Role of LAMMER Kinase: A Fine-Tuner for Stress Responses in Fission Yeast?

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A number of studies have reported that the dual-specificity LAMMER kinases are involved in the regulation of cellular processes such as mRNA splicing, cell signal transduction, and chromatin condensation [1,2]. The LAMMER kinase deletion mutant of *Schizosaccharomyces pombe*, *lkh1Δ,* reveals various phenotypes [3,4], including: abnormality in cell-size control, flocculation in liquid culture, filamentous and adhesive growth on solid media, and sensitivity to oxidative stress, however, the mode of LAMMER kinase action in yeast is largely unknown.

The sensitivity of the *lkh1Δ* mutant to oxidants is caused by reduced levels of $atf1^+$ mRNA, which in turn results in the decrease of antioxidant enzyme activity owing to reduced expression [5]. The Csx1 protein containing three RNA-recognition motifs has been reported to bind directly to the $atf1^+$ mRNA in response to oxidative stress and to maintain normal levels of Atf1 by stabilizing the $atf1^+$ mRNA [6]. We found that Csx1 is phosphorylated by the Lkh1, under oxidative conditions and that the stress-activated binding of the Csx1 to the $atf1^+$ mRNA is also affected by Lkh1 and Spc1 [5].

In *S. pombe, tup11Δtup12Δ* mutant shows pleiotropic phenotypes including: flocculation, defective mating, and defective stress response [7], which mirror those of the *lkh1Δ* mutant. Based on this and the fact that Tup12 was isolated as Lkh1-interacting protein by a pull-down screen, we investigated a functional link between Lkh1 and Tup12 as well as Tup11 in gene expression regulation. Interactions between Lkh1 and Tup proteins were confirmed by binding assays. Tup proteins were phosphorylated by Lkh1 in a LAMMER motif dependent manner. Transcriptional activity assays using promoters negatively regulated by Tup11 and Tup12, showed six- or two-times higher activity in the *lkh1Δ* mutant than the wild-type, respectively. Northern analysis revealed decreased expression of a glucose-repressible gene for fructose-1,6-*bis*-phosphatase, *fbp1*+, in the *lkh1Δ* and the *tup11Δtup12Δ* mutant cells. Microarray and quantitative RT-PCR analysis also showed that a number of genes that were differentially expressed in the *tup11Δtup12Δ* mutant revealed similar pattern of differential expression in the *lkh1Δ* mutant.

Upon testing the effect of nutritional stress, the *lkh1Δ* mutant revealed reduction in vegetative-growth and ascospore-production under nitrogen starvation. Among the proteins involved in response to nitrogen starvation, Rum1, when the gene for it is deleted, shows defect in meiotic cycle and thus reduction in ascospore-production [8]. *In vitro* and *in vivo* pull-down assay confirmed the interaction between Lkh1 and Rum1, and peptide mass

fingerprinting identified Lkh1-mediated phosphorylation site in Rum1. *In vitro* kinase assay with a mutant Rum1, in which the Lkh1-mediated phosphrylation site was substituted, showed no incorporation of ^{32}P by Lkh1. Although further analyses should be conducted, these results suggest that the Lkh1 is involved in cell-cycle progression by regulating Rum1, an inhibitor of cyclin-dependent kinase, in fission yeast.

In conclusion, our results presented here suggest that the LAMMER kinase may act as a fine-tuner for stress-responses by modulating the activity of nucleic acid binding proteins and other regulatory protein(s) in fission yeast.

References

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