# *Saccharomyces cerevisiae* Transcription Elongation Mutants Are Defective in *PUR5* Induction in Response to Nucleotide Depletion

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**IMP dehydrogenase (IMPDH) is the rate-limiting enzyme in the de novo synthesis of guanine nucleotides. It is a target of therapeutically useful drugs and is implicated in the regulation of cell growth rate. In the yeast *Saccharomyces cerevisiae*, mutations in components of the RNA polymerase II (Pol II) transcription elongation machinery confer increased sensitivity to a drug that inhibits IMPDH, 6-azauracil (6AU), by a mechanism that is poorly understood. This phenotype is thought to reflect the need for an optimally functioning transcription machinery under conditions of lowered intracellular GTP levels. Here we show that in response to the application of IMPDH inhibitors such as 6AU, wild-type yeast strains induce transcription of *PUR5*, one of four genes encoding IMPDH-related enzymes. Yeast elongation mutants sensitive to 6AU, such as those with a disrupted gene encoding elongation factor SII or those containing amino acid substitutions in Pol II subunits, are defective in *PUR5* induction. The inability to fully induce *PUR5* correlates with mutations that effect transcription elongation since 6AU-sensitive strains deleted for genes not related to transcription elongation are competent to induce *PUR5*. DNA encompassing the *PUR5* promoter and 5′ untranslated region supports 6AU induction of a luciferase reporter gene in wild-type cells. Thus, yeast sense and respond to nucleotide depletion via a mechanism of transcriptional induction that restores nucleotides to levels required for normal growth. An optimally functioning elongation machinery is critical for this response.**

Nucleotides are essential for DNA and RNA synthesis and therefore for cellular growth. Ribonucleotide pool size is carefully regulated in accordance with cellular growth rate in prokaryotic and eukaryotic cells (10, 13, 16, 18). IMP dehydrogenase (IMPDH) is a rate-limiting enzyme in the de novo synthesis of guanine nucleotides. Changes in IMPDH activity are found in some transformed cells and human tumors, and modulation of ribonucleotide levels can trigger p53-mediated cell cycle arrest (27, 29, 51). IMPDH is a medically important target of antimetabolite drugs aimed at reducing cellular proliferation and is the drug target for antimicrobial, immunosuppressive, and antitumor compounds. Such inhibitors include mycophenolate, 6-azauracil (6AU), tiazofurin, and ribavirin (15, 58). In cultured mammalian cells, IMPDH expression parallels guanine nucleotide levels (7, 22). Addition of guanosine to the growth medium results in a 5- to 10-fold reduction of IMPDH mRNA. In contrast, inhibitors of IMPDH that depress the intracellular guanine nucleotide pools induce a fourfold increase in mRNA levels in mammalian cells (17). Changes in IMPDH levels are regulated during growth and differentiation, and tumor cells can become resistant to the growth-inhibiting effect of IMPDH-directed drugs by mutating and/or overproducing the enzyme (43).

One process in which nucleoside triphosphate (NTP) levels play a critical role is transcription elongation by RNA polymerase II (Pol II). In vitro, elongation is strongly influenced by both NTP levels and regulatory elongation factors (48). When NTP substrate concentrations are low, Pol II's elongation rate is slowed and its propensity to become arrested increases. SII is an elongation factor that releases Pol II from the arrested state and enables it to resume elongation (53). Deletion of the gene encoding SII in *Saccharomyces cerevisiae*, *DST1* (also known as *PPR2*), results in increased sensitivity to the growth inhibitor 6AU (31). This drug reduces intracellular GTP and UTP levels by inhibiting the biosynthetic enzymes IMPDH and orotidylate decarboxylase, respectively (12). One interpretation of the drug-sensitive phenotype is that the elongation stress put upon Pol II by limited intracellular NTP levels causes frequent arrest of Pol II which can be overcome, at least to some extent, by SII (3, 12, 26). In the absence of SII, elongation becomes limiting for growth in the presence of the drug (3). This view is supported by findings that mutations in genes encoding Pol II subunits (*RP021* [also known as *RPB1*], *RPB2*, and *RPB6*) that also confer increased sensitivity to 6AU (6AUˢ) manifest a biochemical elongation defect (24, 37, 57). Mutations in genes encoding elongation factors or proteins implicated in elongation (*ELP1*, *ELP3*, *SPT4*, *SPT5*, *SPT6*, and *SPT16*) also confer increased 6AUˢ to yeast or otherwise modify 6AUˢ phenotypes (21, 33, 35, 55). There are, however, 6AUˢ mutations in genes that lack apparent roles in elongation, including *PPR1*, *SNO1*, and *SNZ1* (30, 36). *PPR1* encodes a DNA-binding transcriptional regulator of the pyrimidine biosynthetic pathway, whereas the latter two genes encode members of a family of stationary-phase proteins that may be involved in pyridoxine biosynthesis (11, 34, 36).

In previous work, we showed that combining a 6AUˢ allele of *rpb2* with a disruption of *DST1* resulted in synergistic 6AU sensitivity (26). This allele, *rpb2-10*, is a point mutation that reduces the average elongation rate of Pol II in vitro (37). The mutant polymerase is also prone to arrest in vitro at well-characterized arrest sites from which it can be rescued by SII (37). Hence, this strain has an elongation-compromised Pol II and lacks an elongation factor that assists arrested Pol II. Drug treatment resulted in a large reduction of total poly(A)⁺ RNA levels and specific transcripts (26). This suggested that SII is

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involved in transcription of many if not all genes in yeast and offered direct evidence that 6AU inhibits mRNA synthesis. This drug-induced shutoff of transcription elongation is similar in magnitude and kinetics to the loss of poly(A)$^+$ RNA seen in yeast defective in transcription initiation due to mutation of *RP021* and other genes encoding the general initiation machinery (8, 19, 39, 46, 47, 52). Interestingly, wild-type cells also showed a strong reduction in mRNA synthesis after 6AU treatment; however, this change was transient, implying that wild-type but not mutant cells can compensate for depressed intracellular NTP pools.

To understand the biological consequence of perturbing the elongation machinery, we have analyzed the impact of 6AU upon gene expression in wild-type and mutant yeast strains. Here we report the novel finding that 6AU treatment provokes a transcriptional induction of *PUR5*, a gene encoding an IMPDH homologue in *S. cerevisiae*. The time course of induction was consistent with an upregulation of IMPDH enzyme and activity and restoration of normal NTP levels, suggesting that yeast cells compensate for depressed intracellular NTP pools, at least in part, via the transcriptional induction of specific genes. Five strains carrying 6AU$^s$ mutations in elongation-related genes were defective in this response. 6AU$^s$ strains not known to be elongation defective retained the ability to induce *PUR5* transcription. *PUR5* sequences upstream of the open reading frame (ORF) conferred 6AU dependence upon a heterologous reporter gene. This suggests that yeast can respond to the intracellular loss of GTP by induction of a rate-limiting enzyme. *PUR5* transcription may be particularly sensitive to lowered GTP levels and an optimally functioning elongation machinery.

## MATERIALS AND METHODS

**Strains and plasmids.** Yeast strains used in these studies are listed in Table 1. Where indicated, 6AU (75 μg/ml) and mycophenolic acid (15 μg/ml) were included in the medium. Strains DY700 and DY706 were generated from Z96 (R. Young, Massachusetts Institute of Technology) and ABG-G11 (D. Kaback, UMDNJ-New Jersey Medical School), respectively, by transformation with pPur5P800luc. Strains DY731 and DY732 were generated from ABG-G11 and ABG-G12, respectively, by transformation with pRS316 (42). Strains DY741, DY742, DY743, DY746, DY760, and DY761 were generated from BY4741, BY4742, BY515, BY11569 (all from Research Genetics, Huntsville, Ala.), FY120 (21), and FY1638 (21), respectively, by transformation with pRS316. DY2050 was generated from DY173 by transformation with pC1016. DY173 was generated from DY100 by a two-step allele replacement of *RP021* with *rpo21-18* after subcloning the mutant allele from the plasmid pYF1504 (J. Friesen, University of Toronto) into pRS306 (42). DY100 was generated from Z96 by disrupting *DST1* using the *hisG* recombination cassette as described elsewhere (1, 26). DY190 was generated from DY100 by transformation with pC1016.

pC1016 was constructed by inserting a PCR product representing the *DST1* locus prepared with the primers 5′-GGCACTGGACTCTAAATCTC-3′ and 5′-AAAGGATTTTACGTGAGACAGAC-3′ into the *Sma*I site of pRS316. The reporter plasmid pPur5P800luc was derived from pGAL-Luc (6) by excision of the *GAL* promoter with *Bam*HI and *Hin*dIII digestion and insertion of a *Bam*HI and *Hin*dIII-digested PCR product generated using the primers 5′-CTGATCAGG ATCAGGATCCGGCCATTGCTTTTGCTACTT-3′ and 5′-GGGGTACCAA GCTTGTTAACAACAAACACAGTCCA-3′, which amplify the upstream region of *PUR5* from genomic DNA.

**RNA analysis.** Total RNA was isolated from thawed cell pellets by the hot phenol extraction method and quantitated by measuring absorbance at 260 nm (4). For Northern analysis, 15 μg of total RNA was resolved on a 1% formaldehyde-agarose gel and blotted onto Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, Calif.). Filters were rinsed with 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), baked at 75 to 80°C for 1 h, and cross-linked in a Stratalinker 1800 (Stratagene, La Jolla, Calif.) for 1 min. Filters were prehybridized for a minimum of 3 h at 42°C in 5× SSC–5× Denhardt's solution (4)–50% (vol/vol) formamide–1% (wt/vol) sodium dodecyl sulfate (SDS)–100 μg of salmon sperm DNA per ml and hybridized under the same conditions with ≈10$^8$ cpm of $^{32}$P-labeled DNA probe for 15 to 18 h. Filters were washed at 22°C twice in 0.2× SSC–0.1% SDS for 5 min each time and twice in 0.2× SSC–0.1% SDS for 5 min each time, followed by two 0.2× SSC–0.1% SDS washes at 42°C for 15 min each, exposed to Kodak X-Omat film, and quantitated with a Fuji BAS1000 imaging system. DNA probes were prepared by PCR using a wild-type yeast

genomic DNA template (S288C; Research Genetics) and the corresponding ORF primer pairs obtained from Research Genetics except for primers complementary to *YHR216W*, which were 5′-GTGGTATGTTGGCCGGTACTACC G-3′ and 5′-TCAGTTATGTAAACGCTTTTCGTA-3′. Probes were labeled to a specific activity of ≈10$^7$ to 10$^8$ cpm/μg with Klenow DNA polymerase (Promega Life Sciences, Madison, Wis.), random hexamer primers (Gibco BRL, Rockville, Md.), and [α-$^{32}$P]dATP (Amersham Pharmacia Biotech, Piscataway, N.J.).

**Luciferase assay.** Cells from a saturated culture were diluted to an optical density at 600 nm (OD$_{600}$) of 0.05 to 0.1 and grown to an OD$_{600}$ of 0.5 at 30°C with aeration. The culture was treated with 6AU, and triplicate samples of 0.5 OD unit of cells were collected at the indicated times and frozen. Cell pellets were stored at −80°C, thawed at 4°C for assay, and lysed by vortexing (six times, 20 s each) with 40 to 50 μl of acid-washed glass beads in 55 μl of luciferase lysis buffer (Promega luciferase assay system; product no. E1501). Samples were spun for 15 s at 15,000 × $g$ in a microcentrifuge. Forty microliters of the soluble cell extract was moved to 100 μl of assay substrate at 22°C, and luminescence was scored in a Optocomp I Luminometer (GEM Biomedical, Pineville, N.C.).

## RESULTS

**6AU induces IMPDH expression.** We have previously shown that both wild-type and 6AU$^s$ yeast strains show a reduction in mRNA levels when challenged with 6AU (26). For wild-type cells, this was a transient reduction with a maximal depression at 30 min. Cells were able to regenerate normal levels of
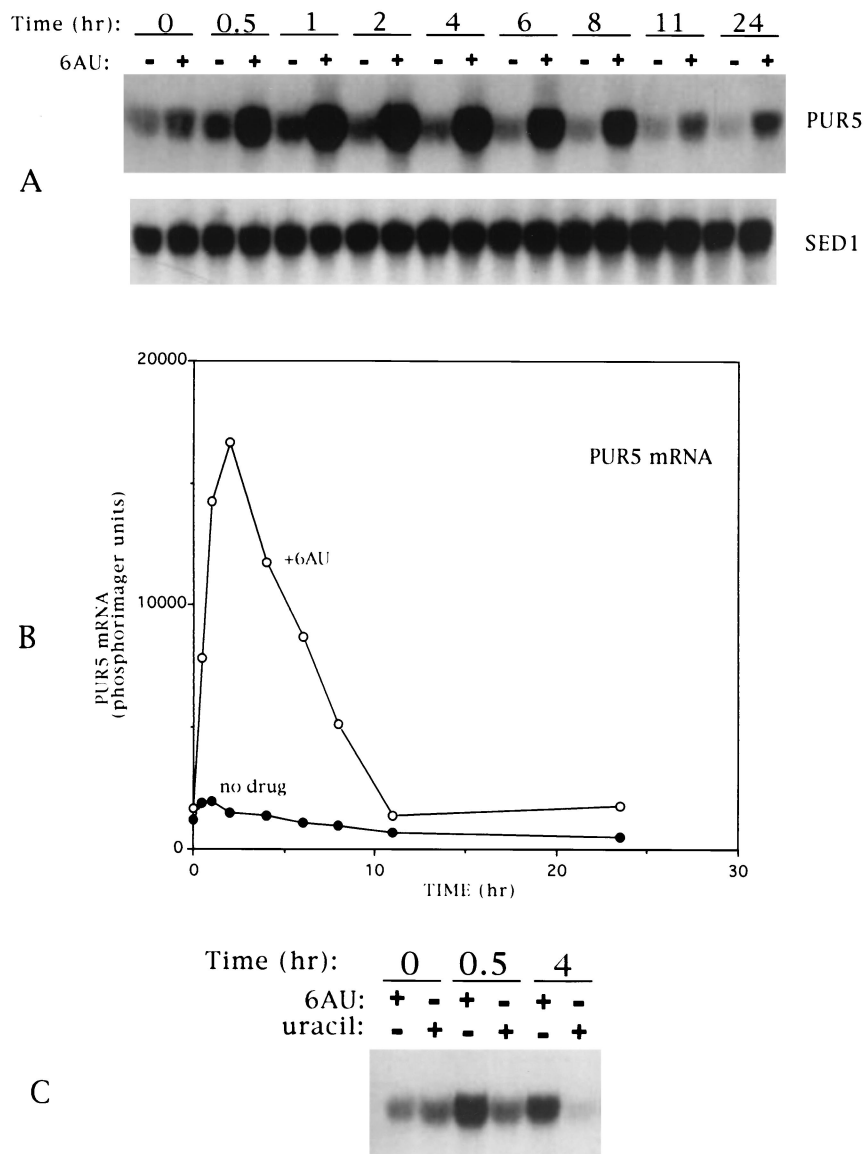
### TABLE 1. Yeast strains used in this study

| Strain | Genotype |
| --- | --- |
| ABGG10$^a$ | *MATa ade1Δ2 LEU2Δ4 HIS3 his5 leu2* |
| DY103$^b$ | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP214 (*LEU2 RPB2*)][pRS316 (*URA3*)] |
| DY105$^b$ | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP2-10L (*LEU2 rpb2-10*)][pRS316 (*URA3*)] |
| DY106$^b$ | *MATα ura3-52 leu2-3, 112 his3Δ200 rpb2Δ297::HIS3 dst1::hisG* [pRP214 (*RPB2 LEU2*)][pRS316 (*URA3*)] |
| DY108$^b$ | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3 dst1::hisG* [pRP2-10L (*LEU2 rpb2-10*)][pRS316 (*URA3*)] |
| DY190 | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3 dst1::hisG* [pRP214 (*RPB2 LEU2*)][pC1016 (*DST1 URA3*)] |
| DY700 | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3* [pRP214 (*LEU2 RPB2*)][pPur5P800luc (*URA3*)] |
| DY706 | *MATα Δ4HIS3 trp1 ura3-1 his3-11,15 leu2-3,112* [pPur5P800luc (*URA3*)] |
| DY731 | *MATα Δ4HIS3 trp1 ura3-1 his3-11,15 leu2-3,112* [pRS316 (*URA3*)] |
| DY732 | *MATα Δ3LEU2 ade1 ura3-1 his3-11,15 leu2-3,112* [pRS316 (*URA3*)] |
| DY741 | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* [pRS316 (URA3)] |
| DY742 | *MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0* [pRS316 (*URA3*)] |
| DY743 | *MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Δyml056c::kanMX4* [pRS316 (*URA3*)] |
| DY746 | *MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 Δppr1::kanMX4* [pRS316 (*URA3*)] |
| DY760 | *MATa his4-912δ lys2-128δ leu2 Δ1 ura3-52* [pRS316 (*URA3*)] |
| DY761 | *MATa his4-912δ lys2-128δ leu2Δ1 ura3-52 rpb1-221* [pRS316 (*URA3*)] |
| DY2050 | *MATα ura3-52 leu2-3,112 his3Δ200 rpb2Δ297::HIS3 rpo21-18 dstl::hisG* [pRP214 (*RPB2 LEU2*)] [pC1016 (*URA DST1*)] |
| MW926$^{c,d}$ | *MATα snz1Δ2 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 can1-100* |
| MW980$^{c,d}$ | *MATa snz1Δ3 sno1Δ3 snz2Δ3 sno2Δ3 snz3Δ3 sno3Δ3 leu2-3,112 trp1-1 ura3-1 ade2-1 his3-11,15 can1-100* |
| MW1072$^c$ | *MATa ade2-1 his3-11 trp1-1* |
| Z196$^e$ | *MATa ura3-52 leu2-3,112 his3Δ200 his4-912 lys2-128 rpb1Δ187::HIS3* [pRP112(*CEN URA3 RPB1*)] |
| Z460$^e$ | *MATa ura3-52 leu2-3,112 his3Δ200 his4-912 lys2-128 rpb1Δ187::HIS3* [pRP1-1L(*CEN URA3 rpb1-1*)] |

$^a$ Obtained from A. Barton and D. Kaback (UMDNJ-New Jersey Medical School).
$^b$ Reference 26.
$^c$ Reference 36.
$^d$ URA$^+$ due to insertion of *URA3* at the *SNZ/SNO* loci (36).
$^e$ Obtained from N. Hannett and R. Young (Massachusetts Institute of Technology).

FIG. 1. (A) Time course of IMPDH mRNA induction by 6AU. Yeast strain DY103 was diluted to an $OD_{600}$ of 0.1 in SC-Ura and grown to an $OD_{600}$ of ≈0.5 at 30°C. The culture was split, and 6AU (75 μg/ml) was added to half; at the indicated times, RNA was harvested and subjected to Northern blotting. (B) Quantitation of Northern blot in panel A. (C) Underivatized uracil does not induces *PUR5* mRNA synthesis. Cells were treated with 6AU or uracil (each at 75 μg/ml); RNA was harvested and subjected to Northern blotting.

poly(A)⁺ RNA by 4 h posttreatment (26). To test the idea that recovery is due to upregulation of the amount of the drug's target, IMPDH, we compared the levels of *PUR5* mRNA in 6AU-treated and untreated wild-type yeast cells. Equivalent total RNA was extracted from an equal amount of cells and subjected to Northern blotting. An ≈10-fold induction of *PUR5* mRNA was observed 2 h after a 6AU challenge (Fig. 1A and B). This level declined to base line by 10 h posttreatment. As a control for RNA loading, we probed for *SED1* mRNA. *SED1* encodes an abundant cell wall protein whose expression continues throughout the growth of a culture and into stationary phase (41). Its level is relatively resistant to change during the otherwise general 6AU-induced transcriptional shutoff seen in elongation mutants (26). As expected, *SED1* mRNA was detected in all samples, at levels that remained relatively unchanged by 6AU (Fig. 1A). Hence, *PUR5* transcript levels dramatically increase in the presence of 6AU during a period

in which levels of GTP and total mRNA synthesis are low (Fig. 1 and reference 26). The response was dependent on the inhibitory activity of 6AU and not simply high extracellular pyrimidine levels, since the addition of uracil did not result in induction (Fig. 1C). No 6AU induction was observed for *DST1* mRNA, which encodes SII, or for *GUA1*, which encodes GMP synthase, the next enzyme in the GTP biosynthetic pathway (data not shown).

**IMPDH induction arises from one of four related genes.** An inspection of the yeast genome revealed that *S. cerevisiae* has four ORFs that encode a set of homologous IMPDH-like proteins. Two of these genes, located on chromosomes I (*YAR073W*) and VIII (*PUR5*; *YHR216W*) are related (96% amino acid identity) by an apparent ancient chromosomal duplication (5, 56). The copy on chromosome I is near the telomere and thought to be transcriptionally silent. The copy on chromosome VIII (*PUR5*), on the other hand, has previously
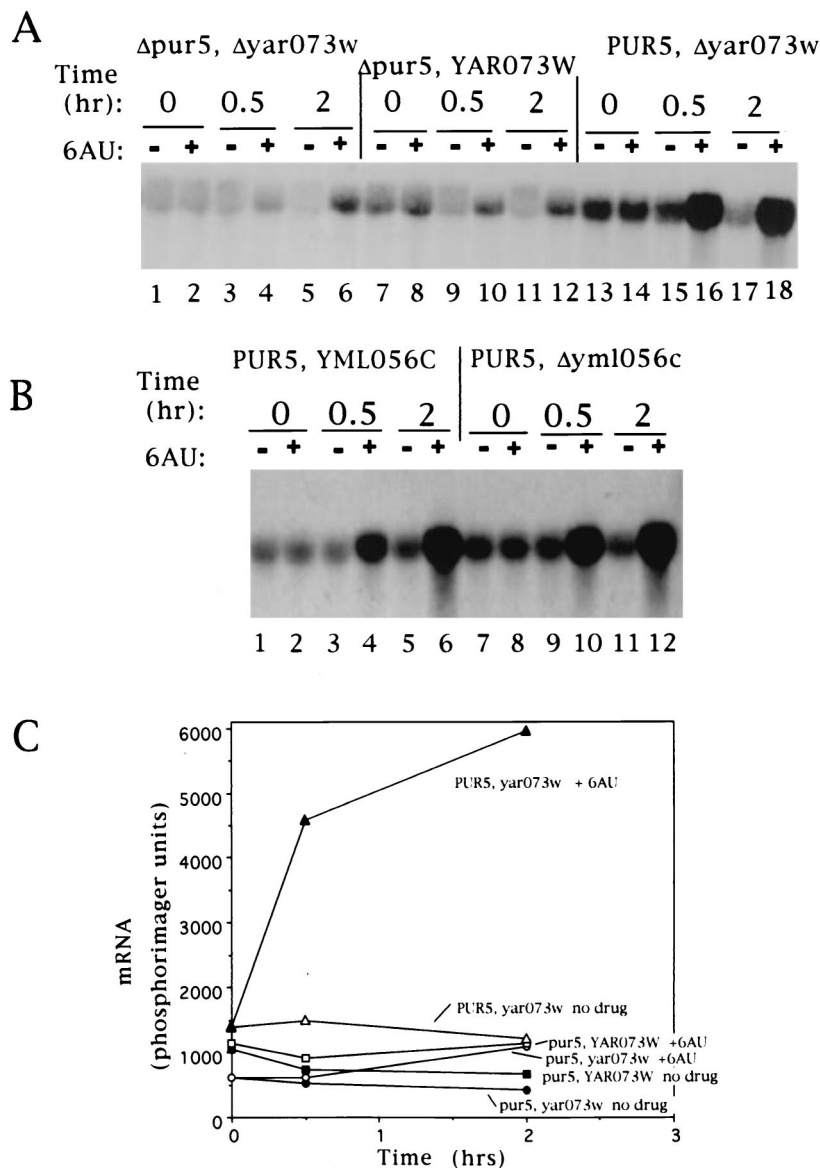
FIG. 2. IMPDH mRNA induction is derived largely from the *PUR5* gene. Strains lacking *YAR073W* and *PUR5* (ABGG10; A, lanes 1 to 6), *PUR5* (DY731; A, lanes 7 to 12), *YAR073W* (DY732; A, lanes 13 to 18), or *YML056C* (DY743; B, lanes 7 to 12) were treated with 6AU (75 μg/ml) for the indicated times; RNA was isolated and subjected to Northern blotting. A control strain (DY741; B, lanes 1 to 6) otherwise isogenic to DY743 was also analyzed. Filters were quantitated by phosphorimaging and plotted (C).

been shown to be transcriptionally active (5). Another pair, on chromosomes XII (*YLR432W*) and XIII (*YML056C*) (89% amino acid identity) are also thought to be related by duplication, but their transcriptional activity has not been examined directly. None of the four genes are essential (5, 54). To learn which of the genes contributed to the observed induction, we examined mutant strains with deletions in these genes. The probe used cross-hybridizes to mRNA from all four genes. Strains with a deletion of a region of chromosome VIII including *PUR5* destroyed most of the response (Fig. 2A, lanes 7 to 12). Induction was readily observed in cells deleted for a region of chromosome I containing *YAR073W* (Fig. 2A, lanes 13 to 18) or in a strain with a deletion of *YML065C* on chromosome XIII (Fig. 2B, compare lanes 1 to 6 with lanes 7 to 12). Trace amounts of mRNA remained detectable in a double deletant lacking *PUR5* and *YAR073W* (Fig. 2A, lanes 1 to 6), suggesting

that the chromosome XII and/or XIII homologues contribute to a small extent to the steady-state levels of mRNA. A slight induction of this residual expression is also apparent after 2 h of 6AU treatment in the double deletant (Fig. 2A, lanes 5 and 6). Since the majority of the IMPDH mRNA induced by 6AU was derived from transcription of *PUR5*, we focused on the regulation of this member of the IMPDH family.

Growth of yeast strains deleted for *DST1* is also sensitive to mycophenolic acid, a well-characterized IMPDH inhibitor that is structurally distinct from 6AU (12). We examined whether *PUR5* transcription in wild-type cells could be activated by exposure to this drug. A strong induction was observed with mycophenolic acid (Fig. 3, wt). Yeast can import free guanine from the medium and convert it to GTP via salvage synthesis. Consequently, addition of guanine to the growth medium obviates the need for IMPDH activity and ameliorates the 6AU[s]
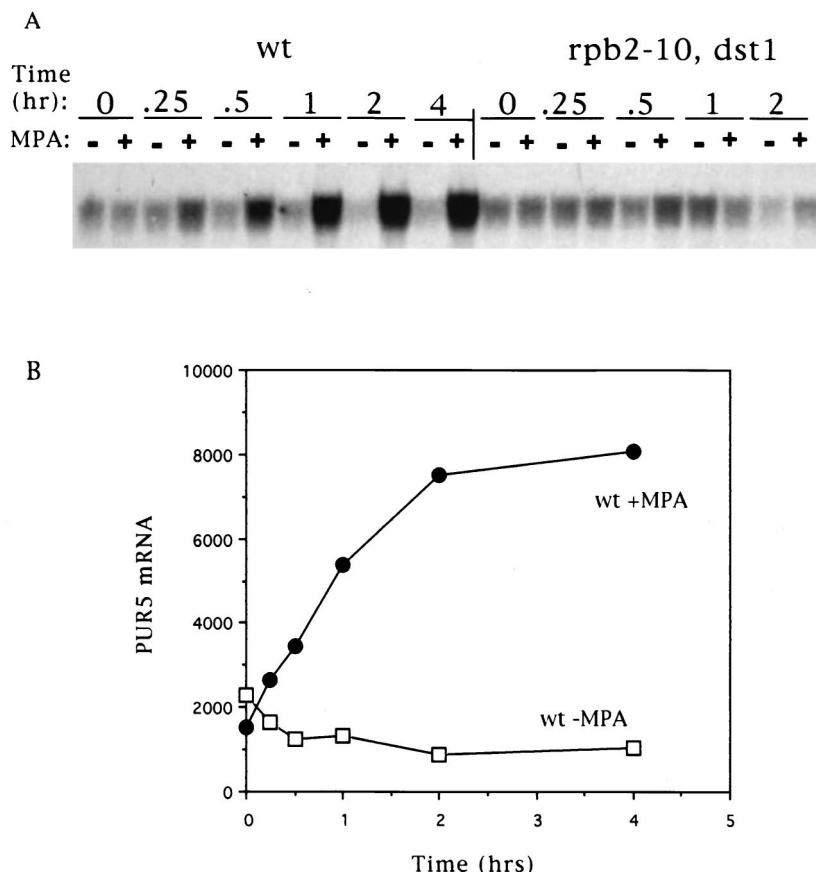
FIG. 3. Induction of *PUR5* transcription by mycophenolic acid. Yeast strains DY103 (wt [wild type]) and DY108 (*rpb2-10*, *dst1*) were treated with mycophenolic acid (MPA; 15 μg/ml); RNA was isolated and subjected to Northern blotting. Results were quantitated by phosphorimaging and plotted (B).

phenotype of *DST1* mutants (3, 12). When cells were pretreated with guanine for 30 min before the addition of 6AU, induction was no longer observed (Fig. 4). Taken together, these data strongly suggest that yeast can sense intracellular GTP depletion and compensate by inducing transcription of *PUR5*.

**6AU$^s$ mutants in the elongation machinery are defective in *PUR5* induction.** The foregoing results suggest that the previously observed loss of poly(A)$^+$ mRNA seen in a yeast strain bearing two 6AU$^s$ mutations, the *rpb2-10* point mutation and a disruption of *DST1*, could be due to the strain's inability to transcribe *PUR5* and therefore to regenerate nucleotide levels. Indeed, strains with either the *rpb2-10* mutation or a disruption of *DST1* were compromised in the ability to induce *PUR5*, although a limited induction capacity remained (Fig. 5). This residual activity was obliterated when both mutations were introduced into a single strain (Fig. 5), consistent with prior genetic data demonstrating supersensitivity to 6AU of the double mutant relative to the single mutants (26). Induction of *PUR5* by mycophenolic acid was also impaired in both the *rpb2-10* and the *dst1* single mutants (data not shown), as well as in the *rpb2-10 dst1* double mutant, which was severely compromised in its response to mycophenolic acid (Fig. 3, *rpb2-10*, *dst1*).

To examine further the correlation between 6AU$^s$ mutations in the elongation machinery and the inability to induce transcription of *PUR5*, we studied the response in two additional strains with 6AU$^s$ mutations in *RP021*. Both exhibited a reduced ability to induce *PUR5* transcription in response to a 6AU challenge (Fig. 6). These two mutations, *rpo21-18* and

*rpb1-221*, are known to be involved in transcription elongation (3, 21, 57). The *rpo21-18* mutation is a linker insertion that results in the addition of five amino acids to the largest subunit of Pol II (3). The resulting enzyme binds SII poorly and confers the 6AU$^s$ phenotype (57). The *rpb1-221* mutation was identified as 6AU$^s$ allele of *RP021* that suppresses a cold-sensitive mutation in elongation factor gene *SPT5* (21). It is synthetically temperature sensitive when combined with a deletion of *DST1*. Induction of *PUR5* was hindered (although still observable) when cells were grown at 30°C (Fig. 6). These data extend the correlation between 6AU$^s$ mutations in the elongation machinery and the inability to induce *PUR5* transcription.

We next wished to determine whether all 6AU$^s$ mutants are defective in the *PUR5* induction response. To address this question, we analyzed 6AU$^s$ strains lacking genes not known to be involved in transcription elongation, *PPR1* and *SNO1* (30, 36). A strain lacking *PPR1* was obtained from the *Saccharomyces* Genome Deletion Project, and its 6AU$^s$ phenotype was confirmed (data not shown). *PPR1* is a DNA-binding transcription factor that activates transcription from genes encoding pyrimidine biosynthetic enzymes such as *URA3*, which encodes orotidylate decarboxylase (12, 38). Loss of *PPR1* function results in the underproduction of orotidylate decarboxylase (2), the need for which becomes more acute in the presence of 6AU (12, 30). *PPR1* deletion strains grow poorly on uracil dropout medium in the absence of drug, consistent with the idea that cells lacking Ppr1p have a limited ability to *trans* activate the pyrimidine biosynthetic genes (2; data not shown). Like the wild-type strain, the *PPR1* mutant was able to induce
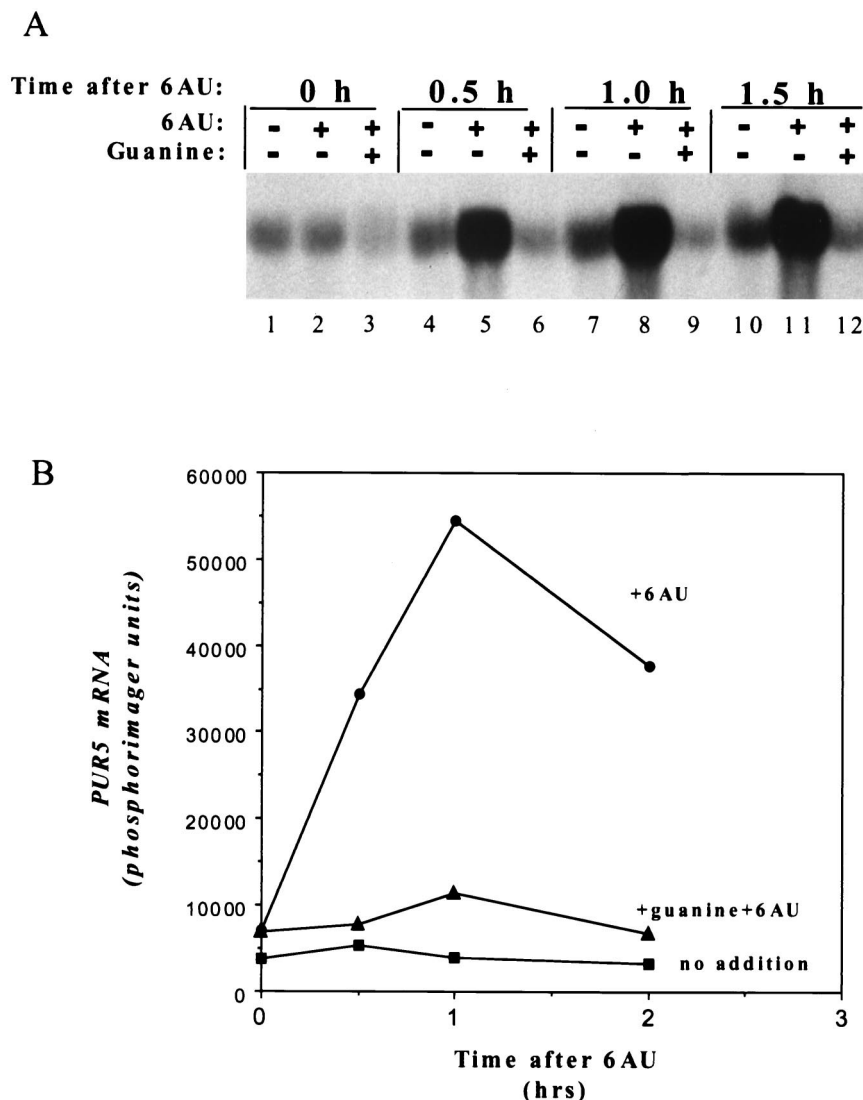
A



B



FIG. 4. Guanine suppresses *PUR5* induction by 6AU. Cultures of DY103 were grown for 30 min in medium containing (lanes 3, 6, 9, and 12) or lacking (lanes 1, 2, 4, 5, 7, 8, 10, and 11) 1 mM guanine; 6AU (75 μg/ml) was added; samples were withdrawn at the indicated times and analyzed by Northern blotting. A control culture lacking guanine and 6AU was also tested (lanes 1, 4, 7, and 10). Filters were phosphorimaged; the data are plotted in panel B.

*PUR5* transcription when challenged with 6AU (Fig. 7). Similarly, deletion of *SNZ1* or all six members of the *SNZ1*-related genes (*SNZ1,2,3* and *SNO1,2,3*) does not affect the ability of 6AU to induce *PUR5* transcription (Fig. 7). The *SNO/SNZ* family of genes are coregulated in response to nutrient limitation and encode proteins with homology to glutamine amidotransferases and enzymes involved in vitamin B$_6$ synthesis (11, 34, 36). Hence, not all 6AU$^s$ mutants are defective in *PUR5* induction, indicating that drug sensitivity does not lead to failure to induce *PUR5* but could be a consequence of a failure of its induction.

As an additional control, we examined the induction of *PUR5* transcription in a strain bearing the well-characterized *rpb1-1* mutation, which is not known to be 6AU$^s$ (32). This strain has a near-wild-type growth rate at 24°C. Although it possesses a reduced constitutive level of *PUR5* mRNA, it is able to induce transcription of *PUR5* following 6AU treatment (Fig. 8). Hence, not all Pol II mutations are equivalent in their impact on *PUR5* induction.

**6AU inducibility is transferable.** To test whether *PUR5* sequences could confer the inductive response to a heterologous gene, we inserted 800 bp of *PUR5* sequence into a selectable episomal plasmid upstream of the firefly luciferase coding sequence (6, 42). This DNA spans almost the entire region between the *PUR5* ORF and the next upstream gene, *PHO12*. Although the transcription start site has not been mapped for *PUR5*, this region must include both the *PUR5* promoter and the 5' untranslated region. The plasmid was used to transform a strain which is wild type at *DST1*, *RPB2*, and *RP021*. Cells were challenged with 6AU, and induction was scored by assaying yeast extracts for luciferase activity. Luciferase activity was strongly induced by 6AU with kinetics comparable to that seen for *PUR5* mRNA by Northern blotting (compare Fig. 9 and 1B). There was no response to 6AU when this strain harbored the same reporter construct lacking *PUR5* sequences (data not shown).

The transient nature of the induction seen for endogenous *PUR5* mRNA (Fig. 1) and reporter activity (Fig. 9) could result
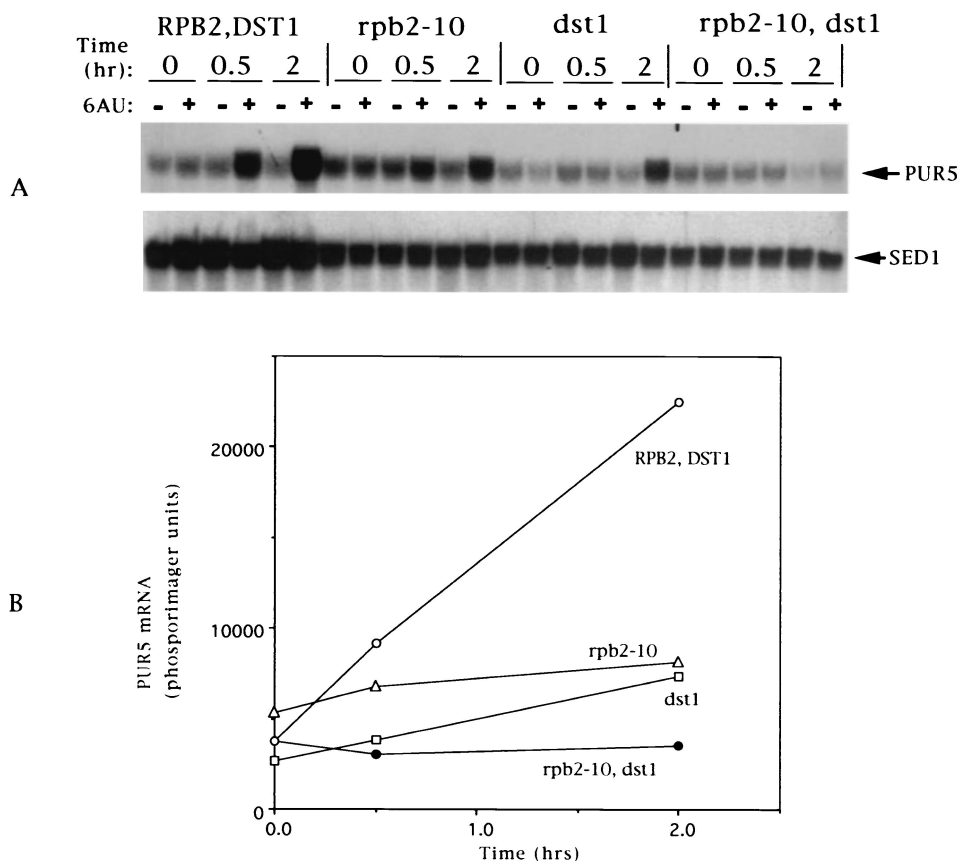
FIG. 5. 6AU$^s$ elongation mutants fail to induce *PUR5* transcription. Strains DY103 (RPB2,DST1), DY105 (*rpb2-10*), DY106 (*dst1*), and DY108 (*rpb2-10, dst1*) were treated with 6AU (75 μg/ml) for the indicated times. RNA was prepared, subjected to Northern blotting (A), and quantitated by phosphorimaging (B). Only the data for drug-treated cells are plotted presented in panel B.

in part from successful negative feedback by GTP after the burst of *PUR5* transcriptional activity that yields Pur5p and IMPDH activity. To test this idea, we placed the reporter plasmid in a strain lacking *PUR5* (Fig. 2A). If feedback from IMPDH activity is involved in shutoff of the reporter, there should be a reduction in turnoff of the luciferase reporter. The luciferase induction kinetics in the *PUR5* deletant were identical to that seen in cells with an intact *PUR5* gene (Fig. 9). However, the strain lacking *PUR5* showed a significant slowing in the time needed for luciferase activity to return to baseline, although the rate of its loss was comparable between strains (Fig. 9). The fact that reporter transcription was not indefinitely sustained in the *PUR5* deletant might be explained by other additional mechanisms that repress the response (see Discussion). This strain may also have residual inductive capacity in the other three IMPDH homologues (Fig. 2A), which could provide a sufficient accumulation of IMPDH activity to regenerate GTP and repress reporter activity. This reporter will be useful in dissecting the sequence requirement of the 6AU response.

## DISCUSSION

We have documented a transcriptional induction system in yeast in which depletion of intracellular nucleotide pools prompts the increased expression of a rate-limiting enzyme. The temporal pattern of expression suggests that a burst of de novo IMPDH synthesis and activity is responsible for the recovery of mRNA levels observed for wild-type cells (26). Re-

markably, yeast mutants altered in the transcription elongation machinery, but not 6AU$^s$ mutations in genes not known to be involved in transcription elongation, failed to activate *PUR5*. This could explain the inability of a previously characterized elongation-defective strain to carry out mRNA synthesis (26). *PUR5* would appear to be unusual in that it is highly transcribed when the intracellular GTP level is lowered to ≈10% of normal, a condition in which cellular mRNA synthesis is depressed (12, 26). Restricted substrate availability has profound effects on elongation in vitro and in vivo (48, 49). This appears to be exacerbated for mutant Pol II such as that bearing the *rpb2-10* substitution (37). The ability of *PPR1* and *SNO/SNZ* deletants to induce *PUR5* is consistent with the idea that the elongation defect is causally involved in the inability to activate this gene during GTP depletion. It also suggests that defective induction of *PUR5* is a primary problem in some elongation mutants and that this is one mechanism that leads to the 6AU$^s$ phenotype. Further analysis of mutant strains will be required to learn how strong this correlation is. The 6AU$^s$ phenotype of *PPR1* mutants is likely due to defects in induction of the enzymes in pyrimidine nucleotide biosynthesis (12). Although the *SNO/SNZ*-encoded proteins resemble enzymes involved in nucleotide and pyridoxine metabolism (11, 34, 36), the molecular basis for the 6AU$^s$ phenotype in the *SNO/SNZ* mutants is less obvious.

Our findings regarding *PUR5* induction are similar to previous results obtained in mammalian cells in which IMPDH mRNA levels increase after treatment with mycophenolic acid
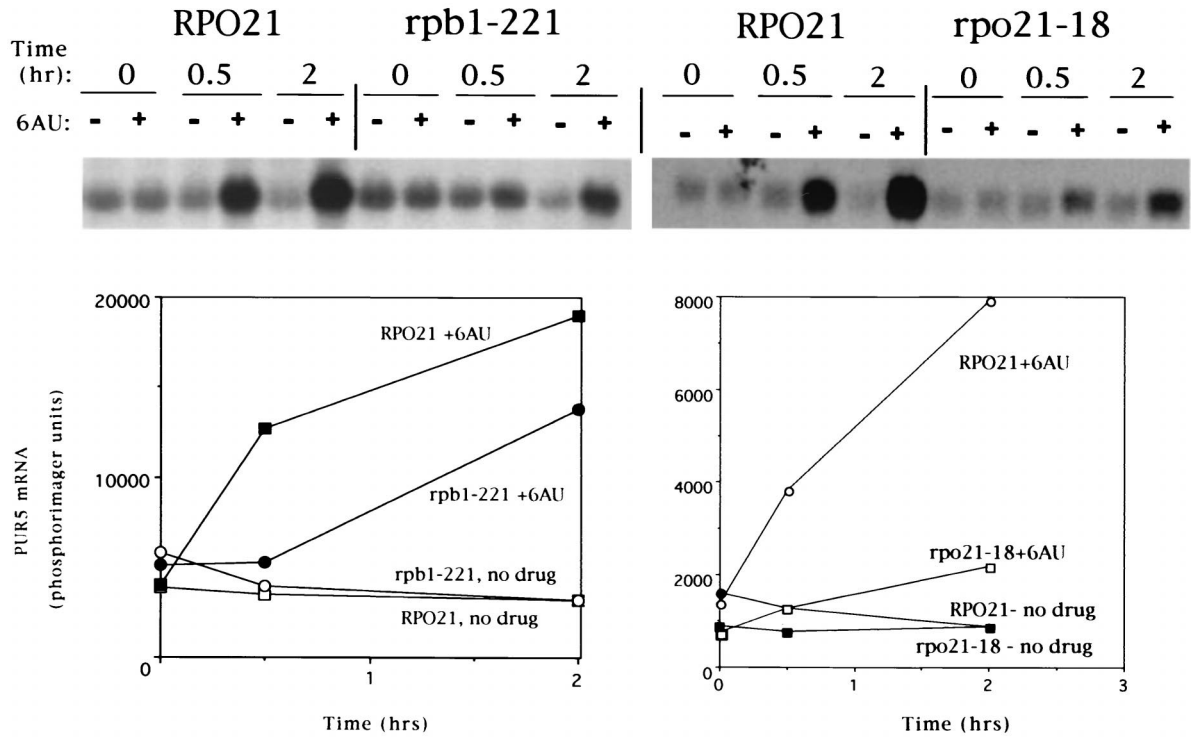
FIG. 6. *PUR5* induction in 6AU[s] strains mutated in Pol II. Strains DY760 (*RPO21*), DY761 (*rpb1-221*), DY190 (*RPO21*), and DY2050 (*rpo21-18*) were grown in the presence or absence of 6AU (75 µg/ml), and RNA was prepared for Northern blotting at the indicated times. Results were quantitated by phosphorimaging and plotted.
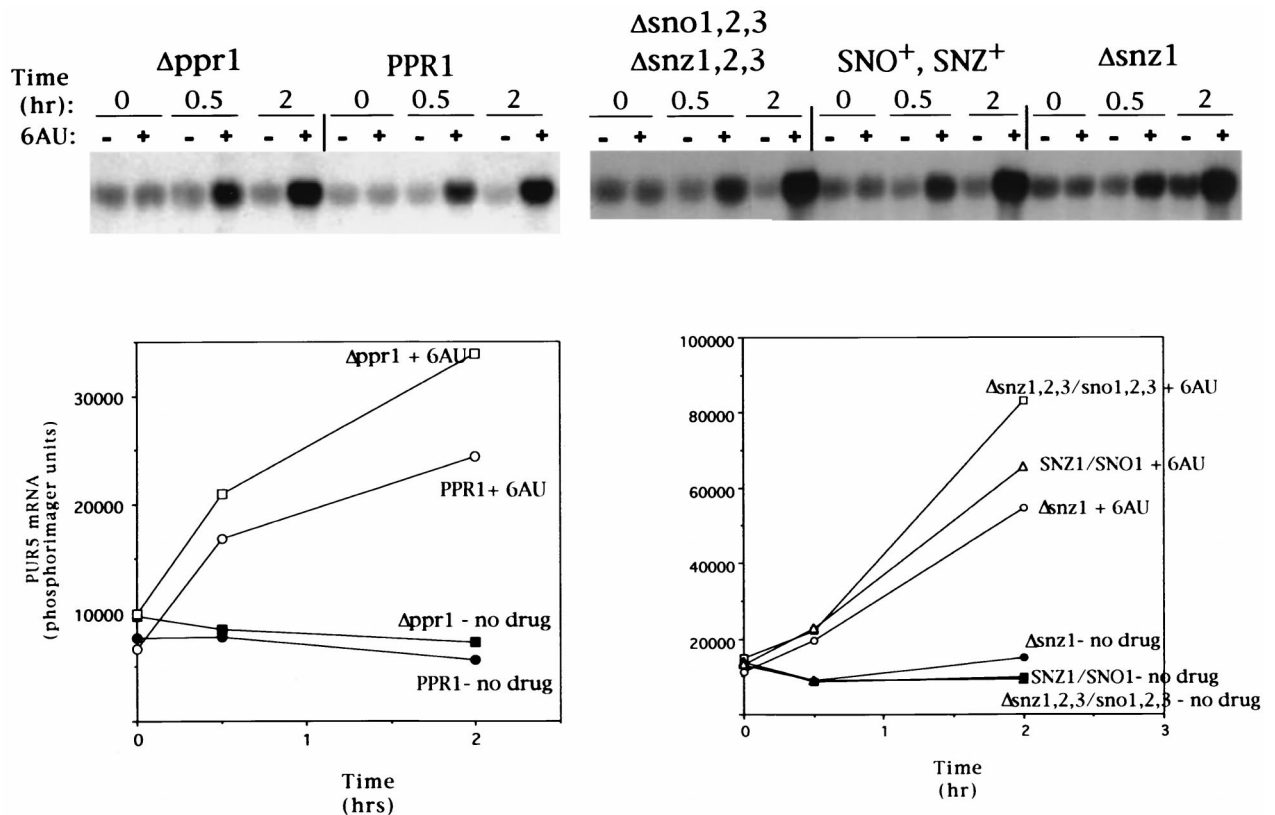


FIG. 7. 6AU[s] mutants in *PPR1* and *SNZ/SNO* can induce *PUR5*. The experiment described for Fig. 6 was repeated with strains DY746 (Δ*ppr1*), DY742 (*PPR1*), MW980 (Δ*sno1,2,3*, Δ*snz1,2,3*), MW1072 (SNO[+], SNZ[+]), and MW926 (Δ*snz1*).
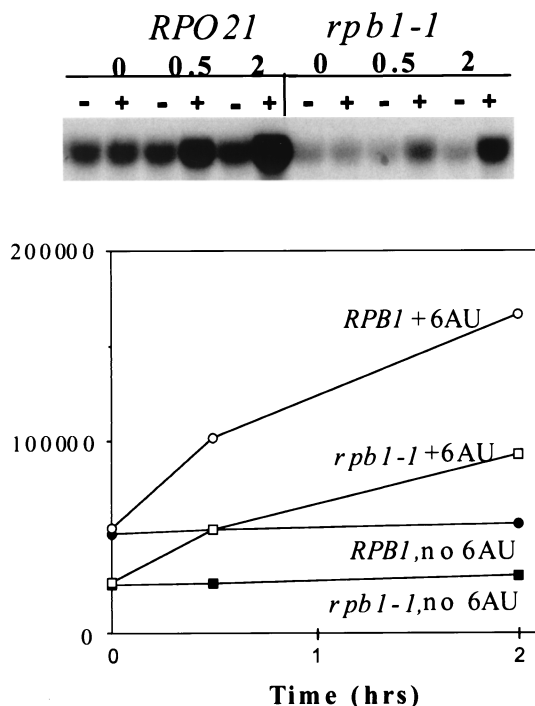
FIG. 8. *PUR5* induction in the *rpb1-1* strain. Strains Z196 (*RPO21*) and Z460 (*rpb1-1*) were grown in the presence or absence of 6AU (75 µg/ml), and RNA was prepared for Northern blotting at the indicated times. Results were quantitated by phosphorimaging and plotted.

and fall when guanine is added to the medium (7, 17). mRNA accumulation in that case was through a posttranscriptional mechanism (17). In humans, two IMPDH genes have been identified (reviewed in reference 58). Stimulation of T cells requires de novo nucleotide synthesis and is accompanied by increased levels of both type I and type II mRNAs through a transcriptional induction (9, 59, 60). The mechanism involved is not known.

Transcriptional regulation of genes encoding enzymes of pyrimidine biosynthesis has been described in yeast (38). Induction involves direct activation of *PPR1* by pyrimidine metabolic intermediates (14). Given this precedent, we must consider that the IMPDH induction that we observe may operate via a purine metabolite sensing mechanism that culminates with the activation of a *PUR5* promoter-binding factor. Neither the *cis*-acting sequences nor transcriptional start sites have been characterized for any of the yeast IMPDH-like genes. The reporter plasmid analysis will serve as a first step in learning if induction operates through upstream promoter sequences, the identity of the regulatory factor(s) that may be involved, and why the other yeast IMPDH homologues are not responsive to the same extent as *PUR5*.

Sensitivity of yeast growth to 6AU has become widely used as a marker for defects in transcription elongation. This is due in part to the spectrum of genes in which most mutations are found, i.e., elements of the transcription elongation machinery. Mutations in *DST1* and *PPR1* were identified through a 6AU<sup>s</sup> screen (23, 30). 6AU<sup>s</sup> alleles of *RP021* and *RPB2* were identified following mutant hunts for conditional mutations and later linked to elongation through in vitro biochemistry (3, 26, 37, 57). Some 6AU<sup>s</sup> mutations, such as *rpb1-221*, were identified through genetic interactions, and they, or other alleles in those genes, were retrospectively shown to display the 6AU<sup>s</sup> phenotype (21). Still other genes, such as human *SPT4*, *SPT5*, and

*SPT16* and yeast *ELP1* and *ELP3*, were shown to encode subunits of elongation factors for which mutant alleles were identified that were 6AU<sup>s</sup> or modified other 6AU<sup>s</sup> mutations (21, 33, 35, 50, 55). A comprehensive search for 6AU<sup>s</sup> mutants has not been completed, and it is possible that only a subset of genes that confer 6AU sensitivity has been identified. The existing set may be biased toward components of the elongation machinery, since those mutants are more likely to be tested for this phenotype. Some reports suggest that 6AU may inhibit enzymes involved in amino acid catabolism such as aminoisobutyrate-pyruvate aminotransferase, albeit at relatively high drug concentrations (25, 44, 45). Nevertheless, there is currently a good correlation between the inability to induce *PUR5* and mutations that affect transcript elongation.

Both Northern and reporter analyses show a sharp peak in the inductive response; i.e., there is a signal terminating the response. Part of this may be transcriptional repression of *PUR5* as the cells approach the diauxic shift ($\approx$10 h [Fig. 1]). This is also suggested by the small but reproducible time-dependent reduction in the constitutive (uninduced) level of *PUR5* mRNA seen in untreated cells (Fig. 1A, 1B, 2C, and 3B). *PUR5* mRNA may also return to baseline due to a loss of the induction signal (low GTP) after adequate IMPDH and GTP levels have been achieved. This is inferred from the broadening of the peak seen when the endogenous *PUR5* gene is deleted (Fig. 9, Δ*pur5*). Additional mechanisms may also contribute to reduced transcription of *PUR5* mRNA.

Is the transcription defect in 6AU<sup>s</sup> elongation mutants specific for *PUR5*? A common phenotype of yeast with a debilitated Pol II elongation machinery is a slowed response to gene induction signals (35, 40, 55). Even in the absence of 6AU, the *rpb2-10* and *dst1* mutants are defective in induction of *GAL1* transcription in response to galactose (M. Wind and D. Reines, unpublished data). The inability of the 6AU<sup>s</sup> strains described here to induce *PUR5* could arise from a pleiotropic gene induction problem at the level of elongation, and therefore tran-
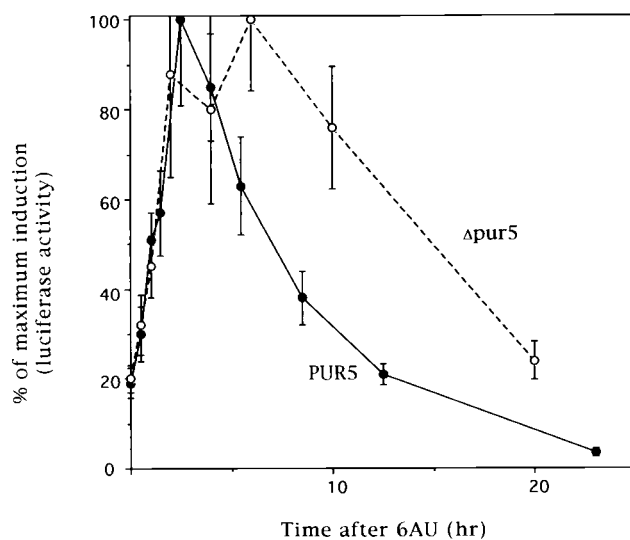


FIG. 9. *PUR5* sequences confer 6AU inducibility on a luciferase reporter gene. Strains containing (PUR5) or lacking (Δpur5) *PUR5* and harboring a reporter plasmid containing 800 bp of DNA of *PUR5* sequence upstream of the firefly luciferase coding sequence were treated with 6AU (75 µg/ml) and frozen at the indicated times. Extracts were prepared and assayed for luciferase activity in a luminometer. Data for each strain were normalized to the maximal amount of relative light units obtained over the time course. Experiments were performed in triplicate, and the means ± standard deviations (error bars) were calculated and plotted.

scriptional output from many or most genes is slowed. Alternatively, it could result from the unusual requirement for *PUR5* to be transcribed during low GTP availability if, for example, Pol II has an unusually low $K_s$ for GTP during transcription of *PUR5*. This property may be important during initiation, promoter clearance, or elongation on the *PUR5* gene. In bacteria, regulation of a pyrimidine biosynthetic operon employs a mechanism in which the magnitude of the NTP pools is sensed by Pol II itself (20, 28). A version of this model can be postulated for *PUR5* induction. Under GTP-poor conditions, promoter clearance or early elongation is possible on privileged genes such as *PUR5*, but the process would be particularly sensitive to elongationally crippled RNA polymerase and would require an optimally functioning enzyme and SII to support efficient elongation through the gene. If transcribed sequences determine the ability of Pol II to elongate through *PUR5* at low GTP levels, such a requirement is not evident in the gross guanine content of the ORF part of the transcript, which is 23% guanine (about average for a yeast ORF [C. Ball {*Saccharomyces* Genome Database}, personal communication). Upstream regulatory sequences may play a sole or additional role in regulation of *PUR5*. A test of this hypothesis must await the identification of the transcription start site in order to assess the relative contributions from promoter elements and the 5′ untranslated region.

## ACKNOWLEDGMENTS

## ADDENDUM IN PROOF

The *Saccharomyces* Genome Database (http://genome-www.stanford.edu:80/Saccharomyces/) has recently updated the recommended gene names for the four IMP dehydrogenase-like genes (YAR073W, YHR216W [*PUR5*], YLR432W, and YML056C) described in this paper to *IMD1*, *IMD2*, *IMD3*, and *IMD4*, respectively.

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