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# The determination of mother cell-specific mating type switching in yeast by a specific regulator of *HO* transcription

**Kim Nasmyth**

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

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**In haploid homothallic budding yeast, cell division gives rise to a mother cell which proceeds to switch its mating type and a daughter cell (the bud) which does not. Switching is initiated by a specific double strand cleavage of mating type DNA by an endonuclease encoded by the *HO* gene. Previous data suggest that the pattern of *HO* transcription is responsible for the mother cell specificity of switching. *HO* is transcribed transiently, at START, during the cell cycle of mother cells but not at all during the cell cycle of daughter cells. The *HO* promoter is complex. Sequences between −1000 and −1400 (called *URS1*) are essential for transcription, whereas sequences between −150 and −900 (called *URS2*) are necessary for cell cycle control. Moreover, 10 *trans*-acting gene products called *SWI*1−10 are necessary for maximum expression. In an attempt to identify the *cis*-acting DNA sequences which are responsible for mother cell specificity and to identify which *SWI* genes are involved, a hybrid *GAL/HO* promoter was constructed in which the upstream activation region putatively involved in mother cell-specific activation (*URS1*) is replaced by the upstream activation region of the *GAL*1−10 promoter. The properties of this hybrid promoter show, for the first time, that: (i) the *HO* promoter is modular since mother cell specificity can be replaced by galactose dependence without compromising cell cycle control or *a*/α repression; (ii) transcription of *HO* is indeed the major rate-limiting event for switching which is absent in daughter cells; (iii) *SWI*1,2,3, 4,6,7,8,9 and 10 are unlikely to be involved in mother cell specificity but *SWI5* probably is.**

*Key words:* mating type/switching/yeast/mother cell/*HO* gene

## Introduction

Mating type switching in homothallic yeast is tightly regulated. It occurs almost every division in certain *a* or α haploid cells but not at all in diploid *a*/α cells (Hawthorne, 1963; Takano and Oshima, 1970; Hicks and Herskowitz, 1976). In addition to its repression in diploids, switching is also tightly regulated during growth of haploid cells. It occurs during a restricted phase of the cell cycle of mother cells but not at all during the cell cycle of daughter cells (Hicks and Herkowitz, 1976; Strathern and Herskowitz, 1979). This paper addresses the question of how switching is confined to mother cells.

Switching is thought to be initiated by a specific double strand cleavage of mating type DNA by an endonuclease encoded by the *HO* gene (Strathern *et al.*, 1982; Kostriken *et al.*, 1983). Two lines of evidence suggest that the failure of daughter cells to switch is due to the absence of *HO* endonuclease in such cells. First, neither the endonuclease activity nor its mRNA are made during the first cell cycle of $G_0$ stationary phase daughters upon their inoculation into fresh medium, whereas they are both produced in a cell cycle-dependent fashion in mother cells under the same circumstances (Nasmyth, 1983). Moreover, neither the endonuclease nor its mRNA are sufficiently stable to be transferred to a daughter cell after their synthesis in a mother cell.

The second piece of evidence concerns the analysis of cells carrying a recombinant *HO* gene whose transcription is driven by the *GAL* upstream promoter element, which is presumably active in both mother and daughter cells (Jensen, 1983; Jensen and Herskowitz, 1984). In the presence of galactose, which activates the *GAL* upstream promoter element (Guarente *et al.*, 1982), daughter cells are now observed to switch as frequently as mother cells. The interpretation of this experiment is complicated, however, by the fact that this *GAL/HO* fusion causes a 50- to 100-fold overproduction of the *HO* endonuclease. Nevertheless, it is a reasonable working hypothesis that the failure of daughter cells to switch their mating type is due to an underlying failure to transcribe the *HO* gene throughout their cell cycle.

The *HO* promoter is subject to at least three forms of regulation: (i) repression in *a*/α diploids (Jensen *et al.*, 1983), (ii) cell cycle regulation and (iii) mother cell specificity (Nasmyth, 1983). Previous analyses suggest that these three forms of regulation are mediated by different *cis*-acting DNA sequences (Nasmyth, 1985a,b). Given this complexity, it is perhaps not surprising that *HO* transcription requires 10 *trans*-acting gene products called *SWI*1−10 (Haber and Garvick, 1977; Stern *et al.*, 1984; L.Breeden and K.Nasmyth, in preparation). It seems likely that the different *SWI* gene products are involved in different aspects of *HO* regulation.

This paper describes an attempt to identify the *cis*-acting DNA sequences which are responsible for mother cell specificity and to identify which *SWI* genes are involved in this activity. To this end a hybrid *GAL/HO* promoter in which an upstream activation region of *HO* putatively involved in mother cell-specific activation is replaced by the upstream activation region of the *GAL*1−10 promoter has been constructed. The properties of this hybrid promoter show that: (i) mother cell specificity can be replaced by

**Table I.** The *SWI5* dependence and pedigree of mating type switching caused by wild-type *HO* and Δ−145 to −901

| | *HO* RNA levels | | Switching per cell division | | |
|---|---|---|---|---|---|
| | *SWI5* | *swi5*Δ | Mothers | Daughters | Spores |
| Wild-type | 100 | < 1.0 | 20/28 | 0/29 | 0/7 |
| Δ−145 to −901 | 142 | < 2.0 | 47/96 | 2/93 | 0/35 |

The pedigree of switching caused by the −145 to −901 deletion was determined as described by Strathern and Herskowitz (1979). An *a*/α diploid homozygous for the deletion (H538) was sporulated and the spores dissected in the vicinity of a heavy streak of α cells on a YEPD plate. *MAT*a spores germinate, undergo $G_1$ arrest, and cell enlargement, whereas *MAT*α spores undergo one or more cell divisions in the presence of α factor supplied by the α cell streak. Switching is detected when an α cell that has divided in the presence of α factor produces a pair of cells that undergo $G_1$ arrest and cell enlargement. The dependence of transcription on *SWI5* was measured in *ho* versions as described by Nasmyth (1985a).

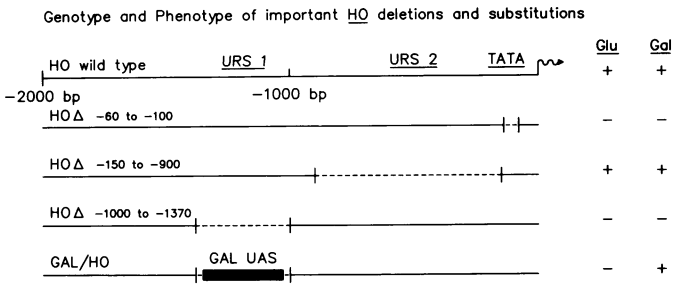


**Fig. 1.** The genotype and phenotype of *HO* deletions and substitutions. On the left hand side of the diagram, *HO* promoter DNA is represented by a solid line, deleted DNA by a dotted line and inserted DNA by a solid bar. On the right hand side of the diagram, the levels of *HO* RNA produced in YEP glucose or YEP galactose are represented by + or − signs (+ being ≥ 100 × −). All constructions were made and transplaced into the *HO* locus in yeast as described by Nasmyth (1985a). The *GAL/HO* hybrid was made by cloning a 330-bp *Dde*I−*Sau*3A fragment containing the *GAL*1−10 UAS (Guarente *et al.*, 1982) with *Sal* linkers attached into the *Xho*I site left by the −995 to −1362 deletion (no. 252.176, Nasmyth, 1985a). The deletion marked Δ−150 to −900 is actually from −145 to −901.

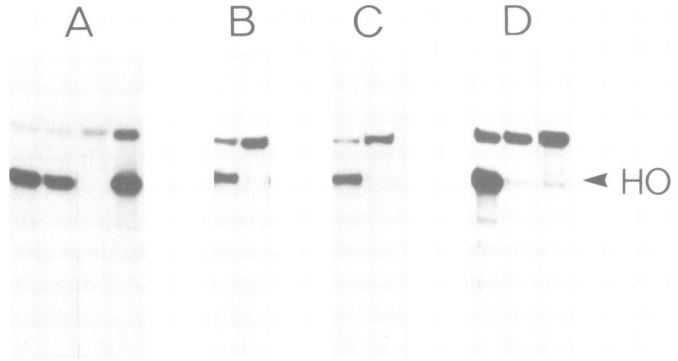


**Fig. 2.** Properties of *HO* transcription from the *GAL/HO* promoter. *HO* RNAs were measured by S1 mapping as described by Nasmyth (1985a). Cells were grown either in YEP glucose (glu) or YEP galactose (gal) as stated. **(A)** *HO* RNAs from *HO* (glu), *HO* (gal), *GAL/HO* (glu), *GAL/HO* (gal). Yeast strains K839 and K1121 grown at 25°C. *MAT*a1 RNAs were also measured (data not shown). *HO* RNA levels were quantitated as described by Nasmyth (1985a). The levels were from left to right: 100%, 83%, < 3% and 137%. **(B)** *HO* RNAs from *GAL/HO* (gal) in the absence (left) and presence (3 μg/ml for 15 min, right) of α factor (Sigma). Yeast strain H407 grown at 30°C. **(C)** *HO* RNAs from *GAL/HO* (gal) at 25°C (left) or after 180 min at 37°C (right) in a *cdc*15-2 strain K1121. **(D)** *HO* RNAs from *GAL/HO* (gal) in wild-type (K1121, 25°C), *swi*4-100 (H685, 30°C), or *swi*2-314 (H687, 30°C) mutants.

galactose dependence without compromising cell cycle control or *a*/α repression; (ii) transcription of *HO* is the major rate-limiting event for switching which is absent in daughter cells; (iii) *SWI*1,2,3,4,6,7,8,9 and 10 are unlikely to be involved in mother cell specificity but *SWI*5 probably is.

## Results

### *A GAL/HO hybrid promoter*

In addition to a TATA-like region at −90, the *HO* promoter requires sequences between −1000 and −1400, which are called *URS*1 (Nasmyth, 1985a). In contrast, the DNA between −900 and −150 (called *URS*2) can be deleted witout loss of promoter function, but it is required for correct cell cycle control (Nasmyth, 1985b). Removal of *URS*2 leaves an active promoter which is still apparently sensitive to the mother/daughter state. This is confirmed by the data in Table I, which show that switching caused

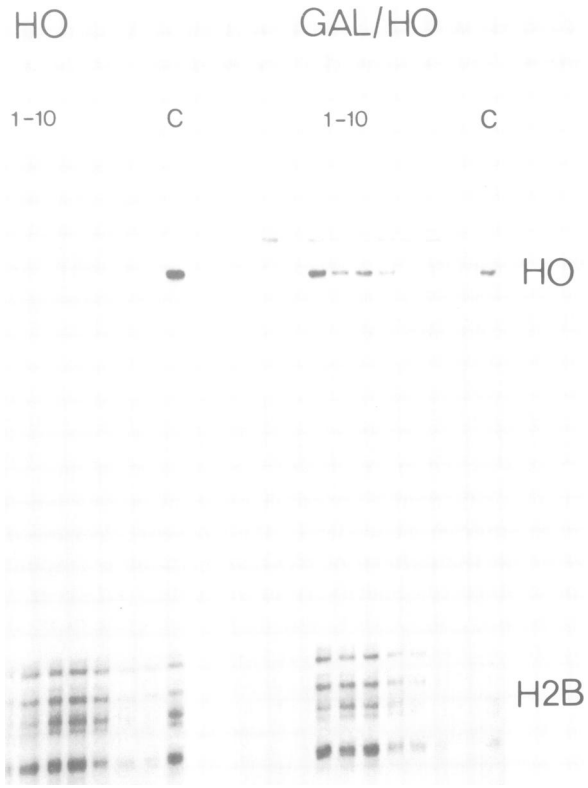


**Fig. 3.** The level of *HO* RNA from *HO* and *GAL/HO* in daughter cells in YEP galactose. The strains bearing *HO* (left, K839) and *GAL/HO* (right, K1121) contained the ts *cdc*15-2 mutation. Stationary phase daughter cells were prepared from cells grown at the permissive temperature 25°C and inoculated into fresh YEP galactose medium at 37°C and samples were collected after 30, 60, 90, 120, 150, 180, 225, 270, 315 and 360 min **(lanes 1−10)**. An aliquot of cells was also grown at 25°C for 580 min (lane marked c). At 37°C the $G_1$ cells will undergo only a single (daughter) cell cycle and will arrest in a late stage of mitosis, whereas at 25°C the cells will proceed into subsequent cell cycles (i.e. daughter and mother cell cycles). The level of *HO* and H2B RNAs are shown.

by such a deletion (Δ from −145 to −901) still occurs predominantly in mother cells. It has been suggested, therefore, that *URS*1 contains DNA sequences which activate *HO* only in mother cells (Nasmyth, 1985a). This hypothesis predicts that replacement of *URS*1 by an upstream activation sequence from another promoter which is not sensitive to the mother/daughter state might cause the *HO* promoter to become active in daughter cells.

Such a hybrid promoter has been made by replacing the 367 bp of *HO* DNA between −995 and −1362 by a 330-bp DNA fragment containing the upstream activation region (UAS) of the *GAL*1−10 promoter (Guarente *et al.*, 1982, see Figure 1). Two types of such a construction have been made. The hybrid promoter has been linked either to an intact *HO* structural gene or to an *ho*-β-galactosidase gene fusion. Both types have been transferred to the *HO* locus using the *SUP*4-0 replacement technique described by Nasmyth (1985a).

The *GAL/HO* promoter gives rise to *HO* transcripts when cells are grown on galactose but not when they are grown on glucose (see Figure 2A). The level of *HO* RNA produced by the hybrid promoter, when induced on galactose (137%), is similar to that produced by a wild-type *HO* promoter in cells grown on either glucose (100%) or galactose (83%) (see Figure 2A). This suggests that the *GAL* UAS, which is only active in the presence of galactose, can fulfill the functional normally executed by *URS*1, the wild-type *HO UAS*.

**Table II.** The number of switches per cell division caused by *HO* and *GAL/HO*

| | Mothers | Daughters | Spores |
|---|---|---|---|
| *HO* | 62/96 (64.6%) | 0/88 (0) | 0/18 (0) |
| *GAL/HO* | 84/162 (51.9%) | 58/152 (38.2%) | 18/58 (31%) |

The pedigrees of strain H413 (*HO*) and H411 (*GAL/HO*) were determined as described in the legend to Table I, except that cells were grown on a plate containing YEP galactose.

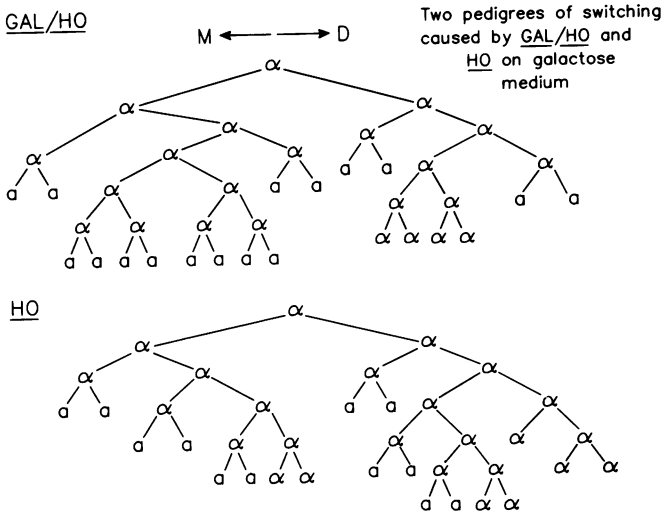


**Fig. 4.** Actual pedigrees produced by the *HO* and *GAL/HO* promoters. This pedigree analysis was performed as described in the legend of Table II. Of each pair of sister cells produced at division, the mother cell is shown on the left and the daughter on the right. Cells were inferred to have an $\alpha$ mating type if they divided in the presence of $\alpha$ factor and *a* if they underwent cell cycle arrest and 'Schmoo'-like enlargement.

**Table III.** Levels of *ho*−β-galactosidase activity from the *HO* and *GAL/HO* promoters in *swi*$^-$ and *SWI*$^+$ strains

| | *HO* (15−20ū) | | *GAL/HO* (50/60ū) | |
|---|---|---|---|---|
| *SWI*$^+$ | 100 | (K1107,K1115) | 100 | (K1127,K1131) |
| *swi*1-2 | ≤1.0 | (Stern *et al.*, 1984) | <5.0 (C.T) | (H474−476) |
| *swi*2-314 | 0.14 | (H206) | 2.5 | (H307,311) |
| *swi*3-1 | ≤1.0 | (Stern *et al.*, 1984) | <5.0 (C.T) | (H477−481) |
| *swi*4-100 | <0.1 | (H378) | <1.0 | (H313,315) |
| *swi*5-100 | 0.69 | (H235) | 105.0 | (H332) |
| *swi*6-399 | <0.1 | (H379) | 2.0 | (H317,319) |
| *swi*7-288 | 2.6 | (H220) | 11.3 | (H427,431) |
| *swi*8-299 | 2.4 | (H236) | 16.0 | (H415,416,419,420) |
| *swi*9-289 | 3.4 | (H237) | 15.7 | (H422,424,425) |
| *swi*10-281 | 0.58 | (H454) | 4.4 | (H433,434,438,440) |

The construction of the *swi* mutant strains used here will be described by L.Breeden and K.Nasmyth (in preparation). In the case of strains containing the *ho*−βgal fusion, cells were grown in YEP glucose. In the case of strains containing the *GAL/ho*−βgal fusion, cells were grown in YEP galactose. β-Galactosidase activities were measured as described by Guarente *et al.* (1982). Yeast strain numbers are in parentheses. The strains in the right hand column are segregants from crosses between *swi* mutant strains and K1127 and K1131. The numbers are averages from the segregants indicated. The effect of *swi*1-2 and *swi*3-1 on *ho*−βgal activities has been previously reported by Stern *et al.* (1984). The effect of *swi*1-2 and *swi*3-1 on *GAL/ho*−βgal was not quantitated (marked C.T). A large (> 20-fold) qualitative effect was noticed using a filter β-gal assay using the chromogenic substrate X gal (L.Breeden and K.Nasmyth, in preparation).

It should be noted that, in addition to inducing RNAs with the correct *HO* 5′ terminus, the *GAL* UAS substitution also induces a lower but significant level of RNAs (10−20% of *HO*) which initiate further upstream. It is not known where these RNAs originate but it is unlikely that they can be translated efficiently to produce *HO* endonuclease (see later).

*Regulatory properties of GAL/HO*

The galactose-dependent *HO* transcription from the hybrid *GAL/HO* promoter is still subject to two of the three physiological controls that affect the wild-type *HO* promoter. Transcription from *GAL/HO* is repressed by the *a*/α state (data not shown) and is cell cycle dependent (see Figure 2B and C). The third major physiological control that affects the wild-type *HO* promoter is its activity in mother cells but not in daughter cells. This property has been previously observed by measuring the level of *HO* transcripts during the first cell cycle of outgrowth of $G_0$ stationary phase daughter cells (Nasmyth, 1983, 1985a). Figure 3 shows that, unlike the wild-type *HO* promoter, the *GAL/HO* hybrid produces abundant *HO* transcripts in a cell cycle-dependent manner in such daughter cells when grown on galactose. A comparison of the *HO* RNA levels found during the first cell cycle of isolated daughter cells with those found in a mixed population of mother plus daughter cells suggests that the level of transcription in daughter cells is similar to that in mother cells. These data are consistent with the hypothesis that the *GAL* UAS, which is presumably active in both mother and daughter cells, is substituting for a mother cell-specific *HO* UAS which normally resides in *URS*1.

*Mating type switching caused by GAL/HO*

Three major rules for yeast mating type switching have emerged from the direct observation of switching cells under the microscope (Strathern and Herskowitz, 1979). The first, and possibly most important, is the rule that *a* cells do not switch their mating type when arrested in $G_1$ by α factor. The second rule is that cells always switch in pairs; i.e. both progeny of a switching cell have the new mating type. The third rule is that only cells that have previously budded (i.e. mother cells) can switch their mating type. The question arises, therefore, as to whether *HO* transcription driven by the hybrid *GAL/HO* promoter causes mating type switching that disobeys any of these rules. To answer this, *a*/α diploids homozygous either for *HO* or *GAL/HO* were sporulated and the spores allowed to germinate on galactose medium. Table II summarizes the types of α to *a* mating type switches observed. In both cases, all switches occurred in pairs and both *a* cells produced remained arrested by α factor for several hours. As expected, the wild-type *HO* promoter failed to cause any spore or daughter cell to switch. In contrast, the *GAL/HO* promoter caused daughter cells (and spores) to switch their mating type almost as frequently as mother cells. For the *HO* strain, mother cells switched their mating type at 65% of their cell divisions whereas daughters and spores not at all. For the *GAL/HO* strain, on the other hand, mother cells switched mating type at 52% of their divisions, daughter cells at 38% and spores at 31%. Examples of the type of pedigrees obtained with the *GAL/HO* strain are shown in Figure 4.

Since the *GAL* UAS replacement of *URS*1 in the *HO* promoter leads to a < 2-fold increase in *HO* transcription which is still cell cycle regulated, it is reasonable to conclude from the above results that the acquisition by daughter cells of the sort of *HO* transcription that normally occurs only in mother cells is sufficient also to cause them to switch their mating types efficiently. In other words, the failure of normal daughter cells to switch their mating types must be mainly determined by their lack of *HO*

endonuclease, which is due to their failure to transcribe the *HO* gene.

*Which regulators are responsible for mother/daughter control of HO transcription?*

The differential transcription of *HO* in mother and daughter cells could be due either to a daughter cell-specific repressor or a mother cell-specific activator (or both). Mutation of a putative daughter-specific repressor will lead merely to transcription in both mother and daughter cells, a phenotype that is difficult to detect in any mutant screen. Mutation of a putative mother specific-activator, on the other hand, should lead to a total defect in *HO* transcription, a phenotype which can be readily detected. So far, 10 unlinked genes called *SWI*1,2,3,4,5,6,7,8,9 and 10 (Haber and Garvick, 1977; Stern *et al.*, 1984; L.Breeden and K. Nasmyth, in preparation) have been identified as being necessary for *HO* transcription. The question is whether any of these *SWI* genes encodes an activator which is present in mother cells but absent in daughters.

One explanation for the replacement of mother cell specificity by galactose dependence in the *GAL/HO* hybrid promoter is that the function of a mother cell-specific activator is performed instead by the *GAL4* gene product (Douglas and Hawthorne, 1972) which is responsible for *GAL* UAS activity. This hypothesis predicts that the *GAL/HO* promoter should be independent of the *SWI* gene which codes for such an activator. A corollary of this is that the *SWI* genes which are still required for *GAL/HO* transcription must encode activators which are capable of functioning in daughter cells; i.e. they cannot be mother cell specific. What then is the dependence of the *GAL/HO* promoter upon the various *SWI* genes?

Table III compares the level of β-galactosidase activity due either to the wild-type *HO* promoter (in glucose) or the *GAL/HO* hybrid promoter (in galactose) in wild-type and mutants in each of the 10 *SWI* genes. It is striking that the *GAL/HO* promoter is still noticeably dependent upon *SWI*1,2,3,4,6,7,8,9 and 10 but not upon *SWI5*. The implication is that all the *SWI* gene products, with the possible exception of *SWI5*, must be to some extent (if not fully) active in daughter cells. *SWI5* is, therefore, the only candidate for a mother cell-specific activator.

The dependence of *GAL/HO* upon *SWI4* and its independence of *SWI5* is also seen if mating type switching is used as a measure of *HO* transcription. α *swi*4-100 *GAL/HO* cells remain predominantly αs upon growth in galactose medium whereas α *swi*5Δ *GAL/HO* cells switch mating types as rapidly as α *SWI5 GAL/HO* cells (pedigree data not shown). Figure 2D shows that the *swi*2-314 and *swi*4-100 mutations greatly reduce the level of *GAL/HO*-induced *HO* RNAs but not the level of RNAs which initiate further upstream. This observation together with the observation that *swi*4-100 reduces *ho*-β-galactosidase activity at least 100-fold (see Table III) implies that there is no significant translation of the upstream RNAs.

## Discussion

*GAL/HO causes daughter cell switching*

This paper describes the construction and characterization of a hybrid promoter composed by replacing the *HO* upstream activation sequence by that of the *GAL* promoter. The hybrid *GAL/HO* promoter is only active in cells grown on galactose, suggesting that the *GAL* UAS is playing an essential role, but it nevertheless retains the transcriptional start points, the *a*/α repression and cell cycle dependence characteristic of the parental *HO* promoter.

A crucial difference between the *HO* and *GAL/HO* promoters is that the latter is active in daughter cells whereas the former is not. *GAL/HO* produces in both mother and daughter cells the sort of *HO* transcription normally (i.e. from *HO*) only seen in mother cells. Daughter cells normally never switch their mating type, but as a consequence of the alteration in *HO* transcription caused by *GAL/HO*, they now switch almost as frequently as mother cells. This implies that transcription of *HO* is the major rate-limiting event for switching which is normally absent in daughter cells. It should be pointed out that the *GAL/HO* promoter appears to cause mother cells to switch slightly more frequently than daughter cells: 52 versus 38%. It is not known whether this is a reflection of some residual asymmetry in *GAL/HO* transcription or due to another process involved in switching being less efficient in daughter cells.

The observation that daughter cells will switch their mating type as efficiently as mother cells when *HO* transcription is deregulated has been reported previously by Jensen and Herskowitz (1984). The crucial difference between that experiment and this one is that the fusion used by Jensen and Herskowitz (1984) causes a 100-fold increase in *HO* RNA levels, whereas the fusion described here produces a level of *HO* RNA in daughter cells that is close to that normally seen in mother cells. In addition, the changed switching pattern due to the present *GAL/HO* promoter cannot be attributed to any changed cell cycle control.

*Identification of a mother cell-specific activator?*

A corollary of the above conclusions is that *HO* is normally never transcribed during the $G_1$ period of log phase daughter cells. How is this asymmetry in *HO* transcription between mother and daughter cell achieved? One possibility is that an activator essential for *HO* transcription is present only in mother cells. The isolation of mutants which are defective in *HO* transcription has identified 10 genes called *SWI*1 − 10, any one of which could in principle encode a mother cell-specific activator. The data presented in this paper appear to exclude, however, nine out of 10 *SWI* genes from this role.

The case against *SWI4* and *SWI6* is the strongest. The 229.102 deletion, which removes *HO* DNA from −145 to −901, is independent of *SWI4* and *SWI6* (L.Breeden and K.Nasmyth, in preparation), yet it is still sensitive to the mother/daughter state. The case against the other *SWI* genes rests on the analysis of the *SWI* dependence of the *GAL/HO* hybrid promoter, which is efficiently transcribed in daughter cells. *GAL/HO* is dependent upon *SWI*1, 2,3,4,6,7,8,9 and 10, suggesting that these genes are to some extent active in daughter cells.

In contrast, our data suggest that mother cell specificity may be due to the role of *SWI5* in *HO* activation. The *GAL/HO* promoter does not require *SWI5*. This implies that the replacement of *HO* DNA between −995 and −1362 (*URS*1) by the *GAL* UAS results in the *GAL4* gene product (the *GAL* UAS activator) replacing the function of *SWI5* in *HO*. Since the replacement causes the substitution of galactose dependence (a property of *GAL4*) for mother cell specificity, it seems likely that it is *SWI5* which normally ensures mother cell-specific activation of *HO*.

Analysis of the DNA sequence of *SWI5* suggests that it encodes a protein which contains, at its C terminus, three regions homologous to the repeated DNA binding domain of the *Xenopus* 5S transcription factor TfIIIa (Miller *et al.*, 1985a). Recent results using a gel retardation assay imply that *SWI5* binds to specific sequences within *URS*1 (D.Stillman, A.Bankier and K.Nasmyth, in preparation). This is of course consistent with the fact that *SWI5* dependence is lost when such sequences are replaced by

the *GAL* UAS. As already mentioned, a deletion removing DNA between −145 and −901 alters the cell cycle control but not the mother cell specificity of *HO* transcription. This deletion retains *URS*1, the region from which we propose that *SWI5* activates *HO* and, as predicted, transcription from the deletion is still strongly *SWI5* dependent (see Table I).

*Implications for combinatorial control*

The regulatory behaviour of the *GAL/HO* fusion has significance for the general question of combinatorial control. The activation of promoters only under a special combination of conditions is clearly a central feature of the differentiation of eukaryotic cells. The *HO* promoter in yeast is a good example: it is only active in certain cell types (*a* or α but not *a*/α), only when the cell undergoes START, and only if the cell is a mother cell. Distinct sequences within the *HO* promoter have been implicated in *a*/α repression (Miller *et al.*, 1985b) and cell cycle regulation (Nasmyth, 1985b). The observation that the *GAL/HO* promoter is insensitive to the mother/daughter state but retains cell type and cell cycle dependence implies that a third set of different DNA sequences are involved in mother/daughter control.

We do not yet know how the three controls are integrated on the *HO* promoter. It seems that the system must be modular because sensitivity to the mother/daughter state can be replaced by sensitivity to the presence of galactose by replacing the *HO* UAS (called *URS*1) by an equivalent region of the *GAL*1−10 promoter, without prejudicing the other two *HO* controls. A modular nature to complex combinatorial controls would of course greatly facilitate their evolution.

One of the striking facts concerning hybrid *GAL/HO* promoters is that the *GAL* UAS does not require *SWI2* or *SWI4* when placed 150 bp upstream of the *HO* RNA 5′ terminus (Stern *et al.*, 1984), yet it acquires a strong dependence when placed 1000 bp upstream (this work). One explanation for this finding is that *SWI2* and *SWI4* are required to overcome a system of repression which is otherwise exerted by the DNA between −150 and −1000. There is, however, an alternative view of this phenomenon. The *GAL* UAS normally functions from 200 to 300 bp upstream of

**Table IV.** Yeast strains used in this study

| Strain | Genotype | | | | | | Source |
|---|---|---|---|---|---|---|---|
| H538 | *HMLα* | *MAT*a/α | *HO*(Δ145−901) | *SWI*$^+$ | | | (K838.K700.1b) |
| H413 | *HMLα*? | *MAT*a/α | *HO* | *SWI*$^+$ | | | (K839.K700.9c) |
| H411 | *HMLα*? | *MAT*a/α | *GAL/HO* | *SWI*$^+$ | | | (K1121.K700.1d) |
| K838 | *hml*a | *MAT*a | *HO*(Δ145−901) | *SWI*$^+$ | *cdc*15-2 | | (from K792) |
| K839 | *hml*a | *MAT*a | *HO* | *SWI*$^+$ | *cdc*15-2 | | (from K792) |
| K1121 | *hml*a | *MAT*a | *GAL/HO* | *SWI*$^+$ | *cdc*15-2 | | (from K792) |
| H407 | *hml*a | *MAT*a | *GAL/HO* | *SWI*$^+$ | | | (K1121.K700,4c) |
| H685 | *HMLα*? | *MAT*a | *GAL/HO* | *swi*4-100 | | | (from H262) |
| H687 | *hml*a | *MAT*a | *GAL/HO* | *swi*2-314 | | | (from H271) |
| K1107 | *hml*a | *MAT*a | *ho*−βgal | *SWI*$^+$ | | | |
| K1115 | *hml*a | *MAT*α | *ho*−βgal | *SWI*$^+$ | *HIS*3 | *his*4 | |
| H206 | *hml*a | *MAT*a | *ho*−βgal | *swi*2-314 | | | |
| H378 | *hml*a | *MAT*a | *ho*−βgal | *swi*4-100 | | | |
| H235 | *hml*a | *MAT*a | *ho*−βgal | *swi*5-100 | | | |
| H379 | *hml*a | *MAT*a | *ho*−βgal | *swi*6-399 | | | |
| H220 | *hml*a | *MAT*a | *ho*−βgal | *swi*7-288 | | | |
| H236 | *hml*a | *MAT*a | *ho*−βgal | *swi*8-299 | | | |
| H237 | *hml*a | *MAT*a | *ho*−βgal | *swi*9-289 | | | |
| H454 | *hml*a | *MAT*a | *ho*−βgal | *swi*10-281 | | | |
| K1127 | *HMLα* | *MAT*α | *GAL/ho*−βgal | *SWI*$^+$ | | | (from K715) |
| K1131 | *hml*a | *MAT*a | *GAL/ho*−βgal | *SWI*$^+$ | | | (from K765) |
| 671.$E_1$[a] | *hml*a | *MAT*α | *HO* | *swi*3-1 | | | (I.H.) |
| 671.$E_2$[a] | *hml*a | *MAT*α | *HO* | *swi*1-2 | | | (I.H.) |
| H278 | *hml*a | *MAT*a | *GAL/ho*−βgal | *swi*1-2 | ? | | (671.$E_2$.K1131.10d) |
| H280 | *hml*a | *MAT*α | *GAL/ho*−βgal | *swi*3-1 | ? | | (671.$E_1$.K1131.3a) |
| H262 | *HMLα*? | *MAT*a | *ho/SUP*4-0 | *swi*4-100 | *HIS*$^+$ | | (K.A.N.) |
| H266 | ? | *MAT*a | *ho/SUP*4-0 | *swi*5-100 | | | (K.A.N.) |
| H269 | ? | *MAT*a | *ho/SUP*4-0 | *swi*6-399 | *HIS*$^+$ | | (K.A.N.) |
| H271 | *hml*a | *MAT*a | *ho/SUP*4-0 | *swi*2-314 | | | (K.A.N.) |
| H474−476 | | | *GAL/ho*−βgal | *swi*1-2 | | | (H278.K1127.5c,7b,8c) |
| H307,311 | | | *GAL/ho*−βgal | *swi*2-314 | | | (H271.K1127.4b,7a) |
| H477−481 | | | *GAL/ho*−βgal | *swi*3-1 | | | (H280.K1131.5a,5d,6b,8b,8d) |
| H313,315 | | | *GAL/ho*−βgal | *swi*4-100 | | | (H262.K1127.3b,6a) |
| H332 | | *MAT*α | *GAL/ho*−βgal | *swi*5-100 | | | (H266.K1127.10c) |
| H317,319 | | | *GAL/ho*−βgal | *swi*6-399 | | | (H269.K1127.4b,6b) |
| H427,431 | | | *GAL/ho*−βgal | *swi*7-288 | | | (H220.K1127.4a,8a) |
| H415,416,419,420 | | | *GAL/ho*−βgal | *swi*8-299 | | | (H236.K1127.2c,2d,10a,10d) |
| H422,424,425 | | | *GAL/ho*−βgal | *swi*9-289 | | | (H237.K1127.4b,5d,6a) |
| H433,434,438,440 | | | *GAL/ho*−βgal | *swi*10-281 | | | (H365.K1131.1b,1c,6b,8b) |
| H365 | *hml*a? | *MAT*α | *ho*−βgal | *swi*10-281 | | | (K.A.N.) |

Unless otherwise stated, all strains were *trp*1, *leu*2, *his*3, *ura*3, *ade*2-1 and *can*1-100.
[a]Strains 671.$E_1$ and $E_2$ did not carry the above mutations but were instead *cry*1-3, *ura*4, *met*$^-$.

the *GAL*1 and *GAL*10 start sites, yet it is still capable of action when placed 1200 bp upstream of the *HO* start site. It is possible that the longer range of *GAL* UAS activation at *GAL/HO* is only possible when facilitated by *SWI*2 and *SWI*4 acting via sequences within *URS*2.

One of the current models for cooperative long-range regulation proposes that stable transcription complexes can be formed through the interaction of different factors via protein–protein contacts while each protein is still bound to specific sites which may be hundreds or even thousands of base pairs apart (Nasmyth, 1986; Ptashne, 1986). Recent observations of the cooperative binding of *lac* (Besse *et al.*, 1986) or λ repressor (Hochschild and Ptashne, 1986) to distant pairs of operators have confirmed that such a model is feasible. It is possible to imagine, therefore, that different *SWI* gene products, e.g. *SWI*4 and *SWI*5, manage to interact directly even though they act via different *cis*-acting DNA sequences at *HO*. In this context, it is worth pointing out, however, that by the same criterion *GAL*4 can also cooperate with *SWI*4 even though there has almost certainly been no previous history of such an interaction. This implies that any interaction between *SWI*5 and *SWI*4 is unlikely to be via highly specific protein–protein contacts and may therefore involve a third party such as an RNA polymerase subunit.

## Materials and methods

The construction of recombinant DNA clones, transplacement into yeast, the analysis of transcription and genetic crosses were performed as described by Nasmyth (1985a).

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## References

Besse,M., Von Wilcken-Bergmann,B. and Müller-Hill,B. (1986) *EMBO J.*, **5**, 1377–1382.
Douglas,H.C. and Hawthorne,D.C. (1972) *J. Bacteriol.*, **109**, 1139–1143.
Guarente,L., Rogers Yocum,R. and Gifford,P. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7411–7414.
Haber,J.E. and Garvick,B. (1977) *Genetics*, **87**, 33–50.
Hawthorne,D.C. (1963) *Abstr. Proc. 11th Int. Congr. Genet.*, **7**, 34–35.
Hicks,J.B. and Herskowitz,I. (1976) *Genetics*, **83**, 245–258.
Hochschild,A. and Ptashne,M. (1986) *Cell*, **44**, 681–687.
Jensen,R. (1983) Ph.D. Thesis, University of Oregon.
Jensen,R.E. and Herskowitz,I. (1984) *Cold Spring Harbor Symp. Quant. Biol.*, **49**, 97–104.
Jensen,R., Sprague,G.F. and Herskowitz,I. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3035–3039.
Kostriken,R., Strathern,J.N., Klar,A.J.S., Hicks,J.B. and Heffron,F. (1983) *Cell*, **35**, 167–174.
Miller,J., McLachlan,A.D. and Klug,A. (1985a) *EMBO J.*, **4**, 1609–1614.
Miller,A.M., Mackay,V.L. and Nasmyth,K.A. (1985b) *Nature*, **314**, 598–603.
Nasmyth,K.A. (1983) *Nature*, **302**, 670–676.
Nasmyth,K. (1985a) *Cell*, **42**, 213–223.
Nasmyth,K. (1985b) *Cell*, **42**, 225–235.
Nasmyth,K. (1986) *Trends Genet.*, **2**, 115–117.
Ptashne,M. (1986) *Nature*, **322**, 697–700.
Stern,M., Jensen,R. and Herskowitz,I. (1984) *J. Mol. Biol.*, **178**, 853–868.
Strathern,J.N. and Herskowitz,I. (1979) *Cell*, **17**, 371–381.
Strathern,J.N., Klar,A.J.S., Hicks,J.B., Abraham,J.A., Ivy,J.M., Nasmyth,K.A. and McGill,C. (1982) *Cell*, **31**, 183–192.
Takano,I. and Oshima,Y. (1970) *Genetics*, **65**, 421–427.

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