Responses of *Chlamydomonas reinhardtii* repair-deficient strains to X-ray irradiation

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SLIVKOVÁ, A., MIADOKOVÁ, E., PODSTAVKOVÁ, S., VLČEK, D., Responses of *Chlamydomonas reinhardtii* repair-deficient strains to X-ray irradiation.- Biologia, Bratislava

Survival of several *Chlamydomonas reinhardtii* repair-deficient strains and mutability following X-ray exposure was compared in order to supplement general characteristics of these mutants. Our observations suggest that the recombination repair mechanism plays an important role in removing of DNA lessions caused by X-ray irradiation.

Key words: *Chlamydomonas reinhardtii*, repair deficient strain, X-ray irradiation

Introduction

Chlamydomonas reinhardtii is a convenient model organism for study of the DNA-repair systems of eukaryotic cells. The study of repair mechanisms requires an adequate collection of repair-deficient mutants. The world collection of algae contains 26 repair-deficient mutants (Davies, 1967; Rosen & Ebersold, 1972; Rosen et al., 1980; Cox & Small, 1985; Vlček et al., 1987; Small, 1987; Podstavková et al., 1991; Vlček et al., 1991; Podstavková et al., 1992; Miadoková et al., 1994; Podstavková et al., 1994; Vlček et al., 1995; Podstavková et al., 1996; Vlček et al., 1997). For better understanding of *Chlamydomonas reinhardtii* DNA repair machinery, it is important not only to enlarge the existing collection of DNA-repair-deficient mutants and to complete general phenotypical characteristics of repair-deficient strains. So far they were characterized mainly on the basis of their response to UV-light and MNNG (Vlček et al., 1987; Podstavková et al., 1991; Podstavková et al., 1992; Miadoková et al., 1994; Podstavková

et al., 1994; Vlček et al., 1997). Now we have supplemented general characteristics with results obtained after X-ray irradiation. Up to date we have analysed mutants deficient in excision repair pathway (uvs12, uvs15) (Vlček et al., 1987; Podstavková et al., 1992), recombination repair pathway (uvs10, uvsE1) (Rosen & Ebersold, 1972; Rosen et al., 1980; Portney et al., 1980; Vlček et al., 1987; Podstavková et al., 1996), and mutants without defined particular mechanism of DNA repair (uvs13, uvs14) (Podstavková et al., 1992; Podstavková et al., 1994; Podstavková et al., 1996).

Material and methods

Strains: The wild type strain of *Chlamydomonas reinhardtii* was obtained from University of Lięge (Belgium). The UV-sensitive strains designated as *uvs10, uvs12, uvs13, uvs14, uvs15* were isolated from wild type 137c, mt^+ in our laboratory. The strain *uvsE1* was obtained from University of South Dakota, Vermillion (USA).

Media: Liquid and solid media were prepared according to Starr (1971). For the experiments on the induction of streptomycin-resistant mutants the medium was supplemented with 100 $\mu g.ml^{-1}$ of streptomycin.

Mutagens: A X-ray tube operated at 320 kV and 10mA delivering a dose of 180,6 Gy/h was used as a source of ionizing irradiation.

Survival curves: For X-ray survival curves, the cells were irradiated in the buffer and immediately after irradiation the cells were spread on the surface of solid minimal medium and put on the light shelf. The method of microscopic survival evaluation was used which enables to determine if the cells that failed to form visible colonies had undergone any cell division.

Induction of streptomycin-resistant mutants: the method of Lee and Jones (1973) was adopted for the isolation of streptomycin-resistant mutants induced by X-ray irradiation. Frequency of mutation was expressed as a number of colonies formed in the presence of 100$\mu g.ml^{-1}$ streptomycin per 10^6 surviving cells.

Results and discussion

The X-ray survival curves of the wild type strain and repair-deficient strains are compared in Fig. 1. The most sensitive strain after X-ray treatment was *uvs15* mutant. This mutant is also the most sensitive one following exposure to UV-irradiation and MNNG (Podstavková et al., 1991). Furthermore, this strain did not mutate after X-ray irradiation (Tab. 1) and UV-treatment and exhibited low rate of mutations after MNNG. Moreover, the deficiency in pyrimidine dimers excision was proved in the strain (Vlček et al., 1997). Up to date the mutant with such properties (excision deficiency with no or very reduced level of mutability) has not been described in heterotrophic organisms.

Strains uvsE1 and uvs10 with impaired recombination repair pathway were also very sensitive to X-ray damage (Figs. 1) and we observed increased frequencies of streptomycin-resistent mutations (Tab. 1). These results indicate a possible role of recombination repair mechanism in removing of DNA lessions caused by X-ray irradiation. Moreover, the higher frequencies of streptomycin resistent mutations suggest that recombination repair in *C. reinhardtii* is an error-free process.

The strain *uvs12* was the most resistent one of all UV-sensitive strains used. Mutant strain *uvs12* is deficient in the nuclear excision-repair pathway (Podstavková et al., 1992). It seems that genes responsible for excision repair do not play a decisive role in the repair of damages induced by X-ray.

Another interesting and rather resistent strain was *uvs14* mutant. This mutant belongs to a group of strains with unknown DNA repair deficiency (Podstavková et al., 1996). In spite of the fact that the strain *uvs14* manifests a mutator phenotype (higher levels of spontaneous and induced mutability), we did not observed significantly increased uvs14 mutability after X-ray treatment (Tab. 1).

The aim of our work was to supplement the general characteristics of several repair-deficient mutant strains in order to speed up the study of repair mechanisms in algae. The adequate general knowledge about the repair-deficient mutants allow to study the specific role of single genes and their product involved in DNA repair process and establishes the base for studies on molecular level.

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Tab.1: Comparison of X-ray mutability (number of streptomycin-resistant mutants / 10^6 survivors).

Strains	X-ray dose (krad)				
	0	4,5	9	18	27
W1 mt$^+$	0,5	2,3	2,4	1,25	0
uvsE1	1,15	37,9	100,88	37,3	3,04
uvs10	1,15	20,06	13,06	7,5	2,33
uvs12	0,4	0	0,5	0,5	0
uvs13	0,65	4,9	7,1	7,95	0
uvs14	4,9	4,6	3,9	2,5	0
uvs15	0	0	0	0	0

Data represent means of five independent experiments with 95 % coinfidence limit.