

The Critical *cis*-Acting Element Required for *IMD2* Feedback Regulation by GDP Is a TATA Box Located 202 Nucleotides Upstream of the Transcription Start Site

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Guanylic nucleotides are essential cellular players, and the critical enzyme in their tightly regulated synthesis in *Saccharomyces cerevisiae* is encoded by the *IMD2* gene. The transcription of *IMD2* is subject to general repression by nutrient limitation through the *cis* nutrient-sensing element. It is also subject to specific feedback regulation by the end products of the guanylic nucleotide synthesis pathway. The critical *cis* element for this latter mechanism is the guanine response element (GRE), a TATAATA sequence which is located 202 nucleotides upstream of the transcription initiation site and which functions as the *IMD2* TATA box. We show that the GRE functions in conjunction with a 52-nucleotide stretch near the transcription start site. This very unusual promoter structure ensures low, basal expression of *IMD2* and the recruitment of TFIID to the GRE in response to guanylic nucleotide limitation.

IMP dehydrogenase (IMPDH) catalyzes the first committed step of the de novo biosynthesis of guanylic nucleotides. The regulation of GMP synthesis plays a crucial role in cell proliferation, since increased activity of IMPDH has been observed in rapidly dividing mammalian cells, including human leukemic cell lines, solid tumor tissues, and B and T lymphocytes. In turn, antagonists of IMPDH activity, such as mycophenolic acid (MPA), serve as immunosuppressive agents. The importance of IMPDH as a target for medical treatments has led to the generation of much interest in the study of the molecular mechanisms governing the regulation of genes encoding IMPDH (see references 1 and 34 for recent reviews).

The yeast *Saccharomyces cerevisiae* has a family of four very closely related genes, *IMD1* to *IMD4*, encoding proteins 80 to 96% identical to each other and also highly homologous (60% amino acid sequence identity) to mammalian IMPDH. It has been demonstrated that the levels of *IMD* transcripts decrease when cell growth ceases in response to nutrient limitation (8, 12, 13, 30). Additionally, in exponentially growing cells, *IMD* transcripts are feedback regulated by pathway end products (10, 29). Indeed, the expression of the *IMD* genes is downregulated when cells are provided with guanine, a GMP precursor that can be directly converted into GMP, allowing bypass of the de novo pathway (Fig. 1). In contrast, when cells are treated with MPA or 6-azauracil (6-AU), which inhibits IMPDH activity and therefore lowers guanylic nucleotide levels, the expression of the *IMD* genes is upregulated.

Various investigators have studied the regulation of the *IMD2* gene in more detail (10, 11, 29, 30). On the one hand, studies of fusions of *IMD* promoters to *lacZ* demonstrated

tighter repression by guanine of the *IMD2-lacZ* fusion than of other *IMD-lacZ* fusions (10). On the other hand, strains lacking one or the other *IMD* gene were tested for resistance to MPA, and only *IMD2* was apparently critical (30), while the overexpression of *IMD2* led to increased resistance of cells to MPA (9, 30). Escobar-Henriques and Daignan-Fornier previously mapped the promoter of *IMD2* and identified an octanucleotide *cis* element, called the guanine response element (GRE), thought to mediate regulation by both MPA and guanine (10). Those findings were in agreement with the findings of studies by Shaw et al. (30), who mapped the response to 6-AU to a DNA fragment including the GRE. In the latter studies, an additional fragment responsible for reduced *IMD2* expression was found close to the transcription start site.

Recently, the regulation of the *IMD2* gene has sparked additional interest, because several transcription elongation mutant strains were found to be sensitive to MPA and/or 6-AU, and in some cases this sensitivity could be suppressed by the overexpression of *IMD2* (references 26 and 30 and references therein). These findings have led to the use of sensitivity to MPA or 6-AU as a typical assay to characterize factors that may be involved in transcription elongation. However, a recent systematic study of the yeast “disruptome” for mutations affecting resistance to MPA led to the identification of a large number of genes (approximately 100) that are required for cells to be resistant to MPA as well as transcription elongation mutants (9).

In this work, we investigated the regulation of *IMD2* in more detail by further characterizing the role of the previously mapped GRE, a TATA box-like sequence required for regulation by MPA and guanine. We made the surprising finding that the GRE is the *IMD2* TATA box, considering that it is located 202 nucleotides from the transcription start site. This long distance from the transcription start site was essential to keep *IMD2* expression levels low; surprisingly, other TATA box-like sequences present in this promoter and closer to the

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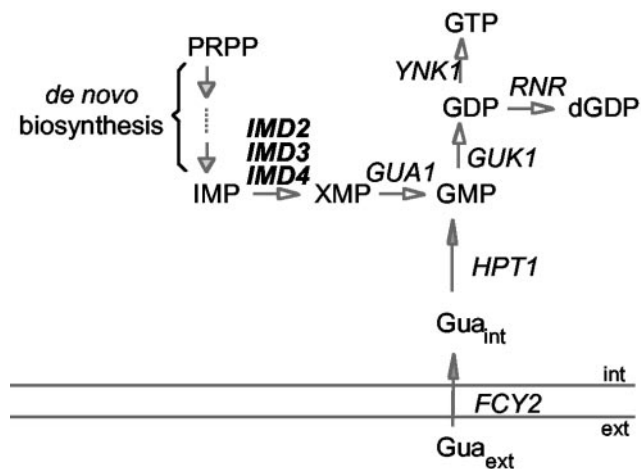


FIG. 1. Schematic representation of the guanylic nucleotide synthesis pathway. Solid lines represent the plasma membrane. ext, extracellular medium; Gua, guanine; int, intracellular medium; PRPP, 5-phosphoribosyl-1-pyrophosphate. Genes are shown in italic type and encode the enzymatic activities: *FCY2*, purine cytosine permease; *GUA1*, guanosine-5'-monophosphate synthetase; *GUK1*, guanosine-5'-monophosphate kinase; *HPT1*, hypoxanthine-guanine phosphoribosyltransferase; *IMD2*, *IMD3*, and *IMD4*, IMPDH; *RNR*, ribonucleotide reductase; and *YNK1*, nucleoside-5'-diphosphate kinase. *IMD1* is not indicated here because it is not expressed (3, 12) and is thought to be a pseudogene.

initiation site could not replace the GRE. Our results are consistent with a model in which the binding of TFIID to the TATA box of the *IMD2* promoter would directly respond to the levels of guanylic nucleotides.

MATERIALS AND METHODS

Yeast strains and media. SD medium is 2% glucose, 0.17% nitrogen base, and 0.5% ammonium sulfate. SDcasa medium is SD medium supplemented with 0.2% Casamino Acids (Difco). Uracil at a final concentration of 200 μ g/ml was optionally added. Guanine was optionally added to a final concentration of 0.13 mM (stock solution of 1.3 mM in 0.05 N HCl). MPA or 6-AU (stock solutions of 10 mg/ml in 0.1 M NaOH) were optionally added to various final concentrations.

Saccharomyces cerevisiae wild-type strain Y350 (*MATa ura3-52 leu2-3,112 lys2 Δ 201*) was used for all experiments except when otherwise indicated. For some experiments (see Fig. 3B), wild-type strain BY4742 (*MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0*) and *ppr2*-disrupted strain Y14411 (*MAT α his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0 ygl043w::kanMX4*) (both purchased from Euroscarf) were used. Strains Y1648, Y1649, Y1651, and Y1652 (see Fig. 4) and strains Y1653 and Y1655 (see Fig. 6) were constructed as follows. Strain Y350 was used to mutate at the endogenous *IMD2* gene the GRE and/or TATA2 boxes, thus generating strains Y1648 (with wild-type GRE and TATA2 boxes), Y1649 (with the GRE box mutated to TACCCATA and with the wild-type TATA2 box), Y1651 (with the wild-type GRE box and with the TATA2 box mutated to TGCAGATTTT), and Y1652 (with the GRE box mutated to TACCCATA and with the TATA2 box mutated to TGCAGATTTT) (mutations are indicated in boldface). These strains were obtained by transforming into strain Y350 plasmids pMAC463B, pMAC464B, pMAC468B, and pMAC469B (*URA3* integration derivatives, constructed as described below) digested with *Cla*I. The *URA3* plasmid DNA was cured from the transformants by streaking on 5-fluoro-orotic acid, and the *ura3* recombinant strains were analyzed by PCR for the presence of the mutated sequences. A *Bam*HI site (for Y1648 and Y1649) or an *Eco*RI site (for Y1651 and Y1652) was created at -311 bp upstream of the *IMD2* ATG in the mutated sequences, and an additional *Bam*HI site was placed -108 bp upstream of the *IMD2* ATG in strains Y1651 and Y1652. Strains Y1653 (with the wild-type GRE box) and Y1655 (with the GRE box mutated to TGTAAATA) were generated in the same manner as strains Y1648 and Y1649 from strain Y643 (*MATa ura3-52 leu2-3,112 lys2 Δ 201 his3 Δ 200 trp1::hisG*) with plasmids pMAC463B and pMAC466B, respectively. For some experiments (see Fig. 7), isogenic strains GY5 (*MATa ura3-52 leu2 trp1 Δ 1 leu2::PET56 gal2 gcn4 Δ taf1::LEU2 pTAF1-*

HIS3) and GY7 (*MATa ura3-52 leu2 trp1 Δ 1 leu2::PET56 gal2 gcn4 Δ taf1::LEU2 paf1-4-HIS3*) (7) were used.

Plasmids. Plasmid P892 or P913, containing the *IMD2-lacZ* fusions and 311 bp upstream of the *IMD2* ATG, corresponding to 205 bp of the *IMD2* promoter, and either a wild-type (TATAAATA) or a mutated (TACCCATA) GRE box, respectively, was described elsewhere (10). The *IMD2-lacZ* plasmid P855 or P1200, with 355 bp upstream of the *IMD2* ATG and either 1,135 bp or only the ATG of the coding region, respectively, was described elsewhere (10, 12). The *lacZ* fusions were constructed in the *URA3* vectors described by Myers and coworkers (25). The *IMD2-lacZ* fusion plasmid P1881, with the GRE replaced by a canonical TATA box (see Fig. 5A), resulted from cloning of the *Bam*HI-*Hind*III fragment of the PCR product obtained with oligonucleotides 311TATAB and IMP22 into the *Bam*HI-*Hind*III sites of vector Yep356. Various other plasmids (see Fig. 6) resulted from cloning of the *Eco*RI-*Bam*HI fragment of the desired PCR product into the *Eco*RI-*Bam*HI sites of plasmid P860 (10). The desired PCR product was obtained by using the promoter-coding strand oligonucleotide Eco311 and the appropriate noncoding strand oligonucleotide. Therefore, to create plasmids P1627, P1625, P1623, P1621, P1619, and P1617, the noncoding strand oligonucleotides Bam160, Bam171, Bam180, Bam188, Bam198, and Bam207, respectively, were used.

The plasmids used to create the endogenous *IMD2* mutant strains were obtained by cloning different fragments into the *Eco*RI-*Hind*III sites of the *URA3* integration vector Y1plac211 as follows. For plasmids pMAC463B and pMAC464B, respectively, used to create strains Y1648 and Y1649, we cloned the PCR product amplified with oligonucleotides 5' *IMD2*-1730 and 3' *IMD2*-312Bam digested with *Eco*RI-*Bam*HI together with the *Eco*RI-*Hind*III fragments from plasmids P892 and P913, respectively. For plasmids pMAC468B and pMAC469B, respectively, used to create strains Y1651 and Y1652, we cloned the PCR product amplified with oligonucleotides 5' *IMD2*-1730 and 3' *IMD2*-312Eco digested with *Eco*RI together with the *Eco*RI-*Hind*III fragments from plasmids P2108 and P2110, respectively. P2108 (with a mutated TATA2 box) and P2110 (with mutated GRE and TATA2 boxes) resulted from cloning of the *Eco*RI-*Bam*HI fragments of the PCR product obtained with the oligonucleotide pairs Eco311-BamTATA2 and Eco311C303-BamTATA2, respectively, into the *Eco*RI-*Bam*HI sites of plasmid P890 (10). To create strains Y1653 and Y1655, we used pMAC463B and pMAC466B, respectively. pMAC463B construction was described above. To construct pMAC466B, we cloned the PCR product amplified with oligonucleotides 5' *IMD2*-1730 and 3' *IMD2*-312Bam digested with *Eco*RI-*Bam*HI together with the *Bam*HI-*Hind*III fragment from plasmid P1691. P1691 (with the GRE replaced by TGTAAATA) resulted from cloning of the *Bam*HI-*Hind*III fragment of the PCR product obtained with oligonucleotides *IMD2*G2B and IMP22 into the *Bam*HI-*Hind*III sites of vector Yep356.

The oligonucleotides used to PCR amplify the *IMD2* fragments from the S288C genomic DNA template were as follows: coding strand—311TATAB, 5' CGG GAT CCA AGT ATA TAA AGT GAA GAC TTT TTC CA 3'; Eco311, 5' GGA ATT CAA GTA TAA ATA GTG AAG ACT T 3'; Eco311C303, 5' CGG AAT TCA AGT ACC CAT AGT GAA GAC TT 3'; 5'*IMD2*-1730, 5' CCG GAA TTC AAC ATA TCC TTG CAA 3'; and *IMD2*G2B, 5' CGG GAT CCA AGT GTA AAT AGT GAA GTC TTT T 3'; noncoding strand—IMP22, 5' TCC CCC GGG CCC AAA TAC CGT A 3'; Bam160, 5' CGG GAT CCA AAA AAA GGT TCC CAG CA 3'; Bam171, 5' CGG GAT CCC AGC ATA CTA GGT AAA 3'; Bam180, 5' CGG GAT CCT AGG TAA AAT GTA TAA GC 3'; Bam188, 5' CGG GAT CCA TGT ATA AGC CCA CGA AA 3'; Bam198, 5' CGG GAT CCC ACG AAA CTG AGA AAA 3'; Bam207, 5' CGG GAT CCT GAG AAA AAG CCA CCA A 3'; BamTATA2, 5' CGG GAT CCT TAT ATA GAA AAT CTG CAG AAA AGT AA 3'; 3'*IMD2*-312Bam, 5' CGG GAT CCT TTT TTT TTT TTA TTT TTT CGT TTT 3'; and 3'*IMD2*-312Eco, 5' CCG GAA TTC TTT TTT TTT ATT TTT TCG TTT T 3'.

Plasmids expressing either wild-type or altered-specificity mutant TATA box binding protein (TBP) (TBPmut3, TRP1, and 2 μ m) were kind gifts from Kevin Struhl.

S1 nuclease assays. Fifty micrograms of total cellular RNA was prepared from cells grown to the desired conditions and analyzed for the presence of specific transcripts as previously described (5). The oligonucleotide used for *IMD2* was *IMD2*S1avantiNSE (5' CCA TCC GGT CTT GGT AGG CTC TTG GTA AAG TCT AGT GCG GTC TTG TAG TCT CTA ATG GCC G 3'); that used for *ACT1* was *ACT1*(S1) (5' CGA GCA ATT GGG ACC GTG CAA TTC TTC TTA CAG TTA AAT GGG ATG GTG CAA GCG CGC C 3'); and that used for *DED1* has already been described (5). Hybridization was performed at 63°C.

β -Galactosidase assays. Cells were grown overnight in medium selective for the reporter plasmids and used to inoculate fresh SDcasa medium supplemented or not supplemented with 0.03 μ g of MPA or guanine/ml. After growth for 6 h (or 12 h [see Fig. 3B]), the cells were collected to measure expression from the

IMD2-lacZ fusions as described previously (10). β -Galactosidase activity was expressed in units of optical density at 420 nm \times 1,000/(optical density at 600 nm \times time [in minutes] \times volume [in milliliters]).

Growth plate tests. Strains Y1648, Y1649, Y1651, and Y1652 transformed with P193 (2 μ m *URA3*) and grown to exponential phase were resuspended in water to an optical density at 600 nm of 1. Ten-microliter drops and four serial 1:10 dilutions of this suspension were spotted on SDcasa medium supplemented with guanine, MPA, or 6-AU and incubated for 3 days at 30°C.

***IMD2* mRNA 5' mapping.** A 0.5-pmol quantity of the labeled *IMD2* probe (*IMD2* prim ext, 5' GGC TCT TGG TAA AGT CTA GT 3') and 20 μ g of total cellular RNA were used to perform 5' extension analysis with hybridization and primer extension reaction steps according to standard protocols, 200 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL), but no actinomycin D. The extension product was ethanol precipitated and loaded onto a 6% sequencing gel.

ChIP analysis. Chromatin immunoprecipitation (ChIP) assays were performed as described previously (7) with exponentially growing Y1648, Y1649, and Y350 cultures treated for 1 h with guanine or 0.03 μ g of MPA/ml. One of the *IMD2* real-time PCR amplifications (see Fig. 5B) was performed with oligonucleotides 5'GREbis (5' CAA AAT TAT TGG TTT TCG TAA CC 3') and 3'GREbis (5' GCC ACC AAG ATT CTC CGG T 3'). Another *IMD2* real-time PCR amplification (see Fig. 7C) was performed with oligonucleotides 5'GREtris (5' AAA AAA AAG TAT AAA TAG TGA AGA CTT 3') and 3'GRE (5' CAG CAA AAA ACT TCC AGC CG 3').

RESULTS

Repression of *IMD2* by nucleotide feedback and repression by nutrient limitation occur through separate *cis* elements.

Previous experiments indicated that *IMD2* gene expression is induced by MPA and repressed by guanine. However, these results were obtained either with *lacZ* fusion constructs, which are subject to artifacts, or by Northern blot analysis, which did not allow discrimination among the very similar *IMD2* to *IMD4* genes. Therefore, S1 nuclease analysis with probes specific to *IMD2* was used to measure *IMD2* mRNA levels unambiguously. This analysis confirmed that both MPA and guanine specifically modulate the levels of transcripts of the endogenous *IMD2* gene (Fig. 2A). In addition to this specific feedback response, various investigators (8, 12, 13, 30) observed that *IMD2* was also repressed under conditions of nutrient limitation (Fig. 2B). Two *cis* elements required for the correct transcriptional regulation of *IMD2* have been mapped. Indeed, the GRE was required for appropriate expression and repression by guanine of an *IMD2-lacZ* fusion gene (10) (Fig. 2C), while repression induced by nutrient limitation was associated with a different *cis* element, located in the coding region of *IMD2* and called the nutrient-sensing element (NSE) (12). Consequently, a fusion containing only the *IMD2* promoter fused to the *lacZ* gene was not regulated when nutrients became limiting, as in postdiauxic phase (12) (Fig. 2D).

The importance of the NSE for the repression and activation of *IMD2* by guanine and MPA was evaluated by analyzing *lacZ* fusion constructs that carried (P855) or lacked (P1200) the necessary coding region (Fig. 2E). It was clear that the coding region was not important for the regulation of *IMD2* through the feedback mechanism induced by guanine. Conversely, mutation of the GRE had no impact on the repression of *IMD2* when cells entered postdiauxic phase (Fig. 2F). Therefore, the two described forms of regulation of *IMD2* clearly take place through distinct *cis* elements.

In the presence of MPA, *ppr2* interferes with *IMD2* expression through the NSE but not the GRE. Even though the repression of *IMD2* by metabolic downregulation and the induction of *IMD2* by MPA occur through different *cis* elements,

the former has been shown to preclude the latter (12). Indeed, once yeast cells engage in the metabolic downregulation of *IMD2*, they can no longer sense the guanylic nucleotide starvation induced by MPA and activate *IMD2* (12). Consequently, any starvation condition is expected to slow down transcription through the NSE and make impossible induction through the GRE in response to guanylic nucleotide limitation. For example, we found that deoxynucleoside triphosphate starvation induced by hydroxyurea treatment resulted in decreased *IMD2* expression (10) (Fig. 3A). As expected, the downregulation of *IMD2* expression due to hydroxyurea required the *IMD2* coding sequence and was abolished in the short *IMD2* fusion devoid of the NSE (Fig. 3A). Similarly, mutations in the gene encoding TFIIS (*PPR2/DST1*) (16), a recognized transcription elongation factor, strongly decreased induction by MPA when the NSE was present in the fusion (P855) but had no effect in the absence of the NSE (P1200) (Fig. 3B). Since *ppr2/dst1* mutants are hypersensitive to MPA, we interpret this result as indicating that in the absence of TFIIS and in the presence of MPA, cells sense some form of metabolic stress. This signal is transmitted to the growth response element within the coding sequence (NSE) of *IMD2*, leading to decreased expression and abolished induction of *IMD2*, most probably contributing to sensitivity to MPA or 6-AU. The twofold induction of P855 still occurring in the *ppr2* mutant at an extremely low MPA concentration (Fig. 3B) was lost at higher MPA doses (data not shown), in agreement with the findings of Shaw and Reines (29). Importantly, even in a wild-type strain, *IMD2* induction was almost precluded at MPA doses of higher than 30 μ g/ml, which strongly affected growth (data not shown).

Taken together, these experiments demonstrate that under certain conditions, such as in strains hypersensitive to MPA, the regulation of *IMD2* by MPA and guanine through the GRE needs to be studied in a context devoid of the NSE.

***IMD2* expression is tightly correlated with MPA resistance.** The *IMD2* GRE is highly unusual. Indeed, it looks like a TATA sequence but is positioned far away (202 bp) from the *IMD2* 5' transcription start site (Fig. 4A). Moreover, its mutation does not abolish *IMD2* expression, as would be expected for a normal TATA box, but rather leads to a loss of *IMD2* regulation by either MPA or guanine and to constitutive expression somewhat higher than the basal level of *IMD2* expression from the wild-type GRE (10). To elucidate the function of the GRE, we first attempted to identify the TATA box in the *IMD2* promoter. We hence mutated other AT-rich sequences, in particular, the three most closely related to TATA boxes, within the *IMD2* promoter region (TATA1, TATA2, and TATA3; Fig. 4A), either alone or in combination with the GRE, and analyzed the expression of *lacZ* fusion constructs (data not shown). None of these elements was important for the regulation of *IMD2* expression by MPA or guanine. However, mutation of TATA2 increased both basal and induced *IMD2* expression, while mutation of TATA3 slightly decreased both basal and activated *IMD2* expression. Thus, the expression of *IMD2* was affected by mutations in TATA2 and, to a lesser extent, in TATA3, but only the GRE was absolutely necessary for the regulated expression of *IMD2*.

The importance of the GRE and the TATA2 element for the appropriate expression and regulation of *IMD2* needed to be confirmed for the endogenous *IMD2* gene. We thus created

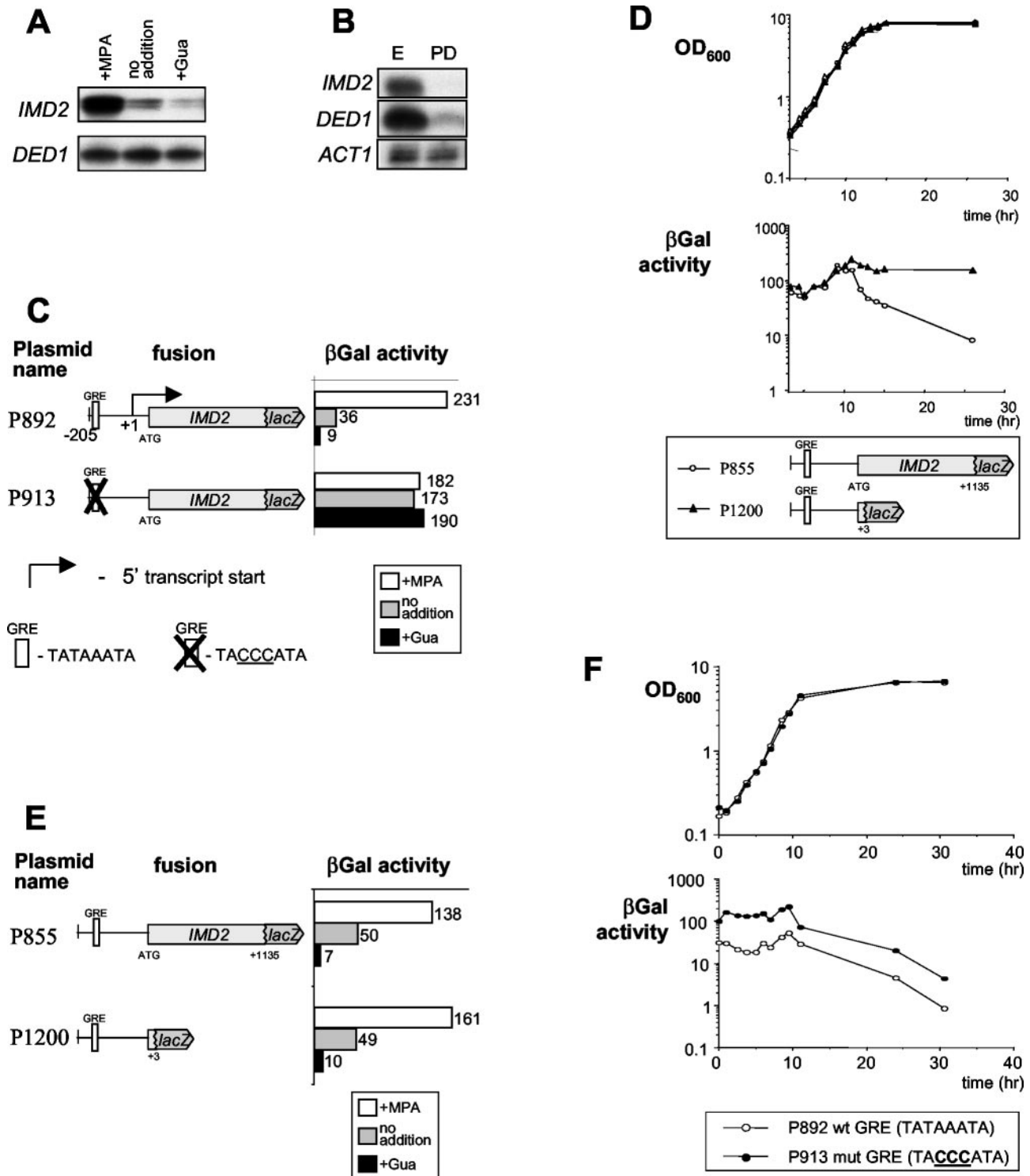


FIG. 2. Repression of *IMD2* by nucleotide feedback or by nutrient limitation occurs through separate *cis* elements. (A and B) Fifty micrograms of total cellular RNA from wild-type Y350 cells grown in the presence of MPA and guanine (Gua) (A) or wild-type Y350 cells grown to exponential phase (E) or beyond the diauxic shift phase (PD) (B) was analyzed by S1 nuclease digestion for the levels of transcripts of *IMD2* and *DED1* or *ACT1* (as internal controls). (C to F) The β -galactosidase (β Gal) activities of the *lacZ* fusions expressed from the indicated plasmids transformed into wild-type strain Y350 grown in the presence or absence of Gua or MPA (C and E) and grown to the indicated optical density at 600 nm (OD_{600}) (D and F) were measured. wt, wild type; mut, mutant.

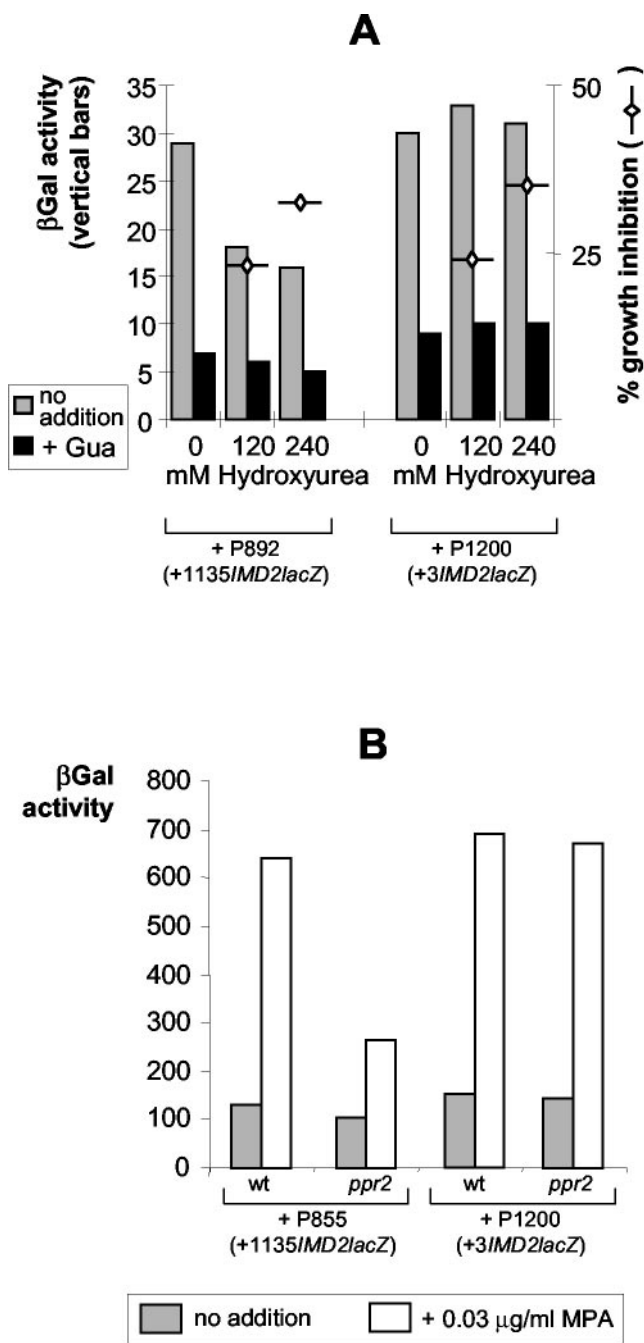


FIG. 3. The impaired MPA induction of *IMD2* in a *ppr2* strain does not involve the GRE. (A) Wild-type cells were transformed with plasmids carrying *IMD2-lacZ* fusions containing (P892) or not containing (P1200) the coding sequences of *IMD2*. Expression from these reporter plasmids was measured for wild-type cells grown to exponential phase and then treated for 12 h with hydroxyurea, an inhibitor of ribonucleotide reductase that affects cellular proliferation. Gua, guanine; βGal, β-galactosidase. (B) Strains BY4742 (wild type [wt]) and Y14411 (*PPR2* disruption [*ppr2*]) were transformed with the indicated plasmids (Fig. 2) and grown from exponential precultures for 6 h in the presence or absence of MPA. Cells were harvested and assayed for βGal activity.

strains in which the endogenous *IMD2* gene carried mutations in the GRE and the TATA2 element, either alone or in combination (see Materials and Methods). S1 nuclease analysis of the *IMD2* mRNA levels confirmed our previous findings with fusion constructs. That is, the GRE box was necessary for the regulation of *IMD2* transcript levels by both MPA and guanine (compare Y1648 with Y1649 in Fig. 4B), while the TATA2 box mutation resulted in increased levels of the *IMD2* transcript (compare Y1648 with Y1651 in Fig. 4B). Importantly, the major transcription start site was the same for all strains (Fig. 4C). Some transcription initiated at several downstream sites, particularly under conditions of higher expression (Y350 or Y1651 in the presence of MPA) or when both the GRE and the TATA2 sequences were mutated (Y1652).

These strains allowed us to determine the importance of *IMD2* expression levels for the resistance to both MPA and 6-AU. Figure 4D shows that strain Y1649, in which the *IMD2* GRE had been mutated, making *IMD2* no longer inducible by MPA, was MPA sensitive and, to a lesser extent, 6-AU sensitive. In contrast, strain Y1651, in which *IMD2* TATA2 had been mutated, resulting in higher expression of *IMD2*, was more resistant than the parental wild-type strain to MPA and, to a lesser extent, to 6-AU. Strain Y1652, in which both the GRE and the TATA2 sequences of *IMD2* were mutated, was sensitive to MPA. Taken together, these results show that the expression of *IMD2* and its capacity to be highly induced in the presence of MPA correlate well with resistance to MPA and, to a lesser extent, to resistance to 6-AU. It is interesting that *IMD2* was maximally induced by 0.1 µg of MPA/ml (data not shown) but that 100 µg of 6-AU/ml was needed to obtain the same induction (Fig. 4D). However, the induction of *IMD2* by both drugs was dependent upon the same GRE (data not shown).

The GRE is a bona fide TATA element of the *IMD2* gene. The results presented so far define the GRE as an element important for the regulation of *IMD2* by guanylic nucleotide levels but do not establish whether it indeed functions as a TATA element. To address this issue, the GRE was mutated to a canonical TATA element; it was clear that this alteration had no effect on GRE function (Fig. 5A).

In a second approach, ChIP experiments carried out with strains either wild type or mutant for the GRE and with TBP antibodies revealed that the modulation of the association of TBP with the wild-type *IMD2* promoter correlated with transcription levels, increasing with MPA and decreasing with guanine (Y1648 in Fig. 5B). In contrast, when the GRE was mutated, no modulation of the low level of cross-linking of TBP to the mutated *IMD2* promoter was detectable in the presence of MPA or guanine (Y1649 in Fig. 5B). The fold regulation of TBP binding to the promoter did not reflect directly the fold regulation of *IMD2* transcript levels, but this finding might reflect a limitation inherent to our ChIP experiments, since we have observed this finding for several promoters under many conditions (data not shown). It was surprising that while the basal expression of *IMD2* was higher in mutant strain Y1649 (Fig. 4B), less TBP was found associated with the sequences surrounding the GRE (Fig. 5B). However, it is very possible that in the mutant strain, the alternative core promoter allowing expression was not located close enough to the GRE to be amplified to any appreciable extent in the ChIP experiments.

Finally, we created a strain carrying a mutated GRE corre-

sponding to a mutated TATA element that can be recognized by an altered-specificity mutant of TBP (32). Using this *IMD2* mutant strain (Y1653) and a wild-type *IMD2* strain (Y1655), we transformed a plasmid overexpressing either wild-type TBP or the altered-specificity mutant. We found that the wild-type *IMD2* strain (Y1655) was normally regulated, regardless of the presence in the plasmid of a gene encoding wild-type or mutant TBP. The presence of endogenous wild-type TBP was most likely responsible for the correct regulation of the wild-type construct, despite the concomitant expression of the mutant TBP. Furthermore, while *IMD2* transcript levels in cells with the mutated GRE and wild-type TBP were not regulated by guanine and MPA, such regulation was restored with the altered-specificity mutant of TBP (Fig. 5C). The fact that the regulation was not totally restored might reflect some form of competition between the endogenous TBP present in the cell (required for viability) and the mutant TBP encoded by the plasmid (possibly for assembly into TFIID).

Taken together, these results clearly establish that the GRE functions as a TATA element in the *IMD2* promoter. Furthermore, this TATA element is necessary for the regulation of *IMD2* gene expression by MPA or guanine.

The distant position of the GRE ensures low levels of *IMD2* expression. Why is the *IMD2* TATA box located so far away from the transcription initiation site? To understand the role of the distance in the regulation of *IMD2*, we removed increasing amounts of sequence between the GRE and the transcription initiation site. We were able to show that shortening of the distance between the GRE and the initiation site gradually increased the expression of *IMD2-lacZ* fusions but did not affect the regulation by guanine (Fig. 6). Thus, the combination of a 100-bp fragment containing the GRE TATA box and the first 52 nucleotides of the *IMD2* promoter was sufficient to create the regulated expression of *IMD2*. However, further deletion of these 52 nucleotides (P1524) abolished guanine regulation (Fig. 6). The importance of this 52-nucleotide region was confirmed with a reporter construct lacking only these 52 nucleotides (P1637), since mRNA levels expressed from this construct were similar whether cells were grown in the presence or in the absence of guanine (data not shown). We characterized the 52-nucleotide region important for the regulated expression of *IMD2*. However, when this region was divided into three largely overlapping segments and used to replace the complete sequence in P1623, none of the segments was sufficient to restore the regulation of *IMD2* (data not shown). Thus, no short element within the first 52 nucleotides is sufficient in combination with the GRE to install regulated expression of the *IMD2* gene. These observations were confirmed by S1 nuclease analysis of transcript levels produced by the reporter constructs (data not shown).

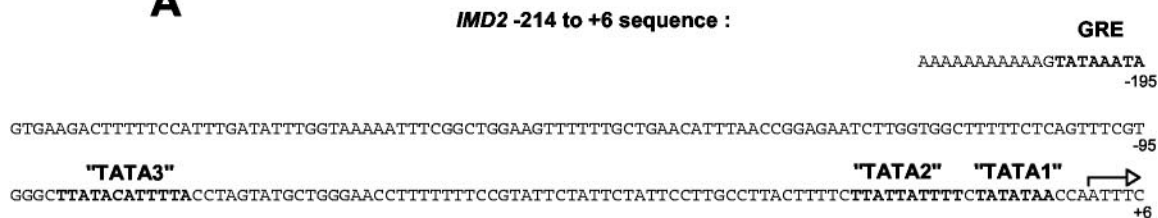
Therefore, the long distance between the *IMD2* TATA box and the transcription start site ensured a low level of *IMD2* expression. In contrast, no single upstream activating sequence (UAS) or upstream repressing sequence (URS) element could be found within the *IMD2* promoter.

TFIID is important for the recruitment of TBP to the *IMD2* promoter in response to guanylic nucleotide starvation. The results presented so far suggest that the regulation of *IMD2* gene transcription involves an interaction among distantly positioned elements: a TATA element (the GRE) located 202 bp upstream of the transcription start site, a 52-nucleotide stretch just upstream of the transcription initiation site, and the NSE located within the *IMD2* coding sequence. These findings led us to investigate the roles of different complexes that affect chromatin structure in the regulation of *IMD2* gene expression. We used strains carrying mutations in *SNF2* (for a review, see reference 28), *ISW1*, *ISW2*, and both *ISW1* and *ISW2* (33). In these strains, both the extent and the kinetics of regulation by MPA or guanine were similar to those in the wild-type strains, except for the basal expression of *IMD2*, which was slightly increased in the *snf2* mutant (data not shown). We next examined the importance of the SAGA complex (15). We found that *IMD2* was appropriately expressed in cells lacking Gen5p (data not shown). Moreover, we analyzed a strain lacking Spt7p, where the SAGA complex is disrupted (data not shown), or a strain lacking Spt3p, which is thought to be important for the SAGA complex interaction with TBP. In these *spt3Δ* and *spt7Δ* mutants (both MPA hypersensitive [9]), *IMD2* induction by MPA was partially impaired but, as for the *ppr2* mutant, this effect was mostly indirect, since it was lost in the *IMD2* reporters devoid of the NSE (data not shown).

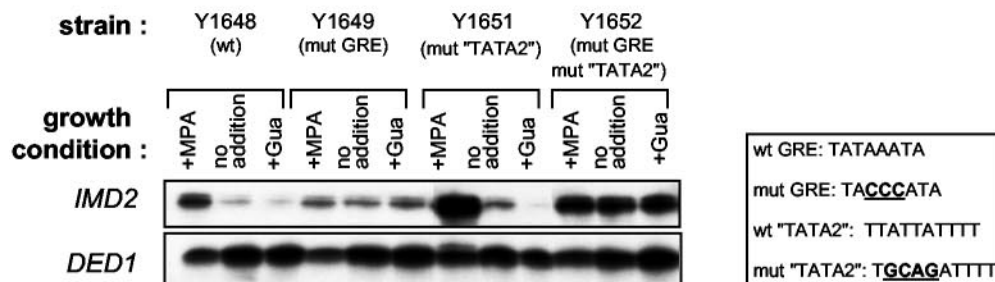
Genome-wide studies with several mutant yeast TAF_{II}s have suggested that basal transcript levels for the *IMD* genes are decreased when TFIID is mutated (18). TAF1 is a component of TFIID (but not SAGA), another complex that contributes to the recruitment of TBP to promoters. We thus analyzed specifically with S1 nuclease assays the regulation of *IMD2* expression in a temperature-sensitive *taf1* mutant (*taf1-4*) (7). *IMD2* transcript levels were decreased in the *taf1-4* mutant at a permissive temperature but were still inducible by MPA, albeit to a lesser extent than in wild-type cells (Fig. 7A, right panel; basal levels were visible only on a longer exposure). However, TFIID function was abolished in this mutant only at a restrictive temperature. Thus, we shifted the *taf1-4* cells to the restrictive temperature for 10 min and then treated the cells with MPA for 1 h. To analyze the inducibility of the *IMD2* promoter in response to guanylic nucleotide levels independently of the dominant metabolic downregulation of *IMD2* by the NSE, we used a reporter construct in which the *IMD2* promoter with its ATG was fused to *lacZ* (P1200; see above).

FIG. 4. GRE and TATA2 mutations affect endogenous *IMD2* transcriptional regulation and resistance of cells to MPA. (A) Sequence of the *IMD2* promoter region, with the GRE and AT-rich sequences shown in bold type. (B) Yeast strains carrying the indicated *IMD2* sequences were treated or not treated with guanine (Gua) or MPA. Total cellular RNA was extracted, and the levels of transcripts of *IMD2* and *DED1* (as an internal control) were measured by S1 nuclease analysis. wt, wild type; mut, mutant. (C) The 5' end of the *IMD2* transcripts was characterized by 5' extension with the indicated yeast strains under various growth conditions. The oligonucleotide used in the extension reactions was also used for sequencing to identify the position of the start site. (D) The indicated yeast strains were grown to exponential phase and plated in serial dilutions on plates containing or not containing Gua, MPA, or 6-AU. Plates were incubated at 30°C for 3 days. (E) S1 nuclease analysis of induction of *IMD2* transcripts by MPA and 6-AU.

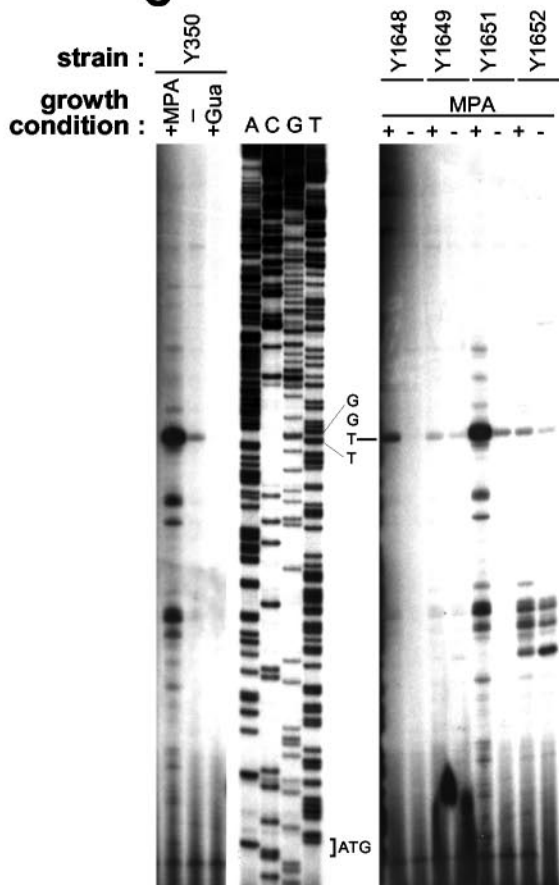
A



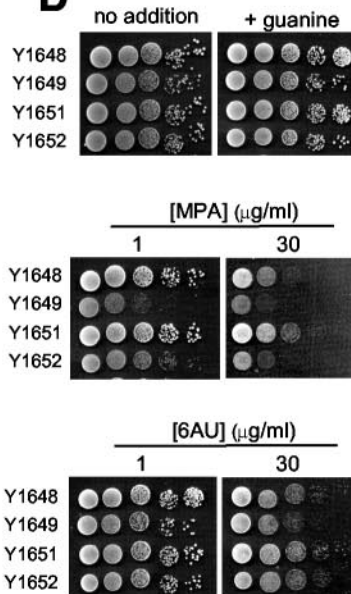
B



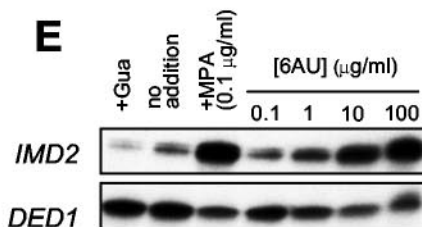
C



D



E



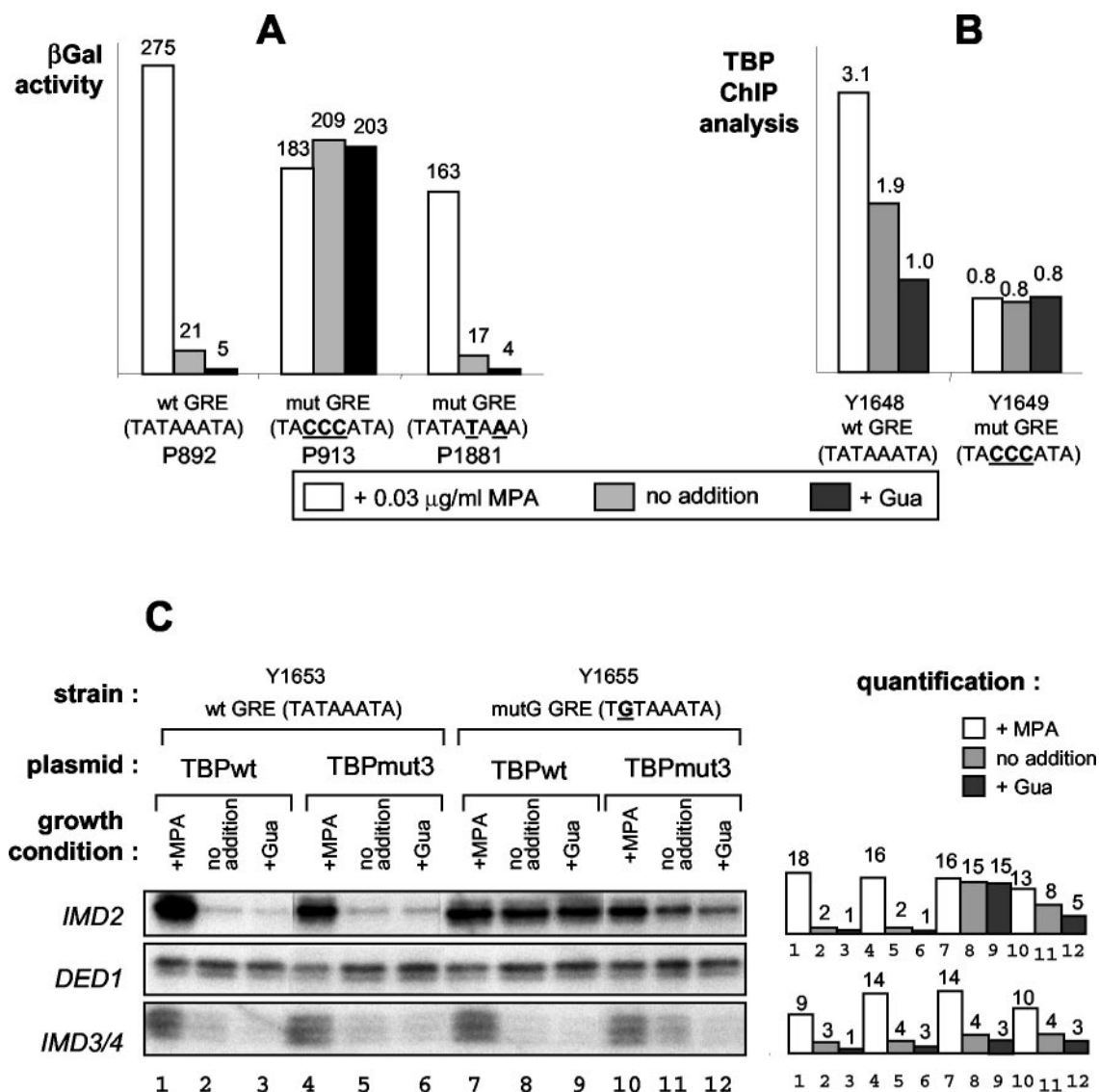


FIG. 5. The GRE is the *IMD2* TATA box. (A) Plasmids carrying *IMD2-lacZ* fusions with the indicated GRE sequences (wt, wild type; mut, mutant) were transformed into wild-type Y350 cells that were treated or not treated with guanine (Gua) or MPA. β -Galactosidase (β Gal) activity was then measured. (B) The TBP occupancy of the *IMD2* promoter was measured by ChIP analysis with isogenic strains carrying either the wild-type or the mutant GRE and treated or not treated with Gua or MPA. The amount of *IMD2* promoter chromatin in the control or TBP immunoprecipitates was normalized to that of input DNA, and the indicated values are the relative levels in TBP versus control immunoprecipitates. An analysis of internal controls, showing no change in TBP occupancy, was also performed by PCR amplification of both the *ACT1* TATA box region and a non-TATA box-containing region of the *IMD2* gene corresponding to its coding sequence (data not shown). (C) A mutant TBP recognizing a TG**T**AATA mutant TATA box (mutation in boldface) is able to restore MPA and Gua regulation to a strain with a TG**T**AATA mutant GRE box at the *IMD2* locus. The indicated strains were transformed with a multicopy plasmid expressing wild-type TBP (TBPwt) or an altered-specificity mutant of TBP (TBPmut3) that can bind the TG**T**AATA sequence. Total cellular RNA was extracted from cells grown in the presence or absence of Gua and MPA, and the levels of transcripts of *IMD2* and *DED1* (as an internal control) were measured by S1 nuclease analysis. A portion of the *IMD2* probe can hybridize to the *IMD3* and/or *IMD4* transcripts, and this hybridization is revealed by a very small labeled DNA fragment protected from S1 nuclease digestion. We included this hybridization product in the figure, since it actually demonstrates that activation by MPA was similar in the different strains. Quantification of the blots (arbitrary units) is shown on the right.

We found that the *IMD2* reporter construct was not at all inducible by MPA at a restrictive temperature in the conditional *taf1-4* mutant strain, while under the same conditions, it was inducible in the wild-type strain (Fig. 7B).

These results suggest that the upregulation of *IMD2* by MPA requires TAF1 and is most likely mediated by TFIID binding to the GRE. To confirm this suggestion, we performed ChIP

experiments. Indeed, the association of TAF1 with the *IMD2* promoter, like that of TBP, was induced by MPA (Fig. 7C).

DISCUSSION

Specific characterization of *IMD2* expression. In this study, we have undertaken a careful characterization of the regula-



FIG. 6. The distance of the GRE from the initiation site is responsible for the low basal expression of *IMD2*. Wild-type cells were transformed with the indicated plasmids, and transformants were grown to exponential phase and then treated or not treated with guanine (Gua). β -Galactosidase (β Gal) activity of the *lacZ* fusions expressed from the indicated plasmids was measured. Similar effects were observed when mRNA from the fusions was analyzed (data not shown).

tion of the *IMD2* gene, one of the four *IMD* genes of *S. cerevisiae*. We used an analysis that allowed us to distinguish among the transcripts originating from the four *IMD* genes (22). In addition, to define elements important in *IMD2* regulation, we introduced mutations in the chromosomal *IMD2* locus. Indeed, in previous work by various investigators (10, 29, 30), conclusions about *IMD2* gene regulation have been obtained by use of fusion constructs with *lacZ* or *luc* reporter genes carried by episomal plasmids. We now know that the enzyme levels produced from some *IMD2-lacZ* reporter constructs (containing internal deletions in the *IMD2* promoter) do not always coincide with transcript levels (data not shown). This fact is apparently due to an "ATG" located at -70 in the promoter region that only in some promoter deletion configurations seems to affect translation of the *IMD2* reporter fusions (data not shown). Furthermore, when endogenous transcript levels were previously analyzed by Northern blot analysis, the probes used could not distinguish among the four highly homologous *IMD* genes (10, 29, 30). This result could have been misleading, since it is clear that *IMD2* and *IMD3* are expressed at similar levels (10, 12) and furthermore that their relative responses to MPA and 6-AU are different. Indeed, the overexpression of *IMD2* leads mostly to MPA resistance, while the overexpression of *IMD3* and *IMD4* leads mostly to resistance to 6-AU (9). Finally, conclusions have also been drawn by use of strains carrying deletions of one or the other *IMD*

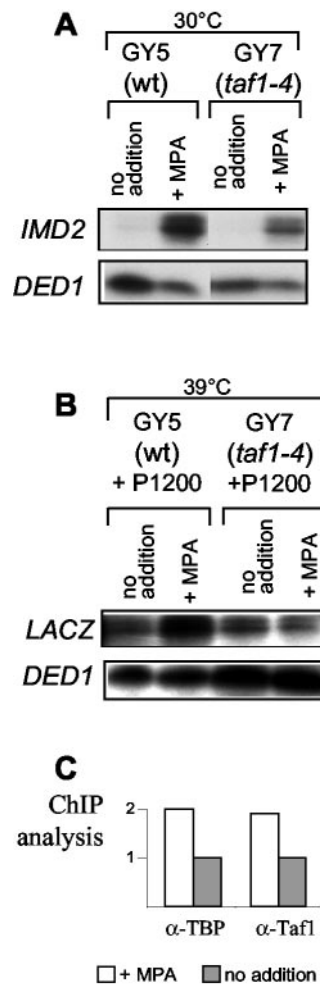


FIG. 7. *IMD2* expression is not inducible in the absence of a functional TFIID. (A) Total cellular RNA was extracted from isogenic strains carrying wild-type *TAF1* (wt) or the temperature-sensitive *taf1-4* allele and treated or not treated with MPA. Cells were grown at 30°C, and the levels of transcripts of *IMD2* and *DED1* (as an internal control) were measured by S1 nuclease analysis. (B) Cells were transformed with a plasmid carrying an *IMD2-lacZ* fusion that does not contain the coding sequences of the *IMD2* gene beyond the ATG (P1200). Transformants were grown to exponential phase and then shifted to 39°C. After 10 min, MPA was added for 1 h. Total cellular RNA was extracted, and *lacZ* and *DED1* (as an internal control) mRNA levels were measured by S1 nuclease analysis. (C) TBP and TAF1 occupancy of the *IMD2* promoter was measured by ChIP analysis with wild-type strain Y350 treated or not treated with MPA. The amount of *IMD2* promoter chromatin in the control or TBP immunoprecipitates was normalized to that of input DNA, and the indicated values are the relative levels in TBP versus control immunoprecipitates. An analysis of internal controls, showing no change in TBP occupancy, was also performed by PCR amplification of both the *ACT1* TATA box region and a non-TATA box-containing region of the *IMD2* gene corresponding to its coding sequence (data not shown).

gene (3). The problem in the interpretation of those experiments is that the *imd2* deletion strain used carried a large chromosomal disruption including many other genes.

The GRE is the TATA box of the *IMD2* gene. We found here that the previously defined GRE (10) is indeed important for regulation of the endogenous *IMD2* gene. This element is essential for the induction of *IMD2* by MPA and the repression

of *IMD2* by guanine. Our very new and exciting finding is that the GRE is in fact the TATA element of the *IMD2* gene. First, we show that the sequence can be mutated into a totally consensus TATA sequence without a loss of function while, in contrast, single point mutations destroying the TATA sequence lead to a loss of *IMD2* gene regulation. Second, regulated binding of TBP to the *IMD2* promoter region, in response to MPA and guanine, requires the GRE sequence. Finally, and most importantly, the regulation of *IMD2* can be restored by a mutated GRE sequence when cells are transformed with a plasmid expressing an altered-specificity TBP that can recognize the mutated GRE sequence.

Several promoter elements determine low levels of *IMD2* expression. The definition of GRE as the TATA element of *IMD2* was unexpected, since it is located 202 bp upstream of the transcription start site. This long distance between the GRE and the transcription start site is not important for the response of *IMD2* to guanylic nucleotide levels but instead ensures a low level of expression of *IMD2*. We have also identified another element that contributes to the low basal expression of *IMD2*, namely, a TATA-like sequence (TATA2) which is localized at nucleotide -20 relative to the transcription start site and which functions as a repressive element for basal, repressed, and induced levels of *IMD2* transcription. This mapped element is certainly responsible for previous observations made by others where a 36-nucleotide deletion of promoter elements around that transcription start site led to increased expression of a reporter construct (30). Moreover, the *IMD2* coding sequence also contains the NSE *cis* element responsible for *IMD2* postdiauxic shift repression. Taken together, our results indicate that *IMD2* is generally in a "repressed" state and can be repressed further under certain conditions. Because we have observed a slight increase in *IMD2* basal expression in an *snf2* mutant (data not shown), we can imagine that the general repression of *IMD2* may be related to a particular structure of the chromatin. More studies will be needed to test such a model.

Considering the distance of the GRE from the transcription initiation site, it is unclear how it functions to recruit the transcription machinery to the initiation site, and it also seems aberrant that no sequence closer to the start site functions instead of this element. In any event, it is clear that when the GRE is mutated, other elements within the promoter can function to mediate transcription initiation, leading to a basal and unregulated level of expression that is higher than that initiated from the GRE. However, this alternate core promoter is obviously not fully functional or accessible when the GRE is intact, perhaps because of the structure imposed upon the *IMD2* promoter by the functional GRE. This masking effect is probably related to the way in which a functional preinitiation complex is recruited to the correct distant initiation site. Whatever the nature or position of this alternate core promoter element, it cannot functionally replace the GRE, since it cannot be regulated by either guanine or MPA. One can expect it to be a TATA-less sequence, similar to the TATA-less core promoters of the *HIS3* and *HIS4* genes which, in contrast to their TATA box promoters, cannot be activated by Gcn4p (21, 27).

The combination of multiple elements within the *IMD2* promoter and the GRE is necessary for appropriate regulation by

guanylic nucleotide levels. In the *IMD2* promoter, besides the TATA box, no *cis*-acting UAS or URS could be found. Many unsuccessful attempts were undertaken to look for *cis* elements and, by defining genetic screens, to identify *trans*-acting factors. Further analysis of specific possible regulators, according to their known functions, such as Rtf1p (31), Mot1p (2, 6, 19), the Ccr4-Not complex (5, 7, 19, 20), or the NC2 regulator (4, 14, 17, 19, 23), was also negative (data not shown). One other unfruitful hypothesis was that the other Imd proteins would have a regulatory role for *IMD2*, which was not the case (data not shown).

Even though our results seem to establish that no specific single *cis*-acting sequence other than the GRE exists within the *IMD2* promoter, the introduction of the GRE into a heterologous *CYC1-lacZ* reporter fusion gene does not lead to guanine and MPA regulation (data not shown). Therefore, the GRE is necessary but not sufficient to confer MPA and guanine regulation.

We have found that the first 52 nucleotides upstream of the transcription start site are necessary and sufficient to mediate *IMD2* regulation in combination with a 100-bp fragment including the GRE. There is clearly something specific about this sequence, since these 52 nucleotides cannot be replaced by other elements of the *IMD2* promoter (data not shown). In addition, we could not define any single element within this sequence that can functionally replace the 52 nucleotides. It seems that multiple elements within this sequence act together to confer specificity. Perhaps a particular structure is conferred by these multiple elements and the establishment of this structure is sensitive to guanylic nucleotide levels. What such a regulatory structure could be is at present totally unclear to us, since neither Snf2p, Isw1p, Isw2p, nor Gcn5p is important for *IMD2* regulation.

The binding of TBP to the GRE and the function of TFIID are essential elements of *IMD2* regulation. Our analyses of mutants have confirmed the crucial importance of TBP binding to the GRE and of TFIID for the regulated expression of *IMD2*. Regulation is unlikely to be mediated by the levels of TBP, since the overexpression of TBP or mutation of the Kap114p importin (rendering TBP mostly cytoplasmic) (24) had no effect on *IMD2* expression or regulation (data not shown). It must be noted that the position of the binding site for TBP at the 5' end of the promoter and the long distance to the site of transcription initiation are very exceptional and suggest that the assembly of the transcription initiation machinery may also be quite exceptional. It seems likely that TBP is recruited in the form of TFIID, since TFIID is necessary for *IMD2* expression and activation, and TAF1 association at the *IMD2* promoter follows that of TBP. The absence of any other identifiable single UAS or URS *cis*-acting element suggests that the recruitment of TFIID to the core promoter essentially depends upon its inherent capacity to bind or the accessibility of its DNA sequence, without the help of *trans*-acting factors bound to other *cis* elements. It is therefore very interesting that the synthesis of guanylic nucleotides with such broad and essential roles in cells seems to have kept regulation features simple, without UAS or URS elements. Whether the levels of guanylic nucleotides affect a chromatin structure, allowing TFIID to bind to the GRE or to form a functional preinitiation complex over the distant site of transcription initiation, or

whether they directly affect TFIID itself is still an open question.

Does regulation by guanylic nucleotide levels require a particular promoter organization? It has been observed by analyzing the yeast proteome after MPA treatment that only the three *Imd* proteins are induced (11), indicating that MPA treatment does not have broad effects on gene expression. Thus, the mechanism leading to the induction of the *IMD* genes in response to guanylic nucleotide levels is likely to be very specific. TFIID obviously is not a component specific to the transcription of the *IMD* genes. This apparent paradox could be tentatively explained by considering the unusual *IMD* gene promoters. *IMD3* is regulated by MPA and guanine, like *IMD2* (data not shown), and the GRE is conserved in the same position in the *IMD3* promoter and is absolutely essential for the regulated expression of *IMD3* (data not shown). Furthermore, the promoters of these two genes are generally very similar (61%), a fact that is rather unusual for noncoding regions, unless it is indeed related to the very specific regulation of these genes. Indeed, if regulation requires multiple elements that act together, the promoters will necessarily be quite conserved overall, rather than just sharing a given short regulatory sequence.

The regulated recruitment of TBP to the GRE is abolished by metabolic shutdown of *IMD2* by the element in the coding sequence. The notion of a particular structure at the *IMD2* promoter that more or less allows TFIID to associate productively with the core promoter is reinforced by our finding that *IMD2* initiation is shut off by the NSE in the coding sequence (12). This NSE, located 260 nucleotides downstream of the transcription initiation site, controls TBP binding to its binding site 462 nucleotides upstream and thereby transcription initiation (12). It seems reasonable to imagine that this entire DNA region is presented in a particular structure that can be either permissive or not permissive for initiation. Exactly how this regulation is achieved is still not known since, as indicated above, proteins implicated in chromatin remodeling, such as *Isw1*, *Isw2*, *Swi2*, or *Gcn5*, are clearly not critical for *IMD2* -regulation (data not shown). The relationship between the unusually distant TATA box and the regulatory element within the coding sequence is certainly an exciting new item to investigate.

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REFERENCES

- Allison, A. C., and E. M. Eugui. 2000. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology* **47**:85–118.
- Auble, D. T., K. E. Hansen, C. G. F. Mueller, W. S. Lane, J. Thorner, and S. Hahn. 1994. *MOT1*, a global repressor of RNA polymerase II transcription, inhibits TBP binding to DNA by an ATP-dependent mechanism. *Genes Dev.* **8**:1920–1934.
- Barton, A. B., H. Bussey, R. K. Storms, and D. B. Kaback. 1997. Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: characterization of the 54 kb right terminal CDC15-Flo1-PHO11 region. *Yeast* **13**:1251–1253.
- Cang, Y., D. T. Auble, and G. Prelich. 1999. A new regulatory domain on the TATA-binding protein. *EMBO J.* **18**:6662–6671.
- Collart, M. A., and K. Struhl. 1994. *NOT1* (*CDC39*), *NOT2* (*CDC36*), *NOT3*, and *NOT4* encode a global negative regulator of transcription that differentially affects TATA-element utilization. *Genes Dev.* **8**:525–537.
- Davis, J. L., R. Kunisawa, and J. Thorner. 1992. A presumptive helicase (*MOT1* gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12**:1879–1892.
- Deluen, C., N. James, L. Maillat, M. Molinette, G. Theiler, M. Lemaire, N. Paquet, and M. A. Collart. 2002. The Ccr4-Not complex and γ TAF1 (γ Taf_{1130p}/ γ Taf_{1145p}) show physical and functional interactions. *Mol. Cell. Biol.* **22**:6735–6749.
- DeRisi, J. L., V. R. Iyer, and P. O. Brown. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**:680–686.
- Desmoucelles, C., B. Pinson, C. Saint-Marc, and B. Daignan-Fornier. 2002. Screening the yeast “disruptome” for mutants affecting resistance to the immunosuppressive drug mycophenolic acid. *J. Biol. Chem.* **277**:27036–27044.
- Escobar-Henriques, M., and B. Daignan-Fornier. 2001. Transcriptional regulation of the yeast GMP synthesis pathway by its end products. *J. Biol. Chem.* **276**:1523–1530.
- Escobar-Henriques, M., A. Balguerie, C. Monribot, H. Boucherie, and B. Daignan-Fornier. 2001. Proteome analysis and morphological studies reveal multiple effects of the immunosuppressive drug mycophenolic acid specifically resulting from guanylic nucleotide depletion. *J. Biol. Chem.* **276**:46237–46242.
- Escobar-Henriques, M., M. A. Collart, and B. Daignan-Fornier. 2003. Transcription initiation of the yeast *IMD2* gene is abolished in response to nutrient limitation through a sequence in its coding region. *Mol. Cell. Biol.* **23**:6279–6290.
- Gasch, A. P., P. T. Spellman, C. M. Kao, O. Carmel-Harel, M. B. Eisen, G. Storz, D. Botstein, and P. O. Brown. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11**:4241–4257.
- Goppelt, A., and M. Meisterernst. 1996. Characterization of the basal inhibitor of class II transcription NC2 from *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **24**:4450–4455.
- Grant, P. A., L. Duggan, J. Côté, S. M. Roberts, J. E. Brownell, et al. 1997. Yeast *Gcn5* functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.* **11**:1640–1650.
- Huber, J. C., A. Guyonvarch, B. Kammerer, F. Exinger, P. Liljelund, and F. Lacroute. 1983. Complete sequence of a eukaryotic regulatory gene. *EMBO J.* **2**:2071–2073.
- Inostroza, J. A., F. H. Mermelstein, I. Ha, W. S. Lane, and D. Reinberg. 1992. *Drl1*, a TATA-binding protein-associated phosphoprotein and inhibitor of class II gene transcription. *Cell* **70**:477–489.
- Lee, T. I., H. C. Causton, F. C. P. Holstege, W.-C. Shen, N. Hannett, E. G. Jennings, F. Winston, M. R. Green, and R. A. Young. 2000. Redundant roles for TFIID and SAGA complexes in global transcription. *Nature* **405**:701–704.
- Lemaire, M., J. Xie, M. Meisterernst, and M. A. Collart. 2000. The NC2 repressor is dispensable in yeast mutated for the *Sin4p* component of the holoenzyme and plays roles similar to *Mot1p* *in vivo*. *Mol. Microbiol.* **36**:163–173.
- Liu, H.-Y., V. Badarinarayana, D. C. Audino, J. Rappsilber, M. Mann, and C. L. Denis. 1998. The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. *EMBO J.* **17**:1096–1106.
- Mahadevan, S., and K. Struhl. 1990. T_C , an unusual promoter element required for constitutive transcription of the yeast *HIS3* gene. *Mol. Cell. Biol.* **10**:4447–4455.
- Maillat, L., C. Tu, Y. K. Hong, E. O. Shuster, and M. A. Collart. 2000. The essential function of *NOT1* lies within the CCR4-NOT complex. *J. Mol. Biol.* **303**:131–143.
- Meisterernst, M., and R. G. Roeder. 1991. Family of proteins that interact with TFIID and regulate promoter activity. *Cell* **67**:557–567.
- Morehouse, H., R. M. Buratowski, P. A. Silver, and S. Buratowski. 1999. The importin/karyopherin Kap114 mediates the nuclear import of TATA-binding protein. *Proc. Natl. Acad. Sci. USA* **96**:12542–12547.
- Myers, A. M., A. Tzagoloff, D. M. Kinney, and C. J. Lusty. 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. *Gene* **45**:299–310.
- Otero, G., J. Fellows, Y. Li, T. de Bizemont, N. M. G. Dirac, C. M. Gustafsson, H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup. 1999. Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. *Mol. Cell* **3**:109–118.
- Pellman, D., M. E. McLaughlin, and G. R. Fink. 1990. TATA-dependent and TATA-independent transcription at the *HIS4* gene of yeast. *Nature* **348**:82–85.
- Peterson, C. L., and J. W. Tamkun. 1995. The SWI-SNF complex: a chromatin remodeling machine? *Trends Biochem. Sci.* **20**:143–146.
- Shaw, R. J., and D. Reines. 2000. *Saccharomyces cerevisiae* transcription elongation mutants are defective in *PUR5* induction in response to nucleotide depletion. *Mol. Cell. Biol.* **20**:7427–7437.

30. **Shaw, R. J., J. L. Wilson, K. T. Smith, and D. Reines.** 2001. Regulation of an IMP dehydrogenase gene and its overexpression in drug-sensitive transcription elongation mutants of yeast. *J. Biol. Chem.* **276**:32905–32916.
31. **Stolinski, L. A., D. M. Eisenmann, and K. M. Arndt.** 1997. Identification of *RTF1*, a novel gene important for TATA site selection by TATA box-binding protein in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **17**:4490–4500.
32. **Strubin, M., and K. Struhl.** 1992. Yeast and human TFIIID with altered DNA-binding specificity for TATA elements. *Cell* **68**:721–730.
33. **Tsukiyama, T., J. Palmer, C. C. Landel, J. Shiloach, and C. Wu.** 1999. Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev.* **13**:686–697.
34. **Zimmermann, A. G., J. J. Gu, J. Laliberte, and B. S. Mitchell.** 1998. Inosine-5'-monophosphate dehydrogenase: regulation of expression and role in cellular proliferation and T lymphocyte activation. *Prog. Nucleic Acid Res. Mol. Biol.* **61**:181–209.