Cell cycle-specific expression of the *SWI4* transcription factor is required for the cell cycle regulation of *HO* transcription

Linda Breeden¹ and Glen E. Mikesell

Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 USA

Expression of the *HO* endonuclease triggers mating-type switching in *Saccharomyces cerevisiae*. Transcription of the *HO* gene is start-dependent and restricted to the late G₁/early S phase of haploid mother cells. The *HO* promoter contains 10 copies of a cell cycle-regulated upstream activation sequence, which is activated by *SWI4* and *SWI6*. *SWI4* mRNA levels vary at least 10-fold throughout the cell cycle and rise sharply just before the rise in *HO* mRNA levels. Constitutive synthesis of *SWI4* mRNA leads to constitutive synthesis of *HO* mRNA. These data suggest that the cell cycle regulation of *SWI4* mRNA is required for the tight cell cycle regulation of *HO* transcription. High-level constitutive synthesis of *SWI4* also suppresses *swi5* and *swi6* mutations, suggesting that *SWI4* is the predominant activator of *HO* transcription and that mutations in negative regulators of *SWI4* could be isolated as suppressors of *swi6* mutations. One recessive suppressor of *swi6* (*ssx1-1*) that allowed high-level expression of *SWI4* during α -factor arrest and constitutive expression of both *SWI4* and *HO* after release from the arrest was isolated. This result suggests that *SSX1* has a negative regulatory role in the cell-cycle regulation of *SWI4* mRNA accumulation.

[Key Words: Cell cycle; transcription; yeast; *HO*; *SWI4*; *SSX1*]

Received January 24, 1991; revised version accepted April 3, 1991.

The *HO* gene encodes an endonuclease involved in mating-type switching in *Saccharomyces cerevisiae*, and it is the expression of this gene that determines when a switch will take place (Jensen and Herskowitz 1983). *HO* expression is regulated by three forms of transcriptional regulation. *HO* is not expressed in diploids because of a diploid-specific repression system (Jensen et al. 1983; Miller et al. 1985). *HO* is transcribed in haploids but only in those that have undergone cell division (mother cells). In haploid mother cells, *HO* transcription is restricted further to a specific period within the cell cycle that is just after and dependent on the commitment to the mitotic cell cycle (Nasmyth 1983). Six genes whose products are absolutely required for *HO* transcription (*SWI1*–*SWI6*) have been identified (Haber and Garvik 1977; Stern et al. 1984; Breeden and Nasmyth 1987a). Four of these gene products act on the URS1 region at about –1200 (*SWI1*–*SWI3* and *SWI5*), where mother/daughter control is exerted (Nasmyth 1985a, 1987; Breeden and Nasmyth 1987a). The other two gene products (*SWI4* and *SWI6*) act specifically on a promoter element (CACGAAA) (Breeden and Nasmyth 1987a) that is repeated 10 times between URS1 and the TATA box (Nasmyth

1985b). This CACGA₄ sequence can act as an autonomous and cell cycle-regulated upstream activation sequence (UAS) (Breeden and Nasmyth 1985) from which transcription is Start dependent and which shows the same periodic fluctuations during the cell cycle as does the intact *HO* promoter (Breeden and Nasmyth 1987a). The *SWI4* protein is part of the CACGA₄–protein complex (Andrews and Herskowitz 1989b), and complex formation depends on the activity of the *SWI6* protein (Andrews and Herskowitz 1989a).

The *SWI4* and *SWI6* genes have been cloned and sequenced. The *SWI6* protein (Breeden and Nasmyth 1987b) is 33% identical over a 420-amino-acid stretch to the *Schizosaccharomyces pombe cdc10* protein, which plays an essential role in starting the cell cycle (Nurse and Bissett 1981). Both the *SWI6* and the *cdc10* proteins contain a 33-amino-acid repeat that can be found in the *Notch* gene of *Drosophila*; the *lin-12*, *glp-1*, and *fem-1* genes of *Caenorhabditis elegans* (Breeden and Nasmyth 1987b; Yochem et al. 1988; Yochem and Greenwald 1989; Spence et al. 1990); a putative proto-oncogene *bcl-3* (Ohno et al. 1990); several viral host range genes; ankyrin (Lux et al. 1990); and a bacterial protein *phlB* (Givskov et al. 1988; L. Breeden, unpubl.). The *SWI4* protein also contains two of these repeats and bears some similarity to both the *SWI6* and the *cdc10* proteins in the

¹Corresponding author.

areas surrounding the repeats (Andrews and Herskowitz 1989b).

This paper explores the mechanism of the cell cycle regulation of *HO* transcription. It reports that *SWI4* mRNA accumulation is also regulated within the cell cycle. *SWI4* mRNA levels increase sharply in G_1 , just before *HO* mRNA levels increase. Constitutive high-level expression of *SWI4* causes high levels of *HO* transcription throughout the cell cycle. When a low level of *SWI4* is expressed constitutively, a corresponding increase in *HO* transcription throughout the cell cycle is observed. These data indicate that the cell cycle regulation of *SWI4* mRNA is required for the cell cycle regulation of *HO* transcription. The overproduction of *SWI4* also suppresses the requirement for *SWI5* and *SWI6* for *HO* transcription, suggesting that *SWI5* and *SWI6* have less direct roles in *HO* transcription than does *SWI4* and that mutations in negative regulators of *SWI4* could be isolated as suppressors of *swi6* mutations. One such mutation (*ssx1-1*), which allowed *swi6-399* cells to produce high levels of both *HO* and *SWI4* mRNAs constitutively in α -factor-synchronized cells, was isolated. This result lends further support to the view that the cell cycle regulation of *SWI4* mRNA is critical for the cell cycle regulation of *HO* transcription. It also identifies a gene product that is required for the periodic expression of *SWI4* mRNA throughout the cell cycle.

Results

SWI4 mRNA levels are cell cycle regulated

SWI4 and *SWI6* are required for cell cycle-regulated transcription from the CACGA₄ sequences in the *HO* promoter. To determine whether either *SWI4* or *SWI6* is expressed differentially during the cell cycle, we monitored the mRNA levels through two synchronous cell

divisions. Wild-type cells were arrested in G_1 with α -factor and inoculated into fresh media. Samples were removed at 5-min intervals through at least two cell cycles, and RNA was extracted and quantitated by S1 nuclease protection. Figure 1A displays *HO*, *SWI6*, and *MATa1* mRNA accumulation through two cell cycles. The *MATa1* message levels remained constant and served as an internal control. *HO* mRNA accumulation showed a typically small peak in the first cycle (15 min) and a large peak in the second cycle (75 min) after the release from α -factor (see also Breedon and Nasmyth 1987a). There was no detectable *HO* mRNA accumulation between these peaks, indicating very tight cell cycle regulation of *HO* and a high degree of synchronization of these dividing cells. Quantitation of these data showed that there was at least a 100-fold difference between the *HO* mRNA levels over the course of the second cell cycle (see Fig. 2).

The *SWI6* message was detectable throughout the cycle; however, it underwent a modest but reproducible variation in level and peaked after the peak of *HO* mRNA (Fig. 1A). The observed delay in *SWI6* expression with respect to *HO* expression was similar to that observed with *SWI5* (Nasmyth et al. 1987b), but the significance of this threefold variation in *SWI6* mRNA levels is not known. Constitutive synthesis of *SWI6* mRNA from the *GAL* promoter has no impact on the cell cycle regulation of *HO* mRNA (K. Neary, unpubl.).

SWI4 mRNA levels were measured in the same RNA samples and also showed periodicity. The levels of *SWI4* mRNA varied at least 10-fold over the cell cycle and began to rise ~5 min before the rise in *HO* mRNA levels (Fig. 1B). Figure 2 is a compilation of the primary data presented in Figure 1, quantitated by video densitometry and normalized to the *MATa1* internal control. The data for the second cell cycle are the most reliable reflection of the mRNA accumulation profile during normal mitotic growth because they are the least likely to be af-

Figure 1. *SWI4* mRNA levels are cell cycle regulated. Wild-type (W303) cells were grown to an OD₅₅₀ of ~0.25 in YEP containing glucose and arrested in G_1 by a 90-min incubation in the presence of 3 μ g of α -factor per milliliter. RNA was collected from the arrested cells and collected again at 5-min intervals after release from the arrest. The levels of mRNA for *HO*, *SWI6*, and *MATa1* (A) and for *SWI4* and *MATa1* (B) were monitored by S1 protection in two separate experiments but with the same RNA samples. The *MATa1* transcript levels serve as an internal control for the amount of total RNA analyzed at each time point. The *MATa1* transcript is spliced, resulting in four different size classes of protected fragments (Miller 1984). The two largest sets of fragments corresponding to RNAs ~490 and 310 bases long are shown (a1).

Figure 2. Profile of *SWI4*, *HO*, and *SWI6* mRNA accumulation during the mitotic cell cycle. The *SWI4*, *HO*, and *SWI6*, and one of the *MATa1* bands on the autoradiographs shown in Fig. 1 were quantitated by video densitometry and whole band analysis with a Visage 2000 system from BioImage Co. The values plotted have been normalized to the level of *MATa1* mRNA to control for experimental error. (▲) Profile of *SWI4* mRNA accumulation; (□) *HO* mRNA; (■) *SWI6* mRNA. The first point reflects the levels of these mRNAs in α -factor-arrested cells.

ected by the method of synchronization. In the second cell cycle, it is clear that the *SWI4* mRNA levels rise and peak immediately before the peak in *HO* mRNA levels. This profile of *SWI4* mRNA accumulation is consistent with the possibility that the regulation of *SWI4* mRNA levels is responsible for the cell cycle regulation of *HO*.

Cell cycle regulation of *HO* is reduced dramatically when *SWI4* is expressed constitutively from the *GAL* promoter

To determine whether the cell cycle regulation of *SWI4* was required for the cell cycle regulation of *HO*, we constructed a hybrid gene in which transcription of the *SWI4* gene was driven by the *GAL1-10* promoter (p*GAL* : *SWI4*). In cells carrying p*GAL* : *SWI4* and grown in galactose, the plasmid-borne *SWI4* gene was transcribed constitutively at high levels throughout the cell cycle (Fig. 3A). When the *HO* mRNA levels were measured in the same samples, they also showed high accumulation throughout the cell cycle (Fig. 3B). The *HO* transcript level was low in α -factor-arrested cells, but 10 min after release it reached a high level that was maintained throughout the first cycle and then increased about threefold in the second cycle. To confirm that these cells continued to carry out other cell cycle-regulated events, we also monitored the histone *H2B* mRNA level and found that it displayed the normal pattern of periodic expression (Hereford et al. 1981) (Fig. 3A). This is a particularly important control, because cells that overproduce *SWI4* are morphologically abnormal (see Fig. 5). The periodic expression of histones confirms that

another cell cycle-regulated process, which occurs after the peak of *SWI4* expression, is unperturbed and is carried out in a synchronous manner in these cells.

Both the timing of *SWI4* mRNA accumulation and the effect of constitutive expression of *SWI4* are consistent with the idea that the cell cycle regulation of *SWI4* is responsible for the cell cycle regulation of *HO*. However, this is not the only interpretation. *SWI4* was originally cloned as a suppressor of a *swi6* mutation (Breedon and Nasmyth 1987b), so it was expected that the 30-fold overproduction of *SWI4* from the *GAL* promoter would suppress *swi6-399* and possibly other *swi* mutations. A survey of the effects of *SWI4* overproduction on the six principle *swi* mutations (Table 1) showed that both *SWI5* and *SWI6* are dispensable for *HO* transcription when *SWI4* expression is fully induced from the p*GAL* : *SWI4* plasmid. This being the case, the loss of the cell cycle regulation of *HO* under these conditions could be due to constitutive expression of *SWI4* and/or to the loss of cell cycle regulation by *SWI5* or *SWI6*. For example, it has been shown that the *SWI5* protein accumulates in the nucleus only during G_1 of the cell cycle (Nasmyth et al. 1990). This accumulation probably contributes to the cell cycle regulation of *HO* in wild-type cells, but this contribution would be less evident in cells overproducing *SWI4*, in which *SWI5* is not required for *HO* transcription.

Figure 3. Constitutive overproduction of *SWI4* by p*GAL* : *SWI4* disrupts *HO* cell cycle regulation. W303 cells carrying p*GAL* : *SWI4* were initially grown in glucose, selecting for the plasmid, and then transferred to YEP containing galactose for 90 min before α -factor addition. RNA was purified from cells sampled during the arrest (left-most lane) and 5, 10, 20, 30 min, etc., through two cell cycles after release. The levels of genomic *SWI4*, *GAL* : *SWI4*, and *H2B* transcripts (A) and *HO* transcripts (B) were monitored by S1 protection and compared with that of the 310-bp protected fragment of the *MATa1* transcript, which is made constitutively and serves as an internal control (IC).

Table 1. *HO* transcription in cells carrying pGAL : *SWI4*

	Raffinose	Galactose
W303 <i>SWI</i>	5.0	ND
BY556 <i>swi1-2</i>	0.1	0.1
BY528 <i>swi2-314</i>	0.8	0.4
BY572 <i>swi3-1</i>	0.1	0.2
BY658 <i>swi4 : LEU2</i>	0.2	4.0
BY562 <i>swi5-100</i>	0.2	2.1
BY660 <i>swi6 : TRP1</i>	0.1	3.9

HO and *MATa1* transcription were measured by S1 protection and quantitated using video densitometry and whole-band analysis with a Visage 2000 system from BioImage Co. These numbers represent the levels of *HO* transcription normalized to the *MATa1* mRNA. ND denotes that transcript levels were not determined.

To determine the effect of constitutive expression of a low level of *SWI4* mRNA, we transformed a *swi4 gal80* strain (BY961) with pGAL : *SWI4*. In this strain the only source of *SWI4* is the pGAL : *SWI4* plasmid, and a low constitutive level of *SWI4* expression can be obtained by growing the cells in glucose. Under these conditions, the *GAL* promoter is neither induced (by galactose) nor repressed (by *GAL80*) and a low constitutive level of *GAL* promoter activity results (Guarente et al. 1982). The level of *SWI4* mRNA production in these cells only partially complements the *swi4* mutation and does not suppress *swi5* or *swi6* mutations (L. Breedon and J. Sidorova, unpubl.). The α -factor synchrony experiment was repeated with these glucose-grown cells, and the results are depicted in Figure 4. Figure 4A shows the low constitutive level of *SWI4* mRNA accumulation in these cells, and Figure 4B displays the effect on *HO* mRNA accumulation. Despite the reduced level of *HO* mRNA overall, the *HO* transcript was observed throughout the cell cycle. The *HO* mRNA level was lower in the first cycle after the arrest than in the second cycle, and peaks of *HO* expression were evident; however, in contrast to the situation in wild-type cells (Fig. 1A), *HO* mRNA accumulated throughout the cell cycle. The hybridization and labeling conditions used in this experiment were the same as those used in Figure 1, and the intensities of the 310-bp *MATa1* control RNAs were roughly equivalent in the two experiments. However, the basal level of *HO* mRNA was much higher in cells that produced a low constitutive level of *SWI4* mRNA (Fig. 4B) than in wild-type cells (Fig. 1). From this result it must be concluded that even a low level of *SWI4* mRNA accumulation throughout the cell cycle is sufficient to disrupt the cell cycle regulation of *HO*. The fact that cell cycle regulation was not abolished in this experiment indicates that the regulation of *SWI4* mRNA accumulation is not the only source of the cell cycle regulation of *HO* transcription.

Cells that overproduce SWI4 are morphologically abnormal

Deletion of the *SWI4* or *SWI6* gene causes cells to grow to twice the size of wild-type cells and to grow 40%

slower, and the *swi4-29ts swi6-399* double mutants die at the nonpermissive temperature (Breedon and Nasmyth 1987b). To determine whether the constitutive 30-fold overproduction of *SWI4* that is induced from pGAL : *SWI4* is also deleterious, we grew wild-type cells carrying pGAL : *SWI4* in raffinose, induced *SWI4* expression by adding galactose, and monitored growth by phase microscopy for several hours. Within 2 hr after the galactose addition, the new buds were visibly elongated, and within another 30 min, some of them were twice the length of the mother cell. The phase-contrast image shown in Figure 5 compares wild-type cells (right) to those carrying pGAL : *SWI4* after 4 hr of galactose-induced overproduction of *SWI4* (left). Most cells have an elongated appearance, and some are three times the length of their parent cells.

SSX1 is a negative regulator of SWI4 expression

The overproduction of *SWI4* leads to the suppression of mutations in *SWI5* and *SWI6* (Table 1) and the constitu-

Figure 4. Low-level constitutive expression of *SWI4* increases *HO* transcription throughout the cell cycle. The synchrony experiment shown in Fig. 3 was repeated with BY961 [*swi4 gal80*] transformed with pGAL : *SWI4* and grown in glucose. Under these conditions, the plasmid-borne *SWI4* gene was expressed at ~50% of the peak levels of genomic *SWI4* transcription and was the only source of *SWI4*. In this experiment, the 310-bp protected fragment of the *MATa1* transcript was used as the internal control (IC). The dark band above the *HO* band is the undigested *HO* probe, which varied in intensity from experiment to experiment.

Figure 5. *SWI4* overproducers have an abnormal elongated morphology. W303 cells transformed with the pGAL : *SWI4* plasmid (left) or the vector alone (right) were grown in raffinose medium, and galactose was added to induce *SWI4* expression. These phase-contrast photographs were taken 4 hr after the addition of galactose.

tive expression of *HO*. This result suggests that mutations in negative regulators of *SWI4* expression might also be isolated as suppressors of either *swi5* or *swi6* mutations. With this in mind, a collection of *ssx* mutations that suppressed the requirement for *SWI6* for *HO : lacZ* transcription (L. Breeden and T. Quinton, in prep.) was screened for those that produced high levels of *HO* mRNA. The most efficient *ssx* mutants were screened for deregulated *SWI4* mRNA accumulation in α -factor-synchronized cells. One mutant that showed a complete loss of the cell cycle regulation of *SWI4* was found (Fig. 6). In contrast to wild-type cells, the *swi6-399 ssx1-1* cells maintained a high level of *SWI4* mRNA during α -factor arrest and for 160 min after release. As in the galactose-induced cells carrying pGAL : *SWI4*, the *HO : lacZ* mRNA level in the *swi6-399 ssx1-1* cells was low during α -factor arrest, suggesting that some other regulation of *HO* is exerted under these conditions. However, after release from the arrest, *HO : lacZ* transcription increased to a high level and varied little throughout the course of the experiment. Histone *H2B* mRNA levels were also monitored in the same samples, and periodic expression of *H2B* mRNA was observed. This result confirms that the cell cycle signals that govern *H2B* expression occurred normally and in a fairly synchronous manner.

The *ssx1-1* mutation is a recessive mutation that allows constitutive expression of *HO* in a *swi6-399* strain. This suppressor phenotype segregates 2 : 2 in tetrads in crosses with an *SSX swi6 : TRP1* strain (BY603), and the suppressor and deregulated *SWI4* phenotypes remain linked after three backcrosses (R. Foster, unpubl.). These results suggest that the suppression of *swi6-399* and the deregulation of *SWI4* mRNA accumulation are both due to mutation of a single gene.

Discussion

The *HO* promoter is an unusual yeast promoter in that sequences that are >1 kb upstream and required in *cis*

for *HO* transcription (Breeden and Nasmyth 1985; Nasmyth 1985a) have been identified. *SWI1*, *SWI2*, *SWI3*, and *SWI5* act through this distal region, called URS1 (Breeden and Nasmyth 1987a), but mutations in all of these *SWI* genes can be suppressed by mutations in other genes (*SIN1*, *SIN2*, *SIN3*) (Nasmyth et al. 1987a; Sternberg et al. 1987). This fact suggests that *SIN1*–*SIN3* exert negative effects on *HO* transcription and that *SWI1*, *SWI2*, *SWI3*, and *SWI5* act indirectly by eliminating this repression. The region between URS1 and the start of *HO* transcription (URS2) contains 10 copies of the sequence CACGA₄ (Nasmyth 1985b), which is a UAS whose activity is both Start-dependent and restricted to the late G₁/early S phase of the cell cycle (Breeden and Nasmyth 1987a). The *SWI4* and *SWI6* proteins are specifically required for this UAS activity (Breeden and Nasmyth 1987a). Efficient suppressors of *swi4* mutations have not been identified (L. Breeden and T. Quinton, in prep.), and overproduction of *SWI4* can bypass the need for both *SWI5* and *SWI6* in *HO* transcription (Table 1). These results suggest that *SWI4* acts directly as the

Figure 6. The *SSX1* gene product is required for the cell cycle regulation of *SWI4* and *HO* transcription. The upper panel shows *SWI4* and *SIR3* [internal control (IC)] mRNA levels in BY178 (*swi6-399 ssx1-1 HO : lacZ46 mat : LEU2 ura3*) synchronized as shown in Fig. 1. With the *SWI4* probe used in this experiment a smaller fragment than those in Fig. 1 was protected (see Materials and methods). Use of this probe allowed the two primary start sites for transcription to be easily visualized. The cell cycle regulation of *SWI4* in wild-type cells (W303) was reconfirmed with this probe (data not shown). The lower panel shows the reprobing of the same RNA preparations with an *HO : lacZ* probe, histone *H2B*, and *SIR3* as the internal control. In this experiment, the left-most lane is a measurement of mRNA levels during balanced growth, the second lane represents the α -factor-arrested cells, and the rest of the lanes represent mRNAs made from cells 5, 10, 20, 30 min, etc., after release.

primary positive activator of *HO* transcription through the CACGA₄ sequence. The role of *SWI6* in *HO* transcription is not yet clear, but *SWI6* may act less directly because *swi6* mutations can be suppressed either by higher levels of *SWI4* (Table 1) or by mutations in *SSX1* (Fig. 5) and several other genes (L. Breedon and T. Quinton, in prep.).

Cell cycle regulation of *SWI4*

The data presented in this paper show that during balanced mitotic growth, *SWI4* mRNA levels are cell cycle regulated. The *SWI4* message levels fluctuate at least 10-fold over the course of a cell cycle and begin to rise just before the rise in *HO* transcription. When *SWI4* mRNA is produced constitutively, even at low levels, the extent of *HO* mRNA accumulation throughout the cell cycle is considerably higher than that in wild-type cells. This result suggests that the *SWI4* protein is normally present only during late G₁, but that when *SWI4* mRNA is produced continuously, *SWI4* mRNA can be translated and the *SWI4* protein can activate *HO* transcription throughout the mitotic cell cycle. If there are any post-translational modifications of *SWI4*, they must either occur in a cell cycle-independent manner or not be absolutely essential for *SWI4* activity.

In the pGAL : *SWI4* plasmid, the *SWI4* open reading frame and its downstream sequences are linked to the *GAL1-10* promoter. The *SWI4* transcript produced from this plasmid displays no cell cycle regulation, whether it is expressed at high or low levels (Figs. 3 and 4). This means that the *SWI4*-coding sequence and 3' mRNA tail are not involved in the cell cycle regulation. It remains to be determined whether the untranslated leader or the *SWI4* promoter itself is the target of cell cycle regulation.

The observation that the overproduction of *SWI4* makes *HO* transcription *SWI6*-independent was exploited to look for mutations in negative regulators of *SWI4*. The *SSX1* gene was identified in this way. A recessive mutation in this gene causes *swi6-399* cells to constitutively express both the *SWI4* and the *HO : lacZ* transcripts. Because these cells show exactly the same pattern of *HO* expression as that seen with constitutive overproduction of *SWI4* from the pGAL : *SWI4* plasmid, the simplest interpretation is that some aspect of the negative regulation of *SWI4* expression is defective in the *swi6-399 ssx1-1* strain and that this loss of *SWI4* regulation leads to the loss of the cell cycle regulation of *HO*. Another possibility that cannot be excluded at this time is that *SWI4* mRNA accumulation and *HO* mRNA accumulation are coordinately regulated and are therefore simultaneously deregulated in *swi6-399 ssx1-1* cells. This possibility is unlikely, because *SWI4* mRNA accumulation and *HO* mRNA accumulation are deregulated to different extents in this mutant strain. Furthermore, *SWI4* and *SWI6*, which are known to be required for the cell cycle-regulated transcription of *HO*, are not required for the accumulation of *SWI4* mRNA in asynchronous cultures (L. Breedon, unpubl.). Studies are under way to determine whether *SSX1* is a direct negative regulator of

SWI4 transcription or whether it acts farther upstream in the signaling pathway that induces *SWI4* expression.

The loss of *SWI4* activity reduces the growth rate of the cells and makes them abnormally large (Breedon and Nasmyth 1987b). Constitutive overproduction of *SWI4* is also deleterious and leads to abnormal cell size and highly elongated cells. This result suggests that the dosage or timing of *SWI4* protein production is also important for balanced cell growth and division. *SWI4* is a transcriptional activator of *HO*, but *HO* is a nonessential gene. Therefore, it must be concluded that *SWI4* regulates the expression of other genes or that it has a second, unknown function. If there are other *SWI4*-regulated genes, they would be of interest because they would be expressed during the G₁-to-S transition, and the absence or continuous overproduction of their gene products might have deleterious effects on cell growth.

Cell cycle regulation of *HO*

There are six *SWI* genes that are absolutely required for *HO* transcription, and there are at least as many putative negative regulators. The data presented in this paper suggest that *SWI4* is the predominant activator of *HO* transcription. Overproduction of *SWI4* resulted in *HO* transcription that was independent of both *SWI5* and *SWI6* and virtually abolished the cell cycle regulation of *HO* transcription. In cells that produced a low constitutive level of the *SWI4* transcript, the *HO* transcript was also easily detectable throughout the cell cycle. Peak accumulation of *HO* mRNA still occurred at the appropriate times, but the tight cell cycle regulation of *HO* was disrupted. In *swi6-399 ssx1-1* cells, an intermediate level of *SWI4* mRNA was produced constitutively and there was a dramatic loss of the cell cycle regulation of *HO*. From these three experiments, it is clear that the cell cycle regulation of *SWI4* plays an important role in the cell cycle regulation of *HO* transcription.

However, in all three of these experiments there was evidence of residual cell cycle regulation. Despite the fact that *SWI4* mRNA was made constitutively, *HO* mRNA levels were low in α -factor and continued to peak in late G₁. This result leads to a second conclusion—that the regulation of *SWI4* mRNA accumulation is not the only source of the cell cycle regulation of *HO*. It is likely that the cell cycle regulation of *SWI5* (Nasmyth et al. 1987b, 1990) and an additional, uncharacterized form of regulation are required to fully account for the cell cycle regulation of *HO* transcription. The nuclear localization of *SWI5* during G₁ could account for the residual peaks of *HO* mRNA when *SWI4* is supplied constitutively, but it cannot explain the reduction in α -factor-arrested cells. *HO* is not transcribed during α -factor arrest, and this repression is exerted upon sequences within URS2. When URS2 is deleted, the resulting (*SWI1*-, *SWI2*-, *SWI3*- and *SWI5*-dependent) promoter is active in α -factor-arrested cells (Nasmyth 1985a). Furthermore, the CACGA₄ UAS, which resides within URS2 and depends on *SWI4* and *SWI6* for activity, is transcriptionally inactive in α -factor-arrested cells (Breedon and Nasmyth

1987a). The fact that high levels of *SWI4* mRNA cannot overcome α -factor repression of *HO* suggests that another form of regulation is exerted upon either the CACGA₄ sequence or another sequence within URS2. Many genes that are induced by α -factor have been identified (for review, see Cross et al. 1988), and two of the G₁ cyclins (*CLN1* and *CLN2*) (Wittenberg et al. 1990) as well as two transcripts of unknown function (Stetler and Thorner 1984) are repressed in the presence of α -factor. The absence of *HO* transcription in α -factor-arrested cells could be due to a common mechanism of pheromone-induced repression. Alternatively, it may be explained by additional regulation or modification of the *SWI4* or *SWI6* proteins.

There are only ~20 nonhistone genes whose message levels fluctuate in the cell cycle of *S. cerevisiae* (for review, see Andrews and Herskowitz 1990; Johnston 1990), and three of the gene products are involved in controlling the expression of *HO* endonuclease, which initiates mating-type switching. It is not surprising that switching activity is carefully regulated within the cell cycle, but there is a surprising degree of complexity in the mechanism of this regulation. Three of the *SWI* genes (*SWI4*–*SWI6*) are cell cycle regulated at the RNA level, and the nuclear localization of the *SWI5* protein is also cell cycle regulated. Despite these four sources of cell cycle regulation, further complexity must be invoked to fully explain the cell cycle regulation of *HO*.

Materials and methods

Strains and plasmids

W303 [*MATa ade2-1 trp1-1 can1-100 leu2-3,115 his3-11,15 ura3 ho*] from R. Rothstein was used as the wild-type strain in these experiments, unless specified otherwise. The *swi4* deletion mutation in BY658 was generated by replacing a wild-type *SWI4* gene in BY87 (an *HO* : *lacZ46* diploid derivative of W303) with the *swi4* : *LEU2*-194 deletion. The *swi4* : *LEU2*-194 deletion allele removes a 2-kb *Bam*HI–*Pst*I fragment of the *SWI4* sequence and replaces it with an oppositely oriented *LEU2* gene on a 2.2-kb *Xho*I–*Sal*I fragment. The *swi6* : *TRP1*-198 deletion in BY660 and BY603 has been described previously (Breedon and Nasmyth 1987b). The other *swi* strains used in this study are listed in Table 1 and have been described previously (Breedon and Nasmyth 1987a). BY961 [*MATa swi4* : *LEU2*-194 *gal80* : *LEU2 ho*] was made by crossing two related strains: DY924 (W303 with the *GAL80* locus replaced by *gal80* : *LEU2*), which was a gift from D. Stillman, and BY658.

BY178 [*mat* : *LEU2 swi6*-399 *HO* : *lacZ46 ura3 ssx1-1*] was isolated in a screen for suppressors of *swi6*-399, which will be described in detail elsewhere (L. Breedon and T. Quinton, in prep.). In brief, cells carrying *swi6*-399 and *HO* : *lacZ46* were mutagenized with ethylmethanesulfonate, and the survivors were screened for the ability to express β -galactosidase activity from the *HO* : *lacZ46* fusion in the absence of *swi6*-399 function. The mutations were tested for dominance and placed in complementation groups essentially as described by Breedon and Nasmyth (1987a). A single mutant allele of *SSX1* was obtained in this screening.

The pGAL : *SWI4* plasmid is a derivative of plasmid 2.2808 (Johnston and Davis 1984) in which the *SWI4* gene replaces the *HIS3* gene. For pGAL : *SWI4* construction, an *Eco*RI site was

generated in the *SWI4* gene by oligonucleotide-directed mutagenesis of an A at position –3 from the ATG (Amersham kit, version 2), and the *Eco*RI–*Pst*I and *Pst*I–*Sal*I fragments containing the *SWI4* open reading frame from BD147 were inserted into *Eco*RI/*Sal*I-cut 2.2808. BD147, a plasmid carrying the *SWI4* gene, was isolated from the M111 *TRP1 CEN3* clone bank (made by Leslie Bell) by complementation of *swi4*-100 (L. Breedon, unpubl.).

Growth conditions

Strains were grown in YEP or minimal media or on plates that were supplemented with 2% glucose, 2% galactose, or 2% raffinose as needed and other essential nutrients as required (Sherman et al. 1982). For synchrony experiments, plasmid-bearing strains were grown overnight in uracil-deficient minimal medium, transferred to rich medium, and grown to an OD₅₅₀ of ~0.2 before α -factor was added (3–5 μ g/ml). α -Factor arrests and synchronous cell cycles were monitored by phase microscopy. For galactose inductions in cells carrying plasmids, overnight cultures were grown in uracil-deficient minimal medium containing raffinose and transferred to YEP plus 2% galactose for ~2 hr before α -factor addition.

Transcript analysis

RNA was prepared and S1 protection experiments were carried out essentially as described by Nasmyth (1983). Uniformly labeled probes homologous to each message were made by primer extension of M13 single-stranded DNA. The *HO*, *HO* : *lacZ*, *H2B* (*HTB1*), *MATa1*, and *SIR3* probes have been described previously (Miller 1984; Miller et al. 1985; Nasmyth 1985a,b; Breedon and Nasmyth 1987a). The *SWI4* probe used in Figures 1 and 2 includes DNA from about –300 to +600 (from the ATG) cloned into M13mp18. The *SWI4* probe used in Figure 5 includes DNA from about –500 to –12 cloned into M13mp19.

Acknowledgments

We thank all of our colleagues at the Fred Hutchinson Cancer Research Center, especially Hal Weintraub, Steve Hahn, and Ginger Zakian, for stimulating discussions and helpful comments on the manuscript. Special thanks are also due to R. Foster, K. Neary, and J. Sidorova, who have allowed us to cite their unpublished data, and David Stillman for providing the *gal80* deletion strain. This work was supported by National Institutes of Health grant GM41073 and American Cancer Society grant JFRA-234.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Andrews, B.J. and I. Herskowitz. 1989a. Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. *Cell* **57**: 21–29.
- . 1989b. The yeast *SWI4* protein contains a motif present in developmental regulators and is part of a complex involved in cell-cycle-dependent transcription. *Nature* **342**: 830–833.
- . 1990. Regulation of cell cycle-dependent gene expression in yeast. *J. Biol. Chem.* **265**: 14057–14060.
- Breedon, L. and K. Nasmyth. 1985. Regulation of the yeast *HO*

- gene. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 643–650.
- . 1987a. Cell cycle control of the yeast *HO* gene: *cis*- and *trans*-acting regulators. *Cell* **48**: 389–397.
- . 1987b. Similarity between cell-cycle genes of budding yeast and fission yeast and the *Notch* gene of *Drosophila*. *Nature* **329**: 651–654.
- Cross, F., L.H. Hartwell, C. Jackson, and J.B. Konopka. 1988. Conjugation in *Saccharomyces cerevisiae*. *Annu. Rev. Cell Biol.* **4**: 429–457.
- Givskov, M., L. Olsen, S. Molin. 1988. Cloning and expression in *Escherichia coli* of the gene for extracellular phospholipase A1 from *Serratia liquefaciens*. *J. Bacteriol.* **170**: 5855–5862.
- Guarente, L., R. Yocum, and P. Gifford. 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulator as an upstream site. *Proc. Natl. Acad. Sci.* **79**: 7410–7414.
- Haber, J.E. and B. Garvik. 1977. A new gene affecting the efficiency of mating type interconversions in homothallic strains of *S. cerevisiae*. *Genetics* **87**: 33–50.
- 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.
- Jensen, R.E. and I. Herskowitz. 1983. Directionality and regulation of cassette substitution in yeast. *Cold Spring Harbor Symp. Quant. Biol.* **48**: 97–104.
- Jensen, R., G.F. Sprague, and I. Herskowitz. 1983. Regulation of yeast mating type interconversion: Feedback control of *HO* gene expression by the mating type locus. *Proc. Natl. Acad. Sci.* **80**: 3035–3039.
- Johnston, H.M. and R.W. Davis. 1984. Sequences that regulate the divergent *gal1-gal10* promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **4**: 1440–1448.
- Johnston, L.H. 1990. Periodic events in the cell cycle. *Curr. Opin. Cell Biol.* **2**: 274–279.
- Lux, S.E., K.M. John, and V. Bennett. 1990. Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell cycle control proteins. *Nature* **344**: 36–42.
- Miller, A.M. 1984. The yeast *MATa1* gene contains two introns. *EMBO J.* **3**: 1061–1065.
- Miller, A.M., V.L. MacKay, and K. Nasmyth. 1985. Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast. *Nature* **314**: 598–603.
- Nasmyth, K. 1983. Molecular analysis of a cell lineage. *Nature* **302**: 670–676.
- . 1985a. At least 1400 base pairs of 5' flanking DNA is required for the correct expression of the *HO* gene in yeast. *Cell* **42**: 213–223.
- . 1985b. A repetitive DNA sequence that confers cell cycle START (CDC28)-dependent transcription of the *HO* gene in yeast. *Cell* **42**: 225–235.
- . 1987. The determination of mother cell-specific mating type switching in yeast by a specific regulator of *HO* transcription. *EMBO J.* **6**: 243–248.
- Nasmyth, K., D. Stillman, and D. Kilping. 1987a. Both positive and negative regulators of *HO* transcription are required for mother-cell-specific mating-type switching in yeast. *Cell* **48**: 579–587.
- Nasmyth, K., A. Seddon, and G. Ammerer. 1987b. Cell cycle regulation of *SWI5* is required for mother-cell-specific *HO* transcription in yeast. *Cell* **49**: 549–558.
- Nasmyth, K., G. Adolf, D. Lydall, and A. Seddon. 1990. The identification of a second cell cycle control on the *HO* promoter in yeast: Cell cycle regulation of *SWI5* nuclear entry. *Cell* **62**: 631–647.
- Nurse, P. and Y. Bissett. 1981. Gene required in G1 for commitment to the cell cycle and in G2 for control of mitosis in fission yeast. *Nature* **292**: 558–560.
- Ohno, H., G. Takimoto, and T.W. McKeithan. 1990. The candidate proto-oncogene *bcl-3* is related to genes implicated in cell lineage determination and cell cycle control. *Cell* **60**: 991–997.
- Sherman, F., G.R. Fink, and J.B. Hicks. 1982. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Spence, A.M., A. Coulson, and J. Hodgkin. 1990. The product of *fem-1*, a nematode sex-determining gene, contains a motif found in cell control proteins and receptors for cell-cell interactions. *Cell* **60**: 981–990.
- Stern, M., R. Jensen, and I. Herskowitz. 1984. Five *SWI* genes are required for expression of the *HO* gene in yeast. *J. Mol. Biol.* **178**: 853–868.
- Sternberg, P.W., M.J. Stern, I. Clark, and I. Herskowitz. 1987. Activation of the yeast *HO* gene by release from multiple negative controls. *Cell* **48**: 567–577.
- Stetler, G.L. and J. Thorner. 1984. Molecular cloning of hormone-responsive genes from the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **81**: 1144–1148.
- Wittenberg, C., K. Sugimoto, and S.I. Reed. 1990. G1-specific cyclins of *S. cerevisiae*: Cell cycle periodicity, regulation by mating pheromone, and association with the p34^{CDC28} protein kinase. *Cell* **62**: 225–237.
- Yochem, J., and I. Greenwald. 1989. *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. *Cell* **58**: 553–563.
- Yochem, J., K. Weston, and I. Greenwald. 1988. The *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila Notch*. *Nature* **335**: 547–549.

Cell cycle-specific expression of the SWI4 transcription factor is required for the cell cycle regulation of HO transcription.

L Breeden and G E Mikesell

Genes Dev. 1991, **5**:

Access the most recent version at doi:[10.1101/gad.5.7.1183](https://doi.org/10.1101/gad.5.7.1183)

References

This article cites 34 articles, 8 of which can be accessed free at:
<http://genesdev.cshlp.org/content/5/7/1183.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).