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Induction of Yeast Histone Genes by Stimulation of Stationary-Phase Cells

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In the cell cycle of the budding yeast *Saccharomyces cerevisiae*, expression of the histone genes H2A and H2B of the *TRT1* and *TRT2* loci is regulated by the performance of "start," the step that also regulates the cell cycle. Here we show that histone production is also subject to an additional form of regulation that is unrelated to the mitotic cell cycle. Expression of histone genes, as assessed by Northern (RNA) analysis, was shown to increase promptly after the stimulation, brought about by fresh medium, that activates stationary-phase cells to reenter the mitotic cell cycle. The use of a yeast mutant that is conditionally blocked in the resumption of proliferation at a step that is not part of the mitotic cell cycle (M. A. Drebot, G. C. Johnston, and R. A. Singer, Proc. Natl. Acad. Sci. 84:7948, 1987) showed that this increased gene expression that occurs upon stimulation of stationary-phase cells took place in the absence of DNA synthesis and without the performance of start. This stimulation-specific gene expression was blocked by the mating pheromone α-factor, indicating that α-factor directly inhibits expression of these histone genes, independently of start.

Cells of the budding yeast *Saccharomyces cerevisiae* proliferate in media that meet nutritional requirements. When the growth medium becomes depleted, cells then undergo a regulated cessation of cell proliferation that leads to a population of nonproliferating cells in the stationary phase (35, 43). Stationary-phase yeast cells express a distinct constellation of properties (35, 43), and upon restoration of a satisfactory nutrient environment, these stationary-phase properties are lost as cells resume proliferation (4, 43). The resumption of proliferation upon nutrient replenishment has been shown to constitute more than a simple restoration of biosynthetic activity and reactivation of the mitotic cell cycle; requirements unique to the resumption of proliferation from the stationary phase have been revealed by mutation (3, 4).

The work presented here describes the expression of histone genes upon the stimulation of stationary-phase yeast cells to resume proliferation. In yeast cells, the histone proteins H2A and H2B are encoded by two genetically unlinked gene sets (11, 13). During the mitotic cell cycle, the expression of these histone genes occurs periodically, with significant accumulation of histone mRNA after performance of the cell cycle regulatory step termed "start" (9) and with maximum levels achieved in the S phase (13). Cells treated with yeast mating pheromone to block performance of start do not contain significant levels of H2A and H2B mRNA (13).

Here we show by analysis of transcript levels in both wild-type and mutant cells (3, 4) that the yeast histone genes are induced when stationary-phase cells are stimulated to resume proliferation and that this induction takes place before cells synthesize DNA or perform start to reenter the mitotic cell cycle. Thus, these genes respond to regulation by the stimulation response of stationary-phase cells as well as by activities within the mitotic cell cycle. Moreover, the expression of the histone genes upon stimulation is inhibited by mating factor independently of start, suggesting a direct effect of mating factor on expression of these genes.

MATERIALS AND METHODS

Strains and plasmids. The wild-type strain *S. cerevisiae* GR2 (*MATa ura1 his6*) has been described previously (17). Strain MD-G3G02 (*MATa gcs1-1 sed1-1 ade6 ura1 his6*) is a yeast mutant that is conditionally defective only for resumption of proliferation from the stationary phase (3, 4). Plasmids YIp5-TRT1 (32) and YIp5-TRT2 (28) were supplied by M. A. Osley. Plasmid YIp5-TRT1 contains the yeast *TRT1* locus, comprising the *HTA1* histone H2A gene, the *HTB1* histone H2B gene, and the neighboring protein 1 (adenylate kinase) (19, 29) gene. Plasmid YIp5-TRT2 contains the *TRT2* locus, comprising the *HTA2* histone H2A gene, the *HTB2* histone H2B gene, and the protein 2 gene. To minimize cross-hybridization between transcripts from the *TRT1* and *TRT2* loci, the *Acc*I-*Hin*dIII fragment of the *TRT2* locus was used to detect the H2B transcript, as recommended by M. A. Osley (personal communication). The *Pst*I-*Sma*I fragment from plasmid YIp5 was used to detect the transcript from the *URA3* gene. The entire plasmid YIp5-TRT1 was used to detect protein 1 and histone transcripts from the *TRT1* locus. The 2.4-kb *Sst*1 fragment and 1.4-kb *Sst1-Bam*H1 fragment from plasmid YIp5-TRT1 were used to detect the protein 1/*HTA1* and *HTB1* transcripts, respectively. Plasmids were maintained in cells of *Escherichia coli* RR1 (18) grown in YT medium (25) and were prepared by the method of Messing (24).

Culture conditions and determination of cellular parameters. Yeast cells were grown in YM1 liquid medium (7) at 29°C or at 14°C, the nonpermissive temperature for strain MD-G3G02, in gyratory shaking water baths (New Brunswick Scientific Co.); for 14°C cultures, the water bath was maintained in a cold room. Cells were maintained in stationary phase for 24 h before stimulation, which was routinely

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accomplished by a >100-fold dilution of stationary-phase cultures with fresh YM1 medium. Procedures for the determination of cell concentration and cell morphology have been described (8). The performance of start was assessed by α-factor sensitivity on solid medium (2).

Macromolecular accumulation. DNA was quantified by a modified diphenylamine procedure (38). For RNA and protein, cells were grown for many generations to stationary phase in medium containing [^{3}H]uracil (1.0 μCi/ml) and [^{14}C]histidine (0.1 μCi/ml) to label stable RNA and protein and then stimulated in medium of the same specific activity. Acid-precipitable radioactivity in 1-ml samples was determined as described previously (17).

RNA procedures. Equal amounts of total RNA, extracted as described by Penn et al. (34) and quantified spectrophotometrically, were resolved by formaldehyde-agarose gel electrophoresis and transferred to GeneScreen hybridization membrane as described by Maniatis et al. (22) and by the manufacturer (New England Nuclear Corp.). After transfer, blots were hybridized with plasmid DNA radiolabeled by use of a nick-translation kit (Bethesda Research Laboratories). Northern blots (RNA blots) were subjected to stringent washes (0.1× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]-1% sodium dodecyl sulfate, 65°C; M. A. Osley, personal communication) and exposed to Kodak XAR-5 X-ray film.

Quantification of hybridization. The intensities of bands in developed autoradiograms were quantified by using a video densitometer (Bio-Rad model 620) and expressed relative to the abundance of transcript in stationary-phase wild-type cells. Densitometry of different exposures of the same hybridized blots (data not shown) indicated that film saturation was not achieved and that high levels of signal were accurately estimated. It was evident that changes in low levels of signal were underestimated; however, this nonlinearity at low intensities does not affect the analysis described below.

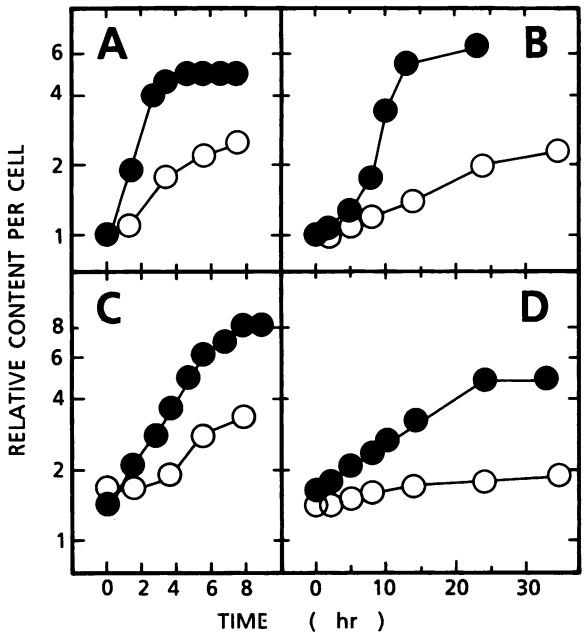

FIG. 1. Kinetics of biosynthetic activation after stimulation of stationary-phase cells. To assess RNA (●) and protein (○) content, cells were grown at 29°C for many generations to stationary phase in medium containing [^{3}H]uracil and [^{14}C]histidine. Stationary-phase cells were then inoculated into fresh medium of the same composition and specific activity and incubated at either 29°C (A and C) or 14°C (B and D), and samples were removed for determinations of cell morphology and macromolecular content. All determinations were normalized to zero-time values. A and B, Wild-type cells; C and D, reentry-mutant cells.

RESULTS

Stimulation of stationary-phase cells to resume proliferation causes prompt induction of histone gene expression. Stationary-phase cells were stimulated to increase biosynthetic activity, reactivate the mitotic cell cycle, and resume proliferation by transfer to fresh nutrient medium. The kinetics of these changes were determined in the experiments reported here by measuring cell protein and RNA and DNA contents and by monitoring cell morphology as an additional marker of cell cycle activity. Protein and RNA levels, on a per cell basis, increased from stationary-phase values (Fig. 1) and became characteristic of those of proliferating cells after 5 h of incubation (at 29°C) in fresh medium, by which time cell proliferation had commenced and cell concentration had increased fourfold. The lag phase was brief in these experiments because cells were maintained in stationary phase for only 24 h before stimulation; maintenance of cells in stationary phase for longer times leads to proportionately longer lag phases (14; unpublished results).

Northern analysis gave another indication of biosynthetic activity upon stimulation. A particularly instructive DNA probe for this analysis was plasmid YIp5-TRT1 (32). This plasmid harbors genes that are regulated in different ways by the cell cycle. The *TRT1* locus contained within the plasmid insert constitutes one of the two pairs of genes encoding the yeast histone proteins H2A and H2B (11). Expression of these histone genes has been shown to occur periodically in the cell cycle in a start-dependent manner (13). Located adjacent to the cloned *TRT1* locus on the same DNA insert is the protein 1 (adenylate kinase) (19, 29) gene that, unlike the neighboring histone genes, shows constant expression throughout the cell cycle (13). Our initial intention was to use this probe to indicate both increased biosynthetic activity, evidenced by protein 1 transcript levels, and cell cycle activity, evidenced by histone transcript levels.

As expected, the stimulation of stationary-phase cells led to the accumulation of mRNA for protein 1 (Fig. 2). Surprisingly, there was also conspicuous accumulation of mRNA for histones encoded by the *TRT1* locus that paralleled the accumulation of protein 1 mRNA (Fig. 2). Unexpectedly, this increase in histone mRNA content preceded indications of initiation of the mitotic cell cycle such as an increase in the proportion of budded cells (Fig. 2). These results introduced the possibility that some histone gene expression in stimulated cells is a direct response to stimulation conditions rather than a consequence of the start event of the mitotic cell cycle.

Histone gene expression occurs without initiation of the mitotic cell cycle. To rule out any involvement of the mitotic cell cycle in the apparent stimulation-dependent expression of the histone genes, levels of mRNA were quantified for a mutant strain that is conditionally defective only for resumption of proliferation from the stationary phase (3, 4). These mutant cells are unaffected in performance of the mitotic cell cycle at all temperatures and resume proliferation normally at 29°C (3, 4) but when stimulated from stationary phase at 14°C are conditionally defective (cold sensitive) for both bud formation and DNA synthesis (Fig. 3). Each of these cell

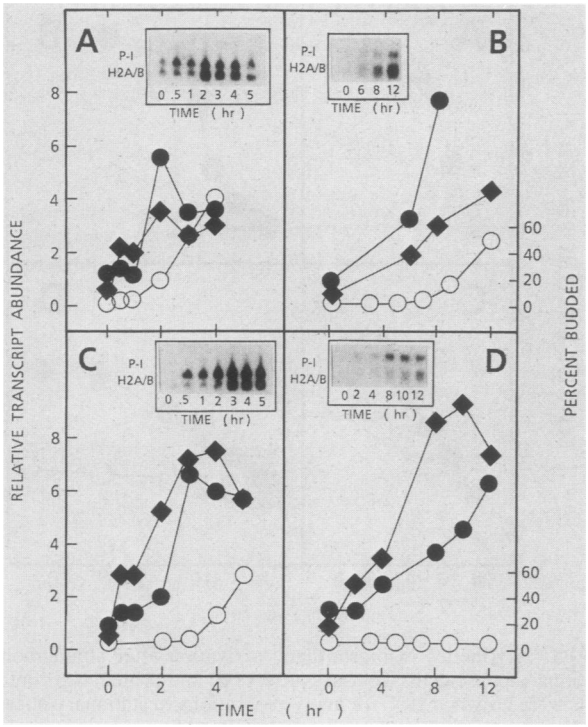

FIG. 2. mRNA levels for histone and protein 1 after stimulation of stationary-phase cells. Stationary-phase wild-type (A and B) and reentry-mutant (C and D) cells were inoculated into fresh medium and incubated at 29°C (A and C) or at 14°C (B and D). Equal amounts of total RNA extracted from cells at the indicated times were resolved by electrophoresis, transferred to a membrane support, and hybridized with radiolabeled YIp5-TRT1 plasmid DNA. Autoradiograms of hybridized RNA (insets) were quantitated by densitometry; levels of histone mRNA (●) and protein 1 mRNA (◆) were normalized to the respective values for wild-type stationary-phase cells. Cell morphologies (○) were determined for parallel samples.

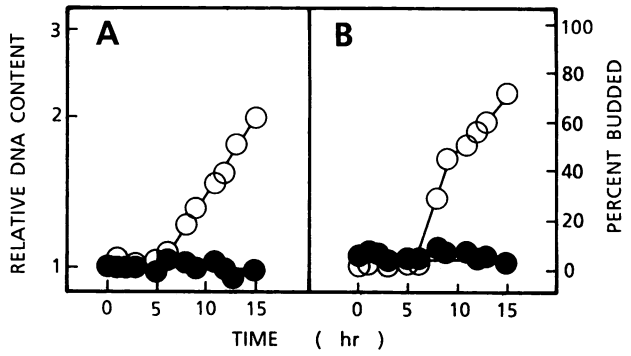

FIG. 3. DNA accumulation after stimulation of stationary-phase cells. Stationary-phase wild-type and reentry-mutant cells were stimulated by addition to fresh 14°C medium and incubation at the restrictive temperature of 14°C. Samples were removed at intervals for determinations of DNA content (A) and cell morphology (B). Values for DNA content were normalized to the value for each initial stationary-phase population. Symbols: ○, wild-type cells; ●, reentry-mutant cells.

cycle events is dependent on the regulatory step start (9), showing that in these mutant cells start is not performed. In fact, order-of-function analysis (12) demonstrated that these mutant cells become blocked in a requirement that must be satisfied before the performance of start and the resumption of proliferation. Despite the inability of these "reentry-mutant" cells to resume proliferation, mutant cells mount a normal physiological response to stimulation conditions, as indicated by the loss of stationary-phase properties at the nonpermissive temperature of 14°C (4).

Stimulation at 14°C caused both reentry-mutant and wild-type cells to accumulate protein and RNA (Fig. 1B and D) but with understandably slower kinetics than those seen at 29°C (compare the time scales in Fig. 1). Both mutant and wild-type cells also accumulated mRNA for protein 1 and for histones detected by the *TRT1* and *TRT2* probes (Fig. 2B and D and see Fig. 5; data not shown). However, the population of mutant cells remained uniformly blocked without activation of the mitotic cell cycle, as evidenced by the absence of DNA synthesis or budding after transfer to fresh medium (Fig. 3); even after extended incubation times of greater than 100 h, the population of reentry-mutant cells remained arrested (data not shown). In agreement with these findings, previous work (4) has demonstrated that stimulated reentry-mutant cells do not perform start at this nonpermissive temperature. Thus, the stimulated reentry-mutant cells induced expression of histone genes under conditions that preclude initiation of the mitotic cell cycle but allow response to stimulation conditions.

Additional confirmation that this histone gene expression occurs before the performance of start was obtained by assessing the timing of histone gene expression and the performance of start that was measured in wild-type cells stimulated to resume proliferation from stationary phase in the absence of any blocking conditions. The performance of start was determined for individual stimulated cells by microscopic inspection, by utilizing the morphological criterion of bud formation and the functional criterion of sensitivity to the yeast mating pheromone α-factor. This oligopeptide blocks the performance of start (9) and therefore inhibits bud formation (9, 36) but is without effect on bud formation for a cell that has already performed start. Sensitivity to α-factor was determined by the examination of individual cells after transfer to solid nutrient medium containing this yeast mating pheromone.

At 6 h after stimulation of wild-type cells at 14°C, a threefold increase in the relative abundance of histone mRNA had occurred when only 3% of the cells had performed start (Fig. 4). Most of this increase in mRNA must be due to cells that had not yet performed start. (For the 3% of the population that had reentered the mitotic cell cycle to be responsible for this net effect on mRNA accumulation, each of those poststart cells would have to experience a 60-fold relative increase in abundance of histone mRNA, a much greater accumulation than was seen for the entire population [Fig. 2].) Even by 8 h after stimulation, only 10% of the cells had performed start, but the relative abundance of histone mRNA had increased by greater than fivefold. Even in wild-type cells stimulated to resume proliferation from stationary phase, histone gene expression apparently begins to increase well before cells perform start to reenter the mitotic cell cycle.

To determine if both histone genes, *HTA1* and *HTB1*, detected with the *TRT1* probe were affected by stimulation of stationary-phase cells, we measured transcript abundance by using specific probes (see Materials and Methods). Upon addition of fresh medium, transcript abundance of both *HTA1* and *HTB1* within the *TRT1* locus increased (data not shown). Thus, both histone genes within the *TRT1* locus respond to stimulation conditions.

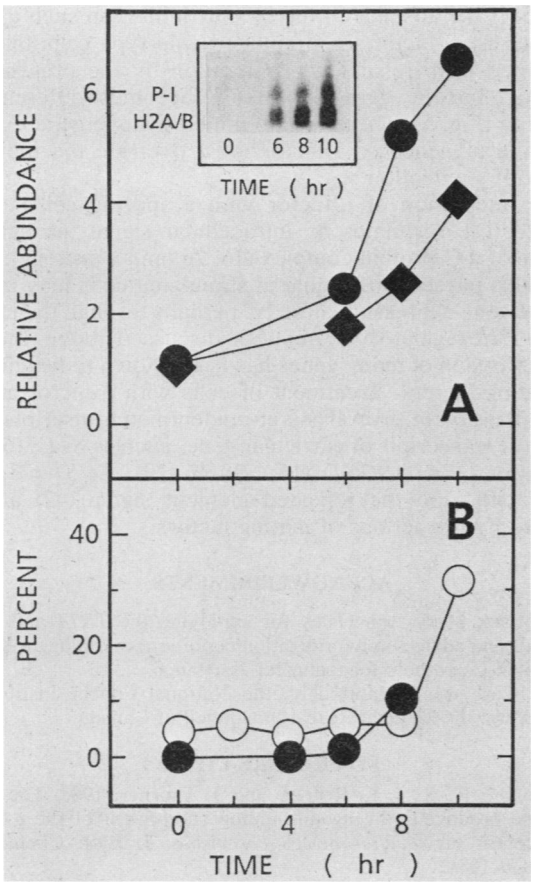

FIG. 4. Kinetics of histone transcription, start, and budding after stimulation of wild-type stationary-phase cells at 14°C. Transcripts (A [inset]) were quantified as in Fig. 2, and start was assessed by sensitivity to α-factor as described in the text. Symbols: (A) ●, histone mRNA; ◆, protein 1 mRNA; (B) ○, percent budded cells; ●, percent cells that had performed start.

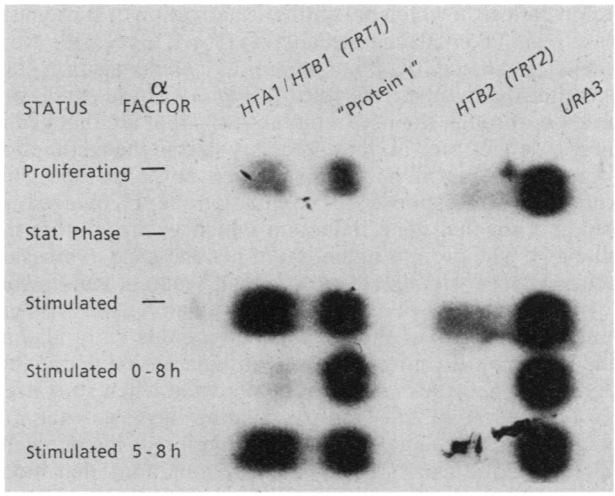

FIG. 5. Inhibition by α-factor of histone mRNA induction upon stimulation of stationary-phase cells. Stationary-phase or proliferating wild-type cells were inoculated into fresh medium and incubated at 14°C. The low temperature of 14°C was chosen to allow a comparison with mutant cells defective for reentry into the mitotic cell cycle, but the same effects of α-factor on gene expression were observed at 29°C (data not shown). To portions of the stimulated stationary-phase population was added sufficient α-factor to prevent completion of start, either at zero time or at 5 h after stimulation with fresh medium, a time when cells were still unbudded. RNA was extracted from all stimulated cells after 8 h in fresh medium and subjected to Northern analysis. Northern blots were probed consecutively, as in Fig. 2, with the indicated histone genes, the protein 1 gene, or the *URA3* gene. The autoradiographs are displayed with the direction of electrophoresis from left to right.

α-Factor inhibits histone gene expression. For cells in the mitotic cell cycle, the inhibition of start also inhibits histone gene expression (13, 44). This inhibition may simply reflect the effects of blocking initiation of the next round of DNA replication. Alternatively, conditions that inhibit start could also exert parallel inhibition of histone expression independently of the effects on start. The situation in stimulated cells, in which histone expression is not dependent on start, offers an opportunity to assay any direct effects of start-blocking conditions on histone gene expression. Therefore, an experiment was carried out to determine the effects of inhibiting start on histone gene expression after stimulation. For these particular experiments, the reentry mutant used above was inappropriate because the defect in a reentry-mutant cell affects the performance of start only indirectly. This fact was shown by an order-of-function analysis (12) indicating that, upon stimulation of stationary-phase cells at 14°C, reentry-mutant cells actually become blocked at a point in the resumption of proliferation before cells are competent to perform start (4). Presumably, start itself is unimpaired in these reentry-mutant cells.

The stimulation of stationary-phase wild-type cells was therefore undertaken in the presence of α-factor. This mating pheromone blocks the performance of start itself (9), with little effect on global biosynthetic activity (40; unpublished data). When α-factor was added to stationary-phase wild-type cells at the time of transfer to fresh medium, the stimulation-dependent induction of histone mRNA was prevented (Fig. 5). In contrast, transcripts from several other genes, including the *URA3* gene and the protein 1 gene within the *TRT1* locus, were unaffected since these transcripts persisted even in the presence of α-factor (Fig. 5). Delayed addition of α-factor to stimulated cells prevented further accumulation of histone mRNA without any effect on other mRNA species (Fig. 5). These findings suggest that α-factor inhibits expression of the histone genes in a way that is independent of the performance of start.

DISCUSSION

Stationary-phase yeast cells undergo many physiological changes when stimulated to resume proliferation, including a general increase in biosynthetic activity (45). The work presented here shows that this stimulation response includes partial induction of the histone genes that are detected by the *TRT1* and *TRT2* loci. Histone genes are expressed periodically in the mitotic cell cycle (10) and are blocked in expression by inhibition of the cell cycle regulatory step start (11, 13, 44), indicating a level of histone gene regulation that is responsive to the cell cycle. Here we have identified an additional level of regulation: histone genes are expressed independently of the mitotic cell cycle but in response to stimulation conditions. Thus these start-responsive genes are also regulated in a way that is unrelated to the mitotic cell cycle.

The regulation of histone gene expression upon stimulation was most clearly observed under a unique set of

circumstances in which biosynthetic activation of stationary-phase cells takes place normally (4) (Fig. 1), yet cells were unable to advance to a point where they could perform start to reenter the mitotic cell cycle (4) (Fig. 3). These conditions were met through the use of mutant cells that are blocked in a newly defined step (4) that precedes start in the resumption of proliferation. Only in this situation could histone gene expression in response to stimulation be recognized as distinct from that gene induction which occurs during the cell cycle. The prompt induction of histone gene expression occurs in both stimulated wild-type and mutant cells, while the unique mutant phenotype allows unambiguous assignment of the status of the cells displaying this early histone gene induction. Furthermore, this stimulation-related induction of histone genes could not be detected when start itself was blocked (Fig. 5). This observation suggests that, for studies of the stimulation-related regulation of histone gene expression, the use of the customary mutations that block start would prove fruitless. The reentry mutant used here thus offers an unparalleled opportunity to investigate stimulation-related responses of genes under complex regulation.

There is evidence from other studies that also suggests that activation of the histone genes upon stimulation takes place. Deletion of the *TRT1* locus but not the *TRT2* locus causes an abnormal sensitivity of the deletion-mutant cells to nitrogen starvation, as indicated by the decreased ability of starved mutant cells to form colonies upon transfer to solid complete medium (28). This starvation sensitivity is most likely an inability of starved cells to resume proliferation after replenishment of the medium, since *TRT1* deletion-mutant cells apparently achieve the usual stationary phase upon nitrogen starvation. Starved deletion-mutant cells display an activated heat shock response (28), one of the characteristics of stationary-phase cells (15). It was therefore suggested that these *TRT1* deletion-mutant cells might be defective in reentering the mitotic cell cycle from the stationary phase (28). The expression of this histone gene set upon stimulation of stationary-phase cells, as detected by use of the *TRT1* probe (Fig. 2), may thus be necessary for resumption of proliferation and may indicate a requirement for a basal level of expression for these genes, as observed in this study.

The timing of histone gene expression from the *TRT1* locus is known to be regulated at the level of transcription in two ways: by 16-bp UAS sequences subject to periodic activation and by an epistatic 19-bp negative regulatory sequence responsible for cell cycle-specific and DNA replication-specific expression (21, 31). *trans*-Acting regulatory proteins encoded by *HIR* genes (33) function primarily through the 19-bp negative regulatory element. Deletion of this negative regulatory sequence or mutation in one of the *HIR* genes alters the regulation of histone genes to allow transcription in the presence of the DNA replication inhibitor hydroxyurea (21, 33) or the cell cycle inhibitor α-factor (33). The increased level of histone gene expression upon stimulation of stationary-phase cells shown in the present study could therefore be brought about through effects on this negative regulatory system. In addition, the periodic expression of these histone genes is affected by posttranscriptional mechanisms (21) that could also influence the stimulation-related histone gene expression seen here.

The induction of the histone genes that occurs upon stimulation of stationary-phase cells is a consequence of two related circumstances. The first of these is, of course, the increased biosynthetic activity that results from the stimulation of stationary-phase cells. The second circumstance (Fig. 5) is the absence of direct start inhibition such as that imposed by α-factor. In stimulated wild-type cells that are prevented from resuming proliferation by the presence of α-factor, histone gene expression becomes differentially inhibited (Fig. 5). Therefore, to inhibit histone gene expression, the presence of α-factor must override the inducing effects of stimulation.

The interaction of α-factor with a specific cell surface receptor (1) modulates an intracellular signal through the actions of a G-protein complex (16, 26) and a protein kinase (39). This intracellular signal in stimulated cells may inhibit histone gene expression directly, perhaps through the effects on the *HIR* regulatory system (33) discussed above. Indeed, the expression of many genes has been shown to be affected by mating factors. Treatment of cells with α-factor affects the expression of several as-yet-unidentified transcripts (37), while the transcripts of particular genes such as *STE2* (6, 27), *STE3* (5), *GPA1* (*SCG1*) (16), *BAR1* (20), *FUS1* (23, 41), *CHS1* (30), and the repeated element sigma (42) are all induced by the actions of mating factors.

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LITERATURE CITED

1. **Blumer, K. J., J. E. Reneke, and J. Thorner.** 1988. The *STE2* gene product is the ligand-binding component of the α-factor receptor of *Saccharomyces cerevisiae*. J. Biol. Chem. **263:** 10836–10842.
2. **Bücking-Throm, E., W. Duntze, L. H. Hartwell, and T. R. Manney.** 1973. Reversible arrest of haploid yeast cells at the initiation of DNA synthesis by a diffusible sex factor. Exp. Cell Res. **76:**99–110.
3. **Drebot, M. A., C. A. Barnes, R. A. Singer, and G. C. Johnston.** 1990. Genetic assessment of stationary phase for cells of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **172:**3584–3589.
4. **Drebot, M. A., G. C. Johnston, and R. A. Singer.** 1987. A yeast mutant conditionally defective only for reentry into the mitotic cell cycle from stationary phase. Proc. Natl. Acad. Sci. USA **84:**7948–7952.
5. **Hagen, D. C., and G. F. Sprague, Jr.** 1984. Induction of the yeast α-specific *STE3* gene by the peptide pheromone a-factor. J. Mol. Biol. **178:**835–852.
6. **Hartig, A., J. Holly, G. Saari, and V. L. MacKay.** 1986. Multiple regulation of *STE2*, a mating-type-specific gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:**2106–2114.
7. **Hartwell, L. H.** 1967. Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. **93:**1662–1670.
8. **Hartwell, L. H.** 1970. Periodic density fluctuation during the yeast cell cycle and the selection of synchronous cultures. J. Bacteriol. **104:**1280–1285.
9. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38:**164–198.
10. **Hereford, L., S. Bromley, and M. A. Osley.** 1982. Periodic transcription of yeast histone genes. Cell **30:**305–310.
11. **Hereford, L. M., K. Fahrner, J. W. Woolford, Jr., M. Rosbash, and D. Kaback.** 1979. Isolation of yeast genes H2A and H2B. Cell **18:**1261–1271.
12. **Hereford, L. M., and L. H. Hartwell.** 1974. Sequential gene function in the initiation of *Saccharomyces cerevisiae* DNA synthesis. J. Mol. Biol. **84:**445–461.
13. **Hereford, L. M., M. A. Osley, J. R. Ludwig, and C. S. McLaughlin.** 1981. Cell-cycle regulation of yeast histone mRNA. Cell **24:**367–375.
14. **Iida, H., and I. Yahara.** 1984. Specific early-G1 blocks accompanied with stringent response in *Saccharomyces cerevisiae*

lead to growth arrest in resting state similar to the G0 of higher eucaryotes. J. Cell Biol. **98**:1185–1193.
15. **Iida, H., and I. Yahara.** 1984. Durable synthesis of high molecular weight heat shock proteins in G0 cells of the yeast and other eucaryotes. J. Cell Biol. **99**:199–207.
16. **Jahng, K.-Y., J. Ferguson, and S. I. Reed.** 1988. Mutations in a gene encoding the α subunit of a *Saccharomyces cerevisiae* G protein indicate a role in mating pheromone signaling. Mol. Cell. Biol. **8**:2484–2493.
17. **Johnston, G. C., R. A. Singer, and E. S. McFarlane.** 1977. Growth and cell division during nitrogen starvation of the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **132**:723–730.
18. **Johnston, S. A., and J. E. Hopper.** 1982. Isolation of the yeast regulatory gene *GAL4* and analysis of its dosage effect in the galactose/melibiose regulon. Proc. Natl. Acad. Sci. USA **79**: 6971–6975.
19. **Konrad, M.** 1988. Analysis and *in vivo* disruption of the gene coding for adenylate kinase (*ADK1*) in the yeast *Saccharomyces cerevisiae*. J. Biol. Chem. **263**:19468–19474.
20. **Kronstad, J. W., J. A. Holly, and V. L. MacKay.** 1987. A yeast operator overlaps an upstream activation site. Cell **50**:369–377.
21. **Lycan, D. E., M. A. Osley, and L. M. Hereford.** 1987. Role of transcriptional and posttranscriptional regulation in expression of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:614–621.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
23. **McCaffrey, G., F. J. Clay, K. Kelsay, and G. F. Sprague, Jr.** 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:2680–2690.
24. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Nakayama, N., Y. Kaziro, K.-I. Arai, and K. Matsumoto.** 1988. Role of *STE* genes in the mating factor signaling pathway mediated by *GPA1* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:3777–3783.
27. **Nakayama, N., A. Miyajima, and K. Arai.** 1985. Nucleotide sequences of *STE2* and *STE3*, cell type-specific sterile genes from *Saccharomyces cerevisiae*. EMBO J. **4**:2643–2648.
28. **Norris, D., and M. A. Osley.** 1987. The two gene pairs encoding H2A and H2B play different roles in the *Saccharomyces cerevisiae* life cycle. Mol. Cell. Biol. **7**:3473–3481.
29. **Oechsner, U., V. Magdolen, C. Zoglowek, U. Häcker, and W. Bandlow.** 1988. Yeast adenylate kinase is transcribed constitutively from a promoter in the short intergenic region to the histone H2A-1 gene. FEBS Lett. **242**:187–193.
30. **Orlean, P.** 1987. Two chitin synthetases in *Saccharomyces cerevisiae*. J. Biol. Chem. **262**:5732–5739.
31. **Osley, M. A., J. Gould, S. Kim, M. Kane, and L. Hereford.** 1986. Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell **45**:537–544.
32. **Osley, M. A., and L. M. Hereford.** 1981. Yeast histone genes show dosage compensation. Cell **24**:377–384.
33. **Osley, M. A., and D. Lycan.** 1987. *trans*-acting regulatory mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol. Cell. Biol. **7**:4204–4210.
34. **Penn, M. D., G. Thireos, and H. Greer.** 1984. Temporal analysis of general control of amino acid biosynthesis in *Saccharomyces cerevisiae*: role of positive regulatory genes in initiation and maintenance of mRNA derepression. Mol. Cell. Biol. **4**:520–528.
35. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast Saccharomyces. Life cycle and inheritance. Cold Spring Harbour Laboratory, Cold Spring Harbor, N.Y.
36. **Singer, R. A., D. P. Bedard, and G. C. Johnston.** 1984. Bud formation by the yeast *Saccharomyces cerevisiae* is directly dependent on "start." J. Cell Biol. **98**:678–684.
37. **Stetler, G. L., and J. Thorner.** 1984. Molecular cloning of hormone-responsive genes from the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **81**:1144–1148.
38. **Storms, R. K., R. W. Ord, M. T. Greenwood, B. Mirdamadi, F. K. Chu, and M. Belfort.** 1984. Cell cycle-dependent expression of thymidylate synthase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2858–2864.
39. **Teague, M. A., D. T. Chaleff, and B. Errede.** 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinases. Proc. Natl. Acad. Sci. USA **83**:7371–7375.
40. **Throm, E., and W. Duntze.** 1970. Mating-type-dependent inhibition of deoxyribonucleic acid synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **104**:1388–1390.
41. **Trueheart, J., J. D. Boeke, and G. R. Fink.** 1987. Two genes required for cell fusion during yeast conjugation: evidence for a pheromone-induced surface protein. Mol. Cell. Biol. **7**:2316–2328.
42. **Van Arsdell, S. W., G. L. Stettler, and J. Thorner.** 1987. The yeast repeated element sigma contains a hormone-inducible promoter. Mol. Cell. Biol. **7**:749–759.
43. **Wheals, A. E.** 1987. Biology of the cell cycle in yeasts, p. 283–390. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, 2nd ed., vol. 1. Academic Press (London), Ltd., London.
44. **White, J. H. M., S. R. Green, D. G. Barker, L. B. Dumas, and L. H. Johnston.** 1987. The *CDC8* transcript is cell cycle regulated in yeast and is expressed coordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. **171**:223–231.
45. **Williamson, D. H.** 1966. Nuclear events in synchronously dividing yeast cultures, p. 81–101. *In* I. L. Cameron and G. M. Padilla (ed.), Cell synchrony. Academic Press, Inc., New York.