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DNA Repair

A *Chlamydomonas reinhardtii* UV-sensitive mutant *uvs15* is impaired in a gene involved in several repair pathways

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Abstract

In this report, three DNA repair-deficient mutants of *Chlamydomonas reinhardtii* (*uvs13*, *uvs14*, *uvs15*) were characterized by using genetic, mutational and biochemical analyses. The mutant strain *uvs15* belongs to the most sensitive repair-deficient mutants following exposure to all agents used. It is deficient in the nuclear excision-repair pathway, whereas *uvs13* and *uvs14* are not blocked in removal of pyrimidine dimers. Mutation study also revealed differences among strains. The mutant *uvs15* does not mutate after UV and X-ray irradiation, and there is very low mutation rate after MNNG. These findings might indicate the involvement of *UVS15* gene product in regulation of several repair pathways. Contrary to this, *uvs14* showed higher mutation frequency, both spontaneous and induced after UV and MNNG treatments. Tetrad dissection proved that the *uvs13* and *uvs14* genes are located on the right arm of the linkage group I in the vicinity of the previously mapped *uvs10* gene. Both mutants belong to the same repair pathway, which is different from that of *uvs10* and *uvs15*.

Keywords: Repair deficient mutant; Repair pathway; Mutation repair; *Chlamydomonas reinhardtii*

1. Introduction

In comparison with some heterotrophic lower eukaryotes, there has been much less progress made in the understanding of the function and organization of repair processes in algae. Knowledge about repair systems concerning this group of organisms has been mostly obtained in *Chlamydomonas reinhardtii* [1–11]. This green alga is, like *Saccharomyces cerevisiae*, a convenient model organism amenable to both genetic and molecular manipulations, and has proven to be an appropriate model system for the study of numerous aspects of molecular and cell biology in photoautotrophs [12]. Following evolutionary trees based on rDNA sequence analysis showing that fungi and metazoa diverged quite recently from the green algae and higher plants [13], this alga may be a convenient model organism for the study of different cell biology topics in both photoautotrophic and heterotrophic eukaryotes [14].

So far, a number of *C. reinhardtii* mutants with sensitivity to DNA-damaging agents, mainly UV-irradiation, have been isolated in our laboratory [15,16]. These repair-deficient strains are used for genetic, biochemical and mutational analysis to con-

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tribute to the knowledge on repair in photoautotrophic microorganisms. Use of this alga as a model system for establishing the mechanisms of DNA repair can contribute to understanding this very important self-guarding processes in higher plants [17].

This paper describes the first *C. reinhardtii* mutant which might be involved in multiple repair pathways, and the genetic, mutational and biochemical analyses of further repair-deficient strains induced in our laboratory.

2. Materials and methods

2.1. Strains

The UV-sensitive mutants designated as *uvs10* [15], *uvs13* [10], *uvs14 and uvs15* formerly (after isolation) designated as uvsN350 [16] were isolated from a wild-type 137c, mating-type plus in our laboratory. The strains: *msr1ac-20y-6 mt*$^-$ (CC-1676) and *arg7mt*$^-$ (CC-1685) used for genetic analysis were obtained from the Chlamydomonas Genetic Center, Duke University, USA.

2.2. Media

Liquid and solid minimal media (HS) were prepared according to Starr [18]. For the experiments on the induction of streptomycin-resistant mutants, the minimal medium was supplemented with 1.2 $g \cdot l^{-1}$ of sodium acetate (HSA) and 100 $\mu g \cdot ml^{-1}$ of streptomycin. For the genetic analysis, the following media were used: minimal medium containing 0.05 $g \cdot l^{-1}$ of NH_4Cl and supplemented with 1.2 $g \cdot l^{-1}$ sodium acetate (HSA1); minimal medium supplemented with 1.2 $g \cdot l^{-1}$ sodium acetate and 4 $g \cdot l^{-1}$ yeast extract (YA); liquid nitrogen-free medium 5 times diluted (HSD); minimal medium supplemented with 1.2 $g \cdot l^{-1}$ sodium acetate plus 100 $\mu g \cdot l^{-1}$ arginine (HSAG), or 4% agar (HSZ), or 500 $mg \cdot l^{-1}$ methionine sulfoximine (MS).

2.3. Mutagens

A 30-W TUV Philips tube was used as the source of UV-irradiation. This tube emits about 95% of the light at 253.7 nm. The energy at the plate surface was 5 $J \cdot m^{-2} \cdot s^{-1}$ as measured with Latarjet dosimeter (no. 81). An X-ray tube operated at 320 kV and 10 mA delivering a dose of 180.6 Gy/h was used as a source of ionizing irradiation. As a chemical mutagen *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG; CAS 70-25-7) purchased from Sigma, was used.

2.4. Survival

The cells were treated with UV-irradiation on the surface of solid minimal medium in Petri dishes at a cell density appropriate for each dose, and then kept in the dark for 24 h to prevent photoreactivation.

For X-ray survival curves, the cells were exposed to radiation from an MG 324 X-ray tube in the buffer and after irradiation, the cells were spread on the surface of solid minimal medium and put on the light shelf.

For MNNG survival curves, the cells were treated with different concentrations of MNNG for 30 min in the dark with continuous shaking. After termination of reaction with 5% sodium thiosulfate (CAS 7772-98-7) purchased from Sigma, the cells were washed in buffer and spread on the solid minimal medium at a cell density appropriate for each dose.

Survival of cells was evaluated by microscopy which enables one to determine if the cells that failed to form visible colonies had undergone any cell divisions [15].

2.5. Induction of streptomycin-resistant mutants

The method of Lee and Jones [19] was adopted for the isolation of streptomycin-resistant mutants induced by UV-irradiation, X-ray and MNNG. After treatments with mutagens aliquots containing about $3–5 \times 10^6$ cells were delivered to test tubes containing 5 ml of 45°C-melted HSA soft agar medium. The mixed contents of each tube were poured into Petri dishes with 20 ml HSA medium containing 100 $\mu g \cdot ml^{-1}$ streptomycin. In each variant of experiment 5 plates with $3–5 \times 10^6$ cells on each of them were evaluated. Frequency of mutations was expressed as the number of colonies formed in the presence of 100 $\mu g \cdot ml^{-1}$ streptomycin per 10^6 surviving cells.

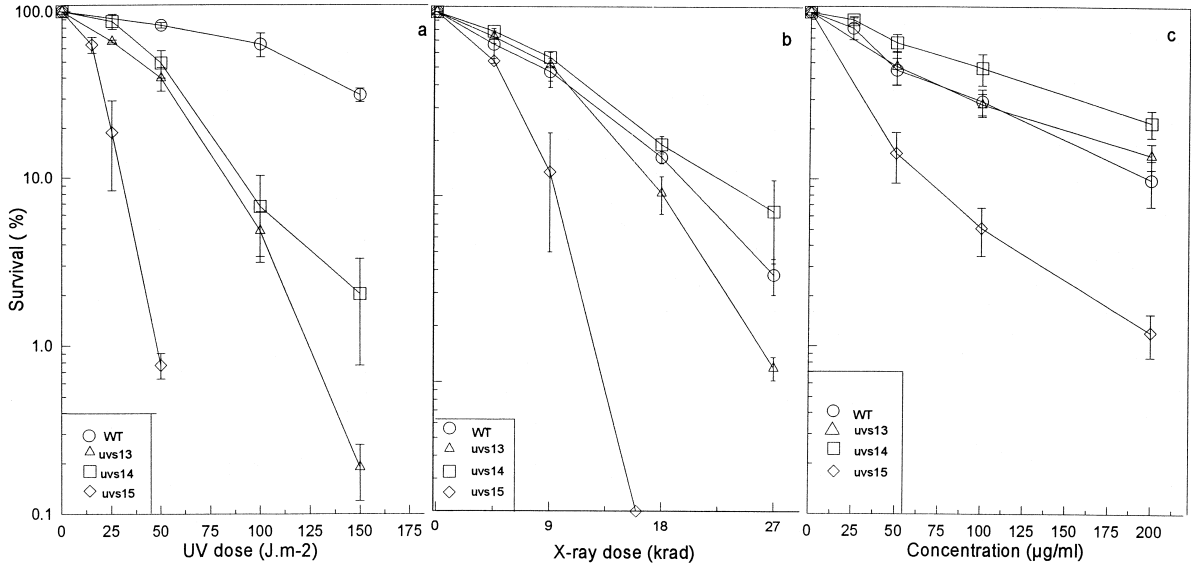

Fig. 1. Survival of wild-type (WT) and UV-sensitive mutants (*uvs13, uvs14, uvs15*) of *Chlamydomonas reinhardtii* following: UV-irradiation (a); X-ray (b); and MNNG treatment (c).

2.6. Excision of pyrimidine dimers

The removal of pyrimidine dimers from DNA after UV-irradiation with 50 $J \cdot m^{-2}$ was determined using a pyrimidine endonuclease assay according to Small and Greimann [4]. The method consists of labeling the cells with radioactive adenine, irradiation, isolation of the DNA and treatment with the UV-specific endonuclease. The number of breaks created by the enzyme was determined by sedimenta-

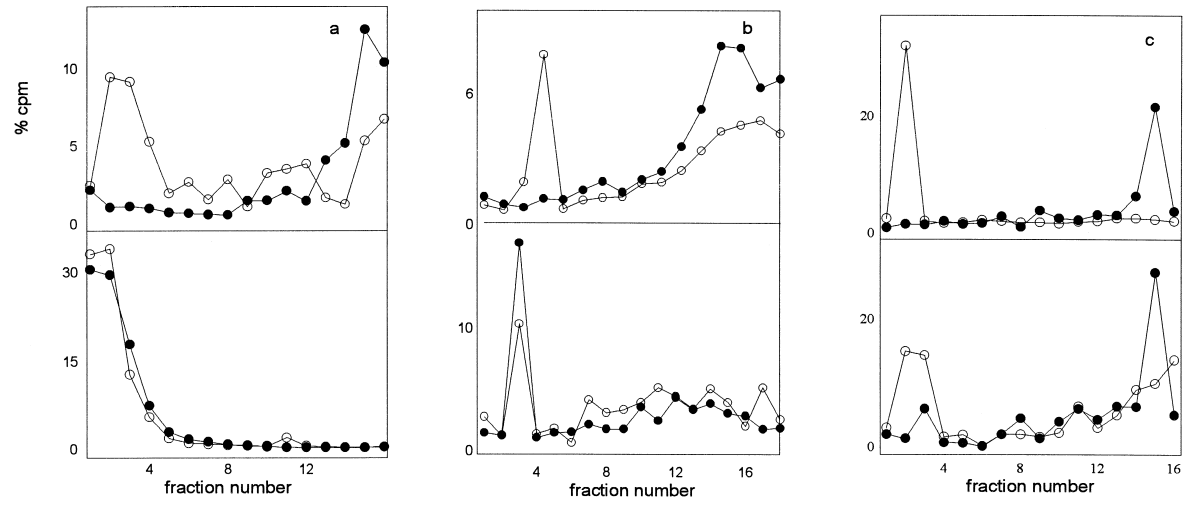

Fig. 2. Removal of pyrimidine dimers from DNA of the *Chlamydomonas reinhardtii* UV-sensitive mutants: *uvs13* (a); *uvs14* (b); and *uvs15* (c). Upper panel: sedimentation analysis of DNA extracted from cells immediately after exposure 50 $J \cdot m^{-2}$ of UV-light. Lower panel: sedimentation analysis of DNA extracted from irradiated cells after 24 h incubation in the dark. ○, no UV-specific endonuclease; ●, treated with UV-specific endonuclease.

tion through alkaline sucrose gradients and evaluated in scintillation liquid counter Rackbeta 1217, LKB Wallace.

2.7. Genetic analysis

For the genetic analysis, the cells were scraped from 7-day-old plates, transferred to HSA1 plates and allowed to grow for 2 days. They were then resuspended in HSD medium (approximately 10^6 cells per ml) and illuminated ($20\ W \cdot m^{-2}$) for 5–6 h. Mating mixtures were plated on HSZ plates and incubated in the light for 20 h followed by 6–7 days cultivation in the dark. Tetrad dissection was carried out as described by Harris [20].

2.8. Data

Each experiment was performed at least three times from independently grown algal cultures. All figures and tables represent the combined results of all experiments.

Table 1
Number of streptomycin-resistant mutants/10^6 survivors following UV, X-ray and MNNG treatment

Strains	UV ($J \cdot m^{-2}$)				
	0	30	50	100	
wt	0.99	1.37	2.69	10.50	
uvs13	0.65	0.75	0.82	2.96	
uvs14	9.92	20.76	26.04	49.61	
uvs15	0.01	0.00	0.00	–	
	X-ray (krad)				
	0	4.5	9	18	27
wt	0.65	2.30	2.40	1.25	0.00
uvs13	0.65	4.90	7.10	9.95	0.00
uvs14	4.90	4.60	3.90	2.50	0.00
uvs15	0.04	0.00	0.00	0.00	0.00
	MNNG ($\mu g \cdot ml^{-1}$)				
	0	30	50	100	
wt	2.02	9.01	13.71	31.66	
uvs13	2.84	10.73	25.35	31.30	
uvs14	9.90	28.16	50.23	66.17	
uvs15	0.05	0.19	3.54	1.10	

3. Results

The UV-survival curves of repair-deficient mutants *uvs13*, *uvs14* and *uvs15* are compared in Fig. 1a. The mutant *uvs15* is the most sensitive and its dose–response curve is very similar to those detected in excision repair-deficient strains [16]. The strains *uvs13* and *uvs14* are approximately equally sensitive to killing by UV-irradiation.

As is shown in Fig. 1b,c, *uvs15* is also very sensitive following exposure to X-rays and MNNG. In comparison to other *C. reinhardtii* repair-deficient strains tested, *uvs15* is one of the most sensitive strains to UV, X-ray and MNNG [16,21].

The rate of pyrimidine dimer removal from nuclear DNA can be assessed by measuring the number of sites sensitive to the UV-specific endonuclease from *Micrococcus luteus*. The ability to remove dimers in three mutants after a fluence of $50\ J \cdot m^{-2}$ is illustrated in Fig. 2a–c. Neither *uvs13* nor *uvs14* is blocked in excision repair. These mutants remove dimers at the same rate as the wild-type cells (data not shown). The third mutant *uvs15* is deficient in the removal of pyrimidine dimers from DNA.

Table 1 summarizes the frequency of streptomycin-resistant mutants following exposure to mutagenes used. The strains *uvs14* and *uvs15* differ distinctly from the wild-type strain. Contrary to the high level of spontaneous and induced mutations in *uvs14*, there were no streptomycin-resistant mutants scored in *uvs15* after UV-irradiation. As can be seen from the Table 1, the *uvs15* did not mutate after X-irradiation and there was a very low mutation yield also after MNNG treatment.

Fundamental information pertinent to understanding the functional relationships of the repair genes emerges from studies on the relative sensitivity of single and double mutants to killing by DNA-damaging agents [22]. When a double mutant does not show additive or synergistic effects with respect to its sensitivity, the corresponding single mutants are thought to be impaired in the same repair pathway, and considered to belong to the same epistasis group. For this purpose, opposite mating types of non-excision strains *uvs10*, *uvs13* and *uvs14* were mated with each other and their responses to UV-irradiation were compared. Sensitivity of UV-treated double mutant *uvs13 uvs14* was the same as that of single mutants indicating that both mutant strains belong to the same repair pathway (Fig. 3). On the other hand, the double mutant *uvs10uvs13* and *uvs10uvs14* revealed additive effects to killing by UV-irradiation,

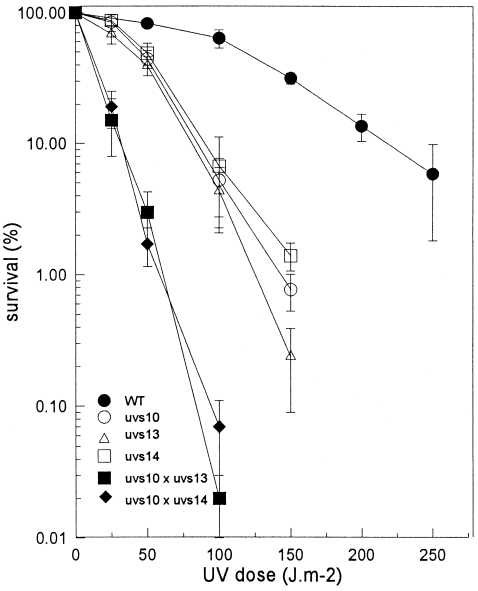

Fig. 3. Survival comparison of single (*uvs10, uvs13, uvs14*) and double mutants (*uvs10uvs13, uvs10uvs14*) of *Chlamydomonas reinhardtii* following UV-irradiation.

suggesting that they can be considered to belong to different repair pathways (Fig. 3).

All three mutants *uvs13*, *uvs14* and *uvs15* showed 2:2 Mendelian segregation in tetrad analysis with respect to their sensitivity to UV. Complementation analysis revealed that *uvs15* is not allelic with the excision-deficient *uvs1* isolated by Davies [1] and *uvs9* isolated by Small [7], and it proved to be non-allelic with *uvs10*, *uvs13* and *uvs14*.

The repair-deficient mutants *uvs13* and *uvs14* were crossed to mapping stocks with markers on linkage group I and with *uvs10*. Both *uvs13* and *uvs14* expressed linkage to all markers used (Table 2). The gene *uvs14* is separated from methionine sulfoximine resistance gene (*msr-1*) 14 map units (m.u.) and map distance between *uvs13* gene and *msr-1* is 8.6 m.u. Since map distance between *uvs14* and *arg-7* is 42 m.u. and *uvs13* is separated from *arg-7* by 31 m.u., *uvs14* gene must be located on the right side of *msr-1* gene and *uvs13* on the left side of *msr-1*.

Table 2
Tetrad analysis of the crosses among strains used

Cross $mt^+ \times mt^-$ (loci)	Tetrad type			Locus–locus map distance (m.u)
	PD	NPD	T	
uvs10–msr1	62	4	37	21.8
uvs13–arg7	65	8	77	31.0
uvs13–msr1	58	0	12	8.6
uvs13–uvs10	109	3	27	11.9
uvs13–uvs14	21	2	9	21.0
uvs14–msr1	49	0	19	4.0
uvs14–arg7	17	7	41	42.3
uvs14–uvs10	58	15	43	29.0

4. Discussion

So far, on the basis of biochemical mutational and genetic analysis, the nuclear Chlamydomonas repair-deficient mutants were tentatively classified into 3 repair-pathways: one involving excision repair; one involving recombination repair; and one or more controlling pathways which have not been precisely defined [7]. Additional mutants have been added to these epistatic groups on the basis of phenotypic similarities to mutant strains already tested [9,10,16]. Following these criteria, *uvs13* and *uvs14* might belong to a group distinct than excision repair pathway. The analysis of pyrimidine dimer removal supported this suggestion (Fig. 2a,b). Concerning *uvs15*, the damage in excision of pyrimidine dimers (Fig. 2c) is again consistent with assumption resulting from phenotypical analysis of this strain. However, in this case, the classification of a mutant strain into specific repair pathway is much more complicated as follows from mutation data (Table 1).

Mutational analysis is one of the fundamental characterizations of repair-deficient strains. While *uvs13* showed only a mild increase of the forward mutations frequency to streptomycin resistance after UV-treatment, the *uvs14* exerted a significantly higher mutability by this agent. The result is interesting due to the fact that uvs14 belongs to the non-excision group of mutants which have usually reduced or unchanged frequency of mutations in heterotrophic microorganisms, such as yeast [22,23]. *uvs14* is the second repair-deficient mutant of *C. reinhardtii* with proficient excision repair in an increase of forward mutations to streptomycin resistance after UV-irradiation has been observed. Previously, we described the high rate of this mutation in *uvs11* [15]. This finding was coupled with the observation that *uvs11* frequently divides at least once before

dying, suggesting that the *uvs11* mutation has resulted in the ability of the replication machinery to proceed past damage in an error-prone fashion much more frequently than the wild-type strain. We can suppose that *uvs14* might also participate in this process and that its repair-deficiency might be connected with damage in mismatch repair. Mismatch repair-deficient cells in other organisms express not only the increased level of mutation, but also resistance to killing by MNNG and perhaps other alkylating agents as well [24]. Higher levels of streptomycin-resistant mutants and resistance to MNNG in the case of *uvs14* could indicate involvement of this mutant's gene in mismatch correction.

Till now, no repair-deficient strain analagous to recA in *Escherichia coli* or to *rad6* in *S. cerevisiae*, has been described in algae. Our findings that *uvs15* does not mutate following exposure to UV-irradiation and X-ray, and exhibits a very low rate of mutations after MNNG indicates the important role of *uvs15* gene in mutagenesis. In comparison with similar mutants in heterotrophic microorganisms, there is striking and interesting difference. This strain belongs to the most sensitive strains following exposure to all agents tested so far and has impaired excision repair. Although several repair-deficient mutants in heterotrophic microorganisms (e.g. *rad6*, *pso1/rev3* in *S. cerevisiae* etc.) exhibit sensitivity to a wide variety of mutagens and have significantly reduced frequencies of spontaneous and induced mutations [25–28], none of them is excision deficient. Nucleotide excision repair is constitutively expressed in *E. coli* and therefore occurs in a recA strain. It is therefore unlikely that *uvs15* encodes a gene controlling the induction of repair pathways. *uvs15* is also more sensitive to both physical and chemical mutagens than these other mutants. This phenotypic characteristic may indicate that the *uvs15* gene product is multifunctional or that there is functional overlap between repair pathways in the Chlamydomonas nucleus. Following these findings, it may be possible to consider that *UVS15* interacts with other repair proteins in the assembly of the repair machinery [29,30] of various repair complexes such as an NER-specific repairosome [31,32] and proposed recombinosome complex [33] described in yeast.

Since the first UV-sensitive mutants of *C. reinhardtii* were reported by Davies [1], only one repair gene determining repair ability in this alga has been mapped [15]. The genes *UVS13* and *UVS14* are linked to the already mapped *UVS10* on the right arm of linkage group I. They form a cluster of non-excision repair genes located on the same chromosome arm. Recombination analysis of excision repair-deficient strains indicate linkage of some genes participating in this mode of repair as well. Localization of them is under analysis at present.

The results of our *C. reinhardtii* DNA repair study obtained so far have indicated that some steps in repair processes are distinct from those in heterotrophs [34–36]. On the basis of results obtained recently in the Chlamydomonas chloroplast genome establishing the role of chloroplast RecA homolog in plastid DNA recombination and survival after exposure to DNA-damaging agents [37] and association of nuclear and chloroplast DNA molecules in synchronized cells of *C. reinhardtii* [38], one may suppose that one reason for these differences may be based on nuclear and chloroplast DNA interactions in the repair of damage following exposure to chemical and physical agents. There is an interesting task to be performed to investigate the role and the involvement of the *UVS15* gene product in these processes.

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