TABLE 2

Comparision of the reactivity(O.D. 405) of clones with TMV

Clone	PVY	TMV
PV08	0.954	0.325
PV19	1.315	0.245
PV24	0.957	0.315
PV31	1.226	0.246
PV41	1.329	0.280
Negative	0.237	0.237

play technology.

To production recombinant antibody fragment, variable (V) gene repertoires are harvested from spleenocytes of mice immunized PVY were used as a source of variable heavy and light chain gene and V genes were combined at random via a flexible linker by overlapping PCR and cloned into pCANTAB5E. Phage carrying scFv that encode binding activities were selected directly with purified PVY by biopanning. The selected five clones were specific for PVY, they did not show cross-reactivity with TMV (Table 2). Boonham and Borker developed single chain variable fragments against PVY from synthetic human scFv library (26). They showed variable light chains of scFv specific for PVY belonging to the V lambda 1 family. But in this study, we used primers specific for variable kappa light chains in the amplification of immunglobulin light chain. So that, single chain variable fragments contained kappa light chain instead of lambda chains.

These results obtaining by using different methodology than previously reported by Bloom et al. and demostrate that phage display technology can be used in production of recombinant antibody against plant virus and these clones (PV08, PV19, PV24, PV31 and PV41) can be used in diagnosis of PVY by immune diagnostic test systems and development of transgenic plants.

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TRANSPOSABLE ELEMENTS IN WHEAT AND TRITICALE SPHAEROCOCCUM MUTANT FORMS

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ABSTRACT

Sphaerococcum mutant forms obtained after EMS treatment in T.aestivum (AABBDD) and 6x-Triticale (AABBRR/2D) were studied at genetical, cytogenetical and molecular level. The initial genetic grouping was based on the existence of two types of sphaerococcum mutation; stable and unstable. Transposable elements like Ac/Ds from wheat and Triticale are described in both Sphaerococcum mutant forms. Cytogeneticaly detectable chromosomal rearrangements including the main briges were established only in unstable mutant forms. Southern hybridization analysis of the unstable mutant forms, 49L sph and 49L aest indicated the presence of Ac/Ds-like transposable elements in their genomes. The stable mutant form MT47 sph possesses Ds-like transposable element. Ac was present in the genome of mutant forms of 613, 6512, 49/202/S1, S.r2x 613 and the control form of T. aestivum (S.ranozreika 2). These observations provide a basis for the isolation and molecular characterization of the sphaerococcum locus in wheat and Triticale.

Introduction

One of the best known transposable element systems in maize, studied genetically in detail by McClintock (17, 18), consists of the autonomous element Activator (Ac) and Ac-dependent element Dissociation (Ds). McClintock showed that Ds can cause mutations in many genes which are unstable in the presence of Ac. McClintock's studies were done with genes whose phenotypes could be scored on kernels, including those controlling anthocyamin pigment syntesis in the aleurone and genes involved in starch biosynthesis.

According to Flavell (4, 5) many of the dispersed repeated sequences in plant genomes are or have evolved from transposable elements (TEs). In this respect we can assume that most plant genomes including those of wheat, contain some latent transposable elements, capable of being activated (to move) and cause mutations. However most of the TEs are silent. The silent elements are either: a/ mutant and incapable to transpose or being activated by another active element in the cell; b/ maintained silent by the methylation of key cytosine residues which prevents them being recognised by proteins facilitating their activation; and/or c/ maintained in highly condensed chromatin inaccessible to the enzymes which facilitate transposition (6).

In the past few years DNA sequences with structural characteristics of transposable elements have been recognized in wheat. An element has been found in an intron of an Adh-1 allele (Mitchell et al. according to Flavell et al./6/) and another close to an a-amylase 1 gene (6). Two new elements were described by Flavell et al. (6) in wheat genome similar to retrotransposones. The first one WIRE-1 was discovered only in wheat genome and was not present in an Aegilops species (6). The second one was insertion (Wis-2) in the Glu-A1 allele of Ch. Spring.

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 53, No 1, 2000

BIOLOGIE
Génétique

UV-C IRRADIATION AFFECTS THE CLEAVAGE OF CHLAMYDOMONAS NUCLEAR DNA BY RESTRICTION ENDONUCLEASE Tru 9I

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(*Submitted by Corresponding Member A. Mehandjiev on July 22, 1998*)

It is acknowledged that cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts are the main lesions produced in DNA by UV light [1]. CPD-induction in naked DNA follows its absorbance maximum – the most effective wavelength appears to be 260 nm, coinciding with the UV-C emission (190–290 nm). Although within the UV spectrum reaching the earth surface UV-C is practically lacking, this wavelength is responsible for 75% of all damage products induced by UV irradiadion [2].

The distribution of CPDs along DNA is sequence-dependent as they are formed at TT, TC-CT and CC dinucleotides at a frequency of 50, 40 and 10%, respectively [3]. The presence of CPDs leads to a local distortion of DNA double-helix [4] which might affect the mode of action of the restriction endonucleases (REs). Initially this proved to be the case when plasmid (pBR322) DNA was irradiated with 254 nm UV light and subsequently digested with appropriate REs. It was suggested that the extent of cleavage block might reflect the dimer formation at the corresponding recognition sequence [5]. Later, it was shown that CPDs formation prevents DNA digestion at gene and chromatin levels inhibiting in addition the chromosome-breaking ability of REs [6,7].

The data concerning the biological consequences of CPD induction are coming mainly from microorganisms and mammalian cells. After identification of several UV-sensitive *Chlamydomonas reinhardtii* strains [8], this unicellular green alga became attractive for such investigations. Our study is aimed to check whether the changes in the cleavage efficiency of REs due to the UV-C irradiation might be utilized as a reliable criterion for induced pyrimidine dimers in *Chlamydomonas reinhardtii*.

Materials and methods. Chlamydomonas strains UVS-1, 137C(+) and AK 9-9 with different UV-C sensitivity were used. The first two are a kind gift from Prof. Tugarinov's Laboratory, Sanct-Petersburg State University, Russia. 137C(+) is a wild type strain. UVS-1 is deficient in dark repair of pyrimidine dimers [9]. Strain AK 9-9 is obtained by chemical mutagenesis (Chankova et al., unpublished results).

IRRADIATION CONDITIONS. A low-pressure BLM-12 Hg germicidal lamp (254 nm wavelength) was used as UV source at a fluency of 1.5 $J/m^2/s$ measured with Intelligent

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 54, No 6, 2001

BIOLOGIE
Génétique

CHARACTERIZATION OF NEW RADIORESISTANT STRAINS OF *CHLAMYDOMONAS REINHARDTII*

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(*Submitted by Corresponding Member A. Mehandjiev on September 18, 2000*)

Radioadaptive elite constituted in chronically irradiated populations could be considered as a result of induced genetic alterations and a selection of more radioresistant individuals [1]. Numerous processes may be involved in cell defence system intended to protect living organisms from environmental damage [2]. Some correlation has been obtained between a spectrum, quantity of specific lesions produced in DNA, the efficiency of DNA repair and both cell survival and chromosome aberrations nevertheless that such correlation has not been found in some radiosensitive cell lines [3-5]. Plants possess numerous biochemical mechanisms protecting cells from the action of various physical and chemical mutagenic factors. Recently it has been proposed that some natural endogenous compounds, such as chlorophyll and carotenoids may inhibit mutagenic processes at various level and play a fundamental role in the process of antimutagenesis [6].

The isolation and characterization of mutants with various level of radioresistance is an appropriate tool for better understanding of cell sensitivity and mutability toward the action of different DNA damaging factors. That is why it is important to enlarge the existing collection of mutant strains of *Chlamydomonas reinhardtii.* The purpose of this work was to investigate 4 new mutant strains of *Chlamydomonas reinhardtii* induced by us in respect to their radioresistance at different levels: cellular, molecular and biochemical. A comparison between radioresistance of the strains, DNA-ssb repair activity and pigment contents (chl "a", chl "b" and carotenoids) was made.

Materials and methods. Strain 137C(+) (wild type) kindly provided by Dr. Tugarinov (Sanct Petersburg University) and 4 mutant strains – GK-1(+), GK-2(+), GK-3(+)and AK-9-9(-) were used in this work. Strains were obtained by the methods of chemical mutagenesis after treatment with 6-hydroxyaminopurine and 2-amino-6-hydroxyaminopurine. It is known that these chemical agents have an action similar to those of base analogues. An orientation test was made for differentiation of the mutants into nuclear or extranuclear. The test was based on evaluation of the growth of isolated colonies on media containing 70 mg/l and 50 mg/l canavanine, 100 mg/l and 500 mg/l streptomycin. Tetrad and complementation analysis were performed according to the method described by HARRIS [7]. Nutrition media-(L2) and cultivation conditions were described earlier [8]. The medium used for genetic analysis was prepared

CARYOLOGIA Vol. 55, no. 2: 99-103, 2002

CHIAS-based positioning of recombination hotspots and Giemsa bands in a multireconstructed barley karyotype

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Abstract -The chromosome complement of reconstructed barley karyotype PK 88 was analyzed by computer-aided Chromosome Image Analysis System (CHIAS). Fine mapping of Giemsa N-bands and regions with increased meiotic recombination activity along each individual chromosome was achieved. It was also found that CHIAS-visualized condensation profiles can be utilized as a reliable criterion for subtle differentiation of hetero- and euchromatin domains within a defined chromosomal regions. Application of CHIAS on karyotypes with distinct chromosome morphology was found to be an appropriate and reliable tool for screening of changes in chromatin compactness and its functional characteristics.

Key words: chromosome banding, *Hordeum*, image analysis, plant chromosomes, recombination, reconstructed karyotype.

INTRODUCTION

Automated chromosome analyses have been initially implemented in order to improve the accuracy and speed of karyotyping in mammalian cells (CASTELMAN and MELNYK 1976). The concomitant development of microelectronics and relevant software enabled the design of a computer-based chromosome image analyses system (CHIAS). In plants this approach was initially utilized for scanning of rye metaphase chromosomes (FUKUI 1986). Barley chromosomal complement was used to reveal the applicability of CHIAS for standardization of karyotyping and for screening of in situ hybridization (FUKUI 1988). The utility of chromosome imaging technique in cultured cells and plants treated with radiation or chemical mutagens was envisaged. CHIAS was proposed as a tool for investigation of the density characteristics of individual plant chromosomes (FUKUI and MUKAI 1988; NAKAYAMA and FUKUI 1997; KATO and FUKUI 1998).

Condensation pattern of chromosomes was also found to be an essential factor for intrachromosomal distribution of chromosomal alterations in mammalian genome. It was shown that condensed chromatin appears to be more resistant to radiation-induced damage than uncondensed one in CHO cells (SLIJEPCEVICH and NATARAJAN 1994). Clear indication was provided that chromosomes with high gene density are preferentially repaired in human cells (SURRALLES *et al.* 1997). At present the link between chromatin structure, DNA repair and chromosome fragility is widely accepted (SURRALLES *et al.* 1998).

Position of the Giemsa bands is thought to be informative for the intrachromosomal distri-

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DNA Repair 2 (2003) 983–990
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Induction and recovery of double-strand breaks in barley ribosomal DNA

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Received 6 February 2003; received in revised form 12 May 2003; accepted 20 May 2003

Abstract

Barley nucleolus organizing regions (NORs) were previously found to behave as prominent aberration hot-spots after treatment with some restriction endonucleases. The ability of MspI for directed induction of double-strand breaks in barley ribosomal DNA was further analyzed. Ionizing radiation-produced strand breakage within the ribosomal gene clusters was also a subject of investigation. Reconstructed barley karyotypes T1586 and T35 with normal and increased expression of rRNA genes were utilized to evaluate the relationship between transcriptional activity and damage induction. Scanning densitometry of the hybridization profiles revealed that MspI is generating double-strand breaks in barley rDNA with efficiency being independent from the NOR activity. Damage induction observed after treatment with γ-rays was also not influenced by the transcriptional status of the ribosomal genes. A tendency towards restoration of rDNA integrity after irradiation of both germinating and dry seeds was observed which is indicative for the efficient recovery of double-strand breaks in barley ribosomal DNA.

Keywords: Barley; Restriction endonucleases; Ionizing radiation; rDNA

1. Introduction

Maintenance of DNA integrity by the natural repair activities in cells exposed to various damaging agents is an essential function of living organisms preserving the genuine status of their heredity. The biological consequences of non-repaired (or miss-repaired) damage are thought to be dependent on the type and frequency of the lesions induced as well as on the functional characteristics and location of the target DNA. There are many data showing that certain types of damage are more effectively removed from transcriptionally active genes than from a silent regions indicating for the existence of intragenomic repair heterogeneity [1–3]. It is now widely acknowledged that transcriptional activity and higher-order chromatin organization are the main factors influencing the repair efficiency within a particular genetic loci or chromatin domains [4,5].

The first decisive evidence for the existence of gene-specific repair was the removal of cyclobutane pyrimidine dimers (CPDs) from the dihydrofolate reductase (DHFR) gene observed after UV-irradiation of Chinese hamster ovary cells (CHO) [6,7]. Subsequently the repair of transcriptionally active genes was referred to the recovery of CPD in the transcribed DNA strand by a process termed "transcription-coupled repair" (TCR) [8].

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doi:10.1016/S1568-7864(03)00095-8

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 57, No 9, 2004

BIOLOGIE
Génétique

LOOP ORGANIZATION OF RIBOSOMAL DNA IN BARLEY

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(*Submitted by Corresponding Member A. Mehandjiev on May 26, 2004*)

Abstract

The relative distribution of ribosomal DNA (rDNA) in loop and matrix-associated fractions isolated from reconstructed barley karyotypes with normal and suppressed NOR activity was analysed. It was found that loop organization of ribosomal RNA genes is probably implicated in the translocation-mediated nucleolar dominance in barley.

Key words: DNA loops, ribosomal RNA genes, nucleolar dominance, barley

Introduction. Barley ribosomal RNA genes in plants comprise DNA sequences coding for 18S, 5.8S and 26S ribosomal RNA (rRNA) separated by an intergenic spacer (IGS). In barley they are organized in repeated units localized at the nucleolus organizing regions (NORs) of chromosomes 6 and 7, respectively [1]. When both NORs are combined by reciprocal translocation on the same chromosome the activity of the transposed NOR is reduced [2]. The mechanisms underlying this phenomenon termed intraspecific nucleolar dominance are far from being understood [3,4]. An attractive but underestimated hypothesis is that the higher order of organization may influence the respective chromatin domains on such a position-mediated ribosomal gene expression.

It is widely accepted that DNA of the eukaryotic nucleus is organized into a series of supercoiled loops attached to a proteinaceous network called nuclear matrix or scaffold by regions designated as MARs (matrix associated regions) or SARs (scaffold associated regions), respectively. These topologically constrained loops are believed to form independent structural and functional domains in mammalian and plant genome [5,6]. It was found that localization of genes is not random in respect to the anchorage points of the loops, transcriptionally active ones being closer to the binding sites [7].

Higher order chromatin organization of rDNA was investigated in different animal species. A number of data shows that insoluble matrix fraction is enriched with rDNA but the character of attachment of the ribosomal genes remains controversial [8]. Our previous investigations have demonstrated that activation of the maize genome during germination correlates with DNA supercoiling and with appearance of defined proteinaceous network [9]. We have also found that matrix DNA is enriched with repetitive DNA [10]. Due to the lack of knowledge on spatial organization of plant rDNA the

This work is partially supported by IAEA research contract No 12608 (RBF).

We show (Figs.1-3) that in liver cells, not only aconitase activity is inhibited, but also the level of IRP1 protein is markedly lowered. IRP1 down-regulation in $SOD^{-/-}$ mice points to the existence of a new control mechanism that maintains a correct balance between iron and oxygen-derived free radicals.

Supported by the State Committee for Scientific Research (KBN) statutory grant for the INCT.

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CHROMOSOMAL ABERRATIONS, SISTER CHROMATID EXCHANGES AND SURVIVAL IN HOMOLOGOUS RECOMBINATION REPAIR DEFICIENT CL-V4B CELLS (Rad51C MUTANTS) EXPOSED TO MITOMYCIN C

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DNA interstrand crosslinks (ICL) pose a major problem for the cell during replication and transcription. The mechanisms of repair of ICL in mammalian cells are presently a topic of intense research.

Rad51C mutated CL-V4B cells [5] and wt cells V79B were exposed to different doses of MMC. The analysed endpoints included chromosomal aberrations, clonogenic cell survival and SCE.

Table. Chromosomal aberrations (per 100 cells) and percent aberrant cells in CL-V4B and V79B cells exposed to MMC. Results are the means and standard deviations of data from 3 independent experiments.

MMC dose [μM]	CL-V4B cells		V79B cells	
	Total aberrations	% aberrant cells	Total aberrations	% aberrant cells
0	1.7 ± 1.3	1.3 ± 1.2	0.3 ± 0.6	0.3 ± 0.6
0.1	40.0 ± 18.2*	21.3 ± 8.5*	0.7 ± 1.2	0.7 ± 1.2

* difference significant at $p<0.05$.

The major pathway of ICL repair appears to be homologous recombination repair (HRR) involving generation of a double strand break at the ICL and a subsequent recombination event with a homologous strand [1,2]. Recent data indicate that recombination independent pathways also exist [3].

Crosslinking agents such as mitomycin C (MMC) are extremely potent inducers of sister chromatid exchanges (SCE). It was estimated that they triple the SCE frequency with one thousandth the concentration required for inducing the same effect with monofunctional alkylating agents. This clearly points towards the ICL as the major lesion responsible for SCE.

Cells deficient in HRR are very sensitive to crosslinking agents (reviewed in [2]). It has been reported that MMC-induced SCE in the chicken B lymphocyte line DT40, mutated to obtain cells deficient in various genes involved in HRR, is lower than in the wild type (wt) line [4]. In the CL-V4B cells, which contain a mutated Rad51C paralog, no MMC-induced SCE can be observed in M1 [5]. These findings suggest that SCE are mediated by HRR [4].

MMC-induced SCE were analysed in the first (M1) and second (M2) post-treatment mitoses.

The analysis of chromosomal aberrations and survival confirmed that CL-V4B cells are very sensitive to MMC due to induction of ICL (Table).

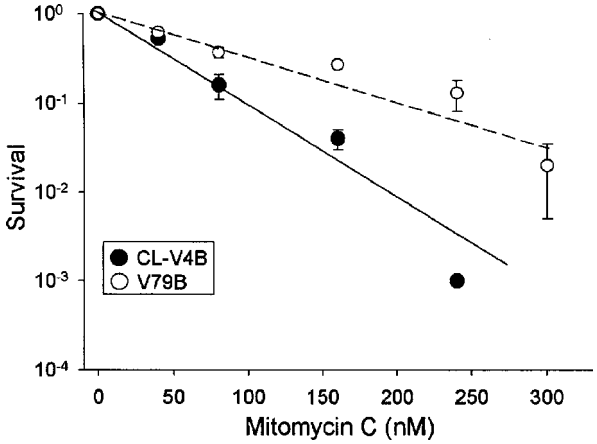

Fig.1. Clonogenic survival of CL-V4B and V79B cells following treatment with MMC. Error bars represent standard deviations from mean values of three independent experiments.

The survival of CL-V4B cells treated with MMC was lower than that of wt cells (Fig.1) but distinctly higher than reported by Godthelp *et al.* [5], possibly due to different culture conditions. The mutant cells showed the same frequencies of MMC-induced SCE as the wt cells (Fig.2). So, mutation in Rad51C did not affect SCE formation after MMC treatment. Additionally, while the wt cells showed the same frequency of MMC-induced SCE in M1 and M2, in CL-V4B cells somewhat more SCE were observed in M2 than M1 (Fig.2). This suggests that in Rad51C mutants ICL induced by MMC are either not removed completely or are transformed into another form of damage, which persists until the next cell cycle. Hence, SCEs may represent a mechanism to bypass MMC-induced ICL without their removal.

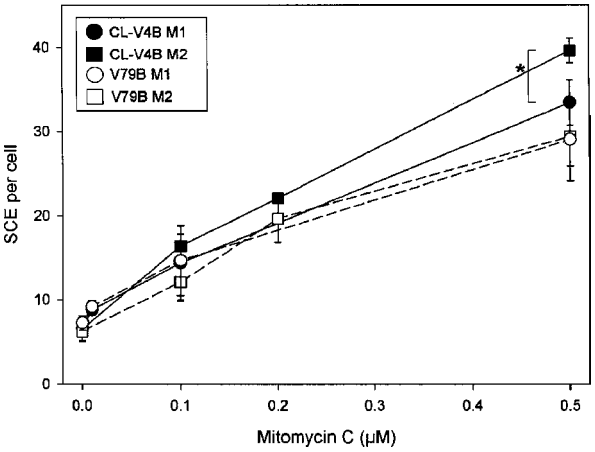

Fig.2. SCEs in first and second mitoses (M1 and M2) following treatment with MMC. Twenty cells were scored per point in each experiment. Error bars represent standard deviations from mean values of three independent experiments. * – difference significant at $p<0.05$ (two-sided, unpaired Student's *t*-test).

Supported by the State Committee for Scientific Research (KBN) statutory grant for the INCT.

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COMPARISON OF SISTER CHROMATID EXCHANGE INDUCTION IN Rad51C MUTANTS TREATED WITH MITOMYCIN C OR UVC

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The mechanisms of sister chromatid exchanges (SCEs) are not known. Cytologically, it is evident that SCE involves a recombination mechanism between sister chromatids. Available data indicate that SCEs arise during S-phase, and it was suggested that the SCE-initiating event is a hindrance of DNA replication by DNA damage [1,2]. A lesion that poses a major problem for the cell during replication is the DNA interstrand crosslink (ICL). One hypothesis is that SCE is a manifestation of Rad51--dependent homologous recombination repair. In order to test this hypothesis, we have compared the frequencies of SCEs induced by mitomycin C (MMC) and 254 nm ultraviolet radiation (UVC) in wild type (wt) V79B and the Rad51C-deficient CL-V4B cells.

In the present study, CL-V4B (mutated in Rad51C) and V79B cells were prelabelled with BrdU for one cell cycle and treated with different doses of MMC. In addition to MMC, we compared the SCE frequencies following exposure of both cell lines to 254 nm UVC. Along with pre-labelling cells with BrdU, the cells were prelabelled with biotin-16-2´-deoxyuridine-5´-triphosphate (biotin-dUTP) in order to exclude the impact of BrdU on the formation of DNA lesions by UVC [3].

SCEs were analysed in the first (M1) and second (M2) post-treatment mitoses. As shown in Fig.1, in M1 MMC induced the same frequencies of SCEs in CL-V4B and V79B cells, while the UVC-induced SCE frequencies were lower in CL-V4B than V79B cells. Following exposure to UVC, less SCEs were observed in CL-V4B than in V79B cells, irrespective of whether cells were prelabelled with BrdU or biotin-dUTP. We have shown recently that the strong sensitizing effect of BrdU towards UVC-induced SCEs may be due to ICL formation [3]. ICLs presumably arise as a result of the formation of bromine atoms and uracilyl radicals in BrdU-prelabelled cells following exposure to UVC. Biotin-dUTP lacks a halogen atom which dissociates upon exposure to UVC. Therefore, it can be assumed that SCEs that arise after exposure to UVC of cells prelabelled with this thymidine analogue are formed by cyclobutane pyrimidine dimers and (6-4) photoproducts. These lesions are efficiently

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Biochemical and Biophysical Research Communications 326 (2005) 805–810
BBRC
www.elsevier.com/locate/ybbrc

Rad51C-deficient CL-V4B cells exhibit normal levels of mitomycin C-induced SCEs but reduced levels of UVC-induced SCEs

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Received 16 November 2004
Available online 7 December 2004

Abstract

The mechanisms of sister chromatid exchanges (SCEs) are not known. One hypothesis is that SCE is a manifestation of Rad51-dependent homologous recombination repair. In order to test this hypothesis, we have compared the frequencies of SCEs induced by mitomycin C (MMC) and 254 nm ultraviolet radiation (UVC) in wt V79B and the Rad51C-deficient CL-V4B cells. SCEs were analysed in the first (M1) and second (M2) post-treatment mitoses. In M1 MMC induced the same frequencies of SCEs in CL-V4B and V79B cells, while the UVC-induced SCE frequencies were lower in CL-V4B than V79B cells. In CL-V4B cells, MMC-induced SCEs were higher in M2 than in M1, suggesting that interstrand cross-links (ICL) are either not removed completely or are transformed into another form of DNA damage that persists until the next cell cycle. We suggest that SCEs may represent a mechanism to bypass MMC-induced ICL without their removal.

Keywords: Sister chromatid exchanges; Homologous recombination; Rad51C; UV radiation; Bromodeoxyuridine; Mitomycin C

Sister chromatid exchanges (SCEs) are induced by a variety of mutagenic compounds [1]. The molecular mechanisms of SCE formation are not known, although a number of mechanistic models have been proposed [2–9]. It appears that SCE induction is not influenced by a defect in mismatch repair [10] and that SCEs occur predominantly within damaged, transcriptionally active regions of the genome [11].

Cytologically, it is evident that SCE involves a recombination mechanism between sister chromatids. Available data indicate that SCEs arise during S-phase [12,13], and it was suggested that the SCE-initiating event is a hindrance of DNA replication by DNA damage [4,5]. A lesion that poses a major problem for the cell during replication is the DNA interstrand cross-link (ICL). The main pathway of ICL repair appears to be homologous recombination repair (HRR) involving the generation of a double-strand break at the ICL and a subsequent recombination event with a homologous strand [14,15]. A key protein in this process is Rad51 [16], although recombination-independent pathways also exist [17].

The data on the involvement of Rad51 in SCE formation are somewhat inconsistent [18]. For example, chicken DT40 cells with knocked-out *Rad51* paralogs express lower spontaneous and MMC-induced SCE frequencies [19,20]. It was recently suggested that this reduction is due to the elimination of highly damaged cells [21]. No MMC-induced SCEs at all were observed in CL-V4B cells, which have a mutated *Rad51C* paralog [22].

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0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.11.113

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 61, No 9, 2008

BIOLOGIE
Biologie moléculaire

RIBOSOMAL RNA GENE EXPRESSION IN RECONSTRUCTED BARLEY KARYOTYPES

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(*Submitted by Corresponding Member I. Pashev on June 18, 2008*)

Abstract

Ribosomal RNA gene expression in barley lines with modulated activity of nucleolus organizers (NORs) caused by three different types of chromosomal rearrangements was studied. The lack of the whole rRNA gene cluster residing in chromosome 6H of the homozygous deletion line T-35 led to a compensatory effect in the expression of the single NOR remained on chromosome 5H, resulting in increased rRNA transcription. The enhanced rRNA gene transcription in this line was accompanied by an increased rate of transcript elongation. The repositioning by translocation of NOR of chromosome 5H to the long non-satellite arm of chromosome 6H did not cause any alterations in the "run-on" transcription, as indicated by the activity of RNA polymerase I, and in the susceptibility of rDNA chromatin to DNase I digestion, thus suggesting that the chromosome reconstruction did not affect the number of active rRNA genes involved in transcription. No alterations were also observed in segment tetraploid D-2946, which contained a duplication of medial region of satellite arm of chromosome 6H.

Key words: RNA polymerase I, DNase I sensitivity, ribosomal RNA genes, interspecies nucleolar dominance, barley

Introduction. In eukaryotes ribosomal RNA (rRNA) genes are organized in tandemly repeated units consisting of 18S, 5.8S and 26S coding region separated

This work is supported by the National Science Fund of the Bulgarian Ministry of Education and Science under Grant No B 1529-05.

Environmental and Molecular Mutagenesis 49:000–000 (2008)

Research Article

Assessment of DNA Strand Breaks Induced by Bleomycin in Barley by the Comet Assay

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Comet assay was applied to study induction and repair of DNA damage produced by bleomycin in barley genome. Experimental conditions were adapted to achieve efficient detection of both DNA single- and double-strand breaks. Substantial increase of the parameter "% of DNA in tail" was observed coupled with almost linear dependence from bleomycin concentration, more pronounced for the induction of DNA double-strand breaks. Data obtained at different recovery periods displayed rapid restoration of breakage, revealing that efficient mechanisms for repair of strand discontinuities induced by bleomycin are functional in barley DNA loop domains. Environ. Mol. Mutagen. 49:000–000, 2008. © 2008 Wiley-Liss, Inc.

Key words: barley; bleomycin; comet assay; DNA strand breaks

INTRODUCTION

Comet assay is a fast, reliable, and sensitive method for detection of single- and double-strand DNA breaks (SSB and DSB) in individual eukaryotic cells [Östling and Johanson, 1984; Singh et al., 1988; McKelvey-Martin et al., 1993; Fairbairn et al., 1995; Koppen and Verschaeve, 1996; Collins, 2004]. Although initially designed for animal cells, it was successfully applied also to isolated nuclei from different plant sources for analyses ranging from environmental monitoring of DNA damage to programmed cell death [Olive and Banath, 1995; Lesniewska et al., 2000].

DNA DSB exhibit substantial influence on genome stability, cell-cycle progression, and development. Such "detrimental DNA lesions" [Dip and Naegeli, 2005] can be generated directly or indirectly by ionizing radiation (IR), ultra violet light, chemotherapeutic or radiomimetic agents, and soil pollutants or endogenously by free radicals, recombination and replication errors, transposition, transduction, transformation and conjugation in bacteria, mating-type switching in yeast, V(D)J recombination in vertebrate, and various endonucleases [Pastink et al., 2001; Jackson, 2002; Migliore and Coppede, 2002; Willers et al., 2004; Karagiannis and El-Osta, 2004]. Incorrect DSB repair can lead to accumulation of mutations, chromosomal rearrangements, and aneuploid daughter cells or cell death due to replication errors [West et al., 2004].

Initial applications of the comet assay in plants were directed toward the impact of IR on seed disinfection and preservation from diseases and pathogens [Cerda et al., 1993, 1997; Koppen and Cerda, 1997]. Studies on DSB induction usually display relatively low sensitivity of the assay under neutral conditions (neutral lyses/neutral electrophoresis, N/N protocol). Koppen and Angelis [1998] found an increase of about 3% in DNA migration after X-ray treatment of *Vicia faba*. Values of the same magnitude were detected when DSB generated during aging were monitored in *Nicotiana tabacum* and *Vicia faba* [Koppen et al., 1999]. N/N comet assay, however, proved to be adequate for estimation of DNaseI-induced DSB in the presence of $MnCl_2$ [Menke et al., 2000a], although being less sensitive than alkaline lyses/neutral gel electrophoresis (A/N), used to follow repair of damage induced by *N*-methyl-*N*-nitrosourea (MNU) and UVC in barley genome [Jovtchev et al., 2001; Armalyte and Zukas, 2003]. Data obtained so far in plants by the N/N protocol display relatively high control levels of % DNA in the tail [Koppen and Angelis, 1998; Angelis et al., 1999; Menke

Grant sponsor: National Science Fund of Bulgaria; Grant number: G-1-01; Grant sponsor: IAEA Research Contract Bul; Grant number: 12608/RO/RBF.

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Received 10 May 2007; provisionally accepted 11 February 2008; and in final form 26 February 2008

DOI 10.1002/em.20396

Published online in Wiley InterScience (www.interscience.wiley.com).

ARTICLE

DOI: 10.2478/V10133-010-0082-7

COMPARATIVE ANALYSIS OF DATA DISTRIBUTION PATTERNS IN PLANT COMET ASSAY

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ABSTRACT

Screening capabilities of the comet assay have a real potential to study the impact of radiation and different mutagenic sources on induction of damage in DNA in plant nuclei. Heterogeneity of DNA damage data obtained by the application of different agents leads to an inconsistency and variation of the experimental outcomes, obtained after assessment of the comet populations. Although the potential of the comet assay technology has been clearly demonstrated, many important and interesting statistical questions remain. In this respect, different types of data need different statistical designs and this usually makes statistical analysis problematic. We advocate here a greater attention to different classical statistical distributions which best fit to plant comet data. We also demonstrate that all distribution patterns of the % of DNA in tail can be fitted by a Johnson SB distribution.

Biotechnol. & Biotechnol. Eq. 2010, **24**(4), 2142-2148

Keywords: comet assay, distribution patterns, DNA damage data, % DNA in tail

Abbreviations: BLM: bleomycin; KS: Kolmogorov-Smirnov; χ^2: Chi-Square; GEV: Generalized extreme value distribution; JSB: Johnson SB distribution; PF: Power Function distribution; D4: Dagum (4P) distribution; D: Dagum distribution; F: Frechet (3P) distribution; GP: Generalized Pareto distribution; LL: Log-Logistic (3P) distribution; P: Pert distribution; GM: Gumbel Max distribution; R: Rayleigh (2P) distribution; T: Triangular distribution; GG: Generalized Gamma distribution; K: Kumaraswamy distribution.

Introduction

The comet assay is a useful and sensitive method for quantification of DNA damage and repair *in vivo* and *in vitro* in eukaryotic and some prokaryotic cells, and a tool to study factors affecting mutagenicity. Comet assay already has been used as versatile and comparatively new approach in genotoxic research, ecotoxicology, radiation and micro beam biology, as well as in biomonitoring and experimental data modelling. Initially studies with this methodology were performed in animal (21) and human model systems (reviewed by 26), but in the last decade of the 20th century the standard comet procedure undergoes changes leading to its use for adequate assessment of the damage of plant DNA. Hence, research interests are directed towards prediction of direct or indirect influence by physical agents, different types of radiation, or genotoxic compounds such as alkylating, crosslinking, and oxidizing agents. (12, 20).

The correct selection of statistical models for experimental data analysis is a key factor in comet assay studies. The choice of directly observed experimental unit (8, 14) is considered as an essence for a successful application of statistical test for comet assay experimental work. The assumptions of the distribution of the values of this experimental unit may cause differences in the receipt of response and to influence the further conclusions. For assessment of the effect, at first was used an empirical method of visual scoring (10, 17) or conversion to pseudo-percentage score (5), followed by advanced image analysis systems (17). The latter enforces more detailed statistical analysis. An essential part of the statistical analysis is the application of different distributions of the experimental comet assay data providing a broad background for unbiased estimates as well as improved estimates of the mean and variance of exposures (7). Moreover, by definition all models for statistical analysis depend on the sample size. A smaller number of records (smaller than 50 comets per slide) in the analysis might lead both to a masking of the significant levels of the effect after treatment with different concentrations of agents and to a bias in outcomes regardless of the statistical approach. In some recent studies, the preferred number of comet formation exceeds 100 per slide.

In most plant studies, experimental unit is selected from the values of the tail moment, the tail length, the tail intensity, or the percentage DNA in tail. These observed parameters are usually presented by their mean values (16, 18, 28) or median ratio (13), comprising the standard deviation for the studied populations.

The diversity of the data in comet populations rarely fit normal distribution, limiting in this manner the usefulness of the parametric statistical tools. Among several approaches intended to solve this problem, the transformation of the data followed by modifying through function (3, 15) emerges as a very popular one.

Molecular variability in barley structural mutants produced by gamma-irradiation

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Abstract

Single Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) markers were used to survey the gamma-rays-induced genetic variation in a set of 13 originally produced structural barley (*H. vulgare* L.) mutants from cv. Freya including 8 single translocation lines, 3 double translocation lines and 2 multiple reconstructed karyotypes. Both marker systems contributed to the evaluation of the radiation-induced DNA alterations and revealed in general 0.49% polymorphisms in the studied genotypes. AFLPs were observed with 3 out of 10 PstI/MseI primer combinations. Transmissible microsatellite instability at loci with perfect (AT)n repeats located in the introns of the *rubisco activase* and *waxy* was documented in 3 mutant lines. The obtained results emphasized that in addition to the point mutations, the steadily transmitted to progeny small indels (2bp) form the major fractions of the gamma-induced DNA alterations in the analyzed barley mutant genotypes.

Keywords: radiation mutagenesis, AFLPs, SSRs, *Hordeum vulgare* L.