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Rapid Report

A high dose of the *STM1* gene suppresses the temperature sensitivity of the *tom1* and *htr1* mutants in *Saccharomyces cerevisiae* ☆

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Abstract

A new gene (*STM1*; suppressor of *tom1*) of *Saccharomyces cerevisiae* was isolated by the ability to suppress the temperature sensitivity of a *tom1* mutant, by increasing its gene dosage. The gene could also suppress the temperature sensitivity of the *htr1* disruptant (Kikuchi et al. (1994) Mol. Gen. Genet. 245, 107–116) and was physically mapped in the region near *PEP3* on chromosome XII R. The predicted gene product (29 999 Da) is basic and partially homologous to various histone H1. The level of the gene expression increased 2-fold when exposed to mating pheromone.

Keywords: STM1; *TOM1*; *HTR1*; Multi-copy suppressor; (*S. cerevisiae*)

We have isolated a temperature sensitive mutant, *tom1* (Trigger of Mitosis) of *Saccharomyces cerevisiae*. At a restrictive temperature the mutant appeared to be arrested at the G2/M transition of the cell cycle (Utsugi et al., unpublished data). It is not clear at present how the gene functions at the molecular level. Hoping to clone genes which interact with *TOM1* genetically, we isolated multi-copy suppressors which allowed the *tom1* mutant to grow at a high temperature. The gene library constructed on YEp24 [2], was introduced into a yeast strain TUY001 carrying *tom1 ura3* mutations, by the alkaline-cation method [3]. Two types of plasmids, pTU106 and pTU113 which were recovered from the transformants, could suppress the growth of the *tom1* mutant at 35°C, but not at 37°C (Fig. 1A) and had an overlapping DNA region (Fig. 2). A responsible DNA region to suppress the temperature sensitive growth, was determined by standard sub-cloning experiments (Fig. 2). The suppressor ability of the 1.7 kb *Eco*RV-*Eco*RV fragment (pTU162) was a little worse than the original, suggesting that the functional region located beyond one or both junctions. When the DNA was inserted into a single-copy vector (pTU161), it hardly suppressed, indicating that the gene was a multi-copy suppressor. Thus we named it *STM1* as a suppressor of *tom1*. As shown in Fig. 1B, *STM1* could also suppress the temperature sensitivity of the *htr1* disruptant. As described previously [1], the *HTR1* gene is required for high temperature growth and recovery from G1 arrest by mating pheromone, but the function of the gene still remains unclear. Among other multi-copy suppressors of the *htr1* disruptant, we have reported the *SMS1* gene, which encodes a serine-rich trans-membrane protein [4].

Fig. 3 shows the nucleotide sequence of the gene, determined by the dideoxy terminator method. The predicted gene product containing 273 amino acids (molecular mass: 29 999 Da) is rather basic and has a weakly homologous region to various histone H1, as shown in Fig. 4. The 5′ upstream region contains eight putative PRE (Pheromone Response Element)-like sequences, although there are no complete consensus sequence (TGAAACA). In order to see whether the *STM1* gene was induced by exposure to mating pheromone, we constructed a plasmid pTU163, by inserting the 1.4 kb *Sca*I fragment carrying its own promoter and N-terminal 204 amino acid residues of Stm1p into the *Sma*I site of YEp357 [5], so that Stm1p was fused in frame to β-galactosidase. The plasmid was introduced into a wild type yeast strain 15Dau (*a bar1 trp1 ura3 leu2*

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☆ The sequence data reported in this paper for *S. cerevisiae STM1* have been submitted to the DDBJ Data Libraries under the accession number D32208.

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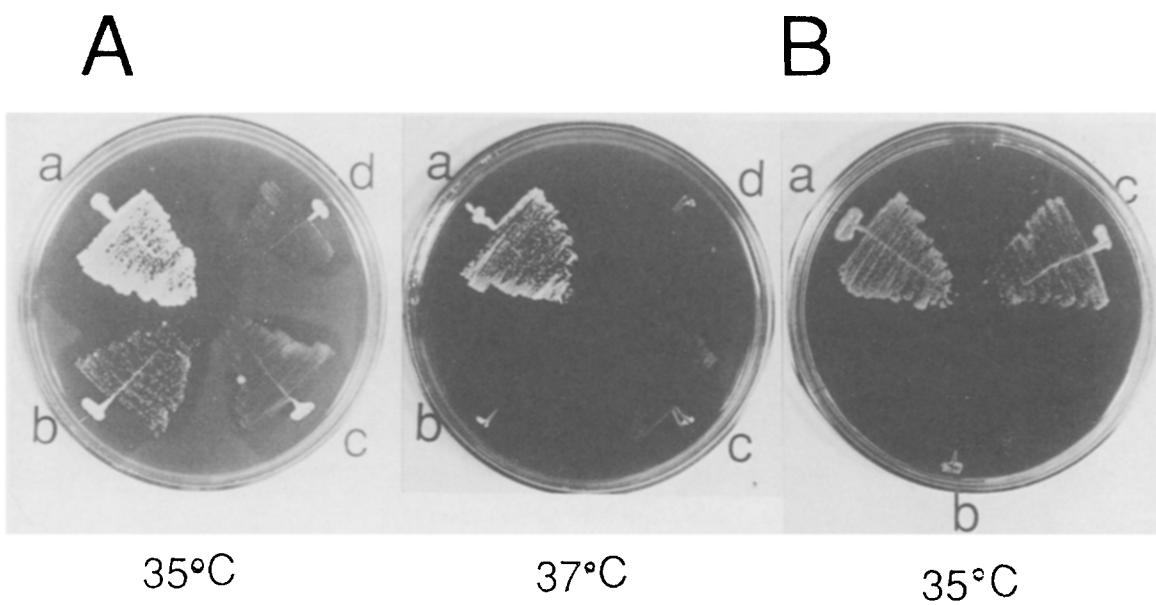

Fig. 1. Suppression of the temperature sensitivity of *tom1* or *htr1* disruptant by high doses of *STM1*. (A) The yeast strain TUY044 *a/α tom1/tom1 ura3/ura3 leu2/leu2 trp1/trp1 His*$^-$, was introduced with following plasmids, (a) pRS316-*TOM1* (Utsugi et al., unpublished data), (b) YEUp3-*STM1* (pTU151), (c) YEUp3, (d) YCUp4-*STM1* (pTU161). (B) The strain W303 a *htr1::HIS3 ura3 leu2 his3 trp1 ade2 can1*, was transformed with (a) YCp50-*HTR1* (pYK690) [1], (b) YEUp3, (c) YEUp3-*STM1* (pTU151). Each transformant was streaked on YPD-plates which were incubated at indicated temperature for 2 days. Composition of medium is described in [8].

his2 ade1), and the β-galactosidase activity was assayed at 2 h after adding 1 μg/ml of α factor to the cultures. In fact, the enzyme level of the cells treated with mating pheromone, was 2-fold, compared to that of the untreated cells, as described in Table 1. The constitutive level of the *STM1* expression was much higher than that of *FUS1* [6], even though the Stm1-lacZ was on a multi-copy plasmid.

The *STM1* gene was physically mapped on the chromosome XIIR: the 1.7 kb *Eco*RV fragment containing *STM1* was hybridized to the lambda phage clones 70619 and 70821 on the ATCC membrane filter by the Standard Southern method.

The *STM1* gene is not an essential gene. We constructed a plasmid pTU166 (the 0.1 kb *Xba*I-*Pst*I fragment within *STM1* was replaced with the 2.0 kb fragment containing *LEU2*), linearized with restriction enzymes and disrupted the gene by the one-step-replacement method [7]. The DNA structure of the disruptant was verified by the Southern method (data not shown). The *stm1* disruptant was not temperature sensitive nor cold sensitive. It was not

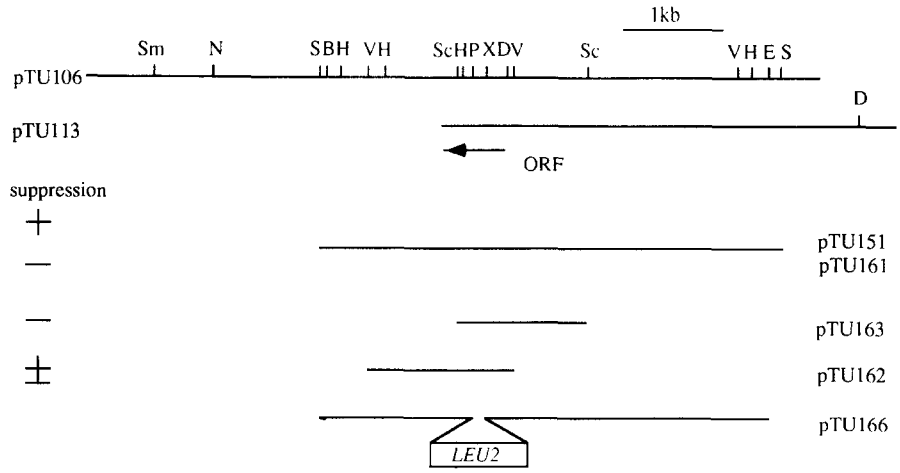

Fig. 2. Restriction map, sub-cloning of *STM1*, and the plasmid structure for its gene disruption. Plasmids pTU106, 113 (YEp24), pTU151, 162 (YEUp3) are multi-copy plasmids. Plasmid pTU161 carries the same DNA fragment as pTU151, but on a single-copy vector YCUp4. pTU163 contains the Stm1-lacZ fused gene. pTU166 (bluescript SK +) was used for the gene disruption. Abbreviations: B; *Bam*HI, D; *Dra*I, E; *Eco*RI, H; *Hin*dIII, N; *Nru*I, P; *Pst*I, S; *Sal*I, Sc; *Sca*I, Sm; *Sma*I, V; *Eco*RV, X; *Xba*I.

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-420 TTGTAGCCGTATAACATATGGCGCTACCATGTTGTTATTTAGACGGGTAACATCACCGTT
-360 TAGAAAGATAAAGGAAAGTGAAATTATTGGAATGGAAAGTGTTCCCAAAAGTTCGCACCA
-300 ACGCAAAAAGAAATTTTTTTTTCAATTTTGATATCATCGTTGCGTAGAGGAAGCCCTAAC
-240 TGAGTAGAGAAATTATATGGTATTTTCCTTGTAATATTTGTTTTGAGTCGATATCCCCTT
-180 TGTTCTTCTTCCTTCCTTCGCTTGTTTTAGTTGTATACTTGGTTTATTGTGGAAGATTTT
-120 TTTTTCTGCTTCGTAGTTTTAAATTAGAAGTTATTTCTTTGCAAATTTCTCTTCCCCCCA
 -60 CAGTATTCTTTTAGAGGTGAAGTAGAAATAAACCAAGAAAGCATACACATTTTATTCTCA
   1 ATGTCCAACCCATTTGATTTGTTAGGTAACGACGTCGAAGACGCTGACGTTGTCGTTTTG
     M  S  N  P  F  D  L  L  G  N  D  V  E  D  A  D  V  V  V  L
  61 CCACCAAAGGAAATCGTCAAGAGCAACACTTCCTCCAAGAAGGCTGACGTCCCACCTCCA
     P  P  K  E  I  V  K  S  N  T  S  S  K  K  A  D  V  P  P  P
 121 TCCGCTGACCCATCCAAGGCTAGAAAGAACAGACCAAGACCTTCTGGTAACGAGGGTGCT
     S  A  D  P  S  K  A  R  K  N  R  P  R  P  S  G  N  E  G  A
 181 ATCAGAGACAAGACCGCTGGTAGAAGAAACAACAGATCAAAGGATGTCACTGACTCTGCC
     I  R  D  K  T  A  G  R  R  N  N  R  S  K  D  V  T  D  S  A
 241 ACCACCAAGAAGTCCAACACCAGAAGGGCCACTGACCGCCACTCTAGAACTGGTAAGACT
     T  T  K  K  S  N  T  R  R  A  T  D  R  H  S  R  T  G  K  T
 301 GACACCAAGAAGAAGGTTAACCAAGGTTGGGGTGATGACAAGAAGGAATTGAGTGCTGAA
     D  T  K  K  K  V  N  Q  G  W  G  D  D  K  K  E  L  S  A  E
 361 AAGGAAGCTCAAGCCGATGCTGCTGCTGAAATTGCTGAAGACGCTGCAGAAGCTGAAGAC
     K  E  A  Q  A  D  A  A  A  E  I  A  E  D  A  A  E  A  E  D
 421 GCTGGTAAGCCAAAGACCGCTCAATTGTCTTTGCAAGACTACTTGAACCAACAAGCTAAC
     A  G  K  P  K  T  A  Q  L  S  L  Q  D  Y  L  N  Q  Q  A  N
 481 AACCAGTTCAACAAGGTCCCAGAAGCTAAGAAGGTTGAATTAGACGCTGAAAGAATTGAA
     N  Q  F  N  K  V  P  E  A  K  K  V  E  L  D  A  E  R  I  E
 541 ACTGCTGAAAAGGAAGCTTACGTTCCAGCAACCAAGGTCAAGAACGTCAAATCTAAGCAA
     T  A  E  K  E  A  Y  V  P  A  T  K  V  K  N  V  K  S  K  Q
 601 TTGAAGACCAAGGAGTACTTGGAATTTGATGCCACTTTTGTTGAATCTAACACTAGAAAG
     L  K  T  K  E  Y  L  E  F  D  A  T  F  V  E  S  N  T  R  K
 661 AACTTCGGTGACAGAAACAACAACAGCAGAAACAACTTCAACAACCGTCGTGGTGGTAGA
     N  F  G  D  R  N  N  N  S  R  N  N  F  N  N  R  R  G  G  R
 721 GGCGCTAGAAAGGGTAACAACACTGCTAACGCTACTAACTCTGCTAACACCGTTCAAAAG
     G  A  R  K  G  N  N  T  A  N  A  T  N  S  A  N  T  V  Q  K
 781 AACCGTAACATTGACGTTTCTAACTTGCCATCTTTGGCTTAAGCCTTATATATTGAATTA
     N  R  N  I  D  V  S  N  L  P  S  L  A  *
 841 ATTCCCAACTTGAA
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Fig. 3. The nucleotide sequence of *STM1* and the deduced amino acid sequence are shown. Potential PRE sequences are underlined. The nucleotide sequences of the *Eco*RV and *Sca*I sites cited in the text, are boxed.

defective in recovery from the G1 arrest by mating pheromone by halo assay (data not shown). In addition, the *tom1 stm1* and *htr1 stm1* double mutants were viable, without any synergic effects; they had similar phenotypic growth properties as *tom1* and *htr1* single mutants, although the permissive temperature of each double mutant was slightly lower than the respective single mutant. Thus, one possibility is that *STM1* functions downstream of *TOM1* or *HTR1* and high level expression of *STM1* could overcome the *tom1* or *htr1* mutant defect, since *STM1* can only suppress the temperature sensitive mutation of *tom1* or *htr1* when present in multiple copies. In this case there must be a functionally redundant gene(s) with *STM1*. It is also possible to speculate that the Stm1p plays a role in chromatin condensation or re-modeling, since the putative Stm1p is similar to histone H1. Expression of some genes may be affected by an overdose of *STM1*, leading to suppress the mutations.

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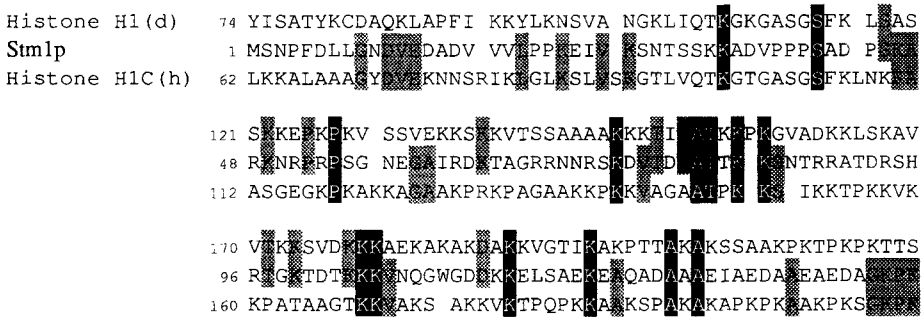

Fig. 4. Sequence alignment of Stm1p with human and chicken histone H1s [10,11]. Identical amino acids among three proteins are black-boxed, the same amino acids of Stm1p with either histone H1 are heavily-shaded and those between two histone H1s are lightly-shaded.

Table 1
Gene induction of *STM1* by exposure to mating pheromone

	β-Galactosidase (units)	
	Stm1-lacZ	Fus1-lacZ
− α factor	268	1.3
+ α factor	574	20.0

The yeast strain 15Dau *a bar1 ura3 trp1 leu2 his2 ade1* (a kind gift from Dr. S. Reed of Scripps) transformed with pTU163 containing the Stm1-lacZ on YEp357 [5], or pSB231 carrying the Fus1-lacZ on a single-copy vector (a generous gift from Dr. G. Fink of MIT) [6], was grown to mid-log phase (OD_{600} = 0.5) in a minimal medium lacking uracil [8], and the cultures were added with 1 μg/ml of α factor (Sigma). 2 h later the enzyme activity was measured, according to the protocol described in [9]. Average values from two independent transformants are described.

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