Transcriptional Regulation by Lge1p Requires a Function Independent of Its Role in Histone H2B Ubiquitination*§

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Saccharomyces cerevisiae cells that have lost their mitochondrial genome (ρ0) strongly induce transcription of multidrug resistance genes, including the ATP-binding cassette transporter gene PDR5. PDR5 induction in ρ0 cells requires the presence of the zinc cluster transcription factor Pdr3p. The PDR3 gene is positively autoregulated in ρ0 cells by virtue of the presence of two binding sites for Pdr3p in its promoter. We identify the novel protein Lge1p as a required participant in the ρ0 activation of PDR3 and PDR5 expression. Lge1p is a nuclear protein that has been found to play a role in ubiquitination of histone H2B at Lys123. This ubiquitination requires the presence of the ubiquitin-conjugating enzyme Rad6p and the ubiquitin ligase Bre1p. Interestingly, ρ0 strains lacking Lge1p failed to induce PDR3 transcription, but induction was still seen in Δrad6, Δbre1, and H2B-K123R mutant strains. Microarray experiments also confirmed that the pattern of gene expression changes seen in cells lacking Lge1p, Bre1p, or Rad6p or containing the H2B-K123R mutant as the only form of H2B share some overlap but are distinct. These findings provide a strong argument that Lge1p has roles in gene regulation independent of its participation in the Rad6p-dependent ubiquitination of H2B.

In eukaryotic cells, coordination of nuclear and mitochondrial gene expression is a critical physiological feature. Alterations in this communication circuit can lead to an array of metabolic consequences, including programmed cell death (reviewed in Ref. 1) and tumorigenesis (2, 3). Studies in the yeast Saccharomyces cerevisiae have provided important insights into the molecular mechanisms underlying mitochondrial/nuclear communication in a signaling pathway called retrograde regulation. Loss of the mitochondrial organellar DNA (ρ0 cells) triggers the induction of a number of nuclear genes, including CIT2 and DLD3, via activation of the transcriptional regulatory proteins Rtg1p and Rtg3p (4–6). A major function of the Rtg1p/Rtg3p-regulated retrograde signaling pathway is to permit adequate synthesis of amino acids that rely on the tricarboxylic acid cycle for their production.

Global analysis of the genome-wide transcriptional profile of ρ0 cells indicates that the expression of a large number of genes is induced, including locus coding for cell-surface components, membrane transporters, and enzymes of the tricarboxylic acid cycle and of glycolysis (7–9). Transcriptional regulation of some of these genes is not strictly dependent on the function of Rtg1p/Rtg3p, indicating that other pathways of retrograde regulation must exist in S. cerevisiae. One of these additional pathways is defined by the Zn2-Cys6 cluster-containing transcription factor Pdr3p.

Pdr3p, along with its homolog Pdr1p, regulates the expression of a group of proteins involved in membrane transport and biogenesis referred to as the Pdr network (see Refs. 10–12 for reviews). Pdr1p and Pdr3p exert their influence by binding to DNA sites termed Pdr1p/Pdr3p response elements (PDREs)1 present in target gene promoters and by increasing gene expression (13–15). An important target gene in the Pdr pathway is the ATP-binding cassette transporter-encoding gene PDR5 (16–18). Pdr5p is a major determinant in multidrug resistance (19) and contains three PDREs in its minimal promoter region (15). Microarray experiments have indicated that PDR5 is one of the most highly induced genes upon activation of the Pdr pathway (20).

A search for genes that negatively regulate the expression of PDR5 led to the finding that PDR5 transcription is dramatically induced in ρ0 cells (21). This retrograde regulation is exclusively dependent upon the presence of Pdr3p and triggers the induction of PDR3 expression via positive autoregulation of this gene. Unlike PDR1, the PDR3 locus contains two PDREs in its promoter and responds to the level of Pdr3p activity in the cell (22). Further analysis of the ρ0 signal provided evidence that although some Pdr target genes such as PDR5 and PDR3 are highly induced, others are not (23). These data indicate the selectivity of the Pdr3p-mediated response to loss of the mitochondrial genome.

To identify proteins that participate in the transduction of the mitochondrial signal to nuclear Pdr3p (24), we carried out transposon mutagenesis of cycloheximide-hyper-resistant ρ0 cells. The LGE1 gene was found to be required for the activation of PDR3 and PDR5 that would normally occur in these cells. Recent work has demonstrated that Lge1p acts with the

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§ The on-line version of this article (available at http://www.jbc.org) contains Supplemental Table I.

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1 The abbreviations used are: PDREs, Pdr1p/Pdr3p response elements; GFP, green fluorescent protein; MRE, metal response element.
ubiquitin ligase Bre1p and the ubiquitin-conjugating enzyme Rad6p to attach ubiquitin to histone H2B, a modification important for gene silencing (25–28). Surprisingly, we found that \( \Delta bre1 \) or \( \Delta rad6 \) mutants, along with cells lacking the Rad6p-dependent ubiquitination site on H2B, can still support \( \rho^0 \)-mediated PDR3 induction. Microarray experiments indicated that Lge1p has both positive and negative effects on gene expression, but only a fraction of these overlap with the effects of Rad6p. These data provide evidence that Lge1p clearly has actions in addition to those executed with Rad6p/Bre1p.

**MATERIALS AND METHODS**

**Strains, Media, and \( \beta \)-Galactosidase Assay**—The \( S. \) cerevisiae strains used in this study are listed in Table I. Yeast cells were grown on rich YPD medium (2% yeast extract, 1% peptone, and 2% glucose) or on synthetic complete medium lacking the appropriate auxotrophic components (29). Yeast strains were transformed by the lithium acetate method (30). Gradient plate assays (31) and standard \( \beta \)-galactosidase assays (32) were performed as described previously. Luminescence \( \beta \)-galactosidase assays (Clontech) were performed following the manufacturer’s recommendations. All assays were done with at least four independent transformants. Disruption mutations in cells lacking mitochondrial DNA were generated by disruption of a given gene in our previously characterized \( \rho^0 \) background with the exception of the H2B mutant strains, YZS276 and YZS277. We produced \( \rho^0 \) derivatives of these strains by integrative disruption of the FZO1 gene. FZO1 is required for normal mitochondrial segregation, and \( \Delta zio1 \) cells become uniformly \( \rho^0 \) in all nucleotides of GFP sequence. The PCR product was purified and transformed into BY4741 along with the Asp718-cut pXTZ164 plasmid. Transformants were selected on synthetic complete plates lacking histidine. Plasmids from His \( ^{+} \) transformants were then retrieved and characterized.

**DNA Manipulation**—The \( LGE1 \) gene was PCR-amplified from genomic DNA isolated from the wild-type \( S. \) cerevisiae strain using primers 5′-GAC GGC ACA AGA AAA AAG and 3′-R GCA CGG CTG AAA GCC TGC GC and cloned into Topo2.1 vector DNA (Clontech), yielding pXTZ161. The sequence of the complete \( LGE1 \) gene was verified by automated sequencing. The plasmid pXTZ164 was constructed by transferring the NotI-SpeI fragment containing the \( LGE1 \) gene from pXTZ161 to the same sites of pRS313 (\( CEN, HIS3 \)) (36).

The green fluorescent protein (GFP) gene fusion with the C terminus of \( PDR3 \) was generated by one-step PCR combined with \( in \) \( vitro \) recombination in yeast. First, a GFP-TRP1 cassette was PCR-amplified with primers C-LGE1GFP70 (TAA ATA TTC AAG TAA CCA ACC AAG AGA AAT TTA TGA TCT TTA ACA TTA G; and 3′-un- underlined). Plasmids from His \( ^{+} \) strains were mapped using homemade microarray slides containing probes for most of the yeast open reading frame. Total RNA used for Northern blot analyses. We used homemade microarray slides containing probes for most of the yeast open reading frame. Western blots were incubated with anti-Pdr5p antibody, anti-hemagglutinin antibody (Covance), or anti-Vph1p antibody (Molecular Probes, Inc.) and were developed using the enhanced chemiluminescence detection system (Pierce). Anti-Pdr5p antibody was provided by Karl Kuchler.

**Immunological Procedures**—Protein concentrations were determined using the Bio-Rad protein assay kit as recommended by the manufacturer. Immunological procedures were performed using cultures grown to an OD \( _{600} \) of 0.8 in minimal medium. Cells were observed at a magnification of \( \times 100 \) using Nomarski optics or fluorescence through a green filter to observe GFP. Images were captured by a Hamamatsu ORCA CCD camera attached to a Nikon Eclipse 800 microscope. The fluorescent microscopy. Images were processed identically using Adobe Photoshop Version 5.0.

| Strain designation | Genotype | Source |
|-------------------|----------|--------|
| BY4742             | MATA leu2 ura3 his3 met15 | Research Genetics |
| BY4742 : Lge1Δ   | MATA leu2 ura3 his3 met15 lge1-Δ (p = 200) kanMX4 | Research Genetics |
| SEY6210            | MATA leu2-3,112 ura3-52 lys2-801 trp1-α901 his3-Δ200 suc2-Δ9 Mel- | Scott Emr |
| SEY6210 Δ w3         | MATA leu2-3,112 ura3-52 lys2-801 trp1-α901 his3-Δ200 suc2-Δ9 mel [pW3] | Ref. 21 |
| AK1                | SEY6210 lge1-Δ:kanMX4 | This study |
| AK2                | SEY6210 lge1-Δ:kanMX4 [pW3] | This study |
| PB2                | SEY6210 pdr3-Δ:hisG | This study |
| TCH3               | SEY6210 pdr3-Δ:hisG [pW3] | This study |
| AK3                | SEY6210 pdr3-Δ:kanMX4 | This study |
| AK4                | SEY6210 pdr3-Δ:kanMX4 [pW3] | This study |
| YZS 276            | MAT a1-Δ : hta1-lacZ LEU2 his2-Δ2 trp1-α2Δ6 his3-Δ200 | Ref. 45 |
| YZS 277            | MAT a1-Δ : hta1-lacZ LEU2 his2-Δ2 trp1-α2Δ6 his3-Δ200 | Ref. 45 |
| AK23               | YZS276 2Δ : natMX | This study |
| AK24               | YZS277 2Δ : natMX | This study |
| AK25               | SEY6210 rad6-Δ : kanMX4 | This study |
| AK26               | SEY6210 rad6-Δ : kanMX4 [pW3] | This study |
| AK27               | SEY6210 ubr1-Δ : kanMX4 | This study |
| AK28               | SEY6210 ubr1-Δ : kanMX4 [pW3] | This study |
| AK29               | SEY6210 bre1-Δ : kanMX4 | This study |
| AK30               | SEY6210 bre1-Δ : kanMX4 [pW3] | This study |
frames (~6000 oligonucleotides) deposited in duplicates. The oligonucleotides were supplied by MWG Biotech (yeast oligonucleotide set).

frames were used to ultragaps from Corning. Four micrograms of mRNA were used for each reverse transcription reaction. Detailed protocols are at www.biologie.ens.frfr/genetiq/puces/protocolespuces.html. The arrays were read by a Genepix 4000 scanner (Axon Instruments, Inc.) and were analyzed with Genepix Version 3.0 software. Each microarray result presented here is an average of at least two independent biological measurements. We excluded artifactual spots, saturated spots, and low signal spots (foreground < background + 2 S.D.). Assuming that most of the genes have unchanged expression, the Cy3/Cy5 ratios were normalized by use of the median of all the ratios for each experiment. We considered ratios >1.8-fold as representing significant gene expression changes. The choice of this threshold is based on an error model constructed from control versus control experiments (data not shown) in which, after filtering and averaging of two independent measurements (see above), no ratio was above 2-fold, and only one or two features per replicate experiment were above 1.8-fold. Northern blotting was performed as described previously using radiolabeled probes for PDR5 and ACT1 mRNAs (9).

RESULTS

Genetic Screen for Regulators of Retrograde Control of Pdr3p Activity—We have previously found that expression of the multidrug-resistant ABC transporter gene PDR5 is strongly induced in cells that have lost their mitochondrial genomic DNA (ρ0) (21) or that have defects in biogenesis of the F0 component of the mitochondrial ATPase (23). This mitochondrial dysfunction leads to activation of the zinc cluster transcription factor Pdr3p, which in turn positively autoregulates PDR3 gene expression. Pdr3p levels rise with accompanying stimulation of the expression of target genes such as PDR5.

To identify participants in the pathway of mitochondrial signaling to nuclear Pdr3p, we carried out transposon mutagenesis with the goal of blocking Pdr3p activation in ρ0 cells that are cycloheximide-resistant. A ρ0 strain was transformed with DNA corresponding to a S. cerevisiae genomic library containing randomly inserted transposons. About 5000 transformants were first selected on minimal plates and then replica-plated onto minimal plates containing 0.25 μg/ml cycloheximide. Five colonies failed to grow on plates containing cycloheximide, and these colonies were retested for PDR5-lacZ expression. Three strains that exhibited both phenotypes, cycloheximide sensitivity and decreased PDR5-lacZ expression, were selected for further study. Additional genetic analyses indicated that the behavior of only one of these mutants could be consistently linked to the presence of the transposon, and we focused on this isolate.

The transposon insertion point in this mutant strain was found 42 bp upstream of the translation start site for the gene LGE1 (YPL055C; GenBankTM/GenBank accession number NP_013270). LGE1 encodes a basic protein of 333 amino acids with no significant homology to known proteins. Lge1p has recently been identified as a component of a protein complex that mediates ubiquitination of histone H2B, although its exact function is still unknown (39).

To confirm that the observed effects of cycloheximide sensitivity and reduced PDR5 expression were indeed due to disruption of the LGE1 locus rather than a special property of the transposon mutant allele, gene disruption forms of LGE1 were constructed in both ρ0 and ρ+ strains. The entire LGE1 open reading frame was replaced with the kanMX4 marker (40) in otherwise isogenic ρ0 and ρ+ cells. These disruption mutants were then tested for their ability to tolerate cycloheximide (Fig. 1).

As seen for the transposon insertion mutant, deletion of the LGE1 open reading frame dramatically reduced cycloheximide resistance in ρ0 cells. A reduction in cycloheximide tolerance was also seen upon elimination of Lge1p production in ρ+ cells. In either case, the cycloheximide-sensitive phenotype elicited by the Δlge1 lesion could be fully complemented by introduction of the wild-type LGE1 gene into a low copy number plasmid.

The effect of the Δlge1 mutation on gene expression was assayed in two different ways. Gene fusions between Escherichia coli lacZ and two different Pdr-regulated genes (PDR5 and PDR15) as well as two genes not responsive to Pdr regulatory signals (TRP5 and HSP12) were tested in these backgrounds. Western blot experiments were carried out to confirm that the expression level of Pdr5p produced from the authentic PDR5 locus also responded to loss of Lge1p.

Whereas expression of both PDR5-lacZ and PDR15-lacZ was strongly induced in ρ0 cells, expression levels were markedly decreased when the Δlge1 mutation was present in these same ρ0 strains (Fig. 1B). In ρ+ cells, the presence of the Δlge1 mutation did not affect expression of either PDR5 or PDR15. HSP12-lacZ and TRP5-lacZ expression was not significantly influenced in these same strains.

Expression of Pdr5p from its normal chromosomal location was also evaluated by Western blotting (Fig. 1C). Immuno-reactive Pdr5p levels were strongly induced in ρ0 cells. This level of induction was sensitive to the presence of Lge1p, as Pdr5p was seen to decrease in abundance upon introduction of the Δlge1 mutation into the ρ0 background. Interestingly, residual induction of Pdr5p was still seen in ρ0 Δlge1 cells, which could be eliminated by removing PDR3 from this background. The decrease in expression of Pdr5p in ρ0 Δlge1 cells could be reversed by introduction of the low copy number LGE1 clone in a fashion that correlated well with the cycloheximide resistance phenotypes of these same strains.

Deletion of LGE1 Decreases PDR3 Expression in ρ0 Cells—As previously reported, autoregulation of PDR3 is critical for induction of PDR5 expression in cells with mitochondrial defects (21, 23). To examine whether PDR3 autoregulation in ρ0 cells is also affected by the deletion of LGE1, a low copy number plasmid containing PDR3-lacZ was transformed into wild-type or Δlge1 cells and their ρ0 derivatives. We also tested expression of a PDR1-lacZ fusion gene in these same backgrounds since this gene is insensitive to mitochondrial status (21).

The activity of the PDR3 promoter was enhanced (7-fold) in the ρ0 background (Fig. 2) (21). However, this induction was nearly abolished in ρ0 cells lacking Lge1p. LGE1 deletion did not have any effect on PDR3 expression in ρ+ cells. This is in line with microarray analyses in which expression of Pdr3p-regulated genes in ρ0 cells was unchanged upon deletion of LGE1 (see Fig. 7 and Supplemental Table I). Together, these data indicate that the Δlge1 mutation impaired PDR3 autoregulation; moreover, this effect was observed only in ρ0 cells. Expression of PDR1-lacZ was not significantly altered in the various backgrounds tested, reflecting the specificity of the Lge1p effect on Pdr3p activity.

Genetic Interaction of Lge1p with Mitochondrial Signals—The presence of Lge1p is crucial for normal transcriptional induction of PDR3 to occur in ρ0 cells. Since normal PDR3 expression appears to rely on Lge1p only in ρ0 cells, we wanted to determine the possible mode of action of Lge1p when mitochondrial function is compromised. To determine whether the subcellular localization of Lge1p is regulated in response to the presence of the mitochondrial genome, we constructed an Lge1p-GFP fusion in a low copy number plasmid. This Lge1p-GFP fusion protein was able to complement Δlge1 defects in both ρ+ and ρ0 strains (data not shown). Transformants were then visualized by fluorescence microscopy to determine the subcellular distribution of Lge1p-GFP (Fig. 3).

In wild-type cells, Lge1p-GFP exhibited localization to a single compartment in the cell that was also strongly stained by 4′,6-diamidino-2-phenylindole. This pattern is typically what is seen for nuclear proteins and did not change upon loss
of the mitochondrial genome. We conclude that Lge1p is a constitutive nuclear factor and that control of the localization of this protein is unlikely to be involved in its activation of PDR3 expression in \( /H9267 \) cells.

Western blot experiments indicated that expression of Lge1p was also not significantly different in \( /H9267 /H11001 \) and \( /H9267 \) cells (data not shown). These data suggest that any influence on Lge1p activity in \( /H9267 \) versus \( /H9267 /H11001 \) cells is most likely to occur at the level of protein function.

To explore the basis for Lge1p regulation of Pdr3p activity, we uncoupled production of Pdr3p and autoregulation of the PDR3 promoter by replacing the PDREs in this promoter with tandem CUP1 Ace1p-responsive elements (metal response element (MRE)) (41). This MRE-PDR3 fusion gene is now induced upon addition of copper to the medium and is no longer responsive to Pdr3p autoregulation.\(^2\) Pilot experiments were done to determine the minimal copper concentration required for production of levels of Pdr3p that resemble those of the wild-type PDR3 locus (data not shown). The MRE-PDR3 gene was introduced into \( \rho^+ \) and \( \rho^0 \) cells, either containing or lacking LGE1, along with a PDR5-lacZ reporter gene. This strain background lacked the chromosomal copy of PDR3 but still contained PDR1. Appropriate transformants were grown in the presence or absence of copper, and \( \beta \)-galactosidase activities were determined (Fig. 4).

Activation of the MRE-PDR3 gene with copper strongly bypassed the block in expression of PDR5-lacZ that would other-

\(^2\) M. Kolaczkowski, unpublished data.
wise occur in \( \rho^0 \Delta lge1 \) cells. In each strain background, some level of induction of \( PDR3 \) expression was seen when the cells were exposed to copper. Interestingly, the effect of the \( \rho^0 \) signal could still be seen, as activation of \( PDR5-lacZ \) was consistently higher in the absence of the mitochondrial genome.

We draw two important conclusions from this experiment. First, the loss of \( Lge1p \) can be suppressed by artificially elevating \( Pdr3p \) expression. This supports the notion that \( \Delta lge1 \) cells have a defect in \( PDR3 \) expression that is manifested at the level of the \( PDR3 \) promoter. This defect can be bypassed by placing \( Pdr3p \) production under the control of a heterologous promoter. Second, these data provide further evidence that \( Pdr3p \) detects a signal from \( \rho^0 \) mitochondria at a post-translational level, as we have suggested previously (21, 23).

**FIG. 2.** \( LGE1 \) is required for proper induction of \( PDR3 \) in \( \rho^0 \) cells. \( LGE1 \) and \( \Delta lge1 \) cells, either containing or lacking the mitochondrial genome, were transformed with low copy number plasmids bearing either a \( PDR1-lacZ \) or \( PDR3-lacZ \) fusion. \( \beta\)-Galactosidase activity was measured, and the values reported are the averages for at least four independent transformants.

**FIG. 3.** \( Lge1p \) is a nuclear protein. A centromeric plasmid containing functional GFP-tagged \( LGE1 \) was transformed into a \( \Delta lge1 \) strain as well as its \( \rho^0 \) derivative. Cells were grown to mid-log phase and examined by fluorescence microscopy. Photographs of the same fields viewed with Nomarski optics and stained with 4',6-diamidino-2-phenylindole (DAPI) to visualize cell nuclei are shown.

**FIG. 4.** \( Ace1p \)-driven \( PDR3 \) can bypass the requirement for \( LGE1 \) to induce \( PDR5 \) expression. PDREs present in the \( PDR3 \) promoter region were replaced with two \( Ace1p \)-binding sites. A centromeric plasmid bearing \( Ace1p \)-driven hemagglutinin-tagged \( PDR3 \) was introduced into \( \Delta pdr3 \) and \( \Delta pdr3 \Delta lge1 \) cells as well as the \( \rho^0 \) derivatives of these strains along with the \( PDR5-lacZ \) reporter plasmid. \( \beta\)-Galactosidase activity was determined in cells grown to mid-log phase in the appropriate media from cultures not induced (solid bars) or induced (hatched bars) with copper.
Two genes encoding proteins resembling dehydrogenases corresponding to oligonucleotides representing microarray (Figs. 5 and 7). RNA was prepared from these ubiquitin acceptor site (H2B-K123R) were analyzed by DNA significance of -fold changes (see "Material and Methods"). were considered as significant. This and all subsequent microarray experiments have been processed using the same criteria for filtering and significance of -fold changes (see "Material and Methods"). WT, wild-type; ABC, ATP-binding cassette.

indicate that Lge1p is necessary to allow induction of PDR3 expression in \( \rho^0 \) cells. Recently, Lge1p was found to form a protein complex with Bre1p, a protein required for resistance to the Golgi-collapsing agent brefeldin A (42). Both Bre1p, a ubiquitin-protein isopeptide ligase homolog, and Lge1p are necessary for histone H2B monoubiquitination (39, 43), which is mediated by the ubiquitin carrier protein Rad6p (44–47). Rad6p functionally cooperates in a variety of cellular processes with multiple ubiquitin-protein isopeptide ligase homologs, including Ubr1p and Bre1p (39, 48). To test whether the system of enzymes catalyzing histone H2B ubiquitination modifies PDR3 expression, microarray experiments were carried out in an isogenic series of strains lacking RAD6, BRE1, or LGE1 in both \( \rho^- \) and \( \rho^0 \) backgrounds. Along with these factors involved in H2B ubiquitination, \( \rho^- \) and \( \rho^0 \) strains lacking the H2B ubiquitin acceptor site (H2B-K123R) were analyzed by DNA microarray (Figs. 5 and 7). RNA was prepared from these strains, differentially labeled, and hybridized to slides corresponding to oligonucleotides representing ~6000 different \( S. \textit{cerevisiae} \) open reading frames. The resulting slides were processed for the relative degree of hybridization as described (49).

Loss of \( \textit{LGE1} \) from \( \rho^0 \) cells blocked the induction of several different Pdr3p-regulated target genes to a degree directly comparable with the block observed previously when the PDR3 gene was eliminated from \( \rho^0 \) cells (Fig. 5) (9). These known Pdr3p-regulated genes include several genes encoding transporter proteins (RSB1, PDR5, and PDR15) and PDR3 itself. Two genes encoding proteins resembling dehydrogenases (\( YKL071w \) and \( YAL061w \)) exhibited a greater transcriptional dependence on \( \textit{LGE1} \) than PDR3 in \( \rho^0 \) cells. Expression of other Pdr3p-responsive genes (\( \textit{SCS7}, \textit{ICT1}, \textit{YLR346c}, \textit{and SNQ2} \)) was modestly reduced in \( \rho^0 \) \( \textit{Lge1} \) cells, similar to their reduced effect seen when PDR3 was deleted from \( \rho^0 \) cells (9).

The block in \( \rho^0 \) induction of expression seen in \( \rho^0 \) \( \textit{Lge1} \) strains is not due to a general defect of the \( \textit{Lge1} \) strain for the \( \rho^0 \) response since many genes, including two encoding mitochondrial proteins (\( \textit{IDH1} \) and \( \textit{IDH2} \)), were still properly induced in the \( \rho^0 \) \( \textit{Lge1} \) strain. On the other hand, the effect of the \( \textit{Lge1} \) mutation is not completely restricted to the Pdr3p-dependent \( \rho^0 \) response since the induction of glycolytic genes that are not Pdr3p-regulated was slightly affected in our experiments (Supplemental Table I).

Strikingly, loss of other components required for H2B ubiquitination did not have an effect on gene expression similar to that of \( \textit{Lge1} \). Deletion of \( \textit{RAD6} \) or \( \textit{BRE1} \) or loss of the H2B acceptor site (H2B-K123R) from \( \rho^0 \) cells had no significant effect on expression of these Pdr3p-responsive genes. For example, introduction of the \( \Delta \textit{rad6} \) allele into \( \rho^0 \) cells only modestly reduced induction of the Pdr-regulated genes (no more than 50%), whereas a similar \( \rho^0 \) strain lacking either PDR3 or \( \textit{LGE1} \) failed to induce these same Pdr-responsive genes (Fig. 5).

This effect is likely to be indirect or at least less specific for Pdr3p activity than the influence of Lge1p, as the deletion of \( \textit{RAD6} \) altered the global \( \rho^0 \) response, including genes encoding mitochondrial proteins (Fig. 5). Unlike \( \textit{Lge1} \), the deletion of \( \textit{RAD6} \) had dramatic effects on gene expression in \( \rho^0 \) cells (almost 300 genes with altered levels of transcription, including many chaperones and stress response protein-encoding genes) (Supplemental Table I) and significantly altered the fitness of

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**Fig. 5.** Microarray analyses of the \( \rho^0 \) response in strains defective in H2B ubiquitination. The results of microarray analyses for a selection of \( \rho^0 \)-responsive genes in different genetic backgrounds are represented using a color scale (green for down-regulation, red for up-regulation, black for unchanged, and gray for no data). The strains used are all in the SEY6210 background with the exception of the H2B-K123R mutant strain. The descriptions of gene function were taken from the \textit{Saccharomyces} Genome Database (available at www.yeastgenome.org). The complete results and numerical ratios of these microarray analyses are listed in Supplemental Table I. The data for the \( \rho^0 \) versus \( \rho^- \) and the \( \rho^0 \) versus \( \Delta \textit{pdr3} \) comparisons are from Devaux et al. (9). Only ratios above 1.8-fold (average of at least two independent experiments) were considered as significant. This and all subsequent microarray experiments have been processed using the same criteria for filtering and significance of -fold changes (see "Material and Methods").
the cells, as shown by their slow growth in rich medium. Similar to the effects observed in $\rho^0$ $\Delta rad6$ cells, loss of either BRE1 or H2B-K123 from a $\rho^+$ strain had negligible effects on induction of the Pdr3p-dependent retrograde response.

To confirm the effects on gene expression seen in our microarray analyses, we analyzed several features of the expression of $PDR5$. First, we compared the cycloheximide resistance of $\rho^+$ and $\rho^0$ strains lacking Lge1p, Bre1p, and Rad6p or con-

**FIG. 6.** Defects in $\rho^0$ activation of $PDR5$ expression elicited in strains lacking Lge1p but not other aspects of H2B ubiquitination. A, shown is the cycloheximide resistance of $\rho^+$ and $\rho^0$ strains. The $\rho^+$ and $\rho^0$ strains containing different defects in the ability to attach ubiquitin to H2B were grown to mid-log phase, and 1000 cells of each culture were placed on medium containing a gradient of cycloheximide (Cyh), with regions of higher concentration indicated by the bars of increasing width. B, shown are the results from Northern blot analysis. Total RNA from each of the indicated strains was electrophoresed, transferred, and hybridized to labeled probes as described (9). Radiolabeled probes were used to detect $PDR5$ or $ACT1$ mRNA. C, the indicated strains were transformed with a $PDR5$-lacZ reporter plasmid and grown to mid-log phase, and $\beta$-galactosidase activities were measured (32).
FIG. 7. Global transcriptional effects of mutants lacking different aspects of H2B ubiquitination. DNA microarray comparison of isogenic ρ0 strains lacking Lge1p (Δlge1), Rad6p (Δrad6), Bre1p (Δbre1) or the ubiquitin acceptor site in H2B (H2B-K123R) was performed. The changes in mRNA levels are presented using a color code, and gene descriptions were obtained as described in the legend to Fig. 5. A, negative effects of H2B ubiquitination. Genes that are up-regulated in response to loss of the indicated components of H2B ubiquitination are shown and
FIG. 8. Overlapping effects of trans-acting mutants on H2B ubiquitination machinery. The Venn diagrams represent the overlaps between the DNA microarray experiments described in the legend to Fig. 8. Global indicates the total number of genes exhibiting a change in expression in response to the relevant disruption mutations, whereas Negative denotes the number of genes that were induced in the absence of each gene product. The probability of these overlaps occurring at random is presented in parentheses.

taining the ubiquitin acceptor site mutant H2B-K123R. Sec-
ond, Northern blot experiments were carried out to assay levels
of PDR5 and ACT1 mRNAs in each sample used in the microar-
ray. Equal amounts of total RNA were electrophoresed through
denaturing gels, transferred to a membrane, and hybridized
with 32P-labeled DNA fragments corresponding to
PDR5 and ACT1 as described (9). Finally, we compared the expression of
PDR5-lacZ in each strain.

Deletion of LGE1 had the most severe effect on the increased
cycloheximide tolerance normally seen in /H92670 strains (Fig. 6A).
Loss of either BRE1 or RAD6 had only minor effects on cyclohexi-
me resistance. This same phenotypic behavior was seen for the
ρ° H2B-K123R mutant (data not shown). PDR5 mRNA was
clearly increased in all ρ° samples with the exception of the ρ°
Δlge1 strain (Fig. 6B). ACT1 mRNA levels were not significantly
different in these RNA samples. Finally, the ρ° Δlge1 strain
exhibited the largest reduction in expression of PDR5-lacZ that
was seen in this comparison. We believe that the reduction seen
in PDR5-lacZ expression may be an underestimate of the effect
caused by loss of Lge1p from ρ° cells because of the accumulation
of the extremely stable β-galactosidase protein (50) since larger
changes were seen by Northern (Fig. 6B) and Western (Fig. 1C)
blotting of native PDR5 transcript and protein levels, respec-
tively. These data are consistent with our microarray experi-
are grouped relative to their behavior in a Δlge1 background. The presence of more than one gene product name next to a given category indicates that multiple mutants influence this group of genes in a similar fashion. B, positive targets of H2B ubiquitination. This set of genes was transcriptionally repressed in the absence of the indicated components of H2B ubiquitination. WT, wild-type; TCA, tricarboxylic acid.
ments and provide further evidence that the role of Lge1p in the control of PDR5 expression cannot be explained by its role in H2B ubiquitination. Along with this analysis, which was focused primarily on the effects of LGE1 and H2B ubiquitination on retrograde regulation of PDR gene expression, we evaluated the global gene expression profiles of these mutants to determine their degree of phenotypic similarity.

The Transcriptional Regulatory Influence of Lge1p Only Partly Overlaps with Mutants Defective in Histone H2B-Ubiquitin Conjugation—We evaluated the transcriptional changes of the disruption alleles tested (Δlge1, Δbre1, and Δrad6) or the H2B mutant (H2B-K123R) by the positive and negative effects on gene expression. We have focused here on genes that exhibit changes in expression as a function of LGE1 status, but it is important to bear in mind that expression of many genes (~250) changes in response to loss of Bre1p or Rad6p but not Lge1p. DNA microarrays were used to compare the relevant disruption alleles or the H2B point mutant with corresponding isogenic wild-type strains for changes in gene expression (Fig. 7).

Most genes influenced by Lge1p, Bre1p, and Rad6p exhibited increased expression upon elimination of any of these factors (negatively regulated targets) (Fig. 7A). In the case of the Δlge1 strain, the most dramatic effects were the derepression of amino acid biosynthesis genes (especially the MET and ARG clusters), of genes involved in mating, and of some MATα-specific genes. All these genes are normally tightly repressed in MATα strains exponentially growing in the rich medium used to grow the cells prior to RNA isolation (reviewed in Ref. 51). These results suggest that, in addition to its positive role in the p0 response, Lge1p has important role(s) in the repression of several categories of genes. It is striking to note that strains lacking Δlge1 and Δbre1 share striking overlaps as far as negative regulatory target genes are concerned. Lge1p and Bre1p repressed a common set of 41 genes, whereas individually each repressed a unique set of 33 or 20, respectively (Fig. 8). Both Lge1p and Bre1p share only ~30% of target genes negatively regulated by Rad6p. These data argue that the transcriptional profiles and likely the function of Lge1p and Bre1p are closer to each other than to Rad6p.

However, there are common negative targets shared by all three proteins. Among these genes is ARG1, for which repression in rich medium was already known to be dependent on Rad6p (52). ARG1 was derepressed in the Δlge1, Δbre1, and Δrad6 strains to a similar degree, although this extent of derepression was not seen in the H2B-K123R mutant strain. In general terms, loss of the ability to ubiquitinate H2B did not elicit transcriptional defects as large as loss of Lge1p, Bre1p, or Rad6p. This suggests the possibility that these factors exert their effects on gene expression via mechanism(s) including but not restricted to ubiquitination of H2B.

The positive effects of Lge1p, Bre1p, and Rad6p on gene expression presented a different degree of overlap (Fig. 7B). Nearly 50% of the genes positively regulated by Lge1p or Rad6p were in common, whereas only approximately one-third of the positive target genes were shared by Lge1p and Bre1p. Similarly, Rad6p and Bre1p shared an overlap of only six positive target genes compared with 49 or 40 unique targets, respectively (Fig. 8). Rad6p and Lge1p seem likely to function more similarly in terms of positive regulation of gene expression than either does with Bre1p. Interestingly, loss of either LGE1 or RAD6 led to reduction in expression of a number of histone genes, consistent with the idea that modification of histones and their expression may be coupled.

Ubiquitination of H2B at Lys123 Is Not Required for Retrograde Regulation of PDR5—Our results indicate that Lge1p modifies PDR expression in p0 cells. Bre1p, Lge1p, and Rad6p are also required for histone H2B ubiquitination at Lys123, a modification that is required for gene silencing at telomeres (43, 45). Although loss of any of these three proteins required for H2B ubiquitination (Bre1p, Lge1p, and Rad6p) completely blocked Lys123 ubiquitination (39, 43, 47), only loss of Lge1p blocked PDR gene induction in p0 cells. To determine whether Lys123 ubiquitination of H2B is involved in retrograde activation of PDR5 expression, we tested the ability of a strain in which this ubiquitination site was removed to respond to p0 signaling.

Since there are two copies of the H2B gene in wild-type S. cerevisiae (53), we employed yeast strains harboring a plasmid-borne functional FLAG-tagged allele of HTB1-HTA1 as the sole source of H2B production (45). Two different versions of this FLAG-tagged allele were used: wild-type or HTB1-K123R. We produced p0 derivatives of these strains, introduced the PDR5-lacZ reporter gene, and determined β-galactosidase activity produced in these transformants. We also determined the expression level of a TRP5-lacZ fusion gene in these same backgrounds to control for the effects of these mutations on global gene expression since TRP5 does not contain PDREs or respond to Pdr signals. The data are presented as a ratio of PDR5-lacZ to TRP5-lacZ determined in same strain backgrounds.

As in other strain backgrounds (7, 9, 21), a p0 version of this wild-type H2B plasmid-containing background strongly induced PDR5 expression (12-fold over TRP5 expression). However, an isogenic p0 derivative of this strain carrying H2B-K123R was still able to induce PDR5-lacZ expression 9-fold above TRP5 expression. Deletion of LGE1 from this background reduced the response of PDR5 to p0 status, arguing for the ubiquitination-independent effect of Lge1p in gene expression. This finding supports our assertion that the effect of Δlge1 cannot be explained in terms of an effect on ubiquitination of H2B since the presence of H2B-K123R as the only source of H2B does not prevent p0-mediated activation of PDR5 expression. The implications of this result are discussed below.

DISCUSSION

Recent experiments have begun to clarify the molecular details linking chromatin modification with alterations in gene expression (see Ref. 54 for a review). Modification of histone H2B by ubiquitin has been recently implicated as an important requirement for gene silencing in S. cerevisiae (44). The ubiquitination of

FIG. 9. PDR5 induction in p0 cells still occurs in the absence of histone H2B ubiquitination. Low copy number PDR5-lacZ and TRP5-lacZ reporter plasmids were transformed into isogenic strains YZS276 and YZS277 carrying wild-type HTA1-FLAG-HTB1 and HTA1-FLAG-HTB1-K123R, lacking the H2B ubiquitination site, as well as p0 derivatives of these same strains. Deletion of the PZO1 gene was used to generate the p0 forms of YZS276 and YZS277. LGE1 was also deleted in the YZS277 (H2B-K123R) background to demonstrate the effect of Lge1p in this strain. Transformants were grown to mid-log phase, and β-galactosidase activity was measured. Enzyme activities are presented as a ratio of PDR5-lacZ to TRP5-lacZ determined in the same strain backgrounds.
H2B at Lys123 triggers subsequent methylation of histone H3 at Lys^4 and Lys^79 by different enzymatic systems (25–28, 55, 56). Current models propose that this methylation acts to mark active or euchromatic DNA and to prevent association of silencing proteins with these regions of the genome (see Ref. 57 for a recent review). Although individual loss of Rad6p, Bre1p, or Lge1p is sufficient to block ubiquitin attachment to H2B, only loss of Rad6p or Bre1p prevents subsequent methylation of H3 at Lys^4 (39, 43). This observation provided the first indication that phenotypic differences exist between strains lacking different components of the H2B ubiquitination machinery.

These experiments described here extend this initial observation in two important ways. First, loss of Lge1p prevents the normal positive regulation of PDR3 gene expression in response to retrograde signals from defective mitochondria. This finding indicates that this factor plays a positive role in controlling gene expression. Second, loss of Rad6p or Bre1p or loss of Lys123 from H2B does not have the same effect on PDR3 expression as deletion of LGE1 (Fig. 5). Together, these observations suggest that although Lge1p works with Rad6p and Bre1p to ubiquitinate H2B at Lys123 to mark active chromatin and to ensure normal gene silencing, Lge1p may act in an additional pathway to ensure normal expression of genes such as PDR3.

At least two different models could explain the influence of Lge1p on PDR3 expression. Loss of Lge1p could prevent the usual chromatin structure of the PDR3 promoter from being produced and lead to a reduction in the normal level of Pdr3p expression seen in response to a P^o signal. This would represent a defect primarily at the level of transcription initiation. However, recent experiments have indicated the possibility that Lge1p (and Bre1p) might influence transcriptional elongation, as mutations eliminating either of these proteins are synthetically lethal to the histone H3 methylase Set2p (58). We favor a role for Lge1p, distinct from that of Bre1p, in the control of PDR3 transcription initiation based on the ability of Ace1p-driven PDR3 transcription to strongly suppress any requirement for Lge1p (Fig. 4). If Lge1p influenced elongation through the PDR3 gene, then we would expect Ace1p transcriptional control to be unable to bypass the reduction in expression that is seen in Lge1 cells, as most upstream activator proteins like Ace1p act primarily to influence transcription initiation (see Ref. 59 for a review). However, in P^o Lge1 cells, Ace1p-driven Pdr3p levels restore PDR5 transcription to 80% of the level seen in P^o LGE1 cells. This is most consistent with Ace1p increasing the level of transcription initiation and bypassing the usual requirement for Lge1p in PDR3 promoter function. We cannot yet eliminate the possibility of Lge1p acting via some other indirect mechanism, but we believe that our data are most consistent with an effect of this protein on an aspect of PDR3 transcription.

A clear role for Lge1p is also seen in PDR5 transcriptional activation upon Ace1p-mediated production of Pdr3p in P^o cells. Copper induction of MRE-PDR3 leads to a 2-fold higher level of PDR5 expression in LGE1 cells compared with an isogenic Lge1 mutant. We explain this functional dependence on Lge1p as a result of the Ace1p-binding sites being inserted in the context of an otherwise wild-type PDR3 promoter. This construction retains the possibility that Lge1p still can modulate the activity of this chimeric promoter even in the absence of Pdr3p autoregulation.

The ability of Pdr3p to respond to P^o signaling in the absence of wild-type Pdr3p autoregulation also supports our earlier model explaining activation of PDR3 transcription in response to loss of the mitochondrial genome (21, 23). Pdr3p receives a post-translational signal from defective mitochondria that stimulates its activity by a modest degree (~2-fold). However, the PDR3 promoter is highly responsive to the presence of this activated Pdr3p and is rapidly up-regulated through recruitment of Pdr3p to the PDR3 promoter (22). We hypothesize that Lge1p acts to sensitize the PDR3 promoter to the presence of activated Pdr3p as a key contributor to the autoregulatory amplification of PDR3 transcription. Once sufficient Pdr3p levels accumulate, induction of downstream genes like PDR5 and PDR15 will occur.

The selective contribution of Lge1p to induction of PDR3 transcription would also explain how the PDR3Es in PDR3 could be stimulated by increasing levels of Pdr3p in the face of a large excess of the closely related factor Pdr1p. Previous experiments from our laboratory (21) and others (24, 60) have established that the expression of PDR1 is anywhere from 10 to 100 times that of PDR3. Appropriate retrograde regulation of PDR3 transcription may be ensured by the influence of Lge1p on the activity of the PDR3 promoter via its impact on Pdr3p function itself. Preliminary chromatin immunoprecipitation analysis demonstrated robust recruitment of Pdr3p to the PDR3 promoter that was dependent on the presence of Lge1p (data not shown). Together with the observation that retrograde regulation of PDR3 occurs normally in the absence of Pdr1p (21), these data support the idea that Lge1p influences recruitment of Pdr3p to the PDR3 promoter and permits the selective induction of transcription of this regulatory protein. It is important to note that the action of Lge1p on PDR3 promoter function can be recapitulated by our PDR3-lacZ fusion gene, which contains only 652 bp of 5’-noncoding DNA from PDR3. This result indicates that the target for Lge1p influence on PDR3 is likely to be the promoter region of this gene.

Along with the participation of Lge1p in retrograde control of PDR3, this work has uncovered the complex nature of the role of Lge1p in transcriptional regulation. Lge1p clearly plays a role in gene repression along with positive regulation of PDR3 and other genes. Cells carrying the Δlge1 lesion exhibited elevated levels of expression of several clusters of genes that would normally be repressed (Fig. 7). As mentioned above, these effects on gene transcription are not reproduced by several other mutants defective in H2B ubiquitination, further supporting the idea that the action of Lge1p on PDR3 is unlinked to this histone modification. Previous work provided a link between Lge1p and Rad6p in histone H2B ubiquitination but did not examine the functional role of these two proteins in the control of gene expression (39). Our data demonstrate that Lge1p has a multiplicity of actions on gene transcription and is a central participant in ensuring accurate expression of the genome.

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Transcriptional Regulation by Lge1p Requires a Function Independent of Its Role in Histone H2B Ubiquitination

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