MOLECULAR AND CELLULAR BIOLOGY, Aug. 1996, p. 4281–4294 Vol. 16, No. 8
0270-7306/96/$04.00+0

SUM1-1, a Dominant Suppressor of *SIR* Mutations in *Saccharomyces cerevisiae*, Increases Transcriptional Silencing at Telomeres and *HM* Mating-Type Loci and Decreases Chromosome Stability

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Received 7 November 1995/Returned for modification 11 December 1995/Accepted 13 May 1996

Transcriptional silencing in the yeast *Saccharomyces cerevisiae* occurs at *HML* and *HMR* mating-type loci and telomeres and requires the products of the silent information regulator (*SIR*) genes. Recent evidence suggests that the silencer- and telomere-binding protein Rap1p initiates silencing by recruiting a complex of Sir proteins to the chromosome, where they act in some way to modify chromatin structure or accessibility. A single allele of the *SUM1* gene (*SUM1-1*) which restores silencing at *HM* loci in strains mutant for any of the four *SIR* genes was identified a number of years ago. However, conflicting genetic results and the lack of other alleles of *SUM1* made it difficult to surmise the wild-type function of *SUM1* or the manner in which the *SUM1-1* mutation restores silencing in *sir* mutant strains. Here we report the cloning and characterization of the *SUM1* gene and the *SUM1-1* mutant allele. Our results indicate that *SUM1-1* is an unusual altered-function mutation that can bypass the need for *SIR* function in *HM* silencing and increase repression at telomeres. A *sum1* deletion mutation has only minor effects on silencing in *SIR* strains and does not restore silencing in *sir* mutants. In addition to its effect on transcriptional silencing, the *SUM1-1* mutation (but not a *sum1* deletion) increases the rate of chromosome loss and cell death. We suggest several speculative models for the action of *SUM1-1* in silencing based on these and other data.

Cytological studies have long suggested that eukaryotic genomes are organized into two distinct types of functional domains that can influence states of gene expression (reviewed in reference 40). In general, lightly staining euchromatic regions contain transcriptionally active or potentially active genes and are replicated early during S phase. In contrast, heterochromatic regions, where chromatin appears more condensed, are typically transcriptionally inactive and late replicating. The repressive effect of heterochromatin has been known and studied genetically for many years: transposition of euchromatic genes to regions next to heterochromatin can result in variable but heritable repression of the euchromatic gene, a phenomenon known as position-effect variegation (reviewed in reference 25). Position-effect variegation not only provides a means to study the nature of heterochromatin but may also reveal ways in which stable transcriptional states are normally established in euchromatic genes (28, 49).

A well-characterized example of position effect, in which the expression of a gene depends on its location in the chromosome, occurs in the yeast *Saccharomyces cerevisiae* at the silent mating-type loci *HMR* and *HML* (reviewed in reference 38). Mating type in this yeast is determined by the information present at the *MAT* locus, near the centromere of chromosome III. The *MAT***a** and *MAT*α alleles encode transcription factors that control the expression of cell-type specific genes, thereby imparting the **a** and α mating phenotypes of haploid cells and the nonmating, sporulation-proficient phenotype of **a**/α diploids. Yeast cells typically have additional copies of **a** and α information stored at loci called *HMR* and *HML*, respectively. These loci, found near the right and left telomeres of chromosome III, are transcriptionally repressed but can be transposed to the active *MAT* locus by a gene conversion event called mating-type switching. Mating-type genes at the *HM* loci (*HML* and *HMR*) contain all of the promoter sequences required for their expression and in fact can become active when any of four *SIR* (silent information regulator) genes are mutated (54).

Repression of the *HM* loci requires a number of *trans*-acting factors (including the four *SIR* genes) and *cis*-acting sequences that flank these regions, called silencers (1, 6, 17). The *HMR-E* silencer is found to the left of the *HMR* locus and is both necessary and sufficient for repression (6). *HMR-E* consists of three partially redundant regulatory sites (A, E, and B), which are binding sites for the origin recognition complex (4), Rap1p (9, 62, 63), and Abf1p (15). Genetic studies have demonstrated a direct role for both the origin recognition complex and Rap1p in silencing at *HMR* (3, 18, 34, 46, 68). Position-effect repression of genes at *HM* loci probably results from a modification of chromatin structure, since it requires the highly conserved N-terminal tails of histones H3 and H4 (29, 45, 51, 71). Repression by the *HMR* silencers is not specific to mating-type genes and can also affect RNA polymerase III transcription (7, 43, 61).

Telomeres in yeast cells are also subject to a position effect similar in many respects to that observed at *HM* loci. Genes placed near a telomere can be transcriptionally repressed (21) by a mechanism that requires *RAP1*, three of the four *SIR* genes (*SIR2*, *SIR3*, and *SIR4*) and the N-terminal tails of histones H3 and H4 (2, 36, 47, 71). The *SIR1* gene, which is required for stable silencing at *HM* loci, appears to play no role in the transcriptional repression of telomere-linked genes (2). Perhaps for this reason, transcriptional silencing at telomeres is normally unstable, resembling position-effect variegation in multicellular eukaryotes such as *Drosophila melanogaster*.

The role of *SIR* genes in silencing has been investigated by

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the isolation and characterization of extragenic suppressors of *sir* mutations (31, 60). One such suppressor gene is *SUM1*, a single allele of which (*SUM1-1*) was isolated as a suppressor of *mar1-1* (*MAR1* is allelic to *SIR2* [31]). *SUM1-1* restores mating to *sir2* strains by restoring transcriptional repression at *HML* and *HMR* (41). *SUM1-1* is unique among suppressors of *SIR* mutations because it is able to suppress mutations in all of the *SIR* genes (37) and thus appears to bypass the requirement for *SIR* gene function in silencing. Significantly, though, *SUM1-1* still requires some *cis* and *trans* elements normally involved in silencing, since it will not bring about repression in strains carrying a deletion of the histone H4 N terminus or a deletion of the *HMR-E* silencer (37).

Initial studies indicated that the *SUM1-1* allele is recessive (31). However, subsequent work using a different strain background showed that the *SUM1-1* allele can be dominant to wild-type *SUM1* (37). These two contrary results have led to different interpretations of the nature of the mutant phenotype and the function of the wild-type protein. Where the mutation was found to be recessive, it was proposed that *SUM1-1* is a loss-of-function allele in a gene encoding an activator required for transcription at *HM* loci but not at *MAT*. In this model, the *SIR* genes were proposed to encode negative regulators of *SUM1*. One problem with this model is that it fails to explain why the *HM* loci should require a special activator (*SUM1*) not needed for transcription from the *MAT* locus. Furthermore, though loss-of-function mutations might be expected to arise frequently, *SUM1-1* was the only allele isolated from a heavily mutagenized culture (31). On the other hand, where *SUM1-1* appeared to be dominant to wild type, it was proposed to be a gain-of-function or altered-function mutation (37). In this case, it was imagined that *SUM1* might encode a component of the repressed chromatin structure at the *HM* loci or an assembly factor involved in its formation. The *SUM1-1* mutation might then alter or increase the protein's function such that *SIR* gene products would no longer be required to assemble repressed chromatin at *HM* loci.

We report here the cloning and characterization of the *SUM1-1* and *SUM1* alleles. Our results show that *SUM1* is involved in both *HM* locus silencing and telomeric position effect. However, we rule out the model that *SUM1* is an activator required for expression of *HM* loci, since a *sum1* null mutation allows full expression from the silent mating-type loci in combination with *sir* mutations. Instead, our results are consistent with the proposition that the *SUM1-1* allele is a partially dominant altered-function mutation that bypasses the need for *SIR* function at *HM* loci and increases repression at telomeres in *SIR* wild-type strains. Interestingly, the *SUM1-1* mutation, but not a *sum1* deletion, increases the rate of cell death and chromosome loss. As expected for a protein directly involved in transcriptional silencing, the *SUM1* gene product is localized to the nucleus. Our results suggest possible molecular models for the function of *SUM1* and *SUM1-1* in both *HM* repression and telomeric silencing.

MATERIALS AND METHODS

Construction of genomic libraries from *SUM1-1* strains. Total genomic DNA from yeast strains JRY2465 and JRY2466 was partially digested with *Sau*3AI. DNA was separated on agarose gels, and fragments within the range of 10 to 16 kb were recovered by electroelution. YCp50 (a *URA3 CENIV* vector [56]) was cleaved with *Bam*HI, which is within the tetracycline resistance gene, and dephosphorylated with calf intestinal phosphatase. This vector DNA was then mixed at a roughly 2:1 ratio (by weight) with the size-fractionated genomic DNA, and the mixture was ligated at a total DNA concentration of either 12 or 25 ng/μl. Ligation mixtures were used to transform *Escherichia coli* DH5α (22) by electroporation. Transformants were collected by scraping cells from the surface of the plates, using LB broth, and pooled. Four libraries were generated, each containing more than 20,000 independent transformants. More than 97% of the plasmids contain inserted yeast DNA, as judged by tetracycline sensitivity of DH5α transformants.

Yeast strains, media, and genetic methods. The genotypes of the yeast strains described in this paper are listed in Table 1. All yeast genetic manipulations were performed as described previously (57). Yeast transformations were performed by the lithium acetate method (26). Mating-type tests of patches of cells were performed as described previously (54), using the tester strains YDS31 (*MAT***a**) and YDS32 (*MAT*α), unless otherwise indicated. Quantitative mating assays were performed as described previously (66). Mating efficiency was calculated as the number of diploid cells (prototrophs) formed divided by the number of viable cells added to the tester strain. The reported efficiencies represent the mean of three independent assays per strain.

Yeast spot assays for tryptophan or uracil prototrophy or 5-fluoro-orotic acid (FOA) resistance (5) were done as follows. Overnight cell cultures were five times serially diluted by a factor of 10. Each dilution (5 μl) was transferred to either control or test plates, and the cells were allowed to grow at 30°C for 2 days before the plates were photographed. Colony-forming ability was assayed by micromanipulating individual cells from overnight liquid cultures onto YEPD agar plates, which were then incubated for 5 days at 30°C.

Plasmids. Marking of the wild-type *SUM1* allele for linkage analysis was done with plasmid DM268, in which a 1.2-kb *Pvu*II-*Spe*I fragment from the *SUM1-1* allele was cloned into pRS405, a *LEU2*-containing integrating plasmid (64). The plasmid was linearized by cleavage within the insert (*Sma*I) and used for yeast transformation. Note that through this integration process, the C terminus of the predicted *SUM1* open reading frame (ORF) was truncated at codon 906. The *sum1*::*URA3* mutation was constructed by inserting a *Hin*dIII fragment containing the *URA3* gene into the *Spe*I sites of an *Avr*II fragment containing the complete *SUM1* gene. This results in the removal of the entire predicted *SUM1* coding region. The *sum1*::*URA3* allele was subcloned into pBluescript II, creating plasmid DM264, and was released by digestion with both *Hin*dIII and *Xba*I before yeast transformation. *sum1*::*LEU2* (DM286) contains the *Nsi*I-*Bgl*II fragment of the *SUM1* gene in pRS405, an integrating vector. The plasmid was used for yeast transformation after cleavage within the *SUM1* insert at a unique *Nru*I site. The integration creates a disruption by the *LEU2*-containing vector with a partial duplication (*Nsi*I-*Bgl*II fragment) of the *SUM1* coding sequence. (The resulting two *SUM1* gene fragments consist of [i] the start codon to codon 581 and [ii] codon 340 to the end of the gene.)

The *SUM1* gene was tagged with an epitope from the influenza virus hemagglutinin (HA) protein for immunofluorescence studies. A 4.9-kb *Avr*II fragment containing the wild-type *SUM1* gene was cloned into the multicopy vector pRS425 (13), creating plasmid DM383, and a *Not*I site was generated just 5′ to the termination codon of *SUM1* by PCR mutagenesis. The *Not*I fragment from plasmid GTEP, encoding an HA triple-epitope tag (72), was inserted to create DM651. The HA-tagged *SUM1* allele was tested for complementation in MC33, as well as expression of the tagged protein on immunoblots, before being examined by immunofluorescence microscopy.

Construction of isogenic *SUM1-1* strains. To replace the *SUM1* gene with the mutant *SUM1-1* allele, we first replaced the wild-type gene with the *sum1*::*URA3* deletion/insertion by one-step gene disruption (58). Subsequently, an *Avr*II fragment containing the *SUM1-1* allele was cotransformed with the 2μm-*LEU2* plasmid pRS425 (13) into the *sum1*::*URA3* strains. Leu^+ transformants were replica plated to FOA plates to select for those cells in which the *SUM1-1* allele had replaced the *sum1*::*URA3* mutation. The putative *SUM1-1* strains were further characterized by Southern blotting analysis with multiple restriction digests to confirm that the *SUM1-1* fragment had replaced the normal *SUM1* locus. Strains MC54, MC57, MC105, and MC113 were constructed accordingly. Other *SUM1-1* strains were derived from crosses with these four parental *SUM1-1* strains. The *SUM1-1* genotype of segregants derived from a *SUM1-1* heterozygous diploid was scored by the following criteria: slow growth, loss of a *Bgl*II restriction site present in the wild-type allele, and the ability to suppress mating defects of *MAT*α *sir2* strains.

Chromosome stability assays. The rates of mitotic chromosome loss at chromosomes III and V were assayed in diploid strains (MC105 × MC113 and GA224 × MCY2675), using fluctuation analysis, as described previously (52). Briefly, individual colonies of independent diploids were grown at 30°C on YPD medium to an average colony size of about 10^7 cells. Colonies were removed, resuspended in water, and plated on YPD plates for viable cells per colony. A portion of the cell suspension (~5×10^5 cells) was mated with approximately 10^7 cells of either mating-type tester strain in YPD broth for 4 h and then plated onto minimal medium. The mating-proficient cells included both **a**- and α-mating cells. The same amount of cell suspension was also plated on synthetic complete medium with canavanine in place of arginine and later replica plated to complete medium lacking threonine. Seven colonies of each strain were assayed, and median chromosome loss frequencies for both chromosome III and chromosome V (number of maters and number of Can^r Thr^- cells in the total cell population) were determined. The chromosome loss rates (number of events per cell per generation) were calculated according to the following formula: rate = (0.4343 × median frequency)/log$N$ − logN_0, where N is the number of cells present in the colony and N_0 (the number of initial cells) = 1 (16). For each genotype, three independent diploids were assayed by fluctuation analysis, and the average loss rate is reported.

TABLE 1. Strains used

Strain	Genotype	Source
JRY2515	*MAT*α *ade2 his3 leu2 sir2*::*HIS3 ura3*	J. Rine
JRY2465	*MAT*α *ade2-101 his3 Δ200 leu2 lys1-1 lys2-801 sir2*::*HIS3 SUM1-1 ura3-52*	J. Rine
JRY2466	*MAT*α *ade2-101 his3 Δ200 leu2 lys1-1 lys2-801 sir2*::*HIS3 SUM1-1 ura3-52*	J. Rine
JRY3138	*MAT***a** *ade2-101 his3 Δ200 leu2 lys1-1 lys2-801 sir2*::*HIS3 SUM1-1 ura3-52*	J. Rine
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 GAL*$^+$	R. Rothstein
W303-1B	Same as W303-1A except *MAT*α	R. Rothstein
W303	W303-1A × W303-1B	R. Rothstein
W1089-1	W303-1A *LEU2*$^+$	R. Rothstein
MC25	JRY2515 *sum1*Δ::*LEU2*	
MC27	W303-1A *sum1*::*URA3*	
MC28	W303-1B *sum1*::*URA3*	
JRY3935	W303-1B *hmr*::*ssΔI*	J. Rine
MC31	JRY3935 *sum1*::*URA3*	
YLS586	W303-1B *hmrΔB*::*ADE2*	
MC33	YLS586 *sum1*::*URA3*	
YLS404	W303-1B *hmrΔA*::*ADE2*	
MC35	YLS404 *sum1*::*URA3*	
MC47	W303-1A *sir2*::*HIS3 sum1*::*URA3*	
MC49	W303-1B *sir2*::*HIS3 sum1*::*URA3*	
MC54	W303-1A *sir2*::*HIS3 SUM1-1*	
MC57	W303-1B *sir2*::*HIS3 SUM1-1*	
YDS712	W303-1A *sir2*::*HIS3*	
YDS714	W303-1B *sir2*::*HIS3*	
AJL275-2a	W303-1B *URA3*/*ADE2-TelVIIL*	A. Lustig
MC51	W303-1A *URA3*/*ADE2-TelVIIL SUM1-1*	
MC52	W303-1B *URA3*/*ADE2-TelVIIL SUM1-1*	
MC53	W303-1A *URA3*/*ADE2-TelVIIL SUM1-1*	
MC60	W303-1B *URA3*/*ADE2-TelVIIL sir2*::*HIS3 SUM1*	
MC61	W303-1B *URA3*/*ADE2-TelVIIL sir2*::*HIS3 SUM1-1*	
YDS631	W303-1B *URA3-TelVIIL*	
MC64	YDS631 *sum1*::*LEU2*	
YDV66	W303-1A *URA3-TelVIIL hmrΔA*::*TRP1*	
MC66	YDV66 *sum1*::*LEU2*	
YDV67	W303-1B *URA3-TelVIIL hmrΔA*::*TRP1*	
MC68	YDV67 *sum1*::*LEU2*	
MC80	W303 (haploid, *MAT* allele not determined) *sir2*::*HIS3 SUM1-1 URA3-TelVIIL*	
MC88	W303-1A *SUM1-1*	
MC89	W303-1B *SUM1-1*	
MC90	W303-1A *URA3 SUM1-1*	
MC91	W303-1B *URA3 SUM1-1*	
MC92	W303-1A *HIS3 SUM1-1*	
MC93	W303-1B *HIS3 SUM1-1*	
YLS506	W303-1B *hmrΔA*::*TRP1 rap1-12*::*URA3*	
MC96	W303-1B *SUM1-1 hmrΔA*::*TRP1 sir2*::*HIS3 rap1-12*::*URA3*	
MC97	W303-1B *SUM1-1 hmrΔA*::*TRP1 sir2*::*HIS3*	
MC98	W303-1B *SUM1-1 hmrΔA*::*TRP1 rap1-12*::*URA3*	
MC99	W303-1B *SUM1-1 hmrΔA*::*TRP1 rap1-12*::*URA3*	
MC100	W303-1B *SUM1-1 hmrΔA*::*TRP1 rap1-12*::*URA3*	
GA224	*MAT*α *can1-100 hom3 his3 leu2-3,112 trp1 ura3*	S. Gasser
MC105	GA224 *SUM1-1*	
MC120	GA224 *sum1*::*URA3*	
MCY2675	*MAT***a** *his3 Δ200 leu2-3,112 ura3-52*	M. Carlson
MC113	MCY2675 *SUM1-1*	
MC122	MYC2675 *sum1*::*URA3*	
YDS31	*MAT***a** *his1*	K. Nasmyth
YDS32	*MAT*α *his1*	K. Nasmyth
MC124	W303-1B *sir3*::*HIS4*	
MC125	W303-1B *sir4*::*HIS3*	
MC130	W303-1A *sum1*::*LEU2 rap1-17 ADE2-TelVIIL*	
MC131	W303-1B *SUM1-1 rap1-12*::*HIS3 hmrΔA*::*TRP1 URA3*/*ADE2-TelVIIL*	
AJL440-1c	W303-1A *HIS3 rap1-17 ura3*/*ADE2-TelVIIL*	A. Lustig
MC132	W303-1A *SUM1-1 rap1-17 ADE2-TelVIIL*	
MC133	W303-1A *SUM1-1 rap1-17 ADE2-TelVIIL hmrΔA*::*TRP1*	

Indirect immunofluorescence of yeast spheroplasts. Indirect immunofluorescence was performed as described previously (39), with the following modifications. Cells with either the HA-tagged or wild-type *SUM1* gene on a 2μm plasmid (DM383 or DM651) were grown overnight to high density in selective medium. The culture were first fixed for 20 min under growth conditions by adding 1/4 culture volume of 0.5 M KP_i (pH 6.5) and 18.5% formaldehyde. The fixed cells were converted to spheroplasts by treatment with Zymolyase (0.125 mg of Zymolyase per ml in wash solution) for 2.5 to 4 h at 30°C. The final pellet was

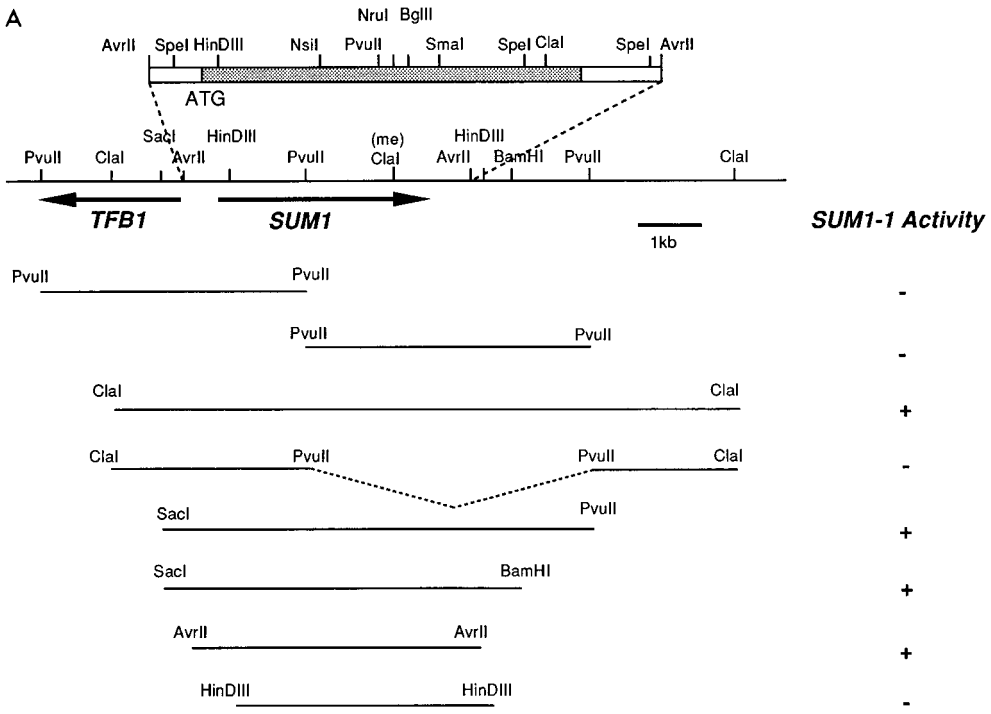

FIG. 1. (A) Restriction map of the *SUM1* locus and the eight subclones used to localize the *SUM1-1* allele. (B) Nucleotide sequence of the *SUM1* gene. The predicted amino acid sequence of Sum1p is shown in the one-letter code below the nucleotide sequence.

washed with and resuspended in 2 volumes of NS^+ azide (NS [20 mM Tris-HCl {pH 7.6}, 0.25 M sucrose, 1 mM EDTA, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM $ZnCl_2$], 1 mM phenylmethylsulfonyl fluoride, 7.2 mM β-mercaptoethanol, 0.02% sodium azide). The slides were incubated in mouse monoclonal anti-HA antibody 12CA5 (6.2 mg/ml; Berkeley Antibody Company, Berkeley, Calif.) diluted 1:500 in PBT for 2 h at room temperature. The slides were washed 10 times with PBT and then incubated with rhodamine-conjugated goat anti-mouse immunoglobulin G (Boehringer Mannheim) according to the manufacturer's instructions. Slides were viewed with a 100×, 1.32-numerical aperture oil immersion objective on a Leitz Dialux fluorescence microscope (Leitz, Rockleigh, N.J.) equipped with a 3.1× projection lens (Diagnostics Instruments, Sterling Heights, Mich.) and a Star-1 cooled charge-coupled device camera (Photometrics, Tucson, Ariz.). Images were processed by the NIH Image program (version 1.55) on a Macintosh Quadra 800 (Apple Computer Inc., Cupertino, Calif.).

Nucleotide sequence accession number. The nucleotide sequence reported in this paper is entered in GenBank with accession number U34832.

RESULTS

Cloning of the *SUM1-1* allele. *SUM1-1* behaves as a dominant mutation in the strain background described in a previous report (37). We decided to use this property as a basis for cloning the *SUM1-1* gene, reasoning that the introduction of this mutant allele into a *MAT*α *SUM1 sir2* strain would restore mating by suppressing the *sir2* mutation. To obtain the DNA encoding *SUM1-1*, we constructed recombinant plasmid libraries that contained genomic DNA from *SUM1-1* mutant strains. Genomic libraries, with inserts ranging from 10 to 16 kbp, were made by using DNA from yeast strains JRY2465 and JRY2466 in the centromeric vector YCp50 (see Materials and Methods). Four libraries were generated, each containing more than 20,000 independent recombinants.

Recombinant plasmid DNA prepared from each library was used to transform yeast strain JRY2515 (*MAT*α *SUM1 sir2*::*HIS3 ura3 leu2*) to uracil prototrophy. Transformants were then mated with strain W1089-1A (*MAT***a** *SUM1 SIR2 ura3 LEU2*) by replica plating directly from transformation plates onto YEPD plates containing a lawn of the tester strain. Mating-competent transformants, which potentially contained a plasmid with the *SUM1-1* allele, were identified by making a second replica from the mating plate onto plates lacking both uracil and leucine. Approximately 25,000 transformants were screened for each library, and 12 that acquired the ability to mate with the tester strain were identified. Plasmids were rescued from these cells and used to retransform strain JRY2515, in order to retest their ability to confer mating competence. Two independent clones tested positive in this rescreening and were found to contain overlapping inserts. Analysis of subclones from a region in common between these two clones revealed that a 4.9-kb *Avr*II fragment could confer mating competence in the *sir2* strain JRY2515 (Fig. 1A). Northern (RNA) blot analysis had previously shown that this fragment encodes an RNA of approximately 3.7 kb (19, 27).

To determine whether the cloned DNA is derived from the *SUM1* locus, we performed a linkage analysis. A *Pvu*II-*Spe*I fragment of the cloned DNA was inserted into pRS405, a *LEU2*-containing integrating plasmid, and integrated into the chromosome of a *sir2 SUM1* strain (JRY2515) by cleavage within the insert at a *Sma*I site (see Materials and Methods for further details). Integration of the cloned DNA at its homologous chromosomal locus was confirmed by Southern blotting analysis (data not shown). The resulting strain, MC25, still mated very poorly, indicating that the *Pvu*II-*Spe*I fragment was not sufficient to confer the *SUM1-1* phenotype. (This result was expected since the fragment contains only the C-terminal part of the ORF contained on the minimal active fragment.) MC25 was made mating proficient by introduction of a *SIR2*-containing plasmid and was then crossed to strain JRY3138 (*MAT***a** *sir2*::*HIS3 SUM1-1 leu2*). After loss of the *SIR2* plasmid, the resulting diploid was induced to undergo meiosis. The haploid segregants from this cross should all contain the *sir2*::*HIS3* mutation. Because *SUM1-1* suppresses mating defects in *MAT*α *sir2* strains better than in *MAT***a** *sir2* strains (37), the segregants

FIG. 1—*Continued.*

should display detectable mating in patch mating assays only when they are *MAT*α *SUM1-1*. We observed that all of the mating-proficient spore colonies from 24 tetrads dissected were phenotypically α mating and Leu$^-$. This result indicates that the cloned DNA is closely linked to the *SUM1* locus.

Cloning and DNA sequencing of the wild-type *SUM1* gene. Several approaches were taken to clone the wild-type copy of the *SUM1* gene. The cloned *SUM1-1* allele was used to probe a yeast chromosome blot and nitrocellulose filters containing an ordered set of DNA clones representing most of the yeast genome (obtained from L. Riles and M. Olsen, Washington University, St. Louis, Mo.). λ clone 6898 hybridized to the probe, placing *SUM1* on chromosome IV-R between *GCN2* and *PEP7* (53). To obtain a full-length clone of the *SUM1* gene, two yeast genomic libraries in plasmid vectors (kindly provided by M. Rose and M. Carlson) (11, 56) were then screened by colony hybridization (59). The *SUM1* sequence (Fig. 1B) is derived from phage λ clone 6898 and genomic clones from each of the two plasmid libraries. The putative *SUM1* gene encodes an ORF of 1,048 codons which predicts a polypeptide of approximately 115 kDa. Analysis of the GenBank (release 88.0) and PIR (release 44.0) databases revealed no significant homologies to known protein sequences. Furthermore, no similarities to known sequence motifs were found (GCG sequence analysis software package; Genetics Computer Group, Inc., Madison, Wis.).

***SUM1* is essential neither for normal growth nor for transcriptional silencing.** To determine whether the product of the *SUM1* gene is important for cell growth or for silencing, a disruption of the *SUM1* gene was constructed in vitro and integrated into the chromosome by the one-step gene replacement method (58). The *sum1*::*URA3* allele (DM264; see Materials and Methods) was introduced into a homozygous diploid strain (W303), and a Ura$^+$ transformant heterozygous for the *sum1* gene disruption was identified by Southern blotting analysis. This *SUM1*/*sum1*::*URA3* diploid was then sporulated, and the phenotypes of the haploid segregants were analyzed. In all 13 complete tetrads examined, the Ura$^+$ phenotype segregated 2:2. There was no obvious growth difference observed between the Ura$^+$ and Ura$^-$ spore colonies on rich medium. Because the *sum1*::*URA3* mutation removes all of the predicted *SUM1* ORF and is therefore presumably a null allele, these data indicate that *SUM1* is not an essential gene, nor is it necessary for normal growth on rich medium. Furthermore, mating defects were not observed in the Ura$^+$ segregants. Thus, a *sum1* null mutation does not seem to affect gene repression at the silent mating-type loci in a wild-type *SIR* background.

To determine whether telomeric repression is affected by the *SUM1* disruption, a *sum1*::*LEU2* mutation was constructed (see Materials and Methods) and introduced into strains that contained a *URA3* reporter gene immediately adjacent to a telomere created at the *ADH4* locus of chromosome VII-L (21) (strains YDS631, YDV66, and YDV67 [Table 1]). The level of telomeric repression in the *sum1*::*LEU2* mutant strains (MC64, MC66, and MC68) was monitored by growth on complete medium, complete medium minus uracil, and FOA medium, which kills cells that are expressing the *URA3* gene product (5). In all strains tested, expression of the telomeric *URA3* reporter was unaffected by the *sum1*::*LEU2* mutation (data not shown).

We also introduced the *sum1*::*URA3* disruption into strains containing the *sir2*::*HIS3* mutation, whereby the *SUM1-1* mutant allele is able to restore mating. The resulting strains (MC47 and MC49) were still defective in mating (data not shown), indicating that a *sum1* null mutation cannot suppress the mating defects of a *sir2* mutation in the W303 background. This result strongly suggests that *SUM1-1* is not a loss-of-function mutation (see below).

***sum1* mutation can slightly weaken repression at *HMR* loci with a mutated silencer.** It is possible that the lack of a silencing phenotype for the *sum1*::*URA3* disruption is due to the complexity of the *HMR-E* silencer element. As stated in the introduction, the three *HMR-E* silencer regulatory elements (A, E, and B) are partially redundant: any two of these three binding sites are sufficient for silencing, though the Rap1p binding site (E) is required for full repression (7, 30). To test for possible synthetic effects of the *sum1*::*URA3* disruption allele, we began with strains YLS404 and YLS586, which contain the *ADE2* reporter gene placed adjacent to an *HMR-E* silencer with mutation in the A and B silencer elements, respectively (*hmr*Δ*A* or *hmr*Δ*B* silencer) (69). The *ADE2* gene provides a colony color marker for the transcriptional state at *HMR*: phenotypically Ade2$^-$ cells accumulate a pigment and yield red colonies, whereas Ade2$^+$ cells are white (55). In these strains, the *ADE2* reporter gene is normally slightly derepressed and yields primarily red colonies with few white sectors. Introduction of the *sum1*::*URA3* allele into these strains (to produce strains MC33 and MC35) caused an increase in the number of white sectors in the colonies, indicating a further decrease in repression (Fig. 2).

Other tests of *sum1* mutations, however, did not reveal effects on the activity of weakened silencers (data not shown). For example, introduction of the *sum1*::*URA3* mutation into a strain containing a synthetic silencer in place of *HMR-E* and a deletion of the *HMR-I* silencer (JRY3935 [44]) did not result in a measurable loss of mating efficiency (strain MC31). (The synthetic silencer differs from wild-type *HMR-E* in that all three silencer regulatory elements are required for complete repression.) In addition, we observed no effect of the *sum1*::*LEU2* allele on repression of a *hmr*Δ*A*::*TRP1* reporter gene (comparing strains YDV66 and MC66), as judged by growth in the absence of tryptophan. We conclude from these results that deletion of *SUM1* has a subtle effect on the *HMR-E* silencer that can be seen only when the functional redundancy of the silencer is eliminated and when a sensitive reporter gene (such as *ADE2*) is present at *HMR*.

We also considered the possibility that the relatively minor effect of *SUM1* deletion on *HM* silencing is due to functional redundancy of Sum1p itself. To begin to determine whether *SUM1* homologs exist, yeast genomic DNA was cleaved with a variety of restriction enzymes and probed with *SUM1* DNA by hybridization at low stringency on Southern blots. This approach failed to reveal any sequences homologous to *SUM1*.

Characterization of the *SUM1-1* mutation and construction of an isogenic set of *SUM1-1* strains. We noticed several restriction fragment length polymorphisms between the *SUM1-1* mutant and wild-type alleles, as well as multiple point mutations and deletions within the 3′ untranslated region. To localize the mutation(s) responsible for conferring the *SUM1-1* phenotype (suppression of the mating defect of a *MAT*α *sir2* mutant), a series of restriction fragment exchanges between the *SUM1-1* allele and the wild-type *SUM1* gene was made (Fig. 3A). The recombined alleles were transformed into strain JRY2515 (*MAT*α *sir2*) and tested for the ability to restore mating. We noted that a C-terminal restriction fragment (*Sph*I to *Bsi*WI, nucleotides 2847 to 3155 [Fig. 3A]), which encodes the terminal 100 amino acids of Sum1p and contains only 8 nucleotides beyond the predicted stop codon, conferred the *SUM1-1* phenotype in the context of otherwise wild-type sequences (Fig. 3B). This fragment was subjected to DNA sequencing and found to have a single missense mutation at

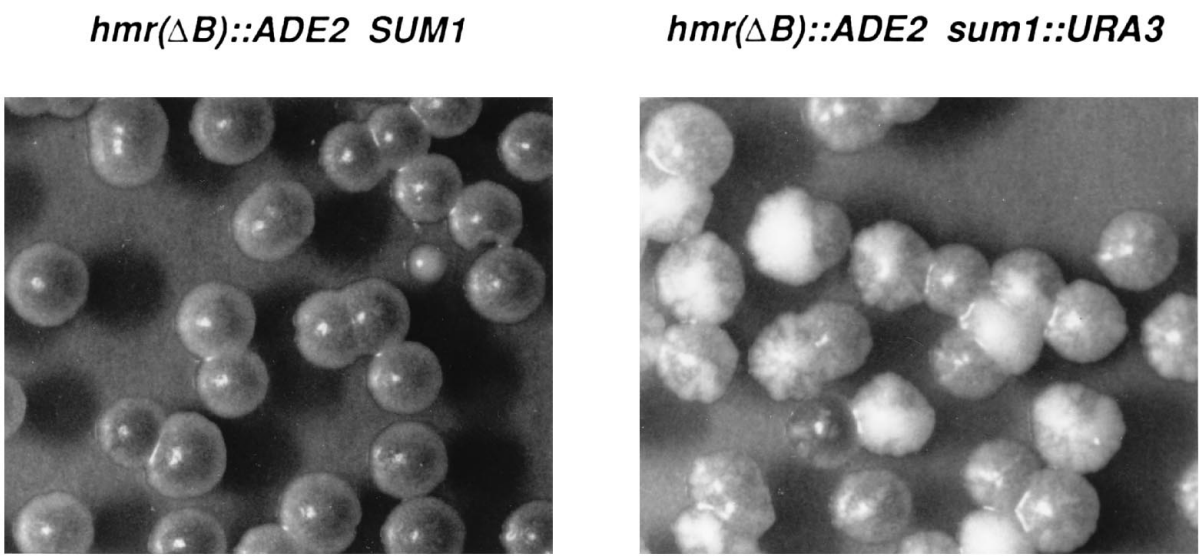

FIG. 2. A *sum1* null mutation weakens repression of *hmrΔB*::*ADE2*. Representative colonies of isogenic *SUM1* and *sum1*::*URA3* strains are shown.

codon 974 in the predicted *SUM1* ORF that would result in a threonine-to-isoleucine change. This mutation maps to a locally hydrophobic part of the predicted protein and would presumably increase the hydrophobic character of this region. The *SUM1-1* allele that contains the point mutation at the codon 974 in the context of otherwise wild-type sequences (DM449) confers a slightly (approximately 2.5-fold) higher mating efficiency to the *sir2* mutant (JRY2515) than does the original *SUM1-1* allele. Our data indicate, therefore, that this mutation in *SUM1-1* is both necessary and sufficient to restore mating in a *MAT*α *sir2*::*HIS3* mutant.

We proceeded to introduce the *SUM1-1* allele by gene replacement into W303-derived strains in order to study the phenotype caused by the mutation in an isogenic background (see Materials and Methods for details). In these experiments, a 4.9-kb *Avr*II fragment containing the entire *SUM1-1* coding sequence was used for gene transplacement experiments. As shown in Table 2, the *SUM1-1* mutation improved mating by a *MAT*α *sir2*::*HIS3* strain by more than 10,000-fold. In contrast, the mating efficiency of a *MAT***a** *SUM1-1 sir2*::*HIS3* strain was improved by 10-fold relative to that of the *MAT***a** *SUM1 sir2* strain. These data are consistent with results from a previous report (37) which showed that *SUM1-1* strongly suppresses the nonmating phenotype of *sir2*::*HIS3* mutations in *MAT*α strains but restores only weak mating in *MAT***a** strains. We confirmed that the *SUM1-1* allele acts to restore transcriptional repression at *HMR* and *HML* by measuring the steady-state levels of **a***1* and α*1* mRNAs in these strains by Northern blotting (Fig. 4). Consistent with the large improvement in mating observed, we did not detect **a***1* transcripts in the *MAT*α *SUM1-1 sir2*::*HIS3* strain (MC57). We also found that α*1* transcripts were fully repressed in the *MAT***a** *SUM1-1 sir2*::*HIS3* strain (MC54), despite the fact that *SUM1-1* restored only weak mating in this strain. This severe drop in α*1* mRNA may be in part due to residual **a***1*-α2 repression. Finally, we found that the *SUM1-1* plasmid also suppressed mating defects in *MAT*α *sir3*::*HIS3* and *MAT*α *sir4*::*HIS3* strains (MC124 and MC125 [data not shown]), as expected (37).

The *SUM1-1* allele is dominant to the wild-type gene in the W303 strain background, since the *SUM1-1* plasmid could suppress mating defects when transformed to a *MAT*α *SUM1 sir2* strain (YDS714 [data not shown]). However, *SUM1-1* is not a hypermorph, since *SUM1* on a 2μm plasmid could not rescue mating defects when it was transformed into a *MAT*α *sir2*::*HIS3* strain (JRY2515 [data not shown]). Furthermore, we found that additional copies of the wild-type *SUM1* gene in a *sir2 SUM1-1* cell (either **a** or α) actually results in a slight (fivefold) decrease in mating efficiency. We also noticed that the *SUM1-1* plasmid suppressed the mating defects of *sir2*::*HIS3* mutation at least 100-fold better in a *sum1*::*URA3* strain than in a *SUM1* strain. These findings argue against the idea that the *SUM1-1* allele is an increased function (hypermorphic) mutation and support the notion that *SUM1-1* is an altered-function mutation.

***SUM1-1* restores *HM* silencing in *rap1*^{s} and *rap1*^{t} mutant strains.** Because *SUM1-1* has been shown to suppress the silencing defects of multiple silencer site mutations at *HMR-E* (37), we were interested in determining whether it could also restore repression in strains with a mutated silencer-binding protein. Alleles of *RAP1*, called *rap1*^{s}, which are defective in silencing at *hmrΔA* loci but are apparently completely unaffected in essential *RAP1* functions have been identified (68). We therefore asked whether *SUM1-1* could suppress a *rap1-12* mutation, the most severely defective *rap1*^{s} allele. The *rap1-12* mutation results in complete derepression of *hmrΔA*::*TRP1*. A *SUM1-1* strain (MC57) was crossed to a strain containing *rap1-12* and the *hmrΔA*::*TRP1* reporter gene (YLS506). The diploid was sporulated, and haploid segregants of relevant genotypes were tested for growth in the presence and absence of tryptophan. We found that *SUM1-1* fully restored repression of *hmrΔA*::*TRP1* in a *rap1-12* strain (Fig. 5). By contrast, *SUM1-1* appeared only to slightly restore repression of *hmrΔA*::*TRP1* in a *sir2* mutant.

Mutations that result in truncation of the Rap1p C terminus (*rap1*^{t}) have been shown to cause complete derepression of telomeres and a more modest silencing defect at *HML*. We therefore examined the effect of *SUM1-1* on the *HML* locus and on the artificial chromosome VII-L telomere in the strains harboring a severe *rap1*^{t} mutation (*rap1-17*). For this purpose, the strain MC131 (*SUM1-1 rap1-12*::*HIS3 hmrΔA*::*TRP1 URA3-ADE2-TelVII-L*) was mated to the strain MC130 (*sum1*::*LEU2 rap1-17 ADE2-TelVII-L*). The diploid was sporulated, and tetrads were analyzed. The *MAT***a** *rap1-17* mutant (AJL440-1c) has a mating efficiency of 2.8×10^{-2} relative to that of the

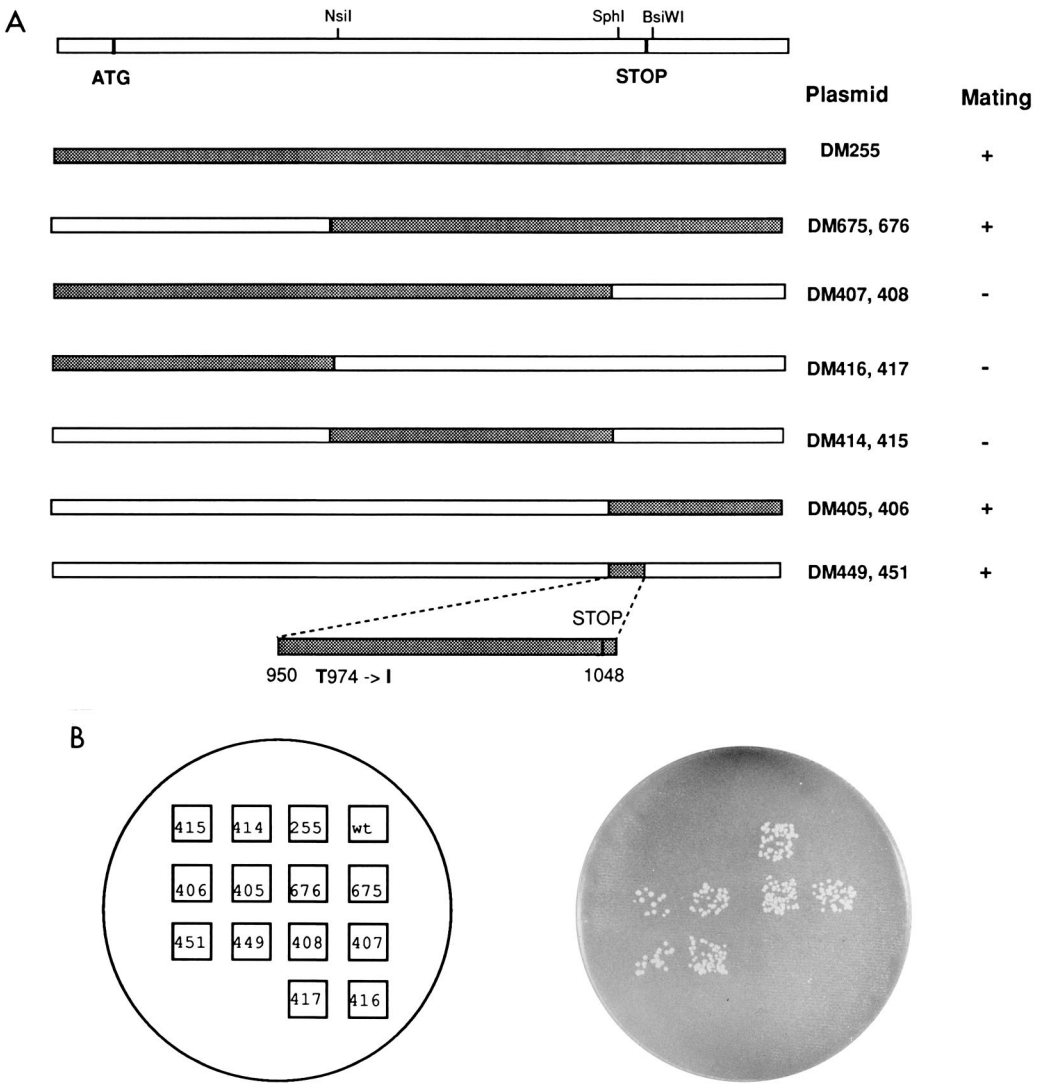

FIG. 3. (A) Schematic representation of *SUM1* alleles constructed by exchanging restriction fragments between the *SUM1-1* mutant allele and the wild-type *SUM1* gene. The stippled boxes represent the sequences derived from the *SUM1-1* allele. The plasmid designations and results of patch mating assays (see panel B) are shown to the right. Two independent constructs were tested for each recombinant allele. (B) Patch mating assays for the *sir2* strain JRY2515 transformed with plasmids containing either wild-type *SUM1*, the *SUM1-1* mutant allele (DM255), or the recombinant alleles shown in panel A.

wild-type *MAT***a** (W303-1A) cells. *SUM1-1* restored wild-type levels of *HML* silencing in the *rap1-17* mutant (MC132 or MC133), as judged by the restoration of efficient mating in *MAT***a** segregants. In contrast, *SUM1-1* appeared to have little or no effect on the telomere repression defect of *rap1-17*. Three *SUM1-1 rap1-17 URA3-ADE2-TelVII-L* segregants were examined, and only one showed a slight improvement in FOA resistance (~10 fold), which has not been examined further (data not shown).

SUM1-1* increases telomeric silencing.** As described above, disruption of *SUM1* did not appear to have any effect on telomeric repression, nor did *SUM1-1* appear to restore telomere position effect in the *rap1-17* mutant. To test whether *SUM1-1* can restore telomeric repression in *sir* mutants, a *SUM1-1 MAT*α *sir2*::*HIS3* strain (MC57) was crossed to a *MATa** strain that contained the *URA3* telomeric reporter (YDV66). In analyzing haploid segregants from this cross, we found that *SUM1-1* could not restore telomeric *URA3* repression in *sir2* mutant segregants (e.g., MC80 [data not shown]). In a separate experiment, a *SUM1-1 MAT***a** *sir2*::*HIS3* strain (MC54) was crossed to a *MAT*α *URA3-ADE2-TelVII-L* reporter strain (AJL275-2a). Again, *SUM1-1* did not restore repression of either the *ADE2* or *URA3* reporter in *sir2* mutant segregants. Surprisingly, however, we observed that *SUM1-1*

TABLE 2. Mating efficiencies of isogenic *SUM1* and *SUM1-1* strains

Strain	Relevant genotype	Relative mating efficiency
W303-1B	*MAT*α *SIR2 SUM1*	1
YDS714	W303-1B *sir2*::*HIS3 SUM1*	$\leq 3.1 \times 10^{-5}$
MC57	W303-1B *sir2*::*HIS3 SUM1-1*	0.37
MC89	W303-1B *SIR2 SUM1-1*	5.3
W303-1A	*MAT***a** *SIR2 SUM1*	1
YDS712	W303-1A *sir2*::*HIS3 SUM1*	$\leq 2.2 \times 10^{-5}$
MC54	W303-1A *sir2*::*HIS3 SUM1-1*	1.9×10^{-4}
MC88	W303-1A *SIR2 SUM1-1*	5.4

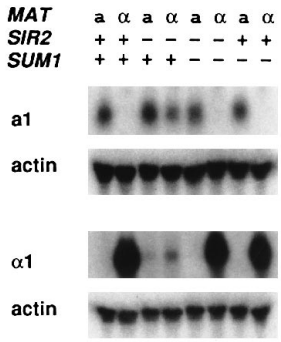

FIG. 4. Northern blot analysis of **a***1*, α*1*, and actin transcripts in isogenic *SUM1* and *SUM1-1* strains. Total RNA was prepared from the indicated strains, size fractionated on a 1% formaldehyde agarose gel, transferred to a Hybond-N filter, and hybridized with either **a***1* or α*1* and actin (control) probes (see Materials and Methods). The relevant genotypes of the strains used are indicated above the autoradiograph. − represents *SUM1-1* (in the case of *SUM1*) and *sir2*::*HIS3* (in the case of *SIR2*).

caused an increase of telomeric repression in SIR^+ segregants. Normally, the telomeric *ADE2* reporter in a W303 (*SUM1*) strain background is only slightly repressed, giving rise to mostly white colonies. However, in the *SUM1-1* segregants from the cross with AJL275-2a, the telomeric *ADE2* gene was further repressed, creating white/red sectored colonies (Fig. 6A). In addition, a *URA3-ADE2-TelVII-L* strain with the *SUM1-1* mutation grew at least 10-fold better on FOA plates than an otherwise isogenic *SUM1* strain, indicating an increase of repression of the telomeric *URA3* reporter in these *SUM1-1* strains (Fig. 6B). The increased telomeric *ADE2* repression could also be observed if *SUM1-1* on a *CEN* plasmid was transformed into the *SUM1 URA3-ADE2-TelVII-L* reporter strain (AJL275-2a), although the effect was weaker in the transformants than in an isogenic *SUM1-1* strain (data not shown).

The *SUM1-1* mutation increases the rates of cell death and chromosome loss. A *SUM1-1* mutant strain grows more slowly than isogenic wild-type or *sum1* mutants. For example, wild-type haploid cells (W303-1A and W303-1B) have a doubling time of ~130 min, whereas isogenic *SUM1-1* mutants (MC88 and MC89) have a doubling time of ~160 min. This slow-growth phenotype of *SUM1-1* mutants can be rescued when copies of the wild-type *SUM1* gene are introduced into these cells. We also noted that although heterozygous *SUM1-1*/*SUM1* diploids show some increase in telomeric repression (as judged by a telomeric *ADE2* reporter), their growth rates appear comparable to those of isogenic wild-type diploids and are clearly much higher than those of *SUM1-1* homozygous diploids (data not shown). Hence, the slow-growth phenotype of the *SUM1-1* mutation, like the silencing phenotype, appears to be partially dominant in the W303 strain background.

We measured cell viability in cultures of *SUM1-1* mutants, since a decrease in cell viability could result in a slow-growth phenotype. The viability was assayed by colony-forming ability in three pairs of isogenic haploid strains (YDS3 and MC89, MC60 and MC61, and YDS712 and MC57). Cell viability of the mutant strain was reduced to about 80% of that of the wild-type cells (Table 3).

We also noted that homozygous *SUM1-1* diploids produce colonies with a much larger size variation than wild-type diploids. Because this phenotype is often associated with mutations that increase chromosome loss, we decided to measure chromosome stability in *SUM1-1* homozygous diploids. We monitored the loss rate of chromosomes III and V in the *SUM1-1*/*SUM1-1* diploid (MC105 × MC113), the *sum1*/*sum1* diploid (MC120 × 122), and the homozygous wild-type diploid (GA224 × MCY2675). Mating assays were used to determine the rate of chromosome III loss, since an **a**/α nonmating diploid cell will acquire the ability to mate if it loses one (or both) of its two copies of chromosome III. This diploid strain also contains a marked chromosome V, with *can1* and *hom3* markers on opposite chromosome arms. A cell that loses the wild-type chromosome V will grow on plates containing canavanine but not on complete media lacking threonine (23). As shown in Table 4, the *SUM1-1*/*SUM1-1* diploid loses both chromosome III and chromosome V at a rate (number of events per cell division per generation) approximately fivefold higher than in the isogenic *SUM1*/*SUM1* and *sum1*/*sum1* strains.

Cellular localization of Sum1p. The results presented above suggest that Sum1p mediates interactions that influence chromosome behavior as well as position effects. To gain further insights into *SUM1* function, we have localized Sum1p by indirect immunofluorescence (Fig. 7), using a version of the protein containing an influenza virus HA antigen tag at its C terminus (see Materials and Methods for details). The HA-tagged *SUM1* allele complemented the partial silencing defect of a *sum1* null mutation in the strain MC33 (data not shown). Nuclear extracts from the cells harboring the tagged allele were analyzed by Western blotting (immunoblotting) using the mouse monoclonal anti-HA antibody 12CA5 as a probe. A protein band of ~125 kDa, specific to the tagged *SUM1* gene, was detected, indicating that intact HA-tagged Sum1p was being made (data not shown). Antibody 12CA5 also recognized a nonspecific band of ~46 kDa in yeast cells. In indirect immunofluorescence of yeast spheroplasts derived from cells lacking the HA tag, we observed weak, slightly punctate staining that appeared to be cytoplasmic, presumably as a result of the 46-kDa cross-reacting protein detected on Western blots. Despite this homogeneous nonspecific staining, we observed an obvious nuclear staining in ~25 to 40% of spheroplasts derived from cells which contained the HA-tagged *SUM1* gene. We therefore conclude that Sum1p is localized to the nucleus, consistent with the effect of *SUM1-1* on both transcriptional silencing and chromosome stability. At present, we do not know why only about one-third of the cells appear to contain

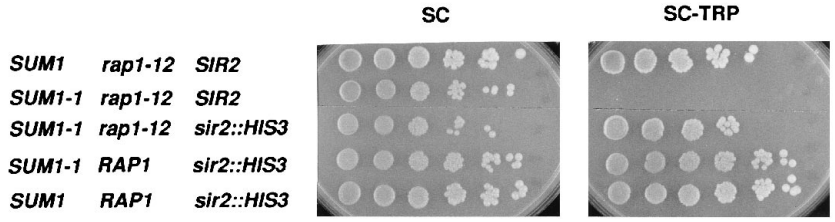

FIG. 5. Effect of the *SUM1-1* mutation on an *hmrΔA*::*TRP1* reporter gene in a *rap1-12* or *sir2* mutant background, as judged by the ability of cells to form colonies in the absence of tryptophan. SC, synthetic complete medium.

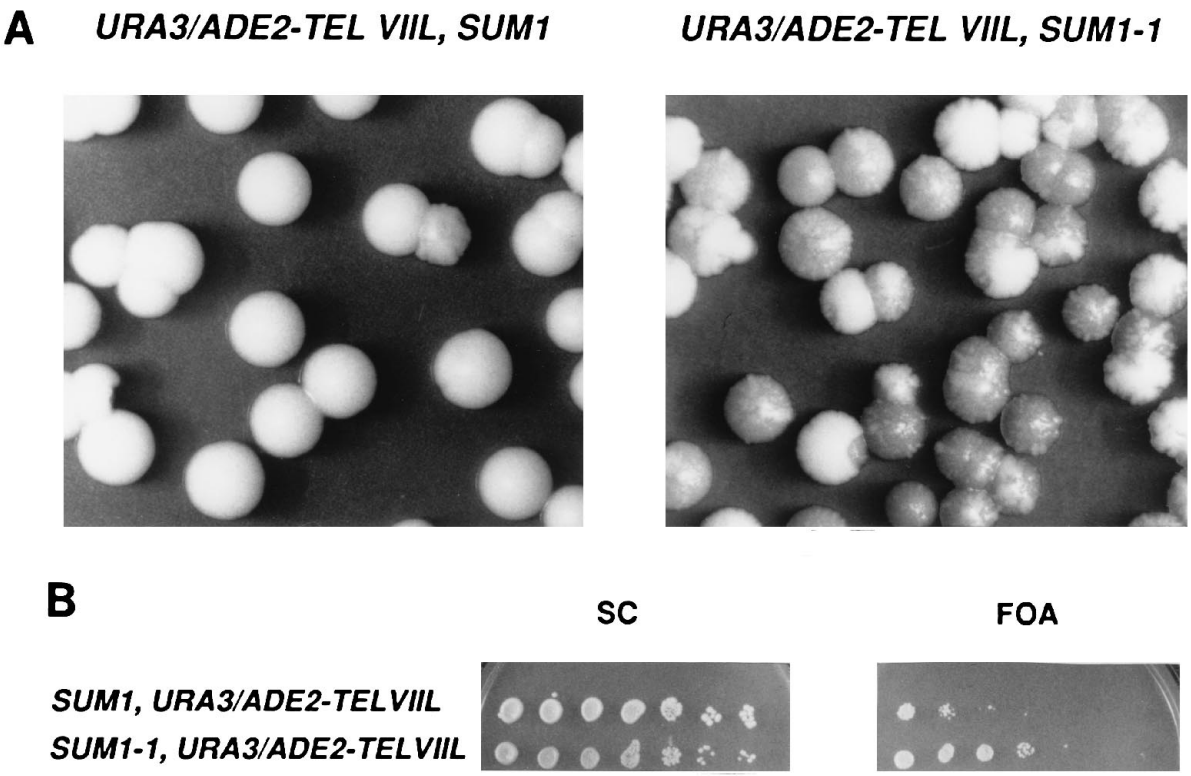

FIG. 6. The *SUM1-1* allele increases telomeric repression. (A) Colonies of *SUM1* and *SUM1-1* strains containing a chromosome VII-L telomeric *ADE2/URA3* reporter are shown. (B) Growth of a *SUM1-1 ADE2/URA3-TelVIIL* strain in synthetic complete (SC) and FOA media compared with that of an isogenic *SUM1* strain.

nuclear Sum1p. This could result from variable permeabilization of the spheroplasts, and hence differences in antibody accessibility, or from differences between cells in Sum1p levels or nuclear localization. We have also constructed an equivalent HA-tagged version of the *SUM1-1* allele. Unfortunately, this modified *SUM1-1* gene does not confer the *SUM1-1* silencing phenotype, perhaps because the epitope is located near the Sum1-1p mutation, and we have not determined its cellular localization.

DISCUSSION

Previous genetic analyses of the *SUM1-1* allele have shown that it has the unique ability to suppress the silencing defects of mutations in a number of *cis*- and *trans*-acting silencer factors, including all four of the *SIR* genes (37). These results have suggested that wild-type Sum1p may play a critical role in silencing. However, lack of a *sum1* null allele and the variable dominance or recessiveness of the *SUM1-1* mutation in different strain backgrounds have prevented a clear understanding of the function of either Sum1p or the *SUM1-1* mutant gene product. As a result, two very different models for Sum1p function have been proposed, one in which the protein is an activator required for the expression of silent mating-type genes which is itself repressed by Sir proteins (31) and another in which Sum1p is directly associated with an altered chromatin structure at *HM* loci (37).

Here we have reported the cloning and initial characterization of both the *SUM1* gene and the *SUM1-1* allele. The availability of these cloned genes has allowed us to examine the null phenotype of *SUM1* and also to study the effects of the *SUM1-1* mutation in a set of isogenic strains. Our results clearly indicate that a *sum1* null mutation does not suppress the effect of *sir* mutations at *HM* loci. Therefore, full expression of *HM* loci apparently occurs in the absence of Sum1p, ruling out the possibility that the protein is an activator required for transcription at the silent mating-type loci. Instead, we found that

TABLE 3. Colony-forming abilities of *SUM1-1* isogenic haploid cells on YEPD medium

Strain	No. of cells	
	Total	No growth
YDS712 (*SUM1*)	50	0
MC54 (*SUM1-1*)	50	9
YDS3 (*SUM1*)	50	3
MC89 (*SUM1-1*)	50	11
MC60 (*SUM1*)	50	0
MC61 (*SUM1-1*)	50	9

TABLE 4. Chromosome stability in homozygous *SUM1*, *SUM1-1*, and *sum1* diploids

Genotype	Loss rate (no. of events [10^{-6}]/cell division/generation)	
	Chromosome V	Chromosome III
SUM1 CAN1 HOM3/*SUM1 can1 hom3*	0.46 ± 0.29	0.86 ± 0.14
SUM1-1 CAN1 HOM3/*SUM1-1 can1 hom3*	2.67 ± 0.49	4.37 ± 1.92
sum1 CAN1 HOM3/*sum1 can1 hom3*	0.57 ± 0.11	2.80 ± 0.78

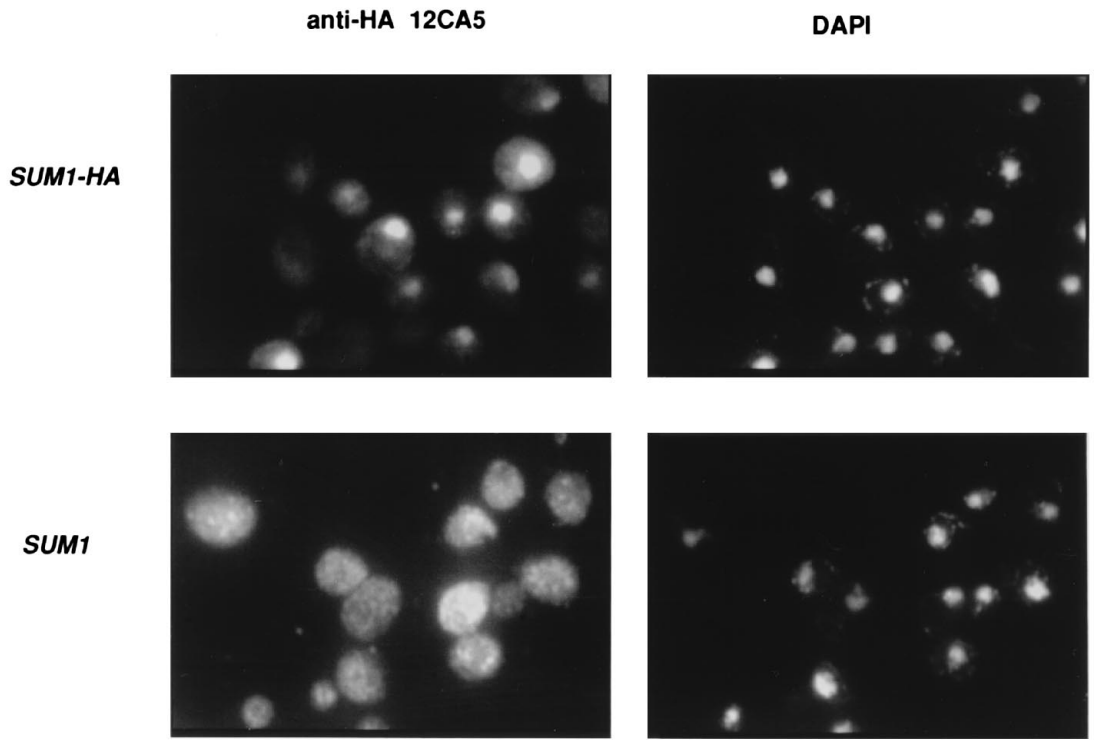

FIG. 7. Indirect immunofluorescence and 4′,6-diamidino-2-phenylindole (DAPI) staining of cells containing either HA-tagged *SUM1* or the wild-type *SUM1* gene. See text for details.

a *sum1* null mutation has a slight derepressing effect on *HM* loci in *SIR*$^+$ strains when the *HMR-E* silencer is weakened by mutation of either the A or the B site. This subtle phenotype of *sum1* null alleles explains why loss-of-function mutations in this gene were never isolated in previous genetic screens for silencing-defective mutants. Taken together, these results suggest that *SUM1-1* is either a gain-of-function (hypermorphic) mutation or an altered-function mutation. This conclusion is also consistent with previous observations (37) and results reported here, all of which indicate that the *SUM1-1* allele is dominant to the wild type. However, our observations that elevated gene dosage of the wild-type *SUM1* gene diminishes the mutant phenotype (suppression of *sir2* mating defects) in *SUM1-1* mutants and that the *SUM1-1* plasmid confers a stronger phenotype in cells devoid of Sum1p than in the wild-type cells lead us to rule out the idea that *SUM1-1* is a hypermorph.

By virtue of having cloned the *SUM1-1* allele, we have been able to examine the effect of this mutation in an isogenic set of strains. This analysis is particularly important given the previously reported strain-to-strain variation in the strength of *SUM1-1* suppression (37) and the observation that *SUM1-1* appears to be recessive to *SUM1* in some strain backgrounds (31). We found a fairly uniform, dominant *SUM1-1* phenotype in isogenic strains of the W303 background. The previously reported variation in the *SUM1-1* phenotype may have been the result of natural strain background differences. Alternatively, the original *SUM1-1* isolate, which was derived from a heavily mutagenized culture (31), may contain other mutations that modify the effect of *SUM1-1*. We noted that *SUM1-1* only slightly restores repression of a *hmr*Δ*A*::*TRP1* reporter in a *sir2* mutant, as measured by the ability to grow in the absence of tryptophan. We do not think that this observation is contradictory to the previous observation (37) that *SUM1-1* restores repression (at least 10-fold better) of *hmr*::*TRP1* in *sir3*::*LEU2* strains. The intact *HMR-E* silencer and the more leaky *sir3*::*LEU2* mutation (12a) might account for the greater suppression observed in this previous study.

With respect to the differential effect of *SUM1-1* on the two *HM* loci, our results are consistent with previous observations that *SUM1-1* more effectively restores silencing at *HMR***a** than it does at *HML*α (31, 37, 41). At present, there seem to be at least two possible explanations for this observation. The first follows from the finding that silencing at *HMR* is generally more resistant than *HML* to mutations in genes (other than *SIR* genes) which have partial effects on silencing. For example, *nat1* or *ard1* mutations have no effect at a wild-type *HMR* locus, whereas they result in partial derepression of *HML* (48). The same is true of a number of different mutations in the histone H4 (*HHF2*) N-terminal tail (29). This difference in the strength of silencing at *HMR* compared with *HML*, which may be due to the redundancy of the *HMR-E* silencer (7, 30) or the specific effect of the neighboring chromosome III-R telomere (70), could explain the apparent differential effect of *SUM1-1*. Alternatively, weak expression of α information in *MAT***a** cells may reduce mating more than weak expression of **a** information in *MAT*α cells. This could explain the observation that **a** information is better repressed than α information (as measured by mating efficiency) regardless of where the genes are located (*HML* or *HMR*) (31).

We also found that *SUM1-1* suppresses two different types of mutations in the silencer-binding protein Rap1 (*rap1*^{t} and *rap1*^{s}). *rap1*^{t} mutants are presumably defective in recruiting Sir3p and Sir4p to the silencers (47), whereas the *rap1*^{s} mutants have been proposed to create a deficiency in Sir4p (and perhaps other factors) available at the *HMR* silencer, as a result of competition by telomeres (10). We imagine that *SUM1-1* sup-

presses these mutations by overcoming the requirement for Sir3p and Sir4p in silencing, thus bypassing the Sir protein recruitment function of Rap1p.

Because many of the same genes involved in *HM* locus silencing are also involved in the variegated silencing phenomenon observed at telomeres, we also tested the effects of *sum1* and *SUM1-1* mutations on telomeric repression. Although we found no effect of a *sum1* null mutation on telomeric silencing, we observed that *SUM1-1* increases telomeric repression of two different telomeric reporters genes (*URA3* and *ADE2*) in *SIR* strains. However, *SUM1-1* does not suppress the telomeric silencing defect caused by a *sir2* mutation. The first observation demonstrates that the effect of *SUM1-1* is not restricted to the silent mating-type loci and suggests that *SUM1-1* should be viewed as a general regulator of position effects in *S. cerevisiae*. The failure of *SUM1-1* to suppress *sir* defects at telomeres may be viewed within the context of several observations which indicate that telomeric silencing is inherently weaker than repression at *HM* loci. To begin with, telomeric silencing is normally unstable, whereas *HM* silencing is not (2, 21). In addition, telomeric silencing is more sensitive to histone H4 and histone H3 mutations, and mutations in the *NAT1* and *ARD1* genes, than are *HM* loci (2, 71). Finally, a *sir3* suppressor mutation which partially restores *HML* silencing in a *hhf2* mutant strain (K16G) fails to restore telomeric repression (2). Taken together, these observations are consistent with the idea that the effect of *SIR* mutations (*SIR2* to *SIR4*) on telomeric silencing may simply be too severe for *SUM1-1* to counteract.

In thinking about how *SUM1-1* suppression might work, it is important to consider current models for silencing at *HM* loci and telomeres. Recent studies indicate that a complex of Sir proteins (containing at least Sir3p and Sir4p) can interact directly with the silencer- and telomere-binding protein Rap1p (47). Sir3p and Sir4p, in turn, are capable of binding in vitro with the N-terminal tails of histones H4 and H3 (24), which genetic studies have shown are involved in both *HM* locus and telomeric silencing (29, 45, 51, 71). These findings have led to a model in which silencing results from the recruitment of a Sir complex to silencers or telomeres and the subsequent assembly of a Sir-nucleosome complex along the chromatin fiber. Silent chromatin appears to be in an altered, more protected structure than nonsilent chromatin, as determined from its decreased accessibility to methylases (20, 36, 65), the *HO*-encoded endonuclease (32, 42, 67), restriction enzymes (42), and thiol-reactive reagents (12). However, a growing number of cell biological studies of *S. cerevisiae* suggests that nuclear localization, or more specifically attachment to the nuclear envelope, may also play an important role in silencing. Indirect immunofluorescence studies using anti-Rap1p antibodies suggest that telomeres are clustered in yeast cells and localized at or near the nuclear periphery (33, 50). Strikingly, this localization and clustering of telomeric Rap1p is lost in *sir3* or *sir4* mutant cells (50), in which telomeric silencing is also abolished.

An intriguing and perhaps informative phenotype of *SUM1-1* mutants is increased chromosome loss. We note that many genes involved in silencing also cause chromosome instability when they are mutated or deregulated. For example, *rap1*^{t} alleles (*rap1-17*) display telomere elongation and elevated chromosome instability (35). Overexpression of the *RAP1* C terminus or of *SIR2* causes increased chromosome instability and cell death, similar to that which we have observed in *SUM1-1* mutants (8, 14), albeit to different degrees. In light of these results and the current working models for silencing, we suggest two models for Sum1p and Sum1-1p function, both of which might explain the chromosome instability and decreased cell viability phenotypes of *SUM1-1* mutants. In the first model, Sum1p is involved in the localization of telomeres and *HM* loci to the nuclear periphery. The altered localization function provided by Sum1-1p is sufficient to allow silencing to occur in the absence of *SIR* gene function. An increase in chromosome loss might be a consequence of this enhanced nuclear envelope attachment function. Alternatively, Sum1p may be a normal (but nonessential) component of heterochromatin in *S. cerevisiae* which is used for both gene silencing and chromosome condensation during mitosis. In this scenario, the mutant Sum1-1p may allow *HM* loci (and perhaps other sites not normally subject to silencing) to form a stably repressed chromatin structure in the absence of Sir proteins. This inappropriate formation of heterochromatin could either directly or indirectly lead to a decrease in chromosome stability. It is important to note, however, that we cannot rule out the possibility that the chromosome loss and decreased viability phenotypes of *SUM1-1* are unrelated to its effect on silencing.

In summary, by cloning and characterizing *SUM1* and the *SUM1-1* mutant allele, we have obtained clear evidence that *SUM1-1* is a dominant altered-function mutation that can either restore or improve silencing at *HM* loci and telomeres in a number of different genetic backgrounds (e.g., *sir*, *rap1*^{t} or *rap1*^{s} mutant or wild type). The nuclear localization of Sum1p, the effect of *SUM1-1* on chromosome stability and cell viability, and the ability of the mutation to bypass the requirement for *SIR* gene function in *HM* locus silencing all point to a role for *SUM1* in chromosome function. Continued study of *SUM1* and the *SUM1-1* allele should provide new experimental approaches to address the precise function(s) of this intriguing gene.

ACKNOWLEDGMENTS

We thank J. Rine, A. Lustig, S. Gasser, A. Mitchell, M. Carlson, and M. Rose for providing strains, plasmids, and/or genomic libraries. We are also grateful to J. Ivy and O. Gileadi for communicating unpublished results. We thank L. Pon and the members of her laboratory for assistance with indirect immunofluorescence, and we thank L. Symington, K. Freeman, and P. Laurenson for critically reviewing the manuscript. M.-H.C. especially thanks P. Laurenson for encouragement and advice.

This work was supported by grants from the American Cancer Society (VM-62) and the National Institutes of Health (GM40094) to D.S.

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