Differential "Liquid Holding Recovery" for the Lethal Effect and Cytoplasmic "Petite" Induction by UV Light in *Saccharomyces cerevisiae*

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Summary. Haploid and diploid wild types and UV-sensitive (uvs_{-}3) strains were exposed to UV light in stationary phase of growth and in log phase. The liquid hold recovery (LHR) was studied in both conditions. 1. It appears that haploid wild type resting cells (1st type of repair) are less capable of repair during dark holding than dividing cells (2nd type of repair). 2. The mutant uvs_{-}3, which behaves like an excision defective strain, has lost the 1st type of repair. In contrast, the 2nd type of repair is still present. 3. The LHR is not additive to photorestoration (PR) for the 1st type of repair. On the contrary LHR and PR are additive for the 2nd type of repair. 4. Caffeine suppresses the 1st type of recovery and has only a slight effect on the 2nd type. 5. Both types of repair are functioning in diploid wild type cells where only the 2nd type of repair is present in UV-sensitive homozygous diploids uvs_{z}/uvs_{z}.

From these data it is tentatively suggested that the 1st type of repair is related to the excision-resynthesis repair mechanism. The 2nd type of repair, active in dividing haploid cells and in diploid cells, may involve chromosomal exchanges.

The effect of storage in the dark for the cytoplasmic "petite" induction by UV was examined in wild type strains. A negative liquid holding (NLH) effect (increase of the frequency of "petites" during storage) was observed for diploid cells and after low doses (up to 1,500 ergs/mm^{2}) for haploid cells. At high doses a recovery is observed in haploid cells. An interpretation of this NLH effect is discussed. This differential response to dark holding for the lethal damage and cytoplasmic genetic damage supports the idea that there is a certain degree of independence between the nuclear and the mitochondrial systems with regard to the repair machinery.

Introduction

Potentially lethal damage induced by ultraviolet or ionizing radiations can be partially reversed if yeast cells are incubated in the dark in distilled water, for a number of days prior to plating (Patrick *et al*., 1964). Although the precise mechanism of this recovery (termed "liquid hold recovery" or LHR) in yeast is unknown, it has been shown that it can be modified by a variety of physical and biochemical parameters after irradiation (Patrick and Haynes, 1964). Moreover the reversibility of the lethal damage of UV in *Saccharomyces cerevisiae* is altered in some of the UV-sensitive mutants (Moustacchi, 1969; Parry and Parry, 1969; Averbeck *et al*., 1970). For instance holding of the irradiated uvs_{2} mutant (Moustacchi, 1969) or UV-sensitive mutants of the groups 3 and 4 in Parry and Parry classification (1969) leads to a decreased survival. In other words the products of some of the loci which control the "immediate" response to UV irradiation inter-
fere with the delayed repair of a fraction of the induced damage. These different observations support the view that the dark recovery in yeast is an enzymatic process.

In *Escherichia coli* B/r the excision-resynthesis type of repair seems to account for the major part of the "liquid hold recovery" of irradiated cells. In UV irradiated excision-sensitive bacteria, B/r, there is a loss of photoreactivability as a function of the time during which the cells stay in the dark. Such an effect is reduced in recombination-deficient (rec<sup>-</sup>) bacteria and is absent in strains deficient in excision-resynthesis dark repair (her<sup>-</sup>) (Harr, 1968).

In yeast, a priori at least two possible mechanisms which are not mutually exclusive may account for the LHR:

1. A direct enzymatic repair of the damaged target molecules of the same type as the excision-resynthesis mechanism of repair in *Escherichia coli*.

2. A genetic exchange between homologous DNA during DNA replication and/or during pairing.

In relation to this question we have reexamined the LHR after UV irradiation of haploid and diploid wild type and *uvs* cells, in stationary phase, as in reported experiments (Patrick et al., 1964; Parry and Parry, 1969; Averbeck et al., 1970), and in comparison to log phase cells. On the one hand, haploid wild type cells and the derived *uvs*<sub>z</sub> (or *uvs<sub>1</sub>-3* see material and methods) mutant, and on the other hand, diploids homozygous and heterozygous for the *uvs<sub>1</sub>-3* marker were examined.

In parallel with the study of the lethal effect, we have examined if a relative independence of cytoplasmic repair can be demonstrated for the LHR. Several *uvs* mutations in *S. cerevisiae* do not affect the dose-effect response for the cytoplasmic "petite" mutation induced by UV light (Moustacchi and Enteric, 1970; Moustacchi, 1969). In other words some of the genes which seem to control the repair of nuclear damage apparently do not interact with the repair of cytoplasmic genetic lesions. Results concerning the delayed response to UV induction of the "petite" mutation in wild type cells will be reported here.

**Material and Methods**

*Strains.* The non-sensitive UV strain is N123 (a hi<sub>1</sub>); it requires histidine for growth. The UV-sensitive yeast mutant is N123-*uvs*<sub>z</sub> referred to in an earlier publication (Moustacchi, 1969); it is derived from N123. The complementation test between this strain and some of the *uvs* mutants, with the same phenotype, identified in other laboratories have been performed. When the mutant *uvs*<sub>z</sub> is mated with the mutant *uvs<sub>1</sub>-3* from Resnick (1968) or *UV<sub>1</sub>* from Nakai and Matsuo (1967), the hybrids formed are UV-sensitive, they do not show complementation. Therefore *uvs*<sub>z</sub> is considered to be allelic to *uvs*<sub>1</sub>. The nomenclature *uvs<sub>1</sub>-3* will be henceforth adopted for this strain.

The *uvs<sub>1</sub>-3* marker was incorporated into the strain 10018 -- 1 (a *ad<sub>1</sub>*) by cross and dissection of asci. Diploids of the following genotypes were synthesized by mating on minimal medium:

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\begin{array}{cccc}
    \alpha & hi1 & AD & uvs<sub>1</sub>-3 \\
    \alpha & HI & ad<sub>1</sub> & UVS \\
\end{array}
\]

(cross N123 -- *uvs<sub>1</sub>-3* × 10018 -- 1 and N123 -- *uvs<sub>1</sub>-3* × 10018 -- 1 *uvs<sub>1</sub>-3*).

*Media and Growth Conditions.* With an inoculum of 5.10<sup>4</sup> cells/ml aerated cultures were grown for 18 hours or 4 days in YEP (yeast extract 1%, bacto-peptone 2%, glucose 2%) at 30°C, 2% agar was added for plates.