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Effects of caffeine and ARG7 locus on mutability of UV-treated photoreactivation-deficient mutants of *Chlamydomonas reinhardtii*

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Abstract

Forward streptomycin-resistant mutations and reverse mutations at the ARG7 locus after UV irradiation were studied in two photoreactivation-deficient mutants of *Chlamydomonas reinhardtii*, Phrl and Phr2. The mutant Phrl was more mutable than Phr2. Caffeine increased survival and reduced mutation rate of streptomycin-resistant mutations induced in both photoreactivation-deficient strains. Two different alleles of ARG7 locus (arg2 and arg7) were introduced into photoreactivation-deficient mutants. It was found that in the presence of both alleles, the frequency of mutants resistant to streptomycin was reduced. The reduction was more remarkable in the presence of arg2. But also under these conditions Phrl was more mutable than Phr2.

Introduction

Up to now only two mutants defective in photorepair of pyrimidine dimers in *Chlamydomonas reinhardtii* cells were isolated and were designed as Phrl and Phr 2 (Cox and Small 1985). Extracts of these mutants had about 17 % of the total photolyase activity of wild-type cells. However, chloroplast photorepair of Phrl and Phr2 was normal and accounted for the residual photolyase activity observed in these strains (Cox and Small 1985, Small 1987). As was suggested previously (Miadoková *et al.* 1991), Phrl and Phr2 are alleles of the same gene, but they differed in their UV-survival and survival of meiosis products of the Phrl × Phr2 cross. In this work we paid attention to a mutability study of photorepair-defective strains, and to the effect of caffeine and "gene background" on the mutation rates after UV-treatment. Selby and Sancar (1990) found that caffeine inhibited *Escherichia coli* photoreactivation by blocking of the photolyase binding to pyrimidine dimers. On the other hand, a stimulation effect of caffeine on survival of UV-irradiated *Chlamydomons reinhardtii* strains in non-photoreactivation conditions was mediated by and increase in recombination repair (Rosen *et al.* 1980).

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It is generally known that the frequency of mutations in some loci can be considerably affected by the gene background (Auerbach 1976). In this article mutability of the Phr1 and Phr2 strains was studied on the basis of forward and reverse mutations. The ARG7 locus as a "gene background" for Phr1 and Phr2 strains was chosen because it had been extensively characterized genetically (Gillham 1965, Lopes and Matagne 1972), and our own experiences indicated that this locus could influence some cell processes. Fine structure analysis revealed that mutants of ARG7 locus fall in five complementation groups, and that two outermost mutations of this locus, arg2 and arg7, are 1.0 - 1.6 recombination units apart (Matagne 1978). After introduction of arg7 and arg2 mutations into photoreactivation-deficient strains, possible relations between photoreactivation-deficiency and reversibility of ARG7 locus, and their possible influence upon induction of strepromycin-resistant mutations, was studied.

Materials and methods

Algal strains: A wild-type of *Chlamydomonas reinhardtii* 137 C (Dr. R. Loppes, Department of Botany, University Liège, Belgium); Phr1, Phr2 (Dr. G.D. Small, Department of Biochemistry, University of South Dakota, Vermillion, USA); arg2 (CC-48), arg7 (CC-1685) (Duke collection, Durham, USA); Phr1 arg2, Phr1 arg7, Phr2 arg2, Phr2 arg7 (Department of Genetics, Comenius University, Bratislava, Slovakia) were used.

Media: Liquid and solid minimal media were prepared according to Starr (1971). Selective media were supplemented with acetate and 2 $\mu g\ cm^{-3}$ arginine, 100 $\mu g\ cm^{-3}$ streptomycin. For experiments with caffeine, minimal medium was supplemented with 1.5 mM caffeine.

Mutagen: A 30 W *TUV Philips* germicidal tube was used as a source of UV irradiation. This tube emits about 95 % of radiation at 253.7 nm. The irradiance at plate surface was 5 $J\ m^{-2}\ s^{-1}$ as measured with *Latarjet dosimeter No. 81* (*IL 254, Germicidal Photometer*, Newsburyport, Massachussets, USA).

Induction of mutations: After introduction of arginine mutations into photoreactivation-deficient strains by crossing the Phr1 strain with the CC-48 strain, and the Phr2 strain with the CC-1685 strain and *vice-versa*, four types of double mutant strains (Phr1 arg2, Phr1 arg7, Phr2 arg2, Phr2 arg7) were obtained. They were UV-irradiated directly on the surface of Petri dishes with minimal medium supplemented with acetate and 2 $\mu g\ cm^{-3}$ arginine in purpose to obtain arginine revertants.

In experiments aimed at induction mutations resistant to streptomycin, photoreactivation-defective strains were UV-irradiated on the surface of minimal agar plates with addition of an acetate, and 100 $\mu g\ cm^{-3}$ streptomycin. A half of plates were supplemented with 1.5 mM caffeine. Each Petri plate was overlayed by a

soft agar (0.7 %) to prevent drying of a medium because the first streptomycin-resistant colonies appeared after two weeks of a cultivation in an illuminator.

Values shown in figures represent mean values of four consecutive experiments with four plates per each UV dose in each experiment.

Results and discussion

Arginine reverse mutations in photoreactivation-deficient strains: Frequency of arg7 and arg2 reversions in Phr1 arg7, Phr1 arg2, Phr2 arg7 Phr2 arg2 strains after UV irradiation was higher in strains with damaged photolyase compared to that of photorepair-proficient strains carrying arg7 or arg2 mutations. There were differences between Phr1 and Phr2. The frequency of arginine reverse mutations was higher in the Phr1 strain. The reversibility of arg7 and arg2 alleles was very similar (Fig.1).

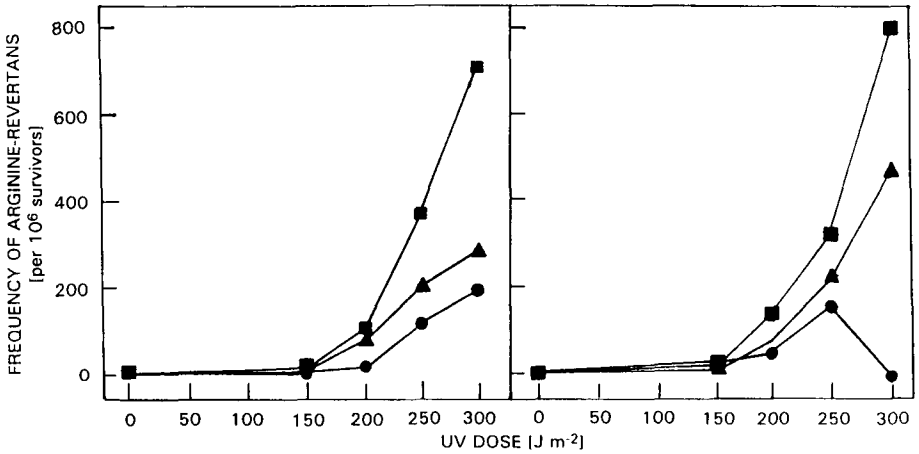

Fig. 1. Comparison of the frequency of arg7 and arg2 revertants induced in double mutants and photorepair-proficient strain of *Chlamydomonas reinhardtii* after UV irradiation. Left: arg2 (*circles*), Phr1 arg2 (*squares*), Phr2 arg2 (*triangles*); right: arg7 (*circles*), Phr1 arg7 (*squares*), Phr2 arg7 (*triangles*).

Effect of caffeine on survival and forward mutations to streptomycin-resistance in photoreactivation-deficient strains: The presence of caffeine increased survival of both photorepair-deficient mutants (Fig. 2). A caffeine stimulation of survival is more expressive in Phr2 strain. A survival stimulation of Phr1 was very similar to that found in wild-type cells (Podstavková *et al.* 1991). Thus, caffeine probably does not inhibit photoreactivation of *Chlamydomonas reinhardtii* as it does in bacteria (Selby and Sancar 1990), but because it stimulates recombination repair (Rosen *et al.* 1980), it causes increasing of survival UV-treated wild-cells and these UV-sensitive strains which have not impaired recombination-repair (Podstavková *et al.* 1991), including photorepair-deficient strains.

Frequency of streptomycin-resistant mutations after UV irradiation in the presence of caffeine is reduced in both mutant strains deficient in photoreactivation (Fig. 3). Streptomycin-resistant mutations are induced in the Phr1 strain in a higher frequency than in the Phr2 strain, both in the absence and in the presence of caffeine. However, there is not any difference in the frequency of streptomycin-resistant mutations induced in a wild-type strain in the absence and in the presence of caffeine under photoreactivating conditions.

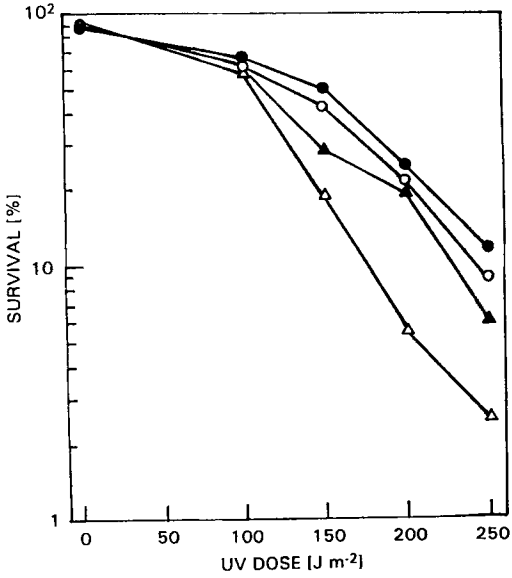

Fig. 2. Comparison of survival of photorepair-deficient strains of *Chlamydomonas reinhardtii* in the absence (Phr1 - *open circles*, Phr2 - *open triangles*) and in the presence of (Phr 1 - *closed circles*, Phr2 - *closed triangles*) of caffeine.

The increase of survival and the reduction of mutation rates of both photorepair-deficient strains in the presence of caffeine can be explained by caffeine stimulation of recombination-repair pathway which might be error-free.

Differences in survival and mutability found between Phr1 and Phr2 in the presence of caffeine could be then explained by a different interaction of remaining activities of their gene products with recombination repair stimulated by caffeine.

Effect of a "gene background" on mutability (streptomycin-resistant mutations) of photoreactivation-deficient strains: The frequency of streptomycin-resistant mutations induced after UV treatment of Phr1 and Phr2 strains is increased only in the presence of arg7 allele, and mutability of Phr1 is higher than that of Phr2 (Fig. 4). In the presence of arg2 mutation the frequency of streptomycin-resistant mutations in Phr1 and Phr2 is not changed as compared to one of strains with non-defective photolyase.

Comparison of data in Figs. 3 and 4 indicates that two outermost alleles of ARG7 locus (Gillham 1965, Loppes and Matagne 1972, Matagne 1978, Debuchy *et al.* 1989) reduce the frequency of streptomycin-resistant mutations induced after UV irradiation of both photoreactivation-deficient strains. But, there is a great difference

in the effects of arg7 and arg2 mutations, because arg2 reduces above mentioned frequency on the level of photorepair-proficient strains.

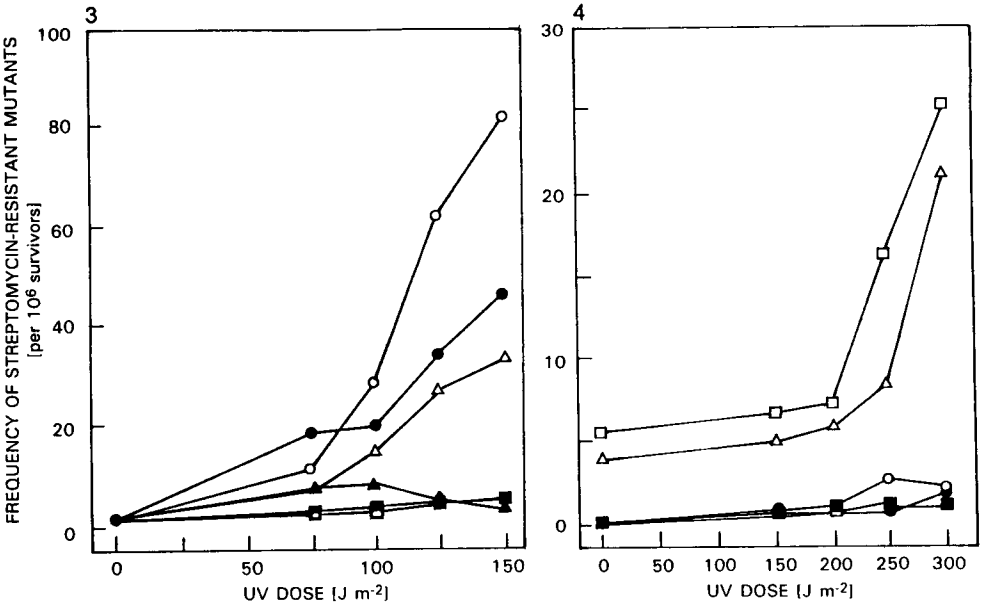

Fig. 3. Comparison of the frequency of streptomycin-resistant mutations induced in photorepair-deficient and photorepair-proficient strains of *Chlamydomonas reinhardtii* in the absence (Phr1 - *open circles*, Phr 2 - *open triangles*, 137C - *open squares*) and in the presence (Phr1 - *closed circles*, Phr2 - *closed triangles*, 137C - *closed squares*) of caffeine.

Fig. 4. Comparison of the frequency of streptomycin-resistant mutations induced in double mutants Phr1 arg2 (*closed squares*), Phr2 arg2 (*closed triangles*), Phr1 arg7 (*open squares*), Phr2 arg7 (*open traingles*) and photorepair-proficient strains of *Chlamydomonas reinhardtii* carrying arg7 (*open circles*) and arg2 (*closed circles*) alleles after UV irradiation.

These findings suggest that a gene background can substantially influence some gene loci (Auerbach 1976). Even different alleles of the same gene locus (ARG7) can not only drastically reduce frequency of streptomycin-resistant mutations induced in photorepair-deficient strains but they are surprisingly different in this effect (Fig. 4). This specific effect of the ARG7 alleles may be connected with its certain role in some cell processes. It follows from our personal experinces that it can interfere with segregation of some genetic markers (mainly mating type).

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