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Intrachromosomal mapping of chromatid aberrations induced by restriction endonucleases in barley

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Abstract The intrachromosomal distribution patterns of chromatid aberrations induced by the restriction endonucleases *Hpa*II, *Msp*I and *Hae*III in a multi-reconstructed barley karyotype were analysed. All of these endonucleases, which differ with respect to their DNA recognition sequences but produce only one type of DNA damage, showed nearly the same pattern of localized chromosome breakage. The most pronounced aberration hot spots proved to be the loci of transcriptionally active and condensed, inactive, rDNA. Possible mechanisms involved in the specific distribution of induced aberrations along the distinct chromosomal regions are discussed.

Key words Restriction endonucleases · Chromatid aberrations · Intrachromosomal distribution · rDNA · *Hordeum vulgare*

Introduction

Non-random patterns of the intrachromosomal distribution of chromatid aberrations have been established for a variety of mutagenic factors, including ionizing radiation (Holmberg and Johansson 1973; Schubert and Rieger 1976), biotic agents (Kato 1967), and substances belonging to different chemical classes (cf. Kihlman 1966; Rieger et al. 1975; Schubert and Rieger 1977).

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In spite of the abundant literature on the subject, the mechanisms underlying this phenomenon, which is known as the regional specificity of mutagens, are far from being understood. Nevertheless, there is clear evidence that mutagens with a non-delayed effect generally show much less-pronounced aberration hot spots than those with a delayed mode of action (Schubert and Rieger 1977). Moreover, such mutagens as maleic hydrazide and mitomycin C, which belong to different chemical classes, have produced similar patterns of aberration distribution (Rieger et al. 1977; Gecheff 1991).

It is generally accepted that chromosomal rearrangements arise as a result of misrepair processes of initially induced DNA lesions, the latter being mainly double-strand breaks. If this is so, the question as to whether a specific distribution of the initial chromosomal lesions along the chromosomes, and/or the heterogeneity of their repair among the chromosomal regions involved, is responsible for the regional specificity of the mutagens seems to be of primary importance. Although the non-random distribution of damage and repair in different chromatin domains has been unequivocally established (Bohr et al. 1987; Terieth et al. 1991; Boulikas 1992; Natarajan et al. 1994), the relationship between the phenomenon mentioned above and the specific clastogenic activity of mutagens along the chromosomes remains obscure at present.

It has already been established that restriction endonucleases (REs) are efficient inducers of chromosomal aberrations both in mammalian (for a review see Bryant 1988; Bryant and Johnston 1993) and plant cells (Stoilov et al. 1996). REs could be effectively utilized in elucidating the mechanisms underlying the regional specificity of mutagens, since they recognize defined DNA sequences and produce only one type of initial DNA damage, namely double-strand breaks. Surprisingly, the available data on this point are rather scarce. Recently a clear association has been found between the occurrence of aberrations and intercalary telomeric

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Inhibition of repair of X-ray-induced DNA double-strand breaks in human lymphocytes exposed to sodium butyrate

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Abstract.
Purpose: Sodium butyrate is known to inhibit histone deacetylase enzymes and to enhance the frequencies of X-ray-induced dicentrics and rings in human lymphocytes. In this study an investigation was made of the mechanisms underlying this enhancement by assessing the effect of sodium butyrate on the extent of X-ray-induced DNA damage and its repair in human peripheral blood lymphocytes.
Methods and materials: Unstimulated G_0 lymphocytes were pretreated for 24 h with sodium butyrate at a final concentration of 5 mM, irradiated with different doses of X-rays and then analysed for different endpoints either immediately or after different repair periods. The frequencies of DNA strand breaks were determined biochemically using nucleoid sedimentation, alkaline elution and immunochemical analysis as well as cytogenetically using the premature chromosome condensation (PCC) technique.
Results: The results show that sodium butyrate pretreatment does not lead to a significant increase of DNA double- or single-strand breaks nor to an increase of alkali labile base damage in G_0 lymphocytes. Moreover, sodium butyrate treatment had no effect on the initial frequency of chromosome breaks. However, PCC analysis clearly showed that the presence of sodium butyrate post-irradiation severely inhibited DNA double-strand break (DSB) repair, which most likely accounts for the increase in X-ray-induced chromosome aberrations.
Conclusions: Sodium butyrate treatment leading to changes in histone acetylation and increased accessibility of chromatin had no effect on the initial levels of X-ray-induced DNA damage. However, sodium butyrate may affect either the chromatin configuration or the enzymatic activities that play a key role in the repair of DSB.

1. Introduction

Sodium butyrate is a short-chain fatty acid that exerts pleiotropic effects in mammalian cells. The main direct effect of this compound, however, is the inhibition of histone deacetylase enzymes leading to hyperacetylation of histone core proteins (Kruh 1982). The hyperacetylation of histones causes modifications of chromatin as manifested by an increased rate of *DNase* I digestion consistent with a more open conformation of chromatin (Simpson 1978, Vidali *et al.* 1978). Among various effects associated with hyperacetylation of chromatin are G_1 arrest and inhibition of cell-cycle progression (Kruh 1982), alteration of transcription activity (Reeves and Cserjesi 1979, Turner 1991) and stimulation of nucleotide excision repair (Smerdon *et al.* 1982, Ramanathan and Smerdon 1989). In addition, evidence has been presented that sodium butyrate increases the induction of DNA damage by chemicals such as daunorubicin (Pani *et al.* 1984) and bleomycin (Lopez-Larraza and Bianchi 1993) in Chinese hamster cells, presumably as a consequence of the enhanced accessibility of chromatin. Enhanced accessibility of chromatin towards repair enzymes has also been implied to account for the marked increase of DNA repair synthesis and removal of cyclobutane pyrimidine dimers after UV-irradiation in butyrate-treated human fibroblasts (Smerdon *et al.* 1982). This increase was found to be confined only to the highly acetylated nucleosomes (Ramanathan and Smerdon 1989).

With regard to ionizing radiation, a role of chromatin structure has been proposed in modulating the frequency and type of DNA lesions (Chiu *et al.* 1982, 1986). Indeed, there is evidence that proteins play a role in protection against induction of DNA damage by radiation because stripping of histones and other proteins from isolated chromatin results in an increase in the frequency of DNA single-strand breaks (SSB) (Ljungman 1991). However, the limited data available from the irradiation of intact cells are contradictory with respect to a modulating effect of chromatin structure on induction of DNA damage by ionizing radiation (Pfeiffer *et al.* 1996). Chiu *et al.* (1986) and Bunch *et al.* (1995) reported that DNA regions containing transcriptionally active DNA were more susceptible to damage by ionizing radiation, but other investigators (Nose and Nikaido 1984,

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*Hae*III induces position-dependent chromosomal breakage in barley (*Hordeum vulgare* L.)

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The pattern of localized chromosomal breakage induced by the restriction endonuclease *Hae*III in reconstructed barley karyotypes T-1586 and T-21 was investigated. It was found that nucleolus organizing regions (NORs) of chromosomes 6 and 7 (segments 46 and 38, respectively), containing actively transcribed ribosomal (r)DNA, as well as segments 39 and 47, both containing condensed rDNA repeats, are the most pronounced aberration hot-spots in T-1586. The number of aberrations observed in these segments was three to five times higher than theoretically expected. The intrachromosomal distribution of chromatid aberrations in karyotype T-21, where the NOR-bearing segments in chromosomes 6 and 7 change their position, revealed a substantial difference in the aberration hot-spot behaviour. A position-specific increase in aberration clustering was observed, most pronounced in segments 38 and 47. On the other hand, segment 46 retained its initial sensitivity, while segment 39 in the new position lost its previous status as a mutation hot-spot. The data are indicative of the expressivity of aberration hot-spots generated after treatment with this restriction endonuclease being influenced by their distinct chromosomal location.

Introduction

There are many studies indicating that the chromatid aberrations induced by various mutagenic agents are not randomly distributed along the chromosomes. The most convincing data in plants were established with experimentally reconstructed karyotypes (Schubert *et al.*, 1986). Amongst the factors influencing the pattern of intrachromosomal distribution of induced aberrations, the karyotype constitution was proved to be of primary importance (Rieger *et al.*, 1977; Schubert *et al.*, 1985; Gecheff, 1991).

Following characterization of the restriction endonucleases as efficient clastogens in both mammalian (Bryant, 1984; Natarajan and Obe, 1984) and plant (Stoilov *et al.*, 1996) genomes, their differential activity along the individual chromosomes was also analysed (Obe *et al.*, 1986; Balajee *et al.*, 1994; Folle and Obe, 1995, 1996; Gecheff *et al.*, 1997).

Analysis of the intrachromosomal distribution of chromatid aberrations induced by restriction endonucleases recognizing different DNA sequences has shown nearly the same pattern of localized breakage (Folle and Obe, 1995, 1996). In a later study it was shown that the rDNA regions are amongst the most pronounced aberration hot-spots produced by different restriction endonucleases in barley (Gecheff *et al.*, 1997).

The repositioning of aberration hot-spot segments was proved to play an essential role in the intrachromosomal distribution of chemically induced structural mutations (Schubert *et al.*, 1986; Gecheff, 1991). In this respect, position-dependent induction of chromosomal damage after treatment with restriction enzymes might also be expected.

The present study is an attempt to shed some light on the problem, taking advantage of the availability of reconstructed barley karyotypes with an altered chromosomal localization of the nucleolus organizing regions (NORs).

Materials and methods

Germinating seeds of reconstructed barley karyotypes T-1586 and T-21 were utilized as the experimental material. Karyotype T-1586, containing a reciprocal translocation between chromosomes 3 (3H) and 4 (4H), permits clear identification of all chromosomes and was used as a control. Karyotype T-21 is identical to T-1586 with respect to the 3–4 translocation and in addition contains a reciprocal translocation between chromosomes 6 (6H) and 7 (5H). As a result, the NOR-bearing segments in chromosomes 6 (6H) and 7 (5H) exchange their positions.

Restriction endonuclease *Hae*III (12 U/μl; Sigma) was used as the inducer of chromosomal damage due to its previously established activity in the barley genome (Stoilov *et al.*, 1996).

Treatment with *Hae*III was performed essentially as previously described (Stoilov *et al.*, 1996). Briefly, after permeabilization of the primary roots with Driselase and conditioning with the respective digestion buffer the material was exposed to 500 U/ml of the enzyme for 3 h at 37°C. Metaphase block, fixation of the material and preparation of Feulgen stained squashes were done as before (Gecheff, 1989). The recovery times used in our experiments (19 and 22 h after application of the restriction endonuclease) were chosen to coincide with the maximal frequency of aberrations produced by *Hae*III, namely 25–28% damaged cells. About 700 cells per recovery time were scored. To analyse the localization of the four types of chromatid aberrations the metaphase chromosomes were subdivided into 53 segments of nearly equal sizes (Figures 1 and 2). The centromeres and secondary constrictions were designated individually. The segments were numbered with respect to their position in the standard barley karyotype. The data are pooled from at least two independently performed experiments and analysed statistically according to the formula of Rieger *et al.* (1975).

Results and discussion

Figure 1 shows the intrachromosomal distribution of *Hae*III-induced chromatid aberrations in karyotype T-1586. The most frequently produced type of rearrangements were isolocus breaks and reciprocal chromatid translocations distributed non-randomly along the individual chromosomes.

Segments 46 and 38, representing the NOR of chromosomes 6 and 7, respectively, as well as segments 39 and 47 [both containing condensed ribosomal (r)DNA], appeared to be the most pronounced aberration hot-spots. The number of aberrations observed in these segments surpassed by more than three times the theoretically expected frequency for a random distribution (for segments 46 and 39 the enhancement was more than five times). It is remarkable that the increased sensitivity of segment 39 in this case is due to its preferential involvement in intercalary deletions. Another distinct feature of the data obtained is that reciprocal translocations are

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SCE formation after exposure of CHO cells prelabelled with BrdU or biotin-dUTP to various DNA-damaging agents

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Formation of sister chromatid exchanges (SCE) is a mechanism of repair or bypass of DNA damage during S phase. Although SCE have been studied for a long time, the types of DNA lesions involved and the role of 5-bromodeoxyuridine (BrdU) in SCE formation are a matter of debate. We have developed a novel method of differential labelling of sister chromatids with biotin-16–2′-deoxyuridine-5′-triphosphate (biotin-dUTP) and could show that a substantial proportion of radiation-induced SCE arise via damage to BrdU-moieties. The present investigations were performed to examine the role of BrdU in the formation of SCE by the endonucleases *Alu*I and DNase I, as well as the alkylating agent mitomycin C (MMC). CHO cells unifilarily prelabelled with biotin-dUTP or BrdU were treated and the frequencies of SCE analysed in the first post-treatment mitoses. *Alu*I induced similar frequencies of SCE in cells prelabelled with BrdU or biotin-dUTP. DNase I induced significantly more SCE in cells prelabelled with BrdU than with biotin-dUTP. MMC induced slightly more SCE in cells labelled with biotin-dUTP than BrdU, but the difference was not significant. The possible mechanisms responsible for the enhanced SCE frequency following DNase I treatment of cells prelabelled with BrdU are discussed.

Introduction

Despite the fact that the phenomenon of sister chromatid exchanges (SCE) has been known for a long time (for a review see Latt, 1981), the mechanisms of their formation are not well understood. Recent investigations have shown that *RAD51*-mediated homologous recombination mechanisms are involved in SCE formation (Sonoda *et al.*, 1999). However, it appears that SCE can also be formed via alternative, *RAD51*-independent pathways (Lambert and Lopez, 2001). The role of topoisomerase II in SCE formation has been discussed, but remains obscure (Dominguez *et al.*, 2001).

Uncertainties also prevail regarding the nature of lesions leading to SCE. It is known that not all types of DNA damage give rise to SCE. S phase-dependent agents such as mitomycin C (MMC) and UV light are among the most effective inducers of SCE (Latt, 1981). It is generally assumed that SCE arise during the S phase of the cell cycle, when damaged DNA is replicated (Painter, 1980). The nature of SCE following treatment of cells in G_1 with agents which induce DNA double-strand breaks (dsb), like ionizing radiation, has been a matter of debate (Littlefield *et al.*, 1979). Ionizing radiation is a poor inducer of SCE and is only effective when applied to cells in G_1 with chromosomes unifilarily substituted with 5-bromodeoxyurdine (BrdU) (Littlefield *et al.*, 1979; Bruckmann *et al.*, 1999b). Therefore, it was proposed that SCE induced by ionizing radiation in G_1 result from chromosomal aberrations, mainly inversions, and are 'false' SCE (Wolff *et al.*, 1974; Mühlmann-Diaz and Bedford, 1995). In a previous study we analysed the frequencies of SCE associated with inversions in the short arm of human chromosome 3 and could show that most radiation-induced SCE do not result from inversions (Wojcik *et al.*, 1999). We have developed a technique to visualize SCE by labelling cells with biotin-16–2′-deoxyuridine-5′-triphosphate (biotin-dUTP) (Bruckmann *et al.*, 1999a), enabling the analysis of the role of BrdU in the formation of SCE. With this technique we have shown that X-rays induce 'true' SCE via radiation-induced damage to BrdU (Bruckmann *et al.*, 1999b).

The present investigations were performed in order to study the role of BrdU in the induction of SCE by DNase I, *Alu*I and MMC. Both DNase I and *Alu*I have been shown to be effective inducers of SCE in CHO cells unifilarily labelled with BrdU and treated in G_1 (Obe *et al.*, 1994). The restriction enzyme *Alu*I induces predominantly dsb (Roberts and Halford, 1993). DNase I induces single-strand breaks (ssb), which, when induced in close proximity, lead to dsb (Lutter, 1997).

Like ionizing radiation, *Alu*I and DNase I induce chromosomal aberrations in an S phase-independent manner. Exposure in G_1 phase leads to chromosome-type aberrations, such as polycentric chromosomes, and exposure in S phase leads mainly to chromatid-type aberrations, such as chromatid interchanges (Obe and Winkel, 1985; Folle *et al.*, 1991; Obe *et al.*, 1992, 1993, 1995). MMC is a DNA crosslinking agent (Iyer and Szybalski, 1963; Ishii, 1981; Borowy-Borowski *et al.*, 1990) and exposure of cells in G_1 phase leads to chromatid-type aberrations in the ensuing metaphase.

Here we describe results which show that following treatment with *Alu*I, similar frequencies of SCE were observed in cells prelabelled with BrdU and biotin-dUTP. DNase I induced significantly more SCE in cells prelabelled with BrdU than with biotin-dUTP. MMC induced slightly more SCE in cells labelled with biotin-dUTP, but the difference was not significant.

Materials and methods

Cell cultures and labelling with biotin-dUTP and BrdU

CHO-9 cells (obtained from A.T. Natarajan, Leiden, The Netherlands) were grown in Petri dishes (Greiner) and McCoy's 5A medium (Gibco) supplemented with 10% fetal calf serum (Gibco) in the presence of 100 U/ml penicillin and 100 µg/ml dihydrostreptomycin sulphate (Gibco) at 37°C and 5% CO_2.

For labelling with biotin-dUTP (Boehringer) and BrdU cells from stock cultures were subcultured for 34 h in 150 mm Petri dishes. Incorporation of

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Repair of DNA double-strand breaks in eukaryotic genome

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ABSTRACT. Double-strand breaks are the most critical lesions leading to genomic instability in eukaryotic cells. Two basic mechanisms are responsible for their repair - homologous recombination and non-homologous end joining. Although both of them are evolutionary conserved ranging from yeast to humans, their contribution varies in lower and higher eukaryotes. Current knowledge on the different aspects of these repair processes in eukaryotes is the subject of this review.

Key words: DNA damage, DSB repair, Homologous recombination, Non-homologous end joining

Abbreviations: Double-strand breaks (DSB); Homologous recombination (HR); Non-homologous end joining (NHEJ); Ionizing radiation (IR); Restriction endonucleases (RE); Apurinic/apyrimidinic sites (AP); Single-strand breaks (SSB); Multiply damaged sites (MDS); Topoisomerase I (TopoI); Gene conversion (GC); Break-induced replication (BIR); Single-strand annealing (SSA); Replication protein A (RPA); Single-stranded DNA (ssDNA); Double-stranded DNA (dsDNA); DNA-dependent Protein Kinase (DNA-PK); DNA-PK catalytic subunit (DNA-PKcs); MAR (matrix attachment regions); Poly(ADP-ribose) polymerase-1 (PARP-1); Methyl methane sulfonate (MMS).

I. Introduction

Eukaryotic genome is continuously exposed to various agents, which induce different types of damage in DNA. Amongst the variety of DNA lesions, double-strand breaks are considered to be the most dangerous as they severely disrupt DNA integrity. Even the persistence of one DSB in the cell can inactivate an essential gene and lead to cell dead. At present it is widely acknowledged that DSB formation is the initial event, leading to the appearance of different types of chromosomal rearrangements, such as translocations, inversions and deletions, associated with increased genomic

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Efficient repair of bleomycin-induced double-strand breaks in barley ribosomal genes

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Abstract

Ability of barley ribosomal genes to cope with damage produced in vivo by the radiomimetic agent bleomycin was investigated. Repair kinetics of bleomycin-induced double-strand breaks in ribosomal and total genomic DNA was compared. Induction and repair of double-strand breaks in defined regions of the ribosomal genes was also analyzed. Preferential sensitivity of barley linker DNA towards bleomycin treatment in vivo was established. Relatively higher yield of initially induced double-strand breaks in genomic DNA in comparison to ribosomal DNA was also found. Fragments containing intergenic spacers of barley rRNA genes displayed higher sensitivity to bleomycin than the coding sequences. No heterogeneity in the repair of DSB between transcribed and non-transcribed regions of ribosomal genes was detected. Data indicate that DSB repair in barley rDNA, although more efficient than in genomic DNA, does not corelate with the activity of nucleolus organizer regions.

Keywords: Ribosomal genes; DSB repair; Barley; Bleomycin

1. Introduction

Eukaryotic cells are continuously exposed to a number of substances with mutagenic nature capable to induce different types of DNA damage. Amongst the variety of DNA lesions double-strand breaks (DSB) are considered to be the most deleterious ones as they severely disrupt genome integrity. It is established that DSB arise in cellular DNA after exposure to exogenous agents such as ionizing radiation, bleomycin or restriction endonucleases. They can also occur naturally in any stage of the cell cycle during replication, transcription, recombination or repair. It is widely acknowledged that generation of DSB is the initial event leading to the formation of chromosomal rearrangements, unequivocally associated with increased genomic instability [1]. Proper maintenance of the genetic information, being essential for the cell survival and normal development of the organisms, has to rely on the effective rejoining of DNA DSB [2].

Non-random distribution of primary lesions and repair after treatment with various mutagenic agents in distinct genomic and chromatin locations has been a matter of substantial interest. Various aspects of the problem have been approached, including the role of mutagen type, chromatin compactness, higher-order chromatin structure, nucleosome positioning, gene and sequence specificity, transcriptional activity as well as cell and cell cycle dependence [3–9]. Crucial

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Adaptive response to DNA and chromosomal damage induced by X-rays in human blood lymphocytes

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Nucleoid sedimentation, single-cell gel electrophoresis (comet assay) and premature chromosome condensation (PCC) technique were utilized to estimate the involvement of DNA strand breaks and chromosomal damage in radio-adaptive response of stimulated human lymphocytes. Conditioning of cells with 0.02 Gy X-rays rendered them more resistant to single- and double-strand DNA breaks produced by 1 Gy challenging treatment as revealed by the sedimentation behaviour of the nucleoids and the comet assay. Nucleoid sedimentation also demonstrated that adaptive reaction towards X-ray-induced DNA damage is favoured in the presence of oxygen. A concomitant decrease in the amount of interphase chromosomal breaks visualized by PCC under the same experimental conditions was observed. Data indicate that adaptation of human lymphocytes to X-rays is tightly linked to the reduced susceptibility towards generation of DNA and chromosomal breaks. It is proposed that the very persistence of DNA strand discontinuities might serve as a triggering signal for the adaptation of human lymphocytes against ionizing radiation exposure.

Introduction

Inducible repair processes as opposed to constitutive ones were initially detected and characterized in bacteria. One type of repair activity observed in *Escherichia coli* after treatment with alkylating agents was termed adaptive response (AR) (1). In general, this phenomenon occurs after treatment of the cells with a low dose of a clastogen. Such a conditioning provokes protective effect against the mutagen employed for the subsequent higher dose (challenging) treatment. Adaptive behaviour was found to be a characteristic feature of both mammalian and plant cells in their response to various mutagenic agents (2–4). Utilizing different biological end points adaptation to low level of alkylation, oxygen species and incorporated ^{3}H-thymidine (^{3}HdThd) or γ-rays has been reported (2,5–7).

The first report that cultured human lymphocytes exposed to low doses of radiation (either from the decay of the incorporated ^{3}HdThd or from external X-rays) became refractory to the induction of chromosomal aberrations by a treatment with higher dose came from Olivieri *et al.* (8). Such a phenomenon has been attributed to the induction of chromosomal break repair. Radio-adaptive response in human lymphocytes was later analysed in a series of studies (9–15, for a review, see 16).

Several distinct features of the protective reaction of human lymphocytes against X-rays have been elucidated. As a rule, adaptation is triggered by a very low dose (the effective range is usually between 0.02 and 0.05 Gy) and manifested not earlier than 4 h after stimulation, i.e. it is not observed in dormant Go cells (17,18). AR is dependent not only on the rate of the initial damage but also on the time gap between adaptive and challenging treatments. AR was found to be effective for a relatively long time, approximately for three cell cycles (17,19). An important feature is also that cross-adaptation is reported for diverse types of initial and challenging treatments (20,21) and there is inter-individual variability among the donors (11,12).

Radio-adaptive response in rodent and human cells has been initially evaluated by the reduction in the frequencies of chromosomal aberrations (both of chromatid and chromosome type) as well as for sister chromatid exchanges (SCEs) and micronuclei induction (7,11,22–24). Up to now, however, the molecular basis of this reaction remains obscure. It was shown that AR is inhibited by 3-aminobenzamide and cycloheximide and there is *de novo* synthesis of several proteins in response to low-dose pretreatment (6,9,25). Although the character of the initial events, presumably DNA damage, is not yet elucidated, there are indications that after the initiating unidentified signal, a subset of components, including various protein kinases and early response genes regulating transcription machinery of the cell, are involved (26). A pivotal role of p53 protein in channelling of radiation-induced DNA double-strand breaks (DSBs) into adaptive repair pathways has been also proposed (27).

Premature chromosome condensation (PCC) is considered to be a reliable tool for studies of interphase chromosomes as they are visualized at different stages of the cell cycle (28). It is one of the most sensitive methods for monitoring initial chromosomal damage following exposure to ionizing radiation (29). As DNA strand scissions are among the major lesions induced from X-rays in DNA with the number of single-strand breaks (SSBs) being much higher than double-strand ones in a ratio of 30–40 : 1 within the low-dose range (30), we aimed our work on the role of DNA and chromosomal breaks in radio-adaptive response. Adaptation of phytohemaglutinin (PHA)-stimulated human lymphocytes was followed via monitoring of changes in DNA integrity by nucleoid sedimentation and comet assays. Chromosomal damage was visualized by PCC technique.

Materials and methods

Experimental material

Lymphocytes from two non-smoking healthy volunteers were obtained the day after the blood was collected from the Blood Bank, Leiden University Medical Centre. The layer with the mononuclear cells was isolated by centrifugation on histopaque-1077 (Sigma, Germany). Cells were washed with phosphate-buffered saline (PBS), suspended in Ham's F10 plus 8% dimethylsulphoxide (DMSO)

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Position-specific effects in the action of mutagenic agents on the chromosomes of barley (*Hordeum vulgare* L.)

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ABSTRACT. A comparative analysis of the intrachromosomal distribution pattern of structural mutations induced by maleic hydrazide (MH) and γ-rays in barley was performed. Two reconstructed karyotypes of barley, T-1586 and T-21, which allow an easy identification of the individual chromosomes of their complements, and differ from each other by the position of the "hot spot" segments, were used as experimental material. No significant differences in the sensitivity of the karyotype variants tested were observed after treatment with both MH and γ-rays. As expected, the two agents clearly differ by the types of aberrations induced; only chromatid type for MH (delayed effect) and both chromatid and chromosome types in the case of γ-rays (non-delayed effect). The most interesting finding in this study was that the chromosome constitution dramatically affected the distribution pattern of aberrations along the individual chromosomes, the effect being especially strongly pronounced in the action of MH. Two segments, namely 32 and 41, located proximally to the nucleolus organizing regions (NORs) of chromosome 5H and 6H, respectively, were found to be the most sensitive sites ("hot spots") of barley karyotype. The effect was mostly expressed for duplication deletions (dd) and especially for intercalary deletions (d). Thus, about 43% of induced intercalary deletions and 35% of duplication deletions were found to be localized at segment 32. The same segments showed an increased sensitivity to the action of γ-rays, as well, but the localized breakage was significantly less pronounced. It is remarkable also that the specific constitution of reconstructed chromosome $5H^{6H}$ (tandem position of the "hot spot" segments) was found to result in the majority of cases in a marked increase of the segment which is involved in intrachromosomal exchanges. To throw an additional light on the nature of the "hot spot" segments and the processes concerning the subtle localization of chromosomal breakpoints and the primary induction and

repair of lesions that may underlie this regional specificity of mutagenic agents, fluorescence in situ hybridization with rDNA probe and analysis of DNA damage by agarose gel electrophoresis under neutral and alkaline conditions were applied.

Key words: chromosomal rearrangement, reconstructed karyotypes, DNA lesions, regional mutagenic specificity, position effect, barley.

Introduction

Mutagenic specificity may occur at different levels of the structural organization of the genetic material. The most convincing evidence for the specific action of mutagens has been demonstrated at chromosome level as a localized breakage. The phenomenon, termed regional mutagenic specificity (Auerbach and Westergaard, 1960), has been established for a great number of mutagenic agents in both higher plants and animals, and was widely explored to investigate the mechanisms of interaction of these agents with the hereditary structures of eukaryotes (for review see Gecheff, 1995).

Barley (*Hordeum vulgare* L.), because of its convenience for cytogenetic studies, opened new possibilities for studying both theoretical and applied aspects of the regional specificity of mutagens. A number of cytologically reconstructed karyotypes were synthesized and used in our laboratory as model systems along this line (Gecheff, 1996). A convincing evidence for the decisive role of chromosome constitution in the processes underlying the specific induction of structural mutations in barley was established using these karyotypes (Gecheff, 1989; 1991; Stoilov et al., 2000). The most important conclusion to be drawn by these studies is that the repositioning of the aberration "hot spots" may dramatically influence both their expressivity and the size of the chromosome segment involved in particular types of structural mutations.

This study was aimed at comparative analysis of the intrachromosomal distribution of structural mutations induced by two mutagenic agents with different mode of action, namely, maleic hydrazide (MH) and gamma-rays, in barley reconstructed karyotypes showing a specific repositioning of aberration "hot spot" segments.

Materials and Methods

Plant materials. Germinating seeds of two reconstructed barley karyotypes — T-1586 and T-21, were used as experimental material. Karyotype T-1586 was produced by gamma-irradiation of the standard variety Freya and contains reciprocal translocation between the short arm of chromosome 3H and the long arm of chromosome 4H. Karyotype T-21 is identical to T-1586 with respect to translocation 3H-4H and involves an additional reciprocal translocation between the short arms of chromosomes 5H and 6H.

Mutagenic treatments. For induction of chromatid aberrations germinating seeds (resting seeds kept on moist filter paper in Petri dishes for 18 hs) were immersed in 5×10^{-3}M aqueous solution of MH for 2.5 h or irradiated by γ-rays (^{137}Cs) with a 5 Gy dose.

Cytological techniques. Feulgen stained squash preparations were used to score chromatid aberrations along the individual metaphase chromosomes and Giemsa N-banding was applied for identification of heterochromatic chromosome regions. Fluorescence in situ hybridization (FISH) with a biotin-labelled rDNA probe pTa71 (wheat clone containing 18S-5S-26S rRNA genes) was used to map the ribosomal gene clusters in reconstructed chromosomes 5H and 6H. More details about the cytological techniques used are given in our previous paper (Georgiev et al., 2001).

Molecular analyses. The formation of single-strand breaks (SSB) and double-strand breaks (DSB) in barley genome after treatment of cell suspension cultures with γ-rays and MH were evaluated by conventional alkaline and neutral agarose gel electrophoresis.

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DNA methylation and chromosomal rearrangements in reconstructed karyotypes of *Hordeum vulgare* L.

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Summary. One standard and two reconstructed barley karyotypes were used to study the influence of chromosomal rearrangements on the distribution pattern of DNA methylation detectable at the chromosome level. Data obtained were also compared with Giemsa N-bands and high gene density regions that had been previously described. The effect of chromosomal reconstruction in barley seems to be decidedly prominent in the repositioning of genomic DNA methylation along metaphase chromosomes. In comparison to the standard karyotype, the DNA methylation pattern was found to vary not only in the reconstructed chromosomes but also in the other chromosomes of the complements not subjected to structural alterations. Moreover, differences may occur between corresponding regions of homologues. Some specific chromosomal bands, including the nucleolus-organizing regions, showed a relative constancy in the methylation pattern, but this was not the case when the two satellites were combined by translocation in chromosome $6H^{5H}$ of line T-30. Our results suggest that epigenetic changes like DNA methylation may play an important role in the overall genome reorganization following chromosome reconstruction.

Keywords: 5-Methyl cytosine; *Hordeum vulgare*; Chromosome reconstruction; Karyomorphometry.

Introduction

Modification of DNA by cytosine methylation is a widespread phenomenon both in prokaryotes and in eukaryotes, including higher plants. DNA methylation is believed to play a decisive role in the defence against invading DNA or transposable elements and is considered to be a key mechanism in the regulation of gene expression (Finnegan et al. 1998, Heslop-Harrison 2000, Attwood et al. 2002), operating most probably at the level of transcription (Tate and Bird 1993). Hypermethylation in regulatory DNA sequences has been found to correlate with reduced levels of gene expression (Sardana et al. 1993, Neves et al. 1995).

In plants, like in many other higher eukaryotes, 5-methyl cytosine (5-mCyt) is the principal modified base and the levels of DNA methylation account for more than 30% of all cytosines in some species (Gruenbaum et al. 1981). The methylation of DNA sequences is an important mechanism for gene regulation in a number of biological processes (Finnegan et al. 2000, Bender 2004) but its variations are often associated with pleiotropic effects on cell differentiation and development (Ronemus et al. 1996).

Compared with animals, plants have a rather complex system for the establishment and regulation of DNA methylation. In addition to symmetrical methylated CpG sites, as in mammals, DNA methylation in plants is found in two other locations: at CpXpG- and CpXpX-containing sequences (Tariq and Paszkowski 2004). This higher complexity is supported by several categories of cytosine methyltransferases. These categories are based on enzyme structure and similarity of conserved amino acid motifs, and on the specificity of their activity. DNA methyltransferase 1 (MET1) and chromomethylase 3 (CMT3) seem to be mainly involved in the "maintenance" of the complementary strand, via transposon DNA methylation at CpG and non-CpG sequences (Finnegan and Kovac 2000, Martienssen and Colot 2001, Tompa et al. 2002). In contrast, the domains rearranged methylase (DRM) class is mostly required for "de novo" methylation at CpGs, CpXpGs and CpXpXs, trans-

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Molecular characterization of structural barley mutants produced by gamma-irradiation

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ABSTRACT. Various molecular marker systems, including STS, CAP, REMAP, RAPD and AFLP were employed to screen for DNA alterations in the genomes of thirteen original barley structural mutants produced by gamma-irradiation. Two out of total 4888 fragments generated in the mutant forms by the applied set of markers were polymorphic which indicate a relatively low occurrence of stable radiation-induced genetic variations in the resulting mutant lines. Among the marker systems AFLP was proven to be the most appropriate for the analysis of radiation-induced DNA alterations in barley. AFLP analysis revealed sequence polymorphism in 2 out of the 13 translocation lines analysed. As far as gross chromosomal rearrangements such as translocations and inversions concern large chromosome domains, the respective specific DNA sequences analysed may not completely cover the areas where subtle mutational changes may have occurred in the mutant genotypes produced. The data obtained in the present study support the notion for the relatively high genetic stability of barley genome.

Key words: structural mutants, gamma-rays, DNA alterations, molecular markers, barley.
Abbreviations: AFLP - Amplified Fragment Length Polymorphism, **CAPS** - Cleaved Amplified Polymorphic Sequences, **RAPD** - Random Amplified Polymorphic DNA, **REMAP** - REtrotransposon-Microsatellite Amplified Polymorphism, **STS** - Sequence Tagged Sites.

Introduction

Genetic diversity is the base for improving plant varieties. In the recent years the improvement of crop plants was done mainly by hybridization to a limited number of dominating in the national and international markets varieties and lines. As a

Restriction Endonucleases as a Tool for *in vivo* Induction of Chromosomal and DNA Damage in Barley Genome

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Abstract

Bacterial restriction endonucleases have been widely utilized to study the significance of DNA double-strand breaks for the formation of chromosomal aberrations based on their ability to produce this particular DNA lesion. Such studies were very scarce in plants until mid-nineties. The stability of maize nuclei towards *in vivo* action of *Eco*RI was investigated, revealing that dry embryo cells were less resistant than meristematic ones actively involved in transcription. Restriction endonucleases were also found to induce structural chromosomal damage in barley genome. They exerted an S-independent mode of action revealing the transition between the G_1 and S phase as the most sensitive stage for aberration induction. Intra-chromosomal localization of chromatid aberrations produced by *Hpa*II, *Msp*I and *Hae*III displayed similar distribution patterns. The most pronounced aberration hot-spots were the Nucleolus Organizing Regions which pointed towards the potential of restriction endonucleases for damage induction in specific genomic locations. Patterns of the localized chromosomal breakage produced by *Hae*III in suitably reconstructed karyotypes showed substantial difference in the aberration hot-spot behavior. Position-specific increase in aberration clustering was found indicating that the incidence of aberration hot-spots generated by restriction endonucleases is dependent on their chromosomal environment. Barley karyotypes with normal and increased expression of rRNA genes were further utilized to evaluate the possible relationship between their transcriptional activity and damage induction. Hybridization profiles obtained after treatment with *Msp*I revealed similar induction kinetics. The potential of barley ribosomal genes to accumulate double-strand breaks with a different structure was also tested by *Alu*I and band intensity reduction followed the pattern found for *Msp*I. Results indicated that the mode of action of restriction endonucleases applied was not substantially influenced by the activity of the nucleolus organizing regions. The data as a whole supports options for the use of restriction endonucleases for directed induction of damage in plant genome.

Introduction

The use of bacterial restriction endonucleases (RE) for induction of chromosomal damage in eukaryotes was the subject of extensive studies during the last two decades of the previous century. This interest was initiated by the need to study the role of DNA double-strand breaks (DSB) in the course of cellular responses to radiation-induced damage and from the necessity to reveal the molecular mechanisms governing the formation of chromosomal damage. The ability of RE to produce one particular DNA lesion, namely DSB, was widely utilized, but data for plants was practically unavailable until the mid-nineties.

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Description of the research

Background studies

Radiation-generated DSBs were found to be the most likely primary lesion leading to the formation of chromosomal aberrations [1, 2]. This suggestion was strongly supported by the successful use of REs as effective inducers of chromosomal damage. The mode of action of REs was found to resemble those of ionizing radiation and radiomimetic chemicals in that it was S-phase independent: chromosome aberrations were induced in G_1 and chromatid in S and G_2 phases of the cell cycle. [3]. It was demonstrated that blunt-ended breaks tended to be more effective, leading to higher frequencies of chromosomal aberrations [4, 3]. Higher efficiency of REs recognizing four bases, and a lower efficiency for those recognizing six bases was also established. Aberration induction was found to also be dependent on a variety of other factors such as cell-cycle progression, cellular repair capacity, DNA methylation and the technique for introduction of REs within the cell nucleus [3, 5, 6] .

It must be pointed out that the majority of the initial studies in the field were performed on mammalian cells, and until recently, the corresponding data from plants was very scarce. The first attempt to analyze the mode of action of RE on plant chromosomes was made by Subrahmanyam and co-authors in 1976 [7]. After treatment of barley root tips with a mixture of the restriction endonucleases *Hind*II and *Hind*III, they found a time-dependent and progressive fragmentation of metaphase chromosomes, as well as a granular appearance of the interphase chromatin accompanied with micronuclei formation. These observations and the ample and decisive evidence of the chromosome-breaking ability of REs in mammalian cells urged us to utilize REs as a tool for induction of chromosomal and DNA damage in the barley genome.

Key findings

Differential response of maize nuclei upon treatment with restriction endonucleases in vivo

It was shown that higher-order chromatin structure in plants resembles that found in other eukaryotes, namely that the nuclear DNA is organized in a series of supercoiled loop domains anchored to a structure termed the nuclear matrix [8]. The germinating maize embryo represents a suitable model system for the study of the transition of an inactive genome of a dry embryo to the higher transcriptional activity of the germinating embryo cells. Electron microscopy studies have shown that the nuclei of dry embryo cells lack internal nuclear matrix, which is however, well-structured in the meristematic cells of the primary roots [9].

Stability towards the action of *Eco*RI of maize nuclei obtained from cells with different transcriptional activity (dry embryo, root tip meristem and epicotyl protoplasts) was investigated. After fluorescent staining of the resulting histone-depleted nuclei, it was found that dry embryo structures are less stable than those actively involved in transcription (**Fig. 1**), most probably due to the absence of a well-defined internal nuclear matrix, which points to the key role of this higher-order chromatin structure in the functioning of the plant genome [10].

Q.Y. Shu (ed.), Induced Plant Mutations in the Genomics Era. Food and Agriculture Organization of the United Nations, Rome, 2009, **235-238**

Genomic and Gene-specific Induction and Repair of DNA Damage in Barley

V Manova*, M Georgieva, B Borisov, B Stoilova, K Gecheff & L Stoilov

Abstract

Repair of DNA damage induced by various mutagenic agents within the barley genomic and ribosomal DNA was the subject of investigation. Reconstructed karyotypes T-1586 and T-35, with normal and increased expression of ribosomal genes respectively, were utilized to evaluate the relationship between the transcriptional activity and the rate of DNA damage induction and their repair. A tendency towards restoration of rDNA integrity after γ-irradiation was observed, indicative for the efficient recovery of double-strand breaks in barley ribosomal DNA. Ability of barley ribosomal genes to cope with damage produced *in vivo* by the radiomimetic agent bleomycin was further analyzed. Preferential sensitivity of barley linker DNA towards bleomycin treatment *in vivo* was established. Fragments containing intergenic spacers of barley rRNA genes displayed higher sensitivity to bleomycin than the coding sequences. No heterogeneity in the repair of DSB between transcribed and non-transcribed regions of ribosomal genes was detected. Data indicated that DSB repair in barley ribosomal genes, although relatively more efficient than in genomic DNA, did not corelate with NOR activity. Repair kinetics of UV-C induced cyclobutane pyrimidine dimers in barley genomic and ribosomal DNA was also studied. Less cyclobutane pyrimidine dimers (CPD) in rDNA in comparison to total genomic DNA was detected. Results showed that UV-C induced CPD in barley ribosomal genes are as efficiently repaired as in the rest of the genome predominantly by light repair mechanisms.

Introduction

Maintenance of DNA integrity by the cellular repair mechanisms is an essential function of living organisms, preserving the genuine status of their genetic information. DNA repair mechanisms are also not fully correct, which increases the genetic diversity and variability of the populations. The biological consequences of non-repaired or miss-repaired DNA damage depend on the type and frequency of the lesions as well as on the functional characteristics and location of the target DNA. Therefore, the investigations on the selective induction and differential efficiency of repair processes in individual genes and defined DNA sequences are of substantial theoretical and practical importance.

Many studies have shown that the heterogeneity of DNA damage induction and repair, dependent on chromatin organization, transcriptional activity and nature of individual DNA sequences, is a widely spread phenomenon in higher eukaryotes. A crucial breakthrough in the topic of differential repair was the finding that actively transcribed genes are more quickly repaired by nucleotide excision repair (NER) than non-expressed ones. It was further demonstrated that such preferential recovery of active genes was mainly due to the accelerated repair of lesions in the transcribed DNA strand [1, 2]. Moreover, intragenic repair heterogeneity, reflecting chromatin alterations along the genes was also established [3, 4]. After the initial observation for strand-specific repair of cyclobutane pyrimidine dimers, the link between DNA repair and transcription for other types of DNA lesions has been extensively studied. Recovery from damage induced by UV, crosslinking and alkylating agents in mammalian ribosomal (rRNA) genes, however, was found to be less effective than in genes transcribed by RNA polymerase II or in the genome overall [5-7]. Efficient, but not preferential, repair was observed for bleomycin and IR-induced strand breaks in mammalian rRNA genes [8, 9] indicating that repair of rDNA might be rather lesion-dependent than tightly linked to transcription. Recently, however, a transcription-dependent repair of UV-induced CPD in yeast rRNA genes accomplished by NER and photoreactivation has been demonstrated [10]. Studies on the gene-specific induction and repair of DNA damage in plants, however, are limited and there is a lack of information about the existence of preferential DNA repair of active plant genes in relation to their transcriptional activity, chromatin structure and genomic location.

Description of the activities performed

Our main research activities were focused on the genomic and gene-specific induction and repair of DNA damage in barley. The induction and repair kinetics of double-strand breaks (DSB) in barley ribosomal DNA (rDNA) after treatment of root tips with ionizing radiation and bleomycin were investigated. The relationship between transcriptional activity of ribosomal genes and the efficiency of induction and repair of these lesions in whole barley repeats, as well as in the transcribed and non-coding rDNA sequences were also analyzed. Formation and repair of CPD in genomic (gDNA) and ribosomal DNA after UV-C irradiation of barley leaves were also a subject of investigation.

Ionizing radiation-reconstructed barley karyotypes T-1586 and T-35 characterized with normal and increased activity of Nucleolus Organizing Regions (NOR) were utilized to study the link between the repair potential of barley ribosomal genes and their expression. Line T-35 is derived from T-1586 and contains deletion of the NOR-bearing segment of chromosome 6H. As a result, a higher activity of the remaining rRNA gene cluster localized in NOR 5H was observed [11, 12]. Barley ribosomal genes are represented by long (9.8 kb) and short (8.8 kb) ribosomal repeats, localized in the NOR of chromosome 6H and 5H respectively (**Fig. 1**).

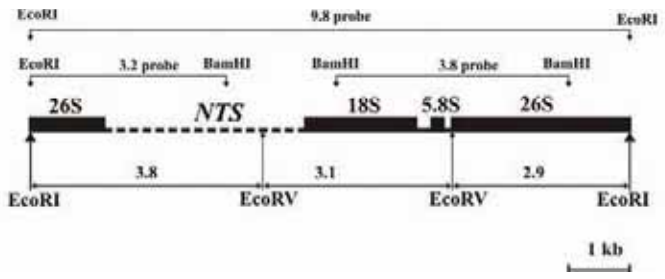

Figure 1 Partial restriction map of the longer 9.8kb barley rDNA repeat (clone HV 014). Solid and dotted lines represent coding regions and non-transcribed intergenic spacer respectively.

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ORIGINAL ARTICLE

DNA methylation pattern in a barley reconstructed karyotype with deleted ribosomal gene cluster of chromosome 6H

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Abstract A reconstructed barley karyotype (T-35) was utilised to study the influence of chromosomal rearrangements on the DNA methylation pattern at chromosome level. Data obtained were also compared with the distribution of Giemsa N-bands and high gene density regions along the individual chromosomes that have been previously described. In comparison to the control karyotype (T-1586), the DNA methylation pattern was found to vary not only in the reconstructed chromosomes but also in the other chromosomes of the complement. Significant remodelling process of methylation pattern was found also in the residual nucleolus organiser regions (NOR) on chromosome 5H as a consequence of deletion comprising the whole NOR of chromosome 6H in T-35. Moreover, differences between corresponding segments of the homologues with respect to some other chromosome locations were also observed. Repositioning of genomic DNA methylation along the metaphase chromosomes following chromosomal reconstruction in barley seems to be essential to ensure correct chromatin organisation and function.

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Keywords 5-Methyl cytosine mapping · rRNA genes · Chromosome reconstruction · Barley

Introduction

DNA methylation represents the main covalent modification occurring at position 5 of cytosine belonging to the DNA in most eukaryotic genome, including higher plants. It is evident that DNA methylation plays a crucial role in the defence against invading DNA or transposable elements and is considered to be a key mechanism in the regulation of gene expression (Finnegan et al. 1998; Heslop-Harrison 2000; Attwood et al. 2002), dynamically operating at transcription level during development (Suzuki and Bird 2008).

In plants, 5-methyl cytosine (5-mCyt) is the main modified base, and in *Arabidopsis*, as many as 20–30% of genes are methylated to some extent (Cokus et al. 2008; Lister et al. 2008).

Compared with animals, plants have a more complex system for the establishment and regulation of DNA methylation: In addition to symmetrically methylated CpG dinucleotide sites, DNA methylation involves two other nucleotide locations: CpXpG- and CpXpX-containing sequences (where X = A, T, C or G; Tariq and Paszkowski 2004). This complexity is supported by several categories of cytosine methyltransferases (Finnegan and Kovac 2000; Wada 2005; Pavlopoulou and Kossida 2007). While in mammals genomic methylation pattern is wiped out during gametogenesis (Rideout et al. 2001), in plants it is transmitted largely intact from parent to offspring (Saze 2008). For this reason, plants have developed a composite system for the removal of surplus 5-mCyt. Genomic DNA methylation patterns can be reshaped in plants either passively, by

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PLANT GENETICS • ORIGINAL PAPER

Genomic diversity of *Ac*-like transposable elements in *sphaerococcum* mutant forms of common wheat (*Triticum aestivum* L.) and triticale (X *Triticosecale* Witt.)

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Abstract DNA sequences homologous to the maize Activator (*Ac*) element are widespread in plant genomes. Nowadays, several reports are available concerning the distribution and characterisation of *Ac*-homologous sequences in natural populations of different cereal species. but these mobile genetic elements still remain to be comprehensively characterised. In this respect, there is a particular lack of information about the dynamics of *Ac*-homologous sequences within mutant germplasm collections. Here, we present data on the genomic diversity and methylation patterns of *Ac*-homologous sequences in ethyl methanesulphonate (EMS)-induced *sphaerococcum* mutant forms of common wheat (*Triticum aestivum* L.) and triticale (X *Triticosecale* Witt.). The results show that the initial EMS treatment has influenced the wheat genome stability by enhancing the dynamics of *Ac* transposon-homologous sequences.

Keywords Transposons · Wheat · Triticale · *Sphaerococcum* mutants · Genomic instability

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Introduction

In many plant species with large and complex genomes, like that of wheat, the mobile genetic elements or transposons comprise more than 50% of the nuclear DNA (Sanmiguel and Bennetzen 1998; Li et al. 2004). Their activity is often associated with changes in the gene or genome structure, accompanied with the modulation of gene expression in both germinal and somatic plant cells (Bennetzen 2000). For this reason, Barbara McClintock named the transposons 'controlling elements' and proposed their role in evolution as a ubiquitous source of hypermutagenicity and for the generation of individuals with increased survival potential within stressed populations (McClintock 1949).

The *hobo*/*Ac*/*Tam3* (hAT) superfamily of transposons is a major group of class II elements (Calvi et al. 1991), which is known to be responsible for diverse morphological and chromosomal mutations. Members of the hAT family (*Ac*-like transposable elements) are widespread in plants, particularly in large cereal genomes (Kunze et al. 1997; Staginnus et al. 2001; Langdon et al. 2003), and can be recognised by structural similarity in their terminal inverted repeats (TIR) or internal transposase coding region. The sequence coding for the maize transposase is the most conservative region of *Ac*-like transposable elements (Rubin et al. 2001). Based on this feature, several approaches (e.g. database homology searches, Southern hybridisation and polymerase chain reaction [PCR] amplification) have been applied for their detection in cereal genomes (Chernyshev et al. 1989; Georgiev et al. 2000; Zale and Steber 2002; De Keukeleire et al. 2004; Altinkut et al. 2006a, 2006b). Beside these reports, there are no data available regarding the structural and functional dynamics of these elements in mutant wheat genomes, as the majority

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