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

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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 a-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 [CACGAAAT] [Breeden and Nasmyth 1987b] that is repeated 10 times between URS1 and the TATA box [Nasmyth 1985b]. This CACGAAAT 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 CACGAAAT-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 GENES & DEVELOPMENT 5:1183–1190 © 1991 by Cold Spring Harbor Laboratory Press ISSN 0890-9369/91 $3.00 1183

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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 G1, 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-I], which allowed swi6-399 cells to produce high levels of both HO and SWI4 RNAs 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 CACGA 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 G1 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 MATα1 mRNA accumulation through two cell cycles. The MATα1 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 Breeden 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 MATα1 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 OD 650 of ~0.25 in YEP containing glucose and arrested in G1 by a 90-min incubation in the presence of 3 μg 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 MATα1 (A) and for SWI4 and MATα1 (B) were monitored by S1 protection in two separate experiments but with the same RNA samples. The MATα1 transcript levels serve as an internal control for the amount of total RNA analyzed at each time point. The MATα1 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].

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Cold Spring Harbor Laboratory Press on July 19, 2018 - Published by genesdev.cshlp.orgDownloaded from
promoter
lated events, we also monitored the histone
is a particularly important control, because cells that
periodic expression (Hereford et al. 1981)(Fig. 3A). This
rain after release it reached a high level that was main-
level and found that it displayed the normal pattern of
accumulation throughout the first cycle and then increased
about threefold in the second cycle. To confirm that
SWI4 overproduce
in galactose, the plasmid-borne
was required for the cell cycle regulation of
SWI4, GAL : SWI4,
and SWI6 are dispensable for HO transcription when
SWI4 expression is fully induced from the pGAL : 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 G1 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 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. (A) Profile of SWI4 mRNA accumulation; (B) HO mRNA; (C) SWI6 mRNA. The first point reflects the levels of these mRNAs in α-factor-arrested cells.

**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 [pGAL : SWI4]. In cells carrying pGAL : 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

**Figure 3.** Constitutive overproduction of SWI4 by pGAL : SWI4 disrupts HO cell cycle regulation. W303 cells carrying pGAL : 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].
Deletion of the 310-bp the regulation of cycle regulation of HO. The fact that cell cycle regulation through¬out the cell cycle is sufficient to disrupt the cell to twice the size of wild-type cells and to grow 40% abnormal only source of the cell cycle regulation of mRNA was much higher in cells that produced a low level of even a low level of 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.

Table 1. HO transcription in cells carrying pGAL : SWI4

|          | Raffinose | Galactose |
|----------|-----------|-----------|
| W303 SWI | 5.0       | ND        |
| BY556 swi1-2 | 0.1     | 0.1       |
| BY528 swi2-314 | 0.8     | 0.4       |
| BY572 swi3-1 | 0.1     | 0.2       |
| BY658 swi4 : LEU2 | 0.2   | 4.0       |
| BY562 swi5-100 | 0.2    | 2.1       |
| BY660 swi6 : TRP1 | 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. Breeden 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 (Breeden 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.

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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 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.
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. Breeden 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 ssx1-1 HO 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. Breeden, 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 [Breeden 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 UR52. When UR52 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 UR52 and depends on SWI4 and SWI6 for activity, is transcriptionally inactive in α-factor-arrested cells [Breeden and Nasmyth.
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 (MATα ade2-1 trp1-1 can1-100 leu2-3,115 his3-11,15 ura3 his3-11.15 hol) 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 gene in BY87 (an HO : lacZ46 diploid derivative of W303) with the swi4 : LEU2-194 deletion. The swi4 : LEU2-194 deletion allele removes a 2.2-kb BamHI–PstI fragment of the SWI4 sequence and replaces it with an oppositely oriented LEU2 gene on a 2.2-kb Xhol–SalI fragment. The swi6 : TRP1-198 deletion in BY660 and BY603 has been described previously (Breeden and Nasmyth 1987a). The other swi strains used in this study are listed in Table 1 and have been described previously (Breeden and Nasmyth 1987b). The other swi strains used in this study are listed in Table 1 and have been described previously (Breeden and Nasmyth 1987b). By961 (MATα swi4 : LEU2-194 gal80 : LEU2 ho) was made by crossing two related strains: DY924 (MATa ade2-1 trp1-1 can1-100 leu2-3,115 his3-11,15 ura3 ade2-1 trp1-1 can1-100 leu2-3,115 his3-11,15 ura3 hol) and BY658. By178 (MATa swi6-399 HO : lacZ46 ura3 sxx1-1) was isolated in a screen for suppressors of swi6-399, which will be described in detail elsewhere (L. Breeden 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 Breeden and Nasmyth (1987a). A single mutant allele of SXX1 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 EcoRI 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 EcoRI–PstI and PstI–SalI fragments containing the SWI4 open reading frame from BD147 were inserted into EcoRI/Sall-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. Breeden, 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 OD650 of ~0.2 before α-factor was added (3–5 μg/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), MATα, and SIR3 probes have been described previously (Miller 1984; Miller et al. 1985; Nasmyth 1985a, b; Breeden 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 I. 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 FRA-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.

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*Genes Dev.* 1991, 5:

Access the most recent version at doi:10.1101/gad.5.7.1183

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