AMP and GMP are synthesized from IMP by specific conserved pathways. In yeast, whereas IMP and AMP synthesis are coregulated, we found that the GMP synthesis pathway is specifically regulated. Transcription of the IMD genes, encoding the yeast homologs of IMP dehydrogenase, was repressed by extracellular guanine. Only this first step of GDP synthesis pathway is regulated, since the latter steps, encoded by the GUA1 and GUK1 genes, are guanine-insensitive. Use of mutants affecting GDP metabolism revealed that guanine had to be transformed into GDP to allow repression of the IMD genes. IMD gene transcription was also strongly activated by mycophenolic acid (MPA), a specific inhibitor of IMP dehydrogenase activity. Serial deletions of the IMD2 gene promoter revealed the presence of a negative cis-element, required for guanine regulation. Point mutations in this guanine response element strongly enhanced IMD2 expression, also making it insensitive to guanine and MPA. From these data, we propose that the guanine response element sequence mediates the repression process, which is enhanced by guanine addition, through GDP or a GDP derivative, and abolished in the presence of MPA.

Purine nucleotides are involved in many important cellular processes, and therefore a balanced synthesis of AMP and GMP is required. Cells can synthesize purine nucleotides through the de novo pathway, a 10-step pathway that produces IMP, which in turn serves as the common precursor for AMP and GMP nucleotide biosynthesis (Fig. 1). In yeast, the regulation of the ADE genes involved in AMP biosynthesis has been characterized (1, 2). Expression of the ADE genes is repressed by extracellular adenine and activated by the transcription factors Bas1p and Bas2p. However, in Saccharomyces cerevisiae, GMP biosynthesis regulation has not received much attention and is therefore poorly understood. IMP dehydrogenase (IMPDH), catalyzing the first step of de novo guanine nucleotide synthesis, has a key role on growth of many cell types, including lymphocytes and rapidly proliferating cells (4), having a key role in cell proliferation, since an increased level of IMPDH activity has been observed in rapidly proliferating cells (4), including human leukemic cell lines (6–9), solid tumor tissues (10), and B- and T-activated lymphocytes (5). Indeed, substances blocking IMPDH activity, such as mycophenolic acid (MPA), act as immunosuppressive drugs and are used to prevent allograft rejection (11–16; see Ref. 17 for recent reviews). Moreover, it was recently shown that a low level of IMPDH activity is necessary for p53-dependent growth suppression. Indeed, constitutive expression of IMPDH abolishes p53-dependent growth suppression (18), although the exact mechanism is not known.

To assess the molecular mechanisms governing guanylic nucleotide synthesis, we have proceeded to study the regulation of GTP synthesis in Saccharomyces cerevisiae. In this yeast, GMP synthetase, GMP kinase, and NDP kinase, required for catalysis of the latter steps of guanylic nucleotide biosynthesis, are encoded by the GUA1, GUK1, and YNK1 genes, respectively (Fig. 1) (19–21). IMPDH, catalyzing the first committed step in guanylic nucleotide biosynthesis, has been isolated from a variety of eukaryotic and prokaryotic sources and exhibits a high level of amino acid conservation (22–27). Molecular mass, kinetic parameters, and reaction mechanism are also similar, demonstrating a high degree of conservation among species (28–30). By using these features and the complete sequencing of the S. cerevisiae genome, we have identified four yeast homologs for IMPDHs, YAR073w, YHR216w, YLR432w, and YML056c, that we named IMD1, IMD2, IMD3, and IMD4 genes, respectively. These genes encode proteins with more than 80% amino acid identity to each other and that share about 60% amino acid residues with human IMPDHs type I and type II.

In this paper we have studied the regulation of the yeast GMP synthesis genes, and we found that GMP synthesis is differentially regulated from IMP and AMP synthesis. We show that the IMD genes transcript levels are impaired by guanine but not by adenine. IMDs transcriptional expression is strongly activated by MPA. Mutational analysis of the sequences upstream of IMD2 ATG allowed us to identify a cis-element (TATAAATA), named guanine response element (GRE), affecting guanine regulation; several trans-mutations affecting regulation of IMDs are also presented. We propose that IMD2 regulation by MPA and guanine takes place through the cis-acting GRE, in response to intracellular concentrations of GDP or a derivative of GDP.

**EXPERIMENTAL PROCEDURES**

Yeast Media—SD is 2% glucose, 0.17% nitrogen base, and 0.5% ammonium sulfate. Adenine or guanine were optionally added to a final concentration of 0.15 and 0.13 mM, respectively. Hydroxyurea (H2U) was used at a concentration of 240 mM. SD casA is SD supplemented with 0.2% casamino acids (Difco).

Yeast Strains—Two sets of isogenic strains were used as follows:

---

*This work was supported by a fellowship from Fundação para a Ciência e Tecnologia (to M. E.-H.), by ARC Research Grant 5259, Conseil Régional d’Aquitaine, and CNRS UMR Grant 5095. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 33 5 56 99 90 55; Fax: 33 5 56 99 90 55; E-mail: B. Daignan-Fornier@ibgc.u-bordeaux2.fr.

‡ The abbreviations used are: IMPDH, IMP dehydrogenase; HU, hydroxyurea; MPA, mycophenolic acid; GRE, guanine response element; PCR, polymerase chain reaction; bp, base pair.

---

Mafalda Escobar-Henriques and Bertrand Daignan-Fornier‡

From the Institut de Biochimie et Génétique Cellulaires, CNRS UMR 5095, 1 Rue Camille Saint-Saëns, F-33077 Bordeaux Cedex, France

---

Received for publication, August 30, 2000, and in revised form, October 12, 2000
Published, JBC Papers in Press, October 16, 2000, DOI 10.1074/jbc.M007926200

---

**Transcriptional Regulation of the Yeast GMP Synthesis Pathway by Its End Products**

---

The Journal of Biological Chemistry
© 2001 by The American Society for Biochemistry and Molecular Biology, Inc.

---

**Vol. 276, No. 2, Issue of January 12, pp. 1523–1530, 2001**

---

Printed in U.S.A.
Regulation of GMP Synthesis in Yeast

Y350 (MATa; ura3-52; leu2-3, 112; lys2Δ201), 216 (MATa; ura3-52; leu2-3, 112; lys2Δ201; his3Δ200), 119 (MATa; ura3-52; leu2-3, 112; lys2Δ201; his3Δ200; fis2Δ206; fcy2-1); and D451-3 (MATa; leu2-3, 112; ura3-52), D451-3Δynk1 (MATa; leu2-3, 112; ynk1Δ:URA3).

Plasmids—The lacZ fusions were constructed in the vectors described by Myers and coworkers (31). Except for P777, all of them are URA3 vectors. The IMD1-lacZ fusion (P677) was constructed as follows: a 389-bp KpnI-BglII restriction fragment, starting 357 bp upstream from the ATG initiation codon, was cloned in YEp353 linearized with BamHI and KpnI. For IMD2-lacZ (P354), a 2321-bp BamHIEcoRV restriction fragment, was constructed as follows: a 588-bp EcoRI-EcoRV restriction fragment, starting 555 bp upstream from the ATG initiation codon, was cloned in YEp356R linearized with SmaI and EcoRI. For IMD4-lacZ, a 2573-bp HindIII-EcoRI restriction fragment, starting 668 bp upstream from the ATG initiation codon, was cloned in YEp356R linearized with HindIII and EcoRI. The URA1-lacZ fusion was constructed by inserting the BamHI-BglII restriction fragment from pFL44/T1-BE (19) in YEp358R linearized with BamHI and HindIII. An IMD2-lacZ fusion (P777) was also constructed in a plasmid carrying the IMD2 promoter, named P909, P963, P911, P915, P917, P919, P921, and P934, and was obtained with the oligonucleotide IMP2-C908, IMP2-C308, IMP2-C602, IMP2-C289, IMP2-C281, IMP2-C283, IMP2-C284, IMP2-C285, and IMP2-C286, respectively. The plasmids containing single C mutations in the -308 to -301 region of the IMD2 promoter, named P1356, P1370, P1372, P1326, P1328, P1330, P1332, and P1334, were obtained with the oligonucleotide IMP2-C1, IMP2-C2, IMP2-C3, IMP2-C4, IMP2-C5, IMP2-C6, IMP2-C7, or IMP2-C8, respectively. The oligonucleotides used to PCR amplify the IMD2 fragments from the S288c genomic DNA template were as follows: IMD2 promoter, coding strand, IMP2-442, 5'-CGGGATCCGGAAGAAAGCGGAAAAATAA-3'; IMP2-311, 5'-CGGGATCCGGAAGAAAGCGGAAAAATAA-3'.

RESULTS

Expression of IMD Genes Is Repressed by Guanine but Not by Adenine—To investigate whether GDP biosynthesis genes are coregulated with the AMP pathway genes, i.e. repressed by
external addition of adenine, fusions between the GDP biosynthesis genes and the lacZ reporter gene were constructed. The β-galactosidase activity of these fusions was measured in the presence or absence of adenine. As shown in Fig. 2, expression of all the tested lacZ fusions was not affected by adenine addition. Expression of the IMD1-lacZ fusion was hardly detectable. In fact, IMD1 is thought to be a silent gene, since it carries a frameshift in its coding sequence, and its transcript was not detectable (33).

Expression of the lacZ fusion genes was also tested in the presence of external guanine. Guanine clearly impaired expression of the IMD2, -3, and -4 lacZ fusions, whereas it did not affect expression of GUA1-lacZ and GUK1-lacZ fusions (Fig. 2A). Thus, only the first step of GDP synthesis appeared to be regulated by guanine. Expression of IMD2 was also monitored in the presence or absence of guanine by Northern blot analysis. Results presented in Fig. 2B and quantification of the blots (data not shown) revealed that the IMD2 transcript was 3-fold less abundant in the presence of guanine. In this experiment, cross-hybridization with IMD3 and IMD4 transcripts cannot be totally excluded, since the IMD2 probe used is 85 and 87% identical to IMD3 and IMD4, and coding sequences of these three genes have approximately the same size. However, if this happens, it further suggests that IMDs are coregulated, in agreement with β-galactosidase results. Because IMD2 is the most repressed gene, we focused on the regulation of this member of the IMPDH family.

Guanine Must Be Metabolized to GDP to Repress IMD2 Expression—Since IMD2 was down-regulated in the presence of extracellular guanine, we investigated the signaling pathway controlling guanine-responsive genes. A IMD2-lacZ fusion was studied in several yeast genetic backgrounds in which guanine utilization is impaired (Fig. 1). The importance of guanine uptake was first analyzed by assaying guanine repression in a strain mutated at the fcy2 locus, encoding purine-cytosine permease. Data presented in Table I show that the fcy2 mutant failed to repress the IMD2 gene expression in response to guanine (compare Y349 to 119), indicating that guanine uptake is strictly required for repression. Once inside the cell, guanine can be metabolized into GMP by the hypoxanthine-guanine phosphoribosyltransferase enzyme, encoded by the HPT1 gene (Fig. 1) (34). Analysis of the IMD2-lacZ fusion expression in a strain mutated at the HPT1 locus (35) showed that the hypoxanthine phosphoribosyltransferase enzyme is necessary to transduce the repression signal (Table I, compare Y350 to 216).

![Fig. 2](http://www.jbc.org/)

**A**

**B**

**Fig. 2. Effect of adenine and guanine on expression of GDP biosynthesis genes.** A, Y350 (wild-type) was transformed with the indicated lacZ fusion plasmids and grown for 6 h in SD case medium supplemented or not with 0.15 mM adenine or 0.13 mM guanine. Cells were harvested and assayed for β-galactosidase (βGal) activity, as described under “Experimental Procedures.” B, total RNAs from the Y350 wild-type strain, grown for 6 h in SD case with or without guanine (0.13 mM), were analyzed by Northern blotting using probes for the gene IMD2 and the control gene ACT1.

**Table I**

| Fusion (plasmid name) | Yeast strain | Relevant genotype | β-Gal activity | DR/R |
|----------------------|--------------|-------------------|----------------|------|
| IMD2-lacZ (P354)     | Y349         | wt                | 34             | 6.8  |
|                      | 119          | fcy2              | 31             | 1.1  |
|                      | Y350         | wt                | 26             | 4.3  |
|                      | 216          | hpt1              | 23             | 1.1  |
|                      | 220          | guk1              | 39             | 1.0  |

The indicated strains, transformed with IMD2-lacZ, were grown for 6 h in SD medium supplemented with casamino acids for derepressing conditions (DR) or in the same medium containing 0.13 mM guanine, for repressing conditions (R). β-Galactosidase (β-Gal) activities were determined as described under “Experimental Procedures.” DR/R is the ratio of the values measured under derepressing versus repressing conditions (repression ratio). wt stands for wild-type.

GMP can then be phosphorylated to GDP. This step is catalyzed by GDP kinase, encoded by the GUK1 essential gene. Expression of the IMD2-lacZ fusion was assayed in the guk1-3 mutant strain strongly impaired for the GMP kinase activity (36). Results presented in Table I clearly show that regulation by guanine of the IMD2-lacZ gene is completely abolished in this strain (compare Y350 to 220). Thus, guanine must be transformed into GDP to repress IMD genes expression.

Further metabolism of GDP involves its reduction to dGDP by ribonucleotide reductase or its phosphorylation to GTP by nucleotide diphosphate kinase. The importance of deoxyribonucleotides in this transduction pathway can be assessed with HU, a drug that specifically inhibits ribonucleotide reductase activity (37, 38). In the presence of 240 mM HU, a dose that resulted in 96% of growth inhibition (see “Experimental Procedures”), guanine provoked a comparable (6-fold) reduction in IMD2-lacZ fusion expression (data not shown). Thus, we found that HU did not affect repression by guanine, even at doses that strongly inhibit cell growth. This result clearly shows that dGDP does not participate in the transduction pathway of guanine repression. Expression of the IMD2-lacZ fusion was assayed in a strain deleted for the nucleotide diphosphate kinase-encoding gene, YNK1, and in an isogenic wild-type strain (21). In the absence of guanine, activity of IMD2-lacZ in the YNK1 and ynk1 strains was 241 and 234 β-galactosidase units, respectively. Guanine

---

**Regulation of GMP Synthesis in Yeast**

1525

---
addition decreased IMD2-lacZ expression to 18 and 30 β-galactosidase units, in the YNK1 and ynk1 strains, respectively. Thus, IMD2 repression by guanine was not severely affected by ynk1 disruption, meaning that the nucleotide diphosphate kinase encoded by the YNK1 gene is not necessary for the transcription of the guanine repression signal. Altogether, these results indicate that the signal molecule responsible for IMD2 repression by guanine could be either GDP itself or a yet unidentified derivative of GDP.

Expression of the IMD2 Gene Is Activated by the Immunosuppressive Drug MPA—We then examined the effect of MPA, a potent and specific inhibitor of euarkyotic IMPDHs and an immunosuppressive drug (see Introduction) on the expression of the IMD2 gene. We reasoned that since GDP or a derivative of GDP represses IMD2 gene expression, MPA inhibition of XMP synthesis should starve the cells for guanine nucleotides, which should result in enhanced expression of IMD2. A range of MPA concentrations was tested for both growth inhibition and expression of the IMD2-lacZ fusion. Results presented in Fig. 3 show that MPA inhibited growth in a dose-dependent manner. In parallel, MPA activated the IMD2-lacZ fusion gene expression, and this response was maximal between 0.03 and 0.3 μg/ml MPA. Addition of guanine completely reversed both MPA toxicity (data not shown) and its effect on IMD2-lacZ expression (Fig. 3), thus confirming the high specificity of this drug. A concentration of 0.03 μg/ml MPA fully activated IMD2-lacZ fusion and only slightly affected growth; therefore, this drug concentration was chosen for further experiments.

Identification of a GRE in the IMD2 Promoter—As a first step toward identification of promoter cis-elements responsible for IMD2 regulation, we mapped the IMD2 transcription start. 5′-Rapid amplification of cDNA ends mapping was done on RNA extracted from cells grown in the presence of either guanine or MPA. In both cases transcription started at the C nucleotide 107 bp upstream from the AGT. The IMD2-lacZ plasmid used in the previous experiments contained 853 bp of 5′-flanking sequence. To map the 5′-boundary of the regulatory region of the IMD2 gene, sequential deletions of the 5′-flanking sequence of IMD2-lacZ were constructed, from 853 to 108 bp upstream from the IMD2 ATG. Expression and regulation by guanine of these constructs were assayed in a wild-type strain (Y350). IMD2-lacZ fusion genes containing 442, 355, and 311 bp upstream of the start codon (Fig. 4, plasmids P853, P855, and P892) were repressed by guanine, as the control IMD2-lacZ plasmid (Fig. 4, P354). In contrast, an IMD2-lacZ fusion gene containing 287 bp of the 5′-flanking sequence (Fig. 4, P895) was insensitive to guanine and was expressed at a much higher level than the control fusion. A similar derepression profile was found for IMD2-lacZ fusion genes containing 442, 263, 234, 197, 159, or 108 bp of the 5′-flanking sequence (Fig. 4, P857, P1527, P1528, P860, and P890), although they were less expressed than the −287 fusion gene. These results suggested the presence of a cis-negative GRE whose 5′-boundary would be between −311 and −287 bp. Positive regulatory cis-elements might also be present between −287 and −108 bp.

To investigate more precisely the importance of the region between −311 and −287 bp in the IMD2 promoter, this region was submitted to site-directed mutagenesis. Triplet CCC mutations were created in the 24 nucleotides spanning the interval −311 to −287. Constructs carrying the clustered mutations were assayed for their effects on β-galactosidase expression under both guanine-repressing and -derepressing conditions. The results shown in Fig. 5 indicate that the CCC substitutions in clusters GTA, TAA, and ATA (plasmids P911, P913, and P915) abolished guanine regulation and enhanced expression of these fusions, as compared with the parental construct. On the other hand, all the other clustered substitutions had no effect, neither on guanine regulation nor on the expression levels of the IMD2-lacZ gene fusion. Comparison of data obtained with plasmids P909 and P963 show that the G to C mutation of the GTA cluster does not affect guanine regulation of the IMD2 gene. In summary, results from this cluster mutations experiment demonstrate that changing the octanucleotide TATAATA into CCTAAATA, TACCCATA, or TATA-
ACCC completely prevents guanine regulation of the IMD2 gene. To identify more precisely the critical nucleotides contained therein, each nucleotide of the TATAAATA box was mutated to a C. The lacZ fusion constructs, each containing a C substitution in the octanucleotide and 311 bp of the 5'-flanking sequence of the IMD2 gene, were tested in guanine-repressing and -derepressing conditions. As shown in Fig. 6, each single mutation introduced led to a guanine-insensitive derepression of the IMD2-lacZ expression, thus revealing that all eight nucleotides of the TATAAATA sequence are required for transcriptional regulation of the IMD2 gene. The TATAAATA box was named GRE. To ensure that this regulation occurs at the transcriptional level, we measured the expression of a wild-type GRE and a mutant GRE IMD2-lacZ fusion in a wild-type strain grown in the presence or absence of external guanine by Northern blot analysis. We first confirmed that regulation by guanine was maintained for the 2311 IMD2-lacZ fusion (Fig. 7). In contrast, an IMD2-lacZ fusion gene containing a mutant sequence in the TATAAATA element was expressed at a much higher level, in a guanine-insensitive manner (Fig. 7). These results confirm that guanine regulation of IMD2 takes place at the transcriptional level, and only in the presence of a functional GRE.

MPA and Guanine Signals for IMD2 Regulation Are Transduced through the Same Pathway—IMD genes are activated by MPA and repressed by guanine. To find out whether these regulations are transduced through the same pathway, IMD2 regulation by MPA was analyzed in hpt1 and guk1 mutant strains, deficient for transduction of the guanine repression signal. Results presented in Table II and Fig. 8 confirmed that in a wild-type strain, MPA addition enhanced IMD2 expression levels. This effect was reversed by guanine addition, which

| plasmid name | -311 to -287 sequence | βGal activity | DR/R |
|--------------|-----------------------|--------------|------|
| P892         | AATAAATA             | 34 | 10 | 3.4 |
| P909         | CATAAATA             | 62 | 26 | 2.4 |
| P963         | CATAAATA             | 60 | 22 | 2.7 |
| P911         | CATAAATA             | 284 | 305 | 0.9 |
| P913         | CATAAATA             | 244 | 240 | 1.0 |
| P915         | CATAAATA             | 290 | 251 | 1.2 |
| P917         | CATAAATA             | 28 | 8 | 3.5 |
| P919         | CATAAATA             | 16 | 6 | 2.7 |
| P921         | CATAAATA             | 26 | 14 | 1.9 |
| P923         | CATAAATA             | 28 | 10 | 2.8 |
| P890         | CATAAATA             | 190 | 236 | 0.8 |

| plasmid name | -308 to -301 sequence | βGal activity | DR/R |
|--------------|-----------------------|--------------|------|
| P892         | AATAAATA             | 17 | 5 | 3.4 |
| P913         | CATAAATA             | 111 | 98 | 1.1 |
| P1369        | CATAAATA             | 97 | 79 | 1.2 |
| P1370        | CATAAATA             | 143 | 120 | 1.2 |
| P1372        | CATAAATA             | 84 | 67 | 1.3 |
| P1326        | CATAAATA             | 122 | 120 | 1.0 |
| P1328        | CATAAATA             | 118 | 115 | 1.0 |
| P1330        | CATAAATA             | 116 | 100 | 1.2 |
| P1332        | CATAAATA             | 106 | 94 | 1.1 |
| P1334        | CATAAATA             | 72 | 53 | 1.4 |

Fig. 5. Triple mutation scanning of −311 to −287 region of IMD2 promoter. Y350 (wt) strain was transformed with the IMD2-lacZ plasmids with the indicated IMD2 promoter sequence in the region between −311 and −287 bp upstream from the ATG. Transformants were grown for 6 h in SD medium supplemented with casamino acids for derepressing conditions (DR) or in the same medium containing 0.13 mM guanine for repressing conditions (R). β-Galactosidase activities were determined as described under “Experimental Procedures.” DR/R is the ratio of the values measured under derepressing versus repressing conditions (repression ratio). In the fusion constructions, underlined nucleotides show the CCC mutations introduced in the wild-type sequence.

Fig. 6. Single mutation scanning of −308 to −301 region of IMD2 promoter. Y350 (wt) strain was transformed with the IMD2-lacZ plasmids with the indicated mutations in the TATAAATA box of the IMD2 promoter sequence. Transformants were grown for 6 h in SD medium supplemented with casamino acids for derepressing conditions (DR) or in the same medium containing 0.13 mM guanine, for repressing conditions (R). β-Galactosidase activities were determined as described under “Experimental Procedures.” DR/R is the ratio of the values measured under derepressing versus repressing conditions (repression ratio). In the fusion constructions, underlined nucleotides show the C mutations introduced in the wild-type sequence.

Fig. 7. Effect of mutations in the GRE on IMD2-lacZ mRNA levels. Y350 (wt) strain was transformed either with plasmid P892, containing the GRE, or with plasmid P913, with a mutant (mut) GRE. Transformants were grown for 6 h in SD medium supplemented with casamino acids or in the same medium containing 0.13 mM guanine. Cells were harvested, and total RNAs were extracted and analyzed by Northern blot using probes for the IMD2 gene or the control gene ACT1.
TABLE II

| Strain         | GRE (plasmid name) | β-Gal activity | (SD + MPA)/SD |
|----------------|-------------------|---------------|---------------|
|                |                   | SD | SD + Gua | SD + MPA | SD + MPA + Gua |                |
| Y350 (wt)      | wt GRE (P892)     | 36 | 9        | 231      | 10            | 6.4            |
|                | mut GRE (P913)    | 173| 156      | 182      | 190           | 1.1            |
| 216 (hpt1)     | wt GRE (P892)     | 33 | 32       | 185      | 187           | 5.6            |
|                | mut GRE (P913)    | 251| 235      | 248      | 229           | 1.0            |
| 220 (guk1)     | wt GRE (P892)     | 86 | 54       | 279      | 309           | 3.2            |
|                | mut GRE (P913)    | 212| 237      | 218      | 208           | 1.1            |

Effect of MPA and guanine on expression of wild-type or GRE mutant IMD2-lacZ fusion in various genetic backgrounds. The indicated strains were transformed with plasmids carrying either wild-type or GRE mutant IMD2-lacZ. Transformants were grown for 6 h in SD medium supplemented with casamino acids or in the same medium containing either 0.03 μg/ml mycophenolic acid or 0.13 mM guanine or both supplements. β-Galactosidase (β-Gal) activities were determined as described under “Experimental Procedures.” (SD + MPA)/SD is the ratio of the values measured in the presence or absence of mycophenolic acid, respectively. wt and mut stand for wild-type and mutant, respectively.

![Fig. 8. Effect of MPA and guanine on expression of IMD2 transcripts in wild-type and guk1-3 backgrounds.](http://www.jbc.org/)

---

decreased IMD2 expression level (Fig. 8 and Table II, line 1). In hpt1 or guk1 mutants, guanine addition was unable to repress IMD2 expression or to reverse MPA activation effect (Fig. 8 and Table II, line 1). We conclude that MPA reversion and guanine repression act through the same pathway. In this hypothesis, a mutation of the GRE box should also disrupt MPA regulation of the IMD2 gene. This was tested by monitoring the effect of MPA on the regulation of an IMD2-lacZ fusion carrying a mutant GRE. Results show that this fusion was expressed at a constant high level, regardless of guanine or MPA presence (Table II, line 2), meaning that IMD2 regulation by guanine or MPA is possible only in presence of the wild-type GRE box. This same fusion, in a hpt1 or guk1 mutant, behaved as in the wild-type strain, making IMD2 insensitive to guanine or MPA addition (Table II, lines 4 and 6).

**DISCUSSION**

In this report, we show that the IMD genes, encoding the S. cerevisiae homologs of IMPDH, are transcriptionally repressed by guanine, which has to be metabolized into GDP or a derivative of GDP to exert its regulatory role. Expression of IMD2 is highly enhanced in the presence of MPA. A cis-negative element, necessary to both guanine and MPA regulation, was found in the IMD2 gene promoter. A simple explanation could be that in response to guanylic nucleotides levels, a repressor could bind to the IMD2 promoter and regulate its transcription. A model for yeast regulation of the guanylic nucleotide synthesis genes is presented in Fig. 9.

**Specific Regulation of the Guanylic Nucleotide Pathway by Its End Product**—We showed that expression of the GDP pathway genes is unaffected by adenine; they are also unaffected by bas1 or bas2 mutations, i.e. the signal and transcription factors involved in regulation of IMP and AMP synthesis pathways (1, 2). These results establish that AMP and GMP synthesis are differentially regulated in S. cerevisiae.

Mechanisms of purine biosynthesis regulation have been elucidated in two bacteria, *Escherichia coli* and *Bacillus subtilis*. In *E. coli*, all the genes for AMP and GMP synthesis are repressed by the PurR repressor in the presence of the corepressors hypoxanthine and/or guanine (39, 40). In *B. subtilis*, synthesis of AMP and GMP is repressed independently by adenine and guanine nucleotides, whereas adenine nucleotides regulate transcription initiation (via the PurR repressor) (41), and guanine nucleotides regulate transcription termination by an anti-termination mechanism (42). Therefore, in these bacteria, regulation of both AMP and GMP biosynthesis pathway genes respond to AMP and GMP precursors, either through a common transcription factor as in *E. coli* or through independent mechanisms as in *B. subtilis*. The situation is clearly different in yeast where AMP synthesis genes is poorly repressed by guanine (35) and where GMP synthesis genes is not affected by adenine (this study). This differential regulation could be a general feature for eukaryotic organisms since it has been shown that expression of human IMPDH is repressed in the presence of guanosine but not adenosine (43).

**Negative Regulation of IMPDH Synthesis by Guanylic Nucleotide Levels Is Conserved among Eukaryotes—** Extracellular guanine led to a 3–7-fold decrease of IMD2 transcription, depending on the experiment. The reasons for such variations of the repression factor, which occurred both with Northern blot and β-galactosidase experiments, are unclear. In the fcy2, hpt1, or guk1 mutant strains, guanine failed to repress the IMD genes, indicating that levels of GDP or a GDP derivative negatively regulate IMD gene expression. Deletion of the YNK1 gene, encoding NDP kinase, did not affect guanine repression of IMD genes. Nevertheless, GTP cannot be totally excluded from the guanine signaling pathway, since the ynk1 disrupted strain retained 10% of GDP to GTP conversion capacity, when compared with wild-type cells (21). Inhibition of dNDPs biosynthesis by a severely growth-inhibiting dose of hydroxyurea did not affect guanine regulation of IMD2 gene. Therefore, whereas guanylic ribonucleotide levels regulate IMDS expression, guanylic deoxycyribonucleotides apparently do not participate in the guanine repression transduction pathway.

In the hpt1 or fcy2 mutant strains, IMD-lacZ fusions were expressed at wild-type levels in the absence of guanine, whereas in the guk1 strain, IMD-lacZ fusions were 2-fold more expressed. We interpret this result as follows: whereas the hpt1 or fcy2 mutations affect only preformed purine utilization, the guk1 mutation affects both salvage and de novo GDP synthesis. Consequently, GDP levels might be more.

---

2 V. Denis and B. Daignan-Fornier, unpublished results.
limiting in a \textit{guk1} mutant than in a \textit{hpt1} or \textit{fcy2} mutant strains. Consistently, a \textit{pur5} mutant, which is allelic to \textit{GUK1} (36), was previously shown to affect IMPDH activity (see the Discussion in Ref. 34).

MPA addition resulted in a 7-fold increase of \textit{IMD2} transcription, suggesting that blocking XMP synthesis severely depletes guanylic nucleotide supplies and consequently increases \textit{IMD} expression. Guanine addition overcomes this effect, since it allows guanine nucleotides supply through the salvage pathway. Accordingly, in the \textit{fcy2}, \textit{hpt1}, or \textit{guk1} mutant strains, guanine can no longer bypass MPA effect on the \textit{IMD} genes.

Therefore, a decrease in guanine nucleotide pools (by MPA or the \textit{guk1} mutation) causes an increase in the expression of \textit{IMD} genes, whereas an increase in these pools (by guanine addition) causes a decrease in \textit{IMD} genes expression.

A similar negative regulation by guanylic nucleotide levels was reported in mammalian cells. Glesne and coworkers (43) have shown that MPA induced a 4-fold increase in the IMPDH mRNA level, whereas guanosine addition decreased it to 20% of the control levels. Moreover, these authors suggested that changes in the levels of guanine ribonucleotide pools, and not deoxyguanine nucleotide pools, are the primary determinant regulating IMPDH gene expression (43). Therefore, regulation of guanylic nucleotide synthesis appears strongly conserved between yeast and mammals.

**The GRE cis-Negative Element of \textit{IMD2}**—Mapping of the regulatory cis-elements present in the promoter of the \textit{IMD2} gene allowed us to identify a GRE, localized 308 to 301 pb upstream of the ATG start codon. Single substitutions of each of the GRE nucleotides to a C prevented guanine regulation of the \textit{IMD2} gene and led to an increased transcription of \textit{IMD2}. Surprisingly, the GRE sequence, TATAAATA, is very similar to the TATA box consensus sequence. Moreover, the TATA-binding protein was shown to bind to the TATAAATA sequence in vitro, although with reduced affinity as compared with binding to the TATAATA consensus sequence (44). Nevertheless, since the GRE in \textit{IMD2} is a negative element, it is not expected to behave as a regular TATA box. Therefore, even if TATA-binding protein binds in vitro to the GRE sequence, it should have a different function than in the basal transcription initiation complex. Interestingly, the GRE sequence is also found in the \textit{IMD3} and \textit{IMD4} genes, at about 300 and 200 pb upstream from their respective ATGs, suggesting that \textit{IMD3} and \textit{IMD4} might also be regulated through a GRE cis-element. Mutations in the GRE sequence lead to an increased expression of \textit{IMD2} gene, suggesting that a repressor transcription factor could bind to the GRE box and repress \textit{IMD2} expression. Mutations in the \textit{TUP1} gene, encoding a corepressor involved in many different transcription processes (45–48), did not affect guanine regulation of the \textit{IMD} genes (data not shown). Also, electrophoretic mobility shift assays attempting to show binding of a trans-acting factor to a wild-type GRE, but not to a mutant GRE, were unsuccessful. This could be due to the low concentration of transcription factors in the crude extract used as a protein source.

In mammals, increased expression of IMPDH is observed in rapidly proliferating and deregulated cells, suggesting that in normal mammalian cells, IMPDH encoding genes are regulated by a repression mechanism. Moreover, it has recently been shown that in murine cells, p53 needs to repress IMPDH to exert its normal tumor suppression role (18). However, it is not yet known whether the p53 factor directly binds to human IMPDH-encoding genes. Even though \textit{S. cerevisiae} does not have any p53 homolog, the overall regulation mechanisms could be conserved between yeast and mammals. Identification of this factor is now necessary to unravel the mechanisms by which \textit{IMD2} transcription is controlled.

**Acknowledgments**—We are grateful to Drs. Fink and Watanabe for sending yeast strains. We thank Dr. Fernandes for suggestions on the manuscript.

**REFERENCES**

1. Daigman-Fournier, B., and Fink, G. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6746–6750
2. Denis, V., Boucherie, H., Monribot, C., and Daigman-Fournier, B. (1998) Mol. Microbiol. 30, 557–566
3. Zimmerman, A. G., Ou, J. J., Laliberte, J., and Mitchell, B. S. (1998) Prog. Nucleic Acids Res. Mol. Biol. 61, 181–209
4. Jackson, R. C., Weber, G., and Morris, H. P. (1975) Nature 256, 331–333
5. Allison, A. C., Hovi, T., Watts, E. W., and Webster, A. D. (1975) Lancet 2, 1179–1183
6. Nakamura, H., Natsumeda, Y., Nagai, M., Takahara, J., Irino, S., and Weber, G. (1992) Leuk. Res. 16, 561–564
7. Nagai, M., Natsumeda, Y., Konno, Y., Hoffmann, R., Irino, S., and Weber, G. (1991) Cancer Res. 51, 3886–3890
8. Nagai, M., Natsumeda, Y., and Weber, G. (1992) Cancer Res. 52, 258–261
9. Kono, Y., Natsumeda, Y., Nagai, M., Yamaji, Y., Ohno, S., Suzuki, K., and Weber, G. (1991) J. Biol. Chem. 266, 506–509
10. Collart, F. R., Chubb, C. B., Mirkin, B. L., and Huberman, E. (1992) Cancer Res. 52, 5826–5828
11. Allison, A. C., and Eguiri, E. M. (1994) Transplant. Proc. 26, 3205–3210
12. Tricot, G. J., Jayaram, H. N., Lapis, E., Natsumeda, Y., Nichols, C. R., Kreebshoe, F., Heerema, N., Weber, G., and Hoffmann, R. (1989) Cancer Res. 49, 3696–3701
13. Nelson, P. H., Eguiri, E., Wang, C. C., and Allison, A. C. (1990) J. Med. Chem. 33, 833–838
14. Sollinger, H. W. (1995) Kidney Int. 51, suppl. 14–17
15. Sollinger, H. W. (1995) Transplantation 60, 225–232
16. Shaw, L. M., Sollinger, H. W., Halloran, P., Morris, R. R., Yatscoff, R. W., Ransom, J., Tzina, I., Kocwin, P., Holt, D. W., Lieberman, B., Jakilitsch, A., and Potter, J. (1995) Ther. Drug Monit. 17, 690–699
17. Jain, A., Khanna, A., Molmenti, E. P., Rishi, N., and Fung, J. J. (1999) Surg. Clin. North Am. 79, 59–76
18. Liu, Y., Bahn, S. A., and Sherrly, J. L. (1998) Mol. Biol. Cell 9, 15–28
19. Dujardin, G., Kermorgant, M., Slonimski, P. P., and Boucherie, H. (1994) Gene (Amst.) 139, 127–132
20. Konrad, M. (1992) J. Biol. Chem. 267, 25652–25655

---

**Fig. 9. Model for \textit{IMD2} regulation by MPA and guanine.** The following abbreviations are used: \textit{ext}, extracellular medium; \textit{int}, intracellular compartment. Gene names are italicized and encode the following enzymatic activities: FCY2, purine cytosine permease (PCP); GUAI, guanosine-5’-monophosphate synthetase (GMPS); GUK1, guanosine-5’-monophosphate kinase (GMPK); HPT1, hypoxanthine-guanine phosphoribosyltransferase (HGPRT).
21. Fukuchi, T., Nikawa, J., Kimura, N., and Watanabe, K. (1993) *Gene* (Amst.) **129**, 141–146
22. Collart, F. R., and Huberman, E. (1990) *Blood* **75**, 570–576
23. Gilbert, H. J., Lowe, C. R., and Drabble, W. T. (1979) *Biochem. J.* **183**, 481–494
24. Kanzaki, N., and Miyagawa, K. (1990) *Nucleic Acids Res.* **18**, 6710
25. Natsumeda, Y., Ohno, S., Kawasaki, H., Konno, Y., Weber, G., and Suzuki, K. (1990) *J. Biol. Chem.* **265**, 5292–5295
26. Sifri, C. D., Wilson, K., Smolik, S., Forte, M., and Ullman, B. (1994) *Biochim. Biophys. Acta* **1217**, 103–106
27. Tiedeman, A. A., and Smith, J. M. (1991) *Gene* (Amst.) **97**, 289–293
28. Okada, M., Shimura, K., Shiraki, H., and Nakagawa, H. (1983) *J. Biochem. (Tokyo)* **94**, 1605–1613
29. Ikemami, T., Natsumeda, Y., and Weber, G. (1987) *Life Sci.* **40**, 2277–2282
30. Holmes, E. W., Pehlke, D. M., and Kelley, W. N. (1974) *Biochim. Biophys. Acta* **364**, 209–217
31. Myers, A. M., Tzagoloff, A., Kinney, D. M., and Lusty, C. J. (1986) *Gene* (Amst.) **40**, 299–310
32. Kippert, F. (1995) *FEBS Microbiol. Lett.* **128**, 201–206
33. Barton, A. B., Bussey, H., Storms, R. K., and Kaback, D. B. (1997) *Yeast* **13**, 1251–1263
34. Woods, R. A., Roberts, D. G., Friedman, T., Jolly, D., and Filpula, D. (1983) *Mol. Gen. Genet.* **191**, 407–412
35. Guetsova, M. L., Lecoq, K., and Daignan-Fornier, B. (1997) *Genetics* **147**, 383–397
36. Lecoq, K., Konrad, M., and Daignan-Fornier, B. (2000) *Genetics*, **156**, 953–961
37. Vitols, E., Bauer, V. A., and Stanbrough, E. C. (1970) *Biochem. Biophys. Res. Commun.* **41**, 71–77
38. Elford, H. L. (1968) *Biochem. Biophys. Res. Commun.* **33**, 129–135
39. Meng, L. M., and Nygaard, P. (1990) *Mol. Microbiol.* **4**, 2187–2192
40. Meng, L. M., Kilstrup, M., and Nygaard, P. (1990) *Eur. J. Biochem.* **187**, 373–379
41. Weng, M., Nagy, P. L., and Zalkin, H. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7455–7459
42. Ebbole, D. J., and Zalkin, H. (1987) *J. Biol. Chem.* **262**, 8274–8287
43. Glesne, D. A., Collart, F. R., and Huberman, E. (1991) *Mol. Cell. Biol.* **11**, 5417–5425
44. Wong, J. M., and Bateman, E. (1994) *Nucleic Acids Res.* **22**, 1896–1896
45. Keleher, C. A., Redd, M. J., Schultz, J., Carlsson, M., and Johnson, A. D. (1992) *Cell* **68**, 709–719
46. Mukai, Y., Harashima, S., and Oshima, Y. (1991) *Mol. Cell. Biol.* **11**, 3773–3779
47. Zhang, M., Rosenblum-Vos, L. S., Lowry, C. V., Boakye, K. A., and Zitomer, R. S. (1991) *Gene* (Amst.) **97**, 153–161
48. Williams, F. E., and Trumbly, R. J. (1990) *Mol. Cell. Biol.* **10**, 6500–6511
