2. CELLULAR RESPONSES OF *SACCHAROMYCES CEREVISIAE* TO DNA DAMAGE

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Several experimental strategies have been used to study responses of *S. cerevisiae* cells to DNA damage. One approach was based on the isolation of novel genes, the expression of which is induced by lesions in DNA. One of these genes, *DIN7*, was cloned and partially characterized previously. The product of *DIN7* belongs to a large family of proteins involved in DNA repair and mutagenesis. This family includes Rad2, Rad27 and ExoI proteins of *S. cerevisiae* and their respective human homologues, all of which are endowed with DNA nuclease activity.To study cellular function of Din7 we constructed the pPK3 plasmid carrying *DIN7* fused to the *GAL1* promoter. Effects of Din7 overproduction on the phenotypes of wild-type cells and of *rad27* and *exoI* mutants were examined. Overproduction of Din7 does not seem to affect the proficiency of wild-type *S. cerevisiae* cells in recombination and mutagenesis. Also, overexpression of *DIN7* does not suppress the deficiency of the *EXOI* gene product, the closest homologue of Din7, both in recombination and in controlling the fidelity of DNA replication . Unexpectedly, we found that elevated levels of Din7 result in a very high frequency of mitochondrial *rho*$^-$ mutants.

A high frequency of production of *rho*$^-$ mutants was also observed in strains defective in the functioning of the Dun1 protein kinase involved in signal transmission in cells exposed to DNA damaging agents. Interestingly, deficiency of Dun1 results also in a significant derepression of the *DIN7* gene. Experiments are under way to distinguish whether a high cellular level of Din7 specifically decreases stability of mitochondrial DNA or affects stability of chromosomal DNA as well.

Analysis of previously constructed *S. cerevisiae* strains carrying random genomic fusions with reporter *lacZ* gene, allowed us to identify the reading frame YBR173c, on chromosome II as a novel damage inducible gene - *DIN8* . We have shown that *DIN8-lacZ* fusion is induced in yeast cells treated with MMS or exposed to UV light. Northern RNA analysis indicates that *DIN8* is induced in response to DNA damage at the transcriptional level. *DIN8* was cloned and the phenotype of cells with disruption of the gene is under study.

POL2-MEC1-RAD53-DUN1-signal transducing pathway has recently been postulated to be involved in the regulation of response of *S. cerevisiae* cells to DNA-damaging agents. We analyzed the expression of a known damage inducible DNA-repair gene, *MAG1*, encoding 3-methyladenine glycosylase, in *S. cerevisiae* strains carrying *MAG1::lacZ* fusion and deficient in either *POL2, MEC1, RAD53* or *DUN1* function. β-galactosidase activity was assayed in cycling cells exposed to MMS or UV light. It was found that, in contrast to model DNA damage inducible *RNR* genes, neither mutation in the sensory C-terminal part of polymerase ε (*pol2-11*) nor the in the Mec1, Sad1/Rad53 or Dun1 cellular kinases blocks the induction of *MAG1* in response to MMS or UV light in cycling yeasts.