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The yeast SRM1 protein and human RCC1 protein share analogous functions

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The *Saccharomyces cerevisiae* protein SRM1 and the mammalian protein RCC1 have amino acid sequence similarity throughout their lengths. *SRM1* was defined by a recessive mutation in yeast that both activates the signal transduction pathway required for mating and leads to arrest in the G1 phase of the cell cycle. *RCC1* was defined by a recessive mutation in hamster cells that causes premature chromosome condensation and other characteristics of entry into mitosis. Despite the seemingly different roles implied by these phenotypes, we suggest that RCC1 and SRM1 proteins have similar functions. In particular, we find that *RCC1* can complement the temperature-sensitive growth phenotype of two independent *srm1* mutations and also complements, at least partially, phenotypes associated with activation of the pheromone response pathway, such as transcription induction of *FUS1*. However, *RCC1* fails to complement an *srm1* null allele. Further characterization of the *srm1* mutant phenotype reveals a defect in plasmid and chromosome stability, suggesting that the mutants have a defect in DNA replication, mitosis, or their coordination. Finally, like RCC1, SRM1 is a nuclear protein. Together, these data imply that SRM1 and RCC1 have a common role in their respective organisms.

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Introduction

Progression through the eukaryotic mitotic cell cycle is controlled at a variety of points, including both the G1-S and the G2-M boundaries. Passage through these points is achieved by a mechanism that is evolutionarily conserved from the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* to mammals. The basic component is a protein kinase, sometimes called histone H1 kinase or mitosis promotion factor, that is encoded in part by the *S. cerevisiae CDC28* and *S. pombe cdc2+* homologues. Progression through the cell cycle occurs by the activation of the CDC28/cdc2 kinase at G1-S and G2-M through its association with G1- and G2-specific cyclins and its modification by other regulators, which may also be cell-cycle specific (Richardson *et al.,* 1989; Broek *et al.,* 1991; Surana *et al.,* 1991).

In *S. cerevisiae,* arrest at the G1-S transition is important for the mating process. As the first step in mating, two haploid cell types, **a** and α, interact via secreted peptide pheromones and cell surface pheromone receptors. Binding of pheromone to receptor activates an intracellular signal transduction pathway that elicits a number of physiological changes in the responding cell. These changes include arrest of the cell division cycle in the G1 phase and increased transcription of many genes required for mating. At least in part, cell cycle arrest is the result of pheromone-mediated reduction in expression of the G1 cyclin genes (Chang and Herskowitz, 1990; Elion *et al.,* 1990; Wittenberg *et al.,* 1990). Thus the pheromone response pathway integrates with the cell cycle machinery at the G1-S control point.

Genetic and physiological studies have identified a number of genes whose products are likely to be components or regulators of the signal transduction pathway. Broadly, these genes fall into two classes—those whose products are required for response to pheromone and those whose products inhibit the response pathway in the absence of pheromone. Loss-of-function mutations in the *STE* genes lead to a nonresponsive phenotype, implying that the *STE* gene products are required for activity of the re-

sponse pathway. These gene products include STE2 and STE3, the pheromone receptors (Burkholder and Hartwell, 1985; Nakayama *et al.*, 1985; Hagen *et al.*, 1986), and STE4 and STE18, homologues to the β and γ subunits of mammalian G proteins (Whiteway *et al.*, 1989). Conversely, loss-of-function mutations in *GPA1* (*SCG1*), which encodes the α subunit of the G protein, lead to pheromone-independent (constitutive) activation of the response pathway (Dietzel and Kurjan, 1987; Miyajima *et al.*, 1987).

In an effort to identify other gene products that, like GPA1, function as negative elements in the response pathway, we and others have identified mutations that restore mating to strains lacking pheromone receptors. The genes defined by these mutations include *SRM1, CDC72, CDC73, CDC36,* and *CDC39* (Reed *et al.*, 1988; Clark and Sprague, 1989; de Barros-Lopes *et al.*, 1990; Neiman *et al.*, 1990). At the restrictive temperature haploid strains harboring temperature-sensitive mutations in any of these genes exhibit a phenotype that suggests that the response pathway has been activated constitutively. This phenotype is evident only if the cells contain an otherwise normal pheromone response pathway; mutant strains that also carry a null mutation of *ste4* remain temperature-sensitive for growth but no longer arrest at a discrete point in the cell cycle nor show elevated transcription of pheromone responsive genes. One interpretation of this observation is that SRM1 (and the CDC products) has another role(s) in the life of a yeast cell and that the particular phenotype of *srm1* mutants with an intact pheromone response pathway reflects an interaction between SRM1 and a component of the pathway. In the absence of the pathway, the inability to carry out this other role contributes to the mutant phenotype.

To gain insight as to the role(s) of SRM1, we have examined in more detail both the properties of the protein and the phenotype of *srm1* mutants. We first report that SRM1 is homologous to the mammalian RCC1 protein. *RCC1* was identified by isolation of a temperature-sensitive hamster cell line in which DNA synthesis was impaired (Nishimoto *et al.*, 1978). When shifted to the restrictive temperature, asynchronous cultures of the *rcc1* mutant arrested with G1 DNA content. In contrast, cultures of the mutant first synchronized in S phase by treatment with hydroxyurea displayed premature entry into mitosis at the restrictive temperature; chromosomes underwent condensation and histones H1 and H3 became phosphorylated, implying that mitosis promotion factor was activated (Nishimoto *et al.*, 1978; Ajiro *et al.*, 1983). The defining *rcc1* phenotype, inhibition of DNA synthesis, is thought to be a secondary consequence of the premature chromosome condensation. Thus, wild-type RCC1 acts at the G2-M control point and behaves as an inhibitor of entry into mitosis, although the possibility that RCC1 also has a positive role at the G1-S transition cannot be ruled out by these analyses. Here we report further analysis of *srm1* mutants that suggests similarities in the function of the two proteins. Most strikingly, *RCC1* complements *srm1* mutations. Additionally, like RCC1, SRM1 is a nuclear protein. Moreover, plasmids and chromosomes show decreased stability in *srm1* mutants, implying a defect in DNA replication, mitosis, or coordination of the two events. One facet of the *rcc1* phenotype, induction of histone phosphorylation, is not observed in *srm1* mutants, however.

Results

SRM1 is homologous to RCC1

Previously we reported the sequence of *SRM1,* which encodes an open reading frame of 482 codons (Clark and Sprague, 1989). A search using the program FASTA (Lipman and Pearson, 1985) of a personal database of about 1600 sequences collected from the literature revealed that the predicted SRM1 protein sequence has homology to the predicted protein sequence of the 421 amino acid human cell cycle protein RCC1 (Ohtsubo *et al.*, 1987) (Figure 1). The two proteins are 28% identical, and 50% of the residues are similar if conservative substitutions are included (Schwartz and Dayhoff, 1978). This similarity extends throughout the lengths of the proteins, although a block of acidic amino acids in SRM1 (residues 130–158) was removed for optimal alignment.

RCC1 complements two independent srm1 mutations

To determine whether the amino acid sequence similarity of SRM1 and RCC1 reflected a functional similarity between the proteins, we asked whether provision of *RCC1* could complement *srm1* mutations. The human *RCC1* cDNA was fused to the *S. cerevisiae ADH1* promoter on a high-copy plasmid, pVT100-U (Vernet *et al.*, 1987), and this construct, pADH-RCC1, was transformed into several *srm1* mutants. The ability of *RCC1* to complement three facets of the *srm1* phenotype was assessed.

We first tested the ability of *RCC1* to allow growth of *srm1-1* mutants at the restrictive

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                        .         .         .         .
SRM1           1 MVKRTVATNGDASGAHRAKKMSKTHASHIINAQE.DYK
                              :  |  |   : |  |
RCC1                       1 MSPKRIAKRRSPPADAIPKSKKVKVS

              .         .         .         .         .
  38 HMYLSVQPLDIFCWGTGSMCELGLGPLAKNKEVKRPRLNPFLPRDEAKII
     |   | :|  :   | | : :||||      |    |  | |      ::
  27 HRSHSTEPGLVLTLGQGDVGQLGLG......ENVMERKKPALVSIPEDVV

              .         .         .         .         .
  88 SFAVGGMHTLALDEESNVWSWGCNDVGALGRDTSNAKEQLKD*PPLAEGH
         |||||: |   ::|:|:|||| ||||||||    ::   : :
  71 QAEAGGMHTVCLSKSGQVYSFGCNDEGALGRDTSVEGSEMVP.GKVELQE

              .         .         .         .         .
 166 KVVQLAATDNMSCALFSNGEVYAWGTFRCNEGILGFYQDKIKIQKTPWKV
     ||||::|:|  : ||  :| |: ||:|| |:|::|       : |:   |
 120 KVVQVSAGDSHTAALTDDGRVFLWGSFRDNNGVIG...LLEPMKKSMVPV

              .         .         .         .         .
 216 PTFSKYNIVQLAPGKDHILFLDEEGMVFAWGNGQQNQL...........G
            :| :|:| ||:: |  :| ::: | |:| ||           |
 167 QVQLDVPVVKVASGNDHLVMLTADGDLYTLGCGEQGQLGRVPELFANRGG

              .         .         .         .         .
 255 RKVMERFRL.KTLDPRPFGLR.HVKYIAS..GENHCFALTKDNKLVSWGL
     |  :||  : | :  :: | | ||::  :  |    ||:::: :: ::||
 217 RQGLERLLVPKCVMLKSRGSRGHVRFQDAFCGAYFTFAISHEGHVYGFGL

              .         .         .         .         .
 301 NQFGQCGVSEDVEDGALVTKPKRLALPDNVVIR.SIAAGEHHSLILSQDG
      :: |  ::    ::  ::     :  :      : ::|:||::  :  :|
 267 SNYHQ..LGTPGTESCFIPQ.NLTSFKNSTKSWVGFSGGQHHTVCMDSEG

              .         .         .         .         .
 350 DLYSCGRLDMFEVGIPKDNLPEYTYKDVHGKARAVPLPTKLNNVPKFKSV
       || ||             :||:   :   |   ::|| :  :|   ||
 314 KAYSLGR.............AEYGRLGLGEGAEEKSIPTLISRLPAVSSV

              .         .         .         .         .
 400 AAGSHHSVAVAQNGIAYSWGFGETYAVGLGPFEDDTEVPTRIKNTATQDH
     | |:  : ||: :|  ::|| |  | :| |  ::|:  |  :     :::
 351 ACGASVGYAVTKDGRVFAWGMGTNYQLGTGQ.DEDAWSPVEMMGKQLENR

              .         .         .
 450 NIILVGCGGQFSVSGGVKLSDEDAEKRADEMDD 482
      :: |: ||| :|     |   | |
 400 VVLSVSSGGQHTV.....LLVKDKEQS 421
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Figure 1. **Comparison of SRM1 and RCC1 amino acid sequences.** Lines (|) indicate identical residues and colons (:) indicate chemically similar residues. The groups of chemically similar amino acids are: S, T, P, A, G; D, E, N, Q; H, R, K; M, I, L, V; F, Y, W; and C (Schwartz and Dayhoff, 1978). Gaps (.) are inserted to maximize sequence homology and the asterisk (*) indicates a large gap of SRM1 from residues 130 to 158.

temperature. As shown in Table 1, the plating efficiency at 37°C of several *srm1-1* strains was similar after transformation with pADH1-RCC1 (pSL1211) or with wild-type *SRM1* (pSL749). A second construct in which *RCC1* expression was driven by the *S. cerevisiae PGK1* promoter also complemented *srm1-1* mutations (data not shown), indicating that complementation is not peculiar to the *pADH1-RCC1* gene fusion. The pADH-RCC1 and pPGK-RCC1 constructs were each present on multicopy plasmids. Expression of *RCC1* from the *GAL1* promoter on a single-copy plasmid also allowed complementation, albeit at a noticeably reduced level (data not shown).

The second *srm1* phenotype examined was induction of *FUS1* transcription at elevated temperatures. *FUS1* is required for efficient cell fusion during mating. In wild-type cells its transcription is increased 10- to 100-fold by pheromone stimulation (McCaffrey *et al.,* 1987; Trueheart *et al.,* 1987). In *srm1* mutants *FUS1* transcription is increased about 10-fold by shifting cells to the restrictive temperature (Clark and Sprague, 1989). When pADH-RCC1 was present in *srm1-1* mutants, no increase in *FUS1* transcription was observed after temperature shifts (data not shown), indicating *RCC1* also complements this *srm1* phenotype.

srm1-1 was identified by its ability to confer mating competence to strains lacking pheromone receptors (*ste3::LEU2* or *ste2::LEU2* mutants) (Clark and Sprague, 1989). Therefore as a final test of the ability of RCC1 to substitute for SRM1, we asked whether expression of *RCC1* reduced the mating capacity of α *srm1-1 ste3::LEU2* mutants. As shown in Table 2, the most dramatic effect was seen when the mating conditions were rather stringent; that is, when the opportunity to mate was limited to 4 h or when *fus1* mutants, which have diminished mating capacity (McCaffrey *et al.,* 1987; Trueheart *et al.,* 1987), were used as the mating partner. Under these conditions, expression of *RCC1* reduced the mating efficiency by 10- to 50-fold. However, in no case did *RCC1* reduce the mating efficiency to the same extent as did *SRM1* (Table 2). Thus by this assay *RCC1* complements *srm1-1,* but only partially.

To extend this analysis we wanted to learn whether *RCC1* could complement other alleles of *SRM1.* For this purpose we were fortunate to find that *SRM1* was identical to a gene described previously, *TSM437* (Hartwell, 1967; Hartwell and McLaughlin, 1968). Genetic crosses revealed that *SRM1* was closely linked to *CYH2* (data not shown) as is *TSM437* (Mortimer and Hawthorne, 1973). An **a**/α *tsm437-1*/*srm1-1* diploid (KCY85 × SY1115) was temperature-sensitive and yielded only temperature-sensitive segregants in 11 tetrads, indicating that the mutations segregate as alleles and fail to complement. *SRM1* and *TSM437* are therefore the same gene.

TSM437 was identified by the isolation of temperature-sensitive mutants. Strains carrying the *tsm437-1* allele exhibited decreased RNA and protein synthesis at the restrictive temperature; however, other *tsm437* mutant alleles exhibited a variety of patterns of macromolecular synthesis (Hartwell and McLaughlin, 1968). Recently, another allele of this gene was identified by isolation of temperature-sensitive mutants defective for RNA processing (*PRP20*) (Aebi *et al.,* 1990). Unlike *srm1-1* mutants, none

Table 1. Complementation of the *srm1* temperature sensitive phenotype by *RCC1*

Strain	Genotype	Plating efficiency at 37°C[a]		
		+pVT100-U	+pADH-RCC1 (pSL1211)	+SRM1 (pSL749)
SY1327	α *srm1-1*	<0.2	87	90
SY1339	a/α *srm1-1/srm1::LEU2*	<0.5	79	102
KCY84	α *tsm437-1*	<0.2	37	92
KCY78	a/α *tsm437-1/tsm437-1*	<0.2	40	97

[a] The plating efficiencies of the strains harboring the indicated plasmids were determined by calculating the percentage of colonies that grew at 37°C relative to 22°C. See Materials and methods for details. Results are the average of two to five experiments.

of the other mutants was reported to arrest at a discrete position in the cell cycle.

The observation that mutation of *SRM1/TSM437/PRP20* can give rise to a wide array of phenotypes highlights the importance of asking whether *RCC1* can complement alleles other than *srm1-1.* The pADH-RCC1 construct was introduced into *tsm437-1* strains by transformation, and the plating efficiency of these transformants was measured at 37°C. As shown in Table 1, pADH-RCC1 complemented *tsm437-1,* although the plating efficiency was slightly less than that observed when the *RCC1* construct was present in *srm1-1* mutants. In addition, Fleischman *et al.* (1991) have observed that *RCC1* complements *prp20* mutations.

RCC1 does not complement deletion of SRM1

To determine whether RCC1 could substitute for all SRM1 functions, we asked whether *RCC1* was able to complement an *srm1* deletion allele. *SRM1* is an essential gene; diploids heterozygous for *srm1::LEU2,* a null allele of *SRM1,* yield tetrads with two viable and two inviable progeny. The inviable progeny typically undergo one to three cell divisions and arrest either as unbudded cells, implying arrest in G1, or as cells with large buds. As it is unclear whether the budded cells have completed cytokinesis, they may be arrested in M or in G1 (Clark and Sprague, 1989). When an *srm1-1/srm1::LEU2* heterozygote, SY1339, was transformed with a plasmid carrying *SRM1* (pSL749), resulting tetrads had two, three, or four viable progeny because *srml::LEU2* spores harboring the *SRM1* plasmid were viable. However, when SY1339 was transformed with pADH-RCC1 (pSL1211), each tetrad still had at most two viable progeny, indicating that *RCC1* was not able to complement deletion of the *SRM1* gene. The inviable segregants from this heterozygote were ex-

Table 2. Mating assays

Strain	Mating efficiency at 33°C		
	SY1229 (6 hr)	SY1229 (4 hr)	KCY23 (6 hr)
α *srm1-1 ste3::LEU2*[a]			
+pVT100-U	5.6×10^{-2}	2.0×10^{-4}	5.6×10^{-4}
+pADH-RCC1 (pSL1211)	1.8×10^{-2}	2.3×10^{-5}	1.6×10^{-5}
+SRM1 (pSL749)	1.7×10^{-6}	6.9×10^{-7}	2.4×10^{-7}
α *tsm437-1 ste3::LEU2*[b]			
+YCp50	2.1×10^{-5}	ND	ND
+SRM1 (pSL749)	7.1×10^{-7}	ND	ND

[a] The mating efficiencies of SY1544 cultures harboring the indicated plasmids were determined using the indicated tester strains for the times shown. SY1229 is a wild-type strain and KCY23 is a *fus1* mutant. Results are the average of two experiments.

[b] The mating efficiencies of KCY108 cultures harboring YCp50 or pSL749 were determined by mating to the tester strain SY1229 for 5 h. Results are the average of two experiments. ND, not done.

amined microscopically and found to be indistinguishable from inviable segregants of the same diploid carrying either the vector, pVT100-U, or no plasmid. Thus RCC1 is unable to confer additional cell division to an *srm1::LEU2* mutant.

SRM1 is a nuclear protein

The finding that *RCC1* can complement presumptive *srm1* point mutations raises the question: do SRM1 and RCC1 perform similar functions in their respective organisms? Although the defining mutations appear to confer different phenotypes, the complementation results prompted us to ask whether features of the *rcc1* phenotype could be observed in *srm1* mutants. We first determined the subcellular location of SRM1 and then examined two facets of mitosis in *srm1* mutants.

The subcellular location of SRM1 was determined by indirect immunofluorescence of spheroplasts using antibodies to bacterially expressed SRM1 protein fragments: either the amino-terminal half, residues 1-263, or the carboxyl-terminal half, residues 222–482. These antisera were purified by affinity chromatography and tested for specificity. In extracts of wild-type cells, both sera recognized a protein of the predicted size of SRM1 (53kDa) (Figure 2 and data not shown). This protein species was modestly more abundant if the cells also harbored a multicopy plasmid carrying *SRM1* (pSL805) (Figure 2, lane 3). Moreover, the protein was absent from a strain that did not express *SRM1.* In particular, a *MATα srm1::LEU2* strain (YY1244) harboring a single-copy pGAL1-SRM1 construct, pSL849, was viable only when grown on galactose medium. On transfer to glucose medium, the *GAL1-SRM1* gene was repressed and growth of the strain slowed and morphologically aberrant cells (shmoos) appeared, similar to those seen on prolonged exposure of wild-type cells to pheromone. Concommitantly, the level of the 53-kDa protein was depleted (Figure 2, lane 1).

Indirect immunofluorescence of wild-type cells or cells overexpressing SRM1 showed that anti-SRM1 staining was coincident with DAPI staining of the nucleus (Figure 3). Greater than 90% of the cells showed nuclear staining with the SRM1 antisera and no consistent pattern of non-nuclear staining was observed. Additionally, there was no specific staining of YY1244 cells grown in glucose to deplete SRM1 (Figure 3). Similar results were obtained with both antibodies. These results indicate that SRM1 is located in the nucleus of *S. cerevisiae* cells, just as RCC1 is located in the nucleus of mammalian cells (Ohtsubo *et al.,* 1989).

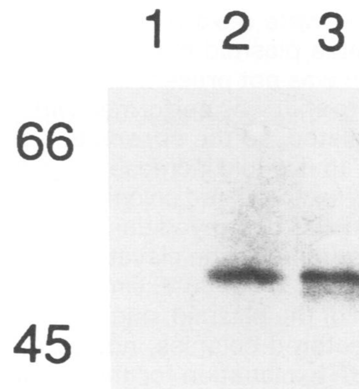

Figure 2. **Immunoblot analysis of SRM1.** Protein extracts from an SRM1-depleted strain YY1244 (lane 1), a wild type-strain SY1187 (lane 2), and an SRM1-overexpressed strain SY1187 + pSL805 (lane 3) were immunoblotted with affinity-purified antibody 330 to SRM1. The positions of protein molecular weight standards, in kilodaltons, are shown.

SRM1 is important for plasmid and chromosome stability

Some of the phenotypes of the *rcc1* mutant hamster cell line suggest that RCC1 is involved in the regulation of mitosis. For example, chromosomes undergo premature condensation and the nuclear envelope breaks down (Nishimoto *et al.,* 1978). It is not feasible to assay these characteristics of mitosis in yeast, but we reasoned that a defect in DNA replication, mitosis, or the coordination of the two might be reflected by a decrease in the stability of plasmids and chromosomes.

Initially we examined the stability of a centromere-containing plasmid, pC689-UAA, that contains the *SUP4-oc* tRNA suppressor (Shaw and Olson, 1984) using a colony color assay as described by Hieter *et al.* (1985) and by Koshland *et al.* (1985). Cells carrying the *ade2-101*^{oc} mutation form white colonies if they also harbor the pC689-UAA plasmid but form red colonies if the plasmid is not present. Thus the frequency of red and red-sectored colonies that arise from an *ade2-101*^{oc}/pC689-UAA strain is a measure of the stability of the plasmid.

When *srm1-1 ade2-101*^{oc} mutants carrying pC689-UAA were incubated for 16 h at 30° or 33°C and then returned to 22°C, the frequency of red and red-sectored colonies increased four to five times relative to the frequency seen with mutants maintained at 22°C or with *SRM1 ade2-101*^{oc} cells that were either maintained at 22°C or shifted to high temperature (Table 3). The

red colonies were auxotrophic for tryptophan, the selectable plasmid marker, indicating that the plasmid was not present in these cells. The temperature shift was performed with cells that had been plated, so the observation that there was a four- to five-fold increase in the frequency of pure (not sectored) red colonies suggests that the plasmid was destroyed during incubation of the *srm1* mutants at the elevated temperature. If incubation at 30° or 33°C simply caused nondisjunction of the plasmid, one would expect to observe sectored colonies, not pure colonies. An alternate explanation for the occurrence of pure red colonies is that suppressor tRNAs are hypertoxic in *srm1* mutants. We therefore examined the stability of natural chromosomes in *srm1* mutants.

To determine whether chromosomes also exhibit increased instability in *srm1* mutants, the frequency with which red sectors or colonies appeared was compared for two isogenic diploids. At the *SRM1* locus one diploid had the genotype *srm1-1/SRM1* and the other diploid had the genotype *srm1-1/srm1::LEU2.* Both diploids were heterozygous at the *ADE2* and *ADE1* loci (*ADE1/ade1 ADE2/ade2*). If either the chromosome XV homologue carrying *ADE2* or the chromosome I homologue carrying *ADE1* were lost during growth of a colony, the colony would have a red sector. No red sectors were produced by the diploid carrying one wild-type *SRM1* allele (Table 4). In contrast, the *srm1-1/srm1::LEU2* mutant diploid had a marked increase in the frequency of red colonies after a pulse of incubation at high temperature (Table 4). In principle, red colonies or sectors could arise as a result of chromosome loss or as a result of mitotic recombination to create a cell homozygous for the *ade1* or *ade2* mutation. To

Table 3. Plasmid stability in *srm1-1* mutants

	% Red colonies[a]			
	22°C		30°C	
Genotype	Sector	Entire	Sector	Entire
SRM1	5.5	3.5	4.0	3.0
srm1-1	4.3	2.3	16.0	15.0

[a] The percentages of colonies that were entirely red (Entire) or at least half-sectored red (Sector) after overnight incubation at the indicated temperatures are presented. The *SRM1* data are from strain SY1546 and the *srm1-1* data are the averaged data from three isogenic mutants, K63A, K63B, and K64A (see Materials and methods). Results from a representative experiment are presented. Similar results were observed at 30° and 33°C (data not shown).

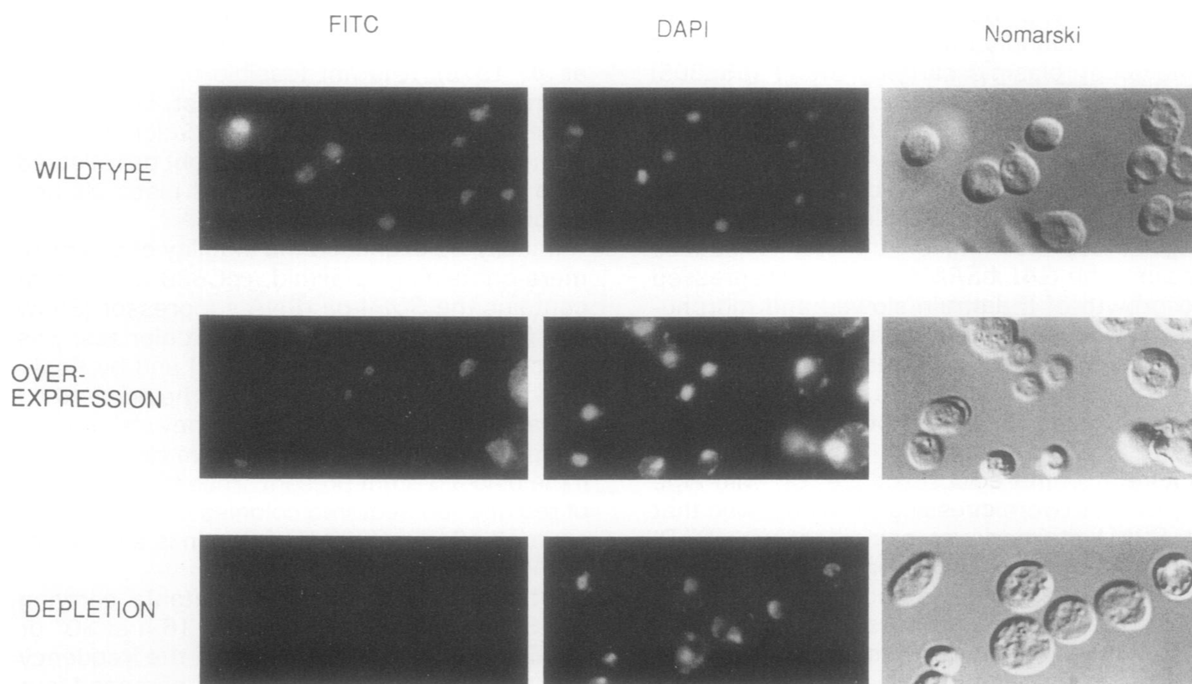

Figure 3. **Immunolocalization of SRM1.** Formaldehyde-fixed spheroplasts were treated with affinity-purified antibody 330 to SRM1 and then examined by Nomarski interference optics or by epifluorescence at the excitation wavelengths of DAPI (for nuclear staining) or of FITC (for SRM1 localization). The strains examined were SY1187 (WILD-TYPE), SY1187 + pSL805 (OVEREXPRESSION), and YY1244 (DEPLETION).

Table 4. Chromosome stability in *srm1* mutants

Strain	Genotype	% Colonies showing red sectors[a]		
		22°C	30°C	33°C
SY1289	*srm1-1/SRM1*	<0.10	<0.08	<0.09
SY1339	*srm1-1/srm1::LEU2*	<0.14	1.3	2.2

[a] The percentages of colonies that displayed any red sectors after an overnight pulse incubation at the indicated temperatures are presented. After a 30°C pulse 0.08% of the SY1339 colonies were at least half-sectored, 0.08% were all red, and 1.1% had small sectors. After a 33°C pulse 1.6% of the SY1339 colonies were at least half-sectored, 0.43% were all red, and 0.2% had small sectors. These are the results of a representative experiment.

distinguish between these possibilities we tested the spore viability of several red and white colonies. If chromosome loss events were responsible for the red colonies, spore viability should be <50%; if mitotic recombination were responsible for the red colonies, spore viability should be the same as seen for the initial diploid. In fact, red colonies had only 20–40% the spore viability of white colonies consistent with the possibility that the genetic constitution of the red colonies was at best 2n-1. Thus the red sectors were the result of improper chromosome segregation. As the frequency of chromosome loss by wild-type *S. cerevisiae* is generally 1 loss per 10^5 generations (Esposito *et al.*, 1982; Hartwell *et al.*, 1982), *srm1-1/srm1::LEU2* mutants display greatly increased chromosome instability.

H1 kinase is not activated in srm1 mutants

rcc1 mutants exhibit another phenotype, increased phosphorylation of histones H1 and H3, that implies that RCC1 regulates mitosis (Ajiro *et al.*, 1983). The increase in histone phosphorylation is presumably the result of activation of H1 kinase, but this has not been tested directly. To determine whether *srm1-1* or *tsm437-1* mutants have elevated H1 kinase activity, we treated the mutant cells with hydroxyurea (HU) to arrest their division cycles at the G1-S boundary (Slater, 1973) and then shifted the cells to the restrictive temperature in the continued presence of HU for 3 h. Extracts prepared from these cells had essentially the same, or perhaps less, H1 kinase activity as did extracts prepared from mutants held at the permissive temperature or as extracts of wild-type cells (Figure 4A). In contrast, extracts of temperature-sensitive *cdc13-1* cells, expected from other studies to have elevated H1 kinase activity (Langan *et al.*, 1989), showed a marked increase in H1 kinase activity when incubated at the restrictive temperature (Figure 4B). The HU treatment did not affect the results because no increase in kinase activity was observed using asynchronous cultures (data not shown). Thus the activity of H1 kinase appears not to be increased in two different *srm1* mutants; in this respect the *srm1* phenotype differs from that seen for *rcc1.*

Phenotypes of tsm437-1 strains

As noted above, *tsm437* mutations are alleles of *SRM1*, yet certain features of the *srm1-1*

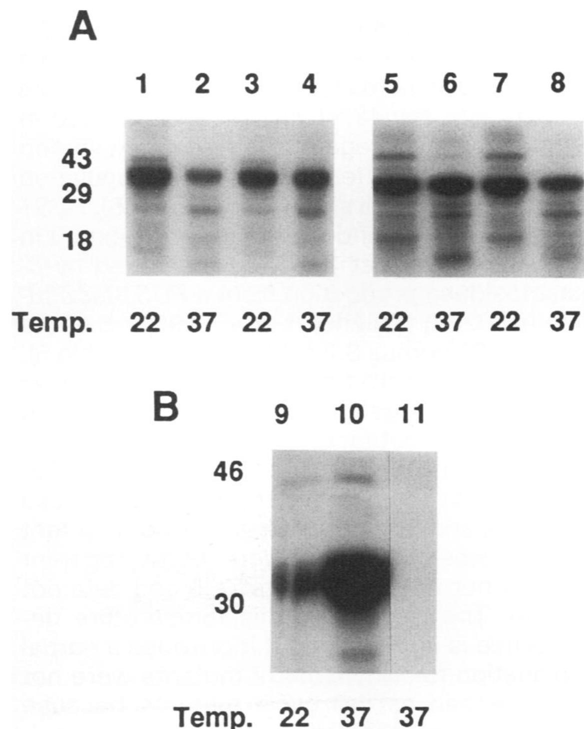

Figure 4. **Histone H1 kinase assay.** Protein extracts from log-phase cells were prepared and assayed for histone H1 kinase activity as described in Materials and methods. Samples in panel A were arrested with hydroxyurea then incubated at the indicated temperature for 3 h in the continued presence of hydroxyurea. Samples in panel B were grown at 22°C then harvested (lane 9) or shifted to 37°C for 3 h (lanes 10 and 11) without any drug treatment. Strains of lanes 1–4 are isogenic: SY1339 (*srm1-1/srm1::LEU2;* lanes 1 and 2) and SY1289 (*srm1-1/SRM1;* lanes 3 and 4). Strains of lanes 5–11 are related to each other: A364A (*TSM437;* lanes 5 and 6), tsm437 (*tsm437-1;* lanes 7 and 8), and H120-17-4 (*cdc13-1;* lanes 9, 10, and 11). Lane 11 is a control lane in which no histone H1 was added. The molecular weight standards are presented in kilodaltons. Comparable results were obtained when the kinase assays were performed with cells incubated at 33°C for 3 h before preparation of extracts (data not shown).

phenotype are not readily apparent in *tsm437* strains. To determine whether the *srm1-1* phenotype is peculiar to that allele, the original **a** *tsm437-1* strain (tsm437) was put through two successive genetic crosses to place the mutation in a genetic background more similar to that used to study *srm1-1.* Temperature-sensitive segregants from these crosses were then examined with respect to the phenotypes we used to characterize *srm1-1* strains: suppression of the mating defect conferred by *ste3::LEU2* mutations, cell cycle arrest in G1 and formation of projections (shmoos), and *FUS1* transcription induction.

The *tsm437-1* strains exhibited each of these phenotypes, although usually to a lesser extent than *srm1-1* strains. For example, an α *tsm437-1 ste3::LEU2* strain (KCY108) showed a 30-fold increase in mating, whereas α *srm1-1 ste3::LEU2* strains showed as much as a 30 000-fold increase relative to α *ste3::LEU2* strains (Table 2). Similarly, *tsm437-1* strains accumulated in G1 and some segregants also exhibited shmoo formation at 33°C (e.g., 78% of the population in G1, including 3% shmoos for KCY105). *FUS1* transcription was induced to a similar extent in the two mutants at 33°C, as measured by β-galactosidase production from a *FUS1-lacZ* fusion (pSB234) (Trueheart *et al.,* 1987): 5.6-fold for KCY105 versus 3.2-fold for YY811 (Table 5). However, the *srm1-1* mutant had a much higher basal level of β-galactosidase activity than did the *tsm437-1* mutant (31 units versus 1.4 units) (Table 5), implying that *srm1-1,* but not *tsm437-1,* is leaky at the permissive temperature (also see Clark and Sprague, 1989). For both mutant alleles, these phenotypes were readily apparent at 33°C but not at 37°C (Table 5 and data not shown). The reason for this temperature dependence is not known but it provides a partial explanation for why *tsm437* mutants were not characterized as cell cycle mutants because those studies were done at 37°C.

Table 5. *FUS1-lacZ* induction in *srm1* mutants

Strain	Genotype	β-Galactosidase units[a]		
		22°C	33°C	37°C
KCY106	α *TSM437*	3.0	2.2	2.4
KCY105	α *tsm437-1*	1.4	7.8	1.2
YY811	α *srm1-1*	31	99	26

[a] The indicated strains harbored pSB234 and were grown at 22°C, shifted to the indicated temperature for 4 h, harvested, and assayed for β-galactosidase activity. Results are the average of duplicate experiments.

Discussion

SRM1 and *RCC1* mutants were isolated by screens designed to identify genes that participate in seemingly unrelated aspects of cell biology, mating in yeast and control of DNA synthesis and mitosis in hamster cells. Analysis of the mutant phenotypes suggested that both gene products are involved in control of the cell cycle: SRM1 in the G1 phase, RCC1 in the G2 phase and perhaps in the G1 phase. As a common mechanism involving the CDC28/cdc2 kinase is thought to be required for progression through both of these control points, it is perhaps reasonable to imagine that other proteins also play crucial roles at both checkpoints. Our findings suggest that SRM1/RCC1 may be such a protein. Alternatively, SRM1/RCC1 may not have a specific role in the cell cycle, but certain *srm1/rcc1* mutations may lead to arrest in G1 or G2 because these are important cell cycle control points. In either event our results imply that SRM1 and RCC1 proteins have analogous functions in their respective organisms.

Foremost, *RCC1* complements three different *SRM1* mutations. Additionally, it has been shown that *SRM1* is able to complement the temperature-sensitive defect of *rcc1* mutant hamster cells (Ohtsubo *et al.,* 1991).

Second, our analysis of *srm1* phenotypes, coupled with the realization that other alleles of the same gene have been studied by others, reveals that *srm1* mutations are pleiotropic, as is the *rcc1-1* mutation. In fact, some of the mutant yeast and hamster cell phenotypes are similar. 1) Under some conditions both mutants arrest in G1. In particular, asynchronous populations of haploid *srm1-1* mutants accumulate as unbudded (G1) cells at the restrictive temperature. Likewise, asynchronous populations of hamster *rcc1* mutant cells accumulate in G1 because they cannot initiate a new round of DNA replication. 2) Bulk protein and RNA synthesis is diminished in hamster cell mutants and in yeast mutants harboring certain *srm1* (*tsm437*) alleles. 3) Both *srm1* and *rcc1* mutants exhibit defects in chromosome metabolism. *srm1-1* mutants have reduced chromosome stability and *rcc1* mutants exhibit premature chromosome condensation, histone phosphorylation, and nuclear envelope breakdown if they are synchronized in S phase before the temperature shift. 4) Both SRM1 and RCC1 are nuclear proteins. Whether each of these similarities reflects similarities in the mechanism of action of SRM1/RCC1 in the two organisms is not known. Some aspects of the mutant phe-

notype, for example G1 arrest, may be only superficially or fortuitously alike, but the ability of RCC1 to substitute for SRM1 (and vice versa) implies that at least some of the similarities reflect a common role for SRM1 and RCC1.

The foregoing discussion emphasized the similarities between SRM1 and RCC1 function. However, the finding that *RCC1* does not complement a null allele of *SRM1* raises the possibility that SRM1 has a function in yeast for which there is no analogue in mammalian cells. Alternatively, it is possible that SRM1 and RCC1 carry out the same constellation of functions in their respective organisms but that RCC1 cannot provide one or more of these functions in the milieu of a yeast cell. Precedent for this possibility is seen in the inability of the human gene for transcription factor IID to complement a null allele of the yeast TFIID gene even though the human factor can substitute in in vitro assays (Gill and Tjian, 1991; Cormack *et al.*, 1991).

Two models can be proposed for the nature of the common SRM1/RCC1 function(s). One is that SRM1/RCC1 is a nuclear protein with a function not directly related to the cell cycle. For example, SRM1/RCC1 may be involved in chromatin structure and affect gene expression in a nonspecific way. Different mutant alleles may alter the pattern of gene expression differently. Some mutations may create a physiology that mimics a condition leading to G1 arrest (e.g., yeast *srm1-1*), whereas others may lead to induction of mitosis (e.g., hamster *rcc1-1*). In support of this model, a protein homologous to RCC1 has been identified as a kinetochore-binding protein in mammalian cells (Bischoff *et al.*, 1990).

A second model is that SRM1 and RCC1 have specific roles at two or more points in the cell cycle, including at the G1-S and G2-M boundaries. The analysis of RCC1 has concentrated on its role in controlling mitosis; other aspects of the *rcc1* mutant phenotype are thought to be a secondary consequence of premature chromosome condensation. However, perhaps the accumulation of asynchronous populations of *rcc1* mutants in the G1 phase actually reflects a requirement for RCC1 function to initiate DNA replication. Similarly, although analysis of SRM1 has concentrated on a potential role in G1, our finding that *srm1-1* mutants display plasmid and chromosome instability points to a role for SRM1 in replication or mitosis. Thus it is tempting to speculate that SRM1/RCC1 regulates both exit from G1 and entry into M phase. Precedent for this idea comes from the requirement for CDC28/cdc2 kinase at both the G1-S and G2-M control points. The mutant *SRM1/RCC1* alleles may confer different phenotypes if the two control points have different sensitivities to the amount or activity of SRM1/RCC1 or if the mutant proteins have altered abilities to interact with cell cycle-specific components.

Materials and methods

Strains and media

The relevant strains are presented in Table 6. Strains were constructed by standard genetic manipulations (Sherman *et al.*, 1987) and by DNA transformation (Scherer and Davis, 1979; Rothstein, 1983). The genotypes of strains constructed by transformation were verified by Southern analysis (Southern, 1975).

The media used were YEPD, SD, SD-URA, SD-TRP, and SD-LEU (Sherman *et al.*, 1987; Clark and Sprague, 1989).

DNA manipulations

Plasmid constructions and bacterial transformations were performed by standard methods (Sambrook *et al.*, 1989). Yeast transformations were performed essentially as described by Beggs (1978) or by Ito *et al.* (1983). DNA and RNA samples were collected and Southern analysis of the DNA or Northern analysis of the RNA was performed as described (Clark and Sprague, 1989). A filter of yeast chromosomes separated by CHEF electrophoresis (Chu *et al.*, 1986) and blotted onto a Zeta-Probe membrane (Bio-Rad, Richmond, CA) was generously provided by E. Louis and J. Haber.

Plasmids

A plasmid to express the human *RCC1* gene from the *S. cerevisiae ADH1* promoter was constructed as follows. A fragment containing the human *RCC1* cDNA was excised from plasmid pSP64.HgiA1.PstI by digestion with *Hin*dIII and *Sal*I. The *RCC1*-containing fragment was cloned into *Hin*dIII-, *Xho* I-cut pVT100-U (Vernet *et al.*, 1987), producing pADH-RCC1. pVT100-U is a 2 μ *URA3* vector with the *Hin*dIII and *Xho*I sites located between the promoter and the terminator of the *ADH1* gene. A construct to express *RCC1* from the *S. cerevisiae PGK1* promoter was constructed in the 2 μ *URA3* vector pCKR201-1 (a gift of C. Raymond). The ends of the *Hin*dIII-*Sal*I *RCC1* fragment were filled in, and the fragment was ligated into the filled-in *Bgl*II site of pCKR201-1 between the *PGK1* promoter and terminator, creating pPGK-RCC1.

Plasmid pSL749 has *SRM1* on YCp50 (Clark and Sprague, 1989). Plasmids pSL805 and pSL839 are fusions of the coding sequence of *SRM1* to the *GAL1/GAL10* promoter (Johnston and Davis, 1984) on a high copy *LEU2* and a centromere-based *URA3* plasmid, respectively. pSB234 is a 2 μ *URA3* plasmid harboring a *FUS1-lacZ* fusion (Trueheart *et al.*, 1987). β-galactosidase assays were performed as described (Jarvis *et al.*, 1988).

Plating efficiency and mating assays

Plasmid-bearing strains were grown overnight at room temperature in SD-URA to select for maintenance of the plasmid. A dilution was plated on SD-URA plates, and the plates were placed at room temperature or 37°C to allow colonies to form. The number of colonies that formed at each temperature was counted after several days. The plating efficiency of a strain was defined as the percentage of colonies that grew at 37°C relative to those that grew at 22°C.

Table 6. Strains

Strain	Genotype	Source
SY1115	*MAT*α *srm1-1 ura3 leu2 trp1 his4*	Clark and Sprague, 1989
SY1327	*MAT*α *srm1-1 ura3 leu2 trp1 ade1 ade2-1*^{oc} *his4 his3* or *HIS3, his6* or *HIS6*	Clark and Sprague, 1989
SY1289	***MAT*a/*MAT*α *SRM1/srm1-1 STE3/ste3::ura3 ura3/ura3 leu2/leu2 ade1/ ADE1 ade2-1*^{oc}*/ADE2 trp1*^{am}*/trp1 LYS2/lys2 met14/MET14 his6/HIS6 HIS3/*(*his3* or *HIS3*) *HIS4/*(*his4* or *HIS4*)	This work
SY1339	Isogenic with SY1289 except *srm1::LEU2/srm1-1*	This work
SY1544	*MAT*α *srm1-1 ste3::LEU2 ura3 leu2 trp1 ade1 his4 his3, his6* or *HIS6*	Sprague lab strain
SY1546	*MAT*α *srm1-1:*YIp5:*SRM1 ste3::LEU2 ura3 leu2 trp1-*Δ*1 ade2-101*^{oc} *his3* or *HIS3, his4* or *HIS4*	This work
K63A	Isogenic to SY1546 except *srm1-1*	This work
YY811	*MAT*α *srm1-1 ste3::LEU2 ura3 leu2 trp1 his3 his4*	Clark and Sprague, 1989
YY1244[a]	*MAT*α *srm1::LEU2 ste3::ura3 ura3 leu2* + pSL839 (*trp1 lys2 his3 his4 his6 met14*)	This work
KCY78	KCY84 × KCY85	This work
KCY84	*MAT*α *tsm437-1 ura3 ade1 ade2 met14* or *MET14*	This work
KCY85[a]	*MAT***a** tsm437-1 ura3 ade1 ade2 trp1 his-(*his6 his7 met14*)	This work
KCY105[a]	*MAT*α *tsm437-1 ura3 leu2* (*trp1 lys2 tyr1 his6 his7 his4 met14*)	This work
KCY106[a]	*MAT*α *ura3 leu2* (*trp1 lys2 tyr1 his3 his4 his6 his7 met14*)	Cosegregant of KCY105
KCY108	Isogenic to KCY105 except *ste3::LEU2*	This work
SY1187	*MAT*α *ura3 leu2 ade2-1*^{oc} *ade1 met14 his6 trp1*^{am} *sup4* or *SUP4-3*	Sprague lab strain
SY1229	*MAT*α *ura3 leu2-3, −112 his3 gal2 can1*	Clark and Sprague, 1989
HR125-5d	*MAT***a** *ura3-52 leu2-3, −112 his3 his4 trp*1	Hagen *et al.*, 1984
KCY23	Isogenic to HR125-5d except *fus1*	Sprague lab strain
A364A	*MAT***a** *ade1 ade2 lys2 tyr1 his7 ura1*	L. Hartwell and McLaughlin, 1968
tsm437	Isogenic to A364A except *tsm437-1*	L. Hartwell and McLaughlin, 1968
H120-17-4	*MAT*α *cdc13-1 ade1 ade2 ura1 leu*$^-$	Langan *et al.*, 1989

Quantitative mating assays were performed essentially as described (Clark and Sprague, 1989). Plasmid-bearing α strains were allowed to mate at 33°C with wild-type **a** cells for 6 or 4 h, or to mate with **a** *fus1* mutants for 6 h. Mating efficiency was calculated as the number of diploids formed divided by the number of plasmid-bearing α haploids.

Antibodies, immunoblotting, and immunolocalization

Two plasmids were constructed to express parts of SRM1 in *E. coli* using the expression plasmids pEXP1 and pEXP2 (Raymond *et al.*, 1990). Plasmid pSL834 has residues 1 to 263 of SRM1 fused to the 15th residue of T4 lysozyme, which is expressed from the *tac* promoter. Three heterologous residues are present at the carboxy-terminus of this fusion. Plasmid pSL831 has residues 222 to 482 of SRM1 fused to the 14th residue of T4 lysozyme.

Extracts of *E. coli* were prepared and the proteins of interest were isolated from sodium dodecyl sulfate-polyacrylamide gels and injected intradermally into female rabbits as described (Roberts *et al.*, 1989). The rabbits were boosted once, and serum was collected at intervals. Serum 327 was raised against protein from pSL834 and serum 330 was raised against the pSL831 product. The sera were affinity-purified twice against the proteins used as antigens as described (Jarvis *et al.*, 1989).

Crude protein extracts were made by the boiling technique described by Jarvis *et al.* (1989). Protein extracts for immunoblotting were made from the same cultures that were used for the immunolocalization experiments. Immunoblotting procedures were performed as described (Jarvis *et al.*, 1989) using a 1:1000 dilution of affinity-purified 330 antibodies.

Immunolocalization of SRM1 was performed with fixed spheroplasts essentially by the procedure of Raymond *et al.* (1990).

Plasmid and chromosome stability analysis

Plasmid stability was assayed by a color assay (Hieter *et al.*, 1985; Koshland *et al.*, 1985) using *ade2-101*^{oc} mutants carrying the plasmid pC689-UAA a gift of M. Olson, Department of Genetics, Washington University, St. Louis, MO. Plasmid pC689-UAA has an *ochre*-suppressing tRNA as well as *TRP1* and *CEN3* (Shaw and Olson, 1984), so *ade2-101*^{oc} mutants that carry the plasmid form white colonies. Cultures were grown overnight at room temperature in SD-TRP to select for maintenance of the plasmid. Dilutions of the overnight cultures were plated on YEPD plates, and these were left at room temperature or incubated at 30° or 33°C for 16 h before being returned to room temperature for several days to allow color development. The percentage of colonies that were entirely red or at least half red indicating that the plasmid had been lost by the first division was determined for each plate.

Chromosome stability was assayed in a similar manner. Strains SY1289 and SY1339 were grown overnight in YEPD at room temperature, and dilutions of the overnight cultures were plated on YEPD plates. Again, the plates were kept at room temperature or they were incubated at 30° or 33°C for 16 h and then returned to room temperature for several days to allow color development. The percentage of colonies that were entirely red, were at least half red, or displayed any red pigment was determined for each plate.

Histone H1 Kinase Assays

A culture of strain H120-17-4 was grown to log-phase at room temperature at which time part of the culture was

shifted to 37°C for 3 h, and the remainder was harvested to prepare crude protein extracts. Exponentially growing cultures of strains A364A, tsm437, SY1289, and SY1339 were first arrested with 0.2 M HU before any additional treatment. Once HU arrest was confirmed by microscopic examination, each culture was split and half was maintained at room temperature for 3 h, whereas the other half was shifted to 37°C for 3 h. Crude protein extracts of all samples were prepared by the procedure of Langan *et al.* (1989), and the protein concentration of each extract was determined with the BioRad Protein Assay (BioRad). Kinase assays using histone H1 kinase as the substrate were performed as described by Langan *et al.* (1989).

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