Variations in UV Induced Lethality and "petite" Mutagenesis in Synchronous Culture of *Saccharomyces cerevisiae*

I. Influence of Post-irradiation Conditions

R. Chanet and M. Heude

Fondation Curie — Institut du Radium, Biologie, Orsay, France

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Summary. Cells of *Saccharomyces cerevisiae* taken at different stages of the mitotic cell cycle exhibit a variation to lethality and "petite" mutation following UV irradiation. The magnitude of the variations encountered for lethality is diminished when the cells are dark held, the maximum liquid holding recovery taking place during G1 phase and the minimum in G2 phase. Liquid holding treatment, on the contrary, increases the magnitude of cyclic variations of "petite" induction.

The illumination of irradiated cells promotes an increase in survival and a restoration of a proportion of "grande" cells at all cell stages. However variations are maintained and the ratio between maximum and minimum resistance is approximately the same.

Introduction

A variation in the lethality and in the cytoplasmic "petite" induction was encountered following UV irradiation at different stages of a synchronized yeast cell population. Lethality was at a minimum during G2 phase and this was not concomittant with the minimum degree of "petite" induction, the latter being at a minimum during the end of G1 (Chanet et al., 1973).

These variations of sensitivity observed at different stages have been interpreted for different organisms as resulting from a variation in efficiency of the repair mechanisms (Davies, 1965; Holliday, 1965; Kimball, 1963; Wood, 1968). The same type of interpretation has been proposed for random cultures to explain the increase of survival when the irradiated cells are liquid held in the dark in a non nutrient medium before plating as compared with the survival after immediate plating (Patrick and Haynes, 1964). Furthermore, in these conditions of liquid holding (LH), differences in sensitivity to lethality and "petite" induction were observed between cells irradiated in stationary or log phase of growth (Moustacchi and Enteric, 1970; Heude and Moustacchi, 1973). We have examined the response of UV irradiated cells at different mitotic stages and noted their responses to LH as compared to immediate plating.

It is known that the number of UV induced pyrimidine dimers is partly responsible for the amount of inactivation of irradiated cells (Setlow and Setlow, 1962). These dimers are split by visible light, promoting a restoration of biological activity (Setlow, 1966). We have tried to determine by the use of visible light treatment whether pyrimidine dimers are involved in the expression of the fluctuations in sensitivity observed during the mitotic cycle following immediate plating.
Material and Methods

Strain. The haploid strain N123 (a\ hii) was used. It has a spontaneous frequency of "petite" mutants of about 1%.

Media. Cells are grown in YEP (Yeast extract Difco 0.5%, Bactopeptone Difco 2%, glucose 2% in distilled water).

Plating was done on YEP solidified by 2% Bacto Agar. For synchronization single cells were isolated by Ficoll gradients; the growth and synchronization conditions, those of irradiation, the ultraviolet and visible light sources, and the "petites" detection methods have been described elsewhere (Chanet et al., 1973; Moustacchi and Enteric, 1970; Ogur et al., 1957).

Aliquots of a cell suspension at specific stages of a synchronized culture were irradiated with several UV doses; a fraction of the cells was plated immediately and another was dark held in distilled water at 30° C for 48 hours prior to plating (LH).

The evolution of the culture was determined by counting the cells with a haematocytometer. We have distinguished the following cell classes: single cells without buds which are in G1, cells with small buds which are in S phase, cells with a bud at about 2/3 of the size of mother cell which are in G2, and the double cells as two cells in G1 stage (Williamson, 1965).

Haematocytometer countings showed that liquid holding conditions did not modify the proportion of different cell classes.

Results

Effects of Dark Holding

Fig. 1 shows the characteristics of the synchronized population, the variation in dose which leaves a 10% surviving fraction (LD 10%), the variations of sensitivity to "petite" induction among survivors (Mutagenic Dose 25%) with or without liquid holding.

Fig. 1 b shows that the liquid holding of irradiated cells leads to a cyclic response. However, it appears that the magnitude of such fluctuations in survival is decreased as compared to the immediate response. In fact, during the G1 phase, which is the most radiosensitive stage after direct plating, we observed the greatest increase of survival following LH. The restoration effect is reduced during S phase and becomes practically non-existent during the G2 phase, which is the most radioresistant stage after direct plating.

On the contrary Figure 1 a shows that such post-irradiation incubation gives rise to an increase in the magnitude of cyclic variation for "petite" induction. Dark holding of the UV sensitive stages, as revealed after immediate plating, resulted in the greatest increase in sensitivity to "petite" induction. On the contrary, following similar post-irradiation incubation the resistant phase exhibited a decrease in induced "petite" frequency.

It can be noticed that, after both immediate and delayed plating after liquid holding, the most resistant stage of the cell cycle for survival is not coincident with that for "petite" mutagenesis. The periodic fluctuations seen for both effects after LH are retained and their pattern during the cell cycle is about the same as after immediate plating. However, the differences in sensitivity to lethal damage between immediate and delayed plating of liquid held cells is decreased, this mainly being due to an increase of resistance in G1 phase. On the other hand, the magnitude of the fluctuations in "petite" mutagenesis is increased.

Effects of Visible Light

Fig. 2 shows that on comparing the LD 10% curve, and the curve for LD 10% followed by photoreactivating light, there is a photoreactivation of UV induced