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120. I dedicate this article to Viktor Hamburger, who promoted and took part in this search, and to whom I am forever indebted for invaluable suggestions and generosity. Without him, the nerve growth factor would never have come to our attention. To my dear friends, Pietro Calissano and Luigi Aloe, I wish to express my deepest gratitude for their fundamental contributions. In this 35-year-long investigation, a large number of colleagues, research associates, and graduate students took part in this scientific adventure. I am particularly indebted and I very gratefully acknowledge the most important work performed by two of them: Drs. Piero Angeletti and Vincenzo Bocchini. To Professor Carlos Chagas, for his generous hospitality in the Biophysic Institute of the University of Brazil, and to Dr. Hertha Meyer, who helped me in devising the tissue culture bioassay of NGF, my warmest thanks.

Transcriptional Regulation in the Yeast Life Cycle

KIM NASMYTH AND DAVID SHORE

The transition from haploid to diploid in homothallic yeast involves a defined sequence of events which are regulated at the level of transcription. Transcription factors encoded by *SWI* genes activate the *HO* endonuclease gene at a precise stage in the cell cycle of mother cells. The *HO* endonuclease initiates a transposition event which activates genes of the opposite mating type by causing them to move away from a silencer element. The activated mating type genes then regulate genes involved in cell signaling such as the mating type–specific pheromones and their receptors. Since *HO* is only activated in one of the sister cells after division (the mother), adjacent cells of opposite mating type are generated which respond to each others' secreted pheromones by inducing genes involved in conjugation. This leads to the formation of a diploid in which many of the genes involved in mating and mating-type switching become repressed due to the heterozygosity of the mating-type locus. This article summarizes what is known about these transcriptional controls and discusses possible parallels in higher eukaryotes.

EVEN RELATIVELY SIMPLE EUKARYOTES SUCH AS THE BUDding yeast *Saccharomyces cerevisiae* exhibit a rich repertoire of developmental events; major insights have been gained concerning these events by the combined application of molecular and classical genetics (*1*). This article describes the role and properties of transcriptional regulation during a crucial phase of the yeast life cycle: from the birth of a haploid spore after meiosis to the reattainment of the diploid state.

The predominant vegetative phase of yeast is a nonmating diploid cell which is heterozygous for the mating-type locus *MAT*; it contains both *MAT***a** and *MAT*α alleles. Upon starvation, these cells undergo meiosis and produce two spores containing *MAT***a** and two spores containing *MAT*α. In heterothallic strains, germination and cell division result in clones of stable haploids of either **a** or α mating type. If these cells encounter cells of the opposite mating type they conjugate to produce a nonmating **a**/α diploid. Communication between **a** and α cells is a prerequisite for conjugation. The α cells secrete a peptide pheromone called α-factor, which not only arrests **a** cells in G1, but also induces genes involved in the conjugation process. Likewise, **a** cells secrete a peptide **a**-factor which has analogous effects on α cells. Both pheromones exert their effect through receptors specific to each mating type (*2–4*).

Most wild yeast strains, however, are homothallic (*5*), which means that a single **a** or α spore will rapidly give rise to **a**/α diploids without having to encounter cells of different clonal origin and mating type (Fig. 1). When germinated in isolation from other cells, homothallic spores with an α mating type, for example, divide by budding to produce a mother cell and a daughter cell, both of which retain the original α mating type. Both cells then undergo a further cell division, during which the mother cell, but not the daughter cell, switches mating type. Consequently, at the four-cell stage there are

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two cells with the original α mating type derived from the daughter and two cells with the **a** mating type derived from the mother (*6*). The spatial pattern of cell division in these haploid cells is such that each **a** cell is in a position to conjugate with an α cell so that a pair of **a**/α zygotes is produced.

If the **a** and α cells produced by homothallic spores are prevented from mating, the pattern of mating-type switching described above is reiterated at subsequent cell divisons (Fig. 2); that is, mother cells switch mating types at almost every cell division to produce a pair of progeny with changed mating type, whereas daughter cells never switch and produce a pair of progeny with their parents' mating type (*7*).

During the developmental sequence which leads from haploid to diploid, homothallic yeast cells (which differ from heterothallic strains only in possessing a functional allele of the *HO* locus) display two phenomena more usually associated with metazoan development (*8*): (i) the ability to undergo cell-autonomous differentiation (the production of **a** cells from the mother and α cells from the daughter of a single cell division), and (ii) differentiation due to inductive processes (the formation of a third cell type, the **a**/α diploid, as a consequence of **a** and α cell interactions). We would like to address the following sorts of questions about these phenomena:

1) How does a cell divide to produce daughter cells which have different developmental fates?

2) How are the different developmental fates initially determined and then maintained, and what is the basis of irreversibility?

3) To what extent can we account for the different behavior of cells by their different patterns of gene expression?

4) How do different cell types interact to produce further differentiation?

Cell-Type Determination: Regulation by *MAT* Genes

In order to understand how a cell switches its cell type and how this process is regulated, it is first necessary to consider the molecular basis of cell type–specific behavior. The three cell types in yeast (**a**, α, and **a**/α) are determined by the *MAT* locus. *MAT***a** and *MAT*α contain different DNA sequences (see below) and thereby direct the transcription of different genes: *MAT*α*1* and *MAT*α*2* or *MAT***a***1* and *MAT***a***2* (*9*). The *MAT*α*1* and *MAT*α*2* genes are required to determine an α mating type, the absence of both of these genes determines an **a** mating type (*10*), and the simultaneous presence of *MAT***a***1* and *MAT*α*2* determines the **a**/α mating type.

It is now clear that the main, if not sole, role of the *MAT* genes is to regulate the genes actually involved in cell behavior that are present in both **a** and α cells (see Fig. 3). *MAT*α*1* is required to activate transcription of the α-factor and **a**-factor receptor genes [the so-called α-specific genes (*11, 12*)], whereas *MAT*α*2* represses transcription of the **a**-factor and α-factor receptor genes [members of a set of **a**-specific genes (*13, 14*)]. *MAT*α cells therefore make α-factor and respond only to **a**-factor. In **a** cells, on the other hand, the **a**-factor gene and α-factor receptor gene are expressed in the absence of *MAT*α*2*. These cells therefore make **a**-factor and respond only to α-factor. In **a**/α cells, the combined action of *MAT***a***1* and *MAT*α*2* represses a set of haploid-specific genes such as *MAT*α*1*, *STE5* (which is required for transcription of pheromone and receptor genes in both **a** and α cells), *HO* (which is required for mating-type switching), and *RME* (which is a repressor of sporulation) (*15*). These cells therefore neither secrete nor respond to pheromones, but are instead capable of sporulation.

There are two main phases of transcriptional control during mating. During the first phase, *MAT* determines which pheromone a cell will make and to which it will respond by regulating the **a**- and α-specific genes. Subsequently, cells respond to pheromones by inducing genes more directly involved in conjugation itself, such as *FUS1* (*16*) (which is required for cell fusion), and *KAR1* (*17*) (which is required for karyogamy, the fusion of cell nuclei after conjugation). Little is known about the intracellular signal transmission leading to the second phase, except that **a** and α cells probably share a common response mechanism (*18, 19*) which involves genes such as *STE4, STE5, STE7, STE11*, and *STE12* (*20*). Phosphorylation may be involved since *STE7* is homologous to protein kinases (*21*). A specific consensus sequence that is found in all inducible promoters is responsible for inducibility, but it is not known which gene products bind to it (*14, 22*).

The mechanism by which *MAT* genes regulate the cell behavior genes is beginning to be understood at the molecular level (Fig. 4). Surprisingly, both *MAT*α*1* and *MAT*α*2* may act, at some promoters, via a common transcription factor that has been called Pheromone and Receptor Transcription Factor (PRTF). PRTF binding sites are required for upstream activation (*23–25*) of both **a**- and α-specific genes (*26, 27*). In the case of the α-factor gene and **a**-factor receptor gene (*26, 27*), α1 (a protein product of *MAT*α*1*) and PRTF bind synergistically to adjacent DNA sequences in vitro (*26, 27*). The α1 protein may therefore function in vivo by recruiting PRTF to α-specific promoters (Fig. 4). In the case of the α-factor receptor gene, α2 binds to sequences which overlap a PRTF binding site that is essential for promoter activity. Therefore, α2 may function at this promoter by excluding PRTF (Fig. 4). PRTF has so

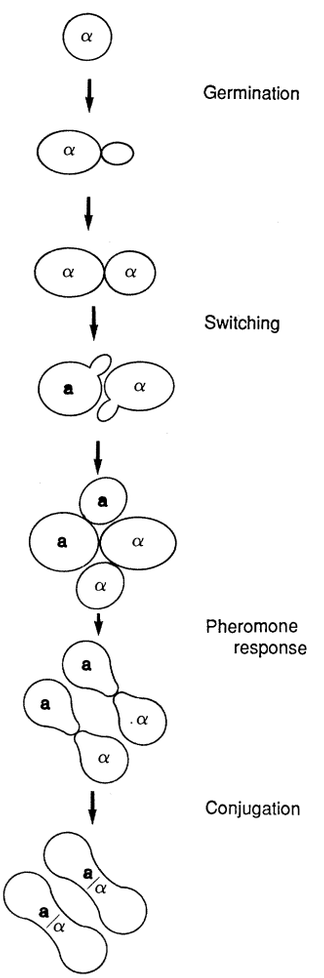

Fig. 1. The role of mating-type switching in the diploidization of homothallic spores. A spore with an initial α mating type (it could equally be **a**) germinates and divides by budding to produce a pair of α cells: a mother on the left and a daughter cell on the right. During their subsequent cell divisions, the mother cell switches mating type and produces a pair of **a** cells, whereas the daughter retains its mating type and produces a pair of α cells (*6*). Cells of opposite mating type now arrest each other in G1 by secreting one pheromone (**a** and α) and by responding to the other. During the pheromone-induced G1 arrest, the cells elongate, becoming pear-shaped (known as schmooing) and eventually fuse to produce a pair of diploids. These diploids are heterozygous for the *MAT* locus and no longer mate nor switch their mating types, but undergo cell division by budding.

far not been identified genetically, suggesting that it may also be required for the expression of essential genes (*26–28*).

The model described in Fig. 4 suggests that *MAT* regulates the **a**- and α-specific genes merely by regulating the binding of PRTF. This is clearly not the whole story since these genes also require other factors such as *STE4, STE5, STE7, STE11*, and *STE12* for activation (*18, 20*). Whether these gene products also bind to the **a**- and α-specific gene promoters, or merely regulate the activity of PRTF, is not known.

A considerable amount of biochemical information has been obtained for α2 since it has been possible to partially purify an active α2–β-galactosidase fusion protein which binds in vitro to a consensus sequence present within all promoters repressed by α2 (*29*). The site-specific DNA binding domain of α2 probably resides at its COOH-terminus since this region is homologous to the helix-turn-helix motif of better characterized bacterial DNA binding proteins, to an equivalent region of *MAT***a***1*, and to the *Drosophila* homeobox (*30*). Point mutations in conserved residues within this region abolish repression (*31*).

Regulation of α2 is perhaps the clearest case of negative control in eukaryotes. For example, the insertion of a single α2 binding site between the upstream activator sequence (UAS) and TATA box of the heterologous *CYC1* promoter causes it to be repressed by α2 (*29*). The mechanism of this repression is not yet clear. Unlike operators in bacteria, the α2 binding site does not have to overlap with the UAS or the TATA box to exert its effect. Moreover, α2 causes much more potent repression of yeast genes than can the binding of LexA, a bacterial repressor protein (*32*). Either α2 binds much more tightly to its operator than does LexA, or α2 repression involves more than just DNA binding. Perhaps α2 bound to its operator specifically prevents the DNA bending required for the interaction of UAS and TATA box factors. In this regard, it will be interesting to see whether there is a domain of α2 required for repression but not for DNA binding per se.

One of the more intriguing phenomena of *MAT* regulation is the repression by *MATα2* of one set of genes in α cells (the **a**-specific genes), but another set (the haploid-specific genes) in **a**/α cells where the *MAT***a***1* gene is also present (*15*). Since a region of **a1** is homologous to the putative DNA binding domain of α2, it has been proposed that **a1** and α2 combine to produce a heteromeric protein whose DNA binding specificity is different from that of α2 (Fig. 4). The operator for such a putative repressor has been identified as a consensus sequence present within all **a1**/α2 repressed genes, and like the α2 site, has been shown to confer **a1**/α2 repression upon heterologous promoters (*33*). The **a1**/α2 consensus sequence within the *MATα1* promoter is situated between the UAS and TATA box, and its deletion causes expression in **a**/α cells (*34*). Repression in **a1**/α2 cells is one of the clearest examples of combinatorial control in eukaryotes. It is vital for the yeast life cycle, ensuring that diploid cells will not mate (due to repression of *STE5* and *MATα1*), will not undergo mating-type switching (due to repression of *HO*), and that they alone will be capable of sporulation (due to repression of *RME*).

Having defined the genes involved in cell type–specific behavior, one would like to establish that a given pattern of gene expression is sufficient to explain a given cell behavior. For instance, how many

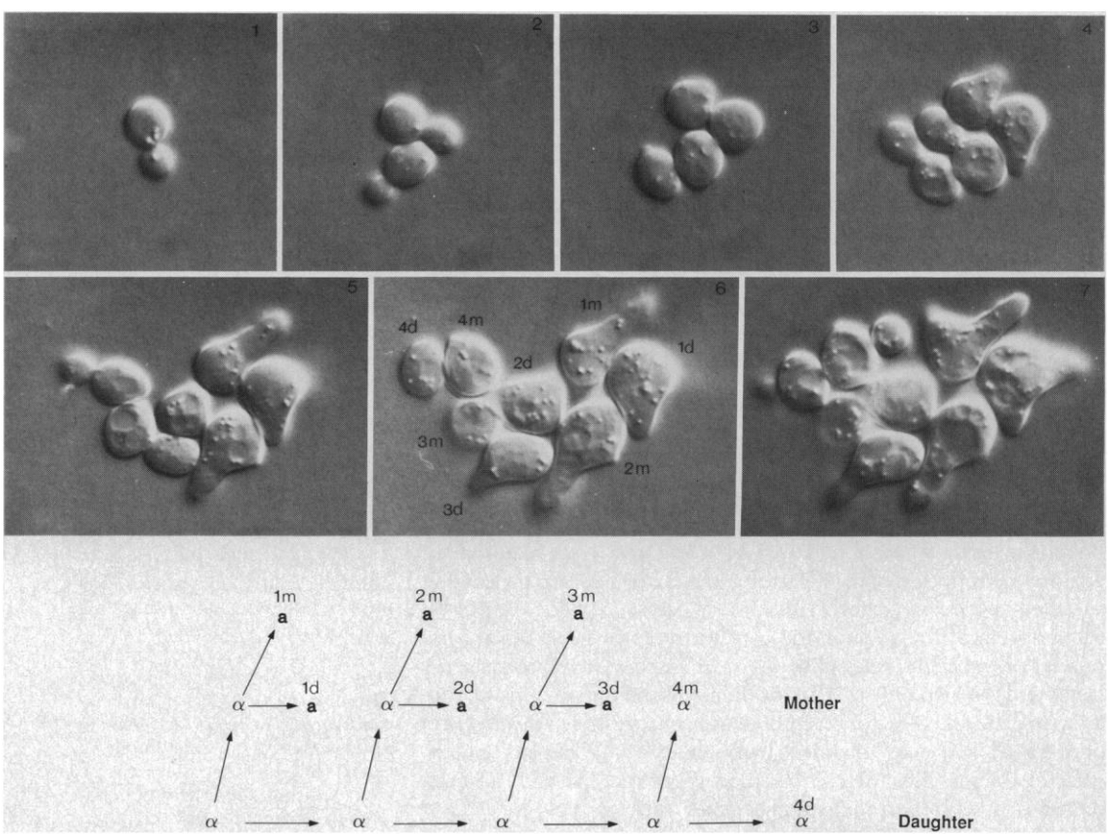

Fig. 2. Direct visualization of a pedigree of mating-type switching. The pattern of mating-type switching is most conveniently monitored by growing cells in the presence of the α-factor pheromone (*6*). This enables one to visualize the mating type of individual cells according to whether they are refractory to α-factor, and therefore continue dividing (if they are α cells), or whether they respond to the pheromone by arresting in G1 and forming large pear-shaped cells known as schmoos (if they are **a** cells). Under these conditions, the pedigree of mating-type switching resembles a stem cell lineage; the daughter cell acts as a perpetually dividing stem cell and gives rise to mother cells which produce pairs of nondividing differentiated progeny (*7*). An α spore was placed on a microscope slide on a thin agar slab containing fresh medium and α-factor and sealed under a cover slip with paraffin wax. The slide was then incubated at 30°C and photographs were taken, using the Nomarski optics of a Zeiss microscope, every 1 to 2 hours (panels 1 through 7). The pedigree of cell divisions and mating-type switches is shown at the bottom of the figure. Each cell division gives rise to a mother (vertical arrow) and a daughter (horizontal arrow). Each mother cell then switches to produce a pair of **a** cells (a mother and a daughter). The identity of each cell in the pedigree is marked on panel 5 (1m and 1d were the mother and daughter cells produced from the first mother cell, and so forth). Cells 1m, 1d, 2m, 2d, and 3d are all clearly seen as **a** cell schmoos in panel 6. Cell 3m has moved out of the plane of focus but was also a schmoo. Cells 4d and 4m are α cells since they both proceed to bud (see panel 7).

genes must be differentially expressed in **a** and α cells to account fully for their different behaviors? In this regard, it has been shown that the specificity of hormone response (whether a cell will respond to **a**- or α-factor by inducing conjugation genes) is due entirely to which pheromone receptor gene is expressed in that cell, suggesting that a common response mechanism is involved (*18, 19*). More specifically, one may ask how many genes must *MAT*α*1* activate and *MAT*α*2* repress to convert an **a** cell into an α cell. In the case of *MAT*α*1*, all its primary targets have been identified, since the mating defect of *mat*α*1* mutants can be rescued by the introduction of constitutive versions of the α-factor and **a**-factor receptor genes (*18, 35*).

Similar questions can be asked about the determination of the **a**/α diploid state. For instance, expression of the **a**- or α-factor receptor in diploid cells (by fusion to a constitutive promoter) is insufficient to cause them to respond to either pheromone (*18, 35*). This is possibly because genes like *STE5*, which are repressed in **a**/α diploids, are required not only for receptor gene transcription but also for the genes induced by the pheromone. Would it be sufficient therefore to express *STE5* and a receptor gene in a diploid to cause it to respond to a pheromone?

Mating-Type Switching: The Regulation of *MAT* Genes

When a cell switches its mating type from α to **a**, it goes from a state in which *MAT*α*1* and *MAT*α*2* are expressed to one in which *MAT***a***1* is expressed. The change in *MAT* expression occurs as a consequence of DNA rearrangement at the *MAT* locus (Fig. 5). *MAT***a** and *MAT*α each contain unique (**a**- and α-specific) DNA sequences which are replaced by ones of the opposite type derived from silent copies situated elsewhere in the genome—at *HML* for α and *HMR* for **a** (*36*). The **a**- and α-specific sequences are flanked by homologous DNA sequences at all loci, and the rearrangement, which can be viewed as a site-specific gene conversion, is initiated by a specific double-stranded cleavage at *MAT* produced by the *HO* endonuclease (*37*). The silent copies are presumably used as templates for DNA synthesis needed to repair the break.

The question of how *MAT* genes are activated during a switch resolves, therefore, to why the silent copies of **a** and α information at *HMR* and *HML* are only expressed when moved to the *MAT* locus. The simplest explanation for this phenomenon, that only *MAT* contains an intact promoter, does not apply—the α*1* and α*2* promoters (and coding sequences) are present in their entirety at *HML*α, and the same is true for **a***1* and **a***2* at *HMR***a** (*9*). Indeed, in certain mutants (see below), the silent copy genes are activated in situ, that is, without movement to *MAT*. Somehow, the sequences flanking *HML* and *HMR*, or *MAT*, influence whether or not transcription is initiated at the mating-type promoters several kilobases away. It is now clear that this phenomenon of long-range control is due to the presence of a "silencer" to the left of both *HML* and *HMR*. Although it has the opposite effect on transcription, the silencer at *HMR* has many of the properties of an enhancer: it will repress genes up to 2.5 kb away; it will repress several different types of polymerase II promoters, and even a polymerase III promoter; and it functions in either orientation and from either side of a locus (*38–40*). The silencers also determine that mating-type information will be switched at *MAT* and donated by the silent loci, apparently by blocking access to the *HO* cleavage sites in the chromatin at *HML* and *HMR* (*41*). In addition to the silencer, repression of *HML* and *HMR* requires at least four trans-acting genes called *SIR1, SIR2, SIR3*, and *SIR4* (*42*).

A particularly intriguing aspect of silencer function is the possible involvement of DNA replication. Both silencers have ARS activity (the ability to confer autonomous replication to plasmids) and may therefore be specific origins of DNA replication (*38*). Moreover, passage through S phase is apparently required to reestablish repression after a locus has been derepressed by means of a temperature-sensitive *sir* mutation (*43*).

The *HMR* silencer has been characterized by analyzing the DNA sequences required for repression of the *HMR***a***1* gene (*44*). Three different regulatory elements have been identified (called A, E, and B) which are all within a 120-bp region. Mutation of E alone results in 15% of full *sir*$^-$ derepression, whereas mutation of A or B alone has no phenotypic effect. Deletion of any two of these three sequences, however, leads to full derepression. The A region contains a perfect match to the ARS consensus sequence, deletion of which abolishes the ARS activity of the 120-bp fragment. The E and B regions contain binding sites for two different factors called RAP1 (*r*epressor/*a*ctivator binding *p*rotein, see below) and SBF-B (*s*ilencer *b*inding *f*actor-B (*45, 46*). RAP1 also binds to a homologous region within the *HML* silencer, whereas SBF-B does not bind to *HML*, but instead binds to other ARS elements (such as the B region of *ARS1*).

Curiously, neither RAP1 nor SBF-B are encoded by any of the four *SIR* genes (*45*). To address the question of the role of RAP1 in silencer function, the protein has been purified by DNA affinity chromatography, and antibodies raised against the purified protein

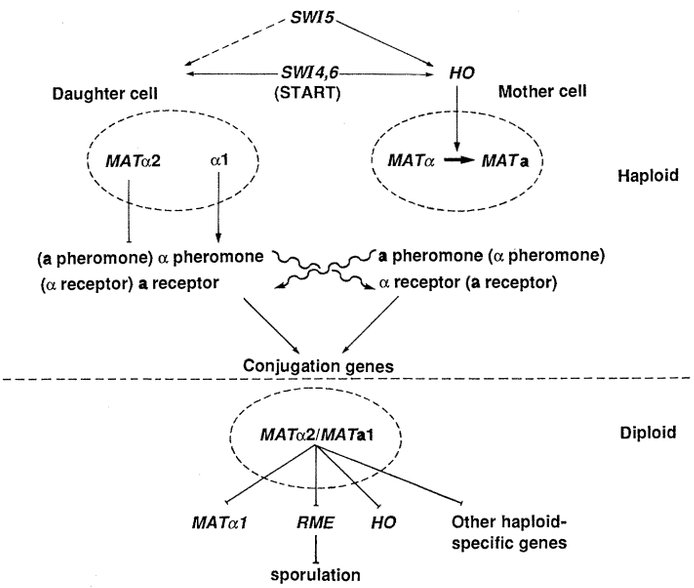

Fig. 3. The hierarchy of transcriptional regulation which is required for the diploidization of homothallic cells. Positive transcriptional regulation is indicated by straight arrows, negative regulation by a straight line with a bar at the repressed gene. Pheromone-receptor interactions are indicated by wavy arrows. The *SWI* genes determine the time and place of *HO* transcription. Differences in the level of *SWI5* ensure that only mother cells transcribe *HO* when activated by *SWI4* and *SWI6* at START. *HO* encodes an endonuclease which causes the α*1* and α*2* genes present at *MAT*α to be replaced by the **a***1* and **a***2* genes (bold arrow), which are activated upon movement away from a "silencer" at the silent copy *HMR***a** (Fig. 5). In the progeny of daughter cells, which do not switch, α*1* activates the α pheromone and **a**-factor receptor genes, whereas α*2* represses the complementary pair. In the progeny of a mother cell, however, the **a** pheromone and α-factor receptor genes are derepressed due to the absence of *MAT*α*2*, whereas the α pheromone and **a**-factor receptor genes fail to be activated in the absence of α*1* (Fig. 4). The **a** and α pheromones, acting via their respective receptors, then cause cells to induce genes required for conjugation, such as *FUS1* (*16*). Conjugation leads to the formation of a diploid cell, in which **a**1 from *MAT***a** and α2 from *MAT*α repress *MAT*α*1*, *RME* (a repressor of sporulation), *HO*, and other haploid specific genes such as *STE5* (*15*).

have been used to clone the gene from a λgt11 genomic library. Gene disruption experiments have shown that *RAP1* is essential for growth (*46*), suggesting a more general role for this factor. A clue to the essential function of RAP1 comes from the observation that its two silencer binding sites are homologous to UAS elements found at both *MAT*α and a large number of ribosomal protein genes (*46, 47*), and in vitro binding studies have shown that RAP1 does indeed bind to these UAS elements. Consistent with this observation is the fact that the two silencer-associated RAP1 binding sites display UAS activity when cloned in front of the *CYC1* TATA box (*44, 46*). These data suggest that RAP1 may be an activator of transcription in one context (for example, the ribosomal protein genes), but a repressor in another (the *HMR* silencer). The observation that RAP1 binding sites from ribosomal protein genes can restore function to a silencer whose normal RAP1 binding site has been deleted supports this notion (*46*). However, direct proof of RAP1 involvement in both activation and silencer functions will require the isolation and characterization of conditional *rap1* mutants (*48*).

How might a transcriptional activation element (the RAP1 binding site) function as part of a silencer? One can imagine several possible models, some of which might be testable. (i) If DNA replication is involved in silencer function, transcriptional activation might be required for replication to initiate at the silencer, by analogy to the requirement for enhancer function in the replication of several animal viruses, such as polyoma and bovine papilloma virus (*49*). (ii) RAP1 binding sites may allow direct silencer-promoter interaction, perhaps via protein-protein contacts between RAP1 molecules bound to the two sites, with the intervening DNA being looped out (*25*). This might target negative regulators also bound to the silencers (such as the SIR proteins) to a specific nearby promoter. Since *MAT***a** (and other promoters which can be subjected to silencer repression) (*39, 40*) appear not to contain RAP1 binding sites, one would have to postulate interactions with other transcription factors bound at these promoters. (iii) The RAP1 binding site may play no direct role in repression, but instead functions as part of a complex recognized by the SIR proteins, which might then be the actual executors of repression. By this hypothesis, the RAP1 binding site, instead of activating transcription, functions as part of a mechanism to mark the silencer to be acted on by other factors (such as the SIR proteins).

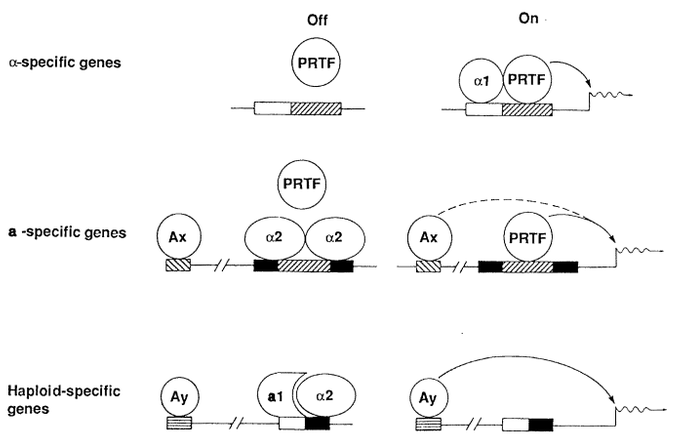

Fig. 4. Regulation of gene expression by *MAT* gene products. Most yeast genes are composed of two types of positive cis-acting elements: a TATA box which is situated 40 to 100 bp upstream of a transcription initiation element, and an upstream activation site (UAS) which may be 100 to 1000 bp further upstream (*23*). UASs exert their effect through the binding of specific transcription factors which are composed of at least two domains: one is responsible for site-specific DNA binding, and the other is required for transcriptional activation (*24*). We know little about how activation domains function, with what other molecules they interact, or even how, in general, activators bound several hundred base pairs away from a TATA box affect the rate of transcription. One possibility is that such activators interact directly with RNA polymerase, even though one is bound to a UAS and the other at or near the TATA box, by virtue of the intervening DNA being looped out (*25*). The genes for the α-factor pheromone and the **a**-factor receptor are only expressed in α cells because α1 helps PRTF to bind to these promoters. PRTF then exerts upstream activation. In **a** cells, the binding of PRTF and/or other factors (labeled Ax) activates transcription. In α cells, on the other hand, α2 represses transcription either by binding to sequences which overlap the PRTF binding site (as may occur in the case of the α-factor receptor gene) or by preventing other factors from exerting upstream activation [as may be the case for the *STE6* gene, which is required for **a**-factor biosynthesis (*13, 14*)]. For haploid specific genes, specific factors (labeled Ay; examples are RAP1 for *MAT*α and SWI5 for *HO*) bind to upstream activation sites and activate transcription. The binding of a putative heteromeric repressor encoded by *MAT***a***1* and *MAT*α*2* somehow prevents this upstream activation. The **a**1/α2 must recognize DNA sequences which are different from those recognized by α2 alone. It is possible that a crucial difference between the α2 and **a**1/α2 operators may be the spacing of related sequence elements (*33*).

The discovery that proteins other than the *SIR* proteins bind to important silencer regulatory elements (E and B) necessitates a reevaluation of the possible role of the SIR proteins in repression. An important question is whether or not they act via the silencer elements A, E, and B. That they do is suggested by the observation that plasmids which replicate autonomously by virtue of ARS sequences within the *HMR* silencer are partitioned much more evenly between mother and daughter cells at mitosis than similar plasmids replicated by other origins such as *ARS1* (*50*). This phenomenon occurs in Sir^+ cells, but not in *sir3* or *sir4* mutants. Although the relationship of this process to silencer function is unclear, the observation provides the first direct evidence that the *SIR* proteins (at least SIR3 and SIR4) act via the silencer DNA. It remains to be seen whether this action involves direct DNA binding, or whether the *SIR* proteins interact with other proteins bound to the silencer (such as RAP1 and SBF-B).

In summary, we now know that the *MAT* genes are activated by their movement away from cis-acting silencers at *HML* and *HMR*. The silencers are composed of multiple regulatory elements, several of which appear to be involved in DNA replication initiation, and at least one of which appears to act as a transcriptional activator in other contexts. The mechanism of repression by the silencers remains a mystery, but their effects on both transcription and transposition, and the apparent involvement of DNA replication, suggest that the localized formation of a higher order chromatin structure may be involved.

Regulation of Mating-Type Switching

The time and place of mating-type switching is crucial for achieving diploidization after only two cell cycles following the germination of an isolated haploid spore. Switching does not occur during the first cycle following germination and after that it occurs in the mother cell, but not the daughter cell, produced by the first cell division [Figs. 1 and 2 and (*6*)]. The switch must be initiated prior to *MAT* replication to ensure that both progeny of a mother cell change mating type. Switching must not occur in G1 cells (which are in the process of conjugation) nor in the zygote and its diploid progeny.

Remarkably, this regulation appears to be solely due to the transcriptional regulation of the *HO* gene, which encodes the endonuclease responsible for initiating the *MAT* gene conversion (*51*). *HO* is transcribed transiently late in G1 (Fig. 6), as haploid mother cells undergo START (that is, upon commitment to the mitotic cell cycle as opposed to other developmental pathways open to a G1 cell, such as conjugation), but never in daughter cells, nor in

a/α diploids. *HO* transcription is therefore sensitive to three parameters: whether a cell is a mother or daughter, its stage in the cell cycle, and whether a cell is haploid or diploid. These three forms of regulation are exerted by different cis-acting regulatory elements within *HO*, whose activity is in turn dependent upon different trans-acting regulatory proteins (Fig. 7).

At least 1400 bp of upstream DNA are required for correct *HO* expression (see Fig. 7). A region between −1000 and −1400 called *URS1* and a TATA box–like region at −60 to −90 are necessary for transcription. The region in between (−150 to −900), called *URS2*, can be deleted without affecting the level of transcription and mother/daughter regulation, but this results in altered cell cycle control, principally the loss of START dependence during G1 (*52*).

The *HO* promoter is unusual, by yeast standards, not only in its size and tripartite structure, but also in the number of its regulatory proteins. At least six genes, called *SWI1* to *SWI6*, are required as activators (*53, 54*). Moreover, the promoter's dependence upon these activators requires the action of repressors such as *SDI1* (also known as *SIN3*) and *SDI2* (*55*), whereas repression in diploids requires the *MAT***a***1* and *MAT*α*2* genes. A major question is: which regulators are responsible for which form of regulation, and via which DNA sequences do they act?

a/α *repression.* Several matches to the consensus sequence for the **a***1*/α*2* operator are found throughout the *HO* promoter and at least two of these have been shown to be sufficient to cause **a**/α repression of heterologous promoters (*33*).

Cell cycle control. The observation that deletion of *URS2* causes START-independent transcription suggests that *URS2* exerts a form of negative control (over activation exerted by *URS1*) which is transiently derepressed as cells undergo START. This interpretation is complicated by the fact that *URS2* contains several copies of a sequence (the $CACGA_4$ repeat) which acts as a START-dependent UAS when placed upstream of the *CYC1* TATA box. This activation is unlikely to be an artifact since, like *HO*, it is dependent upon *SWI4* and *SWI6*. It seems, therefore, that *SWI4* and *SWI6* exert a START-dependent activation on the *HO* promoter via the $CACGA_4$ repeats within *URS2* (*54*). However, this activation is only required if the rest of *URS2* is also present, since deletions lacking *URS2* no longer require either *SWI4* or *SWI6*.

This raises the question of why the intact *HO* promoter requires *SWI4* and *SWI6*. One possibility is that *URS2* also contains negative regulatory sequences which prevent *URS1* from being sufficient for *HO* transcription and which are antagonized transiently in the cell cycle by *SWI4* and *SWI6*. This model is consistent with the observation that the *GAL1–10* UAS, which is normally neither cell-cycle regulated nor dependent upon *SWI4* or *SWI6*, becomes so when placed upstream of *URS2* (*56*).

The transient expression of *HO* at START, before DNA replication, is important for ensuring that cells produce pairs of switched progeny and that cells do not switch during conjugation (when they are arrested pre-START). The molecular nature of the transient activation by *SWI4* and *SWI6* and how it is driven by START are not known.

Mother/daughter control. A remarkable aspect of the *HO* promoter is its differential activity in mother and daughter cells. *HO* is transcribed when mother cells undergo START, but not when daughter cells do (Fig. 6). In fact, the failure of daughter cells to transcribe *HO* is the sole reason why these cells do not switch mating type, since ectopic expression of *HO* is sufficient to cause switching in daughter cells (*56, 57*). Three lines of evidence suggest that a crucial determinant of asymmetric *HO* transcription is the activator *SWI5*:

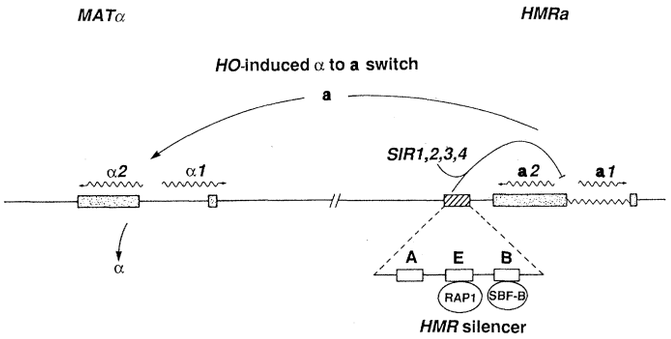

Fig. 5. Activation of *MAT* genes by transposition away from a "silencer." The diagram shows a *MAT*α locus from which *MAT*α*1* and *MAT*α*2* (α*1* and α*2*) are transcribed and a silent *HMR***a** locus at which *MAT***a1** and *MAT***a***2* (**a***1* and **a***2*) genes are repressed by an adjoining silencer. The *MAT*α and *HMR***a** loci share two blocks of homologous sequence (marked by the dotted bars) on either side of α- (straight line) or **a**- (zig-zag line) specific DNA. When a cell switches from α to **a**, the net result is that the α-specific sequence at *MAT*α is replaced by the **a**-specific sequence from *HMR***a**. This creates a *MAT***a** locus from which the **a***1* and **a***2* genes are now transcribed. The *MAT***a***1* and *MAT***a***2* genes and their promoters are present in their entirety at the *HMR***a** locus, but are repressed by the cis-acting silencer to the left of the locus. The silencer is composed of several elements: the ARS consensus sequence (A), the RAP1 binding site (E), and the SBF-B binding site (B). Silencer function requires, in addition, the action of the four *SIR* genes. At present there is no evidence that the *SIR* gene products interact directly with silencer DNA. An analogous process of gene activation occurs when **a** cells switch to become α. Silent locus *HML*α genes are activated by their movement to *MAT* where they replace *MAT***a***1* and *MAT***a***2*. *MAT*α*1* and *MAT*α*2* are kept repressed at *HML*α by a silencer within its left-hand flanking sequences. The *HO* endonuclease initiates the mating-type switch by generating a double-stranded break at the junction between the **a**- or α-specific DNA and the right-hand homology region. This break does not occur at either silent locus, even though the identical sequences are present there, because silencer action reduces their accessibility in the chromatin. Since the break defines a locus as the recipient of the gene conversion, this ensures that the silent copies are themselves never altered during mating-type switching.

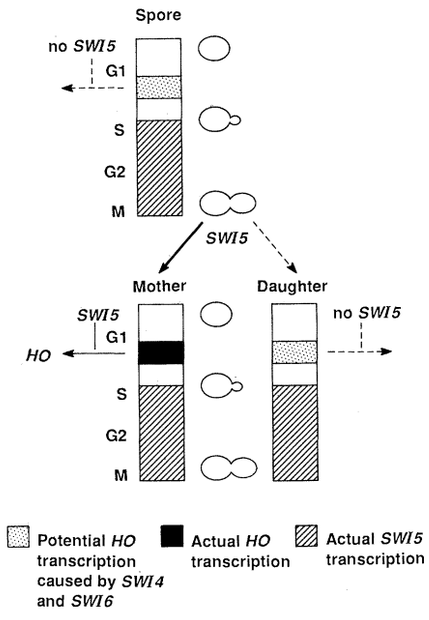

Fig. 6. The pattern of *HO* and *SWI5* transcription during spore, daughter, and mother cell divisions. Each cell cycle is represented by a vertical bar. The approximate window of *HO* transcription in mother cells is marked in black and the approximate window of *SWI5* transcription is striped. The window of potential *HO* transcription, as daughters or spores undergo START, is dotted. The figure shows that SWI5 is only made significantly after the window of potential or actual *HO* transcription, and assumes that SWI5 is asymmetrically segregated at cell division. START is shown occurring somewhat later in the cell cycle in daughters than in mothers, whereas in the presence of hydroxyurea it occurs simultaneously (*60*), without significantly affecting the bias in favor of mother-cell switching. For this reason, it is proposed that SWI5 is asymmetrically segregated at cytokinesis (*59*), but the possibility cannot be excluded that under normal circumstances, the greater length of pre-START G1 in daughter cells also contributes to their failure to express *HO*.

1) Deletion of *URS2* creates an *HO* promoter driven only by *URS1*, which is nevertheless still preferentially active in mother cells. This suggests that *URS1* may exert upstream activation which is mother cell–specific. Replacement of *URS1* alone by the *GAL1-10* UAS creates a hybrid *GAL/HO* promoter in which mother cell–specificity has been replaced by galactose dependence without affecting cell-cycle control (*55*). The *GAL/HO* promoter is still dependent upon *SWI1* to *SWI4* and *SWI6*, but is independent of *SWI5*. This suggests that *SWI5* may be responsible for the mother cell–specific upstream activation normally exerted by *URS1*, which is replaceable by the *GAL* UAS. The properties of the *GAL/HO* promoter show that activation exerted by *SWI1* to *SWI4* and *SWI6* is not necessarily confined to mother cells, whereas that exerted by *SWI5* may be.

2) The analysis of suppressors of a *swi5* mutation has identified a gene called *SDI1* (or *SIN3*) which is required for the *HO* promoter to be fully dependent on *SWI5* (*55*). The *sdi1/sin3* mutations do not suppress the requirement for the other *SWI* genes. They are defective in a repressor which binds to *URS1* and somehow prevents a subset of the *HO* activators (*SWI1* to *SWI4* and *SWI6*) from being sufficient for *HO* transcription. In *swi5 sdi* double mutants, *HO* transcription is about 30 percent of wild type, but occurs equally in mother and daughter cells. Addition of an active *SWI5* gene increases the level of *HO* transcription in mothers, but not in daughters. These results show that the level of mother/daughter switching asymmetry correlates with the degree to which the *HO* promoter is dependent upon *SWI5*. It has therefore been suggested that a difference in the level of *SWI5* in mother and daughter cells as they undergo START is the basis for asymmetry. An alternative interpretation is that *SWI5* is instead responsible for confining a repressor such as *SDI1/SIN3* to daughter cells (*58*).

3) The *SWI5* gene is cell cycle regulated (*59*). Its transcripts are absent during G1 and appear only during the latter half of the cell cycle. This means that if SWI5 were partitioned asymmetrically at cell division, then newly born daughter cells lacking SWI5 would only synthesize it after the decision to transcribe *HO* at START. The cell cycle regulation of *SWI5* is essential to prevent *HO* transcription in daughter cells since constitutive expression of the *SWI5* gene from the *GAL1-10* promoter causes daughter cells to switch their mating types (*59*). This result implies that SWI5 is the only *HO* transcription factor missing when daughter cells undergo START.

The discovery that *SWI5* is only expressed later in the cell cycle than *HO* explains one aspect of the switching pedigree: the fact that a spore or daughter cell undergoes a complete cell division during which it fails to express *HO*, yet then turns on the gene during its subsequent cycle as a mother cell. This delay of greater-than-one cell cycle for *HO* expression is explained if spores and daughter cells are born without SWI5 and cannot synthesize any in time to express *HO* during their first cell cycle. This leaves unanswered, however, why mother cells contain active SWI5, whereas both daughter cells and spores appear to be born without this activator. Two types of hypothesis have been proposed (*55, 59*). The most obvious is that SWI5 is somehow partitioned asymmetrically at cell division. An alternative is that SWI5 is instead partitioned equally between mother and daughter cells at cytokinesis, but that it then decays; asymmetry arises because of a later timing of START in daughter cells (*60*). In order for SWI5 to function, it would have to persist until activation by *SWI4* and *SWI6* occurs at START, and this may occur only in mother cells. This latter model cannot be the sole explanation for the difference between mothers and daughters, since marked switching asymmetry is still seen when differences in G1 length are abolished by growth in hydroxyurea (*59*), and also when *HO* transcription is driven by a promoter deleted for *URS2*, which is no longer START-dependent (*52, 55*).

Insight into the role of *SWI5* as an activator of *HO* has come from the discovery that it codes for a site-specific DNA binding protein of 85 kD, with three tandem copies of the zinc-finger DNA binding motif observed in TFIIIA (*61*) at its COOH-terminus. Furthermore, SWI5 overexpressed in either yeast or *Escherichia coli* binds to specific sequences within the *URS1* region of the *HO* promoter (*62*). What is less clear is how SWI5 manages to exert *HO* activation only in mother cells. A crucial question is whether its NH_2-terminal domain is somehow responsible for its proposed asymmetric segregation at cytokinesis.

An important feature of the *HO* promoter is that it is only active if several conditions are met: it must be in a haploid, mother cell at a specific cell cycle stage. What is the molecular basis for this specificity? An important clue may be the finding that the haploid/diploid control is due to the presence of several operators for the putative **a**1/α2 repressor within the promoter (*33*). In fact, there may be several negative controls acting on *HO*, all of which must be lifted for it to be active. Mother-cell specificity, for instance, is caused by the dependence of the promoter on *SWI5*. However, the *HO* promoter is only fully *SWI5*-dependent because of the binding of the *SDI1/SIN3* repressor. Likewise, *HO* is only dependent on *SWI4* and *SWI6* (and hence START-dependent) if *URS2* is present. One may imagine that, to some extent, *SWI5* exerts its effect by antagonizing *SDI1/SIN3* (Fig. 7) and that *SWI4, 6* exert their effect by antagonizing the binding of an unidentified repressor to *URS2*.

Conclusions and Implications

The transition from a haploid homothallic spore to a diploid cell clearly involves an intricate sequence of transcriptional regulation. The sequence of events can be viewed as a five-tiered hierarchy (Fig. 3). At the top of the hierarchy are the *SWI* genes, which are involved in the spatial and temporal determination of *HO* expression. The *HO* gene in turn regulates the expression of the *MAT* genes by initiating their movement to the active *MAT* locus, away from silencers present at the *HML* and *HMR* loci. Due to differential *HO* expression, two cells at the four-cell stage express *MAT***a** and two express *MAT*α. The *MAT* genes determine mating behavior, principally by regulating the genes involved in cell signaling. Unlike *HO*, which is only transiently required to switch mating type, the *MAT* genes are required continuously to maintain specific patterns of gene expression. Cell signaling involves gene products which act outside the cell or on its surface, such as the pheromones and their receptors; as well as gene products involved in signal transmission within the cell, such as *STE4, STE5, STE7, STE11*, and *STE12* (*13–15, 18, 20, 63*). Then there are the genes involved in conjugation itself, such as *FUS1* (*16*), which are only induced as a consequence of cell signaling. Finally, when a diploid cell has been formed, most of the genes involved in mating and switching are repressed, and sporulation competence is switched on.

It should be stressed that these various transcriptional events must occur at precise times in order to achieve the correct pattern of mating-type switching. The production of *SWI5* must be restricted to the latter half of the cell cycle in order to prevent daughter cells from expressing *HO*. In turn, *HO* must be expressed only transiently post-START (in order to prevent switching during conjugation), but prior to DNA replication (in order to produce pairs of switched progeny). In addition, many of the gene products involved must be very unstable. For instance, *HO* must decay rapidly once synthesized so that none survives to cause switching in G2 and none enters a daughter cell. Likewise, the *MAT* gene products, and the pheromone receptors which they regulate, must also have short half-lives, so that a cell can change its mating type within a single cell cycle.

Clearly, post-transcriptional processes such as messenger RNA degradation and proteolysis may be just as important as the transcriptional regulation (*73*).

It is now clear that more complex developmental processes in metazoa may involve networks of transcriptional control which are analogous to those found in yeast. For example, many early events in *Drosophila* development associated with metamerization involve complex patterns of transcriptional regulation. One can draw certain analogies between the *MAT* genes in yeast and homeotic genes such as *Ultrabithorax* (*Ubx*) and *Antennapedia*, which determine segment identity in *Drosophila* (*64*). Both sets of genes are required continuously (to determine either cell type or segment identity), both are thought to act by regulating other genes which are more directly involved in cell behavior (*65*), and both are switched on only in certain cells. In the case of an α spore, the *MAT***a** genes are only turned on in the two progeny of a mother cell. In the case of *Drosophila*, *Ubx* is turned on in parasegments 5 to 14, but not parasegments 1 to 4. In both organisms, there are genes such as *HO* for *MAT*, and *Krüppel* or *ftz* for *Ubx*, which are required for the correct initiation of gene activation but not for its maintenance (*66*). The pattern of *Ubx* or *MAT* expression is dependent upon the pattern of expression of these "activator" genes, which are themselves spatially restricted by maternally derived determinants [*SWI5* for *HO* and perhaps *bicoid* for *Krüppel* (*67*)].

There are of course crucial differences between the regulation of *Ubx* and *MAT*. DNA rearrangement is the means by which *MAT* genes are switched on by *HO* in an irreversible manner (that is, *HO* is not required to maintain *MAT* expression). No such mechanism is involved in the switching on of *Ubx*. Instead, positive control loops

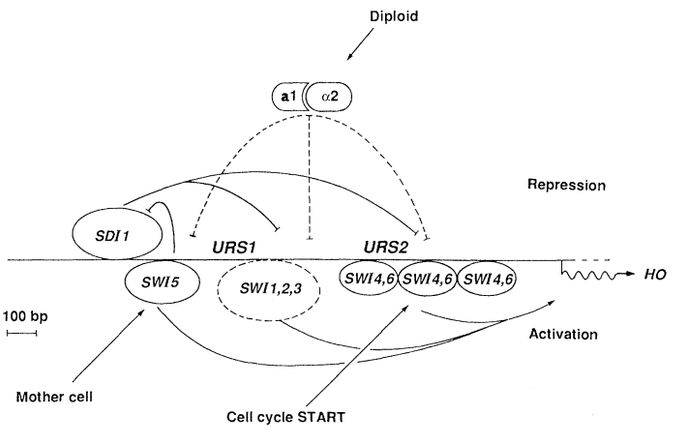

Fig. 7. The *HO* promoter and some of its regulatory proteins. The *HO* promoter requires *SWI1* to *SWI6* for activation, *SDI1*/*SIN3* for causing it to be fully dependent upon *SWI5*, and *MAT***a***1*/*MAT*α*2* for repression in diploids. Differences in the level of the *SWI5* gene product as cells undergo START are responsible for differential activity in mother and daughter cells. The *SWI5* protein has been shown to bind in vitro to sequences within *URS1* (*62*). *SWI4* and *SWI6* exert cell cycle START-specific activation via several copies of the $CACGA_4$ motif within *URS2*. Though *SWI1* to *SWI3* are essential, we do not know what their physiological role is, nor via which exact DNA sequences they act (cells with deletions of *URS2* are still dependent on *SWI1, 2*, and *3*). In the absence of *SDI1*/*SIN3*, *SWI1* to *SWI4* and *SWI6* become partially sufficient for activation; that is, *SWI5* is now partially redundant. *SDI1*, or a protein regulated by it, binds to sequences just upstream of the known *SWI5* binding site in *URS1*. Presumably, *SDI1* somehow down-regulates activation by *SWI1* to *SWI4* and *SWI6*. *SWI5* may perform two tasks within the promoter: direct upstream activation, cooperating with the other *SWI* gene products, and antagonization of *SDI1*/*SIN3*. It should be stressed that there is no evidence that any one of the *SWI1* to *SWI4* or *SWI6* gene products actually binds directly to the *HO* promoter. We do know, though, that *SWI5, SWI1* to *SWI3*, and *SWI4* + *SWI6* all act via different DNA sequences within the *HO* promoter.

are the more likely mechanism by which *Ubx* expression is maintained (*68*) in the absence of gene products such as *ftz* and *Krüppel*, which are required to activate it in the first instance.

This comparative analysis is a useful exercise since it forces one to ask why such different mechanisms are used to perform apparently similar tasks. Perhaps DNA rearrangement is not used for *Ubx* control because its expression is varied from segment to segment, whereas *MAT* expression must be all or none. Moreover, positive and negative control loops may be inappropriate for determining *MAT* expression because the diploidization program must function from two different starting states, that is, an **a** or an α spore.

One of the more surprising discoveries about yeast diploidization is that the number of genes involved in regulating other genes is considerably larger than the number of genes actually involved in cell behaviour. How can the cell afford eight or more genes to regulate *HO*, eight or more to regulate *HML* and *HMR* (*69*), and four or more to regulate the α-factor gene, for example? This situation may be an example of the phenomenon of combinatorial control. To reduce the total number of regulatory genes in a cell, each gene is regulated by a large number of regulatory molecules, many of which are used in a different combination to regulate other genes. This is most strikingly seen in the case of the regulator *RAP1*, which is apparently required for repression of the silent mating-type loci *HML* and *HMR*, but may also be involved in the activation of a large number of genes (such as *MAT*α and ribosomal protein genes). It is clearly also the case for *HO*. The pleiotropic phenotypes of *swi* mutants (*70*) suggest that these genes are also involved in the regulation of other genes. Because of its promoter structure, *HO* may be the only gene which is regulated by all six *SWI* genes, thereby acquiring its unusual form of regulation.

The complex combinations of regulatory elements seen at both *HO* and the silent loci *HML* and *HMR* appear similar to the picture beginning to emerge from studies of gene regulation in animal cells (*71*). As in the yeast systems described here, different promoters in animal cells, which have varying forms of regulation, are often recognized by common sequence-specific DNA binding proteins (for example, SP1 and CTF/NF-I). In addition, a given regulatory protein can have more than one function: the DNA binding factor CTF/NF-I can act as a transcription factor (via the CCAAT sequence) or as a factor required for the initiation of adenovirus DNA replication in vitro (*72*). Although in vitro transcription systems for animal cells have allowed certain factors to be tentatively assigned as positive regulators, it may prove difficult in general to precisely define the role of a given protein in the overall control process, or the way in which the activity of individual regulatory molecules is modulated. In contrast, the regulatory systems discussed here can be studied by genetic analysis, in which it is possible to dissect the individual components of a complex system and to analyze their contribution to the overall regulatory process.

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74. We would like to thank our colleagues at the LMB for stimulating discussions, and those elsewhere for allowing us to cite their unpublished results. We would like to thank G. Ammerer, C. Cabrera, and P. Sorger for their comments on the manuscript.