Genes Controlling the Expression of the HO Gene in Yeast

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Michael Stern

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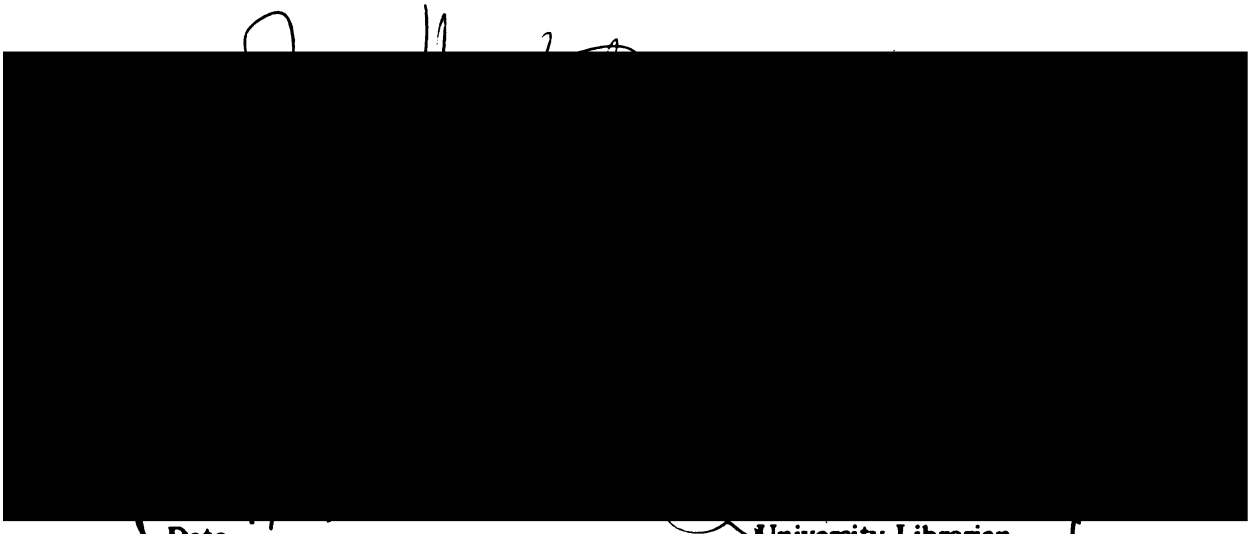

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ABSTRACT

The yeast Saccharomyces cerevisiae can express one of two mating types, denoted $\underset{\sim}{a}$ or α. A third, nonmating cell type ($\underset{\sim}{a}/\alpha$ cell type) results from the mating of an $\underset{\sim}{a}$ and α cell. In most strains, mating type is mitotically stable. However, cells carrying HO can switch mating type from a to α and vice versa as often as every cell division.

Transcription of HO is under three controls. 1) Cell type control: Only $\underset{\sim}{a}$ and α cells, not $\underset{\sim}{a}/\alpha$ cells, can transcribe HO. 2) Cell cycle control: HO is transcribed only in the G_1 phase of the cell cycle. 3) Mother/daughter control: Only mother cells (cells that have budded at least once) can transcribe HO.

From a screen of mutants defective in mating type switching, I showed that five SWI genes (SWI1-SWI5) are required for HO transcription. Because mutations in SWI1-SWI4 are pleiotropic, whereas swi5 mutations are not pleiotropic, I proposed that SWI5 is the most direct activator of HO transcription and that the other SWI genes are required for activity of SWI5 product. Plasmids carrying SWI1 and SWI5 were obtained. SWI1 and SWI5 transcripts are present in mutants defective in each SWI gene. The cloned SWI1 and SWI5 genes were used to construct swi1 and swi5 deletion mutants, denoted swi1Δ and swi5Δ, which behave essentially the same as the swi1 and swi5 mutants isolated in vivo.

To help determine if the SWI genes are responsible for cell type, cell cycle, or mother/daughter control, and to identify genes that act negatively on HO transcription, suppressors of the defect in HO transcription of swi1Δ were obtained. The suppressor mutations define two complementation groups, SHO1 and SHO2. The sho1-1 allele suppresses

mutations in every SWI gene and thus allows bypass of SWI control. One possibility is that SHO1 or SHO2 encode repressors of HO transcription and the SWI genes function to inhibit SHO1 and SHO2 Because HO transcription is still fully subject to a/α repression in swi1Δ or swi5Δ strains carrying sho1-1, neither SWI1 nor SWI5 is required for cell type control of HO transcription.

Cells of different types in a multicellular organism, or cells of the same type facing different environmental conditions, differ in their protein content. For example, yeast cells grown in galactose contain large amounts of three enzymes that convert galactose to glucose-1-phosphate, whereas yeast cells grown in glucose contain virtually none of these enzymes (reviewed in Oshima, 1982). In many cases, changes in the amount of particular proteins reflect changes in the amount of transcript for these proteins. The accumulation of the three enzymes required for galactose utilization in yeast upon shift from glucose medium to galactose medium results from a 1000-fold induction of the transcripts for these enzymes (St. John and Davis, 1979). The amount of a particular transcript can in principle be regulated at a number of levels, such as transcription initiation, termination, stability or splicing. Many of these types of regulation are found in practice as well. In the case of the galactose enzymes described above (and several other yeast enzymes), regulation occurs at the level of transcription initiation (Guarente et al, 1982). In most of the other cases of transcriptional regulation in yeast, the stage at which regulation occurs has not been demonstrated conclusively; however, in these cases, the DNA sequences that confer regulation lie upstream of the gene, in the promoter region, and it is assumed that regulation occurs at the level of initiation. Therefore in yeast, as in all other organisms, the regulation of transcription initiation plays an important role in the response of the cell to internal or external signals.

Transcription initiation is regulated by the interaction of regulatory proteins with specific sequences in or upstream of the regulated gene. The binding of certain regulatory proteins to sequences around

their target gene can stimulate transcription (activators). Other regulatory proteins inhibit transcription when bound to their target genes (repressors). (Gilbert and Muller-Hill, 1966; Ptashne, 1967; Englesberg et al, 1965). The rate of transcription initiation is controlled by controlling the activity of the regulatory proteins, which is accomplished by such diverse means as proteolysis of regulatory proteins (Little et al, 1980) or by the allosteric effect of small molecules on regulatory proteins (Riggs and Bourgeois, 1969). One approach to studying the mechanisms by which transcription is controlled involves the identification of regulatory genes or proteins and the sequences on which the proteins act. Regulatory proteins can be identified in organisms amenable to genetic analysis by recessive mutations that confer defective transcription of the target gene. A recessive mutation in an activator confers uninducible transcription, whereas a recessive mutation in a repressor confers constitutive transcription. In other organisms, regulatory proteins can be identified as factors that activate in vitro gene transcription or bind to specific regulatory DNA sequences. Cis-acting regulatory sequences are identified by mutation or by sequence homologies among genes under similar regulation. This thesis concerns the transcriptional regulation of the yeast HO gene. I describe genetic and biochemical studies that utilize the isolation and characterization of mutants and suppressor mutants. These studies identify genes affecting the transcription of HO and make it possible to infer possible regulatory functions of these genes.

Prokaryotic and Eukaryotic promoters and their regulation

Promoters characterized from several prokaryotic species have similar structure, which is reflected in the ability of wide host-range plasmids and transposons to be expressed in many different prokaryotic species. The prokaryotic promoter consists of two elements, defined both by mutation and sequence homology: the Pribnow box, a hexanucleotide located about ten base pairs (bp) upstream of the transcription start site, and an element located about 35 bp upstream of the transcription start site. RNA polymerase binds both elements *in vitro*.

Negative regulators appear to act on DNA sequences close to the promoter, and thus appear to act by precluding the binding of RNA polymerase to the promoter (Majors, 1975). However, in some cases, simple steric inhibition is insufficient to explain repression. The repressor of the *E. coli* *GAL* genes, encoded by the *galR* gene, needs to act at two distinct sites around the *GAL* promoter, one somewhat upstream of the promoter, and the other early in the first structural gene of the operon (Musso et al, 1977; Irani et al, 1983). The mechanism by which the *galR* product prevents transcription is unknown.

Several systems under positive transcription control have been described in *E. coli*. The λ repressor activates transcription of its own gene, *cI*, from the P_{RM} promoter. The λ repressor has been shown to bind close to polymerase at P_{RM}, and from this it was hypothesized that the λ repressor stimulates transcription by a direct protein-protein interaction. Genetic evidence, involving mutants of *cI* unable to exert positive control and a suppressor mapping to an RNA polymerase gene appears to support this view (Hochschild et al, 1983). The case with

other positive regulators is less clear. At the lac operon, the CAP protein, which exerts positive control, binds close to RNA polymerase and could promote transcription by interacting with polymerase. However, in other operons, CAP binds farther from the promoter (reviewed in de Crombrugghe et al, 1984). Direct interaction between CAP and polymerase in the araBAD operon is unlikely because CAP binds about 100 bp upstream of P_{BAD} (Lee et al, 1981) and another regulatory protein binds in between. Therefore, it is unlikely that CAP acts by binding directly to polymerase.

Promoters of various eukaryotic species are also similar to each other, reflected in the ability of cells (oocytes, for example) to transcribe genes of other species. Eukaryotic promoter elements that have been studied in mammalian cells and their viruses contain three important elements: 1) a proximal element, called the TATA region, located about 25 bp upstream from the transcription start site. This element apparently serves to fix the start of transcription (McKnight, 1982). 2) an upstream element, located 50-100 bp upstream from the transcription start site. This element is required for efficient transcription in vivo, although in vitro requirements for this sequence vary among different systems. 3) an enhancer element (Banerji et al, 1981), which possesses the ability to activate transcription independently of its orientation or distance from the transcription start site, and which can often function either upstream or downstream of the transcription start site. The enhancer can promote a dramatic increase in transcription in vivo and an in vitro requirement for an enhancer has only recently been demonstrated. The enhancer has in several cases also

been shown to be the sequence that confers transcriptional regulation, as will be discussed below.

The enhancer element was first identified as a DNA sequence in SV40 that could increase the transcription of the β-globin gene in an orientation-independent and position-independent manner (Banerji et al, 1981). In addition to stimulating transcription, Chandler et al, (1983) showed that an enhancer also could confer appropriate regulation to the gene on which it acted. A 340 bp fragment from the long terminal repeat of the mouse mammary tumor virus (MTV), which stimulates transcription of MTV sequences or adjacent cellular sequences following treatment with the steroid dexamethasone, was placed upstream of the herpes simplex virus TK gene. In this construction, transcription required the TK TATA box and upstream sequence; transcription initiated from the normal TK transcription start site. This DNA was transfected into a rat cell line and the enhancer was found to stimulate transcription of the TK gene only in the presence of the inducer, the steroid dexamethasone. This 340 bp sequence was also shown to bind steroid receptor protein in vitro (Payvar et al, 1983), raising the possibility that binding of the steroid receptor in the presence of steroid to the enhancer causes a stimulation of transcription of adjacent promoters.

An enhancer located in an intron of an immunoglobulin heavy chain gene was shown to stimulate the transcription either of the homologous heavy chain gene or the heterologous SV40 promoter. However, this stimulation was seen only in the myeloma cell type, in which the immunoglobulin gene is normally transcribed, not in the fibroblast cell type, in which the immunoglobulin gene is not transcribed (Gillies et al, 1983). Stimulation of transcription by this enhancer might require

a tissue-specific factor, one that is present in myeloma cells but not fibroblast cells.

Further work on the interaction of enhancers with putative regulatory proteins has been hindered by the inability to identify these proteins. A requirement for enhancers in in vitro transcription systems has only recently been demonstrated. The steroid receptor, the only protein known to bind at an enhancer, was purified by its ability to bind steroid and not by any function shown at the MTV enhancer. Genes encoding such regulatory proteins have not been identified because mutants defective in enhancer-stimulated transcription have not been identified.

Yeast, as a eukaryote, has promoters that show many features in common with mammalian promoters (Struhl, 1982). Transcription requires a TATA element located from about 20 bp to about 100 bp upstream from the transcription start site, and a second element called an upstream activator sequence (UAS) located at distances from 150 bp to 1500 bp upstream of the transcription start site. The UAS has certain properties in common with the mammalian enhancer; it can act at varying distances from the transcription start site in an orientation-independent manner, and it can stimulate the transcription of a heterologous gene (Guarente et al, 1982). Like a mammalian enhancer, the yeast UAS is the site at which many transcriptional regulators have been shown to act, as will be discussed below.

Some transcriptional regulatory systems in yeast

Many genes and families of genes in yeast are transcriptionally

regulated. As noted above, the transcription of three genes required for galactose metabolism are induced about 1000-fold when cells are shifted from media containing glycerol to media containing galactose. Other examples of genes under transcriptional control include genes required for mating (reviewed in Herskowitz and Oshima, 1981), for biosynthesis of amino acids (reviewed in Jones and Fink, 1982), and utilization of non-glucose carbon sources (Carlson and Botstein, 1982; Guarente et al, 1984). In this section I will describe the transcriptional regulation of three families of genes: the genes that are required for galactose metabolism, the genes that encode amino acid biosynthetic enzymes, and the SUC2 (invertase) gene.

As described above, the transcription of three genes (GAL1, GAL7 and GAL10) required for galactose metabolism is induced by galactose. GAL1 and GAL10 are transcribed divergently from a common promoter sequence (St. John et al, 1981). Induction requires the GAL4 gene; cells carrying a null mutation in GAL4 are uninducible. A negative factor is encoded by GAL80. Cells carrying gal80 mutations transcribe the GAL genes constitutively. Mutations in GAL4 are epistatic to mutations in GAL80, suggesting that the GAL4 product acts more directly on the GAL promoter than does the GAL80 product. It has been proposed that in the absence of galactose, the GAL80 protein prevents the GAL4 protein from inducing transcription, perhaps by a protein-protein interaction (Matsumoto et al, 1980), whereas in the presence of galactose, a small molecule inducer (perhaps galactose or a metabolite of galactose) binds to the GAL80 protein and decreases its binding affinity for GAL4 protein, thus freeing GAL4 protein to induce GAL transcription. This view is supported by the isolation of other kinds

of mutant alleles of GAL4 (the GAL4-constitutive) and GAL80 (the GAL80--uninducible). A dominant GAL4 mutation that confers constitutive expression of the GAL genes is interpreted as being defective in interaction with GAL80 protein, whereas a dominant GAL80 mutation that confers uninducibility is thought to be defective in binding of the small molecule inducer.

Deletion mutation analysis has defined a UAS region between GAL1 and GAL10 that is required for transcription of these genes. When a 365 bp fragment carrying this UAS was inserted upstream of the CYC1 gene in place of the CYC1 UAS, CYC1 transcription became controlled similarly to GAL transcription (Guarente et al, 1982). Transcription was induced by galactose and induction required the GAL4 gene. This result is analagous to the result obtained with the steroid-dependent enhancer upstream of the herpes virus TK gene, and it demonstrates that GAL4 acts on the 365 bp fragment to stimulate transcription and that the site of action of GAL4 is inseparable from the UAS that promotes transcription. It is not possible from experiments of this sort to demonstrate that GAL4 encoded protein itself binds to the 365 bp fragment described.

The SUC2 gene is required for metabolism of sucrose. Transcription of SUC2 is repressed by glucose (Carlson and Botstein, 1982); no other type of regulation has been observed. Although this regulation appears simple, no fewer than seven genes have been identified that affect the expression of SUC2. SNF1-SNF6 encode positive factors; mutants defective in any SNF gene fail to produce invertase. snf1 and snf2 mutants are defective in transcription of SUC2; whereas SUC2 transcription has not been assayed directly in mutants defective in SNF3-SNF6. The SSN6 gene, which encodes a negative factor (Neigeborn and Carlson, 1984) was

identified from a mutation that suppresses snf1. Mutants defective in SNF1, SNF2, SNF4 or SNF5 are pleiotropically defective in growth on galactose or glycerol (which require enzymes that are also under catabolite repression), suggesting that these genes may play a role in regulation of catabolite repression.

Double mutants were constructed with ssn6 and the six snf mutations in order to determine epistasis relationships. Because ssn6 mutations completely suppress the defect in SUC2 expression of snf1, snf3, snf4 and snf6 mutations, it was proposed that the role of SNF1, SNF3, SNF4 and SNF6 is to antagonize, presumably under derepressing conditions, the action of SSN6. The relationships among SSN6, SNF2 and SNF5 are less clear. SSN6 only partially suppresses SNF2 and SNF5, so a more complicated interaction among the three gene products is possible.

Another example of a system in which multiple genes control the expression of other genes is the control of at least 24 genes encoding amino acid biosynthesis enzymes by the general control system. Following starvation for any one of several amino acids, such as histidine or proline, genes encoding enzymes in many different amino acid biosynthetic pathways derepress. This derepression has been shown in several cases to occur at the level of transcription.

Genes required for derepression (GCN2, GCN3 and GCN4) have been identified, as well as one negative regulatory gene (GCD1). Mutations in GCD1 confer constitutive, derepressed level of synthesis of the enzymes under general control. Because gcd1 mutations are epistatic to mutations in GCN2 and GCN3, whereas gcn4 mutations are epistatic to mutations in GCD1, it has been proposed that GCN2 and GCN3 act by antagonizing GCD1, which in turn acts by antagonizing GCN4, which in

turn encodes the direct activator of the genes under general control (Hinnebusch and Fink, 1983). A cis-acting sequence (a hexanucleotide) upstream of HIS4 is also required, in addition to GCN4, for derepression (Donahue et al, 1984). This sequence is also present upstream of several other genes under general control and is thought to be the site of action of GCN4. In addition, this hexanucleotide possesses UAS activity, and this activity is GCN4 dependent (Fink, personal communication). As is the case for the GAL genes, the upstream positive control sequence also contains the site of action of the genetically defined positive activator. No sequence acting "negatively" (defined as a sequence that, when deleted, confers constitutive expression) has been identified upstream of HIS4, suggesting that HIS4 (and by extension, the other genes under general control) is under positive regulation.

Mating type and mating type interconversion in yeast

Yeast can grow vegetatively as either a haploid or a diploid. As a haploid, it expresses one of two mating types, denoted a or α (see Herskowitz and Oshima, 1981). A cell will mate with another cell of opposite mating type, but will not mate with another cell of the same mating type. The mating type of a yeast cell is determined by the allele present at the mating type (MAT) locus. a cells carry the MATa allele at MAT, whereas α cells carry the MATα allele at MAT. Genes of the mating type locus determine the cell's mating type by controlling the expression of unlinked genes required for mating. Cells carrying MATa transcribe genes specifically required for a cells to mate (Wilson and Herskowitz, 1984), as well as genes required for mating by both

mating types. The MATα locus determines the α cell type by inducing the transcription of genes required for α cells to mate (Sprague et al, 1983; S. Fields, personal communication) and by repressing the transcription of genes specific to a cells.

The product of a mating of two cells (called a zygote) is a diploid and is heterozygous at MAT. The zygote can grow vegetatively to form a colony composed exclusively of diploid cells. The two MAT alleles are said to be co-dominant, because the phenotype of the heterozygous diploid differs from the phenotype of either haploid parent. a/α cells, because of transcriptional control by combined activity of the genes at MAT (see Strathern et al, 1981), no longer mate but instead can be induced to undergo meiosis and sporulation. The product of such a sporulation is four haploid spores, two of a cell type, two of α.

Yeast strains differ in the stability of mating type. In most yeast strains, denoted "heterothallic", mating type is a stable, heriditable property of the cell. That is, a cell and its mitotic progeny will almost always express the same mating type. In other yeast strains, denoted "homothallic", mating type is unstable. That is, a cell will frequently express the opposite mating type as its progeny. In homothallic strains, cells can switch mating type from a to α and back to a as often as every cell division. The different behavior of homothallic and heterothallic strains is due to the presence or absence of a particular allele at a single genetic locus, denoted HO (Winge and Roberts, 1949). Homothallic strains carry the HO allele; heterothallic strains carry the ho allele, which is recessive to HO and was therefore thought (and is now known) to represent loss of HO function.

The process of mating type switching, as catalyzed by the HO

product, is now understood in some detail. The mating type switch results from a transposition of genetic information to the mating type locus from one of two loci (unlinked to MAT) denoted HML and HMR (Takano and Oshima, 1967). These loci harbor mating type genes in cryptic form (Rine et al, 1979). Usually, HML harbors the α allele and HMR the a allele, but this need not be the case. HO has been cloned (Jensen et al, 1983), sequenced (Russell et al, 1985), and its encoded product identified (Kostriken et al, 1983; R. Kostriken, personal communication). HO encodes a site-specific endonuclease that catalyzes mating type switching by producing a double-strand break specifically at MAT (Strathern et al, 1982; Kostriken et al, 1983). This double-strand break can give rise to a gap, deleting part or all of MAT. The gap is repaired by the recombinational repair system of yeast, utilizing at least three genes (RAD51, RAD52 and RAD54; Malone and Esposito, 1980; J. Game, personal communication) required for general recombinational repair. Either HML or HMR serves as the donors of homology for this repair. If the previous allele at MAT is opposite from the allele present at the donor locus, then the genes of the opposite mating type locus will be expressed, and the mitotic progeny of the cell will express the opposite mating type.

Analysis of mating type switching in single cells

The determination of the switching pattern in HO containing yeast has been facilitated by two properties of yeast cells (Hicks and Herskowitz, 1976): 1) the mating type of an individual cell can be determined without requiring the cell to undergo mating; therefore, the

mating type of a cell and its progeny can be followed indefinitely; and 2) yeast divides by budding, enabling the two mitotic progeny to be distinguished. The cell arising from the bud is called the daughter cell, whereas the cell that produced the bud is called the mother cell. Observations of the switching patterns of individual cells have revealed that switching obeys the following rules (Strathern and Herskowitz, 1979). First, only mother cells are competent to exhibit switching (denoted asymmetric control). The two progeny of a mother cell can (at a frequency of about 80%, depending on the strain) express the opposite mating type from the mother cell. On the other hand, the two mitotic progeny of a daughter cell always express the same mating type as the daughter cell. Therefore, the capacity to exhibit switching segregates asymmetrically to the mother cell during mitosis. Second, switching always occurs in pairs. At a frequency of approximately 80%, both mitotic progeny of a mother cell exhibit mating type switching. In the other 20% of mitoses of mother cells, neither mitotic progeny exhibit mating type switching. It is never observed, however, that one of the two mitotic progeny of a mother cell has switched and the other has not switched. This observation has led to the prediction that switching occurs only in a particular point in the cell cycle, before the mating type locus has replicated (denoted cell cycle control). Following replication, the same allele is always passed on to both mitotic progeny (Hicks et al, 1977).

A third control of switching is demonstrated by the observation that switching does not take place in the $\underset{\sim}{a}/\alpha$ diploid. As described above, a spore carrying HO will give rise, usually by the four-cell stage, to cells of each mating type. These cells can then mate with

each other, forming a/α cells. Following formation of the a/α cell, switching is shut off (Strathern et al, 1979; Klar et al, 1979). A teleological explanation for this observation is that the function of switching is to diploidize haploid spores. Once the diploid state has been achieved, further switching is no longer needed, and, in fact, is deleterious, because further switching can give rise to polyploid cells by the same mechanism as it gave rise to diploid cells.

The three types of controls of switching are all reflected by control of transcription of HO. Jensen et al. (1983) have shown that the HO transcript is found only in haploids, not in a/α diploids. This observation can explain the stability of mating type, despite the presence of HO, in a/α cells. Furthermore, Nasmyth (1983) has found that the HO transcript is present only in mother cells in G_1 of the cell cycle. In daughter cells, the HO transcript is absent at all phases of the cell cycle. These observations can explain the switching pattern described above. In addition, Jensen (1983) has found that when HO transcription is placed under control of the GAL10 promoter, thus inducing HO production in daughters as well as mothers, switching can occur in daughter cells as often as in mother cells. These results suggest that mother-specific switching is mediated by mother-specific control of HO transcription. Parenthetically, Jensen (personal communication) found that cells carrying the GAL10-HO fusion still switch primarily in pairs. Assuming that HO transcription from this fusion is constitutive throughout the cell cycle, this result suggests that cell cycle control of HO transcription may not be sufficient to account for cell cycle control of switching.

As a gene that is repressed transcriptionally in a/α cells, HO is a

member of a family of genes (including STE5 (V. MacKay, K. Nasmyth and J. Thorner, personal communication), MATα1 (Klar et al, 1981; Nasmyth et al, 1981), and Ty1 (Elder et al, 1981)) repressed transcriptionally in a/α cells. As a gene exhibiting cell cycle transcriptional regulation, HO joins five other genes, those encoding the histones H2A and H2B (Hereford et al, 1981) and CDC9, which encodes DNA ligase (Peterson et al, 1985). Transcriptional studies on the genes encoding H2A and H2B are hindered because the genes are essential, thus mutations in unlinked genes required for expression are likely to be lethal. No other yeast gene is known to be transcribed asymmetrically.

As in the examples of transcriptional regulatory systems described above, the regulation of HO transcription is studied both by the analysis of upstream sequences required for expression or regulation and by the identification of unlinked genes affecting transcription. Preliminary characterization of the sites of the three types of regulation suggests that various control regions are separable. Jensen (1983) has found that about 700 bp upstream from the HO transcription start site can confer complete repression of transcription in the a/α cell type. It has been suggested (Russell et al, 1985; Nasmyth, 1985) that a/α control might be mediated by redundant sequences. Because deletion of these upstream sequences leads to constitutive transcription in the three cell types, these sequences confer negative control. Nasmyth has identified UAS activity about 1000 bp upstream of the start site (Nasmyth, 1985a), and has localized cell cycle transcriptional control (as assayed by CDC28 dependent transcription) to an octanucleotide (which possesses some UAS activity of its own) of sequence CACGAAAA that is repeated ten times. These sequences are distributed between 150 and 900

bp upstream of the start site. Deletion of these 750 bp leads to constitutive, but reduced, transcription throughout the cell cycle (Nasmyth, 1985b), suggesting that these sequences confer negative as well as positive control. However, asymmetric and cell type control remain in this deletion, again suggesting that sequences conferring these controls might be redundant.

The experiments described in this thesis are concerned with genes unlinked to HO that affect HO expression. These genes presumably act at the various control sites upstream of HO. In Chapter II I describe the identification of five genes (SWI1-SWI5) that are required for the transcription of HO. Both in terms of the function of these genes as transcriptional activators, and the large number of genes involved, the SWI system formally resembles the SNF and GCN systems described above. As is the case for these systems, it seems likely that there is a hierarchy of action of SWI genes: some SWI gene products act more directly on HO than other SWI gene products. From analysis of the phenotypes of mutants defective in any of these genes, I propose that SWI5 is the most direct activator of HO transcription, and that SWI5 requires SWI1-SWI4 for its activity. As activators of HO transcription, these genes might be responsible for cell type, cell cycle or asymmetric control. In Chapter III I describe the cloning of SWI1 and SWI5. With these cloned genes, deletion mutations of SWI1 and SWI5 were constructed and SWI1 and SWI5 transcripts were analyzed in mutants defective in each SWI gene to determine if either gene required any other SWI gene for transcription. In Chapter IV, I describe the isolation of suppressors of swi mutants. In these suppressor mutants, the ability to transcribe HO is restored. An interesting possibility is that these mutants

identify genes whose products act negatively on HO transcription. Regardless of their nature, these suppressor mutants can provide the means to determine which, if any, type of control of HO transcription is mediated by SWI genes.

II. Five SWI genes are required for expression of the HO gene in yeast

(this chapter has appeared as Stern, M., Jensen, R. and Herskowitz, I. (1984) J. Mol. Biol. 178, 853-868).

High frequency mating type interconversion in yeast requires the HO gene, which encodes a site-specific endonuclease that initiates the switching process. We have isolated and analyzed switching-defective mutants. These mutants define five complementation and linkage groups, called SWI1 - SWI5. We have shown by two assays, Northern hybridization and β-galactosidase activity in strains containing an HO-lacZ fusion, that mutants defective in any SWI gene fail to express the HO gene. In addition, all of the swi mutants express other phenotypes, the most notable being the inviability of double mutants defective in SWI4 and in either SWI1, SWI2 or SWI3. These results indicate that the SWI genes function in some way as positive regulators of HO expression and have additional cellular roles.

1. INTRODUCTION

Mating type interconversion in Saccharomyces cerevisiae occurs by a unidirectional transposition of genetic information from HML or HMR to the mating type locus (MAT) (reviewed by Herskowitz and Oshima, 1981). The cassette at MAT is removed in this process and is replaced by a cassette originating from HML or HMR. Efficient switching of mating type requires the HO gene: HO strains switch as often as every cell division cycle, whereas ho strains switch at a frequency of 10^{-6} (Hawthorne, 1963; Hicks and Herskowitz, 1976). Strains actively switching mating type produce a site-specific double strand break at MAT in vivo (Strathern et al., 1982) and in vitro (Kostriken et al., 1983); this double-strand break appears to initiate the switching process. Subsequent events must include degradation of the MAT cassette and copying of the cassette from HML or HMR.

Several genes in addition to HO are required for switching. These include three genes involved in DNA repair and recombination: RAD51, RAD52 and RAD54 (Malone and Esposito, 1980; Weiffenbach and Haber, 1981; J. Game, personal communication). Mutants defective in any of these genes are inviable if they carry the HO allele. This lethality is presumably due to the inability of such mutants to repair the HO-dependent double-strand break at MAT. These gene products therefore appear to comprise part of the enzymatic machinery of switching. Two other genes are also required for switching: SWI1 (Haber and Garvik, 1977) and CSM (Oshima and Takano, 1980). The allelic relationship between SWI1

and CSM has not been reported. These genes are not known to play any role in DNA repair or recombination and, unlike the RAD genes, are not required for viability in HO strains. In fact, the swi1-1 mutation restores viability to HO rad52 mutants (Weiffenbach and Haber, 1981), which suggests that the SWI1 product acts before the RAD52 product in switching.

In order to identify other genes required for switching, we have isolated and characterized additional mutants defective in switching. These carry mutations in six genes: HO, SWI1 and four additional genes, SWI2, SWI3, SWI4 and SWI5. Strains defective in any of the SWI genes fail to express HO.

2. Materials and Methods

(a) Media

YEPD medium (complete), SD medium (minimal) and supplements are described by Hicks and Herskowitz (1976). BBMB medium (Sprague et al, 1981) modified from Fink and Styles (1972) is citrate-buffered YEPD medium (0.1 M-citrate, pH 4.5) with 0.003% (w/v) methylene blue (final concentration). Cryptopleurine medium is YEPD medium containing 0.9 μM cryptopleurine (Skogerson et al, 1973).

(b) Strains and crosses

Strains are described in Tables 1 and 6, crosses in Tables 2 and 7. Unless otherwise indicated, strains are HMLα HMRa/ho.

(c) Mating, sporulation, mating type and mating factor assays

Mating, sporulation and mating type assays were performed as described previously (Sprague and Herskowitz, 1981). Production of a-factor was assayed in a fashion analogous to the production of α-factor, except that the strains to be tested were replica plated to a

YEPD agar plate previously spread with strain RC757 (L. Blair, personal communication). RC757 is an α strain containing an sst2 mutation, which confers hyper-sensitivity to mating factors of the opposite mating type (Chan and Otte, 1981).

(d) Determining the presence of HO

The presence of HO in mating proficient strains was determined by mating the strain of interest to an ho tester strain and determining whether the diploid exhibited mating type switching. Because HO transcription is repressed by the products of the MATa1 and MATα2 genes, the ho testers were defective in one of these genes. Testing α cells: Cells that mated as α were mated to ho mata1 strain 866, to form a diploid that is mata1/MATα and hence incapable of sporulation. If the α cell supplies a functional HO gene, then switching will occur and lead to formation of MATa/MATα cells, which are capable of sporulation. If the α cell is defective in HO, the diploid will remain mata1/MATα. Hence, ability to sporulate indicates the presence of HO. Inability to sporulate indicates the absence of functional HO. Testing a cells: Cells that mated as a were analyzed in a similar manner, using a tester strain that is ho matα2-1 (strain 922). This strain also carries the sir1-1 mutation, which allows expression of HMLα and thereby allows 922 to mate as α. Because sir1-1 is recessive to SIR1, the MATa/matα2 diploid is phenotypically Matα2$^-$ and does not sporulate. As before, presence of functional HO in the a cell leads to mating type switching

and the formation of MATa/MATα cells, which are capable of sporulation. Again, sporulation of cells in the diploid colony indicates the presence of functional HO, while lack of sporulation indicates a defect in HO.

(e) Determination of SWI allele by complementation

Strains to be tested were mated to the appropriate swi⁻ tester strains and the genotypes assessed as follows: 1) Complementation of swi2, swi3, or swi5 mutations: a/α diploids defective in any of these genes form colonies that have a distinctive (rough) morphology. Hence, we scored the colony morphology of the diploids for "rough" (indicating lack of complementation) vs. wildtype (indicating complementation). 2) Complementation of swi1 and swi4 mutations: swi1 and swi4 mutants exhibit a wildtype colony morphology, so we monitored switching ability in the diploids formed. Because a/α diploids do not switch mating types, it was necessary to isolate derivatives of these diploids that were homozygous at MAT. MAT homozygotes were isolated by employing diploids heterozygous for the recessive cryptopleurine resistance allele cry1-3, which is tightly linked and centromere proximal to MAT. cry1-3 homozygotes, arising from mitotic recombination, gene conversion or chromosome loss, are selected as cryptopleurine resistant (CryR) derivatives. Approximately 85% of such derivatives are homozygous at MAT as well (R. Jensen and M. Stern, unpublished). The CryR colonies exhibit one of two phenotypes: 1) If the swi mutations complement, mating type switching occurs and all CryR colonies are sporulation proficient and

deficient in mating and production of mating factors. 2) If the swi mutations do not complement, switching will not occur and most CryR colonies will remain homozygous at MAT and thus remain proficient in mating and mating factor production.

In practice, 10^7 cells from four independently isolated diploids of each mating were each plated on cry-YEPD plates. Four colonies from each plating were analyzed for mating and sporulation ability.

(f) Isolation of RNA

Isolation of yeast RNA (R. Elder, personal communication), its agarose gel fractionation (Derman et al, 1981), transfer to nitrocellulose and hybridization with probe (Thomas, 1980) were done as modified by Jensen et al. (1983), except that total (25-30 µg per lane) rather than poly-A-selected RNA was isolated and analyzed. Probe was prepared from YIp5-BH2 (a derivative of YIp5 containing a 870 bp fragment that hybridizes to HO RNA) by nick translation using a New England Nuclear nick translation kit. Washed filters were autoradiographed for seven days at -70° with Kodak XAR-2 film and a Dupont 1 Lightning intensifying screen.

(g) β-galactosidase assays

Stationary phase cells were diluted 1:50 in fresh YEPD and incubated for 4-6 hours (2-3 doublings). 1.5 ml cells were harvested by centrifugation, washed once in Z-buffer and resuspended in 0.15 ml Z-buffer. β-galactosidase assays were performed as described (Miller, 1972) using cells permeabilized with chloroform-SDS. Extracts were incubated for 15' to 30', activities were normalized to cell density (measured at OD_{600}), and cell debris was removed by centrifugation prior to measuring OD_{420}. HR145-4C, a strain isogenic to one of the parents of each cross, but lacking the HO-lacZ fusion, produced a background value (.05 - .20 units) which was subtracted from each sample.

(h) Isolation of mutants

Spores from diploid strain 671 were mutagenized in three different ways: UV mutagenesis (Blair et al, 1979), EMS mutagenesis (Oshima and Takano, 1980), and γ-ray mutagenesis from a Cesium-137 source as described by Chakrabarti et al (1983). In particular, spores were isolated from independent colonies of strain 671 prior to mutagenesis. A-D represent independent colonies that were mutagenized by UV, E-H were mutagenized by EMS, and I-J were mutagenized by γ irradiation. The frequencies of survivors following mutagenesis were approximately 10% for UV, 20% for EMS, and 5% for γ irradiation. The effectiveness of mutagenesis (mutation frequency) was estimated by screening the spore colonies for canavanine resistance (the parent strain carried the

canavanine sensitive CAN1 gene) and was found to be approximately 0.5% for UV mutagenesis, 0.1% for EMS mutagenesis and 0.5% for γ-ray mutagenesis.

3. RESULTS

(a) Mutant isolation strategy

Spores from homothallic diploids give rise to nonmating, a/α colonies (Winge and Roberts, 1949). We isolated mutants defective in switching by screening for colonies that retain the ability to mate. In previous studies, two major classes of mutants were isolated that retain a colony mating type but were not defective in switching (Blair et al, 1979; Oshima and Takano, 1980). These two classes contain changes at HML or HMR or mutations in STE genes. (1) Changes at HML or HMR: Gene conversion of HMLα to HMLa or HMRa to HMRα yields HMLa MATa HMRa HO or HMLα MATα HMRα HO genotypes, respectively. These strains still undergo cassette switching but maintain their original mating type. (2) Mutations in STE genes: Strains that carry a- or α-specific ste mutations switch between mating competent and sterile cells. Such strains cannot diploidize and hence display a colony mating type. We have avoided isolating these classes of mutants by using a parent strain that contains the a allele at both silent loci and screening for colonies that mate as α.

We anticipated that we should obtain three classes of mutations that would allow HMLa MATα HMRa cells to maintain α mating type: 1)

Mutations in the HO gene, 2) mutations at the MATα gene which prevent it from being acted on by the switching machinery (similar to the MATα-inc and STK mutations previously described [Takano and Arima, 1979; Haber et al., 1980]), and 3) mutations in other genes required for switching.

(b) Complementation by ho strains

The 105 mutants isolated as defective in switching were placed into two classes by their behavior in a test for HO function. Each was mated to an ho tester strain and the resultant diploid scored for mating type switching. Mutants containing functional HO and a recessive swi mutation would appear Ho^+ in our test, whereas mutants defective in HO or carrying a dominant swi mutation would appear Ho^-. The complementation test is described in Materials and Methods. Of the 105 mutants isolated, 80 were of the first type (recessive swi^-) and 25 were of the second.

To determine whether the mutants of the second type contained dominant swi mutations or mutations in the HO gene, these mutants were mated to a strain containing HO and the diploids analyzed for ability to switch mating type. All of the diploids were able to switch, demonstrating that the mutations were all recessive and could be complemented by HO (Jensen, 1983). These have not been studied further.

(c) Five SWI complementation groups

Of the eighty recessive mutants able to supply HO function in a complementation assay, fourteen were chosen for further study and were placed into complementation groups by the following method. It was first necessary to cross certain swi mutations into strains of a mating type. One mutant (C10) was chosen at random and crossed to an a strain to yield a MATa ho segregant carrying the swi mutation (subsequently denoted swi4-1). This a segregant was then mated to each of the other mutants (which mate as α), and ability to complement was monitored (as described in Materials and Methods). Three other swi mutants (G22, H16, and H19) contained mutations that were unable to complement swi4-1. These mutants define one complementation group (SWI4). Complementation among the remaining ten mutations was determined using the procedure just outlined until each mutation had been assigned to a complementation group. Five complementation groups were found (Table 1). As shown below, one corresponds to the SWI1 gene previously identified (Haber and Garvik, 1977). Because only fourteen mutants were studied, it is possible that other SWI genes exist. At least two mutant alleles of each gene were identified. None of the mutants contained MATα-inc or STK mutations, which map at MAT, and which would fail to complement all swi mutations in our tests. Such mutants were not found presumably because the target for inc and stk mutations is much smaller than for the five SWI genes.

(d) Allelism tests

Allelism tests have been carried out to determine whether the

complementation groups define different loci. Ten diploids were constructed, each heterozygous for two swi mutations of different complementation groups. As shown in Table 2, tetratype and non-parental ditype tetrads were readily obtained from all crosses. Allelic mutations would show almost exclusively parent ditype tetrads. Therefore we conclude that the five SWI complementation groups define different genetic loci.

(e) Inviability of some double mutants

In seven of the ten crosses described in Table 2, segregants containing both swi mutations were obtained. (The presence of both swi mutations was determined by complementation tests as described in Materials and Methods.) As judged by their mating competence, these segregants were defective in switching. In contrast, in the other three crosses, A14, A24, and A34 (crosses of swi4-1 to swi1-2, swi2-1, and swi3-1, respectively), the segregation results were very different. In these crosses, spore inviability was increased (≥25%) and showed a nonrandom distribution among tetrads. In particular, all tetrads with two wildtype recombinant spores also contained two nonviable spores, and all tetrads with one wildtype recombinant spore also contained at least one nonviable spore. All tetrads with four viable spores were parental ditype. From these observations, we inferred that the double mutant segregants were not viable. We tested this hypothesis by assaying the genotypes (see Materials and Methods) of eight of the mating-proficient segregants in each cross. No segregants were found that contained both

swi mutations. Since the wildtype recombinant appeared in frequencies expected for the segregation of two unlinked genes, we conclude that the double mutants are not viable.

Germinating spores from A24 were observed microscopically in order to determine the terminal phenotype of the inviable segregants. Eight of nine spores inferred to be double mutants (by assaying the genotypes of their sister segregants after the colonies were grown) germinated and grew to be very large. (The ninth spore did not germinate.) Three of these eight changed their shape (see Figure 1). By ten hours, a time at which wildtype
siblings had undergone at least four doublings, only one spore had undergone a cell division; four of the cells had lysed.

We supposed that the nonviability of the three lethal double mutant combinations was independent of HO because death occurred at the germinating spore stage, at which time HO expression is thought not to take place. To test this view, we constructed strain D73 (MATa/MATα HO/ho swi4-1/SWI4 swi2-1/SWI2) and analyzed 23 segregants containing ho, 11 α and 12 a segregants. Four segregants were SWI2 SWI4, nine were SWI2 swi4-1, and ten were swi2-1 SWI4. None was found to be swi2-1 swi4-1, suggesting that the swi2 swi4 double mutant is not viable in ho strains as well.

(f) Other phenotypes of swi mutants

All swi mutations are pleiotropic (summarized in Table 3). Haploid

cells carrying the swi4-1 mutation are clumpy, very large, and bud in a distinctive manner, somewhat similar to the polar budding seen in a/α cells (Hicks et al., 1977). Diploids homozygous for swi2-1, swi3-1 or swi5-1 show a "lacy" colony morphology in contrast to the "smooth" wildtype morphology. This morphology is observed only in a/α cells--it does not occur in a/a or α/α derivatives. Furthermore, swi2-1 haploids containing the sir3-8(ts) mutation are lacy at high temperature (when both α and a information are expressed from the cassettes at HML and HMR) but smooth at room temperature (when only the cassette at MAT is expressed).

Cells carrying the swi1-2, swi2-1, or swi3-1 mutations show several additional phenotypes: (1) They require higher leucine concentrations to satisfy a leucine auxotrophy (leu2) than do SWI^+ leu^- strains: they are unable to form colonies on minimal medium containing 30 µg/ml leucine, which is sufficient for growth of leu^- SWI^+ strains, but grow normally in the presence of 300 µg/ml leucine. For the one swi mutant thus far examined (a swi2 mutant), high leucine is also required to satisfy a leu1 mutation.

(2) They grow poorly on rich medium in the presence of cryptopleurine (cry-YEPD) despite carrying a mutation, cry1-3, that confers resistance to cryptopleurine in SWI^+ strains. The plating efficiency of these mutants on cry-YEPD is reduced approximately 10^5-fold relative to wildtype strains. (3) Diploids homozygous for these alleles fail to sporulate. An ascus has never been observed from a/α cells homozygous for swi2-1, and sporulation is reduced approximately 10^3 in strains homozygous for swi3-1 or swi1-2. (4) As noted above, these mutations confer inviability to swi4 mutants.

All swi mutants are able to grow normally on YEPD containing 0.01% methyl methane sulfonate (conditions under which rad52 mutants [which are methyl methane sulfonate sensitive] are unable to form colonies). The two swi mutants tested, swi2 and swi4, are able to grow normally following irradiation with sufficient ultraviolet light to prevent the growth of ultraviolet-sensitive rad18 mutants. Thus, the swi mutants do not appear to be sensitive to DNA damaging agents.

(g) Defective expression of HO by swi mutants

We suspected that the swi mutants were defective in an early stage in switching because both swi2-1 and swi4-1 were capable of suppressing the inviability of HO strains containing the rad52-1 mutation (M. Stern, unpublished observations). One possibility is that one or more of the SWI genes is required for expression of the HO gene itself. This possibility was tested in two ways: 1) the presence of the HO transcript was assayed in swi mutants by RNA blot hybridization, and 2) β-galactosidase activity was assayed in strains containing a single swi mutation and an HO-lacZ gene fusion present at the HO locus.

(1) RNA was prepared from original isolates of swi mutants (using isolates E2, B2, E1, C10 and I2 for swi1-swi5 respectively), from the parent diploid strain 671 (which does not express HO because it is an a/α cell) and from an HMLa MATa HMRa segregant of 671 (which cannot switch to α, cannot diploidize, and hence expresses HO constitutively). These seven strains comprise an isogenic set. RNA from these strains was probed with plasmid YIp5-BH2, which carries the yeast URA3 gene and

an 870 bp BamHI-HindIII fragment of HO (Jensen et al., 1983). The probe therefore has homology to two yeast transcripts: URA3, used as an internal control, and HO, which is being assayed. As shown in Figure 2, the URA3 transcript is present in similar amounts in all seven strains; however, the HO transcript is present only in the haploid SWI^+ strain. The HO transcript is absent in the a/α diploid and in the five haploid swi mutants. Therefore we conclude that the defect in switching in all five swi mutants is due to lack of the HO transcript.

(2) In order to quantitate the extent of the decrease of HO expression in swi mutants, we utilized a gene fusion of the E. coli lacZ gene to HO (Jensen, 1983). The fusion (HO::lacZ) is an in-frame insertion of lacZ into HO at the first PstI site (which lies 250 bp from the translation start site [D. Russell, M. Smith and R. Jensen, in preparation]) and is present in the chromosome as a replacement of the wildtype HO gene. Strains carrying the HO::lacZ allele are therefore defective in HO. β-galactosidase activity of these strains is regulated similarly to wildtype HO by a1 and α2 (expressed in haploids and not in a/α diploids; Jensen, 1983).

Table 4 describes crosses in which a strain carrying the HO::lacZ fusion was crossed to strains carrying HO and one of the five swi mutations. In each cross, HO:HO::lacZ and swi^-:SWI^+ segregated 2:2. Segregants containing both the HO::lacZ fusion and swi mutations were obtained. In all five crosses, the levels of β-galactosidase activity observed in segregants containing the SWI^+ allele were considerably greater than the activity found in swi^- segregants (Table 4). In particular, swi5 mutants showed a 30-fold reduction in HO::lacZ expression, and mutants defective in the SWI1, SWI2, SWI3 and SWI4 genes

showed ≥100-fold reduction in HO::lacZ expression. These results correlate well with the observed switching properties of the various mutants. Spore clones grown from swi5-1 mutants usually contain some cells of mating type opposite to the mating type of the original spore, indicating that switching, although slow, is not absent. In contrast, cells of mating type opposite to the mating type of the spore are not detectable in spore clones from swi1-2, swi2-1, swi3-1 or swi4-1 mutants.

(h) Allelism of the swi mutation in strain E2 to swi1-1

One of our mutants (E2) contains a swi mutation that is centromere linked (30% tetratype tetrads using LEU2 as a centromere-linked marker). Hence, we wished to determine whether it contains a mutation in the SWI1 gene, which has been reported to be centromere linked (J. Haber, cited by Mortimer and Schild, 1981). Because the original swi mutation (swi1-1) is leaky (see below), we were unable to follow it by conventional assays involving diploidization and mating. Instead, we have monitored the presence of this mutation by its effect on expression of the HO gene using the HO-lacZ fusion as follows: The swi1-1 mutant was crossed to a strain carrying the HO::lacZ allele to form D123 (HO/HO::lacZ swi1-1/SWI1). Of six HO::lacZ segregants analyzed, two had levels of β-galactosidase activity 10-fold lower than the other segregants. One such segregant, D123-1d (MATa HO::lacZ), is assumed to carry the swi1-1 mutation and has been used in further analysis.

To determine the relationship between swi1-1 and the swi mutation of strain E2, D123-1d was mated to a strain carrying the E2 swi mutation

(strain D85-28c) and to a SWI$^+$ strain (D85-16b) to form HC101 and HC102 respectively. α/α mitotic recombinants were selected from these diploids (as described in Materials and Methods) and β-galactosidase activity was assayed. As shown in Table 5, the α/α derivative of HC101 was 30-fold lower in activity than the α/α derivative of HC102, which indicates a failure of the two mutations to complement. Failure to complement was also seen in the sporulation behavior of HC101. Although diploids that are homozygous for swi1-1 sporulate normally and diploids that are homozygous for the swi mutation of E2 show no sporulation, diploids containing swi1-1 and the swi mutation of E2 show an intermediate phenotype in that they sporulate very poorly. These complementation results indicate that the swi mutation originating in strain E2 (designated swi1-2) lies in the same gene as the previously identified swi mutation (swi1-1).

To confirm that swi1-1 and swi1-2 are allelic, diploid D151 (HO::lacZ/HO::lacZ swi1-1/swi1-2) was constructed, and each segregant from six tetrads was assayed for β-galactosidase activity. No segregant showing wild type levels of activity was found: all tetrads were parental ditype. These results confirm that swi1-1 and swi1-2 are allelic.

4. DISCUSSION

(a) The SWI genes and transcription

By screening for mutants that are defective in mating type inter-

conversion, we have shown that in addition to HO, five genes (SWI1-SWI5) are required for switching, and that mutants defective in any of these genes fail to switch mating type because they fail to express HO. The SWI genes are also required for other cellular processes, as all swi mutations are pleiotropic,

The requirement of five SWI genes for expression of HO has been assayed in two ways, by RNA-DNA hybridization and by assaying β-galactosidase activity in strains containing an HO-lacZ fusion. Comparing β-galactosidase activity of sister swi^- and SWI^+ segregants, we find that swi1, swi2, swi3, and swi4 mutations reduce expression of HO at least 100-fold; swi5 mutations reduce expression approximately 30-fold. The effect of the swi mutations is also seen at the level of HO RNA; the HO transcript is not present in strains defective in any SWI gene. We have also found that the previously-isolated swi1-1 mutation affects HO expression, although not as severely as the swi1 mutation reported here: swi1-1 mutants express HO at 5-10% wildtype levels (at least five-fold higher than a swi1-2 mutant). Defective expression of HO in a swi1-1 strain has also been cited by Nasmyth (1983). These studies do not allow us to deduce whether the SWI genes stimulate, for example, transcription initiation or RNA processing or stabilization. Three SWI genes examined thus far (SWI2, SWI4 and SWI5) are not required for expression of HO when the HO gene is read from the GAL10 regulatory region (R. Jensen, unpublished observations). These results provide support for the view that at least SWI2, SWI4 and SWI5 are required for transcription initiation.

(b) Control of mating type interconversion

Mating type interconversion is under many different types of control, several of which are exerted by regulating expression of the HO gene. Because the SWI genes activate expression of HO, we shall consider whether any of these controls might occur via the SWI genes or their products.

Cell type control: HO is expressed in a and α haploid cells but not in a/α diploid cells, in which it is turned off by some action of the a1 and α2 gene products (Jensen et al., 1983). Does a1-α2 regulate HO by inhibiting expression or action of SWI genes in a/α cells? The SWI genes must be expressed and active at some level in a/α cells, because swi mutants exhibit phenotypes in a/α cells (for example, affecting sporulation and colony morphology). However, a1 and α2 could partially inhibit expression of SWI genes to a level which is too low to activate HO expression but sufficient to perform their other cellular functions. a1 and α2 are known to partially inhibit the transcription of several genes, among them α2 (Nasmyth et al., 1981) and Ty1 (Elder et al., 1981).

Periodic expression: Expression of HO appears to occur only in late G1 of the cell division cycle (Nasmyth, 1983; R. Jensen and V. Groppi, unpublished observations). Whether any of the SWI gene products are responsible for triggering periodic expression of HO is not known. The inviability of swi double mutants (discussed below) might be explained if these SWI genes were required in combination to activate

expression of essential genes that are periodically expressed.

Asymmetric expression: Mother cells (cells which have budded at least once) can switch mating type but daughter cells cannot (Strathern and Herskowitz, 1979). It has recently been found that mother but not daughter cells express HO (Nasmyth, 1983; R. Jensen and V. Groppi, unpublished observations). A difference in distribution of one or more SWI gene product between mothers and daughters might be responsible for this asymmetric expression.

(c) The nature of the switching-defective mutations

All switching-defective mutants obtained lacked HO gene product activity, being defective in HO itself or in a gene required for its expression. Although other genes in addition to HO must be required for the cassette transposition process, we did not obtain mutants defective in any of these genes. Our failure to obtain such mutants might be due to their inviability, as is seen for HO strains defective in the DNA repair genes RAD51, RAD52, or RAD54 (Malone and Esposito, 1980; Weiffenbach and Haber, 1981; J. Game, personal communication). These strains are thought to be inviable because they are unable to repair the double-strand break made at the mating type locus by an HO-dependent endonuclease (Strathern et al., 1982; Kostriken et al., 1983). In S. pombe, nine genes required for mating type switching (SWI1 - SWI9) have been identified; three of the SWI genes are required for production of a double-strand break at mat1, and thus may be similar to the SWI genes described here. The other six genes, however, produce wildtype levels

of the double-strand break and are apparently required for proper resolution of recombinational intermediates (Egel et al., 1984).

(d) Other roles of SWI genes

It is apparent that the SWI genes do more than regulate expression of HO; all swi mutations are pleiotropic. The five genes can be placed into three classes based on the types and extent of phenotypes displayed.

Class 1: Mutations in the SWI5 gene are the least pleiotropic: they confer no growth defects and exhibit only an altered colony morphology in a/α cells, in addition to their defect in HO expression. This lack of additional defects might be due to leakiness of the swi5 mutation. A more interesting explanation is that the SWI5 gene product is more immediately concerned with HO expression than the other SWI gene products, as is discussed below. Production of a null swi5 mutation should aid in answering this question.

Class 2: Mutants defective in SWI4 are clumpy, grow slowly, show a distinctive budding pattern, and are inviable in the presence of a swi1, swi2, or swi3 mutation. swi4 mutants share none of the mutant phenotypes with swi1, swi2, or swi3 mutants except with respect to HO expression.

Class 3: Mutations in SWI1, SWI2, and SWI3 result in a virtually identical phenotype. All result in slow growth, inviability when com-

bined with a swi4 mutation, exhibit similar nutritional defects, and do not allow sporulation when homozygous in an a/α diploid. These observations suggest that SWI1, SWI2, and SWI3 might act in the same pathway or at the same step of a pathway. It is not known at present what is the primary function of these genes. The wide variety of apparently unrelated phenotypes expressed by these mutants suggests that these genes act indirectly on most or all of the cellular functions affected. We do not believe, for example, that these genes are directly involved in leucine metabolism or sporulation. In this regard, it can be imagined that these genes act indirectly on HO expression as well. Therefore we propose that the SWI5 gene product acts directly on HO and the other SWI genes are required for its activity.

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Table 1: Complementation groups defined by *swi* mutations

Complementation group	Mutant isolate
SWI1	E2 (*swi1-2*), J1 (*swi1-3*) G21 (*swi1-4*), BW222R-55 (*swi1-1*)
SWI2	B2 (*swi2-1*), B4 (*swi2-2*), B7 (*swi2-3*)
SWI3	E1 (*swi3-1*), H13 (*swi3-2*)
SWI4	C10 (*swi4-1*), G22 (*swi4-2*), H16 (*swi4-3*), H19(*swi4-4*)
SWI5	I2 (*swi5-1*), H8 (*swi5-2*)

Except for BW222R-55B, which carries *swi1-1* (Haber and Garvik, 1977), the *swi* mutants are *MAT*α *swi*$^-$ derivatives of strain 671 (see Table 6). Unless explicitly stated otherwise, *swi1-2*, *swi2-1*, *swi3-1*, *swi4-1* and *swi5-1* were the alleles used for analyses described in this paper.

TABLE 2: The five SWI complementation groups define five separate loci

		Tetrad Type		
DIPLOID	HETEROZYGOUS AT	PD	NPD	T
A12	SWI1, SWI2	1	1	8
A13	SWI1, SWI3	2	6	6
A14	SWI1, SWI4	1	2	11
A15	SWI1, SWI5	2	0	12
A23	SWI2, SWI3	2	3	13
A24	SWI2, SWI4	2	2	6
A25	SWI2, SWI5	2	3	10
A34	SWI3, SWI4	4	4	14
A35	SWI3, SWI5	2	1	9
A45	SWI4, SWI5	3	1	7

Table 2: Presence of a swi mutation was scored by mating ability of segregant. SWI^+ strains diploidized and gave a non-mating colony. For diploids A14, A24, A34: Parental ditype (PD) 4 maters (m) : 0 nonmaters (nm); Non parental ditype (NPD) 2 m : 2 inviable; Tetratype (T) 2 m : 1 nm : 1 inviable. For other diploids, PD 4 m : 0 nm; NPD 2 m : 2 nm; T 3 m : 1 nm.

Table 3: Additional phenotypes of *swi* mutants

	---- Behavior of mutant defective in----				
Phenotype	SWI1	SWI2	SWI3	SWI4	SWI5
Viable with *swi4* mutation	no	no	no	--	yes
Sporulation	no	no	no	yes	yes
Growth on low [leucine]	no	no	no	yes	yes
Resistance to cryptopleurine*	no	no	no	yes	yes
Wildtype colony morphology in *a*/α diploid	yes	no	no	yes	no

Table 3: The *swi* alleles tested are: *swi1-2*, *swi2-1*, *swi3-1*, *swi4-1* and *swi5-1*. Other alleles show differences in some phenotypes. For example, strains homozygous for *swi2-3* sporulate, but with an altered morphology.

* Mutants defective in SWI1, SWI2 or SWI3 fail to form colonies on YEPD containing 0.9 μM cryptopleurine despite the presence of a standard cryptopleurine resistance allele (*cry1-3*).

TABLE 4: Effect of *swi*$^-$ mutations on expression of *HO*

DIPLOID	GENOTYPE		β-galactosidase activity of segregants: *swi*$^-$	β-galactosidase activity of segregants: *SWI*$^+$
D144	*HO*/*HO*::*lacZ*	*SWI1*/*swi1*-*2*	≤ 0.1	10
D101	*HO*/*HO*::*lacZ*	*SWI2*/*swi2*-*1*	≤ 0.1	15
D117	*HO*/*HO*::*lacZ*	*SWI3*/*swi3*-*1*	≤ 0.1	14
D100	*HO*/*HO*::*lacZ*	*SWI4*/*swi4*-*1*	0.1	13
D142	*HO*/*HO*::*lacZ*	*SWI5*/*swi5*-*1*	0.4	14

Table 4: Assays were performed as described in Materials and Methods. Numbers are the averages of activities from at least five segregants from each cross.

TABLE 5: Failure of swi1-1 and swi1-2 to complement

STRAIN	GENOTYPE	UNITS
HC101-1	MATα/MATα ho/HO::lacZ swi1-1/swi1-2	0.3
HC102-1	MATα/MATα ho/HO::lacZ swi1-1/SWI1	9.9
D123-1d	MATa HO::lacZ swi1-1	0.7

Table 5: Assays were performed as described in Materials and Methods and in the legend to Table 4. HC101-1 and HC102-1 are α/α mitotic recombinants derived from HC101 and HC102, respectively.

TABLE 6: Strain list

Strain	Genotype	Source
671	MATa/MATα cry1-3 HMLa HO ura4 met (AH)	L. Blair
671-1b	MATa segregant of 671	
70	MATα thr3-10	F. Sherman
227	cry1 MATa lys1	"
866	mata leu1 ade6 trp5 ura3 can1 rme1	G. Sprague
922	matα2-1 sir1-1 arg4-17 ade6 rme1	G. Sprague
A24-1a	MATa/MATα HO ura3-52 his4 his5 met (AH)	
A24-8a	MATa HO swi2-1 ade5 leu2$^{=}$ ura3-52 his4 his5 met	
A24-11a	MATa HO swi4-1 leu2$^{=}$ ura3-52 his3 his4 his5 met	
BW222r-55b	MATa/MATα HO swi1-1 leu2 lys ade (AH)	J. Haber
D15-34d	MATa swi4-1 leu2$^{=}$ ura3-52 his4 met	
D32-9c	MATa swi3-1 leu2$^{=}$ trp1 his3 his4 met	
D38-31b	MATa swi2-1 ura3-52 leu2$^{=}$ his3 his4 met	
D39-4c	MATα HO swi4-1 ade5 ura3-52 his4 his5 met	
D39-24b	MATα HO swi4-1 ade5 his5 met	
D45-11b	cry1-3 MATa HO swi4-1 ura4 met	

D46-4b	MATa HO swi2-1 his3 his4 his5 ura3-52 leu2$^{=}$ met	
D46-12c	MATα HO swi2-1 leu2$^{=}$ his3 his4 his5 met	
D57-11c	cry1-3 MATa HO swi2-1 ura4 met	
D72-6b	MATa HO swi3-1 his4 his5 ura3-52 leu2$^{=}$ met	
D72-18d	∂∇}α HO swi3-1 his4 his5 trp1 met	
D72-20b	MATα HO swi3-1 his^{-} trp1 met	
D85-16b	MATα cry1-3 ade5 ura4 met	
D85-28c	MATα cry1-3 swi1-2 ura4 met	
D85-41a	MATa swi1-2 his5 met	
D91-4b	cry1-3 MATa HO swi3-1 ura4 met	
D113-10b	MATa HO swi5-1 his^{-} met	
D113-24c	cry1-3 MATα HO swi5-1 ura3-52 his4 his5 met	
D114-23a	MATa HO swi1-2 his5 met	
D114-33b	MATα HO swi1-2 ade5 his5 met	
D123-1d	MATa swi1-1 HO::lacZ his5 leu2 met	
D144-2b	MATα swi1-2 HO::lacZ ura3-52 his5 met	
D149-10a	cry1-3 MATa HO swi1-2 ura4 met	
HR125-5d	MATa ura3-52 leu2$^{=}$ trp1 his3 his4	
HR145-4c-1	MATα HMRα HO::lacZ ura3-52 leu2$^{=}$ his5 ade5	
RC757	∂∇}α met1 his6 can1 cyh2 rme1 sst2	R. Chan
TD41-7b	cry1-3 MATa HO swi5-1 ura4 met	
XMB4-12b	MATa arg9 ilv3 ura sst1	L. Blair
X10-1b	MATa/MATα HO ade5 ura4 his5 met4 met13 (AH)	
X10-1b-1b	MATα segregant of X10-1b	

Table 6: leu2$^{=}$ indicates leu2-3 leu2-112.

Diploids that are AH (all homozygous) result from diploidization of haploid spores through mating type interconversion; hence, they are homozygous at all loci except MAT.

Table 7: Cross list

Diploid	Parents
A12	D114-33b x A24-8a
A13	D114-33b x D72-6b
A14	D114-33b x A24-11a
A15	D113-24c x D114-23a
A23	D72-18d x A24-8a
A24	D39-4c x D46-4b
A25	D113-24c x A24-8a
A34	D72-18d x A24-11a
A35	D113-24c x D72-6b
A45	D113-24c x A24-11a
D15	C10 x HR125-5d
D32	E1 x HR125-5d
D38	B2 x HR125-5d
D39	D15-34d x X10-1b-1b
D45	D39-24b x 671-1b
D46	D38-31b x X10-1b-1b
D57	D46-12c x 671-1b
D72	A24-1a-1b x D32-9c
D91	D72-20b x 671-1b
D100	D45-11b x HR145-4c-1
D101	D57-11c x HR145-4c-1
D113	I2 x A24-1a

D114	D85-41a x X10-1b-1b
D117	D72-6b x HR145-4c-1
D123	BW222R-55b-1b x HR145-4c-1
D142	D113-10b x HR145-4c-1
D144	D114-23a x HR145-4c-1
D149	671-1b x D114-33b
D151	D144-2b x D123-1d
TD42	D113-24c x 671-1b
HC101	D123-1d x D85-28c
HC102	D123-1d x D85-16b

Figure 1: Terminal morphology of nonviable swi2 swi4 double mutants

Asci from diploid A24 were dissected and germinated on YEPD agar slabs. After ten hours, four cells which were presumed to be double mutant on the basis of their cell morphology were chosen for photographing. The smaller, budded cell shown below the other cells is a budding SWI^+ haploid (HR125-5d) of typical size. Analysis of the genotypes of the sister segregants of the presumed double mutants confirmed that they are swi2 swi4.

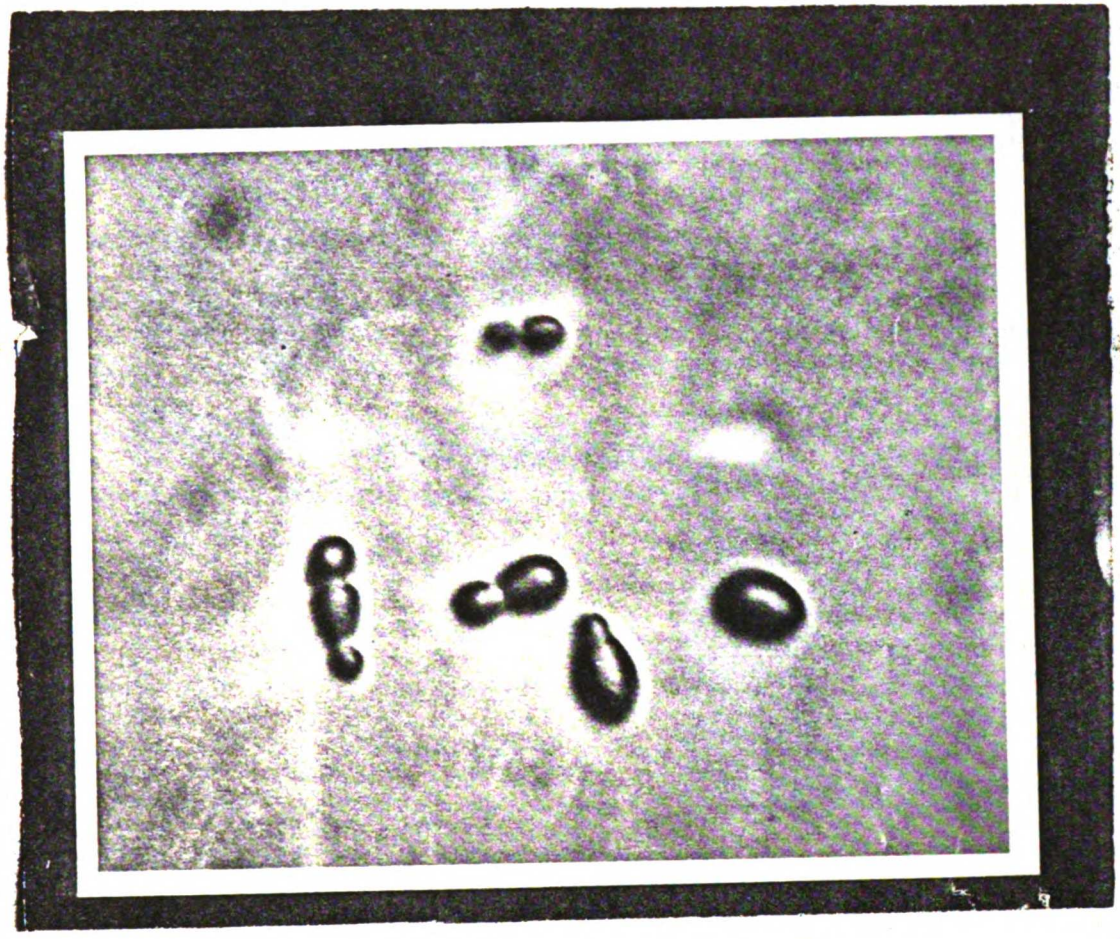
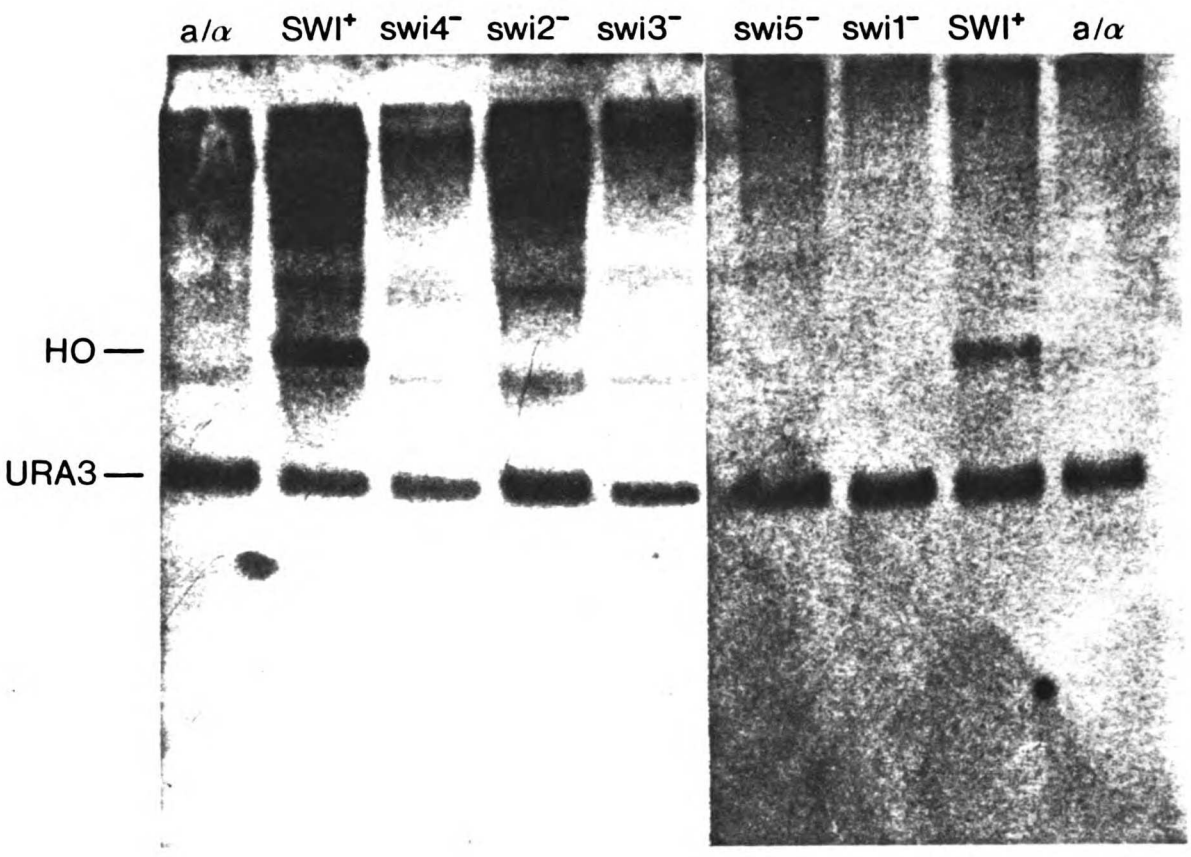


Figure 2: The Five SWI genes are required for HO expression

Total RNA was isolated from the following strains: a/α SWI$^+$/SWI$^+$ (671), SWI$^+$ (671-1b, an a segregant from 671); and swi mutants derived from 671: swi4$^-$ (671-C10), swi2$^-$ (671-B2), swi3$^-$ (671-E1), swi5$^-$ (671-I2) and swi1$^-$ (671-E2). All swi mutants are MATα. 25 - 30μg of RNA from each strain was fractionated by agarose gel electrophoresis, transferred to nitrocellulose and hybridized with radioactively labeled YIp5-BH2 plasmid DNA (see Materials and Methods). Positions of URA3 and HO transcripts are indicated. The faint bands appearing above and below HO co-migrate with ribosomal RNA. The high molecular weight hybridizing material is presumably DNA that has copurified with RNA in these preparations. The figure shows the results from two separate gels.


a/α
SWI+
swi4−
swi2−
swi3−
swi5−
swi1−
SWI+
a/α
HO
URA3

III. The yeast SWI1 and SWI5 genes: cloning, construction of deletion mutations, and transcriptional control

1. Introduction

In chapter II I described the identification of five genes (SWI1-SWI5) required for the expression of the HO gene and considered possible functional relationships among SWI genes. Because swi1, swi2 and swi3 mutants exhibit identical phenotypes, it was proposed that these genes act in the same pathway. The lethality exhibited by swi1 swi4 double mutants suggested that the functions of SWI4 and SWI1 overlap. It was thought to be unlikely that all five SWI genes act independently on the HO promoter to stimulate transcription; rather, we thought it more likely that some SWI genes act indirectly on HO expression by acting via other SWI genes. Because swi1, swi2, swi3 and swi4 mutations are extremely pleiotropic, whereas swi5 mutations are not pleiotropic, we proposed that both SWI4 and SWI1, SWI2 and SWI3 are required in some way for the function of the SWI5 gene product, which then acts more directly on HO (see figure 3). The SWI1-SWI4 genes could act on SWI5 at a variety of levels, being required, for example, for production of SWI5 transcript, or by synthesizing a cofactor that SWI5 protein requires for activity.

Little experimental evidence exists to bear on this model. In order to help collect such evidence, I have cloned the SWI1 and SWI5 genes. These cloned genes provide information in two ways that helps test our model: 1) They allow the construction of SWI deletion (swiΔ) mutations. 2) They provide a direct assay for the presence of SWI transcripts. The ways in which swiΔ mutants and SWI transcript assays can test our model are described below.

Determining the phenotypes of known swiΔ is important in two ways:

First, the swi1 swi4 lethality could be due to overlapping function, as we have proposed, or due to leakiness of mutations in two essential genes. The phenotype of a swi1Δ mutant will distinguish between these possibilities. Second, our belief that SWI5 acts more directly on HO is based on the lack of additional phenotypes conferred by the swi5-1 allele. This lack of phenotypes could be due to more direct action of SWI5 product on HO, as we have proposed, or due to leakiness of the swi5-1 allele. The phenotype of a swi5Δ mutant will distinguish between these possibilities.

Obtaining cloned SWI genes permits the assay of SWI transcripts. We have proposed that the SWI5 product requires the other four SWI genes for activity; the cloned SWI5 gene can be used to determine if this requirement is at the level of transcription. In addition, the isolation of plasmids carrying other SWI genes could reveal possible transcriptional controls that have not been predicted.

In this chapter, I describe the cloning of the SWI1 and SWI5 genes (the cloning of SWI2 is described in Appendix A). These cloned genes were used to construct swi1 and swi5 deletion mutants (denoted swi1Δ and swi5Δ) and to determine if SWI1 or SWI5 transcription requires any other SWI gene. Furthermore, the ability of these cloned genes, when present on multi-copy number plasmids, to complement other swi mutations enabled the testing of certain of our predictions on functional relationships among SWI genes.

2. Materials and Methods

Strains and Genetic Procedures

Strains are listed in Table 8, crosses in Table 9, and plasmids in Table 10. Media and genetic techniques (matings, sporulations, mating type and mating factor assays, scoring HO and scoring SWI alleles were accomplished as described in Chapter II. In addition, SWI alleles were scored in strains containing HO HMRα MATα HMLα by the α-factor halo test described in Blair (1979) and Jensen (1983). In short, this procedure is based on the observation that HO SWI strains containing the α allele at each cassette locus can mate as α but are unable to produce α-factor detectable by "halo" assay. In addition, such strains mate as a at low frequency and produce small amounts of a-factor. Strains that do not exhibit cassette switching, Ho^- and/or Swi^- strains, do not show this effect and behave as normal α cells. SWI alleles were also scored, in strains containing the HO-lacZ allele, by observing colony color on XG medium (Rose et al., 1981). XG medium is synthetic medium buffered to pH 7.0 with 0.05 M phosphate, containing 300 μg/ml leucine and 0.6 mg/ml XG (5-bromo-4-chloro-3-indolyl β-D-galactoside). Isogenic strains constructed for these experiments are derivatives of D117-13a, MATa HO-lacZ ura3-52 $leu2^=$ HMRα. This strain was diploidized by transformation with the cloned HO gene. D117-13a-a/α was sporulated, and a MATα segregant utilized to complete the isogenic set. swi1Δ and swi5Δ mutants were each constructed from D117-13a-α.

DNA techniques

Plasmid DNA was isolated from E. coli by either the rapid, small scale method of Holmes and Quigley (1981) or by cesium banding (Maniatis et al, 1982). Transformations into E. coli and subcloning were performed by standard methods. Plasmid DNA was prepared from yeast as described by Nasmyth and Reed (1980) except that if necessary, plasmid was further purified by passage over an A. 0.5 M agarose sizing column equilibrated in 10 mM tris-HCl 1 mM EDTA pH 7.5. Transformations of plasmid into yeast was performed by the Glusulase method of Beggs (1978) or the lithium method of Ito et al (1983) as modified by Fink (personal communication).

Plasmid curing on non-selective media

Yeast transformants were cured of plasmid by transferring to non-selective medium (YEPD) for approximately 12 generations and then restreaking on YEPD and testing single colonies for loss of plasmid by testing for uracil or leucine auxotrophy. For curing plasmids containing a centromere, single colonies were restreaked on YEPD a second time before testing for loss of plasmid marker. Transformants that resisted loss of marker contained the plasmid integrated in the genome.

Hybridizations

RNA to be used for Northern hybridization was prepared, gel

fractionated, transferred to nitrocellulose and hybridized as described in Chapter II. ^{32}P labelled probes were prepared by nick translation with α-dCTP using a New England Nuclear nick translation kit. Unincorporated label was separated from probe by passage over a 1.5 ml Sephadex G-125 column equilibrated with 10 mM Tris-HCl 1 mM EDTA pH 7.5 Filters were autoradiographed for the indicated times at -70°C with Kodak XAR-2 film and a Dupont Lightning Plus intensifying screen.

β-galactosidase assays

β-galactosidase assays were performed as described in Chapter II, except the background activity was provided by strain D246, isogenic to all strains used in these assays and of a/α cell type, and thus not expressing HO.

Plasmid Integrations

In all cases, plasmids to be integrated into the yeast genome at the SWI1 or SWI5 locus were first digested with a restriction enzyme that cuts within the SWI-containing insert, thus "targeting" integration (Orr-Weaver et al, 1983). Transformants carrying integrated plasmids could be recognized because the plasmid markers in such transformants were mitotically stable.

Isolation of the SWI1 gene

Yeast clone bank DNA, constructed by inserting partial Sau3A

digested yeast DNA into the BamHI site of YEp24 (Carlson and Botstein, 1982), was introduced into strain D143-13c (MATα HMRα HO leu2$^{=}$ swi1-2 ura3-52).

Plasmids able to complement swi1-2 were obtained first by selecting for transformants of D143-13c able to grow on 30 μg/ml leucine. As described in Chapter II, swi1-2 leu2 mutants are unable to form colonies on media containing 30 μg/ml leucine, whereas LEU2 swi1-2 or leu2 SWI1 strains are able to form colonies on this media. D143-13c was transformed with 150 μg of clone bank DNA and plated on media lacking uracil and containing 30 μg/ml leucine, 13 colonies were obtained. Three of these transformants had become leucine prototrophs; plasmids in these transformants apparently contained LEU2. The other ten transformants were Leu^{-}. These were analyzed for the ability to exhibit Ho activity. Because strain D143-13c contains α information at all three cassette loci, it cannot switch to a and thus cannot diploidize regardless of its genotype at SWI. However, Ho activity can be recognized in such strains by the appearance of novel phenotypes characteristic of a strains, as described above. Two transformants, D143-13c-e and D143-13c-s, acquired these phenotypes and thus contained plasmids that complemented the cassette switching defect conferred by the swi1-2 mutation.

Both transformants e and s lost their ability to exhibit cassette switching following curing of plasmid, thus verifying that the phenotype was plasmid dependent. Plasmid DNA was prepared from these transformants and introduced in E. coli strain MH1. One plasmid from transformant e, called e1, and one plasmid from transformant s, called s1, were able to retransform D143-13c to Ho^{+} phenotype.

Isolation of the SWI5 gene

Yeast clone bank in YEp24 was transformed into two swi5-1 strains, D142-2d (MATa ura3-52 HO-lacZ swi5-1) and D142-5a (MATα ura3-52 HO-lacZ swi5-1). Plasmids able to complement swi5-1 were recognized from transformants able to transcribe HO-lacZ and thus form blue colonies on XG. D142-2d and D142-5a were each transformed with 50 μg of YEp24 clone bank DNA. Uracil prototrophs, 10^4 obtained from each strain, were replated on a total of 50 plates lacking uracil but containing 0.6 mg/ml XG. 37 blue colonies, 19 from D142-2d and 18 from D142-5c, were obtained. DNA was prepared from four such transformants, D142-5c-j, D142-5c-ee, D142-2d-f and D142-2d-1, and transformed into E. coli strain MH1. Plasmid DNA was isolated from MH1 transformants and subjected to partial restriction mapping. Plasmid from transformants f and 1 (f5 and 15, respectively) were identical and thus probably not of independent origin. Plasmid from transformant j (called j5) appeared identical to the vector YEp24. Finally, plasmid DNA isolated from transformant ee (called ee5 - figure 7a) carried an insert that was contained within the insert present in f5 and 15; the insert of 15 (15 kb) contains approximately 2.5 kb of DNA flanking both sides of the DNA present in the insert of ee5. The inserts of 15 and ee5 are in opposite orientation with respect to vector sequences. Both of these plasmids, when reintroduced into swi5-1 strains, were able to complement the defect in HO expression conferred.

Construction of swi1Δ

The substitution mutation of SWI1 was constructed as follows: the 4.7 kb SphI-ClaI fragment of 19fΔCla was subcloned into YIp5, to form plasmid 34a. Then, the BamHI-BglII fragment of p34a was replaced with a BglII fragment containing LEU2. The ligation mixture was transformed into E. Coli strain MH6 (pyrF leuB). One transformant containing the desired plasmid was recognized as a leucine and uracil prototroph. To produce a swi1Δ mutant in vivo, p42a was first digested with PstI, for which there are no sites in LEU2, but which cuts in SWI1 sequences 0.6 kb to the left and 1.4 kb on the right of LEU2. This digestion produced a linear fragment of LEU2 carrying SWI1 sequences on both sides. This fragment was transformed into D117-13a-a/α. Leucine prototrophs arise from such a fragment by a double crossover (Rothstein, 1983) with chromosomal SWI1 as diagrammed in Figure 6. Two mitotically stable leucine prototrophs were obtained following transformation.

Construction of swi5Δ

The SWI5 substitution mutation was constructed from ee5 by replacing the four BglII fragments with the LEU2-containing BglII fragment from CV13. The resulting plasmid, p48b, was linearized by digestion with Bam HI, which cuts in insert DNA, and Sph I, which cuts in vector DNA. Linearized p48b was introduced into D117-13a and six mitotically stable leucine prototrophs were obtained.

3. Results

SWI1 and SWI5 were cloned from a yeast genomic clone bank in the plasmid YEp24 (Carlson and Botstein, 1982). YEp24 can replicate both in E. coli, where it confers ampicillin and tetracycline resistance and complements pyrF mutations, and in yeast where it complements ura3 mutations. Plasmids containing SWI1 or SWI5 were identified by their ability to complement the various phenotypes conferred by the appropriate swi mutation. Following isolation, inserts were transferred to the low copy-number plasmid YCp50 to determine if the ability of the insert to complement the swi mutation was an artifact of its high gene dosage when present on YEp24. Inserts were also transferred to the integrating plasmid YIp5 (Struhl et al, 1979) so that the genetic locus to which the insert directs plasmid integration could be monitored. In yeast, plasmid integration into the genome occurs by homologous recombination. If the insert actually contains SWI DNA, it will direct plasmid integration to the SWI locus. If so, the plasmid marker (URA3 in this case) becomes linked to the SWI locus.

Two techniques were used to localize the gene more precisely on the cloned DNA. The maximum extent of the gene was defined by DNA that, when present on YCp50, was able to complement the appropriate swi mutation, whereas the minimum extent of the gene was defined by DNA that, when present on the integrating plasmid YIp5 and integrated at the SWI locus, was able to produce a swi mutation. The latter technique is referred to as the "zapper" technique (described in Shortle et al, 1982). The integration by homologous recombination of a plasmid carrying an internal fragment of a gene creates a duplication of the

internal fragment. One copy of the duplication carries a 5' deletion and the other copy carries a 3' deletion. This event is expected to produce a mutation (see Shortle et al, 1982) and therefore can be used to determine whether a particular fragment is internal to the gene of interest.

The two techniques described above, taken together, permit the localization of the endpoints of the SWI genes to within adjacent restriction sites. Following this localization, substitution mutations were constructed by deleting SWI DNA, including most or all of the DNA defined as internal to the gene, and replacing this DNA with the LEU2 BglII fragment obtained from CV13 (Broach et al, 1979).

Localizing the SWI1 gene on insert DNA

The isolation of plasmid e1, a YEp24 derivative that complements swi1-2, is described in Materials and Methods. A restriction map of e1 is shown in Figure 4a. In order to locate the SWI1 gene and the wild-type allele of the swi1-2 mutation on e1, three deletions of e1 were constructed: e1ΔBam (figure 4b), e1ΔSal (figure 4c) and e1ΔSph (figure 4d). Each plasmid was transformed into D143-13c and the resulting transformants assayed for Swi^- or Swi^+ phenotype. Whereas e1 was able to complement swi1-2, none of the three deletion plasmids was able to do so. This result indicates that SWI1 extends into the DNA defined by the smallest deletion, e1ΔBam.

The nature of the e1ΔBam and e1ΔSal transformants differed from the nature of the e1ΔSph transformants. The former transformants, although Swi^-, gave rise at high frequency (at least ten fold higher than trans-

formants carrying e1ΔSph) to variants able to grow on 30 µg/ml leucine, on which leu2 swi1 mutants fail to grow (Chapter II). In addition, these variants had become Swi^+ because they were able to exhibit cassette switching in a plasmid-independent manner. In contrast, the variants able to grow on 30 µg/ml leucine that were obtained from transformants carrying e1ΔSph did not exhibit cassette switching and thus remained phenotypically Swi^-. Such observations suggest that variants arising from e1ΔBam and e1ΔSal transformants occur via plasmid-directed marker rescue of the chromosomal swi1-2 allele to SWI1. Therefore, the swi1-2 mutation must be located between the unique SalI site of the insert and the rightmost SphI site of the insert. Also, these observations demonstrate that plasmid e1, thought to carry SWI1 on the basis of its ability to complement swi1-2, actually contains SWI1 DNA.

The 5.5 kb Sph I fragment was subcloned into YCp50 and, in both orientations (plasmids 19d and 19f, figure 5a and 5c), was able to complement swi1-2. The ability of this fragment to complement swi1-2 on a low copy number plasmid suggests that this fragment contains the entire SWI1 gene.

In order to further localize SWI1, deletions of 19d and 19f were constructed. Neither 19dΔH3 (figure 5b) nor 19fΔCla (figure 5d) was able to complement swi1-2. These results demonstrate that the SWI1 extends to the left of the ClaI site and to the right of the HindIII site. Using the zapper technique, SWI1 was shown to extend beyond the rightmost EcoRI site. The RS3 fragment (as denoted in Figure 4a) was subcloned into YIp5 and integrated at SWI1. Resulting transformants

were swi1 mutants. Therefore, it is concluded that SWI1 extends to the right of the rightmost EcoRI site.

Cloned SWI1 DNA directs plasmid integration at SWI1

To see if the SWI1 insert directs integration at the SWI1 locus, p27b, a YIp5 derivative containing a 4.0 kb EcoRI-SalI fragment (denoted RS1 in figure 4a) was integrated at SWI1 in strain D117-13a-a. Two transformants were crossed to MATα HO-lacZ swi1-2 ura3-52 strain D144-2b. URA3 segregated 2:2 in all tetrads, demonstrating that the plasmid had integrated at only one locus, and URA3 SWI1:ura3-52 swi1-2 showed 13 parental ditype tetrads, one tetratype tetrad, and no non-parental ditype tetrads. This result demonstrates that URA3 is now linked to SWI1 in these transformants, thus demonstrating that p27b contains sequences tightly linked to SWI1.

Construction of swi1Δ mutation

A swi1Δ mutation was constructed by replacing a 1.5 kb BamHI-BglII fragment, which was demonstrated to be internal to SWI1, with LEU2. The construction of such a plasmid (p42a) is described in Materials and Methods. Because we did not know if the swi1Δ mutant would be viable, and because we knew that at best the substitution mutation would confer growth defects, the substitution was constructed in diploid strain D117-13a-a/α. In the diploid, the expected recessive effects of the substitution would not be expressed, so we would be able to recover the mutation regardless of the phenotype it would confer in a haploid. In

contrast, the LEU2 marker is dominant, so a Leu^+ phenotype could be expressed even in a diploid. Of the two Leu^+ transformants obtained, one (BDY12a) was sporulated and dissected to recover the substitution mutation in a haploid. Spore viability was high (five tetrads with four viable spores and four with three viable spores), although in the tetrads with four viable spores, two colonies were always very small. These small colonies were always Leu^+ and formed white colonies on XG, whereas the large colonies were Leu^- and formed blue colonies on XG.

Three lines of evidence were used to demonstrate that the Leu^+ segregants carry a swi1 mutation: a) the Leu^+ segregants exhibit other phenotypes characteristic of swi1 mutants, b) the Leu^+ segregants fail to complement swi1-2 mutants and c) LEU2 in the Leu^+ segregants is linked to SWI1.

a) Whereas a/α SWI1/swi1Δ strains sporulate normally, a/α swi1Δ/swi1Δ fail to sporulate. Defective sporulation is also observed in swi1-2 mutants (Chapter II). In addition, a/α swi1Δ/swi1Δ cells exhibit a "rough" colony morphology characteristic of a/α swi2-1 or swi3-1 cells. A rough morphology is not observed in a/α swi1-2/swi1-2 cells, and in fact is the only phenotype distinguishing swi1-2 from swi2-1 or swi3-1 mutants. We attributed this phenotypic difference to leakiness of the swi1-2 allele (see Chapter II) and this notion is supported by the observation that a/α swi1-2/swi1Δ mutants exhibit the rough colony morphology.

b) Failure of swi1Δ to complement swi1-2 is observed both by failure of swi1Δ/swi1-2 cells homozygous for the cry1-3 (Cry^R) allele to grow on cryptopleurine medium (Chapter II) and by the failure of a/a swi1Δ/swi1-2 HO cells to exhibit mating type switching. However, at low

frequency, variants able to grow on cryptopleurine were obtained, and these variants were also able to exhibit switching. These variants presumably arise by recombination between the two swi1 mutant alleles to form SWI1, which would suggest that the swi1-2 mutation lies outside the region defined by the deletion used to construct swi1Δ.

c) In cells carrying swi1Δ:LEU2, the LEU2 gene is tightly linked to SWI1. BDY12a-1b (MATα HO-lacZ swi1Δ::LEU2) was crossed to BDY1 (MATa HO-lacZ SWI1-URA3). From nine complete tetrads, LEU2:URA3 showed nine parental ditytpe tetrads. This result demonstrates that LEU2 is now linked to URA3, which was previously shown to be linked to SWI1. Blue:white colonies on XG media segregated 2:2; all blue colonies were Ura^+, all white colonies were Leu^+.

Localizing the SWI5 gene on insert DNA

The isolation of plasmid ee5, a YEp24 derivative that is able to complement swi5-1, is described in Materials and Methods. A restriction map of the insert of ee5 is shown in Figure 7a. Two observations suggest that the insert of ee5 contains the entire SWI5 gene: 1) the insert in ee5 directs integration of ee5 to the SWI5 locus, and 2) the insert in ee5, when present on the low copy number plasmid YCp50, can complement swi5-1.

1) ee5 was integrated into strain D117-13a. To see if ee5 had integrated at SWI5, one mitotically stable transformant was crossed to a swi5-1 HO-lacZ ura3-52 strain (D142-5c). Nine four-spored tetrads were analyzed from this cross. In all nine tetrads, Ura^+:Ura^- segregated 2:2, indicating that ee5 had integrated at one locus. Furthermore, Ura^+

$Swi^+:Ura^-$ Swi^- showed 8 parental ditype tetrads and one tetratype tetrad. Therefore, ee5 directs integration close to the SWI5 locus.

b) To show that the insert of ee5 can complement swi5-1 when present on YCp50, a DNA fragment from the unique SphI site of the vector to the EcoRI site of the insert was subcloned into YCp50. Plasmid 26d (figure 7b) is able to complement swi5-1.

A ClaI deletion of 26d (figure 7c) fails to complement swi5-1, indicating that SWI5 lies on the left side of the insert. An internal fragment of SWI5 was found by the zapper technique. Six plasmids were constructed, each consisting of a fragment from the left side of the insert in YIp5. The six plasmids (p40s, p40e, p40j, p40eΔCla, p40eΔH3 and p40jΔCla, described in Table 10 and Figure 8) were integrated at SWI5 and transformants assayed to see if any had become swi5 mutants. Only p40eΔCla was able to produce swi5 mutants, which was tested both by complementation and linkage with swi5-1. This result suggests that the SalI-ClaI fragment defined by p40eΔCla is internal to SWI5. Parenthetically, it was observed that this SalI-ClaI fragment could confer autonomous replication to YIp5; therefore, this fragment contains an ARS element.

c) The results with the zapper integrations suggested that SWI5 might lie entirely within the 3.3 kb HindIII fragment shown in Figure 9a. In fact, this fragment inserted in YCp50 was able to complement swi5-1, suggesting that SWI5 lies entirely within this fragment. The right end of SWI5 therefore lies between the Cla1 and HindIII sites as shown in Figure 9a. The left end of SWI5 is not as well defined.

Construction of swi5Δ

The four BglII fragments within SWI5 in ee5 were replaced with the 2.6 kb BglII fragment of LEU2 obtained from CV13 (see Materials and Methods) to form p48b. p48b was linearized and integrated into haploid strain D117-13a-α. The use of a haploid strain as recipient in this case was justified because swi5Δ mutants were expected to be viable. If such mutants had not been viable, then all transformants would have resulted from the gene conversion of chromosomal $leu2^{=}$ to LEU2, which is an event that I have observed in other instances (M. Stern, unpublished). This event, however, did not occur in the transformation described here because all six transformants obtained were swi5 mutants.

In order to determine if LEU2 was now linked to SWI5, one transformant was crossed to a derivative of D117-13a that carried plasmid ee5 integrated at SWI5. In this cross, Leu^+ segregated 2:2, which demonstrates that LEU2 integrated at a single locus, and Leu^+ Ura^-:Leu^- Ura^+ showed six parental ditype tetrads of six tetrads analyzed. Therefore, LEU2 was now linked to URA3, and thus had integrated at SWI5. In addition, all Leu^+ segregants were white on XG. These Leu^+ segregants were swi5 mutants because they were unable to complement swi5-1 (monitored both by colony morphology in a/α cells and by switching phenotype). The structure of swi5Δ is shown in Figure 9b. This structure is still presumptive because it has not been checked by Southern analysis.

Phenotype of swi5Δ mutants

As noted in the Introduction to this chapter, we were interested in

determining the phenotypes conferred by a swi5Δ mutation, particularly to see if a swi5Δ mutant had acquired the phenotypes characteristic of a swi1 or swi4 mutant.

First, a swi5Δ strain grows at the same rate as an isogenic SWI5 strain. In cross D246, for example, the two swi5Δ segregants formed colonies of the same size as the two SWI5 segregants. No defect in growth rate as measured in YEPD was observed. These observations are in contrast to the behavior of swi1Δ mutants, which show severe growth defects on YEPD.

Second, a/α cells homozygous for swi5Δ are able to sporulate at the same frequency as a/α cells heterozygous for swi5Δ. A MATa swi5Δ strain (D246-2b) was mated both to a SWI5 strain (D246-2a) and a swi5Δ strain (D246-2d). All strains are isogenic. Both diploids showed an equal frequency of sporulation. Therefore, unlike SWI1, SWI5 is not required for sporulation.

Third, a swi5Δ mutant was crossed to a swi1Δ mutant (BDY12a-1a) and a swi4-3 mutant (D212-6d). In both cases, double mutant segregants were obtained, as verified by complementation tests. Standard spore viability was observed. Therefore, SWI5 is not required for viability in swi1 or swi4 mutants, thus distinguishing SWI5 from the other SWI genes.

We were also interested in observing if swi5Δ mutants resemble swi5-1 mutants with respect to the level of HO expression. swi5-1 mutants produce higher levels of HO, as monitored by β-galactosidase activity, than mutants defective in the other SWI genes. This higher level of expression could be due to leakiness of the swi5-1 allele or by an incomplete requirement of HO transcription for SWI5 function. To answer this question, β-galactosidase activity in isogenic (D117-13a)

SWI, swi1Δ and swi5Δ strains was assayed. Both swi1Δ (average of two assays) and swi5Δ (average of four assays) produced 0.1 units of β-galactosidase. The Swi^+ strain in these experiments produced 5.5 units of β-galactosidase (average of four assays). Therefore, it is concluded that partial HO-lacZ expression in swi5-1 results from leakiness of the swi5-1 allele; a swi5 null allele produces the same, undetectable level of β-galactosidase as a swi1 null allele.

Functional relationships among SWI genes: cross-complementation between swi mutations and plasmids carrying other SWI genes

It is useful to know if a mutation in one gene can be complemented by a second gene present on a multi-copy plasmid. A structural or functional relationship between the two genes is indicated if the presumed overproduction of the second gene product can restore wildtype phenotype to the given mutant. Lack of complementation by the high copy number gene indicates nothing about relationships between the two genes. Because functional relationships among SWI genes have been proposed, it was of interest to determine if any two SWI genes would show cross-complementation. Therefore, original isolates of SWI1, SWI2 and SWI5 genes, present on the multi-copy plasmid YEp24, were each introduced into each swi mutant, and ability of the transformants to express HO was monitored. The results are summarized in Table 11. Cross complementation was observed in two cases: 1) plasmid g2 (YEp24:SWI2) was able to complement swi1-2. Recipient swi1-2 strains showed good growth on 30 μg/ml leucine. By a cassette switching assay in a strain (D143-13c) carrying HO and the α allele at all three

cassette loci (see Materials and Methods), plasmid g2 appeared able to restore levels of switching equal to wildtype strains. However, a swi1-2 HO-lacZ recipient strain (D144-2b) formed only pale blue colonies on XG, indicative of about 10% of Swi^+ expression of HO-lacZ. SWI2 on a low-copy plasmid conferred to swi1-2 mutants a slightly increased ability to grow on 30 µg/ml leucine but not an ability to express HO. Plasmid g2 cannot complement the swi1Δ mutation, as swi1Δ mutants carrying g2 form white colonies on XG. 2) Plasmid e1 (YEp24:SWI1) was able to complement swi4-3. swi4-3 mutants carrying e1 form blue colonies on XG, indicative of approximately wildtype levels of HO-lacZ expression. No other cross complementation was detected.

SWI transcripts in swi mutants

Because all swi mutants exhibit the same effect on HO expression, it seemed possible that SWI genes might act on HO indirectly, perhaps by stimulating transcription of other SWI genes. Because we hypothesized that SWI1-SWI4 act indirectly on HO via SWI5, we were particularly interested in determining if SWI5 transcripts were present in swi1-swi4 mutants. Therefore, RNA was prepared from original isolates of swi mutants (using isolates E2, B2, E1, C10 and I2 for swi1-swi5, respectively) and from an isogenic SWI strain 671-1b. RNA from these strains was probed with p27d (SWI1 probe), p29a (SWI1 probe), and p44f (SWI5 probe). All three plasmids are derivatives of YIp5 and therefore contain homology to the URA3 transcript as well as to SWI transcripts. Furthermore, each plasmid was defined genetically to contain DNA internal to SWI1 and SWI5. Figures 10,11 and 12 show SWI transcripts. In

figure 10 (p44f probe), only one transcript is visible in addition to URA3. This transcript is presumed to be SWI5 transcript. It is present in equal intensities in wildtype and each swi mutant. In Figure 11, the SWI1 probe shows three transcripts in addition to URA3. It is not possible from this blot to determine which, if any, transcript corresponds to SWI1 transcript. However, each transcript is present in equal amounts in all strains tested. In Figure 12, a second SWI1 probe, from another internal EcoRI-SalI fragment neighboring the fragment present in p27d, visualizes only one transcript, the highest molecular weight transcript visualized in Figure 11. This transcript is presumably SWI1 transcript. The other broad hybridizing material visualized on this long exposure co-migrate with rRNA.

4. Discussion

The experiments described here were designed to examine functional relationships among SWI genes and test certain predictions. These predicted relationships are shown in Figure 3. First, by analyzing SWI1 and SWI5 transcripts in mutants defective in each SWI gene, we have shown that SWI1 and SWI5 do not require any SWI gene for transcription. In addition, isolation of the SWI2 gene, described in Appendix A, allowed us to demonstrate that SWI2 also does not require any SWI gene for transcription. Second, the SWI1 gene on a multi-copy plasmid can complement swi4-3, and the SWI2 gene on a multi-copy plasmid can complement swi1-2 but not swi1Δ. Third, swi1Δ and swi5Δ mutants exhibit essentially the same phenotypes as the original mutants isolated in vivo.

We have proposed that the SWI1, SWI2, and SWI3 gene products act in the same pathway, or perhaps are three components of the same protein. Consistent with this proposal is the observation that the SWI2 gene on a high copy number plasmid can partially complement a slightly leaky swi1 mutation but not a null swi1 mutation.

We have also proposed that SWI4 and SWI1, SWI2 and SWI3 have overlapping function. This proposal is based on the observation that the function of either SWI4 or SWI1, SWI2 and SWI3 is required for viability as well as the observation that the function of all four genes is required for HO expression. The double mutant lethality observed between SWI4 and SWI1 could be due to an additive effect of mixing two leaky mutations in two essential genes or due to a non-additive, synergistic relationship between two genes with overlapping function. The latter possibility is supported by the observation that SWI1, at least, is not an essential gene, as the swi1Δ mutant is viable. Overlapping function between SWI4 and SWI1 is further supported by the observation that the SWI1 gene present on a multi-copy plasmid can complement the defect in HO expression conferred by swi4-3. We predict that a swi4 null mutation would also be complemented by the SWI1 plasmid. To test this prediction requires the cloning of SWI4, which has not been accomplished.

Finally, we have proposed that SWI5 acts more directly on HO and that SWI4 and SWI1, SWI2 and SWI3 are both required for the activity of SWI5. This proposal is based on the lack of additional phenotypes conferred by swi5-1. This lack of additional phenotypes could be due to leakiness of the swi5 allele analyzed or more direct action of SWI5 on HO transcription. We have used the cloned SWI5 gene to construct a swi5

deletion mutant. The swi5Δ mutant, as is the case for the swi5-1 mutants, exhibits no additional defects. swi5Δ mutants grow and sporulate as well as SWI5 strains, and the swi5Δ mutation does not confer inviability to mutants defective in any other SWI genes. This observation supports our proposal that SWI5, unlike the other SWI genes, is concerned most directly with HO transcription. The phenotype of the swi5Δ mutant is of interest for a second reason. swi5-1 mutants express HO at a higher level than do mutants defective in the other SWI genes. If SWI1, SWI2, SWI3, and SWI4 act on HO via SWI5, as we have predicted, then the residual expression of HO in swi5-1 must be due to leakiness of swi5-1. In fact, this is the case, as the swi5Δ mutant expresses HO at a lower level than swi5-1 mutants, at a level equal to that of swi1Δ mutants.

To determine if SWI5 required the other SWI genes for transcription, the cloned SWI5 gene was used to probe for SWI5 transcripts. Both SWI5 and SWI1 transcripts are present in all swi mutants, in the same amount as in a Swi^+ strain. Therefore, the putative requirement of SWI5 for the other SWI gene lies at a later stage than transcription. In the GCN system, which was described in the Introduction, it was proposed that GCN4 was the immediate activator of HIS4 transcription, and that two other activators, GCN2 and GCN3, acted indirectly on HIS4 via GCN4. It was found (Hinnebusch, 1984), using a GCN4 probe and GCN4-lacZ fusion, that GCN2 acts on GCN4 at the level of translation, as GCN4 was transcribed but the transcript was not translated in gcn2 mutants. A SWI5-lacZ fusion, which could be used to obtain antibodies to SWI5 protein, could be used to analyze translation of SWI5 message, or SWI5 protein stability in mutants defective in the other SWI genes.

Table 8: Strain List

a) yeast strains

Strain	Genotype
D117-13a-a	MATa HO-lacZ ura3-52 leu2$^{=}$ his4 his5 HMRα can1 met
D117-13a-α	MATα derivative of D117-13a-a
D117-13a-a/α	a/α derivative of D117-13a-a
D117-14c	MATα HO-lacZ ura3-52 leu2$^{=}$ his met ade5
BDY1	p27b integrated at SWI1; isogenic to D117-13a-a
BDY8	ee5 integrated at SWI5; isogenic to D117-14c
BDY12a	p42a (swi1Δ::LEU2) substituted at SWI1; isogenic to D117-13a-a/α
BDY12a-1a	MATα swi1Δ::LEU2 segregant of BDY12a
BDY12a-5a	MATa swi1Δ::LEU2 segregant of BDY12a
BDY12a-1c	MATa SWI1 segregant of BDY12a
BDY12a-5c	MATα SWI1 segregant of BDY12a
BDY19	ee5 integrated at SWI5; isogenic to D117-13a-a
BDY21c	p48b (swi5Δ::LEU2) substituted at SWI5; isogenic to D117-13a-α
D142-2d	MATa HO-lacZ swi5-1 ade5 his met
D142-5c	MATα HO-lacZ swi5-1 his met
D143-13c	MATα HMRα HO swi1-2 ura3-52 leu2$^{=}$ his met ade5
D144-2b	MATα HO-lacZ ura3-52 swi1-2 his5 met
D212-5d	MATα HO-lacZ ura3-52 leu2$^{=}$ swi4-3 ade5 his

D246-2a	MATα SWI5-URA3 segregant of D246
D246-2b	MATa swi5Δ::LEU2 segregant of D246
D246-2d	MATα swi5Δ::LEU2 segregant of D246

b) E. coli strains

MH1 (from M. Hall) araD139 ΔlacX74 galU galK hsr strA

MH6 " " " leuB pyrF otherwise isogenic to MH1

Legend to Table 8: leu2$^{=}$ represents leu2-3 leu2-112. Some strains, described as containing an integrated plasmid, are constructed by targeting plasmid integration to the locus integrated. These constructions were verified by genetic but not biochemical methods. Some strains, described as containing a substituted plasmid, were constructed by transforming the indicated strain with a linear fragment of the plasmid indicated; these constructions were verified by genetic methods.

Table 9: Cross list

Diploid	Parents
D192	D144-2b X BDY1
D193	D144-2b X BDY3
D247	BDY12a-1a X BDY1
D208	D142-5c X BDY19
D246	BDY21c X BDY19
D257	D212-6d X BDY21c
D258	BDY12a-5a X BDY21c

Table 10: Plasmid list

Plasmid	Description
e1	SWI1 original isolate on YEp24
s1	SWI1 original isolate on YEp24
ee5	SWI5 original isolate on YEp24
15	SWI5 original isolate on YEp24
e1ΔBam	e1 containing deletion of 3.3 kb BamHI fragment
e1ΔSal	e1 containing deletion of 5.0 kb SalI fragment
e1ΔSph	e1 containing deletion of 7.0 kb SphI fragments
p19d	SWI1 in SphI site of YCp50, orientation 1
p19f	same as p19d except insert in opposite orientation
p27b	EcoRI-SalI fragment #1 (RS1), from e1
p27d	EcoRI-SalI fragment #3 (RS3), from e1
p29a	EcoRI-SalI fragment #2 (RS2), from e1
p26d	SWI5 in EcoRI-Sph I sites of YCp50
p40c	SalI fragment #2 of ee5 (S2) in YIp5, orientation 2
p40e	SalI fragment #3 of ee5 (S3) in YIp5, orientation 1
p40j	SalI fragment #3 of ee5 (S3) in YIp5, orientation 2
p40s	SalI fragment #2 of ee5 (S2) in YIp5, orientation 1
p40o	SalI fragment #1 of ee5 (S1) in YIp5
p41b	SalI fragment #3 of ee5 (S3) in YCp50
p41c	SalI fragment #1 of ee5 (S1) in YCp50
p40jΔCla	ClaI deletion of p40j
p40eΔH3	HindIII deletion of p403
p40eΔCla	ClaI deletion of p40e

p34a	4.8 kb SphI- ClaI fragment of 19d in YIp5
p42a	BamHI-BglII fragment of p34a replaced with LEU2
p47a	3.3 kb HindIII fragment of SWI5 in YCp50
p48b	2.5 kb BglII fragments of SWI5 replaced with LEU2

Legend to Table 10: Inserts described as present in orientation 1 contain an insert in the orientation relative to pBR322 sequences as found in original SWI isolates e1 or ee5. Inserts described as present in orientation 2 contain an insert in the opposite orientation. Further descriptions of some of these plasmids are present in Figure legends or in the text.

Table 11: Tests for SWI cross-complementations

	SWI1		SWI2		SWI5	
	high	low	high	low	high	low
swi1-2	+	+	+	+/=	-	-
swi1Δ	+	+	-	-	-	-
swi2-1	-	-	+	+	-	-
swi3-1	-	-	-	-	-	-
swi4-3	+	ND	-	-	-	-
swi5-1	-	-	-	-	+	+

Legend to Table 11: This table summarizes the ability of SWI1, SWI2 and SWI5 segments present on high (YEp24) or low (YCp50) to complement the swi mutations indicated. Complementation is monitored by the ability to express HO, measured as the ability of strains to undergo cassette switching or to produce blue colonies on XG. More complete description is found in the text. YCp:SWI2 can partially restore the growth defect of swi1-2 mutants on low leucine, but cannot restore cassette switching to such mutants.

Figure 3: Proposal for the mechanism of action of SWI genes. SWI5 directly stimulates HO transcription. SWI5 requires both SWI4 and SWI1, SWI2 and SWI3 (grouped together because of their common function) for activity. SWI1-SWI4 are required for viability as well as for SWI5 activity, but the requirement is less stringent; viability requires either SWI4 or SWI1, SWI2 and SWI3.

3.

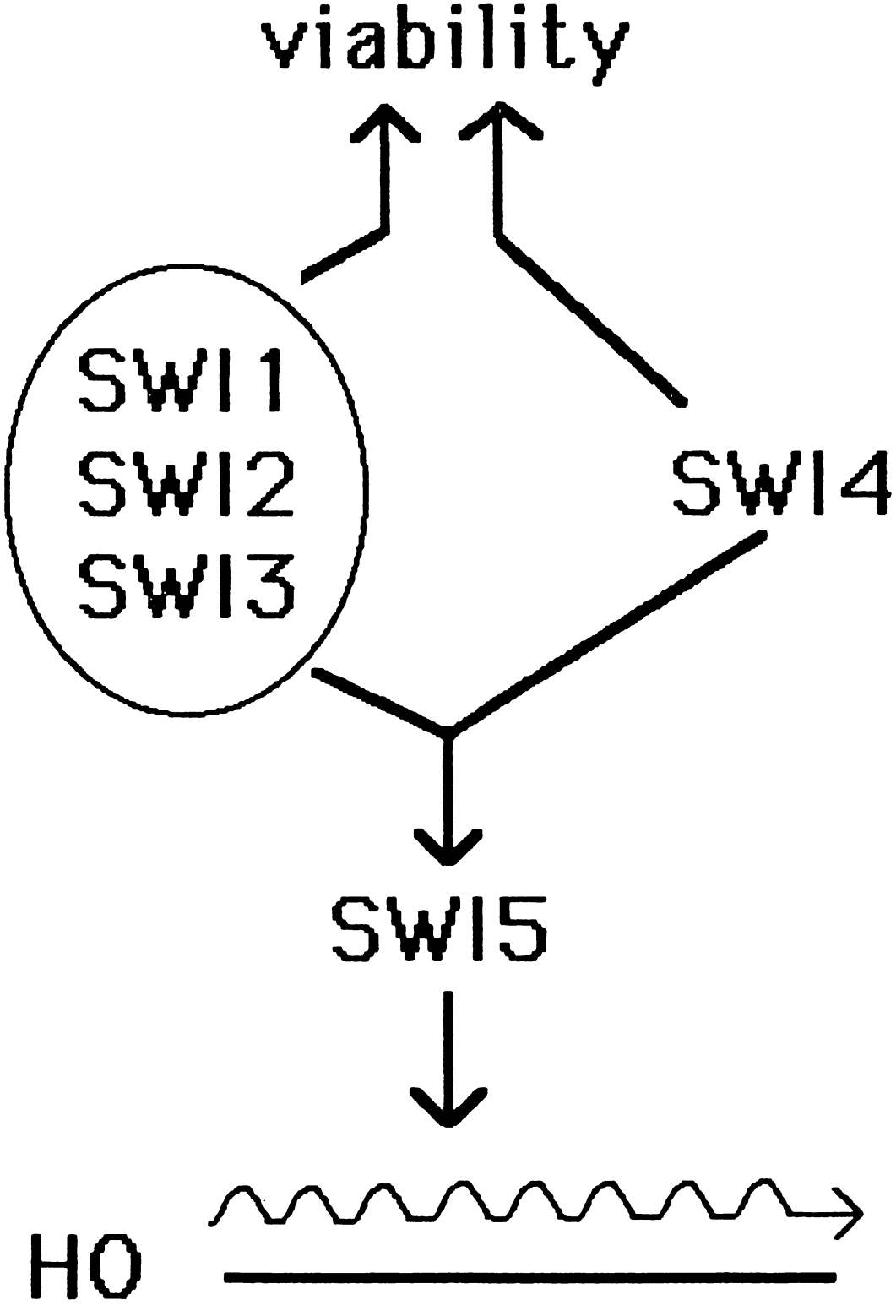
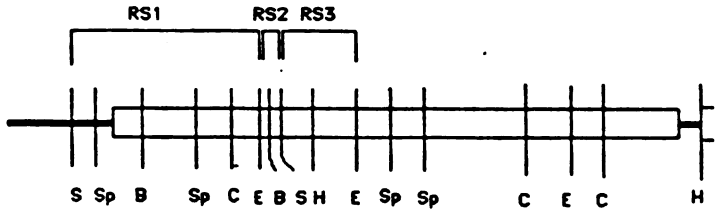


Figure 4. Restriction maps of original SWI1 isolate and three deletion derivatives. Abbreviations of restriction enzymes used: B=BamHI, C=ClaI, E=EcoRI, H=HindIII, S=SalI, S=SphI. Inserts are all present as Sau3A partial digestion fragments inserted in the BamHI site of YEp24. Thick bar represents pBR322 sequences, open box represents yeast sequences. Fragments denoted RS1, RS2, and RS3 are three EcoRI-SalI fragments with which experiments were performed as described in the textExtent of deletions indicated by gap in plasmid map and diagonal lines. a) plasmid e1, original SWI1 isolate, complements swi1-2. b) e1ΔBam, and c) e1ΔSal, cannot complement swi1-2, but can by marker rescue convert swi1-2 to SWI1. d) e1ΔSph can neither complement swi1-2 nor convert swi1-2 to SWI1.

Total size of insert in e1 is 16 kb.

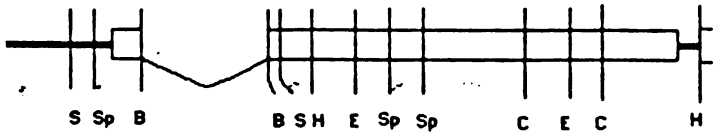
4.

a e1 - original SWI1 isolate



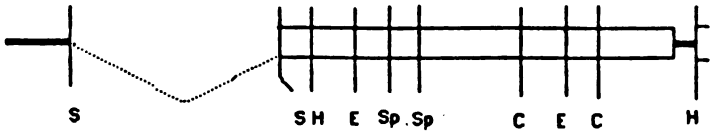
complements	gene converts
swi1-2	swi1-2
yes	ND

b - e1ΔBam



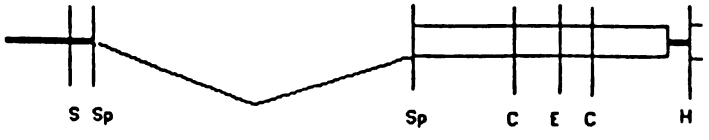
no yes

c - e1ΔSal



no yes

d - e1ΔSph



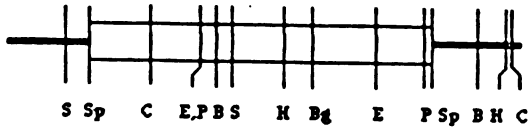
no no

Figure 5. SWI1 subcloned in YCp50 and some deletion derivatives. Abbreviations of restriction enzymes used as in legend to Figure 4 with the addition of Bg=BglII and P=PstI. Plasmid representations as in legend to Figure 4. a) 5.5 kb SphI fragment containing SWI1 (in same orientation relative to vector as in e1) can complement swi1-2 when present on YCp50. b) same as a), except HindIII fragment containing 2.5 kb of insert is deleted. This plasmid cannot complement swi1-2. c) same as a), but insert in opposite orientation. This plasmid can complement swi1-2. d) same as c), except ClaI fragment containing 0.8 kb of insert is deleted. This plasmid cannot complement swi1-2.

5.

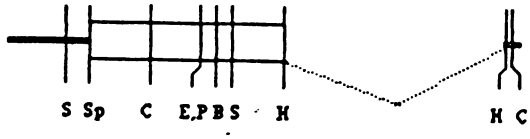
complements swi1-2

a - p19d



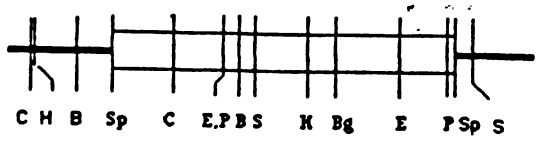
yes

b - p19dΔH3



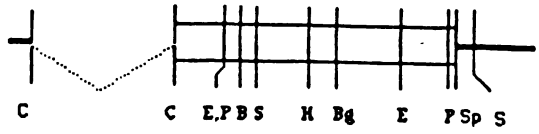
no

c - p19f



yes

d - p19fΔCla

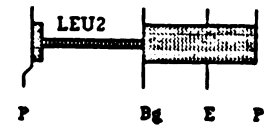


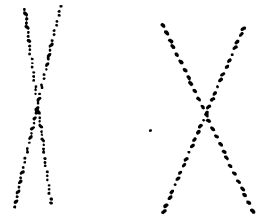
no

Figure 6. Construction of swi1Δ::LEU2 and its replacement of SWI1 in the chromosome. Abbreviations for restriction enzymes are as in legend to Figure 5. Details of construction are described in Materials and Methods. SWI1, with 1.5 kb BamHI-BglII fragment substituted with 2.6 kb Bgl II fragment of LEU2 (thick bar), was linearized with PstI and transformed into $leu2^{=}$ SWI1 strain. A double-crossover event, occuring via regions of homology denoted by shaded boxes, and indicated by X's, is selected by selecting for Leu^{+} transformants. Result is SWI1 sequences replaced by swi1Δ::LEU2.

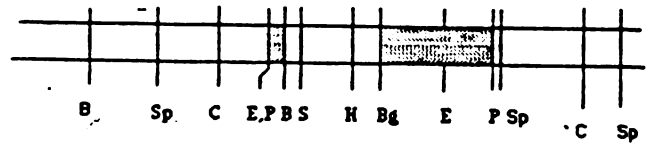
6.

p42a, Pst I digested





SWI1 in chromosome



Select Leu$^+$

swi△:LEU2 in chromosome

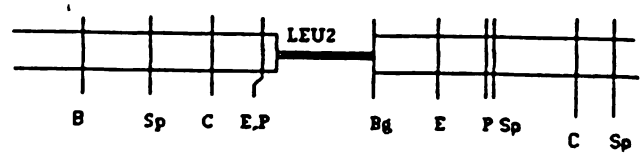
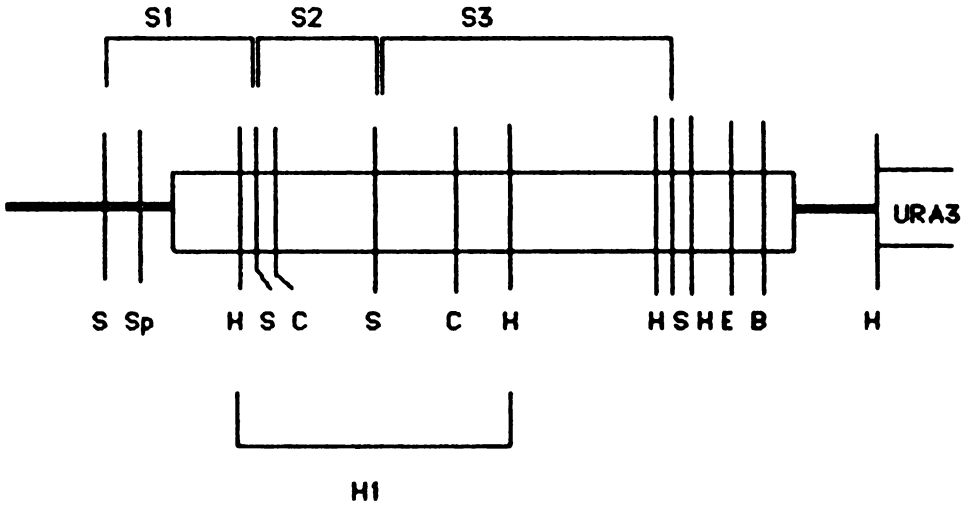


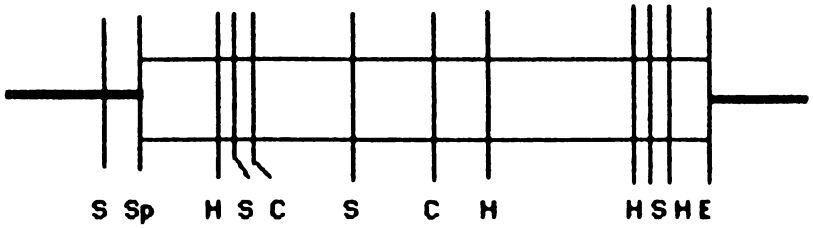
Figure 7. Restriction map of SWI5 clones. Abbreviations for restriction enzymes as in legend to Figure 4. Plasmid representations are as in legend to Figure 4. a) plasmid ee5, original isolate of SWI5. b) 8.5 kb EcoRI-SphI fragment containing most of insert of ee5 is subcloned into YCp50. This plasmid can complement swi5-1. c) same as b), except that a ClaI fragment containing 2.6 kb of insert DNA is deleted. This plasmid cannot complement swi5-1.

7.

a ee5 original SWI5 isolate



b 26d - YCp50-SWI5



c 26d △ Cla

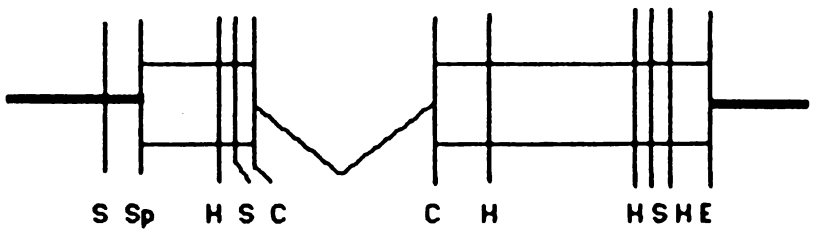
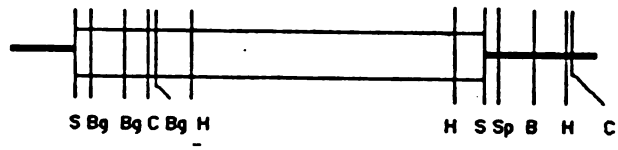


Figure 8. Zapper integrations determine a restriction fragment internal to SWI5. Abbreviations for restriction enzymes as in legend to Figure 5. Plasmid representations are as in Figure 4. Each insert is present in YIp5. Each plasmid described was targeted to integrate at SWI5 by cutting with BglII. Then, transformants were analyzed for genotype at SWI5. If integration could produce a swi5 mutation, then the insert is internal to SWI5. a) 5.0 kb SalI fragment (labelled S3 in Figure 7a) is inserted in YIp5 in the same orientation relative to vector as in ee5. This plasmid does not produce a swi5 mutation following integration into SWI5; therefore, at least one insert endpoint lies outside SWI5. b) same as a), except that HindIII fragments containing 3.0 kb of insert is deleted. This plasmid does not produce a swi5 mutation following integration into SWI5; therefore, at least one insert endpoint lies outside SWI5. c) same as a) except that a ClaI fragment containing 4.0 kb of insert is deleted. This plasmid produces a swi5 mutation following integration into SWI5; therefore, both insert endpoints lie within SWI5. d) same as a), except that insert is in opposite orientation, and a ClaI fragment containing 1.0 kb of insert is deleted. This plasmid does not produce a swi5 mutation following integration into SWI5; therefore, at least one insert endpoint lies within SWI5.

8.

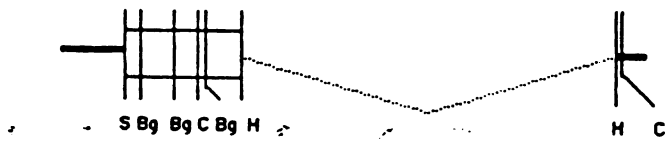
SWI5$^+$ following plasmid integration?

a. p40e



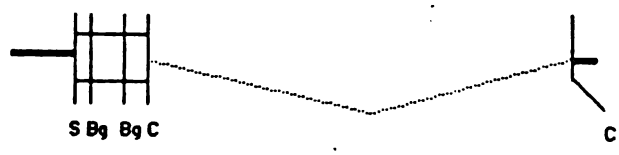
yes

b. p40eΔH3



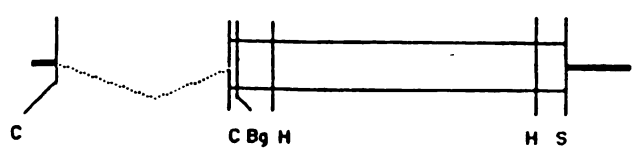
yes

c. p40eΔCla



no

d. p40jΔCla

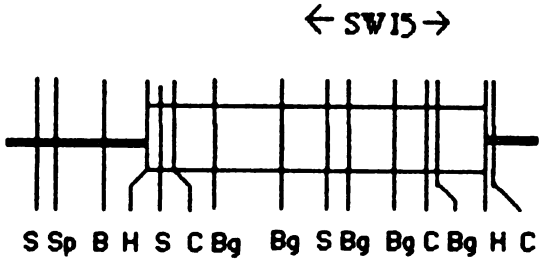


yes

Figure 9. Restriction map of SWI5 and swi5Δ::LEU2 construct. Plasmid representations as in legend to figures 4 and 6. a) plasmid p47a contains a 3.3 kb HindIII fragment (labelled H1 in figure 7a) in YCp50. p47a complements swi5-1. The minimum extent of SWI5, as determined in figure 8, is indicated. b) swi5Δ::LEU2, a replacement of 4 BglII fragments totalling 2.5 kb with the 2.6 kb BglII fragment containing LEU2, is shown. This construct was used to replace wildtype SWI5, as shown in figure 6. Due to technical reasons, the swi5Δ::LEU2 construction was made in ee5, but shown here as it would appear in p47a for simplicity.

9.

a p47a - SWI5 in YCp50



b swi5△:LEU2

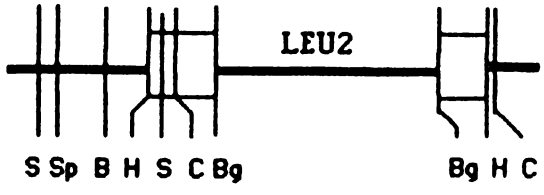


Figure 10. SWI5 does not require any SWI gene for transcription. Total RNA was isolated from the following strains: swi1 (671-E2), swi2 (671-B2), swi3 (671-E1), swi4 (671-C10) and swi5 (671-I2), and SWI (671-1b, MATa, and isogenic to each swi mutant analyzed. All swi mutants are MATα. 15 - 30 μg of RNA from each strain was fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with radioactively labelled p44f plasmid DNA (see Materials and Methods). Positions of URA3 and SWI5 are indicated.

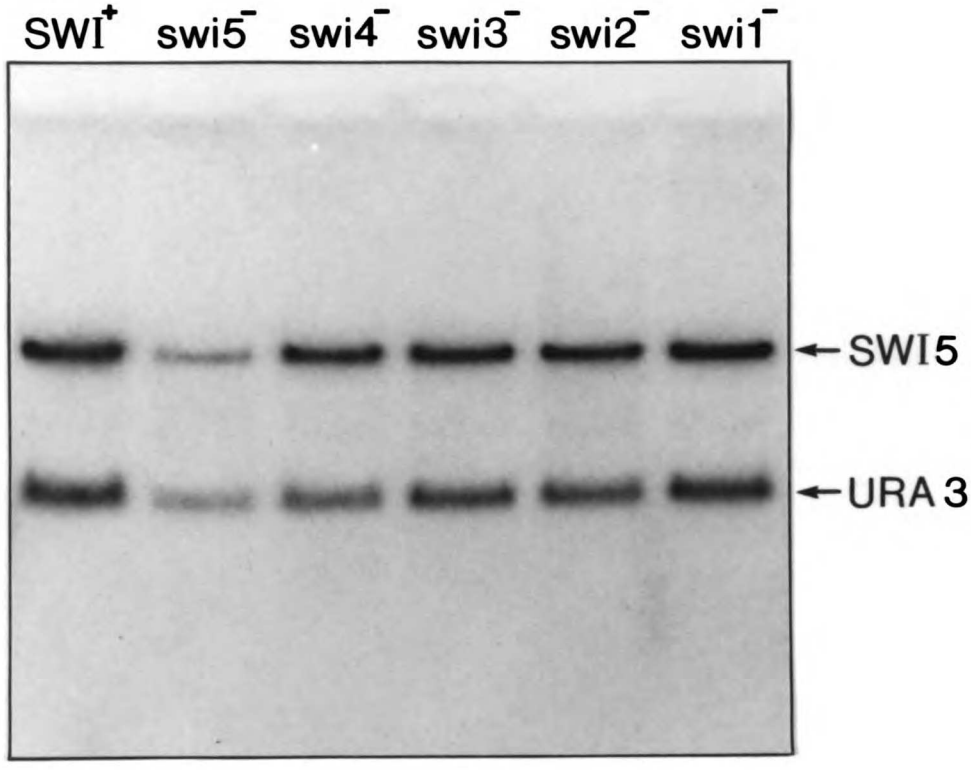

SWI+
swi5-
swi4-
swi3-
swi2-
swi1-
SWI5
URA3

Figure 11. SWI1 does not require any SWI gene for transcription. This blot is identical to that shown in figure 10, except that probe was obtained from plasmid p27d. The position of URA3 is shown. From this blot, it is not possible to determine which of the three higher molecular weight transcripts visualized correcponds to SWI1; however, each is present in equal intensities in each strain.

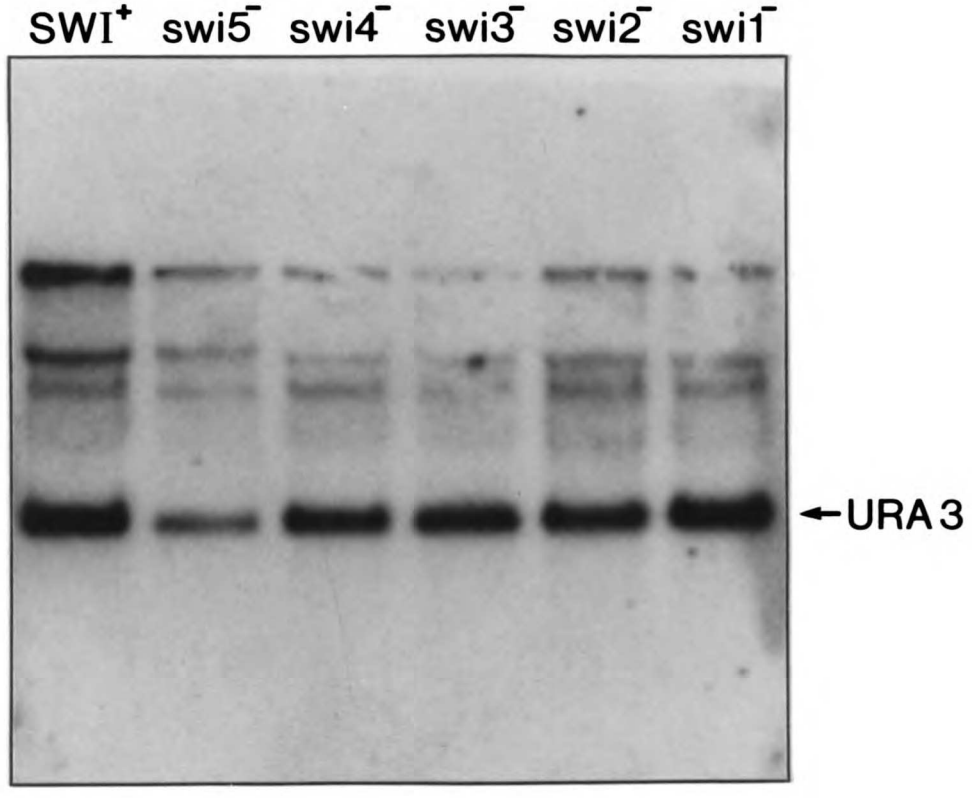
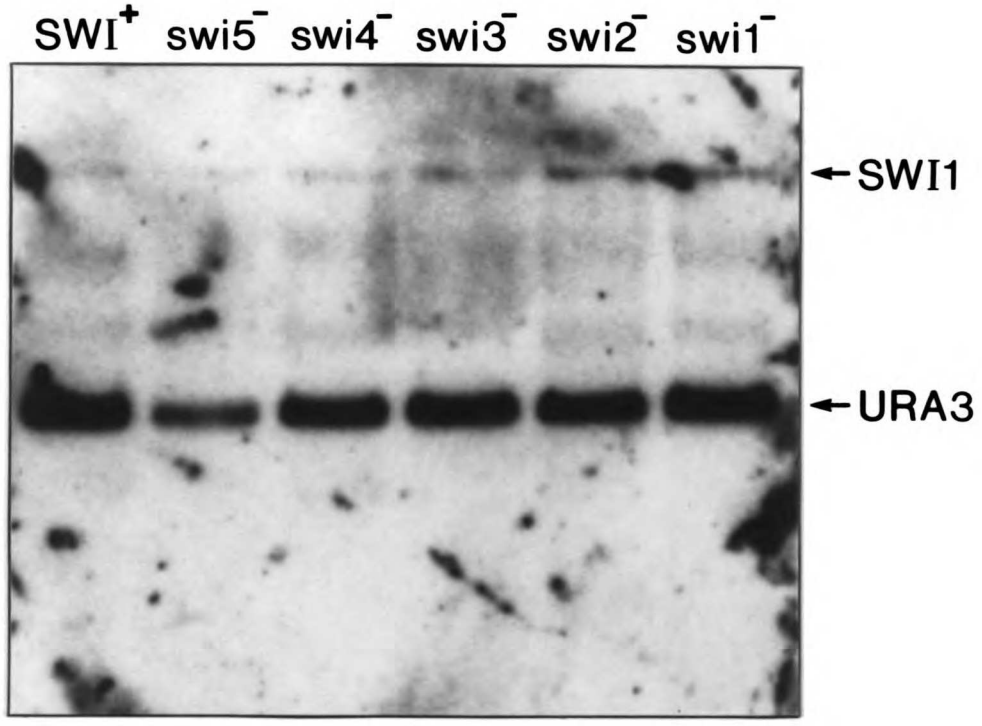

SWI+
swi5-
swi4-
swi3-
swi2-
swi1-
←URA3

Figure 12. The 4 kb transcript probably corresponds to SWI1 message. This blot is identical to that shown in figure 10 except that probe was obtained from p29a (p29a contains an internal fragment of SWI1, neighboring the insert of p27d). The position of URA3 is indicated. Only the highest molecular weight transcript from figure 11 is visualized; the two broad hybridizing bands co-migrate with ribosomal RNA and are visualized here because of the extremely long exposure required to visualize the SWI1 transcript.


SWI+
swi5-
swi4-
swi3-
swi2-
swi1-
SWI1
URA3

IV. Mutations that bypass the requirement of SWI genes for HO expression

1. Introduction

As described in Chapter II, the expression of the HO gene requires an additional five genes unlinked to HO, SWI1-SWI5. The SWI genes are formally activators of HO expression. Rather than act directly on HO, however, the SWI genes might activate HO indirectly, by inhibiting an inhibitor of HO expression. The experiments described thus far would not have detected genes that inhibit HO expression. These genes could be identified by mutations that suppress the defect in HO expression conferred by swi mutations if the suppressor mutations are epistatic to any swi mutation.

In addition to identifying a new set of genes that acts on HO, suppressor mutations might provide information on which aspect, if any, of HO transcription is controlled by the SWI genes. As described in the Introduction, HO transcription is controlled in three ways: the HO transcript is present only in a and α cells but not in a/α cells, only in mother cells but not in daughter cells, and only in the G_1 phase of the cell cycle. Because the SWI genes activate transcription of HO, modulation of HO transcription could result from modulation of activity of a SWI gene product. For example, cell type control of HO transcription might result because SWI activity is high in a and α cells but low in a/α cells. Similarly, mother/daughter and cell cycle control of HO transcription could result from modulation of the activity of a SWI product. Suppressor mutations that allow expression of HO that is independent of a particular SWI gene might make it possible to determine which type of control is mediated by the SWI gene. In a swiΔ strain that can express HO due to the presence of a suppressor, HO expression

should become constitutive for the type of control mediated by the SWI gene, as the activity of the SWI gene (being zero) cannot be varied. The maintenance of a particular type of transcriptional control in such a strain demonstrates that the SWI gene is not required for that type of control.

In this chapter, I describe the isolation of mutants in which the defect of HO expression conferred by swi mutations is suppressed. 18 recessive suppressors of swi1Δ define two complementation groups, both of which suppress swi5Δ as well. In both swi1Δ and swi5Δ strains that carry either suppressor, HO expression is fully repressed in a/α cells, demonstrating that neither SWI1 nor SWI5 is required for cell type control.

2. Materials and Methods

Genetic techniques

Media, matings, sporulations, testing genotypes at SWI and HO were accomplished as described in Chapters II and III. Strains used in this chapter are listed in Chapters II and III, or in Tables 12 and 14. In addition, 30 suppressors of swi5-1 were isolated from D142-3a (MATα HO-lacZ swi5-1 ura3-52 leu2$^{=}$ his met).

Other techniques

β-galactosidase activities were measured as described in chapter III. All strains assayed are isogenic to D117-13a (HO-lacZ ura3-52

leu2$^{=}$, listed in Table 8). Transformations into yeast were accomplished by the lithium method described in Ito et al (1983) as modified by Fink (personal communication). EMS (ethyl methane sulfonate) was used to mutagenize strains. Approximate frequency of survivors was 30% for BDY12a-1b (swi1Δ, Table 8), 15% for D142-3a (swi5-1, Table 8) and 30% for D212-5d (swi4-3, Table8). Efficiency of mutagenesis was assayed in BDY12a-1b (0.05%) and D142-3a (0.1%) by the frequency of appearance of red colonies (ade1 and ade2 mutations) and in D212-5d (0.01%) by the frequency of appearance of canavanine resistant colonies. The apparent low efficiency of mutagenesis of D212-5d probably results from the clumpiness conferred by the swi4-3 mutation.

3. RESULTS

Mutant Isolation Strategy

swi mutants that carry a fusion of the E. coli lacZ gene to HO formed white colonies on XG because such strains fail to express HO. In order to isolate second site revertants that express HO-lacZ, swi mutants were mutagenized and screened for colonies that formed blue colonies on XG. We assumed that any second site mutation able to suppress the swi1Δ mutation would also suppress swi2-1 and swi3-1. Therefore, second site revertants were sought from only three swi mutants: a swi1Δ HO-lacZ strain (BDY12a-1b, isogenic to D117-13a), a swi4-3 HO-lacZ strain (D212-5d) and a swi5-1 HO-lacZ strain (D142-3a). The efficiency of mutagenesis was estimated as described in Materials and Methods. 30,000 colonies from each strain were screened; 30 blue

colonies from D142-3a, 18 blue colonies from BDY12a-1b, and four blue colonies from D212-5d were obtained.

Dominance/recessivity Tests

The suppressor mutations obtained were analyzed in the following way to determine if the mutation was dominant, cis-dominant (such as Ty1 insertions at HO, see Errede et al, 1980), or recessive. Each suppressor mutant obtained from BDY12a-1b was mated to a cry1-3 MATa HO swi1-2 strain (D149-10a). The resultant diploid, of genotype swi1Δ/swi1-2 cry1-3 MATa/CRY1 MATα HO/HO-lacZ SUP^{+}/sup^{-}, was rendered homozygous for MATa by selection for colonies resistant to cryptopleurine (see Materials and Methods, chapter II). Three cryptopleurine-resistant colony phenotypes were possible depending on the nature of the suppressor mutation. 1) If the suppressor mutant carried a dominant suppressor mutation, the expression of HO and thus mating type switching could take place in the diploid described above. Cryptopleurine-resistant colonies from such a diploid would not mate but would be capable of sporulation. 2) If the suppressor mutant carried a cis-dominant suppressor mutation, the expression of HO-lacZ but not HO could take place in the diploid described above. Cryptopleurine resistant colonies from such a diploid would mate as a and form blue colonies on XG. 3) If the suppressor mutant carried a recessive suppressor mutation, neither the expression of HO nor HO-lacZ could take place in the diploid described above. Cryptopleurine resistant colonies from such a diploid would mate as a and form white colonies on XG. Suppressor mutations obtained from D212-5d and from D142-3a were analyzed in a similar way, except that

suppressor mutants obtained from D212-5d were mated to a cry1-3 MATa HO swi4-1 strain (D45-1b), and suppressor mutants obtained from D142-3a were mated to a cry1-3 MATa swi5-1 HO-lacZ strain (D260-9a). In this case it was not possible to distinguish dominant from cis-dominant mutations. All suppressor mutations obtained from BDY12a-1b and D142-3a were recessive. One dominant and three recessive mutations were obtained from D212-5d.

Complementation Tests

Complementation tests among the eighteen mutations that suppress swi1Δ were performed essentially as described for swi mutations in chapter II. First, the suppressor mutation (designated sho1-1) present in mutant strain 1-1 was crossed (D262) to a cry1-3 MATa HO ura4 URA3 SWI1 SHO1 strain (671-1b). The sho1-1 mutation was recovered in a cry1-3 MATa swi1Δ segregant; this segregant was mated back to each swi1Δ suppressor mutant and cryptopleurine-resistant colonies were selected. These colonies were tested for mating ability and for ability to express HO-lacZ. Only the mutations in mutants 1-13 (carrying a mutation designated sho2-1) and 1-15 were able to complement sho1-1. Thus, sho1-1 and the mutations in the fifteen other suppressor mutants define one complementation group. The mutations in strains 1-13 and 1-15 define at least one other complementation group (SHO2). Complementation between sho2-1 and the mutation in 1-15 has not been analyzed.

sho1-1 Segregates 2:2

Because a/α swi1Δ strains do not sporulate, examining the segregation of sho1-1 required the following procedure: the SWI1 gene (plasmid e1, described in Chapter III) was introduced into the sho1-1 original isolate, strain 1-1. Following introduction of cloned SWI1, 1-1 was crossed (D287) to an isogenic MATa HO-lacZ swi1Δ SHO1 strain (BDY12a-5c). Spores from D287 were grown up non-selectively, and segregants were obtained that had lost the plasmid. In seven tetrads from which four viable spores were obtained, Sho^-:Sho^+ segregated 2:2, as assayed by white:blue colonies on XG medium.

With the same method, it was shown (P. Sternberg, personal communication) that sho2-1 (obtained from suppressor mutant 1-13) also segregates 2:2.

sho1-1 does not suppress other swi1Δ phenotypes

The sho1-1 mutation suppresses the defect in HO expression conferred by swi1Δ. However, the other swi1Δ phenotypes are not suppressed. swi1Δ sho1-1 strains grow no faster on YEPD and only marginally faster on cryptopleurine medium than do isogenic swi1Δ SHO1 strains. In addition, an a/α swi1Δ/swi1-1 sho1-1/sho1-1 (constructed by mating D262-1a to strain 1-1 as described above) fails to sporulate. Finally, sho1-1 does not appear to suppress the lethality of swi1Δ swi4-3. When strain 1-1 was crossed to a swi4-3 strain (D212-6c), no swi1Δ swi4-3 segregants were obtained from 27 spore clones analyzed. These experiments indicate that sho1-1 does not act by restoring SWI1

function, but rather by bypassing the requirement for SWI1.

sho1-1 suppresses swi2-1, swi3-1 and swi5Δ

sho1-1 was selected to be epistatic to swi1Δ. To determine whether sho1-1 is also epistatic to swi5Δ, and thus allows bypass of SWI5 control of HO expression as well, strain 1-1 (MATα swi1Δ sho1-1 HO-lacZ) was crossed (D275) to isogenic strain D246-2b (MATa swi5Δ HO-lacZ). β-galactosidase activities of five swi5Δ segregants were measured; two of these five produced 1.9 units of β-galactosidase, an amount equal to about 40% of an isogenic SWI SHO strain and the same value as produced by a swi1Δ sho1-1. Therefore, sho1-1 suppresses swi5Δ as effectively as swi1Δ.

To determine if sho1-1 suppressed swi2-1 and swi3-1, MATa HO-lacZ sho1-1 swi1Δ strain D287-1c was crossed (D296) to D148-6d (MATα HO swi2-1), and MATα HO-lacZ swi1Δ sho1-1 strain D287-3b was crossed (D297) to D117-5b (MATa HO-lacZ swi3-1). Ability of sho1-1 to suppress swi2-1 and swi3-1 was assessed by color on XG medium. In D296, three of six swi2-1 HO-lacZ formed blue colonies on XG, and in D297, 8 of 14 swi3 segregants formed blue colonies on XG. Therefore, sho1-1 suppresses swi2-1 and swi3-1 as well as swi1Δ and swi5Δ

4. DISCUSSION

The experiments described in this chapter identify two additional genes, SHO1 and SHO2, that affect the transcription of HO. Because sho1-1, isolated in swi1Δ background, also suppresses swi2, swi3 and

swi5 mutations, sho1-1 might allow complete bypass of SWI control of HO transcription. sho2-1, also isolated in swi1Δ background, suppresses swi5Δ, but its ability to suppress other swi mutations has not been tested.

sho1 and sho2 mutations in a swi1Δ background restore HO expression to approximately the same level as is found in a SWI strain (see Table 14). It is not known if any of the mutations analyzed are null, so it is not known what the level of HO expression in sho1 or sho2 deletion mutants would be. Furthermore, except for sho1-1 and sho2-1, it has not been demonstrated that the suppressor phenotype is due to a single mutation, so the levels of HO expression in the suppressor mutants could be due to multiple events. sho1 and sho2 null mutations can probably be obtained most easily by cloning SHO1 and SHO2 and constructing deletion mutations in vitro.

The suppressor mutants were obtained from three separate swi strains. We were interested in determining if the suppressor mutations obtained from each swi mutant would lie in the same genes. If so, then each suppressor mutation identified would suppress every swi mutation. Another possibility is that some suppressor mutations would act specifically on particular swi mutations. Although the complementation tests have not been completed, alleles of the two complementation groups identified, SHO1 and SHO2, suppress mutations in all other SWI genes tested. However, the ability of these mutations to suppress swi4 mutations has not been studied adequately, due to difficulties in assaying β-galactosidase in swi4 mutants. Because mutations in SWI5, thought to act more directly on HO than the other SWI genes, are suppressed by sho1-1 and sho2-1, it seems likely that SHO1 and SHO2

act downstream of all SWI genes. Consistent with downstream action, sho1 and sho2 mutations do not suppress any phenotype conferred by swi mutations other than the defect in HO expression.

Although SHO1 and SHO2 function, in a formal sense, downstream of the SWI genes to repress HO transcription, the nature of these genes is unknown. An interesting possibility is that SHO1 and SHO2 are directly concerned with HO expression. For example, either SHO1 or SHO2 might encode a DNA binding protein that specifically represses transcription of HO. In this view, the SWI genes function to inhibit the activity of SHO1 and SHO2, perhaps in a mother-cell, haploid, or G_1 specific fashion. A less interesting possibility is that SHO1 and SHO2 are not directly concerned with HO transcription. For example, sho1 or sho2 mutations might cause the activation of a cryptic UAS near HO, thus allowing bypass of the requirement of HO for the SWI genes. At the present time, these two possibilities cannot be distinguished. However, the first possibility requires that the SWI genes and SHO1 and SHO2 act on the same DNA sequences upstream of HO, whereas if SHO1 and SHO2 act by repressing a cryptic UAS near HO, it is likely that the SWI genes will act on a different DNA sequence from SHO1 and SHO2.

As described in the Introduction to this chapter, regardless of the nature of SHO1 and SHO2, information can be obtained regarding the type of transcriptional control of HO that is mediated by SWI genes. In particular, if a particular type of transcriptional regulation is retained in a suppressed swi mutant, then it is concluded that the SWI gene is not required for that particular type of control. It has recently been found that HO transcription in swi1 or swi5 mutants carrying sho1-1 is still fully repressed in a/α cells (P. Sternberg,

personal communication). Thus, SWI1 and SWI5 do not mediate cell type control. Similar experiments involving cell cycle and mother/daughter control are in progress.

Table 12: Strain list

Strain	Genotype
D45-1b	cry1-3 MATa HO swi4-1 ura4 ade5 met
D212-6c	MATa HO-lacZ ura3-52 leu2$^{=}$ ade5 swi4-3 met his
D260-9a	cry1-3 MATa HO ura4 swi5-1 met his
D261-12d	cry1-3 MATa HO-lacZ swi4-3 ura4 met his
D262-1a	cry1-3 MATa HO swi1Δ ura4 met his
D262-9c	cry1-3 MATa HO ura4 met his
D275-7a	MATα swi5Δ sho1-1, isogenic to D117-13a
D287-1c	MATa swi1Δ sho1-1, " " "
D287-3b	MATα swi1Δ sho1-1, " " "
4-1	isogenic to D212-5d, SUP4-1
4-3	" " " sup4-3
4-4	" " " sup4-4
4-5	" " " sup4-5

Table 13. Cross list

Diploid	Parents
D260	671-1b X D142-3a
D261	671-1b X D212-5d
D262	671-1b X BDY12a-1b
D271	D262-9c X 1-1
D275	D246-2b X 1-1
D282	D246-2b X 1-13
D287	BDY12a-1b X 1-1 carrying e1

Table 14. Expression of HO-lacZ in suppressors of swi1Δ, and comparison of expression of HO-lacZ in SWI, swi1Δ and swi5Δ carrying sho1-1

Strain	β-galactosidase activity	Strain	β-galactosidase activity
BDY12a-1b	0.1	1-9	1.7
BDY12a-5c	1.0	1-10	1.8
1-1	0.4	1-12	0.7
1-2	1.8	1-13	0.4
1-3	1.2	1-15	0.4
1-4	1.3	1-16	0.2
1-5	1.5	1-17	0.5
1-6	1.4	1-19	1.6
1-7	1.5	1-22	1.6
D275-4b (Swi^+)	1.2	D275-7a (swi5Δ)	0.4

Table 14: Assays were accomplished as described in Materials and Methods. A background value provided by D246 (isogenic to all strains assayed, but a/α and therefore not expressing HO-lacZ) was subtracted from all samples. The values listed are normalized to BDY12a-5c, SWI1 SWI5 SHO1.

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Appendix A: Cloning of *SWI2*

Methods

All techniques were performed as described in Chapter III. Yeast strains used: D148-6d (*MATα HMRα HMLα HO swi2-1 ura3-52 leu2*$^{=}$ *ade5 his met*), A24-8a, A24-11b (Chapter II), D117-13a, D117-14c (Chapter III). *E. coli* strain MH1 was described in Chapter III. Plasmids used: g2 (YEp24-SWI2, see Figure 13); p13a, 8 kb SphI fragment of g2 in YCp50, in same orientation as in g2; p14e, 4 kb EcoRI fragment of g2 in YCp50, opposite orientation as in g2; p22d, 4 kb SphI-ClaI fragment of g2 in YCp50; p33j, 4 kb HindIII fragment of g2 in YIp5.

Plasmid g2 was one of seven plasmids that could relieve the growth defect of D148-6d on 30 μg/ml leucine, and one of two plasmids that could restore cassette switching to D148-6d. The insert of the second plasmid, h2, appeared to contain all of the insert of g2 and was not studied further.

Results

Plasmid g2, obtained from a yeast clone bank in YEp24 (Carlson and Botstein, 1982) was identified by its ability to restore both growth on 30μg/ml leucine and cassette switching to D148-6d (*MATα HMRα HO swi2-1 ura3-52 leu2*$^{=}$) as described in Chapter III. The restriction map of g2 is shown in Figure 13. Two lines of evidence demonstrate that g2 contains *SWI2* DNA: 1) an 8 kb SphI fragment containing most of the insert of g2 was able to complement *swi2-1* following subcloning in

YCp50. 2) plasmid p33j, a YIp5 derivative containing the 4 kb HindIII fragment from the insert of g2, directed plasmid integration to SWI2 following subcloning into YIp5. p33j was cut with KpnI and transformed into D117-13a (MATa HO-lacZ ura3-52) and D117-14c (MATα HO-lacZ ura3-52). One transformant from D117-13a was crossed to A24-11b (MATα HO swi2-1 ura3-52, Chapter II) and one transformant from D117-14c was crossed to A24-8b (MATa HO swi2-1 ura3-52). In 22 tetrads analyzed, Ura^+:Ura^- segregated 2:2 and Ura^+ Swi^+: Ura^- Swi^- showed 22 parental ditype tetrads. This result demonstrates that the insert of g2 can direct integration to the SWI2 locus.

In order to assay SWI2 RNA in swi strains, it was first necessary to locate a small restriction fragment likely to carry at least part of SWI2. The following observation suggests that the HindIII fragment labelled H1 (Figure 13) should hybridize to SWI2 transcript: two restriction fragments subcloned into YCp50, p14e (containing 4 kb EcoRI fragment) and p22d (containing 4 kb SphI-ClaI fragment), while not able to complement swi2-1, were able to convert chromosomal swi2-1 to SWI2 (procedure detailed in Chapter III), suggesting that the inserts in both plasmids carry the wildtype allele of swi2-1. Therefore, the YIp5 derivative plasmid p33j, which carries fragment H1, carries the wildtype allele of swi2-1 as well. Thus fragment H1, although not internal to SWI2, ought to hybridize to SWI2 transcript and could be used as probe for SWI2.

In order to determine if SWI2 transcription requires other SWI genes, RNA was prepared from each swi mutant and isogenic Swi^+ strain 671-1b (as described in Figure 14) and hybridized to plasmid p33j. Figure 14 shows that p33j visualizes three transcripts: URA3 and two

high molecular weight transcripts, the minor species running slightly faster than the major species. Each transcript is present in equal amounts in the six strains analyzed. Assuming that one (or both) of the transcripts corresponds to SWI2, it is concluded that SWI2 does not require any SWI gene for transcription.

Figure 13. Restriction map of g2, a YEp24 derivative carrying SWI2. Abbreviations for restriction enzymes as in legend to Figures 4 and 5, Chapter III. In addition, Xb=XbaI, Xh=XhoI, and K=KpnI. Plasmid representations are as in Figure 4. The EcoRI-ClaI fragment labelled EC1 was shown by marker rescue experiments (see text) to contain the wildtype allele of swi2-1. The HindIII fragment labelled H1 was subcloned into YIp5 (forming plasmid p33j) and used as probe for SWI2 message.

13.

g2 - SWI2 original isolate

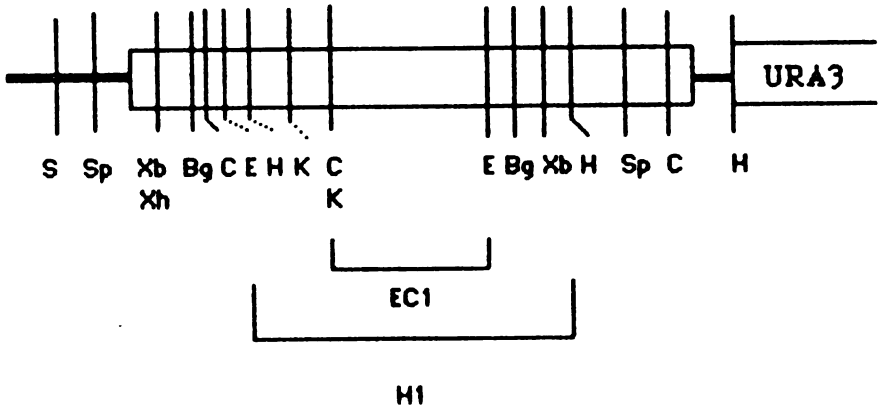
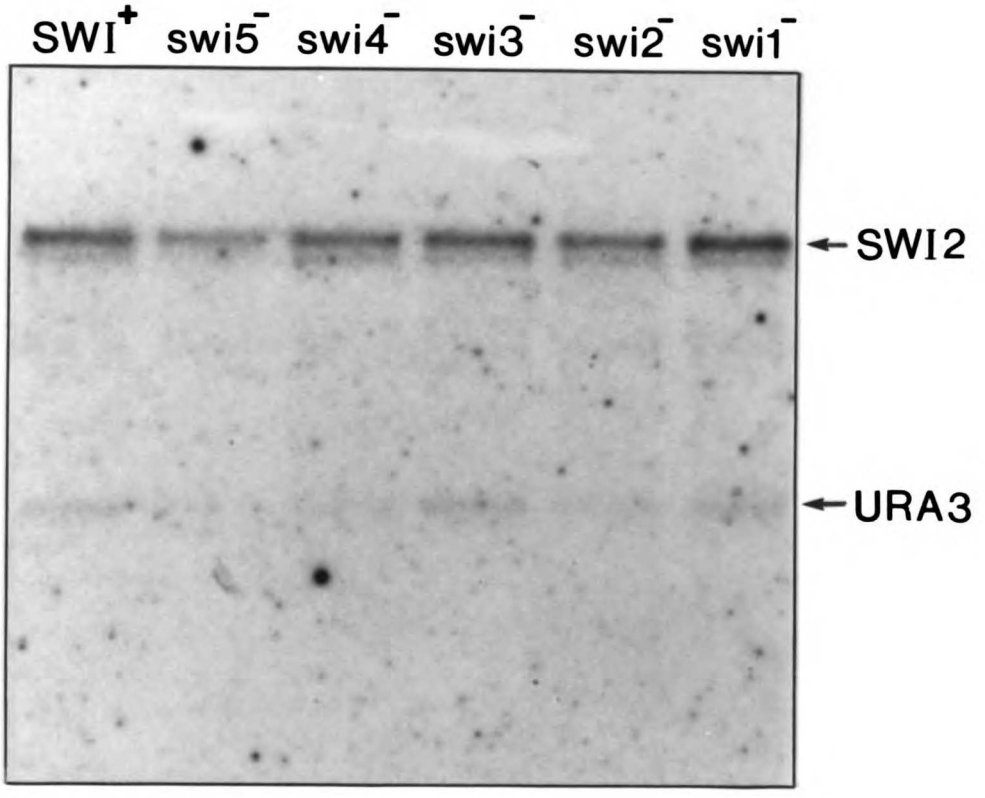


Figure 14: SWI2 does not require any SWI genes for transcription. This blot is identical to those shown in Figures 10,11 and 12, except RNA was hybridized to plasmid p33j (SWI2 probe). The positions of URA3 and SWI2 are shown. It is not possible to determine which high molecular weight transcript corresponds to SWI2; however, each is present in equal intensities in each strain.


SWI+
swi5-
swi4-
swi3-
swi2-
swi1-
SWI2
URA3

Appendix B: Preliminary genetic mapping of SWI2 and SWI5

Strains, plasmids and crosses

Strains: D117-13a-a or D117-13a-α (HO-lacZ ura3-52 his4), D117-14c (HO-lacZ MATα ura3-52) GK1 (MATa cdc6 his2 his6 his7 ade1 ade2 ade6 lys7 lys9 leu1 leu2 ura3 trp1 met2 met14 aro7 arg1 arg4 ilv3 asp5 gal1 mal suc), FW516 (his4-912δ ura3-52 spt3-101) RB1 (MATα rna3 ura thr arg), D246-2a (isogenic to D117-13a-α, carrying ee5 integrated at SWI5), BDY8 (isogenic to D117-14c, carrying ee5 integrated at SWI5), D277-4c (MATα ura3-52 leu2 arg thr rna3), 977 (MATα rad55 trp1) BDY18 (D117-14c derivative carrying g2 integrated at SWI2) D302-5d (MATa rad55 ura3-52), BDY19a and BDY12α (isogenic to D117-13a-a and D117-13a-α, carrying ee5 at SWI5), 950 (MATa his3del1, trp1 ura3 htb2-1), and MH52-3c (matΔ trp1 his3 his4 leu2$^{=}$ ura3-52, carrying CV13) Crosses: D277 (RB1 X BDY19) and D302 (FW516 X 977). Plasmids: ee5 (Chapter III and g2 (Appendix A).

The 2μ mapping method

The SWI2 and SWI5 genes were mapped to chromosome arms XVR and IVR, respectively, with the 2μ mapping method (Falco et al, 1983; Falco and Botstein, 1983). As with most other mapping methods in yeast, use of the 2μ technique maps the gene of interest to a chromosome arm. The procedure utilizes the observation that integration of a plasmid carrying the origin of replication of the 2μ plasmid destabilizes the chromosome on which integration has occurred. The plasmid marker is

lost at high frequency (see Falco and Botstein, 1983, for a model to account for this observation); loss of plasmid marker is associated at high frequency (about 95%) with loss of all markers centromere distal to the site of integration. At lower frequency, loss of plasmid marker is associated with loss of centromere proximal markers; occasionally, loss of all markers on the chromosome occurs as well. Loss of markers is generally monitored in a diploid by uncovering of recessive mutations on the homologous chromosome to which the plasmid has integrated.

To map a gene of interest with this technique, it is first necessary to obtain a plasmid carrying both the gene and the 2μ origin of replication. Because YEp24 carries the 2μ origin and because I obtained both the SWI2 and SWI5 genes in YEp24, I was able to use the SWI2 and SWI5 original isolates, plasmids g2 and ee5, respectively, to map the two genes. After the proper plasmid is obtained, its integration at the locus of the gene of interest is targeted. In the experiments described here, targeting was accomplished with the method of Orr-Weaver et al (1981). A transformant is then mated to a mapping strain carrying at least one recessive mutation on each chromosome. The strain that I used for mapping is strain GK1 (provided by G. Kawasaki, see Methods section for genotype). Following mating, diploids are selected and restreaked for single colonies under permissive conditions for all the phenotypes exhibited by the GK1 parent. Following restreaking, cells from single colonies are diluted and plated (about 500 cells per plate) under permissive conditions. Single colonies are then monitored for loss of plasmid marker. Because GK1 carries ura3, loss of the YEp24 marker can be monitored by acquisition of Ura^- phenotype in the diploid. Colonies that have lost plasmid marker are then screened for expression of any

recessive marker present in GK1.

Following localization of the gene to a chromosome, the 2μ technique can be used to locate the gene more precisely on a chromosome arm. More precise localization can be accomplished because, as described above, loss of plasmid marker is associated at very high frequency with loss of centromere distal markers, but at lower frequency with centromere proximal markers. Therefore, strains carrying integrated 2μ plasmids can be mated to strains carrying markers located all along the chromosome of interest.

Results

To map SWI5, plasmid ee5 (see Figure 7a for restriction map) was integrated into D117-14c (MATα HO-lacZ ura3-52) and D117-13a-a (MATa HO-lacZ ura3-52). Transformants of both strains were crossed to ura3-52 swi5-1 strains, and it was found that in both strains, ee5 had integrated at SWI5 (Ura^+ Swi^+: Ura^- Swi^- showed eight parental ditype tetrads of nine tested, and eight parental ditype tetrads of eight tested, respectively). The transformant from D117-14c was mated to GK1 and diploids were selected. Four independent diploids were restreaked for single colonies and replated on YEPD at 23° (as described in "2μ mapping method", above). Four of the 220 Ura^- colonies obtained had become Trp^- (see Table 15). Therefore, SWI5 lies on chromosome IV.

To determine if SWI5 lay on the left or right arm of chromosome IV, I screened to see if the Trp^- diploids had also lost the HO-lacZ marker present at the distal end of the left arm of chromosome IV. Because the a/α diploids do not express HO-lacZ, it was necessary to select mitotic

recombinants at MAT. This selection was accomplished by rare-mating the four Trp$^-$ diploids to matΔ strain MH52-3c (received from M. Hall). The prototrophs obtained from such a mating are α/α/Δ triploids; these can express HO-lacZ. Because only one of the four Trp$^-$ diploids gave rise to triploids that formed blue colonies on XG, it was concluded that SWI5 lies on the right arm of chromosome IV.

To localize SWI5 more precisely on IVR, transformants carrying ee5 from either D117-14c or D117-13a were mated to strains carrying the following chromosome IV markers: rna3 (D277-4c), spt3 (FW516, provided by F. Winston), and rad55 (D302-5d). In each case, diploids were selected and Ura$^-$ colonies obtained as described. The frequency of Ura$^-$ diploids that had lost the wildtype allele of the marker indicated is described in Table 15. Because loss of URA3 is associated at high frequency with loss of RNA3 and SPT3, whereas loss of URA3 is associated at lower frequency with loss of RAD55, SWI5 probably lies between RAD55 and SPT3, a genetic distance of 200 cM.

To map SWI2, plasmid g2 (see Appendix A) was introduced into strain D117-14c. One transformant from this integration, strain BDY18, was crossed to a ura3-52 swi2-1 strain, and Ura$^+$ Swi$^+$: Ura$^-$ Swi$^-$ showed seven parental ditype tetrads of seven tetrads tested, indicating that g2 had integrated at SWI2. This transformant was mated to GK1. Following non-selective growth on YEPD, these diploids formed red colonies and red sectors at high frequency, indicating loss of ADE1 or ADE2. Four red diploids were subjected to complementation tests versus ade1 and ade2 tester strains; the ade mutation of these diploids complemented ade1, but not ade2, mutations, indicated that g2 was causing loss of ADE2, on the right arm of chromosome XV. As shown in Table 15, of 70

Ura$^-$ colonies analyzed, 17 had become Ade$^-$. Because the ARG1 allele, on the left arm of chromosome XV, was not being lost (zero Arg$^-$ colonies of the 70 Ura$^-$ colonies), SWI2 lies on the right arm of chromosome XV, centromere distasl to ADE2.

To see if SWI2 was centromere distal or proximal to HIS3, which is distal to ADE2 on chromosome XV, strain BDY18 was mated to strain 950 (MATa ura3 his3). Following selection of diploids and restreaking, 33 Ura$^-$ colonies were obtained and 19 of these had become His$^-$ as well (Table 15). This result indicates that SWI2 lies distal to HIS3.

Table 15. Mapping SWI5 and SWI2

Plasmid marker in all cases is URA3

Diploid	Marker uncovered	# Ura$^-$	# of uncovered mutations
1) BDY8 X GK1	TRP1	220	4
2) BDY19a X D277-4c	RNA3	60	57
3) FW516 X BDY8	SPT3	40	39
4) BDY19a X D302-5d	RAD55	60	14
5) BDY18 X GK1	ADE2	70	17
6) BDY18 X 950	HIS3	33	19

Legend to Table 15: For diploids 1 to 4, the plasmid integrated is ee5 (YEp24-SWI5). For diploids 5 and 6, the plasmid integrated is g2 (YEp24-SWI2). "Marker uncovered" lists the gene for which the wildtype allele was lost at high frequency. "# Ura$^-$" lists the number of colonies analyzed that had lost the URA3 marker. "# of uncovered mutations" lists the number of Ura$^-$ colonies that had also lost the wildtype allele of the gene listed under "marker uncovered". Loss of TRP1 was scored by acquisition of tryptophan auxotrophy, loss of RNA3 was scored by acquisition of growth defect at 36°, SPT3 was scored (in a diploid his4/his4-912δ) by acquisition of histidine prototrophy, loss of RAD55 was scored by acquisition of sensitivity to 0.01% methyl methane sulfonate (MMS), loss of ADE2 was scored by adenine auxotrophy and loss of HIS3 was scored by acquisition of histidine auxotrophy.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