SWI5 instability may be necessary but is not sufficient for asymmetric *HO* expression in yeast

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Homothallic haploid yeast cells divide to produce a mother cell that switches mating type and a daughter cell that does not. This pattern is the result of *HO* endonuclease transcription exclusively in mother cells, and there only transiently in late G₁ as cells undergo Start. SWI5 encodes an *HO* transcription factor that is expressed during the S, G₂, and M phases of the cell cycle. The lack of synthesis of SWI5 during G₁ is essential to prevent *HO* transcription in daughter cells. Thus, *HO* must be activated by SWI5 protein synthesized in the previous cell cycle if it is to be properly regulated. SWI5 is inherited by both mother and daughter cells, and we show here that most of it is rapidly degraded during early G₁. More stable mutant SWI5 proteins cause daughter cells to switch mating type, suggesting that SWI5 destruction is necessary to prevent *HO* expression in daughters. We show further that mother cells can still express *HO* when stimulated to undergo Start after arrest in early G₁ for several hours. We propose that a small fraction of the SWI5 protein inherited by mother cells is extremely stable and that the crucial difference between mothers and daughters with regard to *HO* transcription is their differential ability to sequester SWI5 in a stable form, possibly as a component of transcription complexes on the *HO* promoter.

[Key Words: SWI5 instability; *HO* endonuclease; asymmetry; mating type switching]

Received September 17, 1992; revised version accepted December 21, 1992.

Haploid cells of the budding yeast *Saccharomyces cerevisiae* exist in two different forms, a and α, that conjugate to form a/α diploids. Mating specificity is determined by the *MAT* locus whose a and α alleles differ by 700 bp and encode transcription factors that control the expression of a- and α-specific mating pheromones and their receptors (Dolan and Fields 1991). To facilitate conjugation, homothallic (*HO*) strains can switch mating type (Hawthorne 1963). Switching occurs when a double-stranded break at the *MAT* locus, made by the *HO* endonuclease, is repaired using as a template a silent copy of the *MAT* genes held at *HML* (α) or HMR (α) [Nasmyth and Shore 1987; Herskowitz 1989]. The switching process is very tightly regulated, occurring only in the post-Start G₁ phase of mother cells, that is, in cells that have previously produced a bud (Strathern and Herskowitz 1979), and only in haploids [Takano and Oshima 1970; Hicks and Herskowitz 1976]. Regulation is effected at the level of *HO* transcription, which only occurs transiently after Start in haploid mother cells [Jensen et al. 1983; Nasmyth 1983].

This paper concerns the mechanism that confines *HO* transcription to mother cells. *HO* expression requires at least 10 trans-acting genes called SWI1-10 [Stem et al. 1984; Breeden and Nasmyth 1987]. Two of these ([SWI4 and SWI6]) encode components of a transcription factor that confers Start dependence [Breeden and Nasmyth 1987; Andrews and Herskowitz 1989; Taba et al. 1991]. SWI1, SWI2, SWI3, SWI7, SWI8, SWI9, and SWI10 may encode more general transcription factors, mutations in which are pleiotropic [Peterson et al. 1991; Peterson and Herskowitz 1992]. Three lines of evidence suggest that SWI5, which encodes a site-specific DNA-binding protein [Nagai et al. 1988; Stillman et al. 1988], plays a crucial part in the mother cell specificity of *HO*. First, replacement of *HO* DNA between −1000 and −1400 (a region known as URS1) by the *GALI*-10 upstream activation sequence ([UAS]) produces a hybrid promoter, the activity of which is dependent on galactose instead of mother/daughter status and on all known SWI genes except SWI5 [Nasmyth 1987]. Second, mutations in the SIN3/SDI1 gene that allow the *HO* promoter to be transcribed in the absence of SWI5 also allow expression in daughter cells [Nasmyth et al. 1987b; Stemberg et al. 1987]. Third, SWI5 mRNA is very unstable and is only made during the S, G₂, and M phases of the cell cycle [Nasmyth et al. 1987a]. If SWI5 must be present at the time of *HO* expression, the lack of SWI5 transcription during G₁ implies that SWI5 protein used to activate *HO* must be made in the previous cell cycle (i.e., must be of "maternal" origin). Ectopic expression of SWI5 during G₁ causes *HO* to be expressed in daughter cells [Nasmyth et al. 1987a; Lydall et al. 1991].

Maternal SWI5 protein is not inherited solely by
mother cells. Protein made during S, G2 and M phases accumulates in the cytoplasm but translocates suddenly to both mother and daughter nuclei toward the end of anaphase [Nasmyth et al. 1990, Moll et al. 1991]. This paper concerns the fates of the two SWI5 populations. The bulk of SWI5 inherited by both progeny is rapidly degraded soon after [and due to] its nuclear entry, but the fact that HO is transcribed in mother cells suggests that some SWI5 survives in these cells. We propose that it does so as a component of complexes at two widely separated sites within the HO promoter [at −1300 and −1800]. Such “transcription” complexes would appear to be very stable because HO can still be activated by passage through Start even when the preceding G1 period during which there is no SWI5 synthesis is greatly extended. For some reason, SWI5 cannot form stable transcription complexes in daughter cells but can nevertheless activate HO transcription in these cells if its protelysis is prevented by deleting a specific region within the center of the protein.

Results

SWI5-binding sites

SWI5 binds specifically to a sequence within URS1 [marked B in Fig. 1D]. However, a 61-bp deletion that removes this site causes only a 2-fold reduction in promoter activity, whereas mutation of SWI5 causes a 100-fold reduction [Stillman et al. 1988]. To find additional SWI5-binding sites in the HO promoter, we compared the ability of nonspecific and SWI5-specific monoclonal antibodies [Nasmyth et al. 1990] to immunoprecipitate labeled DNA fragments incubated with SWI5-containing extracts [McKay 1981]. Figure 1A shows that only two HindIII-ClaI fragments (401 and 283 bp) are specifically bound by SWI5 in extracts prepared from a yeast strain overexpressing SWI5 from the GAL1-10 promoter (lane 4). The same fragments are also weakly bound by wild-type levels of SWI5 [a longer exposure of lane 2 reveals the same pattern as lane 4], whereas no bound fragments are seen if extracts from swi5 strains are used [data not shown]. This result and a similar one using Satu3AI fragments [Fig. 1A, lanes 5–8] is consistent with the presence of two SWI5-binding sites: one [site A] between −1735 and −1870, and a second [site B] between −1504 and −1222 (the weakly bound 341-bp Satu3AI fragment seen in lane 8 stems from pBR322).

We localized both sites more precisely by methylation interference [Siebenlist and Gilbert 1980]. As shown in Figure 1B, SWI5 binding to site A is inhibited by methylation at any of the G residues on the top strand between −1811 and −1819, whereas binding to site B is inhibited by methylation at any of the G residues on the bottom strand between −1303 and −1307. Thus, site A is a new site and site B corresponds precisely to the previously identified one. We observed no interference owing to methylation of the bottom strand of site A or of the top strand of site B [data not shown]. The fact that SWI5 binds to two apparently dissimilar sequences could reflect interaction with distinct auxiliary factors that mediate DNA-binding or could stem from recognition of short DNA sequences by one or two of the three Zn2+ finger motifs of SWI5. Another yeast transcriptional activator, HAP1, also binds to two distinct sequences [Pfeifer et al. 1987], and mutations within the DNA-binding domain can abolish binding to either or both of these sequences [Pfeifer et al. 1989].

To assess the functional importance of sites A and B, we mutated them separately and in combination in the chromosomal copy of the HO gene and measured the effects on DNA-binding and HO transcription (Fig. 1C, D). Immunoprecipitation experiments in vitro showed that mutants a1 and a2 greatly reduced SWI5 binding to site A, and mutants b1 and b2 reduced SWI5 binding to site B [data not shown]. Mutation of site A did not affect binding at site B or vice versa, showing that there is no cooperativity of binding at the two sites. Mutation of site A alone caused a modest decrease in HO transcription [to 60% wild type; Fig. 1C, lanes 3,4]; mutation of site B had a more severe effect [to 10–30%; lanes 1,2], and the double mutations decreased it >100-fold [i.e., to the level seen in swi5 mutants; lanes 5,6]. It is interesting that mutation of site B has a more severe phenotype than deletion of the site [cf. Stillman et al. 1988]. This may result from the presence of additional regulatory sites within the 61-bp region missing from the deletion. The phenotypes of the mutations show that both sites are important for HO transcription but that they are partially redundant, as mutation of both sites has a much greater effect than would be expected by combining the effects of single mutants. Our results prove for the first time that SWI5 activates HO by binding to specific DNA sequences within its promoter.

Inhibitory domain of SWI5

SWI5 enters both mother and daughter nuclei at the end of anaphase [Fig. 2; Nasmyth et al. 1990] but subsequently activates HO only in mother cells. To test whether a specific part of the SWI5 protein prevents HO expression in daughters we analyzed which parts of SWI5 are dispensable for HO activation and then tested the effect of deleting such regions on the pedigree of mating type switching.

The conclusions of a SWI5 deletion analysis described in Figure 3A and its legend are the following: First, essential for the activity of SWI5 [see deletion series 2] are the zinc fingers and a region immediately amino-terminal to them that is highly conserved between SWI5 and the related transcription factor ACE2 [Butler and Thiele 1991] and that could be involved in stabilizing the first finger [Nakaseko et al. 1992]. Second, there is a broad region at the amino terminus of SWI5 [between residues 24 and 215; deletion series 3] that is probably necessary for activation but not for DNA-binding. There is some redundancy with regard to the function of this region, as deletion of either its amino- or carboxy-terminal halves has little phenotype. From deletion series 4 we conclude that an acidic domain between residues 471 and 502
**Figure 1.** SWI5-binding sites in the HO promoter. [A] Immunoprecipitation of promoter fragments. Plasmid pH0 (Nasmyth 1985), digested to completion with \textit{HinfI + ClaI} (HC) or with \textit{Sau3AI} (SI) and end-labeled, was incubated with yeast extracts prepared from wild type or SWI5-overexpressing \textit{GAL-SWI5} strains and immunoprecipitated with an anti-SWI5 monoclonal antibody (sp-AB) or with a control antibody (mouse IgG), as indicated above the lanes. Lanes 9 and 10 were loaded with the total digests. [B] Methyl-}

ation interference of the two SWI5-binding sites. End-labeled \textit{ClaI-AluI} pH0 fragments containing site A or B [see D) were partially methylated and incubated with SWI5-containing yeast extracts. Free [F] and bound [B] populations were separated by immunoprecipitation with an anti-SWI5 monoclonal antibody, and the nucleic acids were recovered and cleaved at the methylated purines. The top strand of site A and the bottom strand of site B are shown: No interference was detected on the other strands. The sequences of the two sites are given, and the G residues whose methylation interferes with SWI5 binding are marked with a dot. [C] Effects on \textit{HO} transcription of mutations in sites A and B. Yeast strains were constructed in which either or both SWI5 binding-sites in the \textit{HO} promoter were mutated, and the level of \textit{HO} transcription was assayed by S1 nuclease protection using the \textit{MAT\alpha1} transcript as an internal control. [+] Wild-type-binding site or \textit{SWI5} strain. [Lane 7] \textit{HO} transcription in a \textit{swi5} strain. [Lane 9] S1 probe [1% of the amount used in lanes 1-8]. [D] Summary of results. The \textit{Sau3AI} (S), \textit{ClaI} (C), and \textit{HinfI} (H) sites around SWI5-binding sites A and B are shown above. The sequences of the binding sites and the methylation interference data are given below and a consensus site is derived, where U represents a purine and Y a pyrimidine (note that site B is inverted for alignment). The central part describes the effects of mutating site A and/or B. The mutated residues are ringed, and the level of \textit{HO} transcription is given as a percentage of wild type (W.T.).
example Δ8, Δ12, and Δ13. However, when the whole region is missing, as in Δ11, Δ50, and Δ51, ~30% of all daughters switch mating type, and in Δ59 this proportion reaches almost 50%. Δ51 removes 81% of the dispensable regions of SWI5, but the restoration of residues 230–343 to this deletion (Δ33/62) is sufficient to restore mother cell specificity.

**Donor site selection**

It is noteworthy that many of the SWI5 deletions that cause daughter cell switching also increase the frequency of mother cell switching. In wild-type mother cells the proportion of cells that switch mating type in pedigree experiments is typically ~60–70%. This percentage, which in other strains can reach nearly 90%, may reflect imprecision in the choice of the donor site, that is, a MATa cell might repair the break at the MAT locus by copying the MATα information from HML instead of the MATα information from HMR (Hicks and Herskowitz 1976; Strathern and Herskowitz 1979, Klar et al. 1982). In contrast to this, many of the deletions that we tested gave highly efficient switching; and in the extreme case of Δ59, 98% of mother cells switched mating type. This shows that donor site selection is much more precise than believed previously. Δ59 might support such efficient switching because of its [likely] increased stability [see below], but the fact that it enters the nucleus at a time when the wild-type protein is excluded [Nasmyth et al. 1990] might also contribute. It is also noteworthy that all of the strains carrying SWI5 deletions still switched mating type in pairs, suggesting that HO transcription in these strains occurs before the onset of DNA replication.

A surprising feature of the results in Figure 3B is that the level of daughter cell switching never exceeds half that of mother cells. It is conceivable that the HO endonuclease is as active in daughter cells carrying, for example, Δ59 as it is in mother cells but that donor site selection in daughters is random, resulting half of the time in repairs that do not effect a switch of mating type. To test this possibility we analyzed the switching pedigrees of a MATα strain in which both HML and HMR carry MATα. No increase in daughter cell switches was observed over the parent strain [HMLα MATα HMRα; data not shown]. Consequently the asymmetry in switching does not stem from an increased efficiency of donor-site selection in mother cells.

**A role for SWI5 proteolysis**

Unbudded single cells containing nuclear SWI5 are rarely observed (Fig. 2), suggesting that the bulk of SWI5 protein inherited by both mother and daughter cells is rapidly destroyed as cells progress through G1. To test this idea, we measured the level of SWI5 protein by Western blotting in a synchronous culture as cells enter G1. The SWI5 gene continues to be transcribed in cells arrested in late anaphase owing to the temperature-sen-
Role of SWI5 instability in HO expression

Figure 3. A deletion analysis of SWI5. (A) The effect of various SWI5 deletions on ho-lacZ gene activity. Three regions are marked on the wild-type gene at the top: a region of potential α-helicity (AαH), the three zinc fingers, and the nuclear localization sequence (NLS). The end points of each deletion (as an amino acid residue number) are shown next to the gaps that represent the deleted DNA. (+ +) Wild-type-like ho-lacZ activity; (+) a noticeable reduction; (+/-) little activity; - the level seen in null alleles of SWI5. All deletions were transplanted to the SWI5 locus of a strain (H963) whose ho gene had been replaced by ho-lacZ. (B) The effect of SWI5 deletions on the pedigree of mating type switching. The various deletions were transferred to the SWI5 locus of a homothallic (HO) strain (H990), and the patterns of mating type switching were determined. The frequency of switching in mother and daughter cells is shown under M and D, respectively; percentages are at left and the actual numbers of switches per division are within parentheses. The ratio of daughter to mother switching (as a percentage) is shown under D/M.

| M   | D   | D/M (%) |
|-----|-----|---------|
| 66  | 0   | 0       |
| 84  | 0   | 0       |
| 71  | 2   | 2       |
| 83  | 0   | 0       |
| 87  | 1   | 1       |
| 66  | 29  | 29      |
| 70  | 7   | 11      |
| 74  | 11  | 14      |
| 79  | 0   | 0       |
| 72  | 12  | 16      |
| 98  | 47  | 48      |
| 70  | 1   | 1       |
| 83  | 31  | 37      |
| 84  | 17  | 17      |
| 84  | 0   | 0       |

Sensitive cdc15-2 mutation, but the SWI5 protein synthesized is mainly excluded from the nucleus (Nasmyth et al. 1990). Upon return to the permissive temperature, SWI5 transcription is rapidly repressed, SWI5 protein is translocated into the nucleus [by 21 min; Moll et al. 1991], and cells enter G1 with a high degree of synchrony as measured by the disappearance of anaphase spindles (Fig. 4a). ho transcription, which is not detectable at the cdc15 block, peaks at 35 min (Fig. 4e). Most wild-type SWI5 protein is degraded between 16 and 32 min; that is, soon after its entry into the nucleus (Fig. 4b, c). Thus, most SWI5 is unstable during the G1 phase of both
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Figure 4. The degradation kinetics of wild-type and mutant SWI5s following release from a cdc15 cell cycle arrest. (a) SWI5 RNA levels and the percentage of cells containing late anaphase spindles. Both wild-type and Δ11 RNA levels were analyzed and found to be very similar. Only wild-type levels are shown. (b) Representative Western blots: (b1) Wild-type SWI5 and Δ11; (b2) wild-type SWI5 and a version lacking its nuclear localization signal (Δnls). (c) A cross-reactive protein recognized by the rabbit polyclonal anti-SWI5 serum with levels that do not vary significantly during the cell cycle. (c, d) A summary of the data for wild-type SWI5 (●) and Δnls (○) (c), as well as for Δ33 (●), Δ33 (●), and Δ59 (△) (d). The numbers given represent an average from at least three independent experiments. Results with Δ11 were very similar to Δnls and are therefore not shown. (e) The level of ho-lacZ transcripts following release from the cdc15 arrest, assayed by SI mapping. The region implicated in the instability of SWI5 contains the following polypeptide segments that are partially homologous to each other: NITPNNLRLDFKINVED, PFTPKSRVSSATSNSAN, FLSPKRKIS-PASENVED, and LISPKKIRSNPNENLSS.

We then analyzed the stability of various SWI5 deletions. Deletion of the SWI5 nuclear localization signal greatly retards the proteolysis of SWI5 [Fig. 4b, c] as does deletion of the inhibitory domain, as, for example, in Δ59 [Fig. 4d] and Δ11 [Fig. 4b]. The effect of these deletions is not the result of any difference in RNA stability; the profiles of wild-type and Δ11 RNAs were the same [data not shown]. In contrast, Δ33, Δ62, and Δ33/Δ62 [Fig. 4d] mother and daughter cells. [We are confident that the indicated band represents SWI5, as the different deletion mutants that we analyzed give rise to correspondingly faster migrating bands.]
data not shown] are degraded like wild type. These results suggest that the proteolysis of SWI5 is dependent on its entry into the nucleus and that the ability of deletions such as Δ59 and Δ11 to activate HO in daughter cells may be the result of the higher nuclear concentrations of these proteins in late $G_1$ caused by their greater stability (neither Δ59 nor Δ11 is translocated less rapidly to the nucleus than wild-type SWI5; data not shown).

### The fate of SWI5 in mother cells

Our observation that SWI5 is rapidly degraded in $G_1$ cells and that increased stability is associated with HO expression in daughter cells suggests that asymmetric HO expression could arise from the known differences in the length of $G_1$ phase between mother and daughter cells (Hartwell and Unger 1977). According to this timing hypothesis, the expression of HO depends on the outcome of a race between the degradation of SWI5 and the onset of Start. Mother cells may normally undergo Start before SWI5 has been totally destroyed. Daughter cells must spend longer in $G_1$ owing to their smaller cell size at birth, and by the time they grow large enough for Start the SWI5 concentration may be insufficient to bind the HO promoter and activate transcription.

A crucial prediction of the timing hypothesis is that lengthening the pre-Start $G_1$ period of mother cells should abolish HO transcription. To test this, we have manipulated the level of $G_1$ cyclins to arrest cells reversibly in early $G_1$ (Cross and Tinkelenberg 1991). Cells lacking CLN1 and CLN2 genes and kept alive through the expression of CLN3 from the GAL1-10 promoter can undergo Start only in the presence of galactose. An asynchronously growing culture was harvested by filtration and resuspended in medium lacking galactose, causing cells to arrest in $G_1$. After 2.5 hr galactose was readded and cells underwent a series of synchronous divisions during which the levels of $ho$ and SWI5 RNAs were measured. Figure 5A shows that $ho$ was highly expressed immediately after the addition of galactose, long before the appearance of SWI5 RNAs. Transcription was strictly SWI5 dependent (data not shown) and mother cell specific (Fig. 5B). Cells arrested in $G_1$ by galactose removal were separated according to size by centrifugal elutriation. The analysis of bud scars by calcofluor staining (Pringle 1991) showed that the smallest cells were predominately [95%] daughters. This daughter population and a mixed mother plus daughter population composed of the rest of the culture were inoculated into galactose-containing medium (Fig. 5B). Whereas the late $G_1$-specific RNR1 transcript appears with almost identical kinetics in the two cultures during the first cell cycle, $ho$ is only expressed in the mixed culture, the low level of $ho$ transcripts in the daughter culture can be accounted for by its 5% mother cell contamination. In contrast, $ho$ expression was practically identical in the two cultures during the second cell cycle. Note that $ho$ is expressed during the first cell cycle of mother cells even though SWI5 is not transcribed during the previous $G_1$ arrest and most maternal SWI5 protein is degraded (data not shown). For most cells, the arrest in $G_1$ must have lasted between 60 and 90 min, which is much longer than the pre-Start $G_1$ period of a daughter cell's. Moreover, an identical result was obtained with cells that were grown for 3 hr without galactose (data not shown). We conclude that factors other than $G_1$ length determine the mother cell specificity of $ho$.

### Discussion

Our results have important implications concerning the mechanism of the mother cell specificity of HO. We have shown that most of the SWI5 protein inherited by mother and daughter cells is degraded soon after entry into the nucleus at telophase and that removal of a distinct region in the middle of SWI5 (called the inhibitory domain) retards this proteolysis and enables daughters to express HO. Because ectopic expression of the intact SWI5 protein causes a similar rate of daughter cell switching (Lydall et al. 1991), SWI5 deletions lacking the inhibitory domain could activate HO in daughters simply owing to their increased protein concentration in daughter cell nuclei. Thus, rapid SWI5 proteolysis may be necessary to prevent daughter cell switching. Alternatively, the primary function of the inhibitory domain of SWI5 may be to hinder DNA-binding or transcriptional activation in such a way that these events only occur in mother cells. The deleted variants could cause daughter cell switching because SWI5 can now bind stably to both mother and daughter chromatids. If only unbound SWI5 were subject to degradation, the increased overall SWI5 stability owing to removing the inhibitory domain could be a consequence of tighter DNA-binding, even at loci other than HO.

The dependence of the proteolysis of SWI5 on its nuclear localization signal suggests either that the protease responsible is confined to the nucleus or that the nuclear and cytoplasmic forms of SWI5 have different conformations or are associated with different proteins. Changes in phosphorylation occur at the time of the nuclear entry of SWI5, but these changes are implicated in causing translocation rather than being a consequence of it (Moll et al. 1991). The region that confers the instability of SWI5 is rich in X-S/T-P motifs (where X is a hydrophobic residue). Many of these are found within four regions that have, in addition, other sequence similarities to each other and some resemblance to PEST sequences (Rogers et al. 1986; see legend to Fig. 4).

The properties of stabilized SWI5 proteins suggested that the rapid proteolysis of SWI5, coupled with its failure to be resynthesised during $G_1$, could be sufficient for mother/daughter asymmetry, in that mother cells undergo Start before SWI5 is completely degraded but daughter cells cannot. This simple explanation is ruled out by our demonstration that mother cells can still efficiently transcribe ho at Start even when their preceding $G_1$ period is greatly extended. Other observations are also inconsistent with the above hypothesis: Stationary-
phase cells can express ho during the first cell cycle following reinoculation into fresh medium, mother/daughter asymmetry is retained in cells growing in the presence of a sublethal concentration of hydroxyurea, which equalizes the size of mother and daughter cells at birth, and a Start-independent version of the HO promoter can give rise to asymmetric switching (Nasmyth 1987; Nasmyth et al. 1987a).

A possible interpretation of our data is that there are two fates for SWI5 protein molecules that enter mother cell nuclei at the end of mitosis. The majority are rapidly degraded, but a minority are trapped in a more stable
form that evades destruction even during an extended $G_1$ period. According to this hypothesis, the crucial difference between mothers and daughters with regard to $HO$ lies in their differential ability to sequester some SWI5 in a stable form, possibly as stable transcription complexes bound to the $HO$ promoter. We estimate that there are, on average, 400 SWI5 molecules per cell in cycling cultures and that the number sinks below the detection limit (10–40) in cells that express $ho$ after an extended $G_1$ period. An alternative explanation is that all SWI5 molecules are equally unstable and that SWI5 performs a "hit-and-run" function [Rigaud et al. 1991], leaving an activating "imprint" on the $HO$ promoter exclusively in mother cells that persists until cells undergo Start.

The facility of mother cells to express $HO$ after an extended $G_1$ period can be described as "memory." It will be important to determine whether memory corresponds to the inheritance of transcription complexes containing the SWI5 protein or whether it corresponds to an imprint on the $HO$ promoter chromatin left by the prior occupation of SWI5. It is interesting that pheromone treatment destroys memory. Cells cannot express $HO$ during the first cell cycle following release from a $G_1$ arrest induced by $a$-factor [Nasmyth et al., 1987a]. Expression of SWI5 from the $RP39$ promoter during release restores $HO$ expression [Taba et al. 1991], which is consistent with SWI5 being involved in memory.

What might determine the asymmetry in the fate of SWI5 or in its ability to imprint the $HO$ promoter? The differential activity of CLN2 and CLN3 $G_1$ cyclins in mother and daughter cells postulated by Lew et al. [1992] is unlikely to play any part: Expression of CLN3 from the $GAL1$-10 promoter after a $G_1$ arrest leads to $HO$ transcription only in mother cells despite activating $RNR1$ transcription equally in mothers and daughters [Fig. 5B]. There may exist either a mother cell-specific activator that helps SWI5 to form stable complexes or an activating imprint on the $HO$ promoter, or a daughter cell-specific repressor that prevents this. A nuclear component that is segregated preferentially to mother cells would not be without precedent. Autonomously replicating plasmids that lack centromeres are preferentially inherited by mother cells [Murray and Szostak 1983]. The yeast spindle pole body (SPB) appears to be replicated by a conservative mechanism with the old and new SPBs segregating to mother and daughter nuclei, respectively [Vallen et al. 1992]. This organelle could conceivably have a role in the asymmetric segregation of factors that facilitate or antagonize SWI5 function.

A model for $HO$ expression

A model consistent with our results is shown in Figure 6. The bulk of SWI5 that enters the mother cell nucleus at the end of anaphase is degraded, but some can form a stable complex on site A or B [Fig. 1] with the help of MSF, a putative mother cell-specific factor [Fig. 6A]. Because daughter cells lack MSF or possess a daughter cell-specific factor that antagonizes it, SWI5 can only bind transiently to $HO$ with the result that all or most of SWI5 is destroyed by the time cells undergo Start [Fig. 6B]. $HO$ fails to be expressed even in mother cells following release from $G_1$ arrest induced by $a$-factor or following the reincubation of stationary-phase cells into fresh medium if $HO$ promoter DNA between $-816$ and $-1188$ is deleted [Nasmyth et al. 1987a]. We propose that $a$-factor signals the destruction of stable MSF/SWI5 complexes and that the DNA between $-816$ and $-1188$ also affects their long-term stability (as in stationary phase). This region could contain some but not all binding sites for MSF even though it contains none for SWI5.

$HO$ can be expressed in daughter cells either if SWI5 is not rapidly degraded [Fig. 6C] or if it is continually synthesized during $G_1$ [Fig. 6D]. Both situations lead to a higher than normal concentration of SWI5 in late $G_1$, which may be necessary for it to bind in the absence of MSF. The hindering of the nuclear entry of SWI5 has surprisingly little effect on its ability to activate $HO$ in mothers; moreover, it retards degradation without causing $HO$ expression in daughter cells [Moll et al. 1991]. This is also explicable if a higher nuclear concentration of SWI5 were required to activate $HO$ in daughters than in mothers.

It is interesting to note that an $HO$ promoter deletion lacking DNA between $-173$ and $-1300$ ($\Delta125-102$) causes similar levels of mating type switching in mothers (30/102) and daughters (37/99), even though it is still fully SWI5 dependent. Dependence on SWI5 is therefore

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**Figure 6.** A model for asymmetric $HO$ expression. Shown is the hypothetical state of the SWIS-binding region of the $HO$ promoter immediately after the entry of SWI5 into the nucleus at the end of mitosis (left; $t = 0$) and 15 or more minutes later at the time that cells undergo Start (right; $t = 20$). Boxed regions M and D represent the situation in wild-type mother (A) and daughter (B) cells, respectively. Also shown are the hypothetical states in daughter cells that express more stable versions of SWI5 (C) or when wild-type SWI5 is expressed constitutively (D).
not sufficient for asymmetric HO expression. The deletion still just retains both SWI5-binding sites, but site B is now brought to within 40 bp of the TATA box of HO.

It is conceivable that this deletion causes SWI5 to be stabilized in both mother and daughter cells by allowing its interaction with TFIIID or RNA polymerase to form stable transcription complexes. That is, general transcription factors may now perform, though in a cell type- and specific way, the function normally fulfilled by MSF.

Our hypothesis predicts that mutants lacking MSF should never express HO. Should MSF not already have been identified by the extensive screens for such mutants? The dependence on SWI1, SWI2, SWI3, SWI4, SWI6, SWI7, SWI8, SWI9, and SWI10 of a hybrid GAL/HO promoter that is expressed in daughter cells [Nasmyth 1987] suggests that none of these gene products is exclusively restricted to mother cells. It is nevertheless possible that subtle differences in one of their activities in mothers and daughters could constitute MSF. It seems more likely that MSF may have been missed by previous genetic analyses either because of gene redundancy or because it also has an essential function.

**Materials and methods**

**Yeast extracts and DNA-binding**

To prepare yeast extracts, 100-ml cultures [in YEP + 2% (wt/vol) raffinose + 2% (wt/vol) galactose] grown to an OD600 of 2.5 were harvested on ice, and the cells were washed in 5 ml of extraction buffer [400 mM (NH4)2SO4, 200 mM Tris at pH 8.0, 10 mM MgCl2, 1 mM EDTA, 10% (vol/vol) glycerol, 2 mM NaF, 0.1 mM NaVO3], resuspended in 800 μl of extraction buffer, and broken by vortexing with glass beads at 4°C. Glass beads and cell debris were removed by centrifugation, and the ribosomes were removed by a longer centrifugation [1 hr in a microcentrifuge at 4°C]. Six hundred microliters of supernatant was collected, and 400 μl saturated (NH4)2SO4 in buffer E (20 mM HEPES at pH 8.0, 5 mM EDTA) was added. The mixture was incubated on a rotating wheel at 4°C for 30 min, and precipitated proteins were recovered by centrifugation, dissolved in 200 μl of buffer E containing 2 mM NaF and 0.1 mM NaVO3, and frozen in liquid nitrogen. Plasmid pHO [Nasmyth 1988] was restricted with Sac3AI or with Clal and HindIII, and the resulting fragments were end-labeled with [γ-32P]ATP and polynucleotide kinase. Total fragment mixtures [40 ng] were incubated with yeast extract [7.5 μg protein] and poly [dI-dC] [3 μg] in 20 μl of buffer [50 mM KCl, 20 mM Tris at pH 7.5, 1 mM EDTA, 0.4 mM spermidine, 2 mM DTT, 8% (vol/vol) Ficol] on ice for 15 min. Monoclonal antibody SWV12 [Nasmyth et al. 1990] and mouse IgG [Jackson Research Laboratories] were coupled to CNBr-activated Sepharose beads and washed twice with 400 μl of Ipp500 [500 mM NaCl, 10 mM Tris at pH 8.0, 0.1% (vol/vol) NP-40, 0.1% (wt/vol) NaF] and three times with 400 μl of Ipp50 [as Ipp500, but 50 mM NaCl]. The binding reactions were added to the washed beads, vortexed, and incubated on ice for 10 min. Unbound material was removed by washing four times with 1 ml of Ipp50. Protein was digested in 400 μl of HM [300 mM NaCl, 50 mM Tris at pH 7.4, 5 mM EDTA, 1.5% (wt/vol) SDS, 1.5 mg/ml of proteinase K] at 37°C for 30 min, and the DNA was extracted with phenol/chloroform/isoamyl alcohol [25:24:1], ethanol precipitated, and electrophoresed on a 6% nondenaturing polyacrylamide gel.

**Methylation interference**

Plasmid pHO was restricted with Clal, and the fragments were end-labeled with [γ-32P]ATP. Following inactivation of the enzyme, the DNA was cut further with Alul, phenol-extracted, and precipitated. Dimethyl sulfate was used to methylate G residues, and immunoprecipitation reactions were performed as described above (scaled up fivefold). After electrophoresis the precipitated nucleic acids were located by autoradiography, eluted from the excised gel bands by incubation overnight at 37°C in 0.5 M ammonium acetate, 1 mM EDTA, 0.1% (wt/vol) SDS and 10% (vol/vol) methanol, phenol-extracted, ether precipitated, taken up in 200 μl of TE/10 [1 mM Tris at pH 8.0, 0.01 mM EDTA], spermene precipitated, taken up in 50 μl of TE/10, and ethanol precipitated. Cleavage at the methylated purines was effected by dissolving in 10% (vol/vol) piperidine and incubating at 90°C for 30 min, the DNA was then recovered by lyopholization, cleaned by dissolving in water and relyophilizing, and electrophoresed on a 6% denaturing polyacrylamide gel. The control [free] samples were obtained by electrophoresis of DNA treated similarly but without the immunoprecipitation step.

**HO DNA mutagenesis and transplacement**

The HO gene was selected from a yeast library in YCp50 by hybridization. A 6.2-kb XbaI–EcoRI fragment, containing the entire coding region and 4-kb of 5′ noncoding sequence, was cloned into pUC18 to produce construct pG333, which contains only two Clal sites. Site-directed mutagenesis of the 684-bp Clal fragment of the HO promoter (see Fig. 1D) was performed using the Amersham kit, and the mutated fragments were substituted for the Clal fragment of pG333. The constructs were transplanted into the chromosome at the HO locus by recombination in an HO::SUP4-o strain [K765; Nasmyth 1985], and integration was verified by Southern blot restriction mapping. The levels of HO RNA in the resulting strains were measured by counting S1 nuclease protection [Nasmyth 1985] and quantitated by counting the excised bands following autoradiography.

**SWI5 mutagenesis and mutant analysis**

Unidirectional deletions were made in a 3.25-kb HindIII fragment of the SWI5 gene cloned in two orientations in pUC19 [Henikoff 1987]. Each end point was sequenced and in-frame-deleted SWI5 genes were created using an EcoRI site at the end of each deletion. Left HindIII–EcoRI halves and right EcoRI–HindIII halves were combined by recloning into phosphatased HindIII-cut pUC19. Each deletion was transplanted to the SWI5 locus of a strain [P965; derived from K1107, Nasmyth and Dinnick 1991] in which the DNA between two BglII sites had been replaced by a 330-bp BamHI SUP4-o fragment. This strain contains an ho–lacZ fusion at the HO locus, and LacZ activity was measured as described previously [Breeden and Nasmyth 1987]. Selected deletions were transplanted, as described above, to the SWI5 locus of a homothallic strain [MATa, HMαA, HMαa, HO, swi5::SUP4-o, H990], and mating type switching was analyzed as described previously [Nasmyth et al. 1987a].

**Synchronization using cdc15 mutants**

swi5 deletion mutant DNA was used to replace a SUP4-o gene at the SWI5 locus of a cdc15-2 mutant strain [MATa, ho–lacZ, ura3, ade2-1, can1-100, met, his3, trp1-1, cdc15-2, swi5::SUP4-o, K1756]. Transformants growing exponentially at 25°C in YEPD were arrested in late anaphase by shifting to 36°C for 3 hr and...
then returned to 25°C by mixing with an equal volume of fresh YEPD at 14°C. Aliquots were harvested every 7 or 8 min and whole-cell extracts were prepared as described previously [Moll et al. 1991]. Equal amounts of protein were then separated by SDS-PAGE, and SWIS protein was detected using an affinity-purified rabbit polyclonal anti-SWIS serum and 125I-labeled protein A. Protein levels were quantified using a Molecular Dynamics PhosphorImager. SWIS RNA levels were measured by SI mapping [Lydall et al. 1991], and anaphase spindles by in situ immunofluorescence with anti-tubulin monoclonal antibodies [Kilmartin and Adams 1984].

**HO transcription after extended G1**

Cells with the genotype MATa, cln1Δ, cln2Δ, GAL–CLN3, ho, SSD1–K1107 [K2762] were grown at 30°C overnight in YEP medium containing 2% raffinose and 2% galactose until they reached an OD600 of 1.0 (t = −150 min). They were then filtered, washed with at least one volume of fresh YEPRaff medium, resuspended in fresh YEPRaff medium, and incubated for an additional 2.5 hr. Galactose was readded to 2% (t = 0 min). RNA levels were quantified using a Molecular Dynamics PhosphorImager. SWIS protein levels monitored by Western blotting (data not shown) had similar kinetics to SWIS RNA levels. For elutriation, K2762 cells were harvested by filtration and incubated in raffinose medium for 2.5 hr. Cells were then collected by centrifugation, resuspended in fresh YEPRaff medium, and incubated for an additional 2.5 hr. Galactose was readded to 2% (t = 0 min). RNA levels were quantified using a Molecular Dynamics PhosphorImager. SWIS protein levels monitored by Western blotting (data not shown) had similar kinetics to SWIS RNA levels. For elutriation, K2762 cells were harvested by filtration and incubated in raffinose medium for 2.5 hr. Cells were then collected by centrifugation, resuspended in 10 ml of YEPRff, gently sonicated, and loaded into a Beckman J-6M/E elutriation centrifuge. After isolation of a daughter cell population (i.e., the smallest cells), the remaining cells in the elutriation chamber were pumped out by reducing the centrifuge speed. Both sets of cells were collected on ice, and equal amounts were concentrated by centrifugation before inoculating into YEP medium containing both raffinose and galactose and incubation at 30°C. ho and RNRI RNA levels were measured by Northern blotting and quantified using a Molecular Dynamics PhosphorImager.

**Acknowledgments**

We are very grateful to Hannes Tkadletz for help in the preparation of figures and to Irene Acas for help in preparing the manuscript. G.T. was supported by an EMBO long-term fellowship. 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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SWI5 instability may be necessary but is not sufficient for asymmetric HO expression in yeast.

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*Genes Dev.* 1993, 7: Access the most recent version at doi:10.1101/gad.7.3.517