

Screening genes encoding the protein phosphatase, which defend against the cellular senescence.

N. Hayashi, and S. Murakami

Eukaryotic cells must have system to response against ageing stress to keep its life span. A lot of systems, such as replication, cdk-cycline, checkpoint system, metabolic pathway and so on, are controlled for that. Especially, protein phosphorylation should be an important protein-modification to transmit signals. We screened the knocked out series of protein phosphatase genes, whose mutation appeared phenotypes of cellular senescence. We focused telomere length to examine 30 disruptants and 2 mutants encoding protein phosphatases, which were found in the yeast genome project, and examined them by Southern analysis using Y' DNA as probes to detect telomere. In this screening, we found approximately 130 bp short telomere in *sit4* disruptant. *SIT4* gene encodes PP2A, and has been already reported about its functions in G1 cycline pathway. Life span of *sit4* disruptant was shortened comparable to *sgs1* disruptant. We introduced *NOPI-GFP* fusion gene to observe nucleoli structure. Alteration and fragmentation of nucleoli in *sit4* disruptant were also found in major population. Similar phenomena were observed in *pph21 pph22* double mutant. *PPH21* and *PPH22* encode PP2A similarly to *SIT4*. In *sit4* disruptant, Rap1 foci were more dispersed and intensity of Rap1 foci was much weaker than that in wild-type cells. Moreover, *sit4* and *pph21 pph22* lost silencing ability at subtelomere region, suggesting deficiency of the heterochromatin formation. We examined process of cellular death in PP2A disruptants, because cellular death like apoptosis was recently reported in aged yeast cells. Annexine V-FITC staining and TUNEL assay were employed to detect appearance of phosphatidyl serine on cell surface and DNA fragmentation, respectively. In both analyses, some of *sit4* and *pph21 pph22* cells fell into cellular death. These findings suggest that PP2A is required to maintain cellular life span.

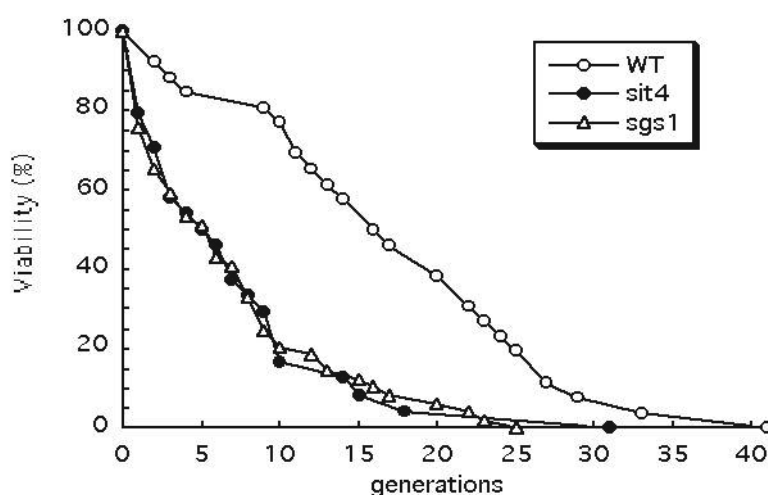


Figure. Life span was shortened in the *sit4* disruptant.