO21*$^+$ strain ranged from 10% (mutant pMA237) to 0.5% (mutant CD19XX) of the activity of wild-type GAL4 protein, as monitored by expression of the *GAL1-lacZ* reporter gene. The relative activation potentials of the GAL4 mutant proteins in combination with the wild-type RPO21 polypeptide differed somewhat from those measured by Ma and Ptashne (16), probably because of differences between our yeast strain and the YM335 strain derivatives used in the original characterization of these mutants.

When we examined each of these *GAL4* mutants in the yeast strain containing the *rpo21-Δ88* allele encoding fewer heptapeptide repeats than wild type, we found that the severity of the GAL4 mutant phenotype was enhanced (Fig.

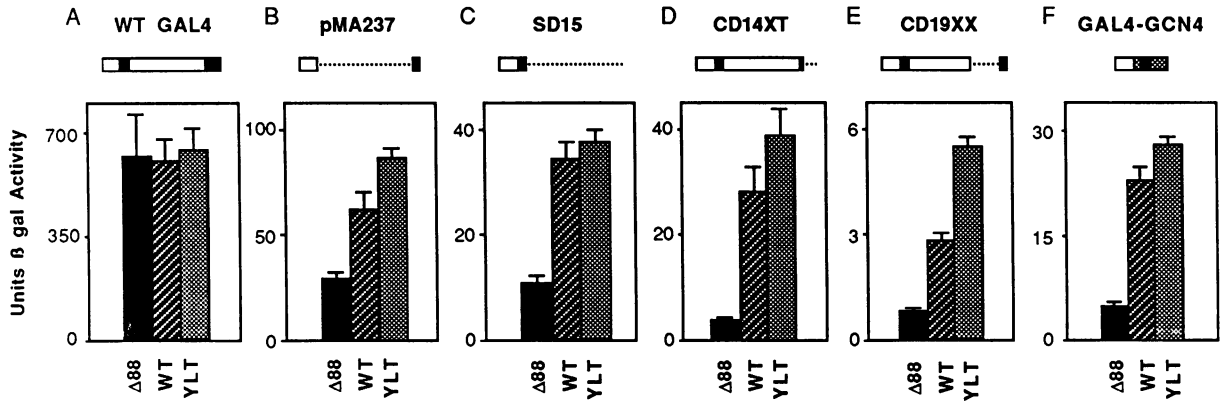

FIG. 3. Transcriptional activation by wild-type, mutant, and chimeric transcription factors in different *RPO21* strains. Each panel shows the transcriptional activity, measured in β-galactosidase units, of a different transcription factor in each of the three strains containing alleles *rpo21-Δ88* (Δ88), *RPO21*$^+$(WT), and *rpo21-ylt*(YLT). (*A*) Wild-type GAL4. (*B–E*) Deletion mutants of GAL4. (*F*) GAL4-GCN4 chimeric transcription factor. Each GAL4 protein derivative is diagrammed above the corresponding histogram: unfilled boxes represent the GAL4 coding sequence, black boxes represent acidic activation regions, the dotted line represents the deleted portion of each protein, and the stippled box represents the GCN4 coding sequence. For each strain, β-galactosidase activity was measured in duplicate for extracts prepared from three colonies arising from each of three independent transformants. The standard deviation of each determination is indicated. These yeast strains, when transformed with the plasmid pMA200 which lacks *GAL4* DNA (16), expressed <0.2 unit of β-galactosidase activity (data not shown).

3 *B–E*). The ability of these mutant GAL4 polypeptides to activate transcription from the reporter gene was reduced to values that ranged from 12% to 47% of that measured for the same mutant GAL4 polypeptides in the presence of wild-type RPO21. Conversely, the mutant phenotype of each of the crippled GAL4 derivatives was partially suppressed when assayed in the *rpo21-ylt* allele-containing strain, which encodes an RPO21 polypeptide containing 50% more C-terminal heptapeptide repeats than are in the wild-type polypeptide. The extent of this suppression was dependent upon the particular GAL4 deletion being examined. For example, there was only minimal suppression of the SD15 mutant phenotype, whereas increasing the length of the RPO21 C-terminal domain increased the transcriptional activation by the CD19XX GAL4 polypeptide 96%.

To ensure that these differences in GAL4 activity were not due to aberrant start-site selection by the mutant RNA polymerases, we have also examined mRNA start sites using primer-extension assays. The three different RNA polymerases each initiate transcription of the *GAL1-lacZ* fusion gene at the same position (data not shown).

Transcriptional Activation by GCN4 Is Also Affected by Mutations in RPO21. To show that the effect of mutations in the RPO21 C-terminal domain was not specific to the activation-domain mutations of the GAL4 polypeptide, we replaced the activation domain of wild-type GAL4 with sequences encoding the transcription factor GCN4 (31). When this chimeric protein that included all of the acidic activating region of GCN4 was assayed for its ability to activate transcription of the *GAL1-lacZ* reporter gene in the *RPO21*$^+$ yeast strain, we observed that it had a weak activating activity. As was the case with the weakly active GAL4 mutant polypeptides, the activity of the GAL4-GCN4 hybrid protein was reduced in the *rpo21-Δ88* strain and increased in the *rpo21-ylt* strain (Fig. 3*F*).

DISCUSSION

We have constructed mutations in an RNA polymerase II subunit that either enhance or partially suppress the phenotype caused by mutations in the activation domains of a DNA-binding transcription factor, GAL4. The activity of defective GAL4 proteins is reduced in the presence of an RPO21 polypeptide containing a shortened C-terminal domain and is increased in the presence of an RPO21 polypeptide containing an extended C-terminal domain. Some trivial explanations for these observations have been ruled out by experiments reported in this paper. A Western blot verified that changing the number of repeats in the RPO21 C-terminal domain did not lead to altered steady-state levels of the largest subunit of RNA polymerase II in yeast cells. In other unpublished experiments, we have established that the mutations in the RPO21 subunit do not result in changes in start-site selection by the mutant RNA polymerase II enzymes on the *GAL1* promoter DNA, nor do these alterations affect start-site selection on the yeast gene *CYC1*, which has a series of well-characterized TATA sequences (32, 33) responsible for positioning multiple start sites of transcription. Finally, it is unlikely that the mutations we have introduced into the RPO21 polypeptide modified the catalytic function of the resulting RNA polymerase II enzyme, since transcription of the reporter gene dependent on wild-type GAL4 was unaffected by these RPO21 alterations.

Two models for the mechanism of action of regulatory proteins in transcription have been discussed recently (17, 18). The first model proposes that promoter-specific transcription factors, such as GAL4, interact with the TATA-binding factor, causing an enhancement of transcription from the adjacent start site(s). A second model postulates a direct interaction between DNA-binding regulatory factors and RNA polymerase II itself. This latter model of transcription regulation seems more analogous to the situation in prokaryotes, where DNA-binding accessory proteins such as the catabolite activator protein CAP (34) and the λ repressor (35) may make direct contact with RNA polymerase. We suggest that the data reported here are consistent with a version of the second model in which the contact between RNA polymerase II and transcription factors is mediated by the acidic domains of transcription factors and the heptapeptide repeats of the RPO21 polypeptide. This model provides the simplest explanation for our *rpo21* suppressor/enhancer data. Deletions in the activation domains of GAL4 and GCN4 result in defective regulatory proteins that are rate limiting for some step in the initiation of transcription. Our engineered RNA polymerase II mutants, while not rate-limiting themselves in combination with wild-type GAL4 (Fig. 3*A*), do change the ability of the mutant regulatory factors to activate transcription of the reporter gene. These results suggest that the RPO21 heptapeptide repeats function at the same rate-limiting step of initiation as do the acidic domains of the transcription factors GAL4 and GCN4. Therefore, these experiments complement biochemical experiments (unpublished) that indicate that the

heptapeptide repeats function during initiation reactions. The C-terminal domain of RPO21 and the activation domains of regulatory proteins could interact, recruiting RNA polymerase II to promoters, where further contacts between polymerase II and the TATA-binding factor may position the enzyme and define the start sites of transcription. In this view the overall strength of a promoter may be set by both the number and ability of different DNA binding regulatory proteins to provide promoter-specific contacts for RNA polymerase II.

It should be noted that our experiments have not excluded the possibility that a third protein is involved in this interaction, nor do they rule out the possibility that the activation domains of transcription factors contact the TATA box factor. Indeed, some evidence in favor of GAL4–TATA box factor contacts has recently been presented (36), and synergistic interactions between GAL4 and the TATA box factor on the one hand and the TATA box factor and RNA polymerase II on the other could provide an explanation for our results. However, the repetitive and redundant nature of the putative amphipathic α helices in the activation domains of some transcription factors (37) and the repetitive and redundant nature of the RPO21 C-terminal heptapeptide domain make direct contacts between transcription factors and RPO21 an attractive possibility. This proposed interaction between RPO21 and transcription factors could involve hydrogen bonding between the many side-chain hydroxyl groups of serine, threonine, and tyrosine residues in the RPO21 repeats and the carboxylic acid side chains of glutamic and aspartic acid residues in the activation domains of transcription factors. Analogous hydrogen bonds are found in proteins that bind carbohydrates, where acidic amino acids hydrogen bond with the sugar hydroxyl groups (38).

Our mutant RPO21 polypeptides are not rate-limiting for transcription of the *GAL1-lacZ* reporter gene in the presence of wild-type GAL4, perhaps because GAL4 is a very strong transcriptional activator. However, this may not be the case with other wild-type transcription factors that do not activate as strongly as GAL4. Indeed, we have shown that the GAL4-GCN4 chimeric transcription factor, although containing all of the acidic activation domain of GCN4, was affected by the heptapeptide repeat length in the RPO21 polypeptide. It is interesting to note in this context that yeast strains with the *rpo21-Δ88* allele are cold sensitive and, in addition, grow more slowly at 30°C than do isogenic strains with wild-type RPO21 (6). This growth defect is more pronounced as even more heptapeptide repeats are deleted from the C-terminal domain (6, 7). Perhaps the growth phenotype is caused by the inability of these mutant polymerases to transcribe adequate levels of some gene whose product is rate-limiting for growth.

A detailed biochemical analysis of the steps involved in the initiation of transcription in yeast cells has not been possible until recently (39) because of the lack of a yeast RNA polymerase II *in vitro* transcription system. These steps in the initiation of transcription are likely to be similar in yeast and other eukaryotic species, since yeast TATA binding factor is functionally analogous to that of mammalian systems (40, 41) and a yeast regulatory protein can activate transcription in *Drosophila* (42), plant (43) and mammalian cells (44, 45), while mammalian (46, 47) and *Drosophila* (12) transcription factors function in yeast. This evolutionary conservation of transcriptional-activation mechanisms is mirrored by a similar conservation of the RPO21 C-terminal domain in eukaryotes; the mammalian and yeast RPO21 heptapeptide repeat domains are functionally interchangeable in both yeast and rodent cells (6, 9). By using yeast or mammalian nuclear extracts, it may now be possible to design biochemical experiments to detect interactions between the heptapeptide repeats of RNA polymerase II and DNA-binding transcription factors. In these experiments it will be important to distinguish between the two possible modes of these proposed interactions: either direct contact or indirect interaction mediated by another component of the transcription machinery.

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