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]

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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* (*a*) (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* (Stern 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 *GAL1-10* 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; Sternberg 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 proteolysis 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 *HinfI*–*ClaiI* 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 *Sau3AI* 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 *Sau3AI* 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 2. In situ immunofluorescence of SWI5 protein in cycling cells. (Top) DNA stained with SWI5; (bottom) DNA stained with DAPI. Wild-type cycling cells are at left (large panel); *swi5* deletion cells are at right (small panel). This experiment has been described previously (Nasmyth et al. 1990). The data are displayed more prominently here to show more clearly the symmetric distribution of SWI5 following mitosis and its apparent destruction as cells enter G₁.

(Stillman et al. 1988) may also contribute to activation. Third and most important, large segments of SWI5 can be deleted without impairing its ability to activate *HO*. It is remarkable that every residue between 8 and 465 can be deleted (deletion series 1), including a region between residues 170 and 220 that very likely forms an extended amphipathic helix (α H) and that is highly conserved between SWI5 and ACE2. It should be noted that protein instability cannot account for the observed inactivity of SWI5 deletions: Western blotting (data not shown) showed the levels of wild type and the inactive $\Delta 20$ to be similar, but $\Delta 52$ was considerably lower than $\Delta 50$. The latter result could have been attributable to the loss of crucial epitopes because the signal obtained with $\Delta 50$ is already very much lower than wild type.

To test whether there is a region of SWI5 that prevents its activity in daughter cells, we have replaced the wild-type SWI5 locus of a homothallic (*HO*) strain with various active SWI5 deletions and followed their pedigrees of mating type switching (Fig. 3B). Our conclusion is that although much of the protein is dispensable for mother cell specificity (see, e.g., $\Delta 33$ and $\Delta 62$), the interval between residues 182 and 326 is required to prevent daughter cell switching. Parts of this interval can be removed without a dramatic rise in daughter cell switching, for

example $\Delta 8$, $\Delta 12$, and $\Delta 13$. However, when the whole region is missing, as in $\Delta 11$, $\Delta 50$, and $\Delta 51$, ~30% of all daughters switch mating type; and in $\Delta 59$ this proportion reaches almost 50%. $\Delta 51$ removes 81% of the dispensable regions of SWI5, but the restoration of residues 230–343 to this deletion ($\Delta 33/62$) 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 *MAT α* cell might repair the break at the *MAT* locus by copying the *MAT α* information from *HML* instead of the *MAT α* 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 $\Delta 59$, 98% of mother cells switched mating type. This shows that donor site selection is much more precise than believed previously. $\Delta 59$ 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, $\Delta 59$ 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 pedigree of a *MAT α* strain in which both *HML* and *HMR* carry *MAT α* . No increase in daughter cell switches was observed over the parent strain (*HML α MAT α HMR α* ; 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-

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 (A α H), 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.

sitive *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 G_1 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 G_1 phase of both

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 (●), Δ62 (■), 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: NITPNNLRDLDFKINVED, PFTPKSRVSSATSNSAN, FLSPKRKIS-PASENVED, and LISPKKIRSNNPENLSS.

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.)

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 Δ33/62 (Fig. 4d,

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 $\Delta 59$ and $\Delta 11$ 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 $\Delta 59$ nor $\Delta 11$ 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 difference 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 predominantly (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 resynthesized 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-

Figure 5. Mother cell-specific *ho* transcription following an extended G_1 period owing to temporary G_1 cyclin deprivation. (A) The levels of *ho* and *SWI5* RNAs before and after galactose readdition shown by autoradiographs (top) and a graph (bottom). The open square indicates *ho* mRNA; the solid diamond indicates *SWI5* mRNA; the stippled square denotes percent buds. (B) The levels of *ho* and *RNR1* RNAs in daughter cells and in mixed cell populations before and after galactose readdition. (○) Daughters; (●) mothers and daughters.

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 α -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

Figure 6. A model for asymmetric *HO* expression. Shown is the hypothetical state of the SWI5-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).

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 α -factor or following the reinoculation of stationary-phase cells into fresh medium if *HO* promoter DNA between –816 and –1188 is deleted (Nasmyth et al. 1987a). We propose that α -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 ($\Delta 125$ –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

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-inspecific 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 1985) was restricted with *Sau3AI* or with *ClaI* and *HinfI*, 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 [d(I-C)] (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% (wt/vol) Ficoll] 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) NaN₃] 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 *ClaI*, and the fragments were end-labeled with [γ -³²P]ATP. Following inactivation of the enzyme, the DNA was cut further with *AluI*, phenol-extracted, and precipitated. Dimethylsulfate 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, ethanol precipitated, taken up in 200 µl of TE/10 (1 mM Tris at pH 8.0, 0.01 mM EDTA), spermine 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 lyophilization, cleaned by dissolving in water and re-lyophilizing, 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 *ClaI* sites. Site-directed mutagenesis of the 684-bp *ClaI* fragment of the *HO* promoter (see Fig. 1D) was performed using the Amersham kit, and the mutated fragments were substituted for the *ClaI* fragment of pG333. The constructs were transplaced 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 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 transplaced to the *SWI5* locus of a strain (H963; derived from K1107; Nasmyth and Dirick 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 (Breedon and Nasmyth 1987). Selected deletions were transplaced, as described above, to the *SWI5* locus of a homothallic strain (*MATa*, *HMLa*, *HMRa*, *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 SWI5 protein was detected using an affinity-purified rabbit polyclonal anti-SWI5 serum and ¹²⁵I-labeled protein A. Protein levels were quantified using a Molecular Dynamics PhosphorImager. SWI5 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 *G*₁

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. SWI5 protein levels monitored by Western blotting (data not shown) had similar kinetics to SWI5 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 YEPD, 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 *RNR1* RNA levels were measured by Northern blotting and quantified using a Molecular Dynamics PhosphorImager.

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

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