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Genetic Relationship between the *SPT3* Gene and *RAS*/cAMP Pathway in Yeast Cell Cycle Control

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The signal transduction pathways through the *RAS* gene product and adenyl cyclase play a critical role in regulation of the cell cycle in yeast, *Saccharomyces cerevisiae*. We examined the genetic relationship between the *spt3* gene and *ras*/cAMP pathway. A mutation in the *SPT3* gene suppressed cell cycle arrest at the G1 phase caused by either an inactivation of the *RAS* or *CYR1* gene which encodes a yeast homologue of human *ras* proto-oncogene or adenyl cyclase, respectively. The phenotypes such as sporulation and heat shock resistancy, that resulted from a partial inactivation of the *RAS* or *CYR1* genes, were also suppressed by the *spt3* mutation. Expression of the *SSA1* gene encoding one of the heat shock proteins (Hsp70) can be induced by heat shock or nitrogen starvation. Expression of this gene is derepressed in *cyr1-2* and *spt3* mutants. The *bcy1* mutation repressed heat inducibility of *SSA1* expression. The high basal expression of *SSA1* in *cyr1-2* was suppressed by the *bcy1* mutation, but not in *spt3* mutants. These results suggest that the *SPT* gene is involved in expression of genes that are affected by the *RAS*/cAMP pathway.

Key words: Cell cycle, *RAS*/cAMP pathway, *SPT3*

Mutations in the *SPT3* gene were initially identified and characterized as suppressors of Ty or solo delta insertion mutations in the 5'-regions of the *HIS4* and *LYS2* genes (1, 29). Further analysis demonstrated that the *SPT3* gene is required for the production of normal Ty transcript that initiates in the 5'-delta and terminates in the 3'-delta sequence (30). In strains that carry *spt3* null mutations, Ty mRNA of full-length is not present but a Ty RNA that is 800 bases shorter at the 5'-end is present at a reduced level. This fact, in conjunction with analysis of *spt3* effects on transcription of genes adjacent to the solo delta insertion mutation, led to the suggestion that *SPT3* is required for initiation of transcription of delta sequence (30). In addition to suppression of Ty and solo delta insertion mutations, the spt3 mutations cause other mutant phenotypes, including defects in mating and sporulation (29).

Yeast mutants defective in sporulation can be classified into three types; 1) one fails to arrest in the G1/Go state and initiation of meiosis/sporulation such as the *bcy1* or $ras2^{val19}$ mutants (15, 11); 2) another is able to arrest in the G1/Go state, but fails to initiate meiosis/sporulation by a deficiency of mating type gene system (12, 13); 3) the other one is able to arrest in the G1/Go state and to initiate meiosis, but fails to complete sporulation such as some *spo* and *spoT* mutants (4, 23). If sporulation deficiency of *spt3* diploids results from an inability of G1/Go arrest in sporulation medium, the *SPT3* gene product may be related to the cAMP pathway, because the initiation of meiosis/sporulation requires the G1/Go arrest in response to nutrient starvation, which is mainly mediated by the *RAS*/cAMP pathway (15, 17). In this paper, we examined a possibility that the function of the *SPT3* gene is related to *RAS*/cAMP pathway in respect to the G1/Go arrest and initiation of meiosis.

Materials and Methods

Strains

The strains used in this study are listed in Table 1. FW516 and L9 strains were obtained from Dr. F. Winston. The designation of *his4-912* refers to strains carrying the sequence of *Ty912* that is inserted at position -161 from the start of translation of the HIS4 gene, and *his4-917* refers to a strain carrying the sequence of *Ty 917* that is inserted at positon -71 from the start of translation. *LYS2-173R2* refers to a strain carrying Ty

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173 that is inserted at 5'-terminus of the open reading frame of *LYS2*. *ras1* and *ras2* represent null alleles constructed by inserting a marker gene into the coding region of *ras1* and *ras2* (10).

Media

Complete medium (YEPD), synthetic complete medium (SC), minimal medium (SD), pre-sporulation medium (YPA), and sporulation medium (SPO) were made as demonstrated by Sherman, Fink, and Hicks (24). SC-leu medium is a complete synthetic medium lacking leucine, and was used to select yeast transformants. LB medium was made as described by Miller (18), and used to grow E. *coli* cells. E. *coli* transformants were selected on LB medium containing 50 μg/ml of ampicillin (14).

General genetic methods and transformation

Standard genetic procedures of cross, sporulation, spore dissection, and tetrad analysis were followed as described by Sherman, Fink and Hicks (24). Diploids were isolated by prototrophic selection where possible. When prototrophic selection could not be employed, diploids were identified after single colony isolation by testing for their ability to sporulate. In the case of sporulation deficient strains, they were selected by their inability for mating. Transformation of yeast cells was performed by the lithium acetate method (9). E. *coli* strain, DH1, was used to transform and amplify the plasmid, and transformed as described by Maniatis, Fritsch and Sambrook (14). A plasmid, pLeSSA1-lacZ, was obtained from Dr. T. Oshima.

Determination of sporulation efficiency, proportion of unbudded cells, and heat shock sensitivity

Cells to be examined were freshly grown on YEPD plates, transferred to pre-sporulation plates (YPA), and incubated for 1 day. They were then transferred to sporulaton plates. After incubating for 3 days, the sporulation efficiency and proportion of unbudded cells were determined under a light microscope. At least 600 cells were counted for each determination. Heat shock sensitivity was determined as described by Shin *et al.* (26). The exponentially growing cells in YEPD medium at 25℃ were exposed to 52℃ for 4 min, in the case of pre-heat treatment, the cells were incubated for 90 min at first 37℃ , and then exposed to 52℃ for 4 min. The heat treated or non-treated cultures were spread onto YEPD plates and incubated at 25℃. After 3 days, colonies on each plate were counted by compairing with the non-treated cultures.

Measurement of β-galactosidase activity

β-galactosidase activity was assayed in cells permeabilized with chloroform and sodium dodecyl sulfate as described by Guarente and Ptashne (5). Units of the enzyme were defined as $(OD_{420}\times 1{,}000)/(\text{cell number}/10^7\times t\times v)$

Determination of trehalase activity

Preparation of cell-free extracts: Yeast cells were grown in YEPD medium to the late log phase, and harvested by centrifugation, washed well with distilled water, and suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 1 mM β-mercaptoethanol and 0.5 mM phenylmethylsulfonyl fluoride (Buffer T). The cell suspension was homogenized with an Aminco French pressure cell press (J5-598A) at 10,000 p.s.i.. The resulting homogenates were centrifuged at 1,000×g for 10 min. The supernatant fluid was obtained by centrifuging the crude extract at 20,000×g for 30 min.

Trehalase assay: Trehalase activity was determined as follows. The reaction mixture (final volume 1.0 ml) containing 0.5% trehalose, 125 mM PIPES buffer (pH 6.2), and enzyme preparation, was incubated at 30 for 15 min. The reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid solution containing 10 g of dinitrosalicylic acid, 16.8 g of NaOH, and 300 g of Rochelle salt/liter, and then the reaction mixture was boiled for 5 min. If the solution was turbid, it was centrifuged to remove the precipitate. The absorbance at 530 nm in the supernatant fluid was measured. One unit of trehalase activity was defined as the amount of enzyme which degraded 1 nmol of trehalose at 30℃ in 1 min. To examine activation of trehalase activity by the additon of cAMP and ATP, the crude extract was incubated with 0.1 mM ATP and 10 μM cAMP at 30℃ for 5 min, and then assayed for trehalase activity.

Measurement of intracellular cAMP level

The cAMP content was measured by the protein binding assay using the cAMP assay kit (Amersham International, Buckinghamshire, England) as described by Uno *et al.* (28).

Results

The *spt3* mutant can not arrest in the G1/Go state of the cell cycle

In response to nutrient starvation, cells undergo G1/Go arrest, or, in the case of a/α diploid cells, they initiate meiosis/sporulation (4, 21, 7, 16). The *bcy1* mutant, which produces cAMP-independent protein kinase, is unable to arrest in the G1/Go state, and consequently they are defective in the initiation of meiosis/sporulation (15, 22). To examine the ability of G1/Go arrest and sporu-

Table 1. Strains used in this study

Strain	Genotype
FW516	MATα *spt3-101 his4-912 ura3-52*
L9	MATα *spt3-101 his4-917 lys2-173R2 trpl-1*
AM221-1D	MATα *cyr1-1*
DE3-32	MATα *cyr1-2 ura3-52*
DE7-11A	MATα *spt3-101*
DE7-11B	MATα *ura3-52*
DE7-11C	MATα *cyr1-2 ura3-52*
DE7-11D	MATα *cyr1-2 spt3-101*
DE60-8A	MATα *cry1-2 spt3-101 leu2 ura ade8*
DE60-8B	MATα *cyr1-2 ura3 ade8*
DE17-1A	MATα *spt3-101 ura3 leu2 trp1*
DE17-1B	MATα *ras2::URA3 ura3*
DE17-1C	MATα *ras2::URA3 ura3 leu2*
DE17-1D	MATα *ura3 trp1*
DE31-9A	MATα *ras1::HIS3 ras2-125 spt3-101 leu2 ura3 trp1*
DE32-10D	MATα *ras1::HIS3 ras2-125 ura3 trp1 ade8*
OL86	MATα *cdc25-5 leu2 trp1 ade2*
DE37-1B	MATα *cdc25-5 his4-912 trp1*
DE37-1D	MATα *cdc25-5 spt3-101 leu2 ura3 ade2*
RA1-1B	MATα *leu2 ura3 trp1 ade8 his3*
DE33-9A	MATα *leu2 ura3 trp1 ade8 his4-912*
HM57-1A	MATα *cyr1-230 ras1::HIS3 leu2 ura3 trp1 his3 met4*
HM57-2C	MATα *cyr1-230 ras1::HIS3 leu2 ura3 trp1 his3*
10-9	MATα *cyr1-230 ras1::HIS3 bcy1-109 leu2 ura3 trp1 his3*
MT1-3B	MATα *bcy1-109 leu2 ura3 met3*
DE37-7A	MATα *spt3-101 leu2 trp1 ade2*
DE66-1B	MATα *spt3-101 leu2 ura3 trp1 ade8 his4-912*
DE70-2A	MATα *bcy1-109 spt3-101 leu2 ura3*
DE70-8B	MATα *bcy1-109 spt3-101 leu2 ura3*
DE-SP-14	MATα *leu2 ura3 trp1 met3 his3/MAT leu2 ura3 trp1 his3*
DE-SP-21	MATα *cyr1-230 ras1::HIS3 leu2 ura3 trp1 met3 his3*/MATα *cyr1-230 ras1::HIS3 bcy1-109 leu2 ura3 trp1 his3*
DE-SP-12	MATα *cyr1-230 ras1::HIS3 bcy1-109 leu2 ura3 trp1 met4 his3*/MATα *cyr1-230 ras1::HIS3 bcy1-109 leu2 ura3 trp1 his3*
DE-SP-31	MATα *cyr1-230 ras1::HIS3 spt3-101 leu2 ura3 trp1 his3*/MATα *cyr1-230 ras1::HIS3 spt3-101 leu2 ura3 met3 his3*
DE-SP-7	MATα *ras2-125 ras1::HIS3 leu2 ura3 trp1 ade8 his3*/MATα *ras2-125 ras1::HIS3 leu2 ura3 trp1 ade8 his3*
DE-SP-34	MATα *ras2-125 ras1::HIS3 bcy1-109 leu2 ura3 trp1 ade8 his3*/MATα *ras2-125 ras1::HIS3 bcy1-109 leu2 ura3 ade8 his3*
DE-SP-51	MATα *ras2-125 ras1::HIS3 spt3-101 ura3 trp1 his3*/MATα *ras2-125 ras1::HIS3 spt3-101 leu2 trp1 his3*
DE-SP-39	MATα *spt3-101 ura3 trp1/MATα spt3-101 leu3 ura3*
DE-SP-5	MATα *spt3-101 his4-912 ura3-52/MATα spt3-101 his4-912 lys2-173R2 trp1-1*
DE-SP-3	MATα *bcy1-1 ura3 trp1/MATα bcy1-1 leu2 ura3 lys2*
DE7	MATα *spt3-101 his4-912 ura3-52/MATα cyr1-2 ura3-52*
DE17	MATα *spt3-101 his4-912 ura3-52/MATα ras2-125 ura3 leu3 trp1*

lation efficiency of the *spt3* mutant, the diploids homozygous for the *spt3* mutation were constructed by crossing between FW516 and L9 (Table 1). The resultant diploids were incubated in sporulation medium for 3 days, and then assayed for the proportion of unbudded or sporulated cells (Table 2). In the culture of wild type diploids, most of cells were arrested in an unbudded state, and sporulated cells were found in 40% of total cells. In contrast, the proportion of unbudded cells in the *bcy1* diploids culture did not increase when compared with that of the growing phase. The sporulated cells were not found in at least 1,000 cells observed (Table 2). The proportion of unbudded cells in the *spt3* diploid culture was not increased. Sporulated cells were rarely found (Table 2). These results indicate that the *spt3* mutant is defective in the ability of G1 arrest and the initiation of meiosis in reponse to the nutrient starvation.

The *spt3* mutation suppresses the G1/Go arrest caused by cAMP deprivation

Table 2. G1/Go arrest and sporulation efficiency of *spt3* mutant in sporulaton medium

Strain	Genotype	YPE medium	SPO medium	
		Unbudded cells(%)	Unbudded cells(%)	Sporulated cells(%)
DE-SP-14	+/+	36	97	42
DE-SP-5	*spt3/spt3*	38	45	0.2
DE-SP-3	*bcy1/bcy1*	32	37	<0.1

The dipoid cells grown in YPD medium were transferred to SPO medium, and incubated for 3 days. The proportion of unbudded or sporulated cells was determined under a light microscope.

Table 3. Suppresion of the G1/Go arrest resulted from a partial inactivation of *CYR1* or *RAS2* genes by the *spt3* mutation

Strain	Genotype	Unbudded cells(%)	
		Permissive condition	Restrictive condition
DE7-11B	WT	45	ND
DE60-8B	*cyr1-1*	49	81
DE60-8A	*cyr1-1 spt3-101*	37	36
DE7-11C	*cyr1-2*	45	84
DE7-11D	*cyr1-2 spt3-101*	48	56
DE32-10D	*ras1 ras2-125*	51	92
DE32-9A	*ras1 ras2-125 spt3-101*	43	42
DE37-1B	*cdc25*	34	81
DE37-1D	*cdc25 spt3-101*	36	41

The temperature-sensitive mutants, *cyr1-2, ras2-125,* and *cdc25,* were grown in liquid YPD medium at 25℃ (permissive condition) were transferred to 37℃ and incubated for 6 hours. The cyr1-1 mutant was grown a YPD medium containing cAMP, transferred to cAMP-free medium, and incubated for 12 hours

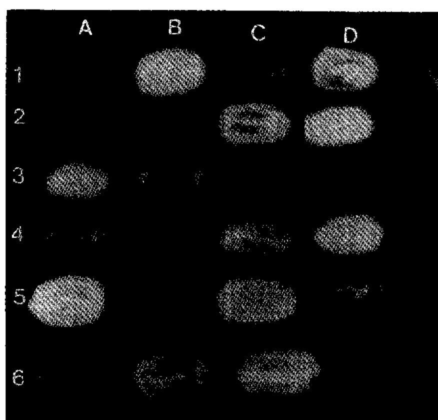

Fig. 1. Segregation of temperature-sensitive phenotype. The diploid cells heterozygous for *cyr1-2* and *spt3* mutations were sporulated and dissected. The six tetrads were incubated in YPD at 37℃ for 2 days. Four patches in row were from one sporulated cell.

The *cyr1-2* mutant has a heat-labile adenyl cyclase and arrests in the G1 phase at non-permissive temperature, 37℃ (16, 17). The bcy1 mutation suppresses the temperature-sensitive growth of *cyr1-2* by producing cAMP-independent protein kinase (15, 22). Since the results presented above indicate the phenotypes of the *spt3* mutant which resemble those of the *bcy1* mutants, we examined whether *spt3* suppresses the cAMP requirement for growth of *cyr1-2* mutant. The *spt3* mutant (FW516) was crossed with cyr1-2 mutant (DE3-32) and the resultant diplopid was sporulated and dissected. The obtained tetrads were incubated at 37℃ for 2 days. The phenotype of temperature sensitive growth was segregated in 2+:2- ratio (Fig. 1).

The deprivation of intracellular cAMP by cyr1-1, *cyr1-2, ras1 ras2-125(ts),* or *cdc25* mutations results in the G1/Go arrest (11, 16, 19, 6). Since it was found that *SPT3* functioned in the G1/Go arrest and the initiation of meiosis, we examined a possibility that *spt3* may suppress mutations related to the *RAS*/cAMP pathway. The *spt3* (FW516) mutant was crossed with *cyr1-1* (AM 221-1D), cyr1-2 (DE3-32), *ras1 ras2-125* (HM-14D), or *cdc25* (OL86) mutants, and the resultant diploids were sporulated and dissected. The *cyr1-1 spt3, cyr1-1 spt3, ras1 ras2 spt3,* and *cdc25 spt3* mutants were obtained from the tetrad analysis, and were determined for the proportion of unbudded cells under the permissive and restrictive conditions. While the proportion of unbudded cells in *cyr1-1, cyr1-2, ras1 ras2-125,* or *cdc25* mutant cultures was increased up to 90%, the proportion of *cyr1-1 spt3, cyr1-2 spt3, ras1 ras2 spt3,* or *cdc25 spt3* mutant cultures was not increased under the restrictive con-

Table 4. Suppression of the sporulation ability of *cyr1-230* and *ras2-125* in nutrient rich media by the *spt3* mutation

Strain	Genotype	Sporulation effciency(%)		
		YPD	YPA	SPO
DE-SP-14	+/+	<0.1	<0.1	40
DE-SP-21	*cyr1-230/cyr1-230*	1	15	46
DE-SP-12	*cyr1-230 bcy1/cyr1-230 bcy1*	<0.1	<0.1	<0.1
DE-SP-31	*cyr1-230 spt3/cyr1-230 spt3*	<0.1	<0.1	<0.1
DE-SP-7	*ras1 ras2-125/ras1 ras2-125*	2	39	52

The sporulation efficiency was determined in the cultures that are incubated for 4 days on YPD plate, or for 1 days on YPD and 3 days on YPA, or for one days on YPA and 3 days on SPO plate under a light microscope.

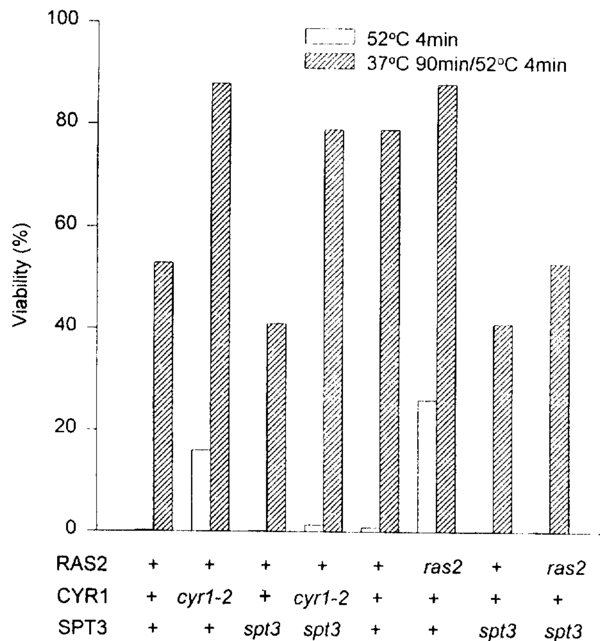

Fig. 2. Suppression of heat resistancy of *cyr1-2* or *ras2* by the *spt3* mutation. The exponentially growing cells at 25℃ were exposed to 52℃ for 4 min or first incubated at 37℃ for 90 min and then exposed to 52℃ for 4 min. The viability was determined as described in Materials and Methods.

dition (Table 3).

The *spt3* mutation suppresses the sporulation ability and heat resistancy caused by a partial inactivation of RAS or adenylyl cyclase

Mutations in the *CYR1* and *RAS* genes, which resulted in the production of low level of cAMP, result in initiation of meiosis even in a nutrient-rich media (10, 17). We examined whether this phonotype is suppressed by the *spt3* mutation. The diploids homozygous for *cyr1-2301, cyr1-230 bcy1-109, cyr1-230 spt3, ras1 ras2-125, ras1 ras2-125 bcy1-109,* and *ras1 ras2-125 spt3* were constructed (Table 1). The sporulation efficiency of these diploids on YPD, YPA, and SPO media was determined (Table 4). While the *cyr1-230* or *ras1 ras2-125* diploids were capable of sporulating on the YPD and YPA media as well as in SPO medium, *cyr1-230 bcy1-109, cyr1-230 spt3, ras1 ras2-125 bcy1-109, or ras1 ras 2-125 spt3* diploids failed to sporulated in the nutrient rich medium and in the SPO medium (Table 4). These results suggest that the *spt3* mutation inhibits the ability of the initiation of meiosis/sporulation that results from the decrease of cAMP level.

cAMP plays a role as a negative control factor of heat shock responses: the acquisition of heat resistance, induction of heat shock proteins and transient G1 arrest to the lethal heat treatment at 57℃ for 4 min (26). And the *bcy1* mutant cells are sensitive to the lethal heat treatment, and fail to acquire heat resistancy after mild heat shock, at 37℃ for 90 min (26). Thus heat sensitivity appears to link to the control of cell cycle by *RAS*/cAMP-dependent protein kinase (26, 11). Tetrads obtained from the diploids heterozygous for *cyr1-2* and *spt3*, or *ras2* and *spt3* mutations were examined for heat sensitivity. The *cyr1-2* or *ras2* mutants acquired heat resistancy at a lethal temperature without a mild temperature pre-heat treatment (Fig. 2). However, the spt3 mutation suppressed heat resistancy of *cyr1-2* or *ras2* mutants at a lethal temperature (Fig. 2).

The *spt3* mutation shows no effect on the cAMP level and trehalase activity

Since the *spt3* mutation suppressed the mutant phenotypes resulted from the deprivation of intracellular cAMP, the effect of the *spt3* mutation on the cAMP production machinery or cAMP-dependent protein kinase system were examined. Since trehalase is phosphorylated and activated by cAMP-dependent protein kinase (27), the intracellular cAMP level and trehalase activity were determined for one set of tetrad obtained from DE7 (*cyr1-2/+, spt3/+*). The *cyr1-2* mutant produced low levels of cAMP and trehalase activity at 25℃ and 37℃ . The levels of cAMP and trehalase activity in the *cyr1-2 spt3* double mutant were approximately the same as those of the *cyr1-2* mutant at both 25℃ and

Table 5. Intracellular cAMP levels and trehalase activity in a tetrad obtained from diploids (DE7) heterozygous for *cyr1-2* and *spt3* mutations

Strains	Genotype	cAMP content(pmol/mg protein)		Trehalase(units/mg protein)	
		25℃	37℃	-ATP, -cAMP	+ATP, +cAMP
DE7-11A	+ *spt3-101*	2.4	2.5	2.19	4.68
DE7-11B	+ +	1.9	2.2	2.85	4.56
DE7-11C	*cyr1-2*+	0.5	0.2	0.78	3.34
DE7-11D	*cry1-2 spt3-101*	0.8	0.2	0.69	3.67

The cells grown at 25℃ or 37℃ were prepared for cAMP binding assay as described (17). An amount of radioactive cAMP bound to binding protein was determined in a liquid scitillation spectrophotometer. Trehalase activity was assayed with or without 0.1 mM ATP and 10 μM cAMP.

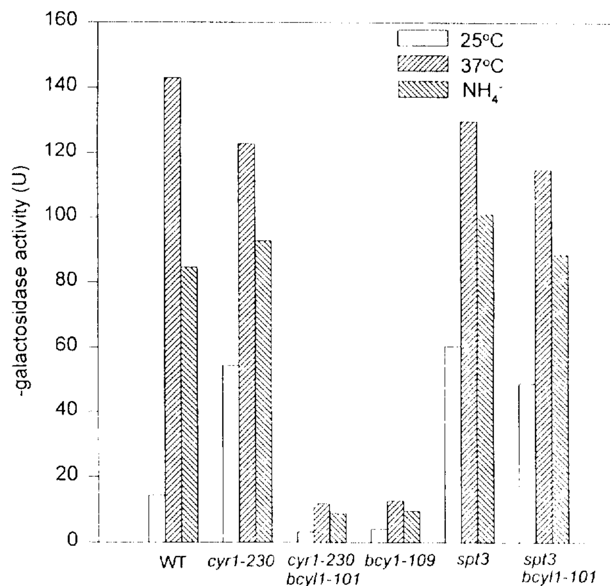

Fig. 3. Regulation of SSA1 promoter activity by *SPT3* and *RAS*/cAMP pathway. The transformants with pLeSSA1-lacZ were grown to late log phase in SD-Leu medium at 25℃, transferred to 37℃, and incubated for 1 hour. The cells were permeabilized by chloroform and sodium dodecyl sulfate, and β-galactosidase activity was determined as described (15).

37°C . The *spt3* mutant produced similar levels of cAMP and trehalase activity as wild type cells (Table 5). These results suggest that spt3 does not alter the cAMP producton machinery and protein kinase activity.

SPT3 and cAMP are negative regulators of *SSA1* gene expression

Synthesis of heat shock proteins (hsp) is a conserved response to environmental stresses that are found in all organisms (8). Among the proteins whose synthesis is stress-induced, the family of polypeptides of relative molecular mass of 70,000 (hsp70s) has been best studied. *Saccharomyces cerevisiae* cells contain at least seven genes encoding hsp70-related proteins (3). The expression of three genes, *SSA1*, *SSA3*, and *SSA4*, is known to be heat-inducible (3).

The *cyr1-2* mutant constitutively synthesizes the heat shock proteins whose molecular masses are 72KDa and 41KDa (hsp72A, B, and hsp41) at a normal temperature, and the *bcy1* mutants are defective in the synthesis of these heat shock proteins (26). We transformed pLeSSA 1-lacZ plasmid into the mutants to examine the transcriptional expression strength of the *SSA1* promoter. Transcriptional activity of *SSA1* promoter was induced not only by heat shock, but also by nitrogen starvation (Fig. 3). The activity of *SSA1* promoter was three fold higher in the *cyr1-2* transformant than in the wild type cells at non-induced condition. Interestingly, the *spt3* mutant also showed a higher activity of the promoter at the non-induced condition. The high basal activity of the promoter in the *cyr1-2* mutant was suppressed by the bcy1 mutation, but was not in spt3 (Fig. 3).

Disscussion

The diploids homozygous for the *spt3* mutation were not arrested in the G1/Go phases in the sporulation medium, which did not contain the nitrogen and fermentable carbon sources (Table 2). Thus, the sporulation deficiency of *spt3* diploids seems to result from the defect of the G1/Go arrest in response to nutrient limitation. This phenotype of *spt3* resembles that of *bcy1* which suppresses cAMP required for growth (15, 11). Further analysis indicated that the *spt3* mutation failed to suppress the cAMP required for growth of *cyr1-2* mutant at the restrictive temperature (Fig. 1). However, it was found that the *spt3* mutation suppresses the G1/Go arrest that resulted from the intracellular cAMP deprivation by mutations in the *CYR1*, *RAS2*, or *CDC25* gene (Table 3). The *spt3* mutation also suppressed the sporulation ability in nutrient rich media and heat resistancy that resulted from the reduction of intracellular cAMP level (Table 4, Fig. 2). However, the *spt3* mutation does

not affect an intracellular cAMP level and trehalase activity (Table 5). These results suggest that *spt3* suppresses the G1/Go arrest and the initiation of meiosis/sporulation resulting from the mutations of *CYR1*, *RAS2* and *CDC25* without altering the machinery for cAMP production or protein kinase system. Thus, the *SPT3* gene product may be related to the regulation of the G1/Go arrest and the initiation of meiosis by cAMP-dependent protein phosphorylation.

The *SSA1* gene is a member of the heat-inducible hsp70 gene family (3). An expression of this gene at the normal growth temperature is derepressed in the *cyr1-230* and *spt3* mutants (Fig. 3). The derepressed expression of SSA1 in the *cyr1-2* mutant was suppressed by the *bcy1* mutation, but was not in *spt3* mutant (Fig. 3). An upstream repression site (URS) was found adjacent to heat shock element (HSE) in the *SSA1* promoter (20). The URS of *SSA1* caused a repression of basal activity of *SSA1* promoter (31). A low level of intracellular cAMP or *spt3* mutation may cause a derepression of *SSA1* promoter through URS.

The *SPT3* gene was initially identified and characterized as a suppressor of Ty and solo delta insertion mutations (30). Further analysis demonstrated that the *SPT3* gene is required for the transcription of Ty and mating pheromone genes, *Mfa1*, *Mfα*, and *Mfα2* (30, 29, 31). However, a consensus sequence of a DNA binding domain was not found in the *SPT3* gene (31, 25). It was suggested that the *SPT3* protein interacts with TFIID, and is involved in transcription of some genes, such as Ty and mating type genes (2). On this line, the *SPT3* protein may mediate the regulation of the G1/Go arrest and the initiation of meiosis by cAMP as a transcription controlling factor.

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