Molecular and Genetic Analysis of the Toxic Effect of *RAP1* Overexpression in Yeast

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Manuscript received May 18, 1995
Accepted for publication September 6, 1995

ABSTRACT

Rap1p is a context-dependent regulatory protein in yeast that functions as a transcriptional activator of many essential genes, including those encoding ribosomal proteins and glycolytic enzymes. Rap1p also participates in transcriptional silencing at *HM* mating-type loci and telomeres. Overexpression of *RAP1* strongly inhibits cell growth, perhaps by interfering with essential transcriptional activation functions within the cell. Here we report a molecular and genetic analysis of the toxic effect of *RAP1* overexpression. We show that toxicity does not require the previously defined Rap1p activation and silencing domains, but instead is dependent upon the DNA-binding domain and an adjacent region of unknown function. Point mutations were identified in the DNA-binding domain that relieve the toxic effect of overexpression. Two of these mutations can complement a *RAP1* deletion yet cause growth defects and altered DNA-binding properties *in vitro*. However, a small deletion of the adjacent (downstream) region that abolishes overexpression toxicity has, by itself, no apparent effect on growth or DNA binding. *SKO1/ACR1*, which encodes a CREB-like repressor protein in yeast, was isolated as a high copy suppressor of the toxicity caused by *RAP1* overexpression. Models related to the regulation of Rap1p activity are discussed.

THE transcriptional regulator Rap1p is an essential sequence-specific DNA-binding protein (SHORE and NASMYTH 1987). Rap1p-binding sites that behave as upstream activating sequences (UASs) are found within the promoters of the majority of ribosomal protein and glycolytic enzyme genes, and at many other genes, including the *MATα* mating-type genes (ROTENBERG and WOOLFORD 1986; SILICIANO and TATCHELL 1986; CHAMBERS *et al.* 1989; NISHIZAWA *et al.* 1989). Studies of temperature-sensitive (ts) *rap1* mutants provide direct proof that Rap1p functions as an activator, at least at the *MATα* locus (GIESMAN *et al.* 1991; KURTZ and SHORE 1991). Rap1p-binding sites are also found within the silencer elements at the *HM* mating-type loci (SHORE and NASMYTH 1987; SHORE *et al.* 1987; BUCHMAN *et al.* 1988a) and within the $C_{1-3}A$ repeats at telomeres (BERMAN *et al.* 1986; BUCHMAN *et al.* 1988a; LONGTINE *et al.* 1989; GILSON *et al.* 1993), where Rap1p plays a role in the repression of transcription (KURTZ and SHORE 1991; SUSSEL and SHORE 1991; KYRION *et al.* 1993).

The specific sequence of a Rap1p-binding site does not determine its function, since a site taken from a UAS element will participate in repression when placed at a silencer, and vice versa (BRAND *et al.*1987; SHORE and NASMYTH 1987; BUCHMAN *et al.* 1988b). Based upon these and other data it has been proposed that the context within which Rap1p binds DNA determines its function, via interactions with nearby DNA-binding proteins and other specific *trans*-acting regulators (SHORE 1994). For example, Rap1p and another DNA-binding protein, Gcr1p, act synergistically to activate transcription of many glycolytic enzyme genes (BAKER 1986; TORNOW and SANTANGELO 1990; BAKER 1991). At *HM* loci a Rap1p site is found in conjunction with an origin recognition complex (ORC)-binding site that is probably essential for the ability of Rap1p, in this context, to promote silencing (MAHONEY *et al.* 1991; RIVIER and RINE 1992; BELL *et al.* 1993; FOSS *et al.* 1993; MICKLEM *et al.* 1993). At silencers and telomeres Rap1p appears to function by recruiting a complex of Sir proteins to the chromosome through direct protein-protein interactions (MORETTI *et al.* 1994).

Three functional domains have been mapped in Rap1p by studies of mutant proteins and fusions of Rap1p to the Gal4p DNA-binding domain (SEE SHORE 1994) (Figure 1A). The first 279 amino acids of the protein can be deleted without affecting any known function of Rap1p (MORETTI *et al.* 1994). The DNA-binding domain has been mapped between amino acids 361 and 596 (HENRY *et al.* 1990; D. BALDERES and D. SHORE, unpublished results). The C-terminal portion of Rap1p contains both activation and silencing functions. An activation domain lies between amino acids 630 and 695, and a silencing domain maps to a partially overlapping region from amino acid 678 to the C-terminus of the protein, amino acid 827 (HARDY *et al.* 1992; BUCK and SHORE 1995). Isolation and characterization of a number of missense and truncation mutations in

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this C-terminal region confirmed that it plays an important role in *HM* locus silencing and is essential for telomere position effect (SUSSEL and SHORE 1991; KYRION *et al.* 1992; LIU *et al.* 1994; MORETTI *et al.* 1994). It is important to note that the activation and silencing domains of Rap1p are genetically separable and that mutations have been isolated that abolish silencing but have no effect on activation (SUSSEL and SHORE 1991).

We were interested in identifying proteins that determine the ability of Rap1p to function as an activator. The essential nature of Rap1p is presumably due, at least in part, to its role in the activation of ribosomal protein and glycolytic genes, which makes a standard genetic analysis of its activation properties difficult. Since the dominant-negative phenotype resulting from *RAP1* overexpression (D. SHORE, unpublished results; CONRAD *et al.* 1990) could be related to its normal essential functions, we decided to analyze this effect in some detail. Growth inhibition by excess Rap1p could be caused by a number of different mechanisms. (1) Increased expression of the Rap1p activation domain may cause squelching, or titration of essential general transcription factors, a phenomenon observed when strong activators such as Gal4p or VP16 are overexpressed (GILL and PTASHNE 1988; PTASHNE 1988; BERGER *et al.* 1990). (2) Increased Rap1p concentration could titrate a specific (and essential) Rap1p-interacting factor. (3) Excess Rap1p might lead to inappropriate DNA binding at low-affinity sites, which could interfere with normal promoter function. (4) Excess Rap1p could result in increased activation of a gene whose product blocks growth or is toxic, or it could cause inappropriate silencing of an essential gene.

MATERIALS AND METHODS

Media and strains: Yeast strains were grown according to standard procedures in media containing either 2% glucose or 2% galactose (ROSE *et al.* 1990). All strains were derived from W303-1A (THOMAS and ROTHSTEIN 1989). Nontoxic *rap1* alleles were selected in YKT124, a *MAT***a** haploid with the N-terminal *RAP1* deletion, *rap1-6* (MORETTI *et al.* 1994), and a Rap1p UAS(5G)-*HIS3* reporter integrated at the *URA3* locus (M. GWADZ and D. SHORE, unpublished results). The Rap1p UAS(5G) is a derivative of a "consensus" Rap1p binding site (GCACCCACACATT) (BUCHMAN *et al.* 1988b) from the *TEF2* promoter in which the C at position 5 is replaced by a G. This mutation abolishes expression of the linked *HIS3* gene as measured by the ability to grow in the absence of histidine (M. GWADZ and D. SHORE, unpublished results). Strains containing the nontoxic *RAP1* alleles, *rap1-32* and *rap1-34*, were constructed by plasmid shuffle of YKT126. YKT126 is a *MAT***a** haploid in which the chromosomal copy of *RAP1* is replaced with *LEU2*, removing all *RAP1* coding sequences up to amino acid 760, and that carries the Rap1p UAS(5G)-*HIS3* reporter at *URA3* and wild-type *RAP1* on a *sup4-o* CEN plasmid. For selection of suppressors of toxicity, *pGAL1:RAP1* was integrated at the *URA3* locus and *pGAL1:lacZ* (YOCUM *et al.* 1984) at the *LEU2* locus of W303-1A (YKT37). For growth assays, cells were grown in 2% glucose to midlog phase. Tenfold serial dilutions were spotted onto plates containing either 2% glucose or 2% galactose, as indicated.

Plasmids: *pGAL1:RAP1* plasmids were constructed by joining an *Eco*RI-*Bam*HI fragment carrying the *GAL1-10* promoter (JOHNSTON and DAVIS 1984) to *RAP1*, using a *Xho*I linker placed 20 nucleotides upstream of the *RAP1* ATG. This promoter fusion was then moved to the integrating plasmid pRS306 for strain constructions, the high copy plasmid pRS425 (D1260) for toxicity assays, or pRS423 (K643) for selection of nontoxic mutations (SIKORSKI and HIETER 1989). *RAP1* deletions were constructed by joining 5′ and 3′ exonuclease deletions (constructed by D. BALDERES) with a *Xho*I linker, thereby inserting four amino acids. These were introduced into the *pGAL1:RAP1* plasmid as *Pst*I/*Xba*I fragments. For selection of nontoxic *RAP1* alleles, the *rap1-35* allele was moved to the *pGAL1:RAP1* plasmid on a *Pst*I/*Bgl*II fragment (K475). The *rap1-35* allele was selected as one that allows the activation of the Rap1p UAS(5G)-*HIS3* reporter and will be described elsewhere (M. GWADZ and D. SHORE, unpublished results). Restriction fragments carrying the mutant alleles were used to replace the wild-type sequences in the plasmid pMG400, a pRS316-based plasmid carrying the *RAP1 Bgl*II-*Xba*I fragment with an *Nhe*I site introduced at nucleotide 1651. The exchanges were confirmed by DNA sequencing. The *pADH1:RAP1* plasmid was constructed as a gene fusion joining *ADH1*, from the plasmid AH10 (AMMERER 1983) to nucleotide 88 of *RAP1*. This fusion construct was then moved to the high copy vector, pRS425.

Selection of nontoxic RAP1 alleles: The *pGAL1:rap1-35* plasmid (K475) was mutagenized by passage through a *mutD5* bacterial strain (ECHOLS *et al.* 1983). Mutagenized plasmids were introduced into strain YKT124 and plated on glucose −Trp medium. Transformants were replica plated to glucose −His and galactose −His media. Those colonies that grew only on galactose −His were selected for further analysis. These strains were subjected to Western blot analysis, and plasmids were isolated from strains that maintained a high level of galactose-inducible Rap1p. The mutation on the plasmid was mapped by restriction fragment swaps with a wild-type plasmid, and the DNA sequence of the fragment conferring the mutant phenotype was determined. The nontoxic mutations were separated from the original *HIS3*-activating mutation by exchanging fragments with the wild-type plasmid, pMG400. These were expressed from either the *GAL1* promoter in K643 or the native *RAP1* promoter in pMG400. To create strains carrying only the mutant allele of *RAP1*, the strain YKT126 was transformed with mutant pMG400 plasmids, and the wild-type plasmid was removed by growth on medium containing canavanine (KURTZ and SHORE 1991).

Electrophoretic mobility shift assays: Electrophoretic mobility shift assays (band shift assays) were performed as previously described (KURTZ and SHORE 1991). The probe was an end-labeled *Hin*dIII-*Eco*RI fragment carrying a Rap1p-binding site from the *TEF2* promoter (TGTTGCACCCACACATTTA) (BUCHMAN *et al.* 1988b). The competitor DNA was the unlabeled parent pUC plasmid carrying this *Hin*dIII-*Eco*RI fragment. In each reaction the total amount of pUC DNA was kept constant at 450 ng by decreasing the amount of pUC vector without insert as the amount of specific (insert-containing) competitor DNA was increased. Each reaction also included 450 ng poly(dIdC), except in Figure 3A, where poly(dIdC) concentration decreases as competitor DNA increases and no pUC DNA is added to the reactions. Protein concentration was measured by the Bradford assay (BRADFORD 1976) and 2.5 μg of extract was used in each reaction.

Identification of high-copy number suppressors of Rap1p toxicity: A YEp24-based *Saccharomyces cerevisiae* genomic DNA library (CARLSON and BOTSTEIN 1982) was introduced into YKT37, and transformants were selected on plates containing glucose. Transformants were replica plated to galactose medium and colonies were identified that showed increased growth. Plasmid dependence for growth on galactose was as-

certained by removal of the library plasmid using 5-fluoroorotic acid selection (BOEKE *et al.* 1984). Finally, the expression level of the *pGAL1:lacZ* reporter was assayed in cells induced in galactose liquid medium (BREEDEN and NASMYTH 1987). Plasmids were isolated from cells that showed plasmid-dependent growth on galactose and no decrease in *pGAL1:lacZ* expression.

Western blot analysis: Cell extracts were prepared as for band shift assays. Fifty micrograms of extract were separated on 8% polyacrylamide, transferred to nitrocellulose and probed with a polyclonal antibody to Rap1p (SHORE and NASMYTH 1987). Amersham ECL was used, following manufacturer's directions, to detect the Rap1p-bound antibody.

RESULTS

Growth inhibition is caused by overexpression of the Rap1p DNA-binding domain and adjacent C-terminal sequences: As Rap1p is a multifunctional protein, able to either activate or repress transcription, it was of interest to determine if overexpression of one particular functional domain of Rap1p was associated with growth inhibition. We therefore constructed internal deletions within *RAP1* coding sequences fused to the *GAL1* promoter and assayed the ability of the resulting mutant proteins to inhibit cell growth on galactose plates, where their expression is induced to high levels (see MATERIALS AND METHODS). Deletion of the N-terminus, removing amino acids 43–345 (Δ43–345), had no effect on toxicity since overexpression of this deletion protein still inhibits cell growth (Figure 1B, rows 2 and 3). However, deletion of more C-terminal sequences, which infringe upon the DNA-binding domain (*e.g.*, Δ208–440), produced a nontoxic protein (Figure 1B, rows 4–6). Deletions within the C-terminus (from amino acids 670 to 806), which remove the silencing domain, had little, if any, effect on toxicity (Figure 1B, row 7). Surprisingly, larger C-terminal deletions, from amino acid 610, that removed both the activation and silencing domains or deletions that removed only the activation domain (*e.g.*, Δ610–669 and Δ622–701) also had no effect on growth inhibition (Figure 1B, rows 9–11). Thus, it would appear that neither the silencing nor the activation functions of Rap1p are required to mediate the toxic effect of overexpression. One exception to this generalization is the Δ619–806 mutant, which resulted in a nontoxic protein. As larger C-terminal deletions retain toxicity (Δ610–806), it is likely that the lack of toxicity of the Δ619–806 mutant is due either to a folding defect or to the masking of a toxic domain still present in the deletion.

Removal of C-terminal sequences past the defined activation domain (Δ599–806 and Δ570–806) produced proteins that were nontoxic when overexpressed (Figure 1B, rows 12, 13). Because a lack of toxicity could be caused by the absence of high levels of protein, each deletion construct was analyzed by Western blotting to confirm that the mutant Rap1p was present at the expected high levels and that the protein product was of the correct size (data not shown). Those deletion constructs that produced unstable proteins were discarded from the analysis and are not included in Figure 1. A number of internal deletions that mapped within the DNA-binding domain produced proteins that were not toxic when overexpressed, suggesting that overexpression of the DNA-binding domain is required for toxicity (Figure 1B rows 5, 6, 13, and 16). Interestingly, deletions removing a short region between the activation domain and DNA-binding domain (Δ595–616 and Δ598–616) and truncation of the C-terminus to amino acid 599 also resulted in proteins that were not toxic when overexpressed (Figure 1B, rows 14, 15, and 12). The smaller of these deletions (Δ598–616), which has a completely nontoxic phenotype, was also expressed from the *RAP1* promoter. This allele, *rap1-31,* was able to completely rescue a *RAP1* deletion strain, implying that all of its essential properties remain intact (data not shown). All three of these nontoxic proteins retain the ability to bind DNA *in vitro,* as assayed by band shift analysis (data not shown and Figure 3A). Row 18 shows that overexpression of a Rap1p protein with deletions of both the N-terminus (43–193 and 273–303) and the C-terminus (610–806) is sufficient to confer the toxic phenotype.

This series of *RAP1* deletions suggests that overexpression of the DNA-binding domain of Rap1p is necessary, but not sufficient, for growth inhibition, and that a domain adjacent to and possibly overlapping the DNA-binding domain is also required. Although one cannot rule out the possibility that the deletions adjacent to the DNA-binding domain have in some way altered protein folding, this would seem unlikely since the mutants can complement a deletion of *RAP1* and must therefore produce functional protein. These sequences could define a protein interaction domain, overlapping the DNA binding domain, through which the function of Rap1p is modulated. Alternatively, binding of Rap1p to DNA may be required to exert the toxic effect.

Isolation of nontoxic *RAP1* point mutations: In an attempt to define further the relationship between DNA binding and toxicity of overexpression, we sought to isolate point mutations in Rap1p that are capable of binding DNA but are no longer toxic. To avoid selection of mutants that destroy Rap1p, we used a strain containing a *HIS3* reporter gene whose expression is dependent upon an upstream Rap1p-binding site (M. GWADZ and D. SHORE, unpublished results). By selecting for expression of this *HIS3* reporter gene, we insured that some level of functional Rap1p protein was being produced. As *RAP1* is essential for viability, we needed to distinguish the overexpressed gene from the native *RAP1* gene. This was accomplished by using a mutant Rap1p-binding site upstream of the *HIS3* reporter gene [UAS(5G)], which results in a His^- phenotype in a *RAP1* wild-type strain, and a corresponding *RAP1* allele that is able to activate the mutant reporter gene, *rap1-35* (D to A change at position 543) (M. GWADZ and D. SHORE, unpublished results). Although

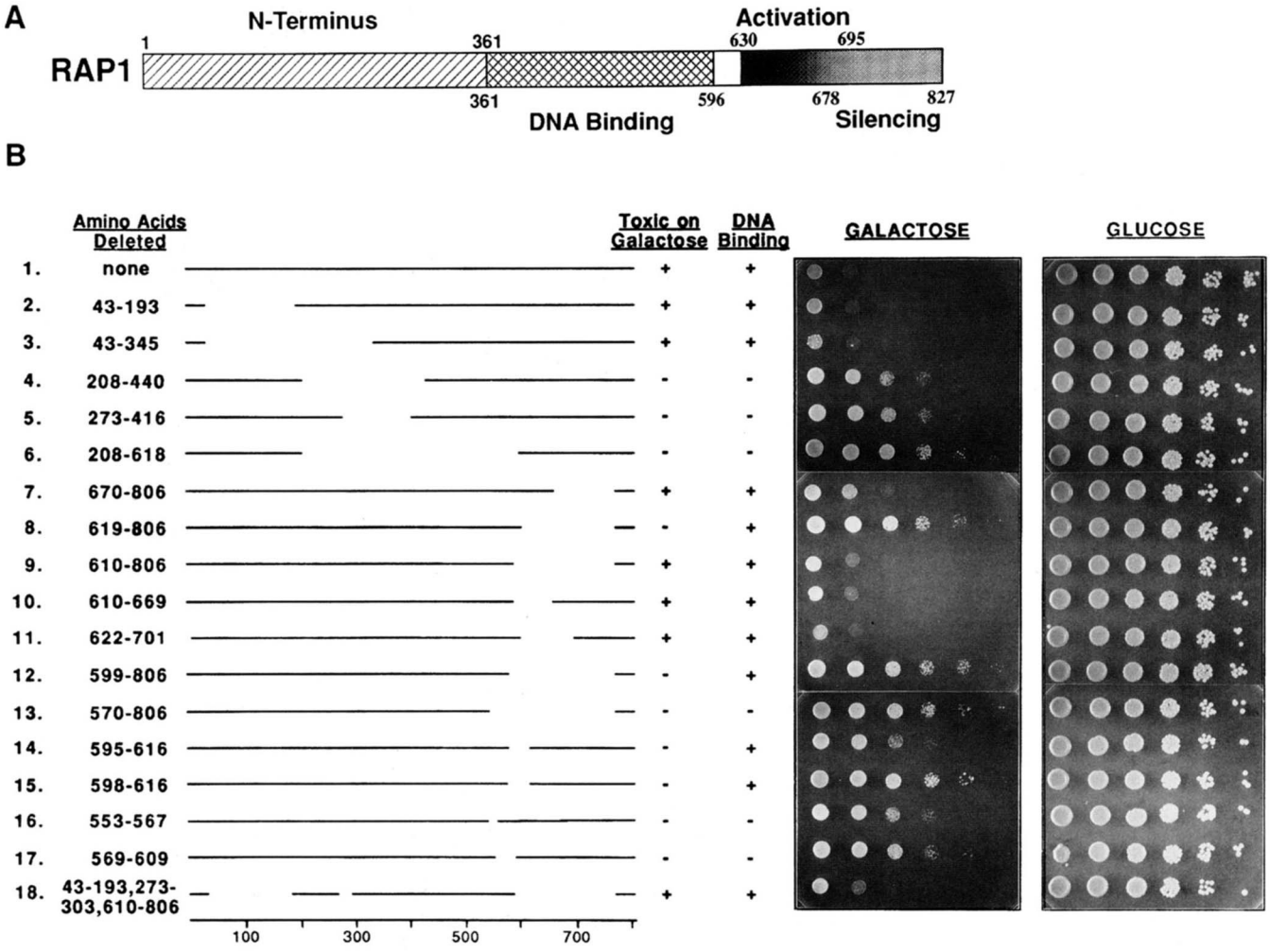


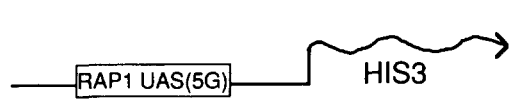
FIGURE 1.—Definition of a minimal toxicity domain. (A) Schematic showing Rap1p protein domains. (B) Amino acid deletions of Rap1p, created by joining 5′ and 3′ deletions with a *Xho*I linker, were expressed from the *GAL1* promoter carried on a 2-μm *LEU2* vector. Cells were grown to midlog phase in selective media containing glucose. Sets of 10-fold serial dilutions were spotted onto selective plates containing either galactose or glucose. DNA binding reflects the ability of each deletion construct to bind a Rap1p UAS based upon our results and previously published data (Figure 3A) (HENRY *et al.* 1990).

the *rap1-35* allele is able to activate the *HIS3* reporter construct, it still binds effectively to a wild-type Rap1p-binding site and is able to complement a *RAP1* deletion strain. When expressed at normal levels, the *rap1-35* mutant allele results in a His^+ phenotype in the reporter strain. However, when this mutant is overexpressed from the *GAL1* promoter, a toxic Rap1p product is produced and cells are therefore unable to grow on media lacking histidine and containing galactose as the sole carbon source (see Figure 2A).

Nontoxic *pGAL1:rap1-35* mutants were selected from a pool of mutagenized plasmids by their ability to grow on galactose plates lacking histidine but not on glucose plates without histidine (the inability to grow on glucose −His assures that the His^+ phenotype is plasmid linked). Potential nontoxic *rap1-35* mutants were examined by Western blot analysis to determine if the mutant proteins were in fact being overexpressed upon galactose induction. In most candidate mutant strains Rap1p protein levels were lower than that of the starting strain. These strains were therefore discarded from the analysis. Mapping and sequencing of the four remaining mutants, all of which contained high levels of the overexpressed protein, showed that they encoded single amino acid missense changes, all within the DNA-binding domain (Figure 2B).

Rap1 alleles were constructed that contained the nontoxic mutations separated from the initial *rap1-35* mutation. These mutant proteins were still nontoxic when expressed at high levels from the *GAL1* promoter but had lost the ability to activate the *HIS3* reporter, indicating that the selected nontoxic phenotype was independent of the *HIS3*-activating mutation. The nontoxic *RAP1* mutant alleles were then expressed from the native *RAP1* promoter and assayed for their ability to support growth in the absence of a wild-type chromosomal copy of *RAP1* (see MATERIALS AND METHODS for details). Two mutant alleles (*rap1-32* and *rap1-34*) were able to support growth, rescuing a *RAP1* deletion strain, whereas a third (*rap1-33*), which was isolated twice, pro-

A



	GLUCOSE - HIS	GALACTOSE - HIS
RAP1	-	-
rap1-35	+	+
pGAL1:RAP1	-	-
pGAL1:rap1-35	-	-
*pGAL1:rap1-35**	-	+

B

ALLELE	MUTATION	VIABILITY
RAP1	NONE	+++
rap1-32	TYR 455 CYS	+++ ts
rap1-33	LEU 549 PRO	+/-
rap1-34	MET 574 LYS	+

FIGURE 2.—Nontoxic *RAP1* mutations map within the DNA-binding domain. (A) Selection scheme: *rap1-35* activates the Rap1p UAS(5G)-*HIS3* reporter. When expressed from the *GAL1* promoter, no growth is observed on glucose plates due to glucose repression of the *GAL1* promoter or on galactose plates due to toxicity. Nontoxic mutations, *pGAL1:rap1-35**, were selected by their ability to restore growth on galactose −His. (B) Nontoxic mutations were mapped and sequenced; the amino acid change is listed for each isolate. *RAP1* deletion strains were rescued with *rap1, URA3,* CEN plasmids. *rap1-32*-rescued strains are temperature sensitive; *rap1-34*-containing strains grow slowly, and *rap1-33*, which was isolated twice, supports only very slow growth and exhibits high reversion rates.

duced only very small colonies that were prone to reversion. Strains rescued with the *rap1-32* allele are temperature sensitive and those rescued with *rap1-34* exhibit a growth rate somewhat slower than that of wild type. The ability to rescue a *RAP1* null strain strongly suggests that the mutant alleles (*rap1-32* and *rap1-34*) retain the ability to bind DNA and to promote transcription of Rap1p-activated genes. As shown in Figure 3B, band shift analysis confirmed that these mutants produce a protein capable of binding to a high affinity Rap1p binding site (referred to as the Rap1p UAS) oligonucleotide probe. However, both Rap1-32p and Rap1-34p bound less probe than did the wild-type protein (approximately nine- and fivefold less, respectively). The Rap1-34p mutant also behaved differently in that its binding to the radiolabeled probe was not competed by the same level of unlabeled probe that could efficiently compete for binding by the wild-type protein (Figure 3B). Western blot analysis showed that equal amounts of wild-type and Rap1-32p were present, while the level of Rap1-34p was slightly higher (data not shown).

To quantify more accurately the efficiency with which the mutant Rap1p proteins bound the Rap1p UAS, cells carrying a chromosomal copy of *RAP1* containing an N-terminal deletion (*rap1-6*) (MORETTI *et al.* 1994) were transformed with a single copy plasmid containing the nontoxic *rap1* alleles. In the resulting strains the binding efficiency of the mutant protein could be directly compared to that of the Rap1-6p since this truncated protein produces a protein-DNA complex of higher mobility on polyacrylamide gels (Figure 3A). We were surprised to find that the nontoxic mutant proteins showed a further decrease in their ability to bind the Rap1p UAS probe when they were expressed in cells containing the *rap1-6* truncation allele (Figure 3C), despite the fact that the binding was carried out in the presence of excess radiolabeled probe DNA. One explanation for these results is that there is a limiting Rap1p-interacting factor or modification that is required for Rap1p to efficiently bind DNA. Since the toxicity assay is performed in the presence of wild-type Rap1p, the nontoxic phenotype of these *rap1* alleles could be due to this decrease in DNA binding in the presence of wild-type Rap1p.

Increased levels of *SKO1/ACR1* suppress Rap1p toxicity: If the growth inhibition observed when *RAP1* is overexpressed were due to the titration of a protein that interacts with Rap1p, it should be possible to suppress this toxicity by increasing the level of the titrated protein. This suppression assay could provide a method for identifying factors that interact with Rap1p and modulate its function. Suppressors were isolated by transforming YKT37, a *pGAL1:RAP1*-containing strain, with a high copy yeast genomic library (CARLSON and BOTSTEIN 1982) and selecting transformants that were able to grow on galactose. These transformants were tested further by ascertaining that growth on galactose was dependent upon the addition of the 2-μm plasmid and by checking that expression of an integrated *pGAL1:lacZ* reporter gene was not decreased (YOCUM *et al.* 1984). From ~10,000 transformants screened, three plasmids containing overlapping inserts were identified that satisfied all of these criteria. The minimal complementing fragment contained a previously identified gene, called *SKO1* or *ACR1,* that was identified as a suppressor of toxicity caused by the overexpression of a cAMP dependent protein kinase (NEHLIN *et al.* 1992) and as a repressor in yeast that binds to (mammalian) cAMP response elements (CREs) (VINCENT and STRUHL 1992). *SKO1/ACR1* encodes a basic leucine-zipper (bZIP) protein similar to the mammalian CREB protein. However, there is as yet no direct evidence that CREs or *SKO1/ACR1* play a role in responding to cAMP in *S. cerevisiae.*

To determine whether *SKO1/ACR1* could suppress *RAP1* overexpression in a promoter-independent manner, we expressed *RAP1* from the strong *ADH1* promoter. Transformation efficiency with this plasmid was very low, suggesting that the cells could not tolerate the high level of Rap1p produced from the *ADH1* pro-

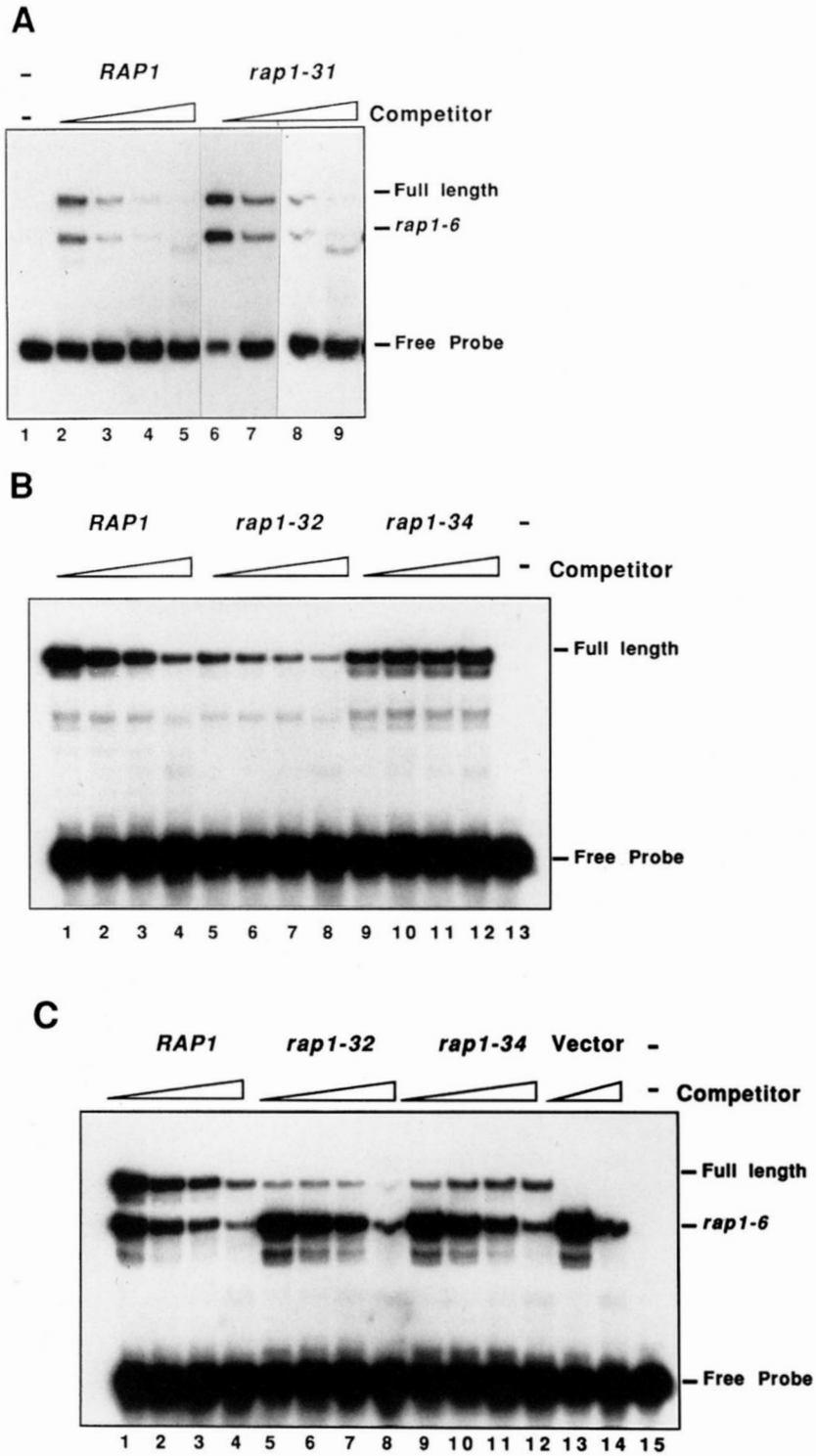


FIGURE 3.—Nontoxic Rap1p mutants are able to bind DNA specifically. (A) Bandshift assay of the nontoxic deletion mutant *rap1-31* using a high-affinity Rap1p-binding site. Extracts from cells containing chromosomal *rap1-6* and either wild-type *RAP1* or *rap1-31* (deletion of amino acids 598–616) on a single copy plasmid were prepared from log-phase cells. Competitor DNA: lane 1, probe only; lanes 2 and 6, 0 ng pUC:TEF2, 450 ng poly(dIdC); lanes 3 and 7, 50 ng pUC:TEF2, 400 ng poly(dIdC); lanes 4 and 8, 150 ng pUC:TEF2, 300 ng poly(dIdC); lanes 5 and 9, 450 ng pUC:TEF2, 0 ng poly(dIdC). (B) Bandshift assay of nontoxic rap1 mutant proteins using a high-affinity Rap1p-binding site. Extracts from cells containing wild-type *RAP1* (YKT126), *rap1-32* or *rap1-34* were prepared from log-phase cells. Competitor DNA: lanes 1, 5 and 9, 0 ng pUC:TEF2, 450 ng pUC; lanes 2, 6 and 10, 50 ng pUC:TEF2, 400 ng pUC; lanes 3, 7 and 11, 150 ng pUC:TEF2, 300 ng pUC; lanes 4, 8 and 12, 450 ng pUC:TEF2, 0 ng pUC; lane 13, probe only. (C) Gel mobility-shift assay of nontoxic *RAP1* mutant alleles in a strain carrying the Rap1p N-terminal truncation, *rap1-6* (YKT124) using the same Rap1p UAS probe as above. Extracts were prepared from cells containing wild-type *RAP1*, *rap1-32*, or *rap1-34* on a single copy plasmid or vector only. Competitor DNA as in B. Lane 13, 0 ng pUC:TEF2, 450 ng pUC; lane 14, 50 ng pUC:TEF2, 400 ng pUC; lane 15, probe only.

moter. Cotransformation with the *SKO1/ACR1* 2-μm plasmid resulted in an increased level of transformation, although the resulting colonies still grew at a rate slightly slower than that of the control cells, as judged by colony size (data not shown). Therefore, *SKO1/ACR1* is able to suppress the toxicity of Rap1p when it is overexpressed from either the *GAL1* or *ADH1* promoters, showing that suppression is not promoter specific.

Suppression by *SKO1/ACR1* might also result from a decrease in the amount of Rap1p present in the cell, due to some effect of *SKO1/ACR1* on *RAP1* at either a translational or posttranslational level. To address this possibility, we assayed Rap1p levels in various *RAP1* deletion strains by Western blot analysis in the presence or absence of increased dosage of *SKO1/ACR1*. As shown in Figure 4, the level of protein from both toxic and nontoxic deletion constructs remained the same in the presence of the increased *SKO1/ACR1* gene dosage.

Alleles of *RAP1* that are not suppressed by *SKO1/ACR1*: To determine whether *SKO1/ACR1* suppression

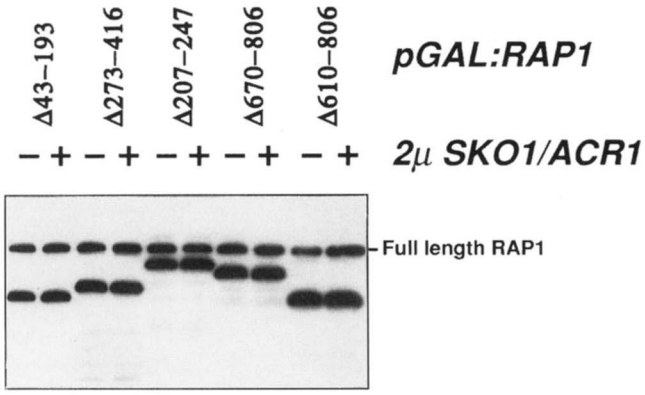


FIGURE 4.—Elevated *SKO1/ACR1* dosage does not affect Rap1p protein levels. Cells transformed with the indicated constructs carried on 2-μm plasmids were grown to early log-phase in 2% glucose, washed, and induced in 2% galactose for 4 hr. Fifty micrograms of extract were separated on 8% polyacrylamide, transferred to nitrocellulose, and probed with a polyclonal Rap1p antiserum. Rap1p-bound antibody was detected using Amersham ECL. Full-length Rap1p is indicated.

functioned by modulation of any particular region of Rap1p, we assayed the ability of *SKO1/ACR1* to suppress the toxic overexpression of the *RAP1* deletion constructs described previously. *SKO1/ACR1* was able to suppress the toxicity of *pGAL1:RAP1* constructs that were deleted for the N-terminus, the activation domain, and the silencing domain (Figure 5A). However, toxicity of the altered-specificity allele, *rap1-35,* used in the screen for nontoxic *RAP1* mutants was not suppressed by increased dosage of *SKO1/ACR1,* nor were *RAP1* mutant alleles with other amino acid substitutions at this position (Figure 5B). It is possible that these *RAP1* alleles have altered their ability to interact with the Rap1p UAS or with associated factors that regulate the ability of Rap1p to bind DNA. Further analysis of these mutant alleles may help to elucidate the mechanism by which *SKO1/ACR1* is able to suppress the toxicity of *RAP1* overexpression.

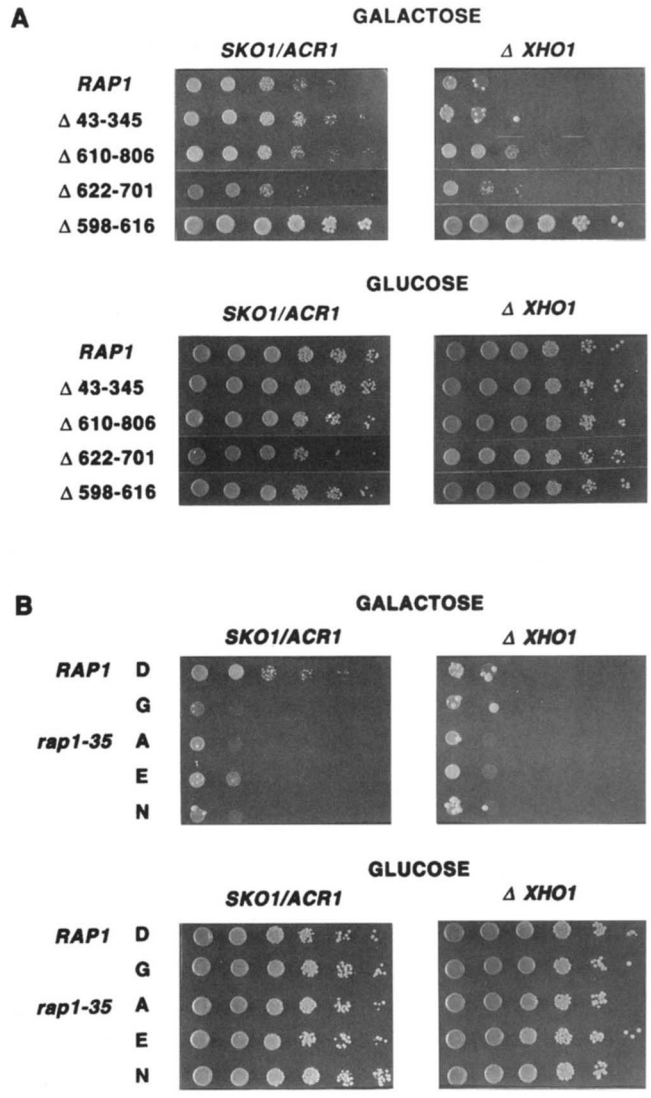


FIGURE 5.—(A) Toxicity of *RAP1* overexpression is suppressed by elevated levels of *SKO1/ACR1.* Wild-type cells were transformed with *pGAL1:RAP1* plasmids with the indicated amino acid deletions and either *SKO1/ACR1* on a 2-μm plasmid or a *Xho*I deletion of this plasmid that removes the *SKO1/ACR1* open reading frame (Δ *Xho*I). Cells were grown to log-phase in glucose-containing media. Tenfold serial dilutions were spotted onto selective media containing either galactose or glucose as indicated. (B) Mutations at amino acid 543 of Rap1p inhibit *SKO1/ACR1* suppression. Wild-type cells were transformed with *pGAL1:RAP1* plasmids that contained different amino acids at position 543 and either *SKO1/ACR1* on a 2-μm plasmid or Δ*Xho*I. Native Rap1p contains an aspartic acid at this position and rap1-35p an alanine. Cells were grown to log-phase in glucose containing media. Tenfold serial dilutions were spotted onto selective media containing either galactose or glucose as indicated.

DISCUSSION

Overexpression of the Rap1p DNA-binding domain and an adjacent region is necessary and sufficient for growth inhibition: We have shown that the Rap1p DNA-binding domain, in conjunction with a short, previously undefined domain immediately downstream of the DNA-binding domain, is sufficient to cause dominant-negative growth inhibition when overexpressed. Our results are in agreement with a previous report showing that removal of a large N-terminal region (amino acids 19–497), which disrupts the DNA-binding domain, renders the overexpressed protein nontoxic (CONRAD *et al.* 1990).

It is important to point out that the mechanism of growth inhibition by deletions containing the Rap1p DNA-binding domain and neighboring sequences could be different from that of the full-length overexpressed protein. However, several observations suggest that this is not the case and point to the DNA-binding domain and adjacent region as the cause of growth inhibition by both full-length and deletion proteins. First, viable point mutations that abolish toxicity in the context of full-length protein map to the DNA-binding domain (discussed in more detail below). Second, a deletion lacking the small region C-terminal to the

DNA-binding domain is still functional, as judged by its ability to restore normal growth to a *RAP1* deletion strain, yet is completely nontoxic. Finally, a large number of internal deletions that contain both the C-terminal activation and silencing domains, but impinge upon the DNA-binding domain and adjacent region from either the N-terminus or C-terminus, all result in a nontoxic phenotype.

Viable mutations within the Rap1p DNA-binding domain and adjacent region are nontoxic: The identification of point mutations within the Rap1p DNA-binding domain that abolish the toxic effect of overexpression provides further support for a direct role of this domain in growth inhibition. The fact that two of these mutations (*rap1-32* and *rap1-34*) support cell growth, clearly indicates that they produce proteins capable of recognizing Rap1p-binding sites *in vivo* and carrying out Rap1p's essential functions. Therefore, these properties are not by themselves sufficient for growth inhibition. Nonetheless, both the *rap1-32* and *rap1-34* mutants display growth defects, and extracts from these strains show clear alterations in Rap1p DNA binding. One simple interpretation of these results is that a slight decrease in DNA-binding affinity is sufficient to relieve growth inhibition by overexpressed protein. However, an additional observation suggests that this explanation may be at best incomplete. When these mutant proteins are examined in extracts of cells that also contain a truncated version of Rap1p that can be distinguished by its different mobility on bandshift gels, we find that the two nontoxic proteins display a further decrease in binding efficiency, even though binding is carried out under conditions of probe excess. This unusual observation suggests that the mutant proteins may not be able to compete as effectively as wild-type protein for interaction with another factor(s) that modulates Rap1p DNA binding, either by directly interacting with Rap1p or by covalently modifying it.

Perhaps the most obvious model to explain growth inhibition by excess Rap1p is that increased concentration of Rap1p leads to indiscriminate DNA binding with a consequent disruption of normal gene regulation. The identification of small deletion mutations immediately downstream of the Rap1p DNA-binding domain that abolish the toxic effect of overexpression, yet have no apparent affect on DNA binding, seems to be inconsistent with this model in its simplest form. We consider two possible explanations for these mutants. One possibility is that the mutations create a subtle defect in DNA binding or protein stability that we are unable to detect but that is sufficient to reduce the activity of overexpressed protein to a nontoxic level. Alternatively, this region, in combination with the adjacent DNA-binding domain, may form a specific protein-protein interaction domain important either for optimal DNA binding or transcriptional activation by Rap1p. If this were the case, toxicity of overexpression might be due to titration of such a putative Rap1p-interacting factor.

Suppression of Rap1p toxicity by elevated gene dosage of *SKO1/ACR1*: Several models can be proposed to explain the relief of growth inhibition by elevated gene dosage of *SKO1/ACR1*. One trivial explanation would be that elevated levels of Sko1p/Acr1p (a CREB-like repressor in yeast) (VINCENT and STRUHL 1992) repress the *GAL1* promoter that is used to drive the overexpression of *RAP1*. However, we detected no effect of *SKO1/ACR1* dosage either on β-galactosidase levels in cells containing a *pGAL1:lacZ* reporter gene or on the levels of *pGAL1*-driven Rap1p. In addition, we showed that increased *SKO1/ACR1* dosage also relieves the toxic effect of *RAP1* overexpression from the unrelated *ADH1* promoter. Lastly, we found that a class of *RAP1* alleles in which the amino acid at position 543 is changed is not suppressed by elevated levels of *SKO1/ACR1* dosage.

A second model to explain the gene dosage effect of *SKO1/ACR1* is based on the proposition that Sko1p/Acr1p is a Rap1p-interacting protein. In this case, elevated gene dosage of *SKO1/ACR1* might allow for the titration of excess Rap1p in such a way that it is no longer toxic. This model predicts that extremely high levels of Sko1p/Acr1p would itself be toxic, due to titration of Rap1p, and in fact such toxicity is observed (NEHLIN *et al.* 1992). However, we were unable to observe a specific interaction between Sko1p/Acr1p and Rap1p either *in vitro*, using GST/Sko1p fusions and radiolabeled Rap1p protein, or *in vivo*, using the two-hybrid system (FIELDS and SONG 1989; CHIEN *et al.* 1991).

Possible modification of Rap1p DNA binding or protein-protein interactions in response to growth conditions: Previous studies have suggested that the affinity of Rap1p for specific DNA-binding sites is regulated by growth conditions via phosphorylation (TSANG *et al.* 1990). When grown in glucose, conditions that induce ribosomal protein gene (RPG) expression, Rap1p was shown to be phosphorylated and to bind DNA more efficiently than when grown in pyruvate, a noninducing carbon source for RPG expression. Other groups have proposed that the DNA-binding capacity of Rap1p is not changed by upshift in carbon source but rather that the activation potential of Rap1p is induced, possibly by interactions with additional factors (KRAAKMAN *et al.* 1993; KLEIN and STRUHL 1994). In addition, deletion of the gene encoding the cAMP-dependent protein kinase regulatory subunit, *BCY1*, results in a Rap1p binding site-dependent increase in RPG expression and in an increase in Rap1p transcriptional activation potential (KLEIN and STRUHL 1994). Therefore, changes in the levels of cAMP, which are usually associated with shifts to glucose growth, can alter the ability of Rap1p to activate RPG transcription (THEVELEIN and BEULLENS 1985; MBONYI *et al.* 1988). The majority of Rap1p-binding sites found in RPG promoters are quite weak with respect to the highest affinity Rap1p-binding sites selected by affinity binding (NIEUWINT *et al.* 1989; GRAHAM and CHAMBERS 1994). Upon carbon upshift, Rap1p

may be induced to bind these sites with higher affinity, or its transcriptional activation potential may be increased. If *SKO1/ACR1* in fact plays a role in mediating the cells response to cAMP, this could be a mechanism by which it is able to suppress Rap1p toxicity.

We suggest that the domain of Rap1p defined here, which overlaps the DNA-binding domain, is involved in modulating the activity of Rap1p. We speculate that this domain is subject to reversible posttranslational modification, most likely phosphorylation/dephosphorylation, that regulates DNA binding, interactions with other proteins, or both. Such effects may play an important role in regulating the activity of Rap1p in response to growth conditions.

We thank the members of the SHORE lab and HANS RONNE for helpful discussions, GEORGE LIVI and DAVID FIGURSKI for comments on the manuscript, and MARIAN CARLSON for the gift of plasmid libraries. This work was supported by grants from the National Institutes of Health (NIH) (GM-40094) and the National Science Foundation (MCB-9220535) to D.S. K.F. was supported as a postdoctoral fellow by a NIH Cancer Biology Grant (CA09503-0) and by a Natonal Research Service Award Institutional Training Grant (5T32AI07161) to the Department of Microbiology.

LITERATURE CITED

AMMERER, G., 1983 Expression of genes in yeast using the *ADC1* promoter. Methods Enzymol. **101:** 192–201.

BAKER, H. V., 1986 Glycolytic gene expression in *Saccharomyces cerevisiae:* nucleotide sequence of *GCR1,* null mutants, and evidence for expression. Mol. Cell. Biol. **6:** 3774–3784.

BAKER, H. V., 1991 GCR1 of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTTCC sequence motif. Proc. Natl. Acad. Sci. USA **88:** 9443–9447.

BELL, S. P., R. KOBAYASHI and B. STILLMAN, 1993 Yeast origin recognition complex functions in transcription silencing and DNA replication. Science **262:** 1844–1849.

BERGER, S. L., W. D. CRESS, A. CRESS, S. J. TRIEZENBERG and L. GUARENTE, 1990 Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61:** 1199–1208.

BERMAN, J., C. Y. TACHIBANA and B.-K. TYE, 1986 Identification of a telomere-binding activity from yeast. Proc. Natl. Acad. Sci. USA **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.

BRADFORD, M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:** 248–254.

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:** 709–719.

BREEDEN, L., and K. NASMYTH, 1987 Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. Cell **48:** 389–397.

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.

BUCK, S. W., and D. SHORE, 1995 Action of a RAP1 carboxy-terminal silencing domain reveals an underlying competition between HMR and telomeres in yeast. Genes Dev. **9:** 370–384.

CARLSON, M., and D. BOTSTEIN, 1982 Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:** 145–154.

CHAMBERS, A., J. S. H. TSANG, C. STANWAY, A. J. KINGSMAN and S. M. KINGSMAN, 1989 Transcriptional control of the *Saccharomyces cerevisiae PGK* gene by RAP1. Mol. Cell. Biol. **9:** 5516–5524.

CHIEN, C.-T., P. L. BARTEL, R. STERNGLANZ and S. FIELDS, 1991 The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:** 9578–9582.

CONRAD, M. N., J. H. WRIGHT, A. J. WOLF and V. A. ZAKIAN, 1990 RAP1 protein interacts with yeast telomeres *in vivo:* overproduction alters telomere structure and decreases chromosome stability. Cell **63:** 739–750.

ECHOLS, H., C. LU and P. M. J. BURGERS, 1983 Mutator strains of *Escherichia coli,* mutD and dnaQ, with defective exonucleotide editing by DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **80:** 2189–2192.

FIELDS, S., and O. K. SONG, 1989 A novel genetic system to detect protein-protein interactions. Nature **340:** 245–246.

FOSS, M., F. J. MCNALLY, P. LAURENSON and J. RINE, 1993 Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae.* Science **262:** 1838–1844.

GIESMAN, D., L. BEST and K. TATCHELL, 1991 The role of RAP1 in the regulation of the *MAT*alpha locus. Mol. Cell. Biol. **11:** 1069–1079.

GILL, G., and M. PTASHNE, 1988 Negative effect of the transcriptional activator GAL4. Nature **334:** 721–724.

GILSON, E., M. ROBERE, R. GIRALDO, D. RHODES and S. M. GASSER, 1993 Distortion of the DNA double helix by RAP1 at silencers and multiple telomeric binding sites. J. Mol. Biol. **231:** 293–310.

GRAHAM, I. R., and A. CHAMBERS, 1994 Use of a selection technique to identify the diversity of binding sites for the yeast RAP1 transcription factor. Nucleic Acids Res. **22:** 124–130.

HARDY, C. J., D. BALDERES and D. SHORE, 1992 Dissection of the carboxy-terminal region of the yeast regulatory protein RAP1 with effects on both transcripitonal activation and silencing. Mol. Cell. Biol. **12:** 1209–1217.

HENRY, Y. A. L., A. CHAMBERS, J. S. H. TSANG, A. J. KINGSMAN and S. M. KINGSMAN, 1990 Characterization of the DNA binding domain of the yeast RAP1 protein. Nucleic Acids Res. **18:** 2617–2623.

JOHNSTON, M., and R. W. DAVIS, 1984 Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **4:** 1440–1448.

KLEIN, C., and K. STRUHL, 1994 Protein kinase A mediates growth-regulated expression of yeast ribosomal protein genes by modulating RAP1 transcriptional activity. Mol. Cell. Biol. **14:** 1920–1928.

KRAAKMAN, L. S., G. GRIFFIOEN, S. ZERP, P. GROENEVELD, J. M. THEVELEIN *et al.,* 1993 Growth-related expression of ribosomal protein genes in *Saccharomyces cerevisiae.* Mol. Gen. Genet. **239:** 196–204.

KURTZ, S., and D. SHORE, 1991 The RAP1 protein activates and silences transcription of mating-type genes in yeast. Genes Dev. **5:** 616–628.

KYRION, G., K. A. BOAKYE and A. J. LUSTIG, 1992 C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **12:** 5159–5173.

KYRION, G., K. LIU, C. LIU and A. J. LUSTIG, 1993 RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae.* Genes Dev. **6:** 1146–1159.

LIU, C., X. MAO and A.J, LUSTIG, 1994 Mutational analysis defines a C-terminal domain of RAP1 essential for telomeric silencing in *Saccharomyces cerevisiae.* Genetics **138:** 1025–1040

LONGTINE, M. S., N. M. WILSON, M. E. PETRACEK and J. BERMAN, 1989 A yeast telomere binding activity binds to two related telomere sequence motifs and is indistinguishable from RAP1. Curr. Genet. **16:** 225–240.

MAHONEY, D. J., R. MARQUARDT, G.-J. SHEI, A. B. ROSE and J. R. BROACH, 1991 Mutations in the *HML* E silencer of *Saccharomyces cerevisiae* yield metastable inheritance of transcriptional repression. Genes Dev. **5:** 605–615.

MBONYI, K., M. BEULLENS, K. DETREMERIE, L. GEERTS and J. M. THEVELEIN, 1988 Requirement of one functional *RAS* gene and inability of an oncogenic *ras* variant to mediate the glucose-induced cyclic AMP signal in the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol. **8:** 3051–3057.

MICKLEM, G., A. ROWLEY, J. HARWOOD, K. NASMYTH and J. F. X. DIFFLEY, 1993 Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. Nature **366:** 87–89.

MORETTI, P., K. FREEMAN, L. COODLY and D. SHORE, 1994 Evidence that a complex of SIR proteins interacts with the silencer and telomere binding protein RAP1. Genes Dev. **8:** 2257–2269.

NEHLIN, J. O., M. CARLBERG and H. RONNE, 1992 Yeast *SKO1* gene encodes a bZIP protein that binds to the CRE motif and acts as a repressor of transcription. Nucleic Acids Res. **20:** 5271–5278.

NIEUWINT, R. T. M., W. H. MAGER, K. C. T. MAURER and R. J. PLANTA, 1989 Mutational analysis of the upstream activation site of yeast ribosomal protein genes. Curr. Genet. **15:** 247–251.

NISHIZAWA, M., R. 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.

PTASHNE, M., 1988 How eukaryotic transcriptional activators work. Nature **335:** 683–689.

RIVIER, D. H., and J. RINE, 1992 An origin of DNA replication and a transcriptional silencer require a common element. Science **256:** 659–663.

ROSE, M. D., F. WINSTON and F. HIETER, 1990 *Methods in Yeast Genetics: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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

SHORE, D., 1994 RAP1: a protean regulator in yeast. Trends Genet. **10:** 408–412.

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. **6:** 461–467.

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, 1986 Identification of the DNA sequences directing the expression of the *MAT*à locus of yeast. Proc. Natl. Acad. Sci. USA **83:** 2320–2324.

SUSSEL, L., and D. SHORE, 1991 Separation of transcriptional activation and silencing functions of the *RAP1*-encoded repressor/activator protein 1: isolation of viable mutants affecting both silencing and telomere length. Proc. Natl. Acad. Sci. USA **88:** 7749–7753.

THEVELEIN, J., and M. BEULLENS, 1985 Cyclic AMP and the stimulation of trehalose activity in the yeast *Saccharomyces cerevisiae* by carbon sources, nitrogen sources, and inhibitors of protein synthesis. J. Gen. Microbiol. **131:** 3199–3209.

THOMAS, B. J., and R. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

TORNOW, J., and G. M. SANTANGELO, 1990 Efficient expression of the *Saccharomyces cereviciae* glycolytic gene *ADH1* is dependent upon a *cis*-acting regulatory element (*UASRPG*) found initially in genes encoding ribosomal proteins. Gene **90:** 79–85.

TSANG, J., Y. HENRY, A. CHAMBERS, A. KINGSMAN and S. KINGSMAN, 1990 Phosphorylation influences the binding of the yeast RAP1 protein to the upstream activating sequences of the *PGK* gene. Nucleic Acids Res. **18:** 7331–7337.

VINCENT, A. C., and K. STRUHL, 1992 ACR1, a yeast ATF/CREB Repressor. Mol. Cell. Biol. **12:** 5394–5405.

YOCUM, R. R., S. HANLEY, R. WEST JR. and M. PTASHNE, 1984 Use of *lacZ* fusions to delimit regulatory elements of the inducible divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **4:** 1985–1998.

Communicating editor: M. JOHNSTON