Regulation of yeast mating-type interconversion: Feedback control of *HO* gene expression by the mating-type locus

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**Genetics**

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ABSTRACT The ultimate product of yeast mating-type interconversion is a stable a/a diploid cell. A haploid cell carrying the *HO* gene gives rise to a diploid cell in a two-step process: first, the cell switches mating type as a result of genetic rearrangement (cassette substitution) catalyzed by *HO*; then, cells of opposite type mate to form a/a diploids. Mating-type interconversion does not occur in a/a diploids despite the presence of the *HO* gene. We have identified a plasmid carrying the *HO* gene by screening a yeast closed bank (constructed in vector YEP13) for plasmids that allow mating-type switching by *ho* cells. The yeast segment responsible for mating-type interconversion integrates by homology at the *ho* locus, thus confirming that it carries *HO*. Using the *HO* gene as a probe, we find that strains with an active mating-type interconversion system produce *HO* RNA, whereas a/a *HO/HO* cells do not and that this inhibition requires products of both the *MATa1* and *MATa2* genes. Thus, mating-type interconversion does not occur in a/a HO/HO cells because the *HO* gene product is not synthesized. These results demonstrate the following: (i) The mating-type locus, proposed on genetic grounds to be a regulatory locus, controls expression of an unlinked gene (*HO*) at the level of RNA production. (ii) The *HO* gene is under negative feedback control: its expression is inhibited after successful completion of diploidization (*formation of a/a diploids*).

The *HO* locus of the yeast *Saccharomyces cerevisiae* governs the frequency of mating-type interconversion (ref. 1; reviewed in ref. 2). Strains carrying *HO* switch between *MATa* and *MATa* as often as every cell division, whereas strains carrying the recessive *ho* allele switch at much lower frequency. *HO* promotes a site-specific, unidirectional transposition of a cassette of genetic information from the *HML* and *HMR* loci (where the cassettes are silent) to the mating-type locus, where the a or a cassette is expressed and determines yeast cell type. As first described by Winge and Roberts (1), the *HO* gene promotes formation of diploid cells from haploids, a process termed "diploidization": haploid *MATa* or *MATa* cells carrying *HO* give rise to sibling cells of opposite mating type as a result of mating-type interconversion, and these cells then mate to form a/a cells. Despite being homozygous for *HO*, the resultant *MATa/MATa* cells remain *MATa/MATa* diploids. The final product of mating-type interconversion is thus a stable a/a diploid derived from a haploid a or a cell.

Mating-type interconversion is controlled by the mating-type locus: switching occurs in *MATa/MATa* and *MATa/MATa* diploids but not in *MATa/MATa* diploids (see ref. 3). Thus, stability of *MATa/MATa HO/HO* cells results not from diploidy per se but rather because of heterozygosity at the mating-type locus. More specifically, inhibition of mating-type interconversion in a/a cells requires the a1 function of *MATa* and the a2 function of *MATa: mata1/MATa* and *MATa/mata2* diploids carrying *HO* exhibit mating-type interconversion and switch cassettes at MAT until the genome contains both *MATa* and *MATa* (3, 4). a1 and a2 of course are also necessary for the more familiar properties of a/a cells—sporulation proficiency and inability to mate.

One explanation for the inability of a/a HO/HO cells to exhibit mating-type interconversion is that *HO* or some other function necessary for mating-type interconversion is not expressed in *MATa/MATa* cells. (A different type of hypothesis is discussed in ref. 3.) Genetic evidence indicates that the mating-type locus controls expression of unlinked genes (Fig. 1) (5-7). Recent studies (8) confirm this hypothesis for at least one gene (*STE3*) that is unlinked to *MAT* and that is required for mating: synthesis of *STE3* RNA occurs only in a cells (and not in a/a or a/a cells) and requires the a1 product of *MATa*. To determine whether *HO* expression is also controlled by the mating-type locus, we have cloned a genomic segment of yeast DNA that carries the *HO* gene and used it as a probe for expression of *HO* in cells that differ in their ability to switch mating-type cassettes. We find that *HO* RNA is not produced in *MATa/MATa HO/HO* cells. Thus, *HO* represents a class of genes that is expressed in haploid cells (or in diploids homozygous for *MATa*) but not in a/a diploid cells.

MATERIALS AND METHODS

Strains and Relevant Genetic Markers. C150-15d (ho *MATa* ste13-1 leu2-3 leu2-112; ref. 8), X10-1b (*MATa/MATa* diploid homozygous for *HO* HMLa HMRa; ref. 9), 3B54 (a UV-induced ste14 mutant derived from X10-1b; ref. 10), AB320 (*MATa/MATa HO/HO; ref. 11), SC3 (*MATa ho ura3-52; R. W. Davis' laboratory, obtained from T. Etcheverry), J12-1A (*MATa/MATa* diploid homozygous for *HO* ura3-1, obtained from J. Kurjan), 341 (*MATa/MATa* diploid homozygous for *HO* HMLa HMRa ura3), HR125-6d (*MATa leu2-3 leu2-112 ura3-52 ste13-1), HR112-1b (*HO* *MATa HMLa HMRa), HR100-1a (*HO* *MATa HMLa HMRa), and 523 (*a*/*a* *MATa* mitotic recombinant derived from *a/a MATa* diploid homozygous for *HO* HMLa HMRa) were constructed for this work by standard methods. HR100-1a and HR112-1b are very closely related to X10-1b: strains carrying the nonstandard alleles at HML or HMR (*HMLa*),

Abbreviations: bp, base pair(s); kb, kilobase(s); kbp, kilobase pair(s).

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* Genetic nomenclature. *MAT, HML, and HMR* are genetic loci that harbor either an a or an a1 cassette. Standard yeast strains carry an *a* cassette at *HML* (*HMLa*) and an a cassette at *HMR* (*HMRa*). *MATa1*, *MATa2*, and *MATa1* are genes that code for products a1, a2, and a1, respectively. Mutations in *MAT* are denoted as *mat*; for example, a mutation in *MATa1* is *matα1*. *Ho* indicates the *HO* gene product or its activity.

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Panels drawn sporulation and asg, a-specific indicates symbols strains were HR100-la XP8-18b strain and XT1172-S245c (matal/MATa G40 HO, ref. transformants of and tween were YEpL3 (13) endonuclease ments to Leu+ genes; is the then HO analyzed mapping obtained a-factor and asg, matal-2 SR2 (14). Ho').

Methods. The strain (12). Cloning and testing for the ability to mate as a by replica-plating with tester strain XR197b-la (matal HMLa HMRa sir1-1 leu2, which mates efficiently with a cells). Plasmid DNA was isolated from yeast as in ref. 11. Transformation of E. coli strains and subcloning were performed by standard methods.

Hybridization Analysis. Yeast DNA was isolated as follows (R. Elder, personal communication): 100-ml cultures of exponentially growing cells were harvested by centrifugation and washed with sterile H2O. Approximately 2 x 10^6 cells were suspended in lysis buffer (0.5 M NaCl/0.2 M Tris-HCl, pH 7.5/0.01 M EDTA/1% NaDodSO4) and added to 4 g of acid-washed glass beads (0.25–0.30 mm). Two milliliters of phenol/CHCl3, 1:1 (vol/vol), was added, and the mixture was vortexed for 2.5 min. After addition of 3 ml of lysis buffer and 3 ml of phenol/CHCl3, the extract was centrifuged to separate phases, and the aqueous phase was reextracted with phenol/CHCl3. RNA was precipitated from the aqueous phase with ethanol. Poly(A)^+ RNA was isolated as in ref. 15 and fractionated on 1.5% agarose/6% formaldehyde as in ref. 16, except that Hepes buffer (pH 7.8) was substituted for borate buffer. RNA was transferred to nitrocellulose and hybridized with probe as in ref. 17, except that dextran sulfate was absent from hybridization solutions. Probe was prepared by nick-translation of plasmid DNA by using a New England Nuclear nick-translation kit. Washed filters were autoradiographed for 48 hr with Kodak XAR-2 film and a DuPont 1 Lightning Plus intensifying screen.

Cloning a Segment Containing ho. A collection of EcoRI DNA fragments (from ho strain S288C) inserted into Agt (18) was obtained from R. W. Davis via F. W. Stahl and was screened with HO probe (obtained by nick-translation of plasmid YIp5-B12) as in ref. 19. A 2.5-kilobase pair (kbp) HindIII fragment corresponding to fragment H2 of YEpHO was inserted into plasmid YIp5 and assayed for Ho activity as described in the text.

RESULTS

Identification and Properties of a Cloned DNA Segment That Allows Mating-Type Interconversion in ho Cells. We have identified a plasmid that carries HO by screening for plasmids with the ability to promote mating-type interconversion in ho strains. The ho recipient used in our transformations was MATa and carried a mutation in an a-specific ST gene (STE13; ref. 7), a gene required for mating by a cells but not by a cells (see Table 1). An ho MATa ste13 cell and the colony derived from it are unable to give a mating response with either a or a tester cells. In contrast, MATa ste13 cells that carry the HO gene switch to MATa; hence a cell that is initially MATa ste13 forms a colony that contains a mixture of two types of cells—those that mate as a (genotypically MATa ste13) and others like the original cell that are unable to mate (genotypically MATa ste13) (ref. 10; see also ref. 20). Therefore, we have screened for plasmids.

Table 1. Phenotypes of ste13 strains carrying ho or HO alleles

<table>
<thead>
<tr>
<th>Genotype of cell</th>
<th>Mating</th>
<th>a-Factor response</th>
<th>Colony, mating</th>
</tr>
</thead>
<tbody>
<tr>
<td>MATa ste13 ho</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MATa ste13 HO*</td>
<td>No</td>
<td>Yes (as a)</td>
<td>Yes (as a)</td>
</tr>
<tr>
<td>MATa ste13 ho</td>
<td>Yes (as a)</td>
<td>Yes</td>
<td>Yes (as a)</td>
</tr>
<tr>
<td>MATa ste13 HO*</td>
<td>Yes (as a)</td>
<td>Yes</td>
<td>Yes (as a)</td>
</tr>
</tbody>
</table>

Mating and a-factor response by individual cells and by colonies grown from these cells were assayed as described in the text.

* HO provided either at its standard chromosomal position or on a plasmid (such as YEpHO).

† These colonies contain two types of cells: MATa ste13 and MATa ste13.
that allow ho MATa ste13 strains to switch to MATa.

An ho MATa ste13 leu2 strain (G150-15d) was transformed with a plasmid pool containing random genomic yeast DNA fragments inserted into the LEU2 vector YEp13. Of 20,000 Leu" transformant colonies screened for mating ability, I contained a plasmid (YEpHO) that allowed the recipient cells to form colonies that mated as a. Direct microscopic observation of individual cells confirmed that cells carrying YEpHO switch between two cell types: cells that were initially sensitive to a factor (phenotypically a, genotypically MATa ste13) gave rise to cells that were resistant to a factor (phenotypically not a, genotypically MATa ste13). These latter cells then gave rise to cells that responded to a factor. Further evidence that YEpHO allowed mating-type interconversion of ho strains came from the following observations: (i) MATa and MATa ho STE" cells carrying YEpHO efficiently gave rise to nonmatting, sporulation-proficient diploid cells—that is, to MATa/MATa cells. (ii) YEpHO allowed cells with a mutation of the mating-type locus (either mata or mata2) to switch readily to MATa and MATa, respectively, and thus to form MATa/MATa cells. The YEpHO plasmid, like a chromosomal HO, thus can heal mutations of the mating-type locus (3, 20). Demonstration that YEpHO carries HO is given in the next section.

To localize the region responsible for HO activity in the original 6-kbp insert in YEpHO, we subcloned restriction endonuclease fragments into the plasmid vector YIp5. When present in either orientation in YIp5, a 2.5-kbp HindIII fragment (denoted "H2"; Fig. 2) provided Ho activity when introduced into ho MATa ste13 ura3 strain HR125-6d. Although YIp5 is incapable of autonomous replication (21), YIp5-H2 yielded unstable transformants at a high efficiency. Thus, H2 apparently contains a sequence (an ARS sequence; ref. 22) that allows autonomous replication of YIp5. A smaller fragment (the 1.1-kbp BamHI fragment, B2) provided Ho activity when inserted in one orientation in YIp5 but not in the other orientation. The 870 base pair (bp) BamHI-HindIII fragment (BH2) representing the overlap between the H2 and B2 fragments lacked Ho activity in YIp5. (The only possible orientation of BH2 in YIp5 is the same as the inactive orientation of B2 in the plasmid.) Taken together, these results indicate that most of the information for Ho activity is carried on the 870-bp BH2 fragment.

YEpHO Carries the HO Gene. To determine whether the insert in YEpHO carries the HO gene itself (and not another gene normally controlled by HO or otherwise limiting for mating-type interconversion), we allowed a plasmid containing the putative HO DNA to integrate into the yeast genome by recombination with homologous sequences and determined its site of insertion. If the cloned DNA segment contains HO, the plasmid should integrate at the HO locus (or its allele ho). For this analysis, we have used the UR3" plasmid YIp5 carrying the B2 fragment. When transformed into a ura3 mutant, stable Ura" transformants arise by integration of the plasmid into the genome (21). (Integration at the defective ura3 locus is greatly decreased by using the ura3-52 mutation in the recipient; ref. 23). Stable Ura" transformants obtained in recipient strain SC3 (ho MATa ura3-52) were crossed to HO MATa ura3 spores (from strains J12-1A and 341), and meiotic products were analyzed. In crosses with three independently isolated Ura" integrants, the Ura" phenotype contributed by YIp5-B2 was tightly linked to the ho locus (within 1 centimorgan): no recombinants were obtained in a total of 87 tetrads (see Materials and Methods). In other crosses, we observed that the Ura" determinant is linked to CDC9 (data not shown) at the same frequency as is HO (G. Kawasaki, cited in ref. 24). These results show clearly that UR3 is now located at the ho locus and thus that the B2 segment contains nucleotide sequences present at this locus.

Additional evidence that YEpHO contains the HO gene comes from analysis of a genomic DNA segment containing the ho allele. A fragment from an ho strain was identified by homology with the BH2 fragment of YEpHO (as described in Materials and Methods), and a 2.5-kbp HindIII subfragment (H2', which corresponds to the H2 fragment of YEpHO) was inserted into YIp5. YIp5-H2' does not promote mating-type interconversion when introduced into an ho MATa ste13 strain. The inability of this segment, derived from an ho strain, to promote mating-type interconversion provides an independent argument that the homologous segment present in YEpHO and derived from an ho strain carries the HO gene.

Control of HO RNA Synthesis by the Mating-Type Locus. To determine whether the mating-type locus controls expression of the HO gene, we used the cloned HO gene as a probe for RNA isolated from HO strains that differ in their ability to switch mating types. Because haploid HO cells diploidize to form MATa/MATa cells, a culture of haploid MATa or MATa HO cells cannot be used as a source of RNA for cells exhibiting mating-type interconversion. Therefore, we have used HO strains in which the interconversion system remains active because these strains do not form a/a diploids. One strain (3B54) contains an a-specific STE mutation (ste14) that prevents mating between daughter cells (19). A second strain (HR112-1b) carries only a cassettes at MAT, HML, and HMR and thus cannot form a/a diploids. Similarly, a third strain (HR100-1a) contains only a cassettes at MAT, HML, and HMR. The mating-type interconversion system is active in such strains (25, 26). These strains were compared with HO strains in which mating-type interconversion does not occur, MATa/MATa HO/HO diploids X10-1b (the parent of the three prior strains) and AB320 (from which the clone bank was derived). We note that X10-1b, HR112-1b, and HR100-1a are isogenic (see Materials and Methods); AB320 and 523 (see below) are from different backgrounds. Poly(A") RNA was isolated from these strains and hybridized with a probe (YIp5-BH2) that contains UR3 and part of HO. As shown in Fig. 3, we observe a species of RNA (≈1.5–1.7 kilobases [kb]) complementary to the cloned DNA probe that is present in the haploid MATa and MATa HO strains, in which mating-type interconversion is active (Fig. 3, lanes A–C); this species is completely absent in HO/HO MATa/MATa diploids, in which mating-type interconversion does not occur (Fig. 3, lanes E and F). This RNA species is also present in diploid, HO/HO MATa/MATa cells that are homoygous for HMLα.
size is identical to that produced by HO strains (Fig. 4, lanes B and C) and that this transcript is controlled in the same manner as in HO strains, expressed in haploids and not in MATα/MATα diploids (Fig. 4, lanes A and B). This RNA is absent from MATα/matal strains (Fig. 4, lane F) and present in MATα/matal2 and mata1/MATα strains (Fig. 4, lanes D and E). Thus, α2 and α1 are required in diploid cells to inhibit production of RNA from the ho locus. The functional significance of the transcript produced in ho strains is unknown.

DISCUSSION

HO and the mating-type locus exert a mutual control over each other: the HO gene product is responsible for switching alleles of the mating-type locus, and the mating-type locus regulates activity of the HO gene. The latter observation has two main implications: first, the mating-type locus controls expression of an unlinked gene at the level of RNA production; second, mating-type interconversion does not occur in α/α cells because HO RNA is not produced. Whether transcription of HO is blocked in α/α cells or the HO transcript is unstable cannot be determined from our analysis.

Because the HO and URA3 segments of the probe are of approximately equal size, the extent of hybridization to the HO and URA3 bands indicates the relative amounts of these RNA species. These bands were excised from the nitrocellulose filter and radioactivity was measured in a liquid scintillation counter, which showed the HO to URA3 ratio to be ~1:2. Thus, the level of stable HO transcript appears to be rather low, only half that of URA3 (which is present in ~5–10 molecules per cell; ref. 27). [We note that 2.5–5 HO transcripts per cell is an average: only certain cells within a clone of HO cells are competent to switch mating types (9, 28), and it is perhaps only these cells that express HO.] By similar quantitative analysis, we find no HO transcript in α/α cells (that is, no hybridization above background). Thus, production of the HO transcript in α/α cells is <1% of the level in expressing cells.

As noted above, physiological studies show that both α2 and a1 products are necessary to inhibit mating-type interconversion. Thus, we anticipated that these products would also be required for inhibiting synthesis of HO RNA. Because MATα/matal2 and mata1/MATα strains carrying HO switch mating-type cassettes efficiently, it is not possible to grow pure cultures with these genotypes to assay HO RNA in such strains. However, we have found that ho strains produce a transcript whose

and HMRα (Fig. 3, lane D). All strains produce similar amounts of URA3 RNA, which is 0.9–1.0 kb (27). These results show a perfect correlation between active mating-type interconversion and production of a RNA that is homologous to a DNA segment carrying HO. Thus, mating-type interconversion does not occur in α/α cells because HO RNA is not produced. Whether transcription of HO is blocked in α/α cells or the HO transcript is unstable cannot be determined from our analysis.

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(29, 30); hence, α-specific genes are not expressed. The level of control exerted by these regulators is known to be at the level of RNA production for two cases: α1 is required for synthesis of RNA from the α-specific STE3 gene (8); and α1–α2 negatively regulates synthesis of RNA from the MATα1 gene (29, 30). We have shown here that α1–α2 negatively regulates the HO gene as well. This conclusion comes from the following observations: (i) MATα/MATα and MATα/MATα (and corresponding haploids) but not MATα/MATα cells produce HO RNA. (ii) mata1/MATA1 and mata1/mata2 strains produce ho RNA. Thus, it is clear that both α1 and α2 are required to inhibit expression of the ho (HO) locus. Haploid strains that are phenotypically a/α because they express a and α cassettes at HML and HMR (due to a mutation allowing expression of these cassettes), as expected, do not exhibit mating-type interconversion (31) and do not express HO (unpublished data).

α1 and α2 inhibit expression of several genes in addition to HO and MATα1. Production of RNA from the STE5 gene (required for mating by both α and α cells) is inhibited in a/α diploids (V. L. MacKay, J. Thorner, and K. Nasmyth, personal communication). Thus, HO and STE5 genes are genes that can be termed “haploid-specific,” expressed in haploid strains (that are phenotypically a or α) but not in diploid strains (that are phenotypically a/α). Production of RNA encoded by the repeated element Ty1 is likewise inhibited in a/α cells (32). Association of a Ty1 element with several different loci (CYC7, DUR, ADR2) places these loci under MAT control: expressed at a higher level in haploids than in a/α diploids (33, 34). Finally, we have proposed (35) that sporulation is triggered in a/α cells because a1–a2 inhibits synthesis of a negative regulator of sporulation.

How does α1–α2 exert its negative regulatory activity at so many different, widely dispersed loci? Obviously, these loci may share a common recognition site for α1–α2. Because genetic rearrangements that place Ty1 adjacent to various genes cause these genes to be under α1–α2 control, Errede et al. (33) have suggested that Ty1 may be naturally associated with certain yeast genes and be responsible for their control by α1–α2. We have no evidence that HO is associated with such a Ty1 element: the cloned HO segment does not hybridize to Ty1 (unpublished data).

Diploidization is a two-step process, mating-type interconversion followed by mating between haploid cells of opposite cell type. If mating-type interconversion were to continue in MATα/MATA1 HO/HO cells (to produce MATα/MATα and MATα/MATα cells), mating between siblings would result in cells of ever-increasing ploidy—for example, production of a/α/α/α tetraploids. However, MATα/MATα cells are stable in the presence of HO because, as shown here, the HO gene product is not synthesized. (We note that mating-type interconversion is turned off immediately after formation of an a/α HO/HO cell; hence, some component of the mating-type interconversion machinery, perhaps the HO gene product itself, may be unstable.) α1–α2 registers the successful formation of an a/α cell and prevents further mating-type interconversion. The a/α diploid state is stable until meiosis and sporulation yield haploid spores, which once again express the HO gene and resume mating-type interconversion and diploidization. Thus, diploidization in yeast demonstrates one mechanism—intracellular feedback control—by which the successful execution of a complex event is monitored. By contrast, successful formation of a λ lysogen (another complex event involving genetic rearrangement) is optimized in a different manner, in this case, by prognostication (36). It is clear that genetic rearrangement is under the types of control that allow it to play a role in a variety of biologically important processes (37, 38).

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