Core promoter elements are essential as selective determinants for function of the yeast transcription factor GAL11

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Abstract **The *GAL11* gene product, which copurifies with RNA polymerase II holoenzyme, is necessary for full expression of many, but not all, genes in yeast. Here we shows that the *GAL11* dependence of a gene for expression is determined by the core promoter structure. In the *GAL80* gene, a *gal11* null mutation caused reduction of TATA-dependent transcription, but exerted no effect on initiator-mediated transcription. *GAL11* stimulated TATA-dependent transcription, but did not affect the TATA-independent transcription in *HIS4*. *GAL11* was also required for transcription mediated by a canonical TATA sequence but not by a nonconsensus TATA sequence of *HIS3*. These results suggest that *GAL11* is specifically involved in the transcription machinery formed on the TATA element.**

Key words: GAL11; Promoter structure; TATA-less gene; Initiator; *Saccharomyces cerevisiae*

1. Introduction

In eukaryotes, two distinct sequences, the 'TATA box' and 'initiator' (Inr), are known as the elements that constitute the core promoter of the protein encoding genes. RNA polymerase II and general transcription factors assemble on the core promoter to generate the 'preinitiation complex' either in a stepwise fashion [1,2] or as a preformed sub-complex in RNA polymerase II holoenzyme [3–5]. The preinitiation complex assembles on the TATA box [1,2], which is involved in determination of the accurate initiation in combination with a preferred sequence in the downstream region in *Saccharomyces cerevisiae* [6,7]. The Inr element, which overlaps the transcription start site, contains all the information required for directing accurate transcription initiation in the absence of the TATA box [8]. It is inferred that Inr alone provides a region for assembly of the preinitiation complex [9,10]. Other promoter elements may exist whose mechanisms of transcription initiation have not been well characterized.

The *GAL11* gene of the yeast *S. cerevisiae* was first isolated as the gene for an auxiliary transcription factor required for full expression of galactose-inducible genes [11]. Although *GAL11* is not essential for cell viability, its loss-of-function mutations result in pleiotropic defects [12], suggesting that the *GAL11*-encoded protein (Gal11p) affects expression of various genes. In fact, *gal11* mutations have also been isolated as *snf* and *spt* (*spt13*) mutations, which are identified as regulatory genes for *SUC2* [13] and yeast transposable elements Ty [14], respectively. We have previously demonstrated that Gal11p stimulates the basal transcription in cell-free transcription systems developed from yeast nuclear or whole cell extracts [15,16]. These results suggest that Gal11p is involved in formation or stabilization of the preinitiation complex via direct interaction with a general transcription factor(s) therein. This notion has been supported by the recent finding that Gal11p copurifies with the RNA polymerase II holoenzyme [17,18]. In fact, immunoprecipitation experiments with anti-Gal11p antibody have demonstrated that Gal11p coprecipitates with the largest subunit of RNA polymerase II [19].

In this study, we analyzed the effect of a null mutation of *GAL11* on the expression of several genes that have two alternative mechanisms for transcription initiation in yeast. Here we demonstrate that the normal function of *GAL11* is necessary for maximal transcription mediated by the TATA box but is dispensable for transcription mediated by TATA-less promoters. These results suggest that Gal11p is a specific component of the preinitiation complex formed on the TATA box but is absent from the complex formed on TATA-less promoters.

2. Materials and methods

2.1. Yeast strains and media

The yeast strains used in this study are listed in Table 1.

Minimal medium (MM) consisted of 2% glucose and 0.67% yeast nitrogen base. Compositions of rich media (YPGlyLac, YPGlyLacGlu, and YPGlyLacGal), synthetic complete medium (SC), and enriched synthetic media (ESGlyLac, ESGlyLacGlu, and ESGlyLacGal) have been described previously [20].

2.2. Plasmids construction

The plasmids used for analysis of *GAL80* expression have been described previously [20], and their structures are shown in Fig. 2A. Tester gene pIG80Tpdm contains mutations of both pIG80Tpm and pIG80Tdm [20]. Tester gene pIGTI, which has a Gal4p-binding sequence (UAS_G)-TATA box-Inr construct, was created as follows (also see Fig. 2A): the *Nhe*I (nucleotide position at −73)-*Ssp*I (at −44) fragment of *GAL7* containing the TATA sequence (nucleotide positions are expressed with respect to the transcription initiation site at +1 [21]) was cloned into the *Xba*I and *Sma*I sites of pSP72 (Promega). The TATA sequence was liberated from the resulting plasmid by digestion with *Sal*I and *Bgl*II, and cloned into the *Sal*I and *Bam*HI sites of pG80-7 [20], such that the distance between the TATA box and Inr of *GAL80* is 60 bp (pSK307). UAS_G liberated by *Nsi*I and *Xho*I digestion from pSK119 [15] was cloned into the *Pst*I and *Sal*I sites of pSK307 (pSK309). The *Hin*dIII (blunt-ended)-*Bgl*II fragment of pSK309 containing the UAS_G-TATA-Inr construct was cloned into the *Sal*I (blunt-ended) and *Bam*HI sites of pIG80V [20] to create pIGTI.

Reporter plasmids of *GAL7-lacZ* were constructed as follows: plasmid pSK145 contains the sequence between −271 and +43 of *GAL7* [16]. Plasmid pSK162, in which the TATA box −68 AAAGATATAAAAG −56 of *GAL7* was changed to −25 CTGAATATATATA −13 of *GAL80*, was constructed by primer chain reaction (PCR) with the template pSK145 and the primer 5′ −77 TTGGCTAGC-

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CTGAATATATATACAGGTCGGAAATATTTATGGGC −34 3′ (changed nucleotides are underlined). In the plasmids pSK364, pSK365, and pSK366, the TATAAA sequence of pSK145 was altered by PCR to CATAAA, TATTAA, and TATTTA, respectively. To construct the integration-type reporter plasmid pI145Z containing the *GAL7-lacZ* fusion and the *URA3* gene, the *CYC1* promoter region encompassing between the *Sal*I and *Bam*HI sites of pSK235 [15] was replaced by the *Sal*I-*Bgl*II fragment of pSK145. The reporter plasmids, pI162Z, pI364Z, pI365Z, and pI366Z, were constructed as *GAL7-lacZ*.

An integration-type *HIS4-lacZ* (pI339Z) was constructed as follows: plasmid p1531, which is a centromeric plasmid harboring *HIS4-lacZ* (fused to *lacZ* at *HIS4* amino acid 11), was a kind gift from A.G. Hinnebusch. The *Nsi*I (at −499, blunt-ended)-*Sac*I (in *lacZ*) fragment of p1531 was inserted between the *Sal*I (blunt-ended) and *Sac*I (in *lacZ*) sites of pSK235 [15]. Plasmid pI357Z, in which the TATA sequence from −51 to −36 was deleted [22], was created by PCR with pI339Z and the primer 5′ CGTATACTGTCCTCGAGGTTCACCTCCGATGTGTGTTGTACA 3′.

2.3. RNA analyses

S1 nuclease mapping was carried out as described [20]. In the case of primer extension analysis, total RNA was dissolved in the annealing buffer [16] containing 60 fmol of 5′ end-labeled primer. After heat treatment at 94°C for 5 min, annealing and extension reactions were carried out as described [16]. The reaction mixture was treated with RNase A, and the extension products were analyzed as described [16].

2.4. Assay of β-galactosidase activity

GAL7-lacZ and *HIS4-lacZ* fusions were digested with *Sma*I and *Stu*I, respectively, and integrated into the *ura3* locus of the yeast cells. Each of the transformants was grown to reach A_{600} of 1.0 in appropriate medium, and the activity of β-galactosidase was assayed as described [15,20]. The specific activity of β-galactosidase is expressed as the amount of orthonitrophenyl-β-D-galactopyranoside hydrolyzed per min per cell mass in arbitrary unit [15]: $A_{420} \times 1000/(A_{600} \times$ time in min). Each assay was performed in triplicate and the standard error was less than 20%.

3. Results

3.1. Effect of GAL11 on the expression of GAL80

Loss-of-function mutations of *GAL11* causes a remarkable reduction in the steady-state amount of transcripts of galactose-inducible genes, such as *GAL1*, *GAL2*, *GAL7*, and *GAL10* [11,12], whose expression is under the control of transcriptional activator Gal4p (see [23] for review). However, an RNA blot analysis indicated that the amount of the *GAL80* mRNA was affected by *gal11* mutations but only slightly in either the uninducing (glucose-grown) or inducing (galactose-grown) conditions [12], despite the fact that the expression of *GAL80* is also regulated by Gal4p [24,25]. These results prompted us to address why the expression of *GAL80* was less responsive to the *GAL11* function than the other galactose-inducible genes.

In contrast to those *GAL11*-responsive genes, whose transcription is exclusively mediated by the TATA box [21,26–29], *GAL80* has two alternative pathways in transcriptional initiation: the constitutive transcription initiated at +1 site is directed by Inr activity, whereas the galactose-inducible transcription which starts from a set of downstream sites at +37, +47, +56, and +67 is regulated by the TATA box [20]. We then analyzed the transcription initiation pattern of *GAL80* in a *gal11* null strain by S1 nuclease mapping technique (Fig. 1).

Table 1
Yeast strains and relevant genotypes

Strain	Relevant genotype
HSY5-3C	*MATα ade1 his1 leu2 ura3 trp1*
HSY5-3B	*gal11::LEU2* of HSY5-3C
NOY396	*MATα ade2-1 ura3-1 his3-11 trp1-1 leu2-3,112 can1-100*
HS16	*gal11::LEU2* of NOY396
HS18	*gal4::HIS3* of NOY396
HS21	*MATα ura3-1 trp1-1 leu2-3,112 can1-100*
HS22	*gal11::LEU2* of HS21
HS23	*gcn4::TRP1* of HS21
HS24	*gcn4::TRP1 gal11::LEU2* of HS21

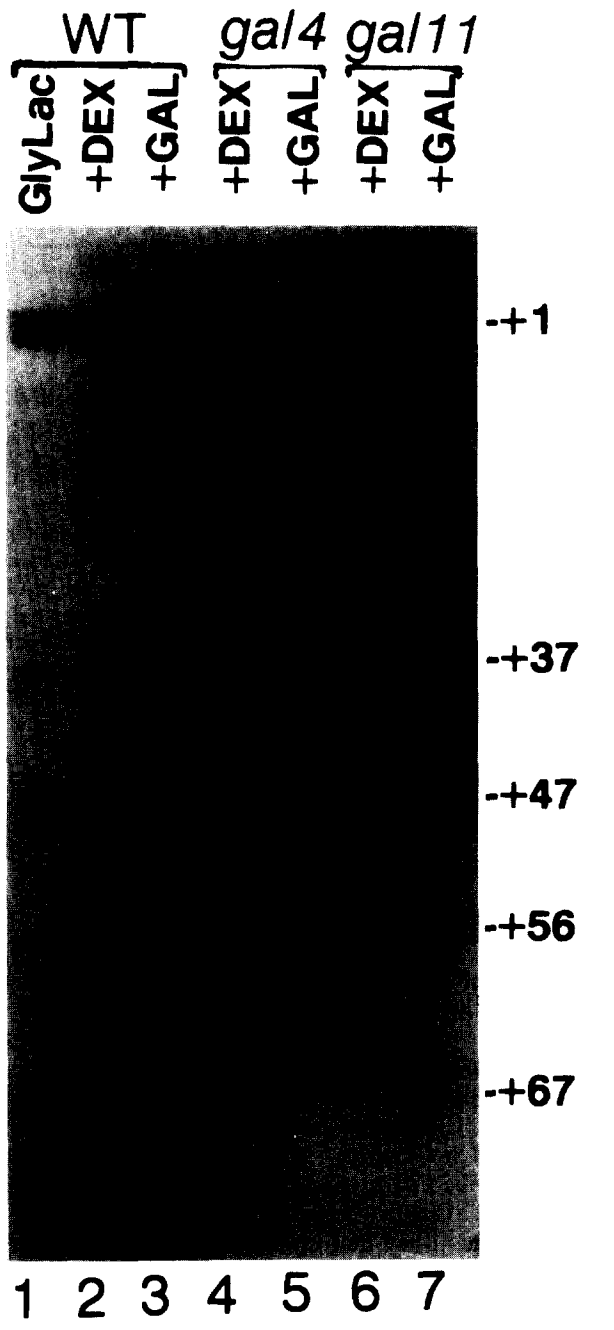

Fig. 1. Transcription of *GAL80* in various strains grown on various carbon sources. The wild-type (NOY396, lanes 1–3), *gal4* null strain (HS18, lanes 4 and 5), and *gal11* null strain (HS16, lanes 6 and 7) were grown in the YPGlyLac medium in the presence of glucose (+DEX; lanes 2, 4, and 6) or galactose (+GAL; lanes 3, 5 and 7). Total RNA (20 μg) was subjected to S1 nuclease mapping with probe DNA labeled at the *Fok*I site (+172) as described [20]. Figures to the right of the panel indicate transcription start sites representing the most upstream initiation site at +1 [20].

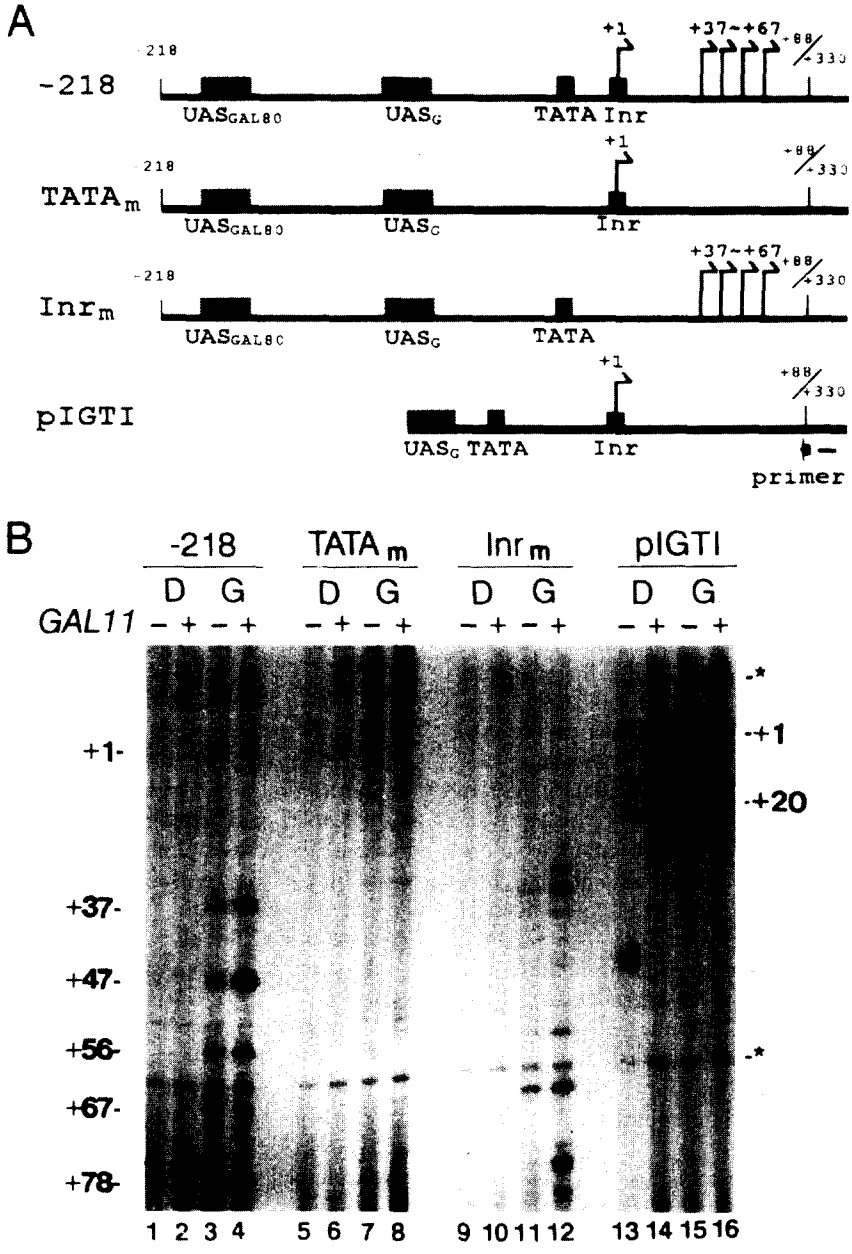

Fig. 2. TATA-dependent but not initiator-dependent transcription is regulated by *GAL11*. A: Schematic representation of the tester genes, pIG80-218 (−218), pIG80Tpdm ($TATA_m$), pIG80Im (Inr_m), and pIGTI. Hatched boxes represent UAS_G and an unknown positive element of *GAL80* (UAS_{GAL80}). Solid boxes represent the TATA box and Inr. Arrows indicate the transcription initiation sites under the inducing conditions (see text, [20]). Position of primer DNA is also shown by bold arrow. B: Primer extension analysis of mRNA from the *GAL80* tester genes. The tester plasmid was integrated into the *ura3* locus of the wild-type (NOY396, even lanes) or *gal11* null strain (HS16, odd lanes). Transformants were grown in the ESGlyLac medium lacking uracil, then glucose (D) or galactose (G) was added to the culture. Primer DNA of *GAL80* 5′ +463 GGCTTGATCTAGTGAACATGCAAGGGCCCA +434 3′ was incubated with total RNA (20 μg) and extended. Tester plasmids are pIG80-218 (−218, lanes 1–4), pIG80Tpdm ($TATA_m$, lanes 5–8), pIG80Im (Inr_m, lanes 9–12), and pIGTI (lanes 13–16). Figures to the left of the panel represent transcription start sites [20]. Asterisks to the right of the panel indicate artifactual bands of primer extension analysis. The amount of sample in lane 13 was seen slightly lower than those in lanes 14 to 16, however, equivalent amounts of RNA were used for primer extension analysis.

When yeast cells were grown in the absence of galactose, the amount of the only transcript initiated at +1 was not appreciably changed in the presence or absence of *GAL11* (lanes 1, 2, and 6). In galactose-grown cells, several transcripts initiated at the downstream sites emerged in addition to the transcript from +1 (lanes 3 and 7). In accordance with previous experiments [20], transcriptional activation was fully dependent on Gal4p (lanes 4 and 5). The amount of transcript from +1 increased in *gal11* null yeast compared to that of the corresponding transcript in the *GAL11* control. By contrast, transcripts from the downstream sites were significantly decreased in the absence of *GAL11* (compare lanes 3 and 7). The results of S1 nuclease mapping analyses therefore successfully explains why the overall amount of *GAL80* transcripts was changed less remarkably than those of *GAL2* or *GAL10* [12] transcripts by the mutational loss of *GAL11* function under either growth condition in the previous Northern analyses. Also, it looks as if the *GAL11* function repressed the Inr-

dependent transcription from +1, but stimulated the TATA-dependent transcription in galactose-grown yeast.

We have previously suggested the existence of competition between the TATA- and Inr-directed transcriptions of *GAL80*, possibly because the distance between two elements is as short as 20 bp [20]. To analyze the effect of *GAL11* on the TATA- and Inr-dependent transcription separately, we constructed two tester genes, bearing a mutation in either the TATA box or Inr (Fig. 2A). A tester gene which has an internal deletion in the open reading frame of *GAL80* [20] was integrated into the yeast chromosome at *ura3*, and its expression was analyzed by primer extension (Fig. 2B). The initiation pattern of pIG80-218 transcription (lanes 1–4), which contains the 5′ upstream sequence to −218, was similar to that of the chromosomal *GAL80* gene (see Fig. 1); transcripts from +37 and +47 under inducing conditions were significantly reduced in the absence of *GAL11*. A mutation of the TATA box resulted in loss of the downstream transcripts in galactose-grown cells, but showed no effect on transcription from +1 ($TATA_m$, lanes 5–8). The Inr-dependent transcription from +1 of this tester gene was not affected by the *gal11* defect. Transcripts from +1 were not seen in the cells harboring pIG80Im which bears a mutation in the Inr sequence, irrespective of the presence or absence of galactose (Inr_m, lanes 9–12). When the cells were grown in the presence of galactose, transcription was predominantly initiated at +67 and +78. This shift of the TATA-directed initiation pattern might be due to loss of interference by the Inr-directed transcription (compare lanes 4 and 12). Transcription from the downstream initiation sites was activated also in the *gal11* mutant, but less efficiently than in the wild-type (lanes 11 and 12). These results suggest that the *GAL11* function is required for TATA-dependent transcription from the downstream sites but dispensable for the Inr-dependent transcription from +1. In the wild-type *GAL80*, the *gal11* null mutation causes a reduction of TATA-mediated transcription, which may result in an apparent stimulation of Inr-mediated transcription due to a decrease of the promoter competition.

3.2. *GAL11 stimulates transcription of an artificial gene which is regulated by the TATA box in cooperation with Inr*

We have just shown that *GAL11* is involved in TATA-dependent transcription but not in Inr-directed transcription in *GAL80*, where the two elements function independently. By contrast, it is known in some genes of higher eukaryotes that the TATA box and Inr cooperatively regulate transcription [1,2,8]. To determine whether or not *GAL11* exerts its function on a gene whose transcription is regulated by the TATA box in cooperation with Inr, we constructed an artificial gene in which the distance between the TATA box and Inr was increased to 60 bp (the TATA boxes are usually located between 40 and 120 bp upstream of the initiation sites in *S. cerevisiae* [6,7]). A set of Gal4p-binding sequence (UAS_G) was also inserted upstream of the TATA box (pIGTI, Fig. 2A). As shown in Fig. 2B, the basal (uninduced) transcription from +1 of pIGTI was higher than that of the gene containing only the Inr element (compare lanes 5 and 6 with 13 and 14). This result strongly suggests that transcription from +1 was controlled by both the TATA and Inr elements in this gene. Note that a new initiation site emerged at +20, whereas those at +37, +47, +56, and +67 seen in the wild-type *GAL80* disappeared. Activator Gal4p also seemed to activate transcription from +1 in galactose-grown cells (lanes 15 and 16). The amount of basal as well as activated transcripts was reduced in *gal11* to 20–30% of the wild-type level judged by the densitometric measurement (lanes 13 and 15). These results suggest the involvement of *GAL11* in the basal and activated transcription mediated by the TATA box irrespective of the presence or absence of Inr.

3.3. *GAL11 stimulates TATA-dependent but not TATA-independent transcription of HIS4*

It is known that *HIS4* also exhibits TATA-independent transcription under certain conditions [22]. Under the repressing conditions (in the presence of sufficient amounts of amino acids), the transcription of *HIS4* is maintained by transcription factors Bas1p, Bas2p, and Rap1p [30]. The transcription is derepressed by the transcriptional activator Gcn4p under amino acid-deficient conditions which are generated by the addition of 3-aminotriazole, a competitive inhibitor of His3p. The TATA box of *HIS4* is required for a high-level activation but is dispensable for a low-level transcription from the correct mRNA start site [22]. Effect of *GAL11* on the TATA-dependent and TATA-independent transcriptions of *HIS4* was tested using the reporter genes *HIS4-lacZ* and TATA-less *HIS4-lacZ* (Table 2). The repressed transcription was studied with the *gcn4* null strain to eliminate completely any residual activity of Gcn4p. The β-galactosidase activity of *HIS4-lacZ* was lowered in the *gal11* null strain under the repressing and derepressing conditions to 7.8% and 36% of the wild-type level, respectively. The expression of TATA-less *HIS4-lacZ* was significantly reduced in comparison to that of the TATA-containing *HIS4-lacZ*. This low-level TATA-independent expression was not appreciably affected

Table 2
TATA-dependent and TATA-independent expression of *HIS4-lacZ*

Reporter	Growth condition (genotype)	β-Galactosidase activity		Ratio
		GAL11	*gal11*	*gal11/GAL11*
HIS4-lacZ	DR (*GCN4*) MM+W,L+3AT	272	99	0.36
	R (*gcn4*) MM+A.A.	166	13	0.08
	R (*gcn4*) MM+A.A.+Ade	140	6.1	0.04
TATA-less *HIS4-lacZ*	DR (*GCN4*) MM+W,L+3AT	3.2	5.2	1.63
	R (*gcn4*) MM+A.A.	1.8	3.1	1.72
	R (*gcn4*) MM+A.A.+Ade	1.2	1.0	0.83

The strains HS21 (*GAL11 GCN4*) and HS22 (*gal11 GCN4*) harboring a reporter gene were grown in MM medium containing tryptophan and leucine in the presence of 10 mM 3-aminotriazole (MM+W,L+3AT) to derepress general amino acid control (DR). The strains HS23 (*GAL11 gcn4*) and HS24 (*gal11 gcn4*) harboring a reporter gene were grown in the MM medium containing amino acids (MM+A.A.) to repress general amino acid control (R) in the presence or absence of 0.1 mM adenine sulfate (Ade). Activity of β-galactosidase was assayed as described in Section 2.

by the *gal11* mutation. Although the values of the enzyme activity were rather low, each measurement was repeated in three independent cultures with the standard errors less than 20%, and the transcripts initiated at the accurate start site were detected by S1 nuclease mapping analysis (data not shown). It has been shown that Bas1p and Bas2p stimulate *HIS4* transcription when cells are grown in an adenine-free medium [22,30]. In the *gal11* null strain, transcriptional activation by Bas1p/Bas2p was similarly observed irrespective of the presence or absence of the TATA box (13 units versus 6.1 units in *HIS4-lacZ* and 3.1 units versus 1.0 unit in TATA-less *HIS4-lacZ*). These results indicate that the *GAL11* function is required for full expression of the TATA-dependent transcription, but is dispensable for the TATA-independent transcription of *HIS4*.

3.4 T_R-dependent but not T_C-dependent transcription of *HIS3* is regulated by *GAL11*

The core promoter of *HIS3* consists of two sequence elements, T_C and T_R, which are responsible for the transcriptional initiation at the +1 and +13 sites, respectively [31]. T_R is a canonical TATA sequence, which responds to transcriptional activation by Gcn4p under the derepressing conditions. T_C is a nonconsensus TATA sequence necessary for constitutive transcription and fails to support activation by Gcn4p [31,32]. As shown in lanes 1–4 of Fig. 3, the *gal11* null mutation showed no effect on transcription from the +1 site of *HIS3* under the repressing and derepressing conditions. However, the amounts of transcripts from +13 decreased in the *gal11* null strain under either growth condition. Therefore, we conclude that the T_R- but not T_C-mediated transcription is regulated by *GAL11* in *HIS3*.

3.5 *GAL11* stimulates transcription mediated by functional variants of the TATA box

The above findings indicated that *GAL11* stimulates transcription mediated by the typical TATA sequences of *GAL80* (TATATA), *HIS4* (TATATA), and *HIS3* (TATAAA). It is known, however, that several other AT-rich sequences also function as a TATA box [33–35]. A question then arises whether or not genes with these functional but atypical TATA sequences also depend on the *GAL11* function. To assess this question, we constructed *GAL7-lacZ* reporters, in which the native TATA box of *GAL7* was replaced by several other TATA-like sequences (Table 3). *GAL7* is a *GAL11* regulated gene (Table 3, see [11,16]) and contains a single functional TATA element (TATAAA) as the sole core promoter element so far as is known [21]. *GAL11* was required for full expression of pI162Z containing the TATA box (TATATA) of *GAL80* [20]. The expression of the reporter gene containing TATTTA (pI366Z), which is characterized as a functional TATA element [34], was similarly reduced in the *gal11* null strain. The TATA sequence of the *TRP3* gene, TATTAA [35], mediated the expression of pI365Z, which was affected by the *gal11* mutation. By contrast, the sequence CATAAA is unable to support high-level transcription not only downstream of UAS_{GCN4} in *HIS3* [33,34] but also downstream of UAS_G in *GAL7* (pI364Z in Table 3). This low-level expression of pI364Z was indifferent of *GAL11* function. These results strongly suggest that functioning of the canonical TATA box is essentially involved in *GAL11* dependence.

Table 3
Expression of *GAL7-lacZ* reporters having TATA-like sequence

Reporter	TATA sequence	β-Galactosidase activity		Ratio
		GAL11	*gal11*	*gal11/GAL11*
pI145Z	AAAGATATAAAAG	205	13	0.06
pI162Z	ctgaATATAtAta	131	2.5	0.02
pI366Z	AAAGATATtAAG	74	2.0	0.03
pI365Z	AAAGATATtAAAG	153	4.0	0.03
pI364Z	AAAGAcATAAAAG	2.0	2.1	1.05

The strains HSY5-3C (*GAL11*) and HSY5-3B (*gal11*) harboring a reporter gene were grown in ESGlyLacGal medium lacking uracil. Activity of β-galactosidase was assayed as described in Section 2. Nucleotide sequences from −68 to −56 of these reporters are indicated. The lower case letters indicate nucleotides different from pI145Z. The TATA sequences are underlined.

4. Discussion

In this study, we have revealed that the *GAL11* dependence of expression of *GAL11*-responsive genes is determined by their promoter structures. The TATA-dependent transcription of *GAL7*, *GAL80*, pIGTI, *HIS4*, and *HIS3* was impaired by a *gal11* null mutation. By contrast, the TATA-independent transcription of *GAL80*, *HIS4*, and *HIS3* was not affected by the *gal11* mutation. In this context, it should be worth noting that the expression of *GAL4*, a TATA-less gene, is independent of *GAL11* function [36,37]. These results indicate that *GAL11* function is required for transcription depending on the TATA box but is dispensable for transcription mediated by Inr or other core promoter elements. We conclude that GAL11 is a novel class transcription factor which regulates transcription depending on the core promoter structures.

We now know that the requirement of general transcription factors varies depending on the promoter structure [38]. In some cases, Inr interacts with specific DNA-binding protein, which generates the preinitiation complex through a pathway different from that of TATA-dependent transcription [9,10]. The Inr element of *GAL80* is recognized by a specific nuclear protein [20], which is required for Inr-dependent transcription in vitro (unpublished data). In the case of TATA-less *HIS4*, the low-level TATA-independent transcription might be maintained by activity of a yet unknown Inr at the +1 region [22]. The T_C element of *HIS3* supports neither activation by Gcn4p or Gal4p in vivo nor accurate transcription initiation in vitro [31,32,39]. In addition, factors that affect T_C- but not T_R-dependent transcription have also been identified [40]. These observations suggest that transcription machinery formed on the canonical TATA box may be different from that formed on Inr or the T_C element of *HIS3*. It has been reported that the holoenzyme of RNA polymerase II, in which Gal11p is a component, enhances basal as well as activated transcription in a reconstituted transcription system [3,4,17,18]. Our recent experiments indicated that Gal11p directly interacts with TFIIE, and further suggested that it is through this interaction that Gal11p regulates the promoter activity [19]. We may therefore suggest that Gal11p in the holoenzyme regulates transcription depending on the presence of the TATA box through interaction with TFIIE.

We have employed an artificial promoter pIGTI, and found that *GAL11* efficiently stimulates the uninduced (basal) expression of pIGTI. In addition, *gal11* mutation caused a sig-

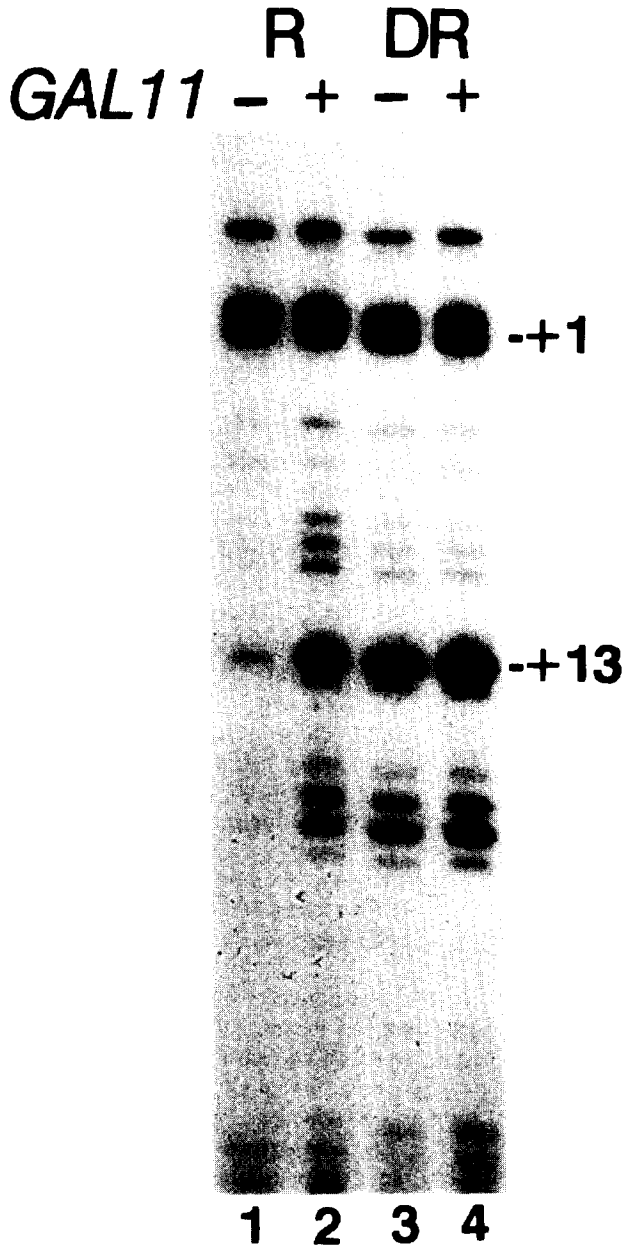

Fig. 3. Transcription of *HIS3* in the wild-type or *gal11* null strain. The yeast strains HS24 (*gal11 gcn4*, lane 1) and HS23 (*GAL11 gcn4*, lane 2) were grown in the SC medium to repress general amino acid control (R). The strains HS22 (*gal11 GCN4*, lane 3) and HS21 (*GAL11 GCN4*, lane 4) were grown in the MM medium containing uracil, tryptophan, and leucine, and general amino acid control was derepressed by the addition of 10 mM 3-aminotriazole (DR). Primer of *HIS3* [33] was annealed with total RNA (15 μg) and extended. Numbers to the right of the panel indicate transcription start sites [31,32].

nificant decrease in the uninduced transcription of *HIS4* as well as the T_R-dependent transcription of *HIS3*, but showed only a modest decrease in their induced (activated) transcription (about 3-fold). These observations are consistent with previous experiments that purified Gal11p stimulates basal transcription in vitro, and that the apparent potentiation of activator-induced transcription by Gal11p is accounted for by the stimulation of basal transcription [15,16]. Thus it is reasonable to assume that the core promoter specific function of *GAL11* may be involved in core promoter structure-dependent regulation of transcription in yeast. In this context, it would be noteworthy that the core promoter structure by itself defines tissue- or differentiation-stage-specific expression of some genes in higher eukaryotes [41–45].

In addition to the genes analyzed here, *GAL11* is required for TATA-dependent transcription of the genes including, *GAL1*, *GAL2*, *GAL10*, *SUC2*, *CYC1*, and *MFα1* [11–15]. However, it is too early to conclude that *GAL11* affects transcription of all the TATA-regulated genes in yeast. In fact, we previously described that expression of a galactose-inducible genes, *MEL1* encoding α-galactosidase appeared unaffected by *gal11* mutations judged by the enzyme determination [12]. Yet *MEL1* has two sets of the canonical TATA box in its promoter region [46]. It is urgent to know what makes this gene apparently unresponsive to Gal11p.

Recently, an alternative mechanism for the differential utilization of the *HIS3* T_C and T_R has been proposed [47]. These authors demonstrated that, under certain circumstances, for example in increasing concentration of Gcn4p in the cell, T_C is preferentially utilized at low levels of transcription, T_C and T_R are equally utilized at moderate levels of transcription, and T_R is preferably utilized at high levels of transcription. Thus they argue that the relative utilization of T_C and T_R depends not only on the quality of the two elements but also on the overall level of transcriptional stimulation. Generalizing this hypothesis, one might argue that Gal11p affects transcription from most promoters except those that are inefficient. In contrast, it is also likely, as suggested in the present studies, that the mechanism for such inefficient transcription is different from that of efficient transcription mediated by the canonical TATA sequence. Further genetic as well as biochemical experiments are needed to understand the exact nature of the promoter specificity of Gal11p.

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