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Cell Viability in G_0-like Stationary Phase of *Schizosaccharomyces pombe*: Roles of Psp1/Sds23 and Ufd2

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Abstract

Under the condition of nutritional deprivation, actively growing cells prepare to enter G_0-like stationary phase. Protein modification by phosphorylation/dephosphorylation or ubiqutination contributes to transfer cells from active cell cycle to dormant stage. We show here that Psp1/Sds23, which functions in association with the 20S cyclosome/APC (1) and is essential for cell cycle progression in *Schizosaccharomyces pombe* (2), is phosphorylated by stress-activated MAP kinase Sty1 and protein kinase A, as well as Cdc2/cyclinB, upon entry into stationary phase. Three serines at the positions 18, 333 and 391 are phosphorylated and overexpression of Psp1 mutated on these sites causes cell death in stationary phase. These modifications are required for the binding of Spufd2, a *S. pombe* homolog of multiubiquitin chain assembly factor E4 in ubiquitin fusion degradation pathway. Deletion of *Spufd2* gene led to increase cell viability in stationary phase, indicating that *S. pombe* Ufd2 functions to inhibit cell growth at this stage to maintain cell viability. Moreover, Psp1 enhances the multiubiquitination function of Ufd2, suggesting that Psp1 phosphorylated by Sty1 and PKA kinases is associated with the Ufd2-dependent protein degradation pathway, which is linked to stress tolerance, to maintain cell viability in the G_0-like stationary phase

Contents

In actively growing eukaryotic cells, the sequential activation of cyclin-dependent kinases (CDKs) control the onset of S phase or mitosis of the cell cycle (3-7). The phosphorylation/dephosphorylataion of CDKs activates the interaction of regulators such as cyclins and CDK inhibitors and is responsible for check point control in the transition of the cell cycle. The phosphorylation status of Cdc2, one of the founding members of CDKs in the fission yeast *Schizosaccharomyces pombe*, primarily controls the progression of the mitotic cell cycle at G_1/S and G_2/M (8) as well as exit from the resting state (G_0) to G_1 (9, 10). When nutrients are exhausted, cells exit active cell cycle and enter stationary phase (11).

The stationary phase is defined as a metabolically dormant state characterized by turning off genes that are required for the mitotic cell cycle, and turning on genes that are required for survival in stressed conditions including starvation, heat shock, and chemical treatment. Disruption of the regulatory subunit of protein kinase A (PKA) renders *S. pombe* cells sterile, and causes a loss of cell viability upon nitrogen or carbon starvation due to an inability enter the stationary phase (12). In addition to controlling the cell cycle and stationary phase switch, PKA plays an important role in response to stress. Gts1p, which is associated with heat tolerance in the stationary phase of budding yeast, is partially phosphorylated at some serine residue(s) when the cells are grown on glucose, and PKA positively regulates the phosphorylation level of Gts1p. In a *pka⁻* mutant, Gts1p does not show any increase in heat tolerance, suggesting that the phosphorylation of this protein by PKA is required for adaptation of the stationary phase (13). Activation of a MAP kinase (MAPK) pathway is also crucial for responding to external stress (14). In fission yeast, the MAPKK homolog $wis1^+$ gene is essential for cell survival under the conditions of stress, and the MAPK homolog Spc1/Sty1 is activated by Wis1 in response to osmotic stress and nutrient limitation. The integrity of the Wis1-Sty1 pathway is required for survival under extreme conditions of heat, osmolarity, oxidation, or limited nutrition (15, 16). In addition to phosphorylation, the ubiquitin-dependent proteolysis of proteins also contributes to the successful completion of cell cycle as well in a switch to the dormant state in response to stressed conditions (17). A multiubiquitin chain assembly factor (E4), such as the yeast UFD2 (ubiquitin fusion degradation) protein, facilitates the polyubiquitination of the target protein and is involved in the degradation of aberrant proteins induced by stress (18). The functions of UFD2 homologs in other organisms have not been well characterized but several reports indicate that they play important roles in development and cell death (19). In humans, *UFD2* is located in chromosome 1p and has been proposed to be a neuroblastoma tumor suppressor candidate gene (20). Thus the proper protein modification throughphosphorylation/dephosphorylation and ubiquitination is essential for cells to respond to changes in stress, such as nutritional limitations, osmotic stress, or heat shock. Here we showed that Psp1/Sds23 phosphorylated by cdc2/cyclin B to enter stationary phase (2), is also phosphorylated by stress activated kinase Spc1/Sty1 kinase and protein kinase A (Figure 1). These phosphorylations are important for maintaining cell viability in a dormant stage, such as the stationary phase, when nutrients are depleted (Figure 2). We also show that *S. pombe* homolog of *UFD2* (ubiquitin fusion degradation protein 2) of *S. cerevisiae*, *Spufd2*, encodes a protein that interacts preferably with the phosphorylated form of Psp1 in the stationary phase (Figure 3 A & B). Moreover, Psp1 enhances multiubiquitination function of Spufd2, indicating that Spufd2 regulates cell viability at the G_0-like stationary phase in association with Psp1 (Figure 3 C). Finally, Psp1 and Ufd2 may have a role in the degradation of proteins to cope with conditions of nutrient depletion stress (Figure 4).

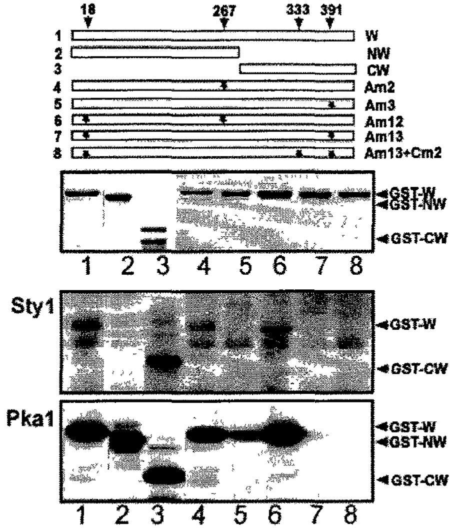


Figure 1. Phosphorylation of Psp1 by Sty1 and Pka1.

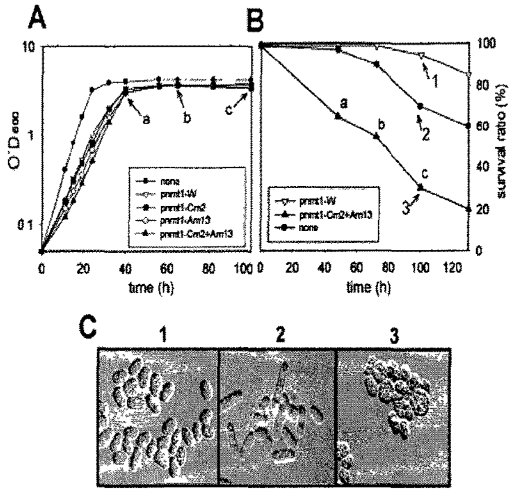
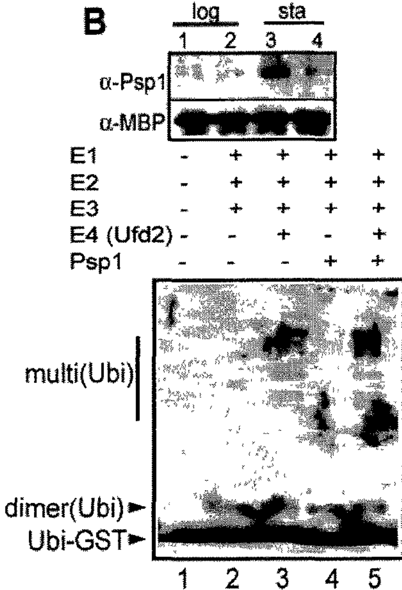


Figure 2. The phosphorylation of Psp1 is important for cell viability



A

Two-hybrid	β-gal activity (units)
pVA3/pTD1	190 0
pGAD/pGBT	0.1
pGAD-Ufd2/pGBT-W	85 0
pGAD-Ufd2/pGBT-Am13	14.6
pGAD-Ufd2/pGBT-Cm2+Am13	8 0

Figure 3. Psp1 phosphorylated interacts and activates the Ufd2 function

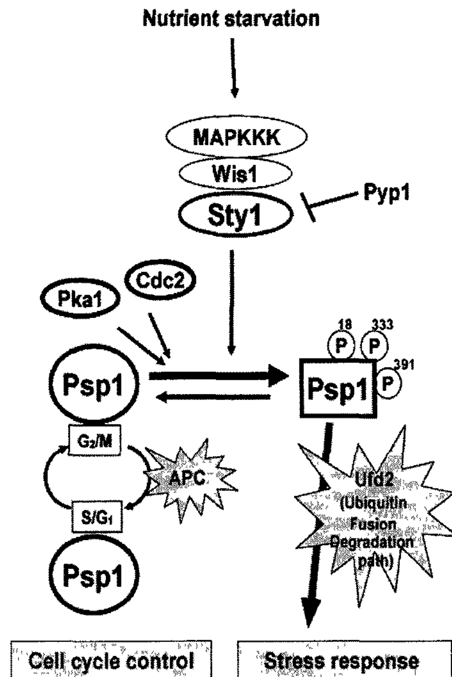


Figure 4. The flowchart about mechanisms. of Psp1 and Ufd2 in stress condition

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