RAP1 protein activates and silences transcription of mating-type genes in yeast

Stephen Kurtz and David Shore

Department of Microbiology, College of Physicians & Surgeons, Columbia University, New York, New York 10032 USA

RAP1 is a sequence-specific DNA-binding protein essential for cell growth. The occurrence of RAPl-binding sites in many promoter regions, the mating-type gene silencer elements, and telomeres suggests that RAPl has multiple functions in the cell. To assess its role in transcription, temperature-sensitive mutations in RAPl were generated. Analysis of raplts strains provides evidence that RAPl functions in both transcriptional activation and silencing of mating-type genes. Several observations indicate that raplts strains are defective in the expression of MATa, whose upstream activation sequence (UAS) contains a RAPl-binding site. At nonpermissive temperatures, decreases in MATa steady-state transcript levels can be detected in MATa raplts strains. Furthermore, these strains are deficient in a-pheromone production and simultaneously express at least two a-specific genes. These phenotypes can be reversed by replacing the RAPl-binding site at MATa with a binding site for the GAL4 transcriptional activator. Certain raplts alleles have an opposite effect on the silent mating-type locus HMR, which becomes partially derepressed at nonpermissive temperatures.

[Key Words: Saccharomyces cerevisiae; transcriptional activation; silencer; mating type; transcription factor]

Received October 19, 1990; revised version accepted February 1, 1991.

RAP1 [TUF/GRF1/TBA] has been independently identified as a sequence-specific DNA-binding protein in studies of ribosomal protein gene promoters [Huet et al. 1985], silencer elements at the nontranscribed mating-type loci HML and HMR [Shore et al. 1987; Buchman et al. 1988a], and the poly[C1_3A] tracts at telomeres [Berman et al. 1986; Dunn 1989]. The RAPl protein has been purified, and its gene has been cloned and shown to be essential for growth [Shore and Nasmyth 1987]. RAPl-binding sites have since been identified in the promoters of a large number of genes in addition to those involved in translation, including many glycolytic enzyme genes and the MATa mating-type genes [for review, see Buchman et al. 1988b; Capieaux et al. 1989]. The location of RAPl-binding sites at both upstream activation sequences (UASs) and silencers has led to the suggestion that RAPl has a dual role in regulating transcription. Recent studies have demonstrated directly that RAPl is also involved in controlling the length of poly[C1_3A] tracts at telomeres [Lustig et al. 1990].

The idea that RAPl functions in vivo as an activator of transcription is supported by a number of deletion analyses of promoter regions containing RAPl-binding sites. Deletion of these sites from promoters typically results in a moderate reduction in expression [Rotenberg and Woolford 1986; Chambers et al. 1988; Elledge and Davis 1989; Hurd and Roberts 1989], although in some cases the effect appears to be more severe [Siliciano and Tatchell 1984; Nishizawa et al. 1989]. Similarly, analyses of silencer elements has suggested a repressor function for RAPl: Deletion of the RAPl-binding site at the HMR silencer causes a partial derepression of this locus [Brand et al. 1987; Kimmerly et al. 1988]. The function of RAPl at a particular locus appears to depend on the context of its binding site within other regulatory sequences rather than the precise sequence of the site, and presumably results from interactions with other regulatory factors. So, for example, UAS-associated RAPl-binding-sites can substitute for the binding site normally found at the HMR silencer [Shore and Nasmyth 1987], and RAPl binding sites taken from either UAS elements, silencers, or telomeres activate transcription when placed upstream of a TATA element [Buchman et al. 1988b].

The presence of RAPl-binding sites at both the MATa promoter and the HML and HMR silencers suggests that RAPl might play a complex role in cell type determination. The MAT locus, which controls cell type (either a or α), is situated near the centromere of chromosome III and can contain either the a or a allele. The MATa allele encodes two regulatory proteins (α1 and α2) that control the expression of a number of cell type-specific genes in haploids (e.g., pheromone and pheromone receptor genes). The MATa protein is an activator of a-specific genes, whereas MATa2 is a repressor of the otherwise constitutive a-specific genes. Therefore, expression of both α1 and α2 represses a-specific genes and activates a-specific genes, leading to the a-mating phenotype. In the absence of MATa expression cells display an a-mating phenotype. The MATa locus has no function in haploids but is required in a/α diploids, which are nonmat-
ing cells capable of sporulation. Two identical, yet non-transcribed, copies of mating-type genes are present near the telomeres of chromosome III, at loci called HMR and HML, which usually contain a and α alleles, respectively. Silencing of these loci is essential for mating. In haploid cells containing a wild-type HO gene, the silent loci are used as donors of information in a mating-type switching event that results in a conversion of the allele present at the MAT locus (for review, see Nasmyth and Shore 1987; Herskowitz 1989).

Several studies have defined DNA sequence elements and genes necessary for maintaining transcriptional silencing. At HMR, a flanking sequence called HMRE is required for repression of the locus (Abraham et al. 1984; Brand et al. 1985). The HMRE silencer is itself composed of three elements (called A, E, and B), one of which (element E) is a RAP1-binding site that is required for full repression (Brand et al. 1987; Shore and Nasmyth 1987; Kimmerly et al. 1988). Element A is an autonomous replicating sequence (ARS) consensus sequence, and element B is a binding site for ARS-binding factor 1 (ABF1) (Shore et al. 1987; Buchman et al. 1988a; Difley and Stillman 1988). Deletions of either A or B alone have no effect on silencing, but either element, in combination with the RAP1-binding site, provides complete repression in the context of HMRE. A number of unlinked trans-acting genes have been identified that are required for silencing. Mutations in the nonessential genes SIR2, SIR3, or SIR4 result in a complete loss of silencer function (Rine and Herskowitz 1987), and mutations in SIR1 appear to affect the establishment, but not the maintenance, of the repressed state (Pillus and Rine 1989). In addition, deletions of the amino terminus of histone H4 lead to derepression of the silent loci (Kaye et al. 1988), and mutations in NAT1 and ARD1, components of an amino-terminal protein acetylase, result in a partial loss of silencing (Whiteway et al. 1987; Mullen et al. 1989). No mutations in the genes encoding the known silencer binding proteins RAP1 and ABF1 have been isolated previously in screens for silencing mutants (Miller and Nasmyth 1984; Rine and Herskowitz 1987), perhaps because their functions are redundant at the silencers and both are essential genes.

To investigate the role of RAP1 in transcriptional regulation, we have isolated conditionally lethal mutations in the RAP1 gene. Strains containing these mutations were then used to examine the requirement for RAP1 in gene expression at MATa and repression at HMR. These experiments provide genetic evidence that RAP1 has a fundamental role in transcriptional activation and repression and demonstrate the importance of RAP1 in establishing and maintaining cell type in yeast.

Results

Isolation and initial characterization of rap1ts mutants

Temperature-sensitive mutations in RAP1 were generated by mutagenesis of the cloned gene. Because the RAP1 gene is essential, we used a plasmid shuffle technique (Boeke et al. 1987; Mann et al. 1987) to introduce a mutagenized copy of the gene into cells deleted for the chromosomal copy. Four independent temperature-sensitive mutants were obtained using this procedure. Plasmids recovered from these strains conferred a temperature-sensitive phenotype upon retransformation of the original strain and subsequent plasmid shuffling, confirming that the temperature-sensitive mutations were plasmid encoded. The mutant alleles were isolated from these plasmids, subcloned into an integrating vector, and used to replace the RAP1 locus. All of the mutations are recessive; they are complemented by plasmids containing the wild-type RAP1 gene, and heterozygous diploids grow normally at 37°C. The integrated alleles segregate as expected 2+ : 2− for growth at 37°C in crosses to a wild-type strain. Strains containing these rap1ts alleles at the genomic RAP1 locus were used in all experiments presented below.

The effect on cell growth of the four rap1ts alleles was assessed at permissive (25°C) and restrictive (37°C) temperatures. As shown in Figure 1A, the mutants display a range of different growth phenotypes relative to the wild-type strain. At 25°C, strains containing the rap1-2 or the rap1-5 mutation grow at near wild-type rates (2 hr doubling time). At 37°C, these strains gradually arrest their growth after 3 to 4 doublings. Strains containing either the rap1-1 or rap1-4 mutation have a more dramatic growth phenotype. At 25°C, both of these strains have a pronounced increase in doubling time (4 hr for rap1-1 and 7 hr for rap1-4) and arrest growth after 1 to 2 doublings when shifted to 37°C. The relative growth differences among the four mutant strains is reflected in their efficiency of plating after a shift to 37°C. The strains that grow poorly at 25°C lose viability at the restrictive temperature more rapidly than the strains that grow normally at 25°C. Strains carrying the rap1-2 or rap1-5 mutations show only a twofold decrease in viability for every 4 hr of incubation at 37°C, whereas the rap1-1 and rap1-4 strains show a more dramatic loss of viability (~10-fold reduction for every 4 hr at 37°C; Fig. 1B). In contrast, wild-type cells show no detectable loss of viability after similar periods of incubation at 37°C.

None of the rap1ts mutants appear to arrest growth at a particular stage in the cell cycle. Strains containing rap1ts mutations have a larger cell size than wild-type cells even at permissive growth conditions. Many of the cells in a population carrying a rapts mutation have pronounced vacuoles and grossly altered morphologies relative to wild-type cells after a temperature shift to 37°C (data not shown).

On the basis of relative differences in growth between the four temperature-sensitive mutations, it seemed likely that each mutant represented a unique rap1 allele. We mapped the mutations to regions in the RAP1 gene by a series of restriction fragment swap experiments (see Materials and methods). Appropriate mutant fragments were then subcloned and sequenced. The four mutants are unique missense mutations in the RAP1-coding sequence distributed over a region of ~250 amino acids in the middle of the coding sequence (Fig. 2).
RAP1 is required for the proper regulation of genes controlled by the MATα locus

In constructing strains for the purpose of isolating rap1 mutants we noticed an unusual property of MATα cells carrying both a deletion of the genomic RAP1 locus and a plasmid containing the wild-type RAP1 gene. In patch mating assays, a portion of these cells were capable of mating to MATα testers, whereas otherwise isogenic strains containing the MATα allele mated normally [Fig. 3]. A quantitative mating analysis indicated that the MATα, rap1 deletion strains mate with the inappropriate cell type [MATα] 200-fold more frequently than the parental wild-type MATα strain [Table 1]. We were able to show by a simple genetic test that this aberrant mating behavior occurred only in cells that had lost the RAP1 plasmid; selection for the URAS gene on this plasmid abolished the ability of this strain to form diploids with the MATα tester strain. This result suggested that loss of the RAP1-containing plasmid was a prerequisite for α-like mating, an event that rescues these cells from death. Because RAP1 binds in vitro to the MATα UAS (Shore and Nasmyth 1987; Buchman et al. 1988a), we suspected that a reduction in RAP1 activity in cells that have lost the RAP1 plasmid might directly affect transcription of the MATα locus. Previous studies have shown that the absence of functional MATα gene products, either due to mutations in both α1 and α2 or promoter deletions that abolish MATα transcription, results in an a-mating phenotype (Strathern et al. 1981; Tatchell et al. 1981; Siciliano and Tatchell 1984).

To test whether RAP1 is required for the expression of the MATα locus, we measured the activities of several gene products known to be directly regulated by MATα.

Figure 1. Growth phenotypes of rap1ts mutants at 25°C and 37°C. [A] Log-phase cultures of wild-type [YDS3] and rap1ts strains [YDS406, 409, 410, 413] were split and maintained in YEPD media at 25°C or shifted to 37°C. Aliquots were removed at regular intervals, and the optical density at 600 nm was determined. OD600 values are plotted vs. time of incubation. [B] Survival of rap1ts mutants after incubation at the restrictive temperature. Log-phase cultures of wild-type [YDS3] and rap1ts strains [YDS406, 409, 410, 413] were shifted from 25°C to 37°C. At various intervals, equal OD600 units were diluted, spread on YEPD plates, and incubated at 25°C. After 5 days, the number of surviving colonies was determined and plotted vs. time of incubation at 37°C.
Role of RAP1 in cell-type determination

RAP1

Bgl II

Pst I

Hind III

Sph I

Bgl II

Xba I

nuc. 1

853

1843

2360

2864

3670

RAP1

Bgl II

I

I

Pst I

I

I

Hind III

Sph I

Bgl II

I

I

Xba I

I

I

3670

1843

2360

2864

Table 1. Relative mating efficiencies of wild-type and rap1 deletion strains

| Strain     | Mating type | x MATa | x MATα |
|------------|-------------|--------|--------|
| YDS 3      | α           | 0.48   | 1.2 x 10⁻⁶ |
| YDS 203-2d | α           | 0.56   | 2.6 x 10⁻⁴ |
| YDS 2      | a           | 1 x 10⁻⁶ | 0.7    |
| YDS 203-6b | a           | 1 x 10⁻⁶ | 0.83   |

The α1 and α2 proteins regulate genes encoding both α- and α-specific functions (Nasmym and Shore 1987; Herskowitz 1989); the α1 protein is an activator of α-specific genes, such as MFα1 and MFα2, which encode α-factor, whereas α2 is a repressor of α-specific genes, including the genes that encode α-factor and barrier, a protease that inactivates α-factor. The presence or absence of the mating factors and the barrier protease, which are all readily detectable in a bioassay, provides a sensitive measure of α1 and α2 activities.

rap1ts strains were tested for the synthesis of α-factor using a halo assay (Julius et al. 1983) in which the production of α-factor by a patch of MATα cells is measured as a zone of growth inhibition in a surrounding lawn of MATα cells. All four MATα rap1ts strains produce halos typical of MATα cells at the permissive temperature (25°C). However, at semipermissive temperatures (30-33°C) these strains fail to produce α-factor halos (Fig. 4; data presented is for the rap1-1 allele). The severity of this phenotype correlates with the overall growth rates of the different rap1ts alleles: rap1-1 and rap1-4 fail to produce halos when transferred directly from 25°C to 30°C tester plates, whereas the rap1-2 or rap1-5 alleles require a 6-hr preincubation at 33°C before the loss of α-factor activity is evident. Because the α2-factor genes require α1 protein for expression, this observation is consistent with the notion that rap1ts strains are defective in the expression of MATα.

Coincident with the decrease in α-factor production in the MATα rap1ts strains is the inappropriate expression of several α-specific genes. At semipermissive temperatures, MATα rap1ts strains express α-factor, a peptide normally secreted by MATα strains and detectable in a halo assay (Fig. 5A; rap1-1 allele is shown). Because α-factor gene expression is repressed in MATα cells by the α2 protein (Brake et al. 1985; Johnson and Herskowitz 1985), this observation suggests that rap1ts strains are also defective in the expression of α2. Consistent with
Elevated temperatures, α-factor production was measured using Figure 4. Fresh colonies of each strain were toothpicked onto plates a halo assay at 25°C (permissive) and 30°C (semirestrictive). MATa MATa at the designated temperature for 2 days. (a) Wild-type spread with a lawn of strain RC634 MATa rapl-1, MATa (YDS3); (b) rapl-1, MATa (YDS406); (c) wild-type RAP1, MATa (YDS2); (d) rapl-1, MATa (YDS407).

Because the aberrant production of barrier in the MATa rapl ts strains could also account for the loss of α-factor activity at semipermissive temperatures, we examined the production of α-factor in these strains in the absence of the BAR1 gene. MATa, rapl ts, bar :: LEU2 strains produce near wild-type levels of α-factor at 25°C; however, at 30°C, these strains produce little or no detectable α-factor (Fig. 6A). Therefore, the aberrant production of barrier in the MATa rapl ts strains cannot alone account for their reduced α-factor production. This result further supports the conclusion that expression of α1, the activator of the α-factor genes, is also reduced in the rapl ts strains following a temperature shift. Finally, the MATa-specific phenotypes of the rapl ts mutants do not result from a derepression of the silent HMRa locus, as they are observed in strains carrying a deletion of the α1 and α2 genes at HMR (data not shown).

Steady-state levels of transcripts from MATα and from the α1-regulated gene encoding α-factor (MFα1) were measured in rap1 ts strains following a shift to nonpermissive conditions. These RNAs were analyzed on Northern blots. A probe specific for URA3, a gene that does not have a RAP1-binding site in its regulatory region, was included as an internal control. There is a temperature-dependent decrease in the amounts of MATα1 and MATα2 mRNAs in strains containing the rap1-1 allele (Fig. 7A) or rap1-4 allele (data not shown) over the course of an 18-hr incubation at 33°C. We note that the decrease in the levels of MATα1 and MATα2 messages is not always as apparent in these strains and is difficult to detect in strains containing either the rap1-2 or rap1-5 alleles (data not shown). The behavior of strains with the rap1-2 and rap1-5 alleles is consistent with properties described above, indicating that they are weak alleles; these strains are defective in α-factor production and aberrantly produce α-factor and barrier only after a preincubation at nonpermissive temperatures.

We also note that the amount of MFα1 message drops significantly following temperature shift (Fig. 7A, MFα1), and this decrease is apparent before there is a detectable drop in MATα1 transcript levels. This may indicate that MFα1 transcription is extremely sensitive to small fluctuations in levels of MATα1 mRNA, the...
Role of RAP1 in cell-type determination

**Figure 6.** A heterologous promoter restores the activation defect in rap1<sup>ts</sup> strains. (A) α-Factor halo assay at 25°C and 30°C on plates spread with a lawn of strain RC634 [MATα, sstl-3]. (a) Wild-type RAP1, MATα (YDS3); (b) rap1-1, MATα (YDS406); (c) rap1-1, MATα bar::LEU2 (YDS407); (d) rap1-1, UASGal-MATα, gal80Δ (YDS558); (e) rap1-1, UASGal-MATα, Δgal80 (YDS447). (B) Barrier assay at 25°C and 30°C on plates spread with a lawn of strain RC634. (a) rap1-1, MATα (YDS406); (b) wild-type RAP1, MATα (YDS2); (c) wild-type RAP1, MATα (YDS3); (d) rap1-1, UASGal-MATα, gal80Δ (YDS447); (e) rap1-1, MATα (YDS407); (f) rap1-1, MATα bar1::LEU2 (YDS540).

MATα1 mRNA produced after temperature shift is non-functional, or RAP1 directly affects transcription of MFA1 (see Discussion). We therefore devised another experiment, described below, to assess the effect of the rap1<sup>ts</sup> mutations on MATα transcription.

**A heterologous promoter at MATα restores α-specific functions in rap1<sup>ts</sup> strains**

Because a known RAP1-binding site has been shown by deletion analysis to be an essential element in the MATα UAS and because mutations in this binding site lead to mating-specific phenotypes very similar to those observed in the rap1<sup>ts</sup> mutants (Giesman et al. 1991), a different experiment was designed to test whether MATα transcription is defective in the rap1<sup>ts</sup> strains. We reasoned that if the mating-specific phenotypes of the rap1<sup>ts</sup> mutants were due to an inability to properly transcribe the MATα genes, then replacing the MATα UAS (RAP1-binding site) with a heterologous UAS element should abolish these phenotypes and restore the α-cell characteristics.

We therefore replaced the RAP1-binding site in the MATα promoter with a single binding site for the GAL4 transcriptional activator (see Materials and methods). This UAS<sub>Gal</sub>-MATα construct was inserted at the MAT locus in a strain that contained the rap1-1 mutation and a gal80 gene deletion. The gal80 mutation was used to facilitate the analysis of the UAS<sub>Gal</sub>-MATα fusion. Because the product of the GAL80 gene is a negative regulator of GAL4 activity in glucose-grown cells (Johnston et al. 1987; Luc et al. 1987; Ma and Pratshpe 1987), deletion of this gene should result in constitutive [galactose-independent] expression from UAS<sub>Gal</sub>-MATα, allowing us to perform halo assays under optimal growth conditions.

rap1<sup>ts</sup> strains containing the UAS<sub>Gal</sub>-MATα gene and the gal80 mutation produce wild-type levels of α-factor at all temperatures [Fig. 6A, strain f] and no longer produce barrier [Fig. 6B, strain d] or α-factor [data not shown] at the elevated temperatures, indicating that the MATα-regulated functions have been restored. The restoration of α-specific functions is not the consequence of overexpression from the heterologous UAS<sub>Gal</sub>-MATα promoter because Northern blot analysis indicates that transcript levels produced from the hybrid gene are similar to those produced from the wild-type promoter [Fig. 7B]. These results suggest that the mating-specific phenotypes associated with the rap1<sup>ts</sup> mutations are due to an inability to properly transcribe the MATα genes, and we conclude from this that one function of the wild-type RAP1 protein is to activate transcription at MATα.

**rap1<sup>ts</sup> alleles affect transcriptional silencing at HMR**

Deletion of the RAP1-binding site in the HMR silencer results in a partial derepression of the locus (Brand et al. 1987; Kimmerly et al. 1988). To determine whether the rap<sup>α</sup>-alleles affect silencing, we measured the transcriptional activity of the UAS<sub>Gal</sub>-MATα construct in the presence and absence of the gal80Δ gene.

**Figure 7.** rap1-1 mutants are defective in MATα transcription. (A) Northern blot analysis of MATα, rap1-1 strain YDS406. RNAs were isolated from a continuous culture at 0, 2, 6, 12, 15, and 18 hr following a temperature shift from 25°C to 33°C. The filter was hybridized successively with probes for the α1, α2, α-factor [MFA1], and URA3 transcripts. (B) Northern blot analysis of MATα transcripts from the UAS<sub>Gal</sub>-MATα construct. RNAs were isolated from YDS439; MATα, rap1-1, Δgal80 grown at 25°C [lane 1] and from YDS447; MATα, rap1-1, Δgal80, UAS<sub>Gal</sub>-MATα grown at 25°C [lane 2] and 8 hr after a shift to 33°C [lane 3]. The filter was hybridized successively with probes for the α1 and α2 transcripts.
tion of HMRa1 after a temperature shift. Because the HMR silencer is only partially dependent on the RAP1-binding site [element E], we also measured HMRa1 transcription in strains carrying deletions of either of the two other silencer regulatory elements, A and B. Both the hmrAΔ and hmrBΔ silencers are totally functional, but unlike the wild-type silencer they are dependent on the RAP1-binding site for repression [Brand et al. 1987]. Therefore, we expected that any effect on silencing in the rap1ts alleles would be more pronounced in the hmrΔA or hmrΔB silencer backgrounds.

RNAs were prepared from strains containing both the rapts alleles and either a wild-type, hmrΔA, or hmrΔB silencer after a shift from 25°C to 37°C. The steady-state level of the HMRa1 transcript was quantified by an S1 nuclease protection assay, using the SIR3 transcript as an internal control [Miller 1984]. In strains containing the rap1-5 allele, this analysis detected a1 transcripts 4 hr after the temperature shift in a strain containing the hmrΔA silencer and 8 hr after the shift in a strain containing the hmrΔB silencer (Fig. 8). Derepression of HMR was also detected in a strain containing the rap1-4 allele and a hmrΔA silencer but not in strains containing the rap1-1 or rap1-2 alleles [data not shown]. No derepression of HMR was detected in strains containing a rapts allele and a wild-type silencer (A, E, and B present). Because a deletion of the RAP1-binding site [hmrΔE] only partially derepresses HMR (~10%), it may be the case that cell death precludes detection of expression at HMR in rapts strains containing a wild-type silencer. Derepression of HMR in strains carrying the rap1-5 allele is the consequence of temperature elevation on this allele; no derepression of these silencer constructions occurs in wild-type RAPI strains at any temperature [see Fig. 8].

DNA-binding activity is heat labile in rapts strains

All of our rap1ts mutants map within or close to a large region in the middle of the protein [from approximately amino acid 360 to 600] that appears to be required for sequence-specific DNA binding [Henry et al. 1990; D. Balderes and D. Shore, unpubl.]. To determine whether the various rapts alleles affected the DNA-binding activity of the mutant proteins, cell extracts were prepared from each of the four temperature-sensitive strains before and after a shift to the restrictive temperature. Strains were maintained in culture at 37°C for 12 hr, and aliquots were removed every 2 hr for preparation of cell extracts. Gel mobility-shift assays were performed at 20°C on the extracts using a high-affinity RAP1-binding site [TEF2] and the binding site for ABF1 [another silencer-binding protein whose activity should be unaffected by these temperature-sensitive mutations]. To reduce the effects of general proteolysis, these strains were made protease deficient by disruption of the PEP4 gene [Ammerer et al. 1986]. Extracts prepared from rapts strains maintained at 25°C produced a protein–DNA complex with the TEF2 probe that was indistinguishable from that produced in extracts containing wild-type RAPI [Fig. 9A]. In strains carrying the rap1-1 or rap1-4 alleles, RAP1-binding activity, although diminished relative to wild-type strains, remains after prolonged incubation at 37°C. Strains containing the rap1-2 and rap1-5 alleles have wild-type levels of RAPI DNA-binding activity under these culture conditions. Although these extracts were prepared from cells maintained at 37°C, the observed levels of RAPI DNA-binding activity may be the consequence of renaturation during the course of the binding assay, which was done at 20°C. To determine whether the temperature-sensitive extracts were capable of binding at nonpermissive temperatures, RAP1-binding activity was assessed at 37°C. The RAP1-binding activity in all of the rapts but not wild-type RAPI strains, is heat labile: Binding reactions performed at 37°C do not produce RAP1–DNA complexes, whereas the binding activity of ABF1 in the same extracts is unaffected [Fig. 9B]. The loss of RAP1-Binding activity in extracts from rapts strains does not appear to be the consequence of proteolysis during the binding reaction, as a RAP1 antiserum detects equivalent amounts of full-length protein in Western blots of extracts incubated at 25°C or 37°C [data not shown].

Discussion

The importance of RAP1 to MATa expression was anticipated from previous studies in which the deletion of a small region of the MATa promoter [containing a RAP1-binding site] was shown to abolish transcription and cause a cells to mate inappropriately, becoming a-like “fakers” [Siliciano and Tatchell 1984]. More recent stud-
Role of RAP1 in cell-type determination

ies in which point mutations were introduced into the RAP1-binding site at MATa have revealed similar mating and transcriptional defects (Giesman et al. 1991). We have presented two types of experiments that, together, strongly suggest that the RAP1 protein mediates transcriptional activation of MATa. First, MATa rap1ts strains fail to produce an α-factor halo and inappropriately express two α-specific gene products, α-factor and barrier, a phenotype characteristic of mata1 mata2 double mutants. Additionally, in the strongest rap1ts mutants, a significant reduction in the steady-state levels of transcripts can be observed. Furthermore, the loss of RAP1 in strains carrying only a plasmid-borne copy of the gene leads to a-like mating in MATa cells, again a property of cells unable to express the MATa genes. In a second set of experiments we have shown that the α-specific defects resulting from the loss of RAP1 activity can be overcome by replacing the RAP1-binding site at MATa with a heterologous UAS element, the GAL4 activator-binding site. This rescue by GAL4 is not an indirect effect owing to overexpression of the MATα genes, as Northern analysis has shown that the steady-state mRNA levels of both α1 and α2 are similar to those produced from the wild-type MATα promoter (Fig. 7B). These experiments indicate that the altered mating phenotypes resulting from diminished RAP1 activity are consistent with a reduced transcription at the MATα locus and support the idea that RAP1 protein functions at the MATα promoter to activate transcription.

A decrease in the steady-state levels of MATα1 and MATα2 mRNAs could account for the defect in α-specific functions observed in rapαts strains. However, we note that this decrease is not always detected in RNAs isolated from rap1-1 or rap1-4 strains and is difficult to observe in the rap1-2 and rap1-5 strains. Furthermore, the levels of Mfa1 transcript decrease before there is a detectable drop in the transcript levels of MATα1 [a known activator of Mfa1]. One explanation for this observation is that a small decrease in MATα mRNA levels can result in a dramatic reduction in Mfa1 transcript levels. Consistent with this explanation, Giesman et al. (1991) have shown that RAP1-binding site mutations at MATα that result in only a partial decrease in MATα steady-state mRNA levels can abolish α-factor production and lead to inappropriate expression of α-specific genes. It is also possible that alterations in transcription start sites or mRNA transport, which are not detected by Northern blot analysis, occur after temperature shift in rap1ts strains and further diminish the pool of functional MATα1 messages. We consider it unlikely that RAP1 is directly involved in Mfa1 expression, as there are no known RAP1-binding sites in the Mfa1 promoter or in the promoter of MCM1, another gene required for Mfa1 expression. Moreover, the restoration of Mfa1 function by altering only the MATα promoter [UASgal-MATα] indicates that the phenotypes observed in the rapαts mutants result from a defect in transcription at MATα. Finally, we cannot exclude the possibility that MATα transcription is deficient in the rapαts strains, for example, during a critical period in the cell cycle required for α1 and α2 function. It is not difficult to imagine how a small drop in MATα2 transcription in an individual cell could lead to a change in cell phenotype. The half-life of α2 protein is <5 min (Hochstrasser and Varshavsky 1990), so this protein is likely to be rapidly depleted from the cell under conditions where MATα expression is not optimal. It is worth noting that the normal process of mating-type switching in homothallic strains also leads to a change in cell type in the course of a single cell cycle.

The relationship between phenotype and mRNA levels that we observe at MATα may have important implications for understanding the cause of death of the rap1ts mutants. We have failed to detect significant temperature-dependent changes in the transcript levels of other genes [RP73, RNR2, PYK1, LSR, and BCY1; data not shown] that contain RAP1-binding sites in their promoter regions. Given that RAP1 is a transcriptional activator at MATα, we think it is likely that it has a similar function at these other genes, particularly in cases where promoter deletion analyses have shown that a RAP1-binding site is a UAS element. As RAP1 becomes limit-
ing in the temperature-sensitive strains following a temperature shift, we imagine that a complex series of events ensues, considering the large number of genes that may be activated by RAP1. It is possible that the function of some essential genes becomes limiting shortly after the temperature shift, for example, due to a stronger dependence on RAP1 for activation, weaker upstream binding sites for the protein, a requirement of full transcription for activity, or a combination of these and other factors. In this case, death may occur before the steady-state mRNA levels of many RAP1-activated genes are affected. As is the case with MATa, a biological assay may be the most effective way of assessing the effect of RAP1 on the transcription of a particular gene. Finally, we have observed that the poly[C1–3A] tracts at telomeres slowly become shorter at semipermissive temperatures for all of the rap1ts alleles (Lustig et al. 1990). Therefore, it is possible that some aspect of chromosome stability (perhaps related to telomere function) is significantly perturbed at the nonpermissive temperature in these mutants and ultimately results in lethality.

The contribution of RAP1 to repression of the silent mating-type loci was suggested by previous studies in which deletions of the RAP1-binding site at HMR were shown to result in partial derepression (Brand et al. 1987; Shore and Nasmyth 1987; Kimmerly et al. 1988). One of the rap1ts alleles (rap1-ts), in combination with a deletion of a silencer regulatory element (either A or B), resulted in a temperature-dependent derepression of the HMR locus. The level of derepression, although not as dramatic as that observed for a complete silencer deletion, may in part reflect the extent of derepression attainable in a population of cells facing impending death. The allele specificity of the derepression we observe suggests that silencing and activation may be two mechanistically different and separable functions of RAP1. For example, the rap1-ts and rap1-5 alleles have similar growth characteristics, yet differ in their effects at HMR. Likewise, the rap1-ts and rap1-1 alleles affect growth more severely than does the rap1-5 allele, yet display little or no derepression of HMR. The separation of essential and silencing functions of RAP1 has been demonstrated directly by the isolation of rap1 point mutants defective in silencing that grow at wild-type rates and display no defect in MATa activation (L. Sussel and D. Shore, in prep.). All of these silencer-specific rap1 mutations map to the carboxy-terminal 100 amino acids of RAP1. It may be noteworthy that the temperature-sensitive mutant that most severely affects silencing [rap1-ts] maps closer to the carboxy-terminal end of the RAP1 protein than the other temperature-sensitive alleles.

We have not examined the effect of the rap1ts mutants on silencing at the HML locus. The cis requirements for silencing at HML differ from those at HMR in that sequences flanking either side of the locus [HMLE and HMLI, left and right flanking, respectively] are by themselves sufficient for silencing (Mahoney and Broach 1989). In addition, the HMLI silencer, unlike either HMLE or the HMR silencer, appears to lack a strong RAP1-binding site (Shore et al. 1987; Buchman et al. 1988a). Therefore, one might predict, that the rap1ts mutants would only affect silencing at HML in the absence of the HMLI silencer.

There is a striking parallel between the role of RAP1 in mating-type gene expression and the role of the MCM1 [PRTE/GRM] protein in the regulation of downstream a- and a-specific genes (for review, see Herskowitz 1989; Sprague 1990). In a cells the MCM1 protein binds upstream of a-specific genes and activates their transcription, whereas in a cells MCM1 functions both as a corepressor [together with α2] to block the expression of a-specific genes and as a coactivator [with α1 protein] to stimulate the transcription of a-specific genes (Bender and Sprague 1987; Kelecher et al. 1988; Tan et al. 1988; Passmore et al. 1989). The ability of sequence-specific DNA-binding proteins to function as both repressors and activators of transcription through combinatorial interactions with other regulatory proteins may be a general theme in eukaryotic transcriptional regulation (Diamond et al. 1990). In the case of MCM1, the factors that determine whether it acts positively or negatively appear to be clearly defined: the presence or absence of the interacting α1 and α2 proteins and sequences flanking the MCM1-binding sites that are recognized by these proteins. The factors that influence RAP1 function are less clearly defined. Although the SIR gene products are required in trans for silencing and the GAL11/SPT13 gene product appears to be required for activation of MATa [Fassler and Winston 1989; Nishizawa et al. 1990], it is still unclear whether any of these proteins interact directly with RAP1 and/or flanking DNA regulatory sequences.

Materials and methods

Yeast media and methods

Strains were maintained at 25°C (fully permissive conditions) in rich medium (YPD) or on minimal media supplemented with the appropriate amino acids, as described in Sherman et al. (1983) [see Table 2]. For the analysis of temperature-sensitive strains, log-phase cultures growing at 25°C were shifted to 37°C [fully restrictive conditions] or 30–33°C [semirestrictive conditions]. The temperature-sensitive phenotype was routinely checked before and after time course experiments to ensure that revertants had not accumulated. Yeast strains were transformed by the spheroplast method [Beggs 1978] or by the LiOAc method [Ito et al. 1983], as indicated.

Quantitative mating analysis was performed according to Sprague and Herskowitz [1981]. Values of relative mating efficiencies were normalized to the number of viable cells plated. Halo assays were done as described in Julius et al. [1983]. All strains tested in halo assays were taken from freshly streaked YEPD plates. Colonies were patched on YEPD plates to which a dilute lawn of responder cells [either strain RC634 or XBHP-2c] had been spread. Plates were then incubated at 25°C or 30–33°C to examine temperature-dependent effects.

Generation of rap1ts strains

Plasmid D130, which contains CEN IV, TRPI, and the wild-type RAPI gene, was mutagenized in vitro by incubating 30 μg
of plasmid DNA in a solution of 1 M hydroxylamine/0.45 N
NaOH for 20 hr at 37°C (Rose and Fink 1987). The mutagenesis
reaction was quenched by the addition of NaCl to a concentra-
tion of 0.1 M and bovine serum albumin to 0.1 μg/ml. The so-
lution was precipitated twice with ethanol, resuspended in 10
mm Tris-HCl (pH 8.0)/1 mM EDTA, and used directly to trans-
lution was precipitated twice with ethanol, resuspended in 10
mm Tris-HCl (pH 8.0)/1 mM EDTA, and used directly to trans-
"CEN"
"RAP1"
"UR3"
"Sup4-o"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"
"URA3"

**Table 2. Strains**

| YDS 2: HMLa MATa HMRa ade2-1 can1-100 his3-11,-15 leu2-3,-112 trpl-1 ura3-1 |
| YDS 3: HMLa MATa HMRa ade2-1 can1-100 his3-11,-15 leu2-3,-112 trpl-1 ura3-1 |
| YDS 31: MATa his1 |
| YDS 32: MATa his1 |
| YDS 36: YDS3 hmrAΔ (silenter deletion 352–358, Brand et al. 1987) |
| YDS 37: YDS3 hmrAB (silenter deletion 300–256, Brand et al. 1987) |
| YDS 39: YDS3 hmrAB (silenter deletion 331–324, Brand et al. 1987) |
| YDS203-2d: YDS3 rap1 :: LEU2, pD145[YCP50, RAP1, URA3, sup4-o] |
| YDS203-6b: YDS2 rap1 :: LEU2, pD145[YCP50, RAP1, URA3, sup4-o] |
| YDS38: YDS3 hmrABΔB (silenter deletions 331–324 and 274–256, Brand et al. 1987) |
| YDS406: YDS3 rap1-1 |
| YDS407: YDS2 rap1-1 |
| YDS408: YDS2 rap1-2 |
| YDS409: YDS3 rap1-2 |
| YDS410: YDS3 rap1-5 |
| YDS411: YDS2 rap1-5 |
| YDS412: YDS2 rap1-4 |
| YDS413: YDS3 rap1-4 |
| YDS438: YDS3 Δgal80 |
| YDS439: YDS406 Δgal80 |
| YDS444: YDS438 UASGal-MATa |
| YDS447: YDS439 UASGal-MATa |
| YDS39: YDS3 bar1 :: LEU2 |
| YDS540: YDS406 bar1 :: LEU2 |
| YDS544: YDS406 hmrAΔ |
| YDS545: YDS406 hmrAB |
| YDS546: YDS409 hmrAΔ |
| YDS547: YDS409 hmrAB |
| YDS548: YDS413 hmrAΔ |
| YDS549: YDS413 hmrAB |
| YDS550: YDS410 hmrAΔ |
| YDS551: YDS410 hmrAB |
| YDS558: YDS406 UASGal-MAT |
| YDS60: YDS3 pep4 :: URA3 |
| YDS61: YDS406 pep4 :: URA3 |
| YDS62: YDS409 pep4 :: URA3 |
| YDS63: YDS410 pep4 :: URA3 |
| YDS64: YDS413 pep4 :: URA3 |
| RC334: MATa sst1-3 ade2 his6 met1 ura1 mel (from R. Chan) |
| XBH8-2c: MATa sst2-4 ceryl his6 ura1 met1 (from L. Blair) |

**Mapping and sequencing of rap1Δ alleles**

The location of each mutation was determined by complementa-
tion of the temperature-sensitive lethality with hybrid RAP1
genes constructed by interchanging fragments of the mutant
allele with homologous fragments from the wild-type RAP1
gene. Fragments containing the entire coding region of the mu-
tant rap1 alleles (from the PstI site at nucleotide position 853 to
the XhoI site at position 3670) were subcloned into pRS315, a
CEN vector that contains the LEU2 selectable marker (Sikorski
and Hieter 1989). These plasmids were then digested with PstI
and SphI or PstI and BglII. Vector fragments isolated from these
digests, and now lacking the respective 1507- or 2011-bp frag-
ments from the rap1Δ coding region, were then ligated with the
corresponding fragments isolated from digestion of the wild-
type RAP1 gene. Plasmids containing the hybrid genes were
then introduced into the temperature-sensitive strains
(YDS406, 409, 410, 413) by transformation, and growth at 37°C
was assessed. Complementation of the temperature-sensitive
phenotype, indicated by growth at 37°C, correlated with a par-
ticular wild-type fragment for each rap1 allele. Smaller frag-
ments of the wild-type RAP1 gene (generated from the HindIII
sites at positions 1843 and 2837) were used to further define
the region complementing the temperature-sensitive phenotype to
~500 bp. The corresponding fragments obtained from the mu-
tant alleles were then subcloned into the M13 vectors mp18 and
mp19 and sequenced using the Sanger dideoxynucleotide
method (Bankier et al. 1987). Mutations were confirmed by se-
quencing both strands of the appropriate fragment.

**Construction of MATa UAS replacement**

A 4-kb fragment containing the MATa gene, deleted in the pro-
moter from base pair 1602 to 1618, was removed by digestion
with HindIII from a YRP7-based plasmid provided by K. Tatch-
el [an XhoI linker had been inserted at the site of the deletion]
and inserted at the HindIII site in pIC20 to form plasmid D1446.
This plasmid was then digested with XhoI, treated with calf alkaline phosphatase, and ligated to a 22-bp oligonucleotide 5′-TTCGAGCTCTATCCGCCTC-3′ and its complement 5′-CTCGAGCTCTATCCGCCTC-3′ containing a GAL4-binding site to form UASGal-MATα in plasmid D1452. The 4-kb UASGal-MATα fragment from D1452 was isolated after digestion with HindIII and inserted into pRS306, a integrating vector that also contains the yeast URA3 gene. This plasmid was used to transform the rap1Δ strains. Stable Ura+ prototrophs were analyzed by Southern blotting to verify that the integration occurred at the MAT locus. Thus, these transformants contain a duplication at the MAT locus in which the UASGal–MATα construct is adjacent to the endogenous MATα locus. Transcription of the UASGal–MATα construct is constitutive in strains that contain a deletion of the GAL10 locus (YDS444, YDS447) and repressed in glucose-grown strains that contain a wild-type GAL10 gene (YDS558, see Fig. 6). Strains that express the UASGal–MATα construct produce wild-type levels of MATα1 and MATα2 transcripts at 25°C and at 33°C as determined by Northern blots.

Isolation and analysis of RNA

RNAs were isolated from aliquots of log-phase cultures maintained at 25°C, 30°C, 33°C, or 37°C for various times by breaking cells with a vortex blender with glass beads and with phenol and chloroform [Kurtz and Lindquist 1984]. RNAs were precipitated in ethanol and analyzed by Northern blotting with probes (labeled with [α-32P]dATP) containing RAP1-binding sites or ABFl-binding sites. Note that the RAP1-binding sites at the MATα locus in which the UASGal–MATα construct is adjacent to the endogenous MATα locus. Transcription of the UASGal–MATα construct is constitutive in strains that contain a deletion of the GAL10 locus (YDS444, YDS447) and repressed in glucose-grown strains that contain a wild-type GAL10 gene (YDS558, see Fig. 6). Strains that express the UASGal–MATα construct produce wild-type levels of MATα1 and MATα2 transcripts at 25°C and at 33°C as determined by Northern blots.

Gel mobility-shift assay

Extracts were prepared from strains grown in rich media at 25°C or at various times after a shift to 37°C. Cells were collected in a vortex blender with glass beads and with phenol and chloroform [Kurtz and Lindquist 1984]. RNAs were precipitated in ethanol and analyzed by Northern blotting with probes (labeled with [α-32P]dATP) containing RAP1-containing TEF2 or ABFl-binding sites. Note that the RAP1-binding sites at TEF2 and MATα are nearly identical matches to the consensus-binding site [see Buchman et al. 1988a]. These sites have binding affinities for RAP1 that are similar, and these sites compete similarly for RAP1 binding.

Acknowledgments

We thank Aaron Mitchell for many fruitful discussions and for comments on the manuscript; Mary Ann Osley and Art Lustig for comments on the manuscript; Janet Schultz for guidance with halo assays and for comments on the manuscript; Lori Sussel for comments and assistance with the preparation of figures; Dina Balderes for assistance with DNA sequence analysis; Dominique Giesman and Kelly Tatchell for communicating results prior to publication; George Sprague, Kelly Tatchell, Manny Ares, John Warner, Holly Hurd, Brian Gallay, and Kim Huang for strains and plasmids. This work was supported by grants from the National Institutes of Health (GM40094), Searle Scholar’s Program—Chicago Community Trust, the Irma T. Hirschl Caritable Trust, and the American Cancer Society [FRA-231] to D.S., and by a fellowship from the Jane Coffin Childs Memorial Fund for Cancer Research (to S.K.).

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Abraham, J., K.A. Nasmyth, J.N. Strathern, A.J.S. Klar, and J.B. Hicks. 1984. Regulation of mating-type information in yeast. J. Mol. Biol. 176: 307–331.
Ammerer, G., C.P. Hunter, J.H. Rothman, G.C. Saari, L.A. Valls, and T.H. Stevens. 1986. PEP4 gene of Saccharomyces cerevisiae encodes protease A, a vacuolar enzyme required for processing of vacuolar precursors. Mol. Cell. Biol. 6: 2490–2499.
Bankier, A.T., K.M. Weston, and B.G. Barrell. 1987. Random cloning and sequencing by the M13/dideoxynucleotide chain termination method. Methods Enzymol. 155: 51–92.
Beggs, J.D. 1978. Transformation of yeast by a replicating hybrid plasmid. Nature 285: 185–187.
Bender, A. and G.F. Sprague, Jr. 1987. MATα1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. Cell 50: 681–691.
Berman, J., C.Y. Tachibana, and B.-K. Tye. 1986. Identification of a telomere-binding activity from yeast. Proc. Natl. Acad. Sci. 83: 3713–3717.
Boeke, J.D., F. LaCroute, and G.R. Fink. 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. 197: 345–346.
Boeke, J.D., J. Trueheart, G. Natsoulis, and G.R. Fink. 1987. 5-Fluoro-orotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. 154: 164–175.
Brake, A., C. Brenner, R. Najariz, P. Laybourn, and J. Merry. 1985. Structure of genes encoding precursors of the yeast peptide mating pheromone a-factor. In Transport and secretion of proteins, pp. 103–108. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
Brand, A.H., G. Micklem, and K. Nasmyth. 1987. A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell 51: 709–721.
Brand, A.H., L. Breeden, J. Abraham, R. Sernglanz, and K. Nasmyth. 1985. Characterization of a “silencer” in yeast: A DNA sequence with properties opposite to those of a transcriptional enhancer. Cell 41: 41–48.
Buchman, A.R., W.J. Kimmerly, J. Rine, and R.D. Kornberg. 1988a. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in Saccharomyces cerevisiae. Mol. Cell. Biol. 8: 210–225.
Buchman, A.R., N.F. Lue, and R.D. Kornberg. 1988b. Connections between transcriptional activators, silencers, and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol. Cell. Biol. 8: 5086–5099.
Capeux, E., M.-L. Vignais, A. Sentenac, and A. Goffeau. 1989. The yeast H+ ATPase gene is controlled by the promoter binding factor TUF. J. Biol. Chem. 264: 7437–7446.
Role of RAP1 in cell-type determination

Chambers, A., C. Stanway, A.J. Kingsman, and S.M. Kingsman. 1988. The UAS of the yeast PGK gene is composed of multiple functional elements. Nucleic Acids Res. 16: 8245–8260.

Chan, R.K. 1977. Recovery of Saccharomyces mating type a cells from G1 arrest by a factor. J. Bacteriol. 130: 766–774.

Diamond, M.I., J.N. Miner, S.K. Yoshinaga, and K.R. Yamamoto. 1990. Transcription factor interactions: Selectors of positive or negative regulation from a single DNA element. Science 249: 1266–1272.

Difley, J.F.X. and B. Stillman. 1988. Purification of a yeast protein that binds to origins of DNA replication and a transcriptional silencer. Proc. Natl. Acad. Sci. 85: 2120–2124.

Dunn, B. 1989. “Telomerases of Saccharomyces cerevisiae.” Ph.D. thesis, Harvard University, Cambridge, Massachusetts.

Elledge, S.J. and R.W. Davis. 1989. Identification of the DNA damage-responsive element of RNR2 and evidence that four distinct cellular factors bind it. Mol. Cell. Biol. 9: 5373–5386.

Fassler, J.S. and F. Winston. 1989. The Saccharomyces cerevisiae SPT13/GAL11 gene has both positive and negative regulatory roles in transcription. Mol. Cell. Biol. 9: 5602–5609.

Giesman, D., L. Best, and K. Tatchell. 1991. Role of RAP1 in the regulation of the MATα locus. Mol. Cell. Biol. 11: 1089–1097.

Henry, Y.A.L., A. Chambers, J. Tsang, A.J. Kingsman, and S.M. Kingsman. 1990. Characterisation of the DNA binding domain of the yeast RAP1 protein. Nucleic Acids Res. 18: 2617–2623.

Herskowitz, I. 1989. A regulatory hierarchy for cell specialization in yeast. Nature 342: 749–757.

Hochstrasser, M. and A. Varshavsky. 1990. In vivo degradation of a transcriptional regulator: The yeast α2 repressor. Cell 61: 697–708.

Huet, J., P. Cottrelle, M. Cool, M.-L. Vignais, D. Theile, C. Marck, J.-M. Buhler, A. Sentenac, and P. Fromageot. 1985. A segment of positive regulatory silencer and UAS binding protein RAP1 in regulation of the yeast life cycle. Science 250: 549–553.

Ma, J. and M. Ptashne. 1987. The carboxy-terminal 30 amino acids of GAL4 are recognized by GAL80. Cell 50: 137–142.

Mahoney, D.J. and J.R. Broach. 1989. The HML mating-type cassette of Saccharomyces cerevisiae is regulated by two separate but functionally equivalent silencers. Mol. Cell. Biol. 9: 4621–4630.

Mann, C., J.-M. Buhler, I. Treich, and A. Sentenac. 1987. RPC40, a unique gene for a subunit shared between yeast RNA polymerases A and C. Cell 48: 627–637.

Miller, A.M. 1984. The yeast MATα1 gene contains two introns. EMBO J. 3: 1061–1065.

Miller, A.M. and K.A. Nasmyth. 1984. Role of DNA replication in the repression of silent mating-type loci in yeast. Nature 312: 247–251.

Mullen, J.R., P.S. Kayne, R.P. Moerschell, S. Tsunasawa, M. Griswold, M. Colavito-Shepanski, M. Grunstein, F. Sherman, and R. Stenånglanz. 1989. Identification and characterization of genes and mutants for a N-terminal acetyltransferase from yeast. EMBO J. 8: 2067–2075.

Nasmyth, K. and D. Shore. 1987. Transcriptional regulation in the yeast life cycle. Science 237: 1162–1170.

Nishizawa, M., Y. Araki, and Y. Teranishi. 1989. Identification of an upstream activating sequence and an upstream repressible sequence of the pyruvate kinase gene of the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. 9: 442–451.

Nishizawa, M., Y. Suzuki, Y. Nogi, K. Matsumoto, and T. Fukasawa. 1990. Yeast Gal11 protein mediates the transcriptional activation signal of two different transacting factors, Gal4 and general regulatory factor I/repressor/activator site binding protein I/translation upstream factor. Proc. Natl. Acad. Sci. 87: 5373–5377.

Passmore, S., R. Ebble, and B.-K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. Genes & Dev. 3: 921–935.

Pillus, L. and J. Rine. 1989. Epigenetic inheritance of transcriptional states in S. cerevisiae. Cell 59: 637–647.

Rine, J. and I. Herskowitz. 1987. Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. Genetics 93: 877–901.

Rose, M.D. and G.R. Fink. 1987. KARI, a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell 50: 1047–1060.

Rotenberg, M.O. and J.L. Woolford. [1986]. Tripartite upstream promoter element essential for expression of Saccharomyces cerevisiae ribosomal protein genes. Mol. Cell. Biol. 6: 674–687.

Sherman, F., G.R. Fink and J.B. Hicks. 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Shore, D. and K. Nasmyth. 1987. Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell 51: 721–732.

Shore, D., D.J. Stillman, A.H. Brand, and K.A. Nasmyth. 1987. identification of silencer binding proteins from yeast: Possible roles in SIR control and DNA replication. EMBO J.
Sikorski, R. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 122: 19–27.

Siliciano, P.G. and K. Tatchell. 1984. Transcription and regulatory signals at the mating type locus in yeast. *Cell* 37: 969–978.

Sprague, G.F., Jr. 1990. Combinatorial associations of regulatory proteins and the control of cell type in yeast. *Adv. Genet.* 27: 33–62.

Sprague, G. and I. Herskowitz. 1981. Control of yeast cell type by the mating type locus I. Identification and control of expression of the a-specific gene BAR1. *J. Mol. Biol.* 153: 305–321.

Strathern, J.N., J.B. Hicks, and I. Herskowitz. 1981. Control of cell type in yeast by the mating-type locus: The $a_1$-$a_2$ hypothesis. *J. Mol. Biol.* 147: 357–372.

Tan, S., G. Ammerer, and T.J. Richmond. 1988. Interactions of purified transcription factors: Binding of yeast MATa1 and PRTF to cell type-specific, upstream activating sequences. *EMBO J.* 7: 4255–4264.

Tatchell, K., K.A. Nasmyth, B.D. Hall, C. Astell, and M. Smith. 1981. In vitro mutation analysis of the mating type locus in yeast. *Cell* 27: 25–35.

Whiteway, M., R. Freeman, S. Van Arsdell, J. W. Szostak, and J. Thorner. 1987. The yeast ARD1 gene product is required for repression of cryptic mating-type information at the HML locus. *Mol. Cell. Biol.* 7: 3713–3722.
RAP1 protein activates and silences transcription of mating-type genes in yeast.

S Kurtz and D Shore

Genes Dev. 1991, 5:
Access the most recent version at doi:10.1101/gad.5.4.616