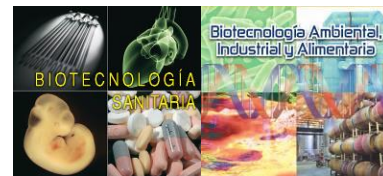

Poster

Isolation of conditional mutations using hypersensitivity to formamide in *Schizosaccharomyces pombe*



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ABSTRACT

Motivation: Conditional mutations are very powerful tools to study the function of the majority of genes in single-cell eukaryotes. High or low temperature are the most commonly used conditions so far. However, some other options such as drug sensitivity, have been proved useful strategies to find new alleles that might not be isolated otherwise. Thus we have searched for new cell cycle mutations that confer sensitivity to Formamide (HCONH₂). This is a small molecule, which presumably enters the cells and is not metabolized. This molecule is widely used as an ionizing solvent in vitro that destabilizes non-covalent bonds. In this study, we have used these properties in vivo to search for mutations in the fission yeast *Schizosaccharomyces pombe* that lead specifically to chromosome segregation defects in the presence of this molecule.

Methods: We performed a traditional mutagenesis using four different mutagens and searched for formamide sensitive mutants (fsm), as a novel conditional phenotype in *S. pombe*. The sensitivity of fsm strains to other conditions was also tested in a qualitative drop assay. The initial characterization of these mutants was made with a chromatin and cell wall staining. In addition, we have also screened for sensitivity to formamide in a genome-wide *S. pombe* non-essential gene deletion library.

Results: We have isolated 72 conditional mutations that show differential or no sensitivity to other traditional conditions used. Several of these mutants display morphological alterations, cell cycle defects and genomic instability. We have also described that there is a clear enrichment of RNA metabolism deleted genes that are hypersensitive to formamide. In order to confirm these result, we tested sensitivity to formamide of a selected group of splicing mutants (prp: pre-mRNA processing, a key process in RNA metabolism). In agreement with our prediction, all prp mutant strains are hyper-sensitive to formamide.

Conclusions: Viable *S. pombe* deletions which are sensitive to formamide have a special predominance of RNA metabolism related genes. Consistently, all prp alleles tested are hypersensitive to formamide. In vivo effect of formamide can efficiently be used as a condition for the search of new alleles of interest with a special focus on RNA related processes that may have escaped other screenings in fission yeast.

REFERENCES

- Aguilera, A. (1994) Formamide sensitivity: a novel conditional phenotype in yeast. *Genetics*. 136(1):87-91.
Dong-Uk, K. et al. (2010) Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol*. 28(6): 617–623.
Potashkin, J., et al. (1989). Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. *The EMBO journal*, 8(2), 551.