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Review Article

"Adaptive response" - Some underlying mechanisms and open questions

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Abstract

Organisms are affected by different DNA damaging agents naturally present in the environment or released as a result of human activity. Many defense mechanisms have evolved in organisms to minimize genotoxic damage. One of them is induced radioresistance or adaptive response. The adaptive response could be considered as a nonspecific phenomenon in which exposure to minimal stress could result in increased resistance to higher levels of the same or to other types of stress some hours later. A better understanding of the molecular mechanism underlying the adaptive response may lead to an improvement of cancer treatment, risk assessment and risk management strategies, radiation protection, *e.g.* of astronauts during long-term space flights. In this mini-review we discuss some open questions and the probable underlying mechanisms involved in adaptive response: the transcription of many genes and the activation of numerous signaling pathways that trigger cell defenses - DNA repair systems, induction of proteins synthesis, enhanced detoxification of free radicals and antioxidant production.

Key words: adaptive response, oxidative stress, DNA repair up-regulation, DNA-binding proteins, antioxidant defense system.

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The adaptive response

Organisms are affected by various different physical and chemical genotoxic agents, some of them natural (*e.g.* solar ultraviolet light, ionizing radiation) and others released in the environment as a result of human activity (anthropogenic environmental pollutants). Many defense mechanisms have evolved to minimize genotoxic damage. One of these is induced radioresistance or adaptive response (AR). The term "adaptive response" usually means that a relatively small "conditioning" radiation dose induces increased radioresistance when the cells are irradiated with higher doses several hours later (Hillova and Drasil, 1967). Thus, radioadaptive response induction expresses the ability of low dose radiation to induce cellular changes that alter the level of subsequent radiation-induced or spontaneous damage. The AR could be considered a nonspecific phenomenon - the exposure to minimal stress inducing a very low level of damage can trigger an AR resulting in increased resistance to higher levels of the same or of other types of stress (Joiner *et al.*, 1996; Wolff, 1998; Joiner *et al.*, 1999; Patra *et al.*, 2003; Asad *et al.*, 2004; Girigoswami and Ghosh, 2005; Yan *et al.*, 2006).

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The AR has been observed in many different organisms: bacteria, yeast, the algae *Oedogonium cardiacam*, *Chlamydomonas reinhardtii*, *Closterium monoliferum* and *Chlorella pyrenoidosa*, in higher plants, insect cells, mammalian cells, human cells *in vitro*, and in animal models *in vivo* during a protracted (low dose-rate) exposure prior to an acute dose treatment (Horsley and Laszlo, 1971, 1973; Bryant, 1974, 1975, 1976, 1979; Howard and Cowie, 1976, 1978; Olivieri *et al.*, 1984; Santier *et al.*, 1985; Wolff *et al.*, 1988; Boreham and Mitchel, 1991; Rieger *et al.*, 1993; Mahmood *et al.*, 1996; Salone *et al.*, 1996; Panda *et al.*, 1997; Asad *et al.*, 1997, 1998; Wolff, 1998; Nikolova *et al.*, 1999; Wang and Cai, 2000; Sawant *et al.*, 2001; Tiku and Kale, 2001, 2004; Venkat *et al.*, 2001; Assis *et al.*, 2002; Chankova and Bryant, 2002; Gajendiran and Jeevanram, 2002; Rubinelli *et al.*, 2002; Schlade-Bartusiak *et al.*, 2002; Sedgwick and Lindahl, 2002; Jovtchev and Stergios, 2003; Patra *et al.*, 2003; Savina *et al.*, 2003; Ulsh *et al.*, 2004; Zhou *et al.*, 2004; Atanasova *et al.*, 2005; Chankova *et al.*, 2005, 2007; Coleman *et al.*, 2005; Friesner *et al.*, 2005; Lanza *et al.*, 2005; Rohankhedkar *et al.*, 2006; Seo *et al.*, 2006).

Different endpoints have been used to demonstrate an AR: cell survival, gene mutations, repetitive DNA loci mutations, chromosome aberrations and micronuclei induction, neoplastic transformation *in vitro*, microarrays

showing gene expression changes, DNA single- and double-strand breaks, biochemical analyses of enzymatic and/or non-enzymatic antioxidant defence system (Hillova and Drasil 1967; Bryant, 1975, 1976, 1979; Rieger *et al.*, 1993; Ikushima *et al.*, 1996; Rigaud and Moustacchi, 1996; Panda *et al.*, 1997; Nikolova *et al.*, 1999; Robson *et al.*, 2000; Wang and Cai, 2000; Tiku and Kale, 2004; Venkat *et al.*, 2001; Assis *et al.*, 2002; Chankova and Bryant, 2002; Guo *et al.*, 2003; Jovtchev and Stergios, 2003; Somers *et al.*, 2004; Ulsh *et al.*, 2004; Zhou *et al.*, 2004; Atanasova *et al.*, 2005; Chankova *et al.*, 2005, 2007; Lanza *et al.*, 2005; Biryukova *et al.*, 2006; Chen *et al*., 2006; Ko *et al.*, 2006; Otsuka *et al.*, 2006; Bercht *et al*., 2007).

An adaptive response to radiation also occurs in human lymphocytes (Shadley and Wolff, 1987; Wojewózka *et al.*, 1996; Stoilov *et al.*, 2007). This was first demonstrated by Olivieri *et al.* (1984) when peripheral blood lymphocytes were irradiated with low doses of X-rays or exposed to tritium labeled thymidine and a lower than expected frequency of chromosomal aberrations was found after a subsequent higher test (or challenge) dose. However, other authors reported a diversity in response of lymphocytes; in some cases showing additive effects or no response at all (*e.g.* Mortazavi *et al.*, 2003c). Sawant *et al.* (2001) also found an adaptive response to low dose gamma irradiation of 10T1/2 cells that were subsequently exposed to microbeam alpha-particle irradiation. Other test systems under some experimental conditions may also not show an AR (Boreham and Mitchel, 1993; Colombi and Gomes, 1997; Zasukhina *et al.*, 2000; Pelevina *et al.*, 2003; Joksic and Petrovic, 2004).

A popular hypothesis presented in Figure 1 postulates that the AR could be induced by reactive oxygen species (ROS) (Feinendegen *et al.*, 1996, 1999; Jones *et al.*, 1999; de Saint-Georges, 2004; Shankar *et al.*, 2006). ROS are generated in organisms during metabolism and/or formed after exposure to different biotic and abiotic stimuli (UV-irradiation, ionizing radiation, ozone exposure, heavy metals), damaging some cell constituents and producing oxidative stress (Joiner *et al.*, 1996, 1999; Mendez-Alvarez *et al.*, 1999; Bolwell *et al.*, 2002; Neill *et al.*, 2002; Vranová *et al.*, 2002; Babu *et al.*, 2003; Asad *et al.*, 2004; Verschooten *et al.*, 2006; Wang *et al.*, 2006). Ionizing radiation (IR) can damage DNA both by direct ionization and by indirect processes in which DNA is affected by numerous radiolytic reactive products. Free radicals can attack biomolecules such as DNA, proteins and lipids and initiate lipid peroxidation and generate intermediates that can react with DNA (Halliwell and Gutteridge, 1989; Marnett *et al.*, 2003). ROS could also induce multiple localized lesions consisting in base damage, single- and double-strand breaks (SSBs and DSBs), DNA-DNA cross-links and DNA-protein cross-links (Goldberg and Lehnert, 2002; Marnett *et al.*, 2003; Asad *et al.*, 2004). For example, it has been found that administration of heavy metals could reduce subsequent thymus lymphocyte DNA lesions and lipid peroxidation in gamma irradiated mice (Osipov *et al.*, 2003) and many bacteria species have adaptive responses which protect them against the toxicity and mutagenicity of DNA alkylating agents (Sedgwick and Lindahl, 2002).

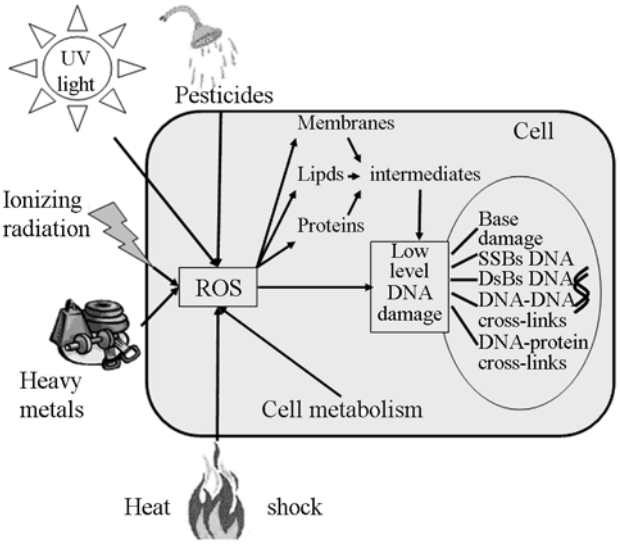

Figure 1 - Scheme of a popular hypothesis for the induction of the adaptive response (AR) via reactive oxygen species (ROS) (Feinendegen *et al.*, 1996, 1999).

Molecular mechanisms of the adaptive response

Little is currently known about the precise mechanisms of AR. There is evidence that different stress conditions can activate similar defense mechanisms in various biological systems (Joiner *et al.*, 1996, 1999; Babu *et al.*, 2003). The AR probably involves the transcription of many genes and the activation of numerous signaling pathways that trigger cell defenses (Figure 2): more efficient detoxification of free radicals, DNA repair systems, induction of new proteins in irradiated cells with a conditioning dose, and enhanced antioxidant production (Bryant, 1979; Wolff, 1998; Mendez-Alvarez *et al.*, 1999; Pajovic *et al.*, 2001; Assis *et al.*, 2002; Chankova and Bryant, 2002; Neill *et al.*, 2002; Sasiadek *et al.*, 2002; Sedgwick and Lindahl, 2002;

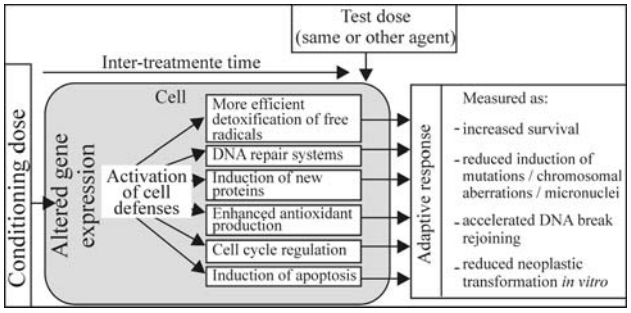

Figure 2 - Some underlying mechanisms probably involved in the adaptive response.

Coleman *et al.*, 2005; Girigoswami and Ghosh, 2005; Lanza *et al.*, 2005).

Sakamoto-Hojo *et al.* (2003) showed that the cell responses to ionizing radiation in lymphocytes of radiation workers involved altered expression of genes associated with cell cycle regulation, DNA repair, signal transduction, apoptosis induction/tumorigenesis and damage response/maintenance of genetic stability (P53-related functions). Similar results were obtained for human lymphoblastoid cells *in vitro* and the authors proposed that certain low dose-induced alterations in cellular functions could be predictive of the subsequent genomic damage risk (Coleman *et al.*, 2005). Other recent molecular studies suggested that alternative dose-specific pathways of radioadaptive response could exist in mammalian cells: one response activated at low doses by the protein kinase C through p38 MAP kinase resulting in P53 activation and another activated at higher doses resulting in activation of ERK and JNK kinases and WIP phosphatase (Lanza *et al.*, 2005). AR is known to require a certain minimal dose for activation (Leonard, 2007). Low levels of damage could be triggering events that signal the activation of DNA repair systems (Boreham and Mitchel, 1991; Wolff, 1998; Matsumoto *et al.*, 2004). For example, the persistence of DNA strand discontinuities could serve as a triggering signal for the adaptation of human lymphocytes against ionizing radiation exposure (Stoilov *et al.*, 2007). The magnitude of the AR has been shown to increase with the dose of radiation up to a certain threshold (Bryant, 1976). A specific dose of UVB was required to induce AR in *Euglena* (Takahashi *et al.*, 2006). Induction of AR by methylating agents has been reported in eukaryotic cells as well. For example, Mahmood *et al.* (1996) reported that in murine cells the AR was induced by the methylating agent methyl methanesulfonate and was stronger than that induced by the ethylating agent ethyl methanesulfonate. Schlade-Bartusiak *et al.* (2002) showed a more pronounced AR in human lymphocytes after treatment with bleomycin, which generates DNA breaks, than with the alkylating agent mitomycin.

Experiments with restriction enzymes indicated that DNA DSBs with blunt or cohesive ends were capable of inducing an AR (Wolff, 1996, 1998). Some radiosensitive DSB repair-deficient mutants were found to exhibit no induced radioresistance, suggesting the involvement of DSB rejoining (Skov *et al.*, 1994). Changes in chromatin conformation could result in less sensitivity of chromatin to damage by indirect effect of a test dose or in increased accessibility of damaged sites to repair enzymes (Belyaev *et al.*, 1996; Kleczkowska and Althaus, 1996). Experiments with repair inhibitors suggested that poly-ADP-Ribose Polymerase-1 (PARP) is also involved in the AR (Kleczkowska and Althaus, 1996; Wolff, 1998; Marples and Joiner, 2000; Patra *et al.*, 2003) possibly interfering in the cell cycle control (Tang *et al.*, 2005) or in the damage-sensing process (Marples *et al.*, 2004). This was confirmed and it has been postulated that the AR can be interpreted in terms of increased non-homologous end-joining of DSB or increased homologous recombination (Vaganay-Juery *et al.*, 2000; Marples *et al.*, 2004; Raaphorst *et al.*, 2006). On the other hand PARP may not be involved in the induction of AR after treatment with alkylating agents in mouse bone marrow cells (Guruprasad *et al.*, 2002). An AR was found to be absent in some radiosensitive tumor lines and ataxia telangiectasia patients cells (Lambin *et al.*, 1994). In our experiments the highly radioresistant *Chlamydomonas reinhardtii* strain H-3 surprisingly showed a clear adaptive response (Figure 3). These results show that the already enhanced ability of strain H-3 to repair radiation damage, evidenced by its radioresistance to single doses of radiation, does not prevent this strain from 'adapting' still further following a priming dose of radiation (Chankova *et al.*, 2005). In contrast, the level of AR from Bloom syndrome (human autosomal recessive disorder, characterized by chromosomal instability and increased risk of malignancy at an early age) patients blood cells has been shown to be the same as that in control cells from healthy donors (Zasukhina *et al.*, 2000). The hamster cell line EM9, which is SSB repair-deficient, can also develop an AR (Skov *et al.*, 1994). CHO cells mutated for different components of the nucleotide excision repair pathway do not express mutation and/or survival AR (Hafer *et al.*, 2007). In spite of the variation in AR in different systems, it has been postulated that AR analysis could be used for assaying DNA repair capacity (Sasiadek *et al.*, 2002). It has also been suggested that membrane damage may switch on some of these responses (Skov *et al.*, 1994) and/or be reduced as a result of AR (Girigoswami and Ghosh, 2005; El-Tayeb *et al.*, 2006).

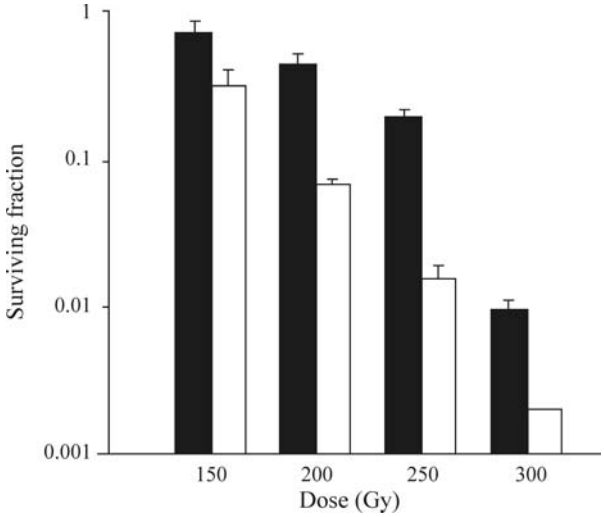

Figure 3 - Adaptive response measured as cell surviving fraction in the radio resistant strain *Chlamydomonas reinhardtii* H-3. The effect of a conditioning dose (150 Gy) on the response of cells given a series of test doses four hours later is shown. (❑) with conditioning dose (150 Gy); (■) without conditioning dose. Modified from Chankova *et al.* (2005).

Does the AR operate via up-regulation of DNA repair?

DNA damage due to hydroxyl radicals derived from the radiolytic decomposition of H_2O produces lesions that strongly induce DNA repair mechanisms (Boreham and Mitchel, 1991). Experiments in various biological systems have been performed to test the hypothesis that DNA repair up-regulation could be involved in the AR. There is evidence that DNA repair underlies the AR induced by low radiation doses in human and plant cells (Lambin *et al*, 1994; Patra *et al.*, 2003) by increasing the amount and rate of DNA repair (Joiner *et al.*, 1996; Joiner *et al.*, 1999). For example, when peripheral blood mononuclear cells from residents of Ramsar (a high natural background radiation area) were irradiated with a challenging dose of gamma rays, Mohammadi *et al.* (2006) detected lower levels of micronuclei, higher numbers of apoptotic cells and enhanced DNA repair. It has been proposed that these effects could be related to the induction of an AR. The AR induced by conditioning UVB exposures in *Euglena* may not be due to biosynthetic UV-absorbing compounds, but to the induction of photolyase enzymes (Takahashi *et al.*, 2006). Activation of UVB-induced AR in human skin cells could involve a p53-dependent gene program with p53-induced cell cycle arrest and DNA repair (Decraene *et al.*, 2005). The study of repair kinetics of DNA damage in Chinese hamster V79 cells showed that the radioadaptive response could be a result of DNA repair mechanisms which lead to less residual DNA damage, but not from the induction of protective mechanisms that reduce the initial DNA damage (Ikushima *et al.*, 1996). An adaptive response was observed through micronuclei formation and neoplastic transformation in murine 10T1/2 cells and the authors postulated that this adaptive response resulted from an enhanced DSB repair (Azzam *et al.*, 1994). This would be in agreement with our finding of accelerated DSB rejoining in *C. reinhardtii* following a conditioning dose of gamma rays (Chankova and Bryant, 2002) or radiomimetics (Chankova *et al.*, 2007) presented in Figures 4 and 5. A reduction in deletion-type mutants in adapted cells may also be a result of DSB DNA repair in various cell systems (Rigaud and Moustacchi, 1996).

However, as Szumiel (2005) pointed out, the view that DNA repair is stimulated in the 'primed' and challenged cell is not supported by all the available data. For instance, at least in some cases the AR may have no connection with modification of repair processes (Tskhovrebova and Makedonov, 2004). In such cases, the AR could be partly due to diminished fixation of DSBs (Szumiel, 2005). For example, induction of AR has been observed in terms of reduced initial DNA damage as well, which could be due to increased oxidative defense processes or to other undefined molecular processes, *e.g.* perturbation of cell cycle progression (Atanasova *et al.*, 2005; Cramers *et al.*, 2005).

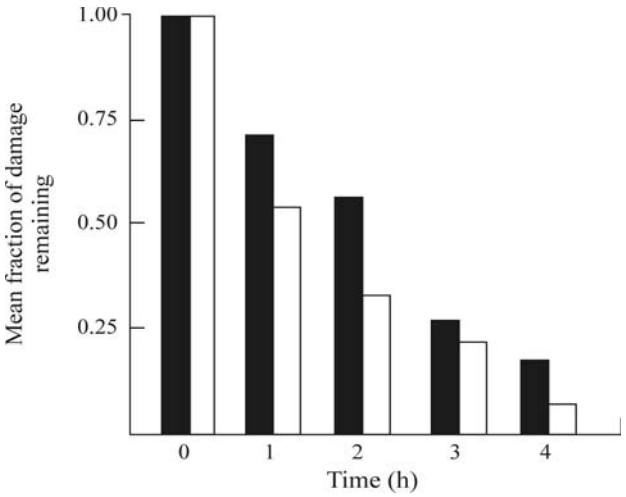

Figure 4 - Rejoining kinetics of DNA double-strand breaks in *Chlamydomonas reinhardtii* CW15 following a test dose of 500 Gy, with (❑) or without (■) a conditioning dose (50 Gy), given four hours before the test dose. Error bars are not visible because the standard error is too small. Modified from Chankova and Bryant (2002).

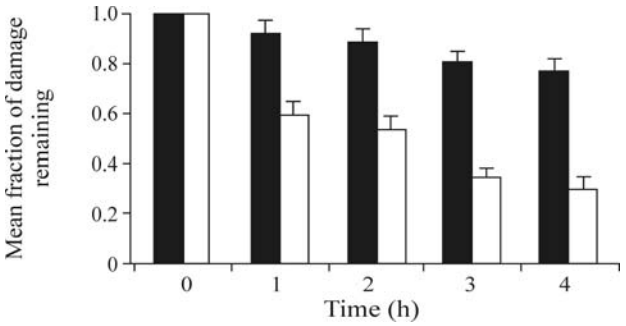

Figure 5 - Rejoining kinetics of DNA double-strand breaks in *Chlamydomonas reinhardtii* CW15 following a test dose 300 μg mL^{-1} zeocin, with (❑) or without (■) a conditioning dose (10 μg mL^{-1}), given four hours before the test dose. Modified from Chankova *et al.* (2007).

Some evidence indicates that the H_2O_2 induced AR in cultured human retinal pigment epithelium (RPE) cells involves increased nuclear DNA protection but no adaptive benefit for mtDNA protection or repair (Jarrett and Boulton, 2005). Hence, it has been suggested that the AR could be an important antioxidant defense for cells located in inherently oxidizing microenvironments. However, mitochondria have been viewed as a weak link in this defense mechanism which would contribute to aging and age-related disease (Jarrett and Boulton, 2005).

Does the AR involve induction of new proteins synthesis?

Little is known about the proteins and genes involved in adaptive responses in cells. Changes in gene transcriptional levels have been found after exposure to ionizing ra-

diation with low doses that result in the induction of AR (Wolff, 1998; Coleman *et al.*, 2005; Lanza *et al.*, 2005). A clue as to the nature of the underlying process was provided by results showing a dependence on *de novo* protein synthesis. Treatment of *Oedogonium*, *Chlamydomonas* and *Closterium* cells with protein synthesis inhibitors (cycloheximide and chloramphenicol) after the first 'conditioning' dose prevented the induced repair responses in these organisms (Horsley and Lazlo, 1971; Bryant, 1975; Howard and Cowie, 1978; Chankova and Bryant, 2002). The synthesis of DNA-binding proteins (MWs 50, 74 and 130 kdal) was found in radiation-conditioned cells of *C. reinhardtii* (Bryant, 1979). Our previous work showed an up-regulation of DNA DSB rejoining four hours after irradiation of *C. reinhardtii CW15* (a Cell-Wall-less mutant with WT radiation response) that was strongly reduced when cells were treated with the protein synthesis inhibitors cycloheximide and chloramphenicol (Chankova and Bryant, 2002) (Figure 6).

The induction of new protein synthesis by low doses could be caused by an effect of low doses on chromatin conformation near genes coding for DNA repair proteins (Belyaev *et al.*, 1996). The AR to alkylating agents in *Escherichia coli* is thought to be related to an increased expression of genes which encode DNA repair proteins (aidB, ada, alkA, alkB) (Rohankhedkar *et al.*, 2006). The AidB component of *E. coli* AR to alkylating agents has been identified as a flavin-containing DNA-binding protein and has been predicted to catalyze the direct repair of alkylated DNA (Rohankhedkar *et al.*, 2006).

Robson *et al.* (1999) isolated a novel gene from L132 cells that is down-regulated in response to ionizing radiation (*DIR1* gene) which they postulated played a regulatory role in the AR. Later research on cells showing low-dose hypersensitivity (V79, RT112 and UM-UC-3) showed that antisense oligonucleotides against the *DIR1* gene resulted in an increased rate of rejoining of DNA single-strand breaks coupled with an increase in cell survival after a dose of 2Gy (Robson *et al.*, 2000). However, ataxia telangiectasia cells (ATBIVA), which do not show low-dose hypersensitivity, did not show such enhanced repair and survival. The authors concluded that radiosensitive cells such as those from AT patients lack the ability to switch on the *DIR1* gene. Not all examples of induced AR involve *de novo* protein synthesis. For example, there are earlier observations that hydrogen peroxide induced a cross-adaptive response to cumene hydroperoxide in *E. coli* which did not require novel gene products but involved modification of the small subunit of Ahp, a protein involved in the protection against alkyl hydroperoxides (Asad *et al.*, 1998).

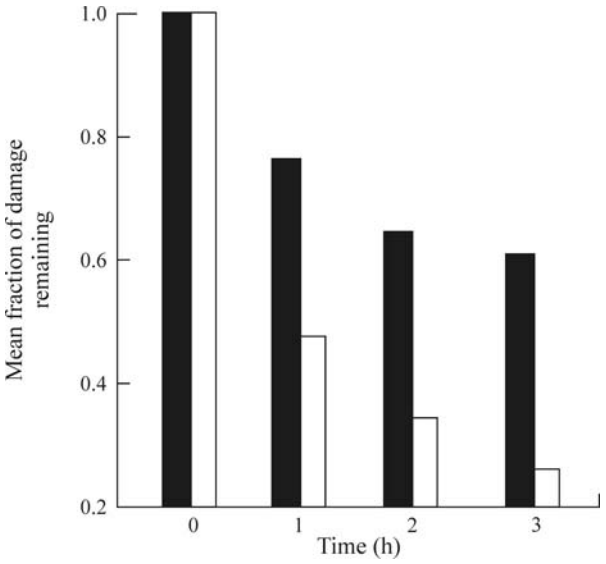

Figure 6 - DNA double-strand breaks rejoining in *Chlamydomonas reinhardii* CW15 after irradiation (500 Gy) in the presence (■) or absence (❑) of cycloheximide (10 µL mL^{-1}) in combination with chloramphenicol (100 µL mL^{-1}). Error bars are not visible because the standard error is too small. Modified from Chankova and Bryant (2002).

Could activation of antioxidant systems have a role in induced resistance?

Mendez-Alvarez *et al.* (1999) proposed that the cell ability to induce AR could be affected by altering cellular oxidative stress levels. Enzymes are considered as a very important component of cell defense mechanisms which protect organisms from the harmful action of ROS damaging DNA and other biomolecules. It is assumed that enzymatic, non-enzymatic and indirect antioxidant defense systems could be involved in the formation of AR to oxidative stress (Mendez-Alvarez *et al.*, 1999; Chen *et al.*, 2006; Yan *et al.*, 2006; Tosello *et al.*, 2007). Joksic *et al.* (2000) suggested that oxidative stress can trigger an antioxidant response that includes changes in the activity of enzymatic defense system, mainly SOD. Radioresistant variants isolated from MCF-7 human carcinoma cells following fractionated radiation or overexpression of MnSOD demonstrated dose-modified factors at 10% isosurvival (Guo *et al.*, 2003). The authors speculated that maybe the induction of MnSOD after fractionated doses caused a redox alteration that resulted in the up-regulation of stress response genes and radiation induced AR. Similarly, enhancement of the antioxidative capacities (catalase and MnSOD) probably played an important role in the reduction of initial DNA damage by low-dose-rate radiation in mice spleen (Otsuka *et al.*, 2006). DNA microarray analysis has revealed that GPX1, CAT, SOD1 and several other genes involved in peroxidase activity were up-regulated after low-dose X-ray exposure of HUVEC cells (Lanza *et al.*, 2005). The AR of yeast cells induced by the oxidants H_2O_2, menadione and juglone was associated with an increase in the activity of cellular catalase, SOD, glucose-6-phosphate dehydrogenase, and glutathione reductase, the main enzymes involved in cell defense against oxidative stress (Biryukova *et al.*, 2006). Enhancement of these antioxidant

activities could be involved in menadione-induced AR to menadione and to H_2O_2 in *Bacillus* sp. F26 (Yan *et al.*, 2006). Leisinger *et al.* (1999) described a glutathione peroxidase homologous gene in *Chlamydomonas reinhardtii* whose expression is up-regulated after treatment with different oxidative stress inducing agents (Leisinger *et al.*, 2001). Other studies indicated that some non-enzymatic antioxidants could affect the AR through binding and detoxification of the genotoxic chemical. For example, it has been recently demonstrated that the AR induced by sublethal concentrations of some oxisterols and prostaglandins in PC12 cells is mediated through elevation of cellular glutathione contents (Chen *et al.*, 2006). Similarly, pretreatment of murine and human cells with alkylating or 8-oxoG inducing agents prior to a test dose resulted in a twofold shift of cellular glutathione levels as an AR (Bercht *et al.*, 2007). *Chlamydomonas reinhardtii* has been reported to produce metal-binding peptides in response to stress induced by different heavy metals (Cd, Hg, Ag) (Howe and Merchant, 1992) and the cadmium-induced AR in *Allium cepa* was prevented after inhibition of phytochelatin synthesis (Panda *et al.*, 1997). Similarly, inhibition of metallothionein synthesis prevented the AR after heavy metal (Cu, Pb) conditioning treatment in *Vicia faba* (Rieger *et al.*, 1993). Inhibition of cytoplasmic protein synthesis prevented the AR induced by Cd in *Allium cepa* (Panda *et al.*, 1997). Inhibition of *de novo* protein synthesis by cycloheximide probably inhibited the Cu^{2+}-dependent metallothionein synthesis in human peripheral blood lymphocytes thereby eliminating the AR triggered by copper sulphate (Nikolova *et al.*, 1999).

On the other hand, ROS (*e.g.* hydrogen peroxide and nitric oxide) could serve as signal transducers in plant and animal cells (Neill *et al.*, 2002; Vranová *et al.*, 2002; Babu *et al.*, 2003; Matsumoto *et al.*, 2004). As signaling molecules, ROS might affect the development of AR through participation in the damage-sensing process after conditioning dose exposure. For example, in UV-irradiation experiments with human skin fibroblasts the addition of antioxidants reduced the cellular oxidative stress and adaptive response in a concentration-dependent manner (Jones *et al.*, 1999). Thus, it is possible that in some cases the increased scavenging of ROS by the antioxidant system might reduce the induced damage resulting in AR. The contribution of the antioxidant system for the development of AR could be further complicated by the fact that certain ROS are known to serve as signal transducers in plant and animal cells.

Miura (2004) showed an insignificantly increased level of the activity of CAT, GPx, GR and glutathione content after low dose and subsequently given higher dose of X-rays irradiation in rat glial cells. He therefore concluded that antioxidant defense can contribute only partly to the radiation induced AR in tested cells. The AR in fibroblasts derived from transgenic mice overexpressing the Cu/ZnSOD gene appeared to be unrelated to the amount of SOD in the cells and, hence, independent of superoxide radicals (Wolff, 1996).

Kinetics of the adaptive response

As summarized by Feinendegen (2005), adaptive protection develops with a delay of hours, may last for days to months, decreases steadily at doses above about 100 mGy to 200 mGy and is not observed anymore after acute exposures to more than 500 mGy. Indeed, there is abundant evidence that the adaptive response depends on the experimental design. The adaptive response was shown to be both dose and time-dependent with a maximal effect occurring several hours later, for example between four to six hours after exposure for the unicellular green alga *Chlamydomonas reinhardtii* (Bryant, 1976; Chankova *et al.*, 2005, 2007). The experimental design used by us for induction of AR by different genotoxic agents in the green alga *Chlamydomonas reinhardtii* as a test system is shown in Figure 7. We also observed a dose-dependence of the radiation-induced AR in *C. reinhardtii* when DSB DNA rejoining was used as an indicator (Chankova and Bryant, 2002). A small conditioning dose of gamma rays irradiation led to a small increase in the rate of DSB rejoining but when the magnitude of the conditioning dose was progressively increased there was a corresponding decrease in the fraction of damage remaining (Figure 8). Heat shock protection against induction of chromatid aberrations by clastogens in *Vicia faba* was found to be dependent on the time span (from less than ten minutes up to four hours) between the test dose and the adaptive treatment (Rieger and Michaelis, 1988). The time course of the adaptive response in lymphocytes was found to be similar to that in plant systems, reaching a plateau after about six hours (Shadley and Wolff, 1987). In murine leukocytes the minimum adaptive dose lies between 0.005 and 0.01 Gy of gamma rays and the early AR to a test dose of 1.0 Gy is induced as early as 30 min after the exposure and persists for at least 18 h (Morales-Ramírez and Mendiola-Cruz, 2004). Venkat *et al.* (2001) observed a maximum AR when a test dose of 100 cGy was given four hours after an adaptive dose, 30 h following the mitogenic stimulation of lymphocytes. The

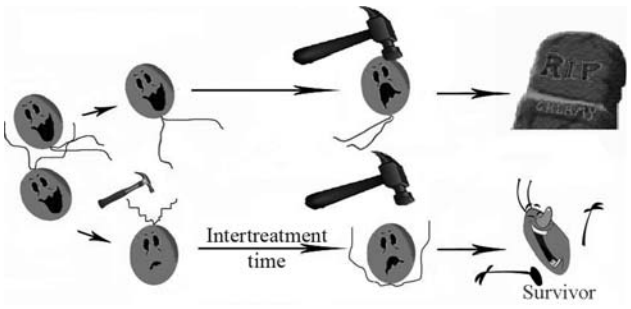

Figure 7 - Experimental design used for induction of the AR in *Chlamydomonas reinhardtii* as a model system.

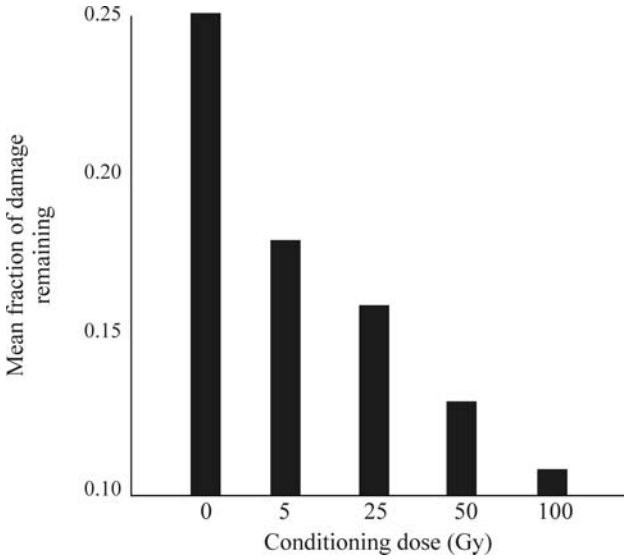

Figure 8 - The effect of increasing the magnitude of the conditioning dose on the fraction of DNA double-strand breaks remaining after a test dose of 500 Gy and four hours incubation in *Chlamydomonas reinhardtii* CW15. Error bars are not visible because the standard error is too small. Modified from Chankova and Bryant (2002).

protective effect against N-methyl-N'-nitro-N-nitrosoguanidine induced by prior treatment with H_2O_2 in *E. coli* is also time dependent, decreasing 15 min after the pretreatment and almost abolished after 30 min (Asad *et al.*, 1997).

Besides being brief, the AR to irradiation could also be modulated (Joiner *et al.*, 1996, 1999; Raaphorst *et al.*, 2000; Tiku and Kale, 2004), *e.g.* AR could be induced more effectively when a conditioning dose was given in small fractions (Tiku and Kale, 2004). It has been proposed that such fractionated irradiation of human fibroblasts could result in elevated survival due to repair of sublethal damage (Raaphorst *et al.*, 2000). The efficiency of cellular defense reactions that are activated can vary and depend on the level and type of the impact. It has been more recently reported that in some instances the conditioning dose can act synergistically, thereby increasing the frequency of chromosomal aberrations seen following the test dose (Matsumoto *et al.*, 2004; Zhou *et al.*, 2004). Different agents may have different impacts which could result in adaptive reactions of variable efficiency (Boreham and Mitchel, 1991; Marples and Joiner, 1993; Joiner, 1994; Schlade-Bartusiak *et al.*, 2002; Marples *et al.*, 2004; Matsumoto *et al.*, 2004). For example, acute doses of alpha-particles and other high Linear Energy Transfer (LET) radiations, such as neutrons, appear to be less efficient in eliciting an adaptive response than low LET radiations (*e.g.* X-rays) (Boreham and Mitchel, 1991; Marples and Joiner, 1993; Joiner, 1994) presumably since the level of local DNA damage is more severe and therefore immediately activates the G2 sensing systems (Marples *et al.*, 2004). However, some reports indicated that AR following neutron exposure could be observed in V79 Chinese hamster cells (Marples and Skov, 1996) or human lymphocytes *in vitro* (Gajendiran *et al.*, 2001).

Remarkably, it has recently been reported that in prostate cells of pKZ1 transgenic mice X-ray-induced 'reverse' AR could be observed when the high damaging dose preceded the low dose (Day *et al.*, 2007). This 'reverse' AR was of similar magnitude to the AR observed when the low dose was given first. These results may indicate that the mechanisms underlying AR may not be due to prevention of damage induced by the high dose but to modulation of the cellular response to this damage.

Relevance of the adaptive response

Low-dose radiation may have some beneficial effects, *e.g.* adaptive protection causing DNA damage prevention and repair and immune stimulation (Upton , 2001; Liu, 2003; Ghiassi-nejad *et al.*, 2004; Feinendegen, 2005). Low to intermediate doses of ionizing radiation have been observed to enhance growth and survival, augment the immune response, and increase resistance to the mutagenic and clastogenic effects of further irradiation in plants, bacteria, insects and mammals (Upton, 2001). Stimulation of immunity has been observed in human populations after long-term exposure to high level natural background radiation (Ghiassi-nejad *et al.*, 2004). Ghiassi-nejad *et al.* (2004) observed a significant increase of CD69 expression on TCD4+ stimulated cells and a significant increase of total serum IgE in Ramsar residents. Removal of damaged cells occurred *in vivo* by way of a low dose-induced immune competence (reviewed in Feinendegen, 2005). Nevertheless, some scientists argue that the AR does not appear to be a relevant mechanism for radiation protection because the worst outcome for the cell (cell death) is probably the best outcome for the organism as a whole since the low (conditioning) dose could also generate a risk of cellular transformation (Hofseth, 2004; de Saint-Georges, 2004). Indeed, there is abundant experimental evidence that chronic low-dose occupational exposure to ionizing radiation could result in adverse health effects (Lin and Mao, 2004) and increased DNA damage (Joksic and Spasojevic-Tisma, 1998; Cardoso *et al.*, 2001; Maffei *et al.*, 2002; Sari-Minodier *et al.*, 2002; Hadjidekova *et al.*, 2003; Zakeri and Assaei, 2004; Güerci *et al.*, 2006; Sari-Minodier *et al.*, 2007). Similarly, increased levels of DNA damage have been obtained for chromium platers (Benova *et al.*, 2002), offset printing workers (Aksoy *et al.*, 2005), welders (Iarmarcovai *et al.*, 2005; 2007), pathologists/anatomists exposed to formaldehyde (Iarmarcovai *et al.*, 2007), and patients taking certain phytopharmaceuticals (Lazutka and Mierauskiene, 2001) or after long-term low-dose antimicrobial prophylaxis (Slapyte *et al.*, 2002). An increased frequency of micronuclei has also been detected after *in vitro* irradiation of domestic animals lymphocytes (Danika and Dunja, 2007) but not in the lymphocytes of cattle raised in the vicinity of a nuclear power plant (Lee *et al.*, 2007). Conversely, very low-dose

rate chronic gamma irradiation induced a marked cytogenetic adaptive response to a subsequent higher dose in mouse germ cells and probably did not cause any risk of damaging effects to the offspring of the irradiated male animals (Cai and Wang, 1995).

Some authors consider that it is misleading to conceive the AR in terms of radioprotection because the AR is highly dependent on the genetic constitution and its measurement depends on the experimental design (Salone *et al.*, 1996). For instance, the genotoxic effect of radiation in occupationally exposed persons may or may not vary depending on alcohol consumption, age and gender (Maffei *et al.*, 2002; Hadjidekova *et al.*, 2003; Zakeri and Assaei, 2004; Iarmarcovai *et al.*, 2007; Sari-Minodier *et al.*, 2007). The increased frequencies of chromosomal aberrations and sister chromatid exchanges in radiation workers could indicate a cumulative effect of low level chronic exposure to ionizing radiation, pointing to the relevance of conducting cytogenetic analysis in addition to physical dosimetry in such cases (Cardoso *et al.*, 2001). Although cytokinetic and cytostatic effects have been detected in heavy and moderate smokers (Calderón-Ezquerro *et al.*, 2007), smoking habits may or may not affect the genotoxic effect of chronic radiation exposure (Maffei *et al.*, 2002; Hadjidekova *et al.*, 2003; Sari-Minodier *et al.*, 2007).

Nevertheless, a better understanding of the molecular mechanisms underlying the AR may lead to an improvement in radiation protection, *e.g.* of astronauts during long-term space flights, risk assessment and management, and cancer treatment strategies (Delone *et al.*, 1991; Wang and Cai, 2000; Upton, 2001; Takahashi *et al.*, 2002; Bonner, 2003; Mortazavi *et al.*, 2003a, 2003b; Liu, 2003; Schaffer *et al.*, 2004; Preston, 2005). In preliminary cytogenetic studies, after *in vitro* irradiation with a test dose of gamma rays, a strong cytogenetic AR was induced in the lymphocytes of residents of the very high background radiation areas of Ramsar (Ghiassi-nejad *et al.*, 2002). A study of the residents living in radioactively contaminated buildings in Taiwan suggested that chronic irradiation could actually be an effective prophylaxis against cancer (Chen *et al.*, 2004). In support of this idea, lifelong low-dose irradiation accompanied by immune activation was shown to result in suppression of thymic lymphoma induction in mice (Ina *et al.*, 2005). Low-dose irradiation has been used successfully for cancer therapy without causing significant symptoms or presenting significant risk (Cuttler and Pollycove, 2003). In line with these observations, some authors proposed additional coefficients reflecting the protective role of low-dose radiation to be introduced in the mathematical dose-response model for estimation of radiation risk (Scott, 2004; Feinendegen and Neumann, 2006).

In conclusion, a better understanding of AR could open up new approaches for protection of cells. The AR and the ability to modify it may play important roles in fractionated radiotherapy and can help to verify whether this phenomenon affects the estimation of the risk of low level radiation exposure. In order to more fully understand the AR, at least two hypothesis need to be tested: firstly, that radiation-induced AR may differ depending on the cell type and genotype; and secondly, that altering cellular oxidative stress levels have an impact on the ability of the cells to initiate the AR.

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