Talk

Generation of thermo-sensitive allele of the TPR like protein Nup211by PCR mutagenesis

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ABSTRACT

Motivation: TPR proteins are conserved large coiled-coil proteins that localize at the nucleoplasmic side of the nuclear pore complex and participate in multiple aspects of DNA metabolism. The protein Nup211, fission yeast homolog of Mlp1/Mlp2/Tpr, participate in the mRNA export and is essential for vegetative growth. The aim of this work is to create a collection of thermo-sensitive alleles of nup211.

Methods: To create the collection, we have generated a new strain with the nup211 gen tagged with GFP at the amino terminal extreme and confirmed by fluorescent microscopy that the protein Nup211 localized in the nuclear envelop. Then, we have carried out a Taq PCR-based Random Mutagenesis with reduced concentration of dATP. The PCR products were transformed into a wild type strain to generate conditional mutants. The transformants obtained whose growth was impaired at 36ºC were preselected as thermo-sensitive mutants. To confirm the growth deficiency of these clones, a drop assay was performed and the best candidates were selected. These thermo-sensitive mutants were cultivated at 25ºC as well as 36ºC and both cultures were subjected to various experiments in order to study any changes in the localization of Nup211.

Results: Up to now, we have demonstrated by fluorescent microscopy that the thermo-sensitive mutants show a modified nuclear distribution of Nup211 and different cellular phenotype, suggesting that the differents clones might represent differents nup211 thermo-sensitive alleles. These alleles are going to be subjected to various experiments to clarify the role of the protein in the mRNA export.

REFERENCES

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