**HSM6 Gene Is Identical to PSY4 Gene in Saccharomyces cerevisiae Yeasts**

D. V. Fedorov, S. V. Kovaltsova, T. A. Evstuhina, V. T. Peshekhonov, A. Yu. Chernenkov, and V. G. Korolev

Konstantinov Petersburg Nuclear Physics Institute, Gatchina, 188300 Russia; e-mail: lge@omrb.pnpi.spb.ru

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**Abstract**—Previously, we isolated mutant yeasts Saccharomyces cerevisiae with an increased rate of spontaneous mutagenesis. Here, we studied the properties of HSM6 gene, the hsm6-I mutation of which increased the frequency of UV-induced mutagenesis and decreased the level of UV-induced mitotic crossover at the region between the centromere and ADE2 gene. HSM6 gene was mapped on the left arm of chromosome II in the region where the PSY4 gene is located. The epistatic analysis has shown that the hsm6-I mutation represents an allele of PSY4 gene. Sequencing of hsm6-I mutant allele has revealed a frameshift mutation, which caused the Lys218Glu substitution and the generation of a stop codon in the next position. The interactions of hsm6-I and rad52 mutations were epistatic. Our data show that the PSY4 gene plays a key role in the regulation of cell withdrawal from checkpoint induced by DNA disturbances.

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**INTRODUCTION**

Genomic DNA must maintain stability during the cell’s lifetime. However, various disturbances, which are induced both by the process of normal cell metabolism and in response to environmental factors, emerge continuously. This damage to the DNA structure can result in cell death (lethal disturbances) or in the generation of a mutation. Mutations are a primary cause of hereditary diseases and cancer, and contribute to the aging process [1].

Eukaryotic cells have a specialized response to DNA damage, called a “checkpoint,” which delays cell cycle progression and facilitates damaged DNA repairation. The activation of the checkpoint is carried out by a cascade of phosphorylated proteins, and is initiated in the yeast cells by two protein kinases, Mec1 and Tel1 [2]. These kinases phosphorylate checkpoint mediatorsRad53 and Rad9, as well as histone H2A [3, 4]. In S. cerevisiae Mec1 and Rad53 are indispensable as they control phosphorylation and activation of checkpoint kinase Dun1 [5]. A major function of Mec1, Rad53, and Dun1-dependent pathway is the maintenance of the adequate deoxynucleotide triphosphate (dNTP) level by the regulation of ribonucleotide reductase (RNR) activity during the cell cycle [6, 7]. RNR is responsible for the synthesis of all the four types of dNTP and contains four subunits. In yeast cells, RNR is highly regulated at many levels by the cell cycle and DNA disturbances [5, 8–10]. The full activation of RNR results in a six- to eightfold increase in the dNTP concentration [11]. This increase in the amount of dNTP correlates with tolerance to DNA damage. Changes in the concentration or dNTP ratios are accompanied by an increase or decrease in the level of spontaneous mutagenesis [12].

At present, significant progress is attained in understanding the initiation and the course of the checkpoint process. However, little is known about the mechanisms of its deactivation. As Ser/Thr-kinases play a central role in the initiation of a checkpoint response, it is likely that Ser/Thr-phosphatases should be involved in its termination. The checkpoint deactivation process is important for the reinitiation of DNA replication and cell survival. This process requires Rad53 and γH2A dephosphorylation. Other studies have shown that some phosphatases specifically produce various forms of Rad53 modifications, which are required for an adequate response to definite DNA disturbances [13].

It has been shown that Pph3 phosphatase is involved in the dephosphorylation of key checkpoint kinase Rad53 in the yeast cells [14]. Pph3 forms a complex with a Psy2 subunit, which binds to Rad53 kinase and dephosphorylates it without the involvement of a third subunit. Another complex, which consists of three subunits Pph3–Psy2–Psy4, dephosphorylates γH2A [15]. The deletion of any gene that encodes the triple complex subunits results in the sensitivity of the mutant cells to cis-platin. Rad53 dephosphorylation occurs normally in psy4Δ mutant cells during the exit from the checkpoint, which was induced by the methylmethane sulfonate (MMS), while in various checkpoint mutants, the PSY4 deletion does not affect MMS sensitivity [14]. In contrast,
the inactivation of any subunit of the Pph3–Psy2–Psy4 triple complex results in a similar defect of γH2A histone dephosphorylation [15]. Thus, it has been suggested that Pph3–Psy2 complex dephosphorylates Rad53, while Pph3–Psy2–Psy4 complex dephosphorylates γH2A [16].

At the Genetics of Eukaryotes Laboratory in the Petersburg Nuclear Physics Institute, a collection of hsm (high spontaneous mutagenesis) mutants has been made [17]. hsm mutants were isolated according to the trait of increased frequency of spontaneous mutations of canavanine resistance. One of these mutants, hsm6, was studied in this study. In the course of these studies, we have conducted genetic and physical mapping of the HSM6 gene and determined the nature of the hsm6-1 mutation. We showed that this gene is identical to the PSY4 gene. Point mutation hsm6-1 has a more pronounced phenotype compared to deletion mutant psy4Δ.

### MATERIALS AND METHODS

**Strains.** Saccharomyces cerevisiae yeast strains used in the study are shown in Table 1.

**Media.** For culture growth, survival and crossover registration the full medium was used [18]. Minimal medium with standard additives was used for auxotrophy testing [18]. Alcohol-containing medium was used in studies of the induced mutagenesis [19]. Spontaneous mutations of canavanine resistance (CanS→CanR) were registered using minimal medium with standard additives and 40 mg/mL canavanine. Sensitivity to cis-platin in a qualitative test was registered using full medium containing 2 mM cis-platin.

**Methods.** A BUF-30-P lamp with a dose power of 1.4 J/m² s served as a UV source. Sensitivity to the lethal effect of cis-platin and UV irradiation was determined using survival curves related to the dose [20]. Sensitivity to the mutagenic effect of UV light was registered by induction of direct mutations in five ADE