Note
Identification and Characterization of a Novel Gene, hos3+, the Function of Which Is Necessary for Growth under High Osmotic Stress in Fission Yeast

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hos3 mutants of the fission yeast Schizosaccharomyces pombe showed the phenotype of high osmolarity sensitivity for growth. An S. pombe strain carrying the hos3-M26 allele cannot form colonies on agar plates containing 2 M glucose, but the parental strain can do so very well, as demonstrated previously. The hos3+ gene was cloned and identified as one that encodes a small protein of 94 amino acids, which shows no sequence similarity to any other proteins in the current databases. A hos3 strain, which we then constructed, had the phenotype of high osmolarity sensitivity, as in the case of the original hos3-M26 mutant. More interestingly, when these hos+ cells were grown in the non-permissive growth condition in the presence of 2 M glucose, we found that unusually many septated cells were accumulated after a prolonged incubation. A multicopy suppressor gene for hos+ mutations was also isolated and identified as the dsk1+ gene encoding a protein kinase, which was previously suggested to be implicated in a process of the mitotic regulation of S. pombe. The function of the hos3+ gene is discussed from these results.

Key words: osmoregulation; Schizosaccharomyces pombe; mitotic control

In general, exposure of cells to high environmental osmolarity leads to dehydration and decrease in cell viability. Accordingly, the ability of cells to adapt to external osmotic stress is a fundamental biological process. In fact, many types of both prokaryotic and eukaryotic cells have developed mechanisms to adapt to severe osmotic stresses in their environment.1-3) The accumulation of osmoprotective compatible solutes inside cells up to the concentrations necessary to counteract the elevation of external osmolarity is a general aspect of osmoregulation.4) In the fission yeast Schizosaccharomyces pombe, glycerol appears to be the major compatible solute, for which the key enzyme is the NADH-dependent glycerol-3-phosphate dehydrogenase (GPD).5) We have cloned the gpd1+ gene, encoding an osmo-inducible GPD, which was indeed demonstrated to be crucially responsible for osmoregulation in S. pombe. A mitogen-activated protein (MAP) kinase cascade is also essential for the underlying responsive signal transduction.5,6) In this particular stress-activated signaling cascade, the Sty1 MAP kinase (also known as Spc1 and Phh1),7-9) the Wis1 MAP kinase (MEK),10) and Wak1 MAP kinase kinase (MEKK)11) constitute a set of central elements. In short, the osmo-inducible transcription of gpd1+ is greatly reduced both in wis1 and sty1 mutants, and consequently, these mutants as well as gpd1 mutant have a high osmolarity sensitive phenotype.5,6)

To gain new insight into molecular mechanisms underlying the osmoregulation in S. pombe, recently we have isolated a set of new mutants (named hos1, hos2, and hos3), each of which show a phenotype of high osmolarity sensitivity for growth.12) It was shown that these hos mutants retain the ability to induce gpd1+ mRNA and accumulate glycerol as normally as in the case of the wild-type cells in response to the external high osmolarity. In this sense, they appeared to be novel mutants which have a defect in some process other than the Sty1 MAP kinase-dependent glycerol synthesis. Amongst them, we previously identified the hos1+ gene, which encodes a small GTP-binding protein.12) In the hope of identifying another gene, here we characterized the hos3 mutant carrying hos3-M26 allele.

From the results of our previous study, it is known that the hos3-M26 mutation is a recessive and single one. We searched for genes that can complement the hos3-M26 phenotype of high osmolarity sensitivity, from a genomic DNA library with a versatile multicopy-number plasmid (pLBDblect).12) The selection was done on SD high osmolarity plates containing 2 M glucose. Among about 104 colonies thus screened, three colonies appeared on the selection plates. Plasmid DNAs were isolated, and confirmed to have the ability to complement the hos3-M26 mutation. Ex-

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tensive restriction analyses of these plasmids showed that two of them (named p42 and p12) carry an overlapping genomic DNA, as schematically shown in Fig. 1. We first characterized these clones, and the other (named p2) will be described later. We constructed and characterized two sub-clones from p12 (named p12-2.4 and p12-3.0). The results showed that the gene in question appears to be located around the HindIII site in Fig. 1. The nucleotide sequence around the HindIII site was analyzed. This result together with an inspection of S. pombe sequence databases showed that, within this region, there is an open reading frame (ORF) of 94 amino acids, which is a hypothetical one (gene name: SPCC417.02 in cosmid c417, GenBank accession No. AL035076). To clarify that this small ORF is indeed responsible for the complementation, the minimum ORF sequence was amplified by polymerase chain reaction (PCR), and then it was placed under the versatile nmt1 promoter on the pREP1 plasmid. The resultant plasmid, named pKA009, was able to complement the hos3-M26 phenotype (Fig. 1). Note that we also succeeded in isolating a corresponding cDNA from a cDNA library (data not shown). From these results, we concluded that the ORF, identified here, is the functional gene responsible for the complementation of hos3-M26.

The crucial question is; whether or not this ORF does indeed correspond to the hos3+ gene. This is the case, as demonstrated below. On the chromosome, this ORF was knocked out by an ura4+ marker-insertion at the Stu1 site (see Fig. 1), with use of standard yeast genetic procedures. The resultant strain carrying the ORF::ura4+ allele was found to show the phenotype of high osmolarity sensitivity. As far as we found, the phenotypes of ORF::ura4+ mutant were indistinguishable from that of the original hos3-M26 mutant. The mutational lesion was complemented by p42 carrying the ORF in question (Fig. 2A). Furthermore, when the ORF::ura4+ (Osm3) and hos3-M26 (Osm8) strains were genetically crossed, no segregant with the Osm8 phenotype appeared (data not shown). We thus succeeded in identifying the hos3+ gene, which encodes a small protein of 94 amino acids. The strain carrying the ORF::ura4+ allele was hereafter used as a hos3A mutant.

The identified Hos3 protein has no significant similarity to any other proteins in the current databases. To gain insight into the function of the hos3+ gene, we further characterized a presumed multicopy suppressor gene for hos3-M26. As mentioned above, plasmid p2 was isolated as the one that was also able to complement the hos3-M26 mutation. This plasmid was potent also toward the hos3A mutant (data not shown). These results indicated the plasmid p2 most likely contains a multicopy suppressor gene for hos3+. Extensive subcloning analyses of this particular plasmid allowed us to identify the single known dsk1+ gene as the multicopy suppressor, as shown in Fig. 2B. The multicopy DNA segment containing only the dsk1+ coding sequence on plasmid pds1 was fully potent to complement the osmosensitive phenotype of hos3A.

Fig. 1. Schematic Representation of S. pombe Genomic DNA Fragments Encompassing the hos3+ Gene and Its Ability to Complement the hos3 Mutant.
A. The DNA fragment cloned in each plasmid is shown. Complementation ability of each plasmid is indicated in parenthesis. B. hos3 mutant (M26; h+ leu1-32 ade6-M210 hos3-M26) was transformed with each indicated plasmid, and a parental strain YJ333 (h leu1-32 ade6-M216) was used as the wild type. These cells were streaked on an SD agar plate and an SD agar plate with 2.2 M glucose. After 4 days of incubation at 30°C, the plates were photographed.
The *dsk1* gene that encodes a protein kinase is interesting, because it was originally identified as a multicopy suppressor for the *dis1* mutants which shows a defect in sister chromatid separation.\(^{13}\) In the *dis1* mutant, chromosomes condense and the elongated spindle forms, but it fails to separate the chromosomes.\(^{14}\) The *dis1* mutant was thus explained by assuming that Dis1-defective cells have an ability to enter mitosis (G2 to M progression), but cannot exit from it. The complementation by the *dsk1* gene was interpreted by assuming that the overexpressed Dsk1 kinase in the *dis1* mutant has a positive effect on the exit from mitosis. In any event, it is likely that the function of Dsk1 is, at least in part, relevant to mitotic cell-cycle control in *S. pombe*.\(^{13}\) Although the physiological importance of Dsk1 is not well known, the facts mentioned above all together led us to envisage that the *hos3* gene might also be implicated in a process of mitosis. Finally, we addressed this intriguing issue.

To directly address the above issue, both the *hos3-*M26 and *hos3* mutant cells were examined under a microscope, particularly under the non-permissive (high osmolarity) growth conditions (Fig. 3A and data not shown). Interestingly we noticed that a number of septated cells were unusually accumulated in the calcofluor-stained mutant cells, after incubation for 6 hr in the non-permissive growth medium, while such an event did not happen in either the wild-type cells (Fig. 3A) or other high osmolarity sensitive mutants such as *ryh1A* and *atif1A* (data not shown).\(^{12,15-18}\) We then quantitatively analyzed this particular phenomenon, by measuring the septation index along with the incubation time. The observation was quantitatively confirmed (Fig. 3B). This apparent defect of a process of the cell cycle, observed for *hos3* mutants, was completely complemented by the introduction of the wild-type *hos3* gene. More importantly, this phenotype was suppressed also by the introduction of the *dsk1* gene at its multicopy state, as shown in Fig. 3B. This suggested that the observed defect in a septum-segregation process is an intrinsic feature of *hos3* mutations, which has an interplay with the mitosis-related gene, *dsk1*.

In this study, a high osmolarity sensitive mutant of *S. pombe* was characterized genetically, and we succeeded in identifying the relevant gene, named
hos3'. This gene encodes a novel small protein of 94 amino acids. Unfortunately, its amino acid sequence is ispid, and did not give us any hint with special reference to the osmoregulation in S. pombe. However, our results from the isolation of a multicopy suppressor gene for the hos3 mutant shed new light on the function of the hos3' gene. As mentioned above, this gene may be relevant to a process of the mitotic cell cycle in S. pombe. Many septated cells were accumulated, when the hos3 mutants were incubated in a non-permissive high osmolarity medium. This phenotype can be suppressed by the presumed overexpression of the dsk1' gene, whose gene product was previously suggested to be a mitosis-related protein kinase. At present, we cannot explain these events at the molecular level, because the mechanisms underlying septum formation and segregation in the mitotic cell cycle progression in S. pombe are obviously very complex, and they are not fully understood. Nonetheless, the hos3' gene, newly identified in this study, may give us a hint for better understanding of the fundamental eukaryotic cellular event, i.e., osmotic adaptation and the control of the mitotic cell-cycle progression.

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