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Biochemistry

Yeast Gal11 protein mediates the transcriptional activation signal of two different transacting factors, Gal4 and general regulatory factor I/repressor/activator site binding protein 1/translation upstream factor

(protein–protein interaction/transcriptional regulation/mating/DNA-binding proteins)

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ABSTRACT **_GAL11_ was first identified as a gene required for full expression of some of the galactose-inducible genes in the yeast *Saccharomyces cerevisiae*. A null mutation within the _GAL11_ locus causes defects in mating, growth on nonfermentable carbon sources, and sporulation of _gal11_ homozygotes. The mating defect was observed only in _MATα gal11_ strains. Northern hybridization analysis revealed that a _gal11_ mutation impaired transcription of α-specific genes (_MFα1_ and _STE3_) but not of an a-specific gene (_STE2_). Furthermore, this mutation reduced expression of the _MATα_ locus, suggesting that a deficiency in MATα1 protein is responsible for the reduced expression of α-specific genes. Since general regulatory factor I (GRFI)/repressor/activator site binding protein 1 (RAP1)/translation upstream factor (TUF) is believed to be an activator of _MATα_ expression, we examined whether _PYK1_, which is known to be regulated by GRFI/RAP1/TUF, is also affected by the _gal11_ mutation. It was determined that the level of _PYK1_ message was significantly lowered by the mutation. The requirement for functional _GAL11_ in transcriptional activation was bypassed when either the upstream activating sequence of galactose-inducible genes or of _PYK1_ was placed very close to the TATA box, suggesting that one of the Gal11 protein functions is to mediate the activation signal of Gal4 and GRFI/RAP1/TUF, when the respective binding site is situated at the naturally occurring distance from the TATA box.**

Investigations over the last few years have generated a large body of information concerning transcriptional regulation of eukaryotic genes in terms of DNA–protein interactions. It appears that for even the simplest promoters, an activator protein binding to a site far upstream of a gene called an upstream activating sequence (UAS) can cause stimulation of transcription of the gene. One of the most intriguing problems in the field is the basis of this activating mechanism, which is able to tolerate manipulation of the distance separating the UAS and TATA box regions over a wide range of distances without affecting the efficiency of activation. The activation is now known to be mediated by binding of a common factor, transcription factor IID (TFIID), to its target site, the TATA box. There are a number of indications that interaction between TFIID and the UAS-binding activator protein requires at least one other protein (for review, see ref. 1 and references therein). This paper deals with a candidate for this hypothetical protein in the yeast *Saccharomyces cerevisiae*.

The regulatory circuit for galactose-inducible genes in yeast may be the best studied of all eukaryotic genes of this type. Two regulatory proteins, Gal4 and Gal80, have been identified, while a UAS has been found upstream of all galactose-inducible genes (for review, see ref. 2). Gal4 binds to the UAS of galactose-inducible genes (UAS_G) to stimulate transcription of the gene, and Gal80 is proposed to bind Gal4, resulting in a block of its action (3). Previously we suggested involvement of another regulatory gene *GAL11* in this circuit (4): yeast with a recessive mutation in that locus ferment galactose only weakly, and the weak fermentation was caused by reduced transcription of the *GAL1*, *GAL7*, and *GAL10* genes (5). However, not all of the galactose-inducible genes require *GAL11* function for their expression: *GAL80* and *MEL1* are transcribed in *gal11* mutants as efficiently as in the wild type (5). Furthermore, a *gal11* null mutant exhibits pleiotropic phenotypes such as slow growth on nonfermentable carbon sources, poor sporulation of *gal11* homozygotes, and inefficient mating. These results imply that *GAL11* function is not specific to galactose-inducible genes but is involved in the regulation of many other genes.

We have found that only α-type yeast cells carrying a *gal11* mutation exhibited a mating defect, which was attributed to reduced expression of the *MFα1* gene. We further determined that the reduction was due to a deficiency in the level of MATα1 protein, an activator of the *MFα1* gene. This line of study has led us to the conclusion that another UAS-binding protein, general regulatory factor I (GRFI)/repressor/activator site binding protein 1 (RAP1)/translation upstream factor (TUF) (6–8), requires *GAL11* for its action. Based on our results, a possible role for *GAL11* in transcriptional activation by Gal4 and GRFI/RAP1/TUF is discussed.

MATERIALS AND METHODS

Strains. *S. cerevisiae* YS12-1 (*MATa ade his3 leu2 ura3 trp1 gal11::URA3*) was constructed by introducing the pYM1256 plasmid (5) into *S. cerevisiae* MT8-1 [*MATa ade his3 leu2 ura3 trp1* (9)]. MT8-2 (*MATα*) is isogenic to MT8-1, and YS12-2 (*MATα gal11::URA3*) is isogenic to YS12-1.

Plasmids and DNA Probes. The pYS125 and pYS226 plasmids were constructed by inserting a synthetic oligonucleotide with the 17-base-pair (bp) consensus sequence of UAS_G

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Abbreviations: UAS, upstream activating sequence; GRFI, general regulatory factor I; RAP1, repressor/activator site binding protein 1; TUF, translation upstream factor; PRTF, pheromone responsive transcription factor; GRM, general regulator of mating; TFIID, transcription factor IID; yTFIID, yeast TFIID; UAS_G, UAS of galactose-inducible genes; UAS_{PYK}, UAS1 of *PYK1*.

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(see ref. 2) into the −125P and −226P plasmids (9), respectively. Construction of pKY56 and SY652 was described elsewhere (10). DNA probes used for a bandshift assay were a 127-bp *Xho* I–*Hin*fI fragment of plasmid X716 containing UAS1 of the *PYK1* gene (10) and a 323-bp *Dde* I fragment containing UAS of *MATα* (11). Probes used for Northern hybridization analysis were as follows: *MFα1*, a 1.7-kilobase *Eco*RI fragment encompassing the whole gene (12); *STE3*, a 1475-bp *Eco*RI fragment [nucleotides 813–2288 (13)]; *STE2*, a 1395-bp *Sal* I–*Hin*dIII fragment [nucleotides 359–1753 (13)]; *STE7*, a 530-bp *Eco*RV fragment of plasmid pSTE7.1 [ref. 14; nucleotides 830–1352 (15)]; *STE12*, a 590-bp *Bam*HI–*Sac* I fragment [nucleotides 1863–2453 (16, 17)]; *MATα1*, a 700-bp *Eco*RV fragment [nucleotides 1913–2612 (18)]; *MATα2*, a 454-bp *Hin*cII–*Rsa* I fragment [nucleotides 668–1121 (18)]; *MATa1*, a 583-bp *Alu* I–*Bgl* II fragment [nucleotides 1427–2009 (18)]; and *PYK1*, an 1880-bp *Eco*RI fragment [nucleotides −476 to 1402 (19)].

DNA-Binding Assay. Yeast whole-cell extracts were prepared as described (20) except that the disruption buffer contained 20 mM Hepes (pH 7.6), 20 mM KCl, 1 mM EDTA, 10% (vol/vol) glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 2 μM pepstatin A, 1 μM leupeptin, and 1 μM antipain. DNA-binding reactions were performed in a 20-μl reaction mixture composed of 0.1–0.5 ng of 5′ end-labeled DNA probe, 10–20 μg of protein (1 μl of extract) and 1 μg of poly(dI-dC) in the disruption buffer supplemented with 0.1 mM spermidine. After incubation on ice for 15 min followed by incubation at 25°C for 10 min, the mixture was loaded onto low-ionic-strength polyacrylamide gels and electrophoresed.

Enzyme and Protein Assays. The β-galactosidase assay (21) and the catechol 2,3-dioxygenase assay (10) were performed as described. Protein was determined by the method of Peterson (22).

Northern Hybridization Analysis. Isolation of yeast total RNA was described previously (5), and electrophoresis and blotting were performed as described (23). ^{32}P-labeled DNA probes were prepared by using a random-primer DNA labeling kit (Takara Shuzo, Kyoto) and [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq; Amersham); and ^{32}P-labeled RNA probes were synthesized by using the SP6 system kit (Amersham), [α-^{32}P]UTP (3000 Ci/mmol, Amersham), and pSP65 plasmid derivatives containing appropriate DNA probe fragments. Hybridization was carried out at 42°C for 18 hr.

RESULTS

Cell-Type-Specific Mating Defect Caused by a *gal11* Null Mutation. As described previously (5), yeast cells bearing a *gal11* null mutation exhibit a defect in the induction of the galactose-inducible genes and growth on nonfermentable carbon sources. In addition, we found that *MATα gal11* cells mate inefficiently with *MAT***a** cells, whereas *MAT***a** *gal11* cells do so normally with cells of the opposite mating type. This observation prompted us to examine whether the *gal11* mutation has an effect on the production of α factor, which is required for mating of *MATα* cells. Production of mating pheromone can be assayed by growth inhibition of a mutant strain [*sst1* (24)] that is supersensitive to the pheromone. As shown in Fig. 1, *MATα gal11* mutant yeast yielded a much smaller growth inhibition zone than the wild-type cells, indicating that the level of α-factor production was greatly reduced. On the other hand, *MAT***a** *gal11* cells inhibited growth of *MATα sst2* cells as efficiently as GAL11$^+$ cells (data not shown).

Expression of α- and a-Specific or Cell-Type Nonspecific Genes in a *gal11* Null Mutant. To determine if the reduced production of α factor was due to the level of transcription, Northern hybridization analysis was performed to examine

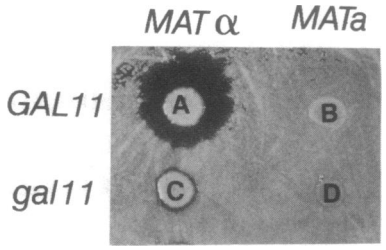

FIG. 1. Effect of a *gal11* mutation on α-factor production. Tested strains (*MATα* and *MAT***a** cells carrying either the *GAL11*$^+$ or the *gal11* mutation) were spotted onto a lawn of *MAT***a** *sst1* cells. The size of the clear zone around the spots indicates the amount of α factor produced. Spots: A, MT8-2; B, MT8-1; C, YS12-2; D, YS12-1.

the expression of both the *MFα1* gene, which encodes the majority of the mating pheromone in the cell (12), and another α-specific gene, *STE3*, which encodes a receptor for **a** factor (13). A *gal11* null mutation diminished expression of both genes (Fig. 2*A*), suggesting that the mating defect was caused by reduced transcription of the *MFα1* gene and that the effect of the Gal11 protein deficiency was exerted on α-specific genes in general. On the other hand, expression of an **a**-specific gene, *STE2*, encoding an α-factor receptor (13) was reduced only slightly (Fig. 2*B*). Expression of cell-type nonspecific genes, *STE7* (14, 15) and *STE12* (16, 17), was not affected by the *gal11* null mutation (Fig. 2*C*).

Expression of *MATα* Locus Is Reduced by a *gal11* Null Mutation. Since both **a**-specific and nonspecific genes require the product of the *MCM1* gene, Mcm1 protein (25), also known as PRTF (pheromone responsive transcription factor,

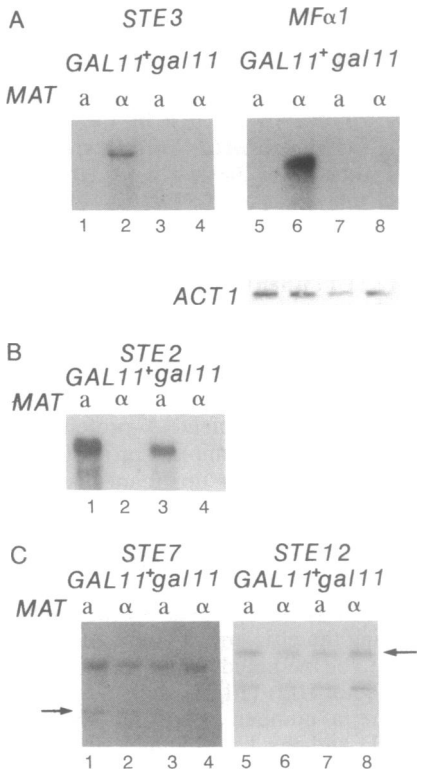

FIG. 2. Effect of a *gal11* mutation on transcription of α-, **a**-, and nonspecific genes. Transcription of *MFα1* and *STE3* (*A*), *STE2* (*B*), and *STE7* and *STE12* (*C*) is shown in *GAL11*$^+$ and *gal11* strains of both mating types. RNA levels were normalized by stripping the filter followed by rehybridization to the *ACT1* message (shown below the *MFα1* filter). In *C*, transcripts of *STE7* and *STE12* are indicated by arrows. The presence of extra bands was also reported previously (14, 16).

ref. 26) or GRM (general regulator of mating, ref. 27), and Ste12 protein (16, 17) for their expression, a *gal11* mutation did not seem to affect the function of either of these two factors. Transcription of the *STE12* gene was not perturbed by the loss of *GAL11* function (Fig. 2*C*, lanes 7 and 8). Expression of α-specific genes requires the *MATα1* gene product (28) in addition to Mcm1 protein and Ste12. We therefore tested expression of the *MATα* locus in *MATα gal11* cells. Northern hybridization analysis showed that transcription of the *MATα* locus was reduced to <5% of the wild-type level in the *gal11* null yeast, whereas the degree of *MAT***a** transcription was decreased to approximately half the level of wild type (Fig. 3). Expression of *MATα1* could be repressed by **a**1/α2 complex (28) if *MAT***a***1* was aberrantly expressed in *MATα* cells. The results shown in Fig. 3, however, indicate that this is not the case in *MATα gal11* cells (Fig. 3, lane 12). From these results, we conclude that a deficiency in production of MATα1 positive factor is responsible for the defective expression of α-specific genes in *gal11* mutants, which ultimately results in α-specific mating deficiency.

Expression of *PYK1* Requires Normal *GAL11* Function. Transcription of the *MATα* locus is believed to be activated by GRFI/RAP1/TUF (6–8), which binds specifically to an upstream sequence (6, 7). This sequence is known to function as a UAS *in vivo* (29). We therefore assumed that GRFI/RAP1/TUF exerts its action through the Gal11 protein. To test this idea, we studied whether transcription of the *PYK1* gene, known to be activated by GRFI/RAP1/TUF (refs. 10 and 29; M.N., unpublished data), is affected by a *gal11* mutation. Indeed *PYK1* appeared to be transcribed poorly in the *gal11* null mutant (Fig. 4*A*). These results suggest that the Gal11 protein is also involved in transcriptional activation of the GRFI/RAP1/TUF-dependent genes. Alternatively, one might argue that a *gal11* mutation causes low production of GRFI/RAP1/TUF. To rule out this possibility, we conducted a bandshift assay using whole-cell extracts from *gal11* or wild-type yeast and a ^{32}P-labeled DNA fragment containing the respective UAS of *MATα* and *PYK1*. No appreciable difference was observed between the mutant and wild-type extracts (Fig. 4*B*) in the intensity of the shifted band due to binding of GRFI/RAP1/TUF to the probe. This result indicated that the loss of *GAL11* function appreciably affected neither the amount of GRFI/RAP1/TUF nor its binding activity to DNA under our experimental conditions. In this regard, it should be noted that *GAL4* is transcribed in *gal11* mutant cells as efficiently as in the wild type (5).

Placing the UAS Close to the TATA Box Bypasses the *GAL11* Requirement. Among the galactose-inducible genes that require Gal4 protein for their transcription, *GAL80* does not require *GAL11* function for its expression (5). We have found that the UAS of *GAL80* is located 65 bp upstream of the TATA box (T.F., unpublished work). By contrast, the distance between the two elements is more than 160 bp in *GAL1*,

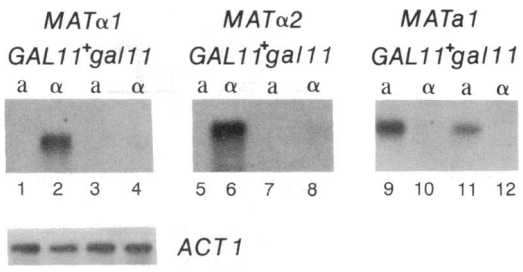

FIG. 3. Effect of a *gal11* mutation on expression of the *MAT* locus. Transcription of *MATα1*, *MATα2*, *and MAT***a***1* is shown. The filter hybridized with a *MATα1* probe was stripped and rehybridized with an *ACT1* probe to normalize RNA levels. The result is shown below the *MATα1* filter.

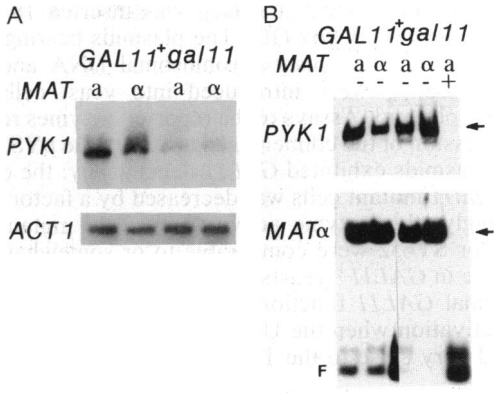

FIG. 4. Effect of a *gal11* null mutation on transcription of the *PYK1* gene and on binding activities of proteins to the UAS of *MATα* and *PYK1*. (*A*) Transcription level of *PYK1* and *ACT1*. The filter hybridized with a *PYK1* probe was stripped and rehybridized with an *ACT1* probe. (*B*) Bandshift assay using whole-cell extracts prepared from *GAL11*$^+$ or *gal11* strains and ^{32}P-labeled probes. An ≈50-fold molar excess of unlabeled probe was added to the reaction mixture as a competitor. The + and − signs above the lanes indicate the presence or absence of competitor DNA. Arrows indicate DNA–protein complexes. F designates the position of free probe, and the top panel shows only the shifted bands.

GAL10, or *GAL7* (2). We theorized that the *GAL11* dependency was related to the distance separating the UAS and the TATA box of an individual gene, such that genes that are normally *GAL11* dependent should be able to bypass the *GAL11* requirement for their transcriptional activation when the respective UAS was placed close to the TATA box element. To examine this idea, we constructed two sets of chimeric genes: one set utilizing a synthetic UAS_G of 17 bp and the other set utilizing the UAS1 of *PYK1* (UAS_{PYK}, ref. 10). As shown in Fig. 5, UAS_G was placed either 25 bp (pYS125 plasmid) or 126 bp (pYS226 plasmid) upstream of the TATA box of the *PHO5–lacZ* fusion gene, which had been depleted of its own UAS (9). Activity of the UAS_{PYK}-bearing promoter was monitored by expression of the *Pseudomonas putida xylE* gene fused downstream of the transcription initiation site of *PYK* (10). Plasmid pKY56 possesses the original upstream sequence in which the distance between UAS_{PYK} and the TATA box is 432 bp long (10),

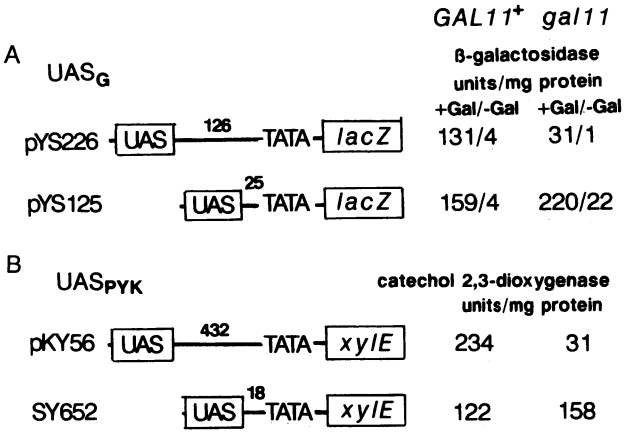

FIG. 5. Effect of a *gal11* mutation on transcriptional activity of the UAS_G (*A*) and the UAS_{PYK} (*B*). Schematic representation of the structure of each plasmid (pYS125, pYS226, pKY56, and SY652) and specific activities of the reporter enzymes expressed in *GAL11*$^+$ and *gal11* strains harboring each plasmid are shown. Numbers above the bar indicate the distance between the UAS and the TATA box in base pairs. In *A*, β-galactosidase was assayed in the presence (+Gal) and in the absence (−Gal) of galactose in the medium.

while in plasmid SY652, UAS_{PYK} was inserted 18 bp upstream of the TATA box (10). The plasmids bearing UAS_G were integrated into yeast chromosomal DNA and those bearing UAS_{PYK} were introduced into yeast cells on a multicopy plasmid. Assays of the reporter enzymes revealed that expression of the chimeric genes in both the pYS226 and pKY56 plasmids exhibited *GAL11* dependency: the enzyme level in *gal11* mutant cells was decreased by a factor of 4–5. Surprisingly, the enzyme activities in *gal11* mutants with pYS125 or SY652 were comparable to or somewhat higher than those in $GAL11^+$ yeasts (Fig. 5). These results indicate that normal *GAL11* function is not required for transcriptional activation when the UAS of *GAL11*-dependent genes is placed very close to the TATA box.

DISCUSSION

A null mutation in the *GAL11* locus confers yeast cells with pleiotropic phenotypes, including reduced expression of some *GAL* genes, defective growth on nonfermentable carbon sources, poor sporulation, and an α-specific mating deficiency. In this paper, we focused our attention on the mating defect and demonstrated that the defect was caused primarily by reduced expression of the *MATα* locus, which provides a positive factor, MATα1, required for *MFα1* expression. The product of the *MCM1* gene (Mcm1 protein/PRTF/GRM) is known to function as an activator for α-specific and a-specific genes in *MATα* and *MAT***a** cells, respectively (30). The same protein also functions as a corepressor of MATα2 protein to repress a-specific genes in *MATα* cells (27). We have not tested the effect of a *gal11* mutation on *MCM1* expression, but a bandshift assay with a DNA fragment containing the Mcm1 protein/PRTF/GRM binding site (25) showed no difference in shifted pattern between $GAL11^+$ and *gal11* cell extracts (data not shown). In addition, we found that a *gal11* mutation did not affect transcriptional activity of Mcm1 protein/PRTF/GRM (data not shown). By taking these observations into account, we conclude that a *gal11* mutation does not appreciably affect the Mcm1 protein/PRTF/GRM function on a detectable scale and that reduction in *MFα1* expression is due mainly to a deficiency in MATα1 protein levels.

We cannot definitely rule out the possibility that the observed low production of α factor was due partly to aberrant expression of *BAR1*, an a-specific gene encoding a protease that degrades α factor (31). Since MATα2 protein, a repressor for the a-specific genes, could be expressed poorly in a *gal11* mutant (Fig. 3), it would lead to derepression of the a-specific genes in *MATα* cells. Although the *STE2* transcript was not detected in *MATα gal11* cells, *BAR1* could be expressed if more α2 protein was required to repress *BAR1* than to repress *STE2*. In this regard, it is noteworthy that an α-specific mating defect observed in an *ssn6* null mutant was caused by aberrant expression of *BAR1* but that transcription of *MFα1* or *MATα2* was not reduced in that mutant (32). A *gal11* null mutation also appeared to influence expression of *STE2* and *MAT***a** loci, but to a lesser extent than that of the *MATα* locus. This suggests that *GAL11* may be involved in the regulation of expression of a-specific genes and the *MAT***a** locus as well by an as yet unknown mechanism.

We demonstrated that normal *GAL11* function is required for the transcriptional activation process invoked by binding of GRFI/RAP1/TUF to the UAS of *PYK1* (10, 29) and of *MATα* (7, 29). GRFI/RAP1/TUF also binds to upstream regulatory regions of many other genes (7, 8, 29), leading to either activation or repression of their expression. This could account for pleiotropic phenotypes of *gal11* mutants.

The Gal11 protein function was dispensable for full expression of both the Gal4-dependent and GRFI/RAP1/TUF-dependent genes when the respective UAS was located close to the TATA box. This result would explain why expression of *GAL80* is independent of *GAL11* function. A similar observation was reported in a mammalian system: Horikoshi *et al.* (33) reported that binding of Gal4 derivatives influences interaction of yeast TFIID (yTFIID) with the TATA box in a HeLa cell nuclear extract. The experiment was performed with a DNA template in which the distance between UAS_G and the TATA box was 20 bp. When the distance was extended to 180 bp, the level of transcription was significantly reduced (34). These results suggest that UAS_G has to be placed close to the TATA box for Gal4 to activate transcription in their *in vitro* system, where, in light of our results, a mammalian counterpart of Gal11 protein is missing. Chasman *et al.* (35) also reported that transcriptional activation in a yeast *in vitro* system depends on the relative distance between the UAS and the TATA box. They demonstrated that fusion proteins of Gal4 and Herpesvirus VP16, known to stimulate transcription from promoters with UAS_G in mammalian cells (36), were also potent activators in an *in vitro* transcription system utilizing yeast nuclear extracts. They further showed that the stimulatory effect decreased by a factor of 3 when UAS_G was moved from 50 bp to 150 bp upstream of the TATA box. These observations raise the possibility that a "strong" activator such as Gal4–VP16 may not need an auxiliary factor like Gal11 protein for maximum function.

How does Gal11 protein exert its function? The presence of a possible helix–turn–helix motif in Gal11 protein (5) suggests that the protein binds to DNA and functions as a coactivator of UAS-binding proteins. However, this possibility seems to be unlikely since no significant alteration in shifted pattern was observed when cell extracts from *gal11* mutant cells were used for a bandshift assay (Fig. 4*B*), unless we failed to detect Gal11 protein binding under our assay conditions. We rather favor the model illustrated in Fig. 6: Gal11 protein is a transmitter of the activation signal from UAS-binding proteins to yTFIID or other general transcription factors through protein–protein interaction (Fig. 6*A*). Alternatively, Gal11 protein may create a circumstance *in vivo* that facilitates interaction between UAS-binding proteins and yTFIID (Fig. 6*B*). When the distance between the UAS and the TATA box is shortened as in *GAL80* or in the

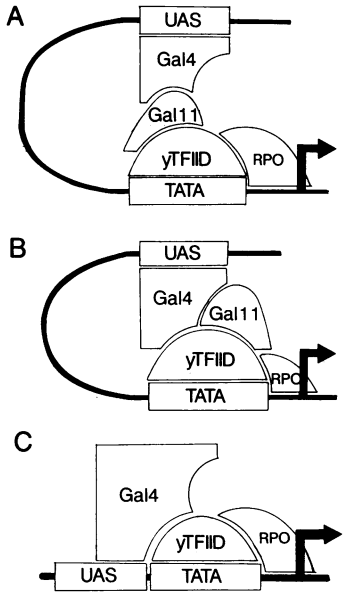

FIG. 6. Proposed model for function of Gal11 protein in transcriptional activation of *GAL11*-dependent genes. RPO, RNA polymerase II. For simplicity, only TFIID and RNA polymerase are shown as components of an initiation complex.

chimeric genes used in our experiment, Gal11 protein is dispensable since UAS-binding protein can interact directly with yTFIID or other general transcription factors (Fig. 6*C*).

While we were preparing this manuscript, we became aware of the paper by Fassler and Winston (37). They reported that *SPT13*, the gene identified as a suppressor for auxotrophs induced by the Ty insertion, was identical to *GAL11* and that loss of *SPT13/GAL11* function lowered transcription of *MATα1* and/or *MATα2*. However, they did not find that transcription of *PYK1* was impaired in *spt13/gal11* cells. The discrepancy may be due to the fact that they used a point mutant or a Tn*10* insertion mutant, which possibly retained some function of *GAL11/SPT13*. They argue that Gal11/Spt13 functions as a repressor of a gene adjacent to the Ty-insertion (37). We assume that Gal11 protein interacts with another yet unknown UAS-binding protein, resulting, in some cases, in repression of transcription.

The genetic analysis of Gal11 protein described in this paper, together with the biochemical approaches using *in vitro* transcription system of the yeast that is now available, provides an opportunity to reveal the molecular mechanism of transcriptional regulation in eukaryotic cells.

We are indebted to Mr. Makoto Igarashi for the synthetic UAS_G, to Dr. Stanley Fields for a *STE12* probe, and also to Dr. Denis Heck for carefully reading the manuscript.

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