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REVIEW

Chlamydomonas reinhardtii: a convenient model system for the study of DNA repair in photoautotrophic eukaryotes

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Abstract The green alga *Chlamydomonas reinhardtii* is a convenient model organism for the study of basic biological processes, including DNA repair investigations. This review is focused on the studies of DNA repair pathways in *C. reinhardtii.* Emphasis is given to the connection of DNA repair with other cellular functions, namely the regulation of the cell cycle. Comparison with the results of repair investigations that are already available revealed the presence of all basic repair pathways in *C. reinhardtii* as well as special features characteristic of this alga. Among others, the involvement of *UVSE1* gene in recombinational repair and uniparental inheritance of chloroplast genome, the specific role of *TRXH1* gene in strand break repair, the requirement of *PHR1* gene for full activity of *PHR2* gene, or encoding of two excision repair proteins by the single *REX1* gene. Contrary to yeast, mammals and higher plants, *C. reinhardtii* does not appear to contain the ortholog of *RAD6* gene, which plays an important role in DNA translesion synthesis and mutagenesis. Completed genome sequences will be a basis for molecular analyses allowing to explain the differences that have been observed in DNA repair of this alga in comparison with other model organisms.

Keywords DNA repair · *Chlamydomonas reinhardtii* · Repair pathways · DNA repair-cell-cycle regulation

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Introduction

Cellular genomes are relatively stable but can be changed in a number of ways, for example, spontaneously, via errors in the replication process or the action of by-products of cellular metabolism and particular chemicals or radiation. The damage can modify the DNA both chemically and structurally leading to mutagenic and toxic effects or to cell cycle disorders. Cells usually respond to the genome damage by activating a DNA damage response pathway to induce/activate cell cycle arrest/checkpoint, DNA repair pathways and/or apoptosis (Carr 2002). To prevent the deleterious consequences of DNA injury and to maintain genome integrity, several strategies have evolved to either reverse, excise or tolerate the persistence of DNA adducts. It has been suggested that much of the DNA repair machinery has evolved to cope with the endogenously generated DNA damage caused by reactive oxygen species, endogenous alkylating agents and DNA single- and double-strand breaks resulting from the oxidative destruction of deoxyribose residues or collapsed DNA replication forks (Lindahl and Wood 1999; Lindahl 2000; Hsu et al. 2004). Thus, the DNA repair/tolerance systems play the most important role in maintaining damage at a level that is tolerable in terms of retaining DNA stability.

The field of DNA repair in green unicellular alga, *Chlamydomonas reinhardtii*, (Davies 1965, 1967) was established almost simultaneously with that of yeast *Saccharomyces cerevisiae*. However, the study of DNA repair in algae as well as higher plants has been in less progress in comparison to yeast and some other eukaryotic organisms. Yet *C. reinhardtii* has several advantages as a model system for the study of DNA damage repair. Above all, it is amenable to genetic and molecular biological experiments (Harris 1989; Tam and Lefebvre 1993;

Asamizu et al. 1999) and to procedures for transformation of nuclear and chloroplast genomes (Kindle 1990; Boynton and Gillham 1993; Shimogawara et al. 1998). The power of molecular genetic approaches using *C. reinhardtii* is increased by developing a molecular map aligned with a genetic map (Kathir et al. 2003) and sequencing of its genome (Grossman et al. 2003; Shrager et al. 2003; Jain et al. 2007) as well as by functional genomics analyses using RNA interference (RNAi) triggered by the genome integrated transgenes (Rohr et al. 2004; Schroda 2006).

A major advantage of using *C. reinhardtii*, as a model for plant cell biology, to investigate DNA repair, is the ease of induction and isolation of repair-deficient mutants in various repair pathways. This is more difficult to perform in higher plants due to demanding and time consuming procedures in particular. In addition, as compared with the most often used and best understood eukaryotic model organism concerning DNA repair, yeast *S. cerevisiae*, there is an additional organelle/chloroplast genome in addition to the mitochondrial one (Rochaix 1995; Harris 2001). It provides the possibility for investigation of the DNA repair in all three autonomous genetic systems located in the nucleus, chloroplast and mitochondria and searching for interactions between nuclear and both organellar genomes. The DNA of the single large chloroplast has the GC content of 40% compared to 65% for nuclear DNA enabling the two DNA species to be easily separated by CsCl density gradients (Sager and Ishida 1963), and together with the ability to selectively label chloroplast DNA allows the investigation of repair systems separately in both nuclear and chloroplast compartments (Small and Greimann 1977a, b). Last but not least, the results of DNA repair investigations obtained in *C. reinhardtii* till now revealed also some differences compared with yeast, higher plants and animals that might bring new knowledge on DNA repair systems after more detailed investigation as it will be discussed in chapters "Photoreactivation", "Nucleotide excision repair" and "DNA damage tolerance and mutagenesis".

In this review, we summarize current knowledge on *C. reinhardtii* DNA repair systems, particularly on UV-induced damage and repair. Furthermore, we list the putative DNA repair genes that resulted primarily from the sequencing of *C. reinhardtii* genome (http://genome.jgi-psf.org/chlre3/chlre3.home.html). These sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov).

DNA repair pathways

Eukaryotic cells have multiple mechanisms for the repair of damaged DNA molecules. They are usually included into three basic groups in accordance with the mechanism that is used by a cell to cope with damage in its DNA—the direct reversal of damage, excision repair and replication-coupled repair/tolerance mechanisms (Friedberg et al. 2006; Ishino et al. 2006).

Direct reversal of DNA damage

The cell uses this strategy to repair the DNA damages induced with factors permanently present in the environment or endogenously generated (e.g., UV-irradiation, alkylation agents). The damage is repaired very effectively in one step in situ, thereby regenerating the normal base. Of course, only such damage can be repaired in this way when it chemically and structurally fulfils the requirements for this kind of repair.

Photoreactivation

Photoreactivation is the major repair pathway for pyrimidine dimers [cyclobutane pyrimidine dimers and (6–4) photoproducts], which are the major sources of base damage following the exposure of living cells to UV radiation (Brash 1988; Todo 1999; Weber 2005). It is a light-dependent DNA repair mediated by a substrate-specific photolyase, which reverses the effect of ultraviolet light when the organisms are exposed concomitantly or subsequently to blue light (Sancar 1992, 1994; Zhao and Mu 1998). The direct damage repair carried out by photolyase consists of breaking the chemical bond(s) constituting the damage to restore normal nucleotides. This protein belongs to DNA photolyase/blue light photoreceptor family that consists of three groups—cyclobutane pyrimidine dimers photolyase (CPD photolyase), (6–4) photolyase and cryptochrome (CRY)/blue-light photoreceptors.

CPD photolyases are classified into two classes (class I and class II CPD photolyases) by their primary structure (Yasuhira and Yasui 1992; Yasui et al. 1994). Class I photolyases are closely related to cryptochromes and the 6–4 photoproduct-specific photolyase family (Sancar 1994; Todo et al. 1993, 1997; Nakajima et al. 1998). For the structure of photolyases and the course of photorepair see Sancar (1994), Weber (2005) and Kao et al. (2005).

The photoreactivation of UV-induced DNA damage in *C. reinhardtii* is currently the best-characterized DNA repair pathway in this alga (Cox and Small 1985; Petersen and Small 2001; Petersen et al. 1999). *C. reinhardtii* cells have been shown to have photolyase activity in both the nucleus and the chloroplast (Small and Greimann 1977a, b). So far, two photoreactivation-deficient mutants, *phr1-1* and *phr1-2*, were isolated in *C. reinhardtii* (Cox and Small 1985) (Table 1). Genetic analysis shows these mutants to be allelic (Munce et al. 1993).

Table 1 DNA repair-deficient mutants and repair pathways in *Chlamydomonas reinhardtii*

Repair pathways	DNA repair-deficient mutants	Allelic strains	References
Photoreactivation	*phr1-1*	*phr1-1* = *phr1-2*	Cox and Small (1985)
	phr1-2		Munce et al. (1993)
Nucleotide excision repair			
Completely blocked excision	*uvs1*, *uvs9*, *uvs12*, *uvs15*, *rex1*	*uvs1* = *uvsN371*	Davies (1967)
	uvsN132, *uvsN351*, *uvsN371*	*uvs9* = *uvsN351*	Small (1987)
		uvs12 = *uvsN132*	Vlček et al. (1997)
Partialy blocked excision	*uvs2*, *uvs3*, *uvs4*, *uvs6*, *uvs7*		Cenkci et al. (2003)
Mismatch repair (probably)	*uvs14*, *uvs13*		Vlček et al. (1997)
Recombinational repair			
First epistatic group	*uvsE1*, *uvs10*		Rosen and Ebersold (1972)
Second epistatic group	*uvsE5*		Portney and Rosen (1980)
Third epistatic group	*uvsE6*		Vlček et al. (1987)
	ble-1 (without genetic analysis)		Sarkar et al. (2005)
Error-prone repair	*uvs 15*		Vlček et al. (1997)
DNA repair/checkpoint	*uvs1* (isolated from A19 wild type)		Vlček et al. (1981)
	uvs11		Vlček et al. (1987)
	uvsX1, *uvsX2*		Slaninová et al. (2003)
Not identified	*uvsE4*, *uvs5*, *uvs8*		Davies (1967) Rosen et al. (1980) Small (1987)

Several attempts to isolate *phr1* gene by using an oligonucleotide probe consisting of a conserved sequence of the known *PHR* genes were unsuccessful (Small et al. 1995), but using an oligonucleotide probe representing a conserved amino acid sequence in the class II DNA photolyase, a gene named *PHR2* was cloned and characterized by Petersen et al. (1999). This represented the first example of a class II DNA photolyase isolated from a unicellular eukaryote. The results of further experiments suggested that *PHR2* is the structural gene for the photolyase targeted to both the nucleus and the chloroplast, and that the *PHR1* gene product is necessary for the full activity of Phr2 protein (Petersen and Small 2001). The requirement for the second protein component for full activity of DNA photolyase has not been found in any other organisms. The Phr2 protein was the first example of the class II DNA photolyase being targeted to two organelles, and the only photolyase that has been localized to both the nucleus and the chloroplast. The *PHR2* gene was isolated in *C. reinhardtii* as the second member of the DNA photolyase/blue light photoreceptor family. The first member, isolated in this organism, *Chlamydomonas* photolyase homologue (*CPH1*), belongs to the class I family (Small et al. 1995). It encodes two proteins that function as cryptochromes in *C. reinhardtii*. These proteins are the first cryptochromes, the degradation of which is known to be induced also under red light. Although the exact function of Cph1 proteins is unknown it is hypothesized that they might function as transcriptional regulators (Reisdorph and Small 2004).

The *C. reinhardtii* genome project has revealed DNA sequences homologous to the repair genes of other eukaryotes including five predicted DNA photolyase genes (Table 2) two of which have been experimentally verified (Small et al. 1995; Petersen et al. 1999). Most of them are the members of the class II photolyases.

Reversal of alkylation damage in DNA

The most frequently induced mutagenic lesions in DNA following alkylating agents are O^6-alkylguanine and O^4-alkylthymine. For this reason, most organisms produce O^6-alkylguanine-DNA alkyltransferases which transfer the methyl group in a single step to the specific cysteine residue of the protein resulting in its inactivation (Mishina et al. 2006; Sedgwick et al. 2007). The protein has been identified, its function studied and its gene cloned ranging from bacteria (Sedgwick 1983; Demple et al. 1985) through yeast (Xiao et al. 1991; Brozmanová et al. 1994; Slaninová et al. 1995) to mammals (Rydberg et al. 1990). *C. reinhardtii* like higher plants (Angelis et al. 1992; Hays 2002) seemed to lack the alkyltransferase protein activity (Frost and Small 1987), but unlike higher plants a homolog of DNA-methyltransferase gene has been found in *Chlamydomonas* genome (Table 2).

Table 2 Homologs of known DNA repair genes in *Chlamydomonas reinhardtii*

Repair pathway	DNA repair genes involved in various repair pathways (bacteria,[a] yeast,[b] human,[c] others[d])	Protein and/or function	Homologs of DNA repair genes in *C. reinhardtii*		
			Presumed enzymatic function	Assigned gene name	Protein ID
Photorepair	*phr*$^+$, *Sc PHR1*	CPD-DNA photolyase class I, removal of CPD	DNA photolyase, FAD binding		110957
	AtPHR1/UVR2	CPD-DNA photolyase class II, removal of CPD	CPD photolyase class II	PHR1	32865
			CPD photolyase class II	PHR2	154289
			DNA photolyase, FAD binding	Unassigned	172460
			DNA photolyase, FAD binding	Unassigned	123069
	AtCRY	Cryptochrome/blue light receptor	Blue-light photoreceptor/cryptochrome	CPH1	60001
			Similar to the photolyase/blue-light receptor	CPH-like-3	147164
			Cryptochrome DASH	Unassigned	111597
			Putative cryptochrome DASH	Unassigned	171766
			Cryptochrome like protein	Unassigned	131659
Reversal of alkylation base damage	*ada*$^+$, *ScMGMT1*, *hMGMT*	O^6-alkylguanine-DNA alkyltransferase, removal of alkyl groups from DNA	Methylated-DNA-[protein]-cysteine S-methyltransferase	Unassigned	150247
Base excision repair	*ung*$^+$, *ScUNG1*, *hUNG*	Uracyl-DNA glycosylase, removal of uracil from DNA	Uracyl DNA-glycosylase	UNG1	108734
	alkA$^+$, *ScMAG1*, *hMPG*	3-mA-DNA glycosylase II, removal of 3-methyladenine, 3-methylguanine, 7-methylguanine, and 7-methyladenine	DNA-3-methyladenine glycosylase II	Unassigned	105620
	mutY$^+$, *hMYH*	MutY DNA glycosylase, removal adenine opposite 8-oxoG	A/G specific adenine DNA glycosylase	Unassigned	141355
	fpg$^+$, *ScOGG1*, *hOGG1*	FaPy-DNA glycosylase, removal of formamidopyrimidine moieties, or 8-oxoG	8-oxoguanine DNA glycosylase	Unassigned	107185
			Formamidopyrimidine-DNA glycosylase	Unassigned	103375
			Formamidopyrimidine-DNA glycosylase	Unassigned	165863
	nth$^+$ *ScNTG1,2 hNTH1*	Endonuclease III, removal ring saturated or fragmented pyrimidines	Endonuclease III/Nth	Unassigned	153844
			Endonuclease III/Nth	Unassigned	43318
	xthA$^+$, *Sc APN2*, *hAPEX1,2*	Exonuclease III, 5′ AP endonuclease, 3′ phosphatase, or 3′ exonuclease	Exonuclease III/Xth	Unassigned	181643
			AP endonuclease	Unassigned	172466
			AP endonuclease	Unassigned	143864
	hPARP1	Poly (ADP-ribose) polymerase	ADP-ribosyltransferase	Unassigned	189972
Nucleotide excision repair	*uvrB*$^+$	Catalyzes unwinding in preincision complex	UvrB/UvrC protein	Unassigned	140837
			UvrB/UvrC protein	Unassigned	141656
	uvrC$^+$	3′ and 5′ incision nuclease	Excinuclease ABC, C subunit	Unassigned	171346
	polA$^+$	Repair sythesis of DNA in bacteria	*E.coli* PolI-like DNA polymerase	POL1A	175585
			DNA polI-like	POL1B	142360

Table 2 continued

Repair pathway	DNA repair genes involved in various repair pathways (bacteria,[a] yeast,[b] human,[c] others[d])	Protein and/or function	Homologs of DNA repair genes in *C. reinhardtii*		
			Presumed enzymatic function	Assigned gene name	Protein ID
	ScRAD4, *hXPC*	Binds distorted DNA	RAD4 component of NEF2	Unassigned	96394
	ScRAD23, *hRAD23B*	Binds distorted DNA	RAD23 component of NEF2	Unassigned	80907
	ScRAD14, *hXPA*	Binds DNA and proteins in preincision complex	XPA	Unassigned	186489
	ScSSL2, *hXPB*	3′–5′ DNA helicase	XPB	Unassigned	188959
		TFIIH subunit	XPB	Unassigned	181520
	ScRAD3, *hXPD*	5′–3′ DNA helicase TFIIH subunit	XPD, helicase c2	Unassigned	100548
	ScTFB1, *hGTF2H1*	TFIIH subunit p62	Similar to TFIIH subunit p62	Unassigned	187817
	ScSSL1, *hGTF2H2*	TFIIH subunit p44	Transcription factor TFIIH, 44 kD subunit	TF2H2	127751
	ScTFB4, *hGTF2H4*	TFIIH subunit p34	TFIIH, 34 kD subunit	TFB4	169016
	ScTFB2, *hGTF2H4*	TFIIH subunit p52	Transcription factor Tfb2	Unassigned	138157
	ScTFB5, *hGTF2H5*	TFIIH subunit p8	Transcription factor Tfb5	REX1S	195671
	ScKIN28, *hCDK7*	TFIIH, kinase subunit	CDK activating kinase	CDKD1	137457
	ScCCL1, *hCCNH*	TFIIH, kinase subunit	Cyclin H-1	Unassigned	172948
	ScRAD2, *hXPG*	3′ incision nuclease	DNA-repair protein XPG	Unassigned	152382
			XPG	Unassigned	96792
	ScRAD10, *hERCC1*	5′ incision nuclease subunits	DNA repair protein Rad10	Unassigned	102433
	ScRAD1, *hXPF AtUVH1*		DNA repair endonuclease Rad1	Unassigned	169954
	ScRAD28, *hCSA*	Cockayne syndrome A, needed for TC-NER	Similar to DNA repair protein CSA	Unassigned	136439
	ScRAD26, *hCSB*	Cockayne syndrome B, needed for TC-NER	Putative CSB/Rad26	Unassigned	112504
			Related to DNA repair protein Rad26	Unassigned	173984
	CrREX1	Nucleotide excision repair	REX1-DNA repair	Unassigned	24388
	CrREX1B	Probably involved in DNA repair	REX1B protein	REX1B	195705
	hDDB1	Damage-specific DNA binding complex	Damaged DNA binding complex subunit 1 protein	DDB1	133572
	hDDB2		Damaged DNA binding complex subunit 2 protein	DDB2	168655
	ScRAD16	E3 ubiquitin ligase and damage binding	Nucleotide excision repair protein Rad16	Unassigned	115102
			NER protein Rad16	Unassigned	120362
	ScCDC9, *hLIG1*	DNA joining	DNA ligase 1 (ATP)	LIG1	133143
			DNA ligase (ATP)	Unassigned	176829
	ScRFA1, *hRPA1*	Binds ssDNA intermediates in recombination, NER and gap-filling pathways	Replication protein A 70kD	RPA70A	123566
			Replication protein A 70kD	RPA70B	193171
	ScRFA2, *hRPA2*		Replication protein A 39kD	RPA30	186935
	ligA$^+$, *ScCDC9*, *hLIG1*	DNA joining	DNA ligase	LIG1	133143

Table 2 continued

Repair pathway	DNA repair genes involved in various repair pathways (bacteria,[a] yeast,[b] human,[c] others[d])	Protein and/or function	Homologs of DNA repair genes in *C. reinhardtii*		
			Presumed enzymatic function	Assigned gene name	Protein ID
Mismatch repair	*mutS*$^+$ family, *ScMSH1*	Required for the repair of yeast mitochondrial DNA	DNA mismatch repair MutS protein	MSH1	142479
			Mismatch repair protein MutS	Unassigned	171477
	ScMSH2, *hMSH2*	Mismatch and loop recognition together with Msh3 and Msh6	DNA mismatch repair protein Msh2	Unassigned	106717
			DNA mismatch repair protein Msh2	Unassigned	175628
			DNA mismatch repair protein Msh2	Unassigned	181107
			Mismatch repair protein Smr/MutS2	Unassigned	186391
	ScMSH3, *hMSH3*		DNA mismatch repair protein Msh3	Unassigned	174349
	ScMSH5, *hMSH5*	Involved in meiotic recombination	DNA mismatch repair protein Msh5	Unassigned	121781
	ScMSH6, *hMSH6*		DNA mismatch repair protein Msh6	Unassigned	120228
	mutL$^+$ family, *ScMLH1*	Involved in mismatch repaire	DNA mismatch repair protein Mlh1	Unassigned	116380
	ScMLH2, *hPMS2*		Mismatch repair protein Mlh2/Pms1/ Pms2 family	Unassigned	101121
	ScMLH3, *hMLH3*		DNA mismatch repair protein Mlh3	Unassigned	106832
Modulation of nucleotide pools	*MutT*$^+$, *hMTH1*	8-oxoGTPase	MutT protein	Unassigned	107572
Homologous recombination	*ruvA*$^+$	ATP-dependent branch migration	RuvA, bacterial DNA recombination protein	Unassigned	110568
	ruvC$^+$	Endonuclease, resolves Holliday junctions	RuvC, crossover junction endodeoxyribonuclease	Unassigned	889
	recA$^+$	Involved in DNA recombination and repair	Bacterial recombination protein	RECA	145381
			Chloroplast RecA recombination protein	RECA	182459
			RecA bacterial DNA recombination protein	RECA-like	170565
	ScRAD51, *hRAD51*	Formation of protein filament to mediate homologous pairing	DNA recombination protein, RecA homologue	RAD51	190735
	hRAD51B	Rad51 paralog	Putative Rad51B protein	Unassigned	56268
	hRAD51C	Rad51 paralog	Rad51C protein	Unassigned	155567
	hRAD51D	Rad51 paralog	Putative Rad51D protein	RECA-like	11294
	ScDMC1 hDMC1	RAD51 homologue in meiosis	Rad51 homologue involved in meiotic recombination	DMC1	196696
			Meiotic recombination protein Dmc1	Unassigned	669
	ScRAD50, *hRAD50*	ATP-dependent DNA-binding activity	DNA repair protein	RAD50	13480
			DNA repair protein Rad50	Unassigned	172615
	ScRAD54, *hRAD54*	dsDNA unwinding activity	SNF2/RAD54 family protein	Unassigned	7539
	ScMRE11, *hMRE11*	3′–5′ dsDNA exonuclease and ssDNA endonuclease activity	DNA damage signaling and repair protein	MRE11	180252
			Homologous to MRE11	MRE11	123286

Table 2 continued

Repair pathway	DNA repair genes involved in various repair pathways (bacteria,[a] yeast,[b] human,[c] others[d])	Protein and/or function	Homologs of DNA repair genes in *C. reinhardtii*		
			Presumed enzymatic function	Assigned gene name	Protein ID
Nonhomologous end-joining (NHEJ)	*ScYKU70*, *hKU70*	DNA end binding	Ku70 autoantigen homologue	Ku70	205970
			DNA-binding subunit of DNA-dependent protein kinase (Ku70 autoantigen)	Unassigned	152226
	ScYKU80, *hKU80*	DNA end binding	Ku80 autoantigen homologue	Ku80	205969
			DNA-binding subunit of DNA-dependent protein kinase (Ku80 autoantigen)	Unassigned	186327
	DNA-PKcs	DNA-dependent protein kinase catalytic subunit	DNA-dependent protein kinase	Unassigned	15365
			Putative DNA-dependent protein kinase catalytic subunit DNA-PKcs	PRKDC	174337
	ScLIG4, *hLIG4*	Ligase	DNA ligase IV	Unassigned	165757
DNA polymerases (catalytic subunits) and accessory factors	*ScCDC2*, *hPOLD1*	Pol δ, NER and MMR	DNA polymerase delta subunit one	POLD1	189721
			Pol delta small subunit	POLD2	103391
	ScPOL2, *hPOLE1*	Pol ε, NER and MMR	DNA polymerase epsilon catalytic subunit A/1	POLE1	9481
	umuC$^+$	Translesion DNA synthesis in bacteria	UMUC-like DNA repair protein	Unassigned	179460
	hPOLI	Polι, lesion bypass	DNA polymerase iota	Unassigned	104547
			DNA polymerase iota	Unassigned	121584
			DNA polymerase iota	Unassigned	95975
	ScREV3, *hREV3L (PSO1)*	Pol ζ, lesion bypass	DNA polymerase zeta	POLZ1	117843
			Putative DNA polymerase zeta, catalytic subunit	Unassigned	117850
	ScREV1, *hREV1*	dCMP transferase	Deoxycytidyl transferase	REV1	173359
	hPOLQ	Pol θ, lesion bypass	DNA polymerase theta	POLQ1	179410
	ScRAD30, *hPOLH*	Pol η, lesion bypass	DNA polymerase eta	POLI1	178197
			DNA polymerase eta	POLI2	176206
	dinB$^+$, *hPOLK*	Pol κ, lesion bypass	DNA polymerase kappa	POLK1	111898
	AtPOL γ	Substitute for DNA pol β in BER	Pol lambda-related class X DNA repair polymerase	POLL1	19822
	dnaN$^+$, *ScPOL30*, *hPCNA*	Sliding clamp	PCNA proliferating cell nuclear antigen	PCN1	140580
	dnaX$^+$, *CDC44*, *hRFC1*	Clamp loader, large subunit	DNA replication factor C complex subunit 1	RFC1	150793
				RFC2	185418
				RFC3	173896
				RFC4	195837
				RFC5	519

Table 2 continued

Repair pathway	DNA repair genes involved in various repair pathways (bacteria,[a] yeast,[b] human,[c] others[d])	Protein and/or function	Homologs of DNA repair genes in *C. reinhardtii*		
			Presumed enzymatic function	Assigned gene name	Protein ID
Processing nucleases	*ScRAD27*, *hFEN1*	5′ nuclease	Nuclease, Rad2 (class 2) family	FEN1	130834
	ScMUS81, *hMUS81*	Structure-specific nuclease subunit	Crossover junction endoribonuclease	Unassigned	173442
Other genes related to DNA repair	*ScPSO2*, *hPSO2*	DNA cross-link repair nuclease	Artemis-related DNA-crosslink repair exonuclease	PSO2	146944
	AtSNM1	DNA cross-link repair protein	DNA repair metallo-beta-lactamase	Unassigned	154372
DNA repair and checkpoint pathways	*ScDDC1*, *hRAD9*	PCNA-like damage sensor	DNA damage checkpoint protein	RAD9	170734
	ScMEC3, *hHUS1*	PCNA-like damage sensor	DNA damage checkpoint protein	HUS1	192621
	ScRAD24, *hRAD17*	RFC1-like damage sensor	DNA damage checkpoint protein	RAD17	13563
		DNA replication factor C complex	DNA damage repair and chromosome cohesion protein RFC1-like	CTF18	11559
	ScTEL1, *hATM*	Ataxia-telangiectasia	ATML1/TEL1 DNA damage sensing related kinase	ATM1	8959
	ScMEC1, *hATR*	Ataxia-telangiectasia related protein	DNA damage sensing PI3 kinase	ATR1	8906
			DNA damage sensing protein kinase related to ataxia telangiectasia	ATR	187830
			Plant specific cyclin dependent kinase CDKC subfamily PITAIRE motif	CDKC1	111873
			Cyclin dependent kinase *Chlamydomonas* specific PVTSIRE motif	Unassigned	103369
			Cyclin dependent kinase *Chlamydomonas* specific AASTLRE motif	Unassigned	153970

The data presented in the Table 2 are based on homologs of C. reinhardtii genes annotated in the JGI database v3. The column "Presumed enzymatic function" represents the DefLine/Description from an annotated gene

[a] DNA repair genes of *E. coli* (lower-capital letters)

[b] DNA repair genes of *S. cerevisiae* (Sc)

[c] Human DNA repair genes (h)

[d] DNA repair genes of *A. thaliana* (At); DNA repair genes of *C. reinhardtii* (Cr) special for this alga (see text)

Excision repair pathways

The excision repair systems correct different kinds of single base damages (monoaducts), pyrimidine dimers (biaducts) and other serious damage-induced distortions of the DNA helix. The systems work both before DNA replication [base excision repair (BER), nucleotide excision repair (NER)] and after DNA replication (mismatch correction). Contrary to direct reversal of damage, in this case the DNA damage is repaired in a succession of several steps catalysed by means of many proteins and protein complexes.

Base excision repair

Cells use this repair to remove, usually, non-bulky DNA lesions (e.g., uracil, thymine glycol, 8-oxoguanine, N3-methyladenine, etc.) from DNA (Berti and McCann 2006). The damaged base is removed by BER glycosylases which cleave the glycosylic bound between the specific damaged base and the deoxyribose, with subsequent incision by AP-endonuclease (apurinic/apyrimidinic endonuclease) at the resulting abasic site. The remaining sugar fragment is excised by exonucleases as well as enzymes with specific DNA-deoxyribophosphodiesterase activity and an undamaged nucleotide is installed by a DNA polymerase (Almeida and Sobol 2006). The steps involved in the recognition of damaged bases are still unclear, however, several studies suggest that a multi-step base-recognition process is a common strategy used by BER glycosylases to guarantee the accurate base excision (Jiang et al. 2004; Banerjee et al. 2005; David 2005).

Some of the *C. reinhardtii* UV-sensitive mutants were found to be also sensitive to the lethal effects of alkylating agents (Sweet et al. 1981; Miadoková et al. 1994). This stimulated the effort to examine the removal of chemically induced methylated bases through the BER in this organism. It was found that the 3-methyladenine and 7-methylguanine were removed from DNA in vivo and cycloheximide inhibited this process suggesting that protein synthesis is required for the repair of these damages from *C. reinhardtii* DNA (Sweet et al. 1981).

Several attempts to detect 3-methyladenine DNA glycosylase in extracts of *C. reinhardtii* have been unsuccessful, but the major AP endonuclease specific for apurinic/apyrimidinic sites has been partially purified and characterized (Frost and Small 1984). Some enzymatic activity with possible role in DNA repair was also described by Tait and Harris (1977a, b). They identified exonuclease activity operative on single-stranded, linear DNA, able to work from both the 3′ and 5′ ends and with elevated concentrations in cells of some *uvs* mutants and increased concentrations at S phase in wild-type cells. Therefore it is uncertain whether its primary function is in replication or in repair and recombination. Altogether, only little knowledge on BER is currently available in *C. reinhardtii* and mechanisms for removing damaged bases remain to be determined.

Sequencing of *C. reinhardtii* genome has revealed the collection of homologs of glycosylase genes participating in the DNA repair (Table 2). However, it must be emphasized that the data described below resulted only from *in silico* studies. Like *E. coli*, fungi, higher plants and animals, *C. reinhardtii* may remove uracil incorporated opposite adenine during replication. It is indicated by the presence of uracil DNA glycosylase ortholog in *C. reinhardtii* genome. As for recognition and excision of damaged bases, there are the homologs of 3-methyladenine DNA glycosylase and glycosylase genes specific for oxidative damages in DNA (DNA formamidopyrimidine glycosylase and endonuclease III). Of the principal components in BER only DNA polymerase beta (Pol β) is missing, similarly as it is in the *Arabidopsis thaliana* genome. The role of Pol β can substitute Pol λ, which may be the only member of the X-family in higher plants, as was observed by Uchiyama et al. (2004). The mammalian X-family DNA polymerases (DNA polymerase beta, lambda, mu and TdT) contribute to BER and double-strand break repair by virtue of their ability to fill short gaps in DNA (Nick McElhinny et al. 2005; Moon et al. 2007). Uchiyama et al. (2004) revealed that Pol λ had activities of DNA polymerase, terminal deoxyribonucleotidyl transferase and deoxyribose phosphate lyase, a marker enzyme for the BER. Nothern blot analysis showed that the level of plant Pol λ in rice (*Oryza sativa*) was induced by DNA-damaging treatments. These properties suggested that plant Pol λ is a DNA repair enzyme that can substitute Pol β and terminal deoxyribonucleotidyl transferase. The similar situation may be expected in *C. reinhardtii* where homolog of Pol λ-related DNA repair polymerase gene was found.

Comparing the data resulting from higher plants genome projects with those in *C. reinhardtii*, it seems that, unlike higher plants (Kimura and Sakaguchi 2006) the BER genes in this unicellular green alga have not undergone such the extensive duplication observable in higher plant model organisms.

Nucleotide excision repair

The NER involves enzymes, which are able to remove the several types of DNA damage especially those that induce large double helix distortion (Gillet and Schärer 2006). This pathway involves the recognition of the damage with subsequent strand separation and dual incision around the lesion, followed by excision of an oligonucleotide containing the lesion, and subsequent repair by DNA synthesis. The mode of repair and excision genes and proteins are

conserved in eukaryotes ranging from *S. cerevisiae* to humans (Wood 1996; Hoeijmakers 2001; Tuteja et al. 2001; Christman et al. 2003).

First UV-sensitive mutants in *C. reinhardtii* with deficiency in "dark repair" (Table 1) were isolated by Davies (1967, 1969). Later, Small (1987) characterized five of these mutants as deficient in excision of pyrimidine dimers. One mutant strain, *uvs1*, was completely blocked and *uvs3*, *uvs4*, *uvs6* and *uvs7* excised pyrimidine dimers more slowly than a wild type. Searching for other repair deficiencies in *uvs1*, e.g., DNA single strand breaks (SSB) induced by acute γ-irradiation, showed the equal level of SSB-repair efficiency as the wild type (Chankova et al. 1994). A second mutant (*uvs9*) that is also completely blocked in excision has been isolated by Small (1987) and two others (*uvs12*, *uvs15*) in our laboratory (Podstavková et al. 1992; Vlček et al. 1997). Genetic analysis confirmed that *uvs9*, *uvs12* and *uvs15* are different loci (Podstavková et al. 1992). The survival of mutant strains with complete blockage of dimer removal is much lower under photoreactivating conditions than that of *phr1*, while the survival of other repair-deficient mutants was higher under photoreactivating conditions than that of *phr1* and did not differ from that of the wild-type strain (Podstavková et al. 1992). This may be used as an additional diagnostic marker for the isolation of mutants defective in excision-repair pathway in this model organism.

One of the most interesting mutants of this group is *uvs15* (Vlček et al. 1997). This strain belongs to the most sensitive repair-deficient mutants following an exposure to all agents used and has impaired excision repair. Mutation study analysis revealed that *uvs15* does not mutate after exposure to UV and X-rays, and there is very low mutation rate after treatment with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). In comparison to similar mutants in yeast, there is a striking and interesting difference. Although several repair-deficient mutants in *S. cerevisiae* (e.g., *rad6*, *pso1*/*rev3*) exhibit sensitivity to a wide variety of mutagens and have significantly reduced the frequencies of spontaneous and induced mutations (Haynes and Kunz 1981; Prakash 1989; Henriques and Brendel 1990) none of them is deficient in excision repair. This phenotype may indicate that the *uvs15* gene product is multifunctional or that there is a functional overlap between several repair pathways in the *C. reinhardtii* nucleus. It is possible that *UVS15* interacts with other repair proteins in the assembly of the repair machinery, similarly as was shown in other organisms (Hays et al. 1995; Habraken et al. 1996; Wang et al. 1997). Thus, the *UVS15* gene product might be involved in the regulation of several repair pathways.

So far, only one excision repair gene (*REX1*) and its protein has been analysed in more detail in *Chlamydomonas*. Mutant strain (*rex1*), which was isolated using insertional mutagenesis, is blocked in the excision of cyclobutane pyrimidine dimers and is also sensitive to the alkylating agent methyl methanesulphonate (MMS) (Cenkci et al. 2003). Cloning of *REX1* revealed that it is an unusual gene in eukaryotic organisms because it encodes two different proteins, a small protein Rex1-S (8.9 kDa) and a large one Rex1-B (31.8 kDa) (Cenkci et al. 2003). The homologs of both proteins exist in other organisms, but these two proteins are not encoded by the same gene. For example, in *A. thaliana*, the gene for the *REX1-S* homolog is on chromosome I and the *REX1-B* homolog gene on chromosome V, respectively (Kunz et al. 2005). First, it seemed the proteins were not homologous to the known DNA repair proteins, but recently it has been found that the *REX1-S* gene in *C. reinhardtii* has sequence homology to *S. cerevisiae TFB5* (http://cbi.labri.fr/Genolevures/fam/GLR.1222.htmol) found as a "new component" (the tenth member) of the DNA repair/basal transcription factor TFIIH participating in NER and conserved throughout evolution (Ranish et al. 2004). Yeast lacking *TFB5* is sensitive to UV irradiation, and mutations in the human ortholog account for the DNA repair-deficient form of the trichothiodystrophy disorder called TTD-A (Giglia-Mari et al. 2004, 2006).

Other *C. reinhardtii* excision repair-deficient mutants so far isolated in our laboratory (Table 1) were allelic with the ones described. *uvs1* was not analysed in detail because of problems with the number of vital zygotes. In summary, at least eight loci (because we do not know if *uvs1* is allelic with *uvs9* or *uvs12*) that affect the rate of excision of pyrimidine dimers have been already known in *C. reinhardtii*.

Two distinct pathways related to the way the lesions are recognized and repaired by NER were identified. The global genome repair (GG-NER) responds to a genome-wide damage (Sugasawa et al. 1998) and the transcription-coupled repair (TC-NER) is specialized exclusively in the repair of lesions on the transcribed strand of actively transcribed genes, particularly those transcribed by RNA polymerase II (Svejstrup 2002). TCR was originally thought to be the specific subpathway of NER for the repair of the UV induced DNA damage. In the meantime it has become clear that several lesions removed by the BER pathway are also repaired through TCR (Leadon and Cooper 1993; Le Page et al. 2000). Therefore, TCR might be defined as a discrete pathway for initiating the rapid removal of transcription-blocking lesions by either NER or BER.

As in higher plants, the occurrence of transcription-coupled repair in *C. reinhardtii* is only hypothetical. On the other hand, the finding of the homologs of *CSA* (Cockayne syndrome A) and *CSB* (Cockayne syndrome B) genes (Table 2), which play the essential role in TCR of mammal cell, may indicate the presence of this repair also in this green alga.

The NER in *C. reinhardtii* appears to contain the similar complement of NER components to that found in other

eukaryotes. The search for its genome sequence reveals that this alga possesses the homologs of several, though not all, of the eukaryotic genes required for NER (Table 2). These include the eukaryotic factors participating in the first steps of NER in vitro: (1) the homologs of *RAD23* and *RAD4* genes products of which form a nucleotide repair factor NEF2 required for damage recognition in *S. cerevisiae* (*XPC-HR23B* in humans), (2) the homologs of DNA-binding protein coding genes (*DDB1*, *DDB2*) involved in damage recognition, and (3) contrary to higher plants, *Chlamydomonas* appears to contain a homolog of *XPA* (*RAD14*) which, together with replication protein A (RPA), may control the proper assembly of the NER preincision complex. The damaged strand is in NER incised 5′ to the lesion by Rad1–Rad10 (XPF-ERCC1) and 3′ to the lesion by Rad2 (XPG) endonucleases. *C. reinhardtii* encodes two orthologs of *RAD2* (*XPG*) endonuclease coding gene and single ortholog of the *RAD1* and *RAD10* which also has an important function in recombination in *A. thaliana* (Dubest et al. 2004) and in some other eukaryotes (Prado and Aquilera 1995; Melton et al. 1998).

C. reinhardtii also possesses the homologs of components of the TFIIH basal transcription factor that are essential for both NER as well as transcription (Drapkin et al. 1994; Svejstrup et al. 1996). Among others, homologs of the $5' \rightarrow 3'$ ATP-dependent DNA helicase gene *XPD* (*RAD3*) and similar to higher plants two copies of $3' \rightarrow 5'$ ATP-dependent DNA helicase gene *XPB* (*SSL2*/*RAD25*) the activities of which are required for the second step of NER, local unwinding of DNA and formation of an open complex, that is further recognized by XPA-RPA proteins (Winkler et al. 2000; Missura et al. 2001).

Unlike higher plants, there are homologs of *E. coli* excinuclease genes, *uvrB*$^+$, *uvrC*$^+$ and DNA polymerase *polA*$^+$ participating in NER in bacteria which might be involved in excision repair in the *C. reinhardtii* chloroplast, inferring from the involvement of *RecA*$^+$ gene in recombinational repair of chloroplast genome. However, one cannot exclude their potential involvement in mitochondrial or nuclear DNA repair.

Several lines of evidence have been obtained with respect to the role of repair genes products in more than one repair pathway (Leadon and Avrutskaya 1997; Smith et al. 2005; D´Errico et al. 2006). Some of them have a pleiotropic effect and their products are also essential for other cell functions (Feaver et al. 1993; Guzder et al. 1994; Helt et al. 2005). One of the first findings indicating the pleiotropic effect of some repair gene products resulted from the work of Yamamoto et al. (1983) and Sancar et al. (1984) who established that photolyase can participate in the light-independent repair of UV-induced damages in *E. coli*. Both in bacteria and yeast it was demonstrated that the photolyase enhanced survival of excision proficient strains in the dark via the specific stimulation of excision repair (Yamamoto et al. 1984; Sancar and Smith 1989). We tried to ascertain whether the interaction between photolyase and dark repair processes might be generalized to photoautotrophic organism such as the single-celled green alga *C. reinhardtii* (Vlček et al. 1995). Our results indicated that the role of photolyase in dark repair might be different in *C. reinhardtii* to that in yeast. In contrast to bacteria and yeast, the photolyase gene enhanced the survival of excision deficient *C. reinhardtii* strains in the dark (Vlček et al. 1995). This indicated that photolyase might play a role in the dark repair following UV irradiation in *C. reinhardtii* also via the stimulation of repair pathway(s) not related to the excision.

Mismatch repair

This system recognizes mismatched base pairs after DNA replication, excises the incorrect bases, and then carries out repair synthesis (Jiricny 2006).

The general mismatch repair is understood best of all in bacteria. In *E. coli*, the products of three genes, *mutS*$^+$, *mutL*$^+$, and *mutH*$^+$ participate in the initial stages of mismatch repair. The MutS protein binds to the mismatch and later forms a complex with the MutL and MutH proteins to bring the unmethylated GATC sequence of the newly replicated DNA strand close to the mismatch. The MutH protein then nicks the unmethylated DNA strand at the GATC site, the mismatch is removed by the exonuclease, and the gap is repaired by DNA polymerase III and ligase (Hsieh 2001). Although the MutS and MutL proteins are highly conserved, MutH is found only in Gram-negative bacteria and no functional homologue has been identified in other organisms (Jiricny 2006).

Mismatch repair was later described in both single- and multicelled eukaryotes (Jiricny 1998) and it was also reasonable to suppose its presence in green algae. The general phenotypic and mutational analysis of UV-sensitive strains *uvs13* and *uvs14* in *C. reinhardtii* brought results indicating that one of them might have undergone damage in a mismatch repair pathway. While *uvs13* showed only a mild increase in the forward mutation frequency to streptomycin resistance after UV-treatment, the *uvs14* exerted a significantly higher mutability by this agent (Vlček et al. 1997), suggesting that the *uvs14* mutation has resulted in the ability of the replication machinery to proceed past the damage in an error-prone fashion much more frequently than the wild type. Together with resistance to killing by MNNG it indicates that the *uvs14* gene function might be involved in mismatch correction systems which repair DNA replication errors and also act on certain types of DNA damage that resemble base mismatches (Karran and Bignami 1994). However, the function of this gene product has not yet been studied in more detail in *C. reinhardtii*.

The strains *uvs13* and *uvs14*, with the NER proficiency, belong to the same repair pathway since the sensitivity of UV-treated double mutant *uvs13uvs14* is the same as that of single mutants (Vlček et al. 1997). Both genes were mapped to the linkage group I (Vlček et al. 1997).

In eukaryotes, there are multiple homologs of *E. coli* general mismatch repair genes (Friedberg et al. 2006). In accordance with findings in other eukaryotes, searching for the homologs of *mut* genes in *C. reinhardtii* resulted in the identification representatives of both basic *MSH* and *MLH* gene groups (Table 2), indicating the presence of this DNA repair system in green alga.

DNA damage tolerance and mutagenesis

DNA damage tolerance mechanisms belong to the cellular responses to DNA damage used by cells to avoid death as a consequence of unrepaired or unrepairable damage to their genomes (Cox 2001; Courcelle and Hanawalt 2003; Friedberg et al. 2006). They rescue the cell from the effect of damages causing the arrest of DNA replication by means of recombination or translesion DNA synthesis. Some of these cell response systems generate mutations due to infidelity connected with the bypass of arrested replication (Veelen et al. 2006; Livneh et al. 2006).

Recombinational repair

Endogenous and exogenous factors such as errors during the DNA replication, ionizing radiation or some chemicals can induce DNA double strand breaks (DSBs) and single strand gaps (SSGs). They are repaired by two main pathways—homologous recombination (HR) and nonhomologous end-joining (NHEJ) (Dudáš and Chovanec 2004; Symington 2005; Ishino et al. 2006). However, there are distinct subpathways depending on the presence of one or two ends at the break site, or flanking homologies surrounding the DSB (e.g., single-strand annealing).

There are more UV-sensitive mutants isolated in *C. reinhardtii* that exhibit the standard rate of removal of pyrimidine dimers from nuclear DNA. This fact suggests that these mutants may be defective in a pathway different from the NER. Some of these mutants in *C. reinhardtii* (e.g., *uvsE1*) were shown to be deficient in recombination during meiosis and sensitive to radiation (Rosen and Ebersold 1972). These stimulated the search for recombination repair deficient mutants in this alga and methods for their selection and isolation.

Rosen et al. (1980) observed that treatment of UV-irradiated *C. reinhardtii* with caffeine caused a significant increase in survival in recombination-proficient strains but had no effect on the recombination-deficient strain. The effect of caffeine on various cell systems including repair pathways has been studied extensively but no clear pictures emerged. Its effect on various repair systems is variable depending on explored organism (Rosen et al. 1980). It has been suggested that caffeine binds to DNA, perhaps with higher affinity to damaged regions and thus interferes with binding of repair enzymes (Selby and Sancar 1990; Kramata et al. 2005). Rosen et al. (1980) also demonstrated that caffeine stimulated the frequency of recombination in *C. reinhardtii*. Sublethal concentrations (1.5 mM) of caffeine increased the frequency of recombination in wild type and also increased the survival of UV-irradiated cells, but had no effect on the survival of *uvsE1*, *uvsE5* and *uvsE6* (Portney and Rosen 1980; Rosen et al. 1980) and *uvs10* (Vlček et al. 1987). The absence of caffeine effect in the case of *uvs10* as in *uvsE1*, *uvsE5* and *uvsE6* might suggest a defect in a recombinational repair pathway as suggested by Portney and Rosen (1980) and Rosen et al. (1980). Genetic analysis demonstrated that these mutant genes belong to different loci (Podstavková et al. 1994). The gene *UVS10* was mapped to linkage group I as the first UV-sensitive mutant ever mapped in *C. reinhardtii* (Vlček et al. 1987). Currently *UVSE1*, *UVSE5*, *UVSE6* and *UVS10* are the genes with established participation in recombinational repair following the analysis of mutant strains in *C. reinhardtii*.

Fundamental information pertinent to understanding the functional relationship of the repair genes emerges from studies on the relative sensitivity of single and double mutants to killing by DNA-damaging agents (Friedberg 1988). 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 are considered to belong to the same epistasis group. The survival of the double mutants *uvsE1*/uvsE5, *uvsE1*/uvsE6, *uvsE5*/*uvsE6* (Portney and Rosen 1980) and *uvsE5*/*uvs10*, *uvsE6*/*uvs10* (S. Podstavková and D. Vlček 1995, unpublished results) was lower than that in any single mutant with the exception of *uvsE1*/*uvs10*. This indicates the participation of the above genes in three different recombinational repair mechanisms (Table 1).

The mutant strain *uvsE1* is interesting in that its cells show the promotion of chloroplast genome transmission from a mt^- parent. The meiotic progeny of *C. reinhardtii* normally receives chloroplast genomes only from the mt^+ parent. There are also exceptional zygotes, which transmit the chloroplast genomes from both parents and, rarely, only into the mt^- parent, but they arise at a low frequency (Harris 1989). Mutations within the mt^+-linked *mat3* locus elevate the transmission of chloroplast genomes from the mt^- parent (Gillham et al. 1987). The findings of Rosen et al. (1991) indicated that the product of *UVSE1* gene contributes, together with *MAT3* gene, to the uniparental inheritance of

the chloroplast genome probably by facilitating activation or synthesis of the wild-type *mat3* gene product in the zygote. This is the only example of a repair gene, the product of which exerts pleiotropic influence on DNA repair and transmission of organellar genomes to meiotic progeny.

C. reinhardtii mutant strains sensitive to DNA-damaging agents including alkylation compounds were also isolated by Sarkar et al. (2005). One of them, *Ble-1*, was shown to carry chromosomal deletion that includes gene encoding thioredoxin h1 (*TRXH1*). The authors found that the wild type copy of *TRXH1* complemented a defect in the repair of MMS-induced DNA strand breaks and alkalilabile sites. Moreover, *TRXH1* suppression by RNAi in a wild type strain resulted in enhanced sensitivity to MMS and DNA repair defects but nothing increased cytotoxicity to H_2O_2 (Sarkar et al. 2005). Similarly to some other cases (requirement of *PHR1* for full activity of *PHR2* gene or interaction between photolyase and dark repair processes, Petersen and Small 2001; Vlček et al. 1995) thioredoxin seems to have a specific role in *C. reinhardtii* DNA repair as compared with other experimental model systems. Contrary to its implication in oxidative-stress in many organisms, in *C. reinhardtii* it participates in DNA strand breaks repair and is dispensable for a response to oxidative agents (Sarkar et al. 2005).

The genome sequencing project and *C. reinhardtii* expressed sequence tag (EST) and genomic libraries allow users to find the orthologs of known recombination repair genes and to reconstruct and clone some of them. One of them is the homolog of *RAD51* recombination/repair gene, which could have a similar role in the recombination process in *C. reinhardtii* as was observed in other eukaryotes. Rad51 recombinases are central to the process of HR in eukaryotes. The recombinases promote homologous pairing and strand exchange leading to the recombination between the two interacting DNA molecules. The first gene—which is involved in recombinational repair, coding a protein, which is a member of the subfamily of higher eukaryotic Rad51-like recombination proteins, *RAD51C*, participating in HR and repair—was cloned in *C. reinhardtii* by Shalguev et al. (2005). It is the only *RAD51C* ortholog, which has been isolated and cloned in this alga so far. These authors demonstrated that the product of *C. reinhardtii RAD51C* is a typical member of the subfamily of higher eukaryotic Rad51C-like proteins. Furthermore, the conservation of functions of *RAD51* paralogs between vertebrates and plants was supported (Bleuyard et al. 2005), so one can suppose the same function of *RAD51C* gene also in this green alga. Similarly to higher plants, the *RAD52* gene encoding a central HR protein in *S. cerevisiae*, is missing in the *C. reinhardtii* genome.

Searching for *C. reinhardtii* genome sequences also revealed some other very important participants in recombination and recombinational repair such as the *DMC1* homolog of *RAD51* involved in meiosis as well as the homologs of *RAD50*, *RAD54*, *MRE11* and *RPA*. The Mre11 and Rad50 proteins together with Xrs2 (NBS1 in humans) are transferred to DSBs in yeast, followed by Rfa (RPA in humans), Rad52, Rad51, Rad55 and the Rad54 (Lisby et al. 2004). The Mre11 complex needed for proper functioning of DSB repair is thought to tether DNA ends together and remove end-blocking lesions by Mre11 nuclease activity and to signal through the ATM kinase to downstream effectors (Chen et al. 2001; Jazayeri et al. 2005).

HR is apparently a very important repair process at DSB sites in *C. reinhardtii*. This follows from the observed highest sensitivity of *uvsE1* to X-ray irradiation among all repair mutants in *C. reinhardtii* (Slivkova et al. 1998) and higher sensitivity of *uvsE1* and *uvs10* to alkylation agents that induce DNA strand breaks (Miadoková et al. 1994), similar to the case of recombination repair-deficient strains in other organisms, e.g., yeast *S. cerevisiae* (Prakash and Prakash 1977).

Unlike the single-celled eukaryote, e.g., yeast *S. cerevisiae*, the repair of DSB by NHEJ is mainly used in higher eukaryotes as compared with HR (Symington 2005). The induction of radioresistance of haploid *C. reinhardtii* cells in the G1-phase (cells in G1-phase can not use HR to repair DSBs) and their response in a manner analogous to cells responsible for recombinational repair described by Boreham and Mitchel (1993) may indicate the implication of NHEJ in the repair of DSBs also in this alga. Both *Ku70* and *Ku80* homologs were found in its genome and, contrary to higher plants, the catalytic subunit of DNA-dependent protein-kinase (DNA-PKcs), which in mammalian cells phosphorylates some NHEJ-mediating proteins, is apparently also present in *Chlamydomonas*. The role of *Ku80* in DSBs repair in *C. reinhardtii* cells was proved by Rohr et al. (2004) using transformants containing integrated copies of the *Ku80* IR (inverted repeat) transgene inducing RNAi gene silencing. Transformants were sensitive to radiomimetic DNA damaging agents such as methylmethane sulfonate and zeocin. This sensitivity correlated with the level of reduction of the *Ku80* transcripts (Rohr et al. 2004). One can infer that, unlike higher plants, the repair of DSB by NHEJ may proceed in this unicellular green alga similarly to those in higher heterotrophic eukaryotes.

Translesion DNA synthesis and mutagenesis

Under normal circumstances, DNA repair processes such as NER and BER remove blocking lesions before the replication machinery encounters them (Hoeijmakers 2001). If, however, damage remains unrepaired, there are mechanisms enabling the cell to bypass the lesion in order to allow continued DNA replication. Such bypass can be

accomplished in a relatively error-free manner via HR or template switching involving the sister chromatid. Alternatively, a lesion can be bypassed by translesion synthesis, which employs low-fidelity DNA polymerases to replicate across the damage, often in an error-prone fashion resulting in increased frequency of mutations (Kunkel 2003). This repair mechanism is involved in the increasing rate of mutations.

The isolation of UV-sensitive strains with a low mutation frequency after treatment with physical and chemical agents (Vlček et al. 1997) indicated the existence of genes with important roles in *C. reinhardtii* mutagenesis. However, the mutation repair pathway has not yet been studied in detail. Searching the *C. reinhardtii* genome database resulted in the identification of genes homologous to those involved in translesion synthesis and mutagenesis in other eukaryotes (Table 2). The most important genes of this pathway are those coding low fidelity polymerases which deal with noncoding lesions blocking the forward progression of the replication fork, resulting in a cell cycle arrest and/or cell death. They belong to the Y family of DNA polymerases, whose members include polymerases involved in the SOS response of *E. coli* (Ohmori et al. 2001).

The homologs of genes encoding known eukaryotic translesion polymerases—DNA Pol eta/iota, Rev1, DNA Pol ζ, DNA Pol κ and DNA Pol θ have been found in the *C. reinhardtii* genome. In *S. cerevisiae*, the use of low fidelity polymerases in bypassing DNA lesions appears to be regulated by the members of the *RAD6* epistasis group that consists of an error-prone and an error-free subpathway, both of which are under the control of *RAD6* and *RAD18* genes (Prakash 1981; Torres-Ramos et al. 2002; Minesinger and Jinks-Robertson 2005). The *C. reinhardtii* genome analysis showed the lack of *RAD6* and probably also *RAD18* homologs, which play an important role in the error-prone pathway in *S. cerevisiae* (Bailly et al. 1997). The *RAD18* homolog is also missing in higher plants but *RAD6* is specifically lost in *C. reinhardtii*. It is possible that another gene plays a role like *RAD6* in the regulation of "postreplication repair" in this alga. One candidate gene might be *UVS15* (see above), the product of which seems to have an effect on several repair pathways (Vlček et al. 1997).

Repair pathways in organelles

Reports focused on DNA repair in organelles have shed light on DNA repair pathways particularly in the mitochondria of animals. All basic repair pathways, except the NER, have been established in this organelle (Croteau and Bohr 1997; Lakshmipathy and Campbell 1999; Zhang et al. 2006). The chloroplast DNA (cpDNA) repair has not been investigated so thoroughly as that in mitochondria.

In *C. reinhardtii*, the presence of a repair activity was established in both nucleus and chloroplast. The first repair pathway demonstrated to operate in the chloroplast of this alga was photoreactivation (Small and Greimann 1977b). Similarly to other repair proteins targeted to more than one compartment (Slupphaug et al. 1993; Larsen et al. 2005) *C. reinhardtii* photolyase also splits pyrimidine dimers in both nucleus and chloroplast (see above).

Swinton and Hanawalt's (1972) observation that thymidine is incorporated specifically into *C. reinhardtii* cpDNA eliminated the necessity of physically separating nuclear and chloroplast DNA in examining the presence of repair pathways in chloroplasts. Using this observation and modifying the previous methods, Small and Greimann (1977b) revealed that wild-type cells removed over 80% of the dimers from cpDNA after 24 h of incubation under non-photoreactivation conditions. The mutant *uvs1* which is completely blocked in the excision of pyrimidine dimers from nuclear DNA, was able to remove these lesions from cpDNA nearly as rapidly as the wild-type. This finding demonstrated that the genetic control of the removal of pyrimidine dimers from nuclear DNA under non-photoreactivating conditions is separate from that of cpDNA. The unique finding of bacterial $uvrB^+$ and $uvrC^+$ homologs in *C. reinhardtii* genome that have not been tracked down in other eukaryotes suggests their possible role in chloroplast NER processes and the reason for its separate genetic control from nuclear DNA. However, it should be taken into account that, what was actually measured was the average distance between the pyrimidine dimers, which increased with time (Small 1987). It is possible that some recombinational events are at least partially responsible for increasing the distance between the pyrimidine dimers.

The occurrence of HR in chloroplast is well documented and the role of organelle recombination in survival and repair has been established (Cerutti et al. 1995; Durrenberger et al. 1996). cpDNA recombination is very active in vegetative cells of *C. reinhardtii*, as indicated by the dispersive labelling of plastid DNA in density transfer experiments (Turmel et al. 1981). The identification of the nuclear gene (*REC1*) encoding a chloroplast-localized protein homologous to *E. coli* RecA, which is supposed to be not a *RAD51* orthologue, in *C. reinhardtii* (Nakazato et al. 2003) and higher plant species (Cerruti et al. 1993; Cao et al. 1997) suggests that the plastid recombination system is related to its eubacterial counterpart.

To verify the existence of a RecA-mediated recombination system in chloroplast the dominant negative mutants of *E. coli* RecA were used for the transformation of *C. reinhardtii* to interfere with the activity of their putative endogenous homolog in the chloroplast (Cerruti et al. 1995). The results revealed that transformants expressing mutant RecA protein showed reduced survival rates when exposed to

DNA damaging agents, the deficient repair of cpDNA, and diminished plastid DNA recombination. Together with the observation that the steady-state level of the endogenous plastid RecA protein is enhanced by exposure to genotoxic agents (Cerruti et al. 1993) these authors deduced that, at least in vegetative cells of *C. reinhardtii*, the primary biological role of the chloroplast recombination system is in the DNA repair, most likely needed to cope with the photooxidative and environmental damage.

Recently, the homolog of bacterial RecA protein targeted to plant mitochondria, which exhibits the highest sequence homology to chloroplast as well as proteobacterial RecA protein, has been found in the *A. thaliana* genome (Khazi et al. 2003). This gene partially complemented a *recA* deletion in *E. coli*, in the same way that the RecA protein complemented the repair deficiency in various eukaryotic species (Vlčková et al. 1994; Slaninová et al. 1995, 1996; Reiss et al. 1996; Morais et al. 1998; Shcherbakova et al. 2000) and enhanced survival after exposure to DNA-damaging agents. This suggests a possible role for *A. thaliana* mt*recA* in HR and/or repair in mitochondria (Khazi et al. 2003) similarly to that in chloroplast (Cerruti et al. 1993).

The results of GuhaMajumdar and Sears (2005), who determined directly the frequency and types of spontaneous base substitutions that occur in chloroplast DNA of *C. reinhardtii*, revealed that DNA repair might be rather efficient in this organelle. Taking into account an abundance of reactive oxygen species generated as bioproduct of oxygenic photosynthesis one could expect efficient excision repair system(s) which protect cell chloroplast against lesions caused by oxygen radicals. Contrary to higher plants, the genome-sequencing project revealed homolog of *mutT*$^+$ gene in *C. reinhardtii* genome which might help to explain the relatively efficient repair of above mentioned base damages. The product of *mutT*$^+$ gene hydrolyzes 8-oxo-dGTP in nucleotide pool back to monophosphate form 8-oxo-dGMP, preventing its incorporation into DNA (Maki and Sekiguchi 1992).

DNA repair in the framework of other cellular processes

The growth of DNA repair research with new data obtained has demonstrated its connection with other cellular functions, e.g., replication, transcription, recombination and cell cycle regulation, which is achieved by the activity of many overlapping surveillance and repair mechanisms (Foiani et al. 2000). In response to the DNA damage or replication block, cells coordinate the activation of DNA repair pathways and cell cycle checkpoint pathways to allow time for DNA repair to occur in order to maintain genomic stability and integrity.

The study of DNA damage-dependent cell cycle checkpoint in *C. reinhardtii* is complicated by the unorthodox mode of cell cycle progression (Harper 1999). *C. reinhardtii* cells normally grow to many times their original size during a prolonged G1 phase and then undergo the multiple alternating rounds of DNA replication and mitosis to produce the daughter cells of uniform size. In each cell cycle a cell must make two size-dependent decisions—(1) whether and (2) how many times to divide. First decision, termed "commitment", takes place in G1. It is equivalent to "start" in yeast or "restriction point" in mammalian cells, and it is defined as the point at which the withdrawal of nutrients and/or light will not prevent the completion of at least one round of cell division (Spudich and Sager 1980; Donnan and John 1983). Following the commitment, the cells maintained in nutrients and light can continue to increase in size before the DNA replication and mitosis begin (Craigie and Cavalier-Smith 1982; Zachleder and Vandenende 1992). The second size-based control mechanism then ensures that mother cells undergo the correct number of S phases and mitoses (reproductive cycles) to produce daughter cells with uniform size distribution (Lien and Knutsen 1979; Donnan and John 1983).

The first UV-sensitive mutant (*uvs1*), which also exhibited temperature-sensitive cell cycle defective phenotype traits was isolated in *C. reinhardtii* more than 20 years ago (Vlček et al. 1981). In addition to the above, the second mutant resistant to UV-irradiation and with prolonged cell cycle in comparison with the wild type, as observed in experiments dealing with cell volume growth, cell numbers, DNA, RNA and protein synthesis during the synchronous cycle, was selected (Vlček et al. 1981). However, both mutants were obtained from the wild-type strain *C. reinhardtii* A19 which was later found to be defective in mating, and therefore not amenable to genetic analysis. Because of this, they were not characterized in more detail concerning their UV sensitivity/resistance. This was the reason why we started isolating UV-sensitive mutants from another wild-type strain (137C$^+$). One selected mutant, *uvs11*, behaved as a single nuclear mutation, proficient in the excision of pyrimidine dimers from nuclear DNA and frequently dividing at least once before dying (characteristic of mutants unable to stop dividing on purpose to extend time needed for DNA repair) (Vlček et al. 1987). The delayed death of cells after their treatment with UV-irradiation was in contrast to wild type and other UV-sensitive mutants. This is very similar to the *S. cerevisiae* mutants of DNA damage-dependent cell cycle checkpoints (e.g., *rad9*) failing to stop dividing; this leads to the formation of nonviable microcolonies.

To investigate the *uvs11* in more detail we observed the response of both, the wild type cells and the *uvs11* mutant cells, to UV-irradiation in the course of the synchronous cell cycle (Slaninová et al. 2003). We found that, if the wild-type cells were irradiated before or during the DNA replication, the cells failed to progress through the cell cycle, leading to the delay of mitosis and cell division. Interestingly, the irradiated *uvs11* mutant does not display any change in its dependence on the cell cycle phase—cells do not stop cell cycle progression, they do not exhibit any change in CDK-like kinase activity, which eventually leads to cell death. However, if the cell cycle progression of mutant cells was artificially delayed by the inhibitor methyl benzimidazole-2-yl-carbamate (MBC), the survival of mutant cells significantly increased (Slaninová et al. 2002) similarly to *rad9* mutant of *S. cerevisiae* (Weinert and Hartwell 1988). Our results showed that the green alga *C. reinhardtii* possessed control mechanisms allowing for stopping the cell cycle in response to DNA damage indicating the occurrence of a major checkpoint, activated after the DNA damage and prior to the mitosis. Together with the recently obtained *sog1* mutant in *A. thaliana* (Preuss and Britt 2003), which was also unable to arrest cell cycle before the mitosis in response to the DNA damage, these findings provided evidence that plants, just like animals, possess the classic checkpoint response. At present, two other strains (*uvsX1*, *uvsX2*) mutant genes of which participate in DNA repair-cell-cycle-connected regulation are under investigation in our laboratory (B. Sviežená et al. manuscript in preparation).

Studies of the cell cycle in *C. reinhardtii*, using the wild type and cell cycle mutants (Howell and Naliboff 1973; Harper 1999; Umen and Goodenough 2001), have demonstrated that its regulation is based on cyclin-dependent kinases (CDKs), as it is also in other eukaryotic organisms (John et al. 1989; Zachleder et al. 1997; Bišová et al. 2000). Recently it has been found that *C. reinhardtii* encodes orthologs of the major plant CDKs and cyclin families (Bišová et al. 2005). Contemporaneously, several homologs of genes involved in the coordinated activation of the DNA repair pathways and the cell cycle checkpoints pathways were identified in *C. reinhardtii* genome (Table 2) (reminder: the *C. reinhardtii* homologs of known checkpoint genes described below resulted only from *in silico* studies). Among others, protein kinases of the PIKK family, also denoted as the PI-3 family (phosphoinositide-3-kinase-related protein kinase family), e.g., homologs of Tel1 and Mec1 or ATM (*ataxia telangiectasia* mutated—protein defective in *ataxia telangiectasia*) and ATR (*ataxia telangiectasia* and Rad 3 related) which are the core checkpoint proteins and, together with DNA-PKcs, relay and amplify the damage signal triggering the cell cycle checkpoints and the DNA repair (Rouse and Jackson 2002; Shiloh 2003). ATM and ATR are required for the phosphorylation of Rad17 (homolog found in *C. reinhardtii* genome) in response to DNA damage although they have also other targets (Bao et al. 2001).

The recruitment of DNA damage-associated PIKKs to DNA lesions is thought to be a principal step in their activation in checkpoint signaling and DNA repair (Melo and Toczyski 2002; Meek et al. 2004). Even when PIKKs have an affinity for DNA, the recruitment to DNA lesions is facilitated by specific partner proteins. For example, the DNA-PKcs recruitment to DNA double-strand breaks (DSBs) is mediated by the Ku70–Ku80 heterodimer (Gottlieb and Jackson 1993), and the ATM recruitment to DSBs needs Mre11-Rad50-Xrs2/NBS1 protein complex (Carson et al. 2003). The homologs of genes encoding Mre11, Rad50 and Ku (see above) have been identified not only in higher plants (Gallego et al. 2003) but also in *C. reinhardtii* as representative of green algae (Table 2).

PIKKs along with other gene products (Rad17, Ddc1, Mec3, Rad24, Ctf18 in yeast and Rad1, Rad9, Hus1, Rad17 in humans), that are homologous to replication proteins (RFC-clamp loader and PCNA-sliding clamp) are involved in the DNA damage recognition and in the initiation of the checkpoint (Nyberg et al. 2002; Qin and Li 2003). Rad24 and Ctf18 are members of RFC (replication factor C)-like complexes (RLCs) that share four common subunits (Rfc2–5) and each of them carries a unique large subunit (Rad24 or Ctf18) replacing the Rfc1 subunit of RFC. Most recently, the third complex with Elg1 subunit was described by Aroya and Kupiec (2005). The homologs of representatives of this group (*RAD17*/*RAD24*, *HUS1*, *RAD9*, *CTF18*) were found in *C. reinhardtii* (Table 2). The ortologs of this pathway are also present in higher plants (*A. thaliana*) (Kimura and Sakaguchi 2006) and their products participate in the DNA repair. Mutations in some of the genes (*RAD17*, *RAD9*) do not cause a general defect in DNA repair in higher plants, but lead mainly to a deficiency in a non-HR repair (Heitzeberg et al. 2004). It will be interesting to find out whether there is a correlation between the function of these genes in higher plants and algae.

Several homologs of checkpoint protein genes found in *C. reinhardtii* comprise BRCT domain ("BRCA1 C-terminal") that is also present in budding yeast *RAD9*, the function of which is required for the recruitment and activation of downstream signal transduction kinases (Blankley and Lydall 2004). It may indicate their participation in DNA damage-induced G_2 arrest in *C. reinhardtii*.

Recent reports describe the direct participation of checkpoint proteins in the DNA repair. This was inferred from identified complexes, which contained checkpoint proteins and several repair and replication proteins, e.g., BASC (BRCA1-associated genome surveillance complex) (Wang et al. 2000). Most recently, direct participation of individual

checkpoint proteins in different repair pathways, e.g., BER and recombinational repair, has been described (Helt et al. 2005; DeMase et al. 2005; Yang et al. 2005) indicating a narrow and continuously running interconnection among such basal cell processes as the DNA replication, recombination, DNA repair and cell cycle.

Conclusion

Although some progress has been made, the data obtained from the DNA repair studies in *C. reinhardtii* clearly point to our still poor understanding of the DNA repair pathways in this model organism. Up to the present only four repair genes have only been cloned and analysed for their ability to complement repair-defective mutants from other species. On the other hand, *C. reinhardtii* is amenable to both classical and molecular genetic approaches to DNA repair studies and considering its role of "green yeast" among model systems for photoautotrophic organisms, it is reasonable to expect greater interest and faster progress in future DNA repair research.

The investigation of DNA repair in *C. reinhardtii* revealed several unique features of this genome maintaining system characteristic of this alga. Particularly, as regards (1) regulation of photolyase activity, (2) pleiotropic effect of recombinational repair gene upon transmission of organellar genomes, (3) participation of gene implicated in oxidative stress in DNA strand breaks repair, (4) regulation of NER and error-prone repair by the same gene, or (5) in contrast with other explored organisms (bacteria and yeast), interaction of photolyase with other repair pathway than excision one. All these and other aspects of *C. reinhardtii* DNA repair would deserve additional research in next period. With the rapid development of new molecular and cell biological techniques it is expected to unravel these interesting phenomena in near future.

As primarily photoautotrophic organism it is much more exposed to UV irradiation and photooxidative stress comparing to heterotrophic organisms. Combination of different living strategies (photoautotrophic, heterotrophic and mixotrophic) might cause evolving of various "repair strategies" and unique features of this system to cope with DNA attacking factors in variable growth conditions.

The completion of the *C. reinhardtii* genome will help to address many interesting questions for algal repair researchers. First of all, there will be an opportunity to study the functions of the algal homologs of various repair proteins. Interesting results can be expected from the comparison between the repair systems of algae and higher plants. The results of DNA repair research might show whether some differences in repair systems observed between higher plants and animals result not only from differences in their developmental strategies but also from various food and environmental strategies in photoautotrophic and heterotrophic organisms.

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