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DNA damage processing and aberration formation in plants

I. Schubert, A. Pecinka, A. Meister, V. Schubert, M. Klatte and G. Jovtchev

Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben (Germany)

Abstract. Various types of DNA damage, induced by endo- and exogenous genotoxic impacts, may become processed into structural chromosome changes such as sister chromatid exchanges (SCEs) and chromosomal aberrations. Chromosomal aberrations occur preferentially within heterochromatic regions composed mainly of repetitive sequences. Most of the preclastogenic damage is correctly repaired by different repair mechanisms. For instance, after N-methyl-N-nitrosourea treatment one SCE is formed per >40,000 and one chromatid-type aberration per ~ 25 million primarily induced O^6-methylguanine residues in *Vicia faba.* Double-strand breaks (DSBs) apparently represent the critical lesions for the generation of chromosome structural changes by erroneous reciprocal recombination repair. Usually two DSBs have to interact in *cis* or *trans* to form a chromosomal aberration. Indirect evidence is at hand for plants indicating that chromatid-type aberrations mediated by S phase-dependent mutagens are generated by post-replication (mis)repair of DSBs resulting from (rare) interference of repair and replication processes at the sites of lesions, mainly within repetitive sequences of heterochromatic regions. The proportion of DSBs yielding structural changes via misrepair has still to be established when DSBs, induced at predetermined positions, can be quantified and related to the number of SCEs and chromosomal aberrations that appear at these loci after DSB induction. Recording the degree of association of homologous chromosome territories (by chromosome painting) and of punctual homologous pairing frequency along these territories during and after mutagen treatment of wild-type versus hyperrecombination mutants of *Arabidopsis thaliana*, it will be elucidated as to what extent the interphase arrangement of chromosome territories becomes modified by critical lesions and contributes to homologous reciprocal recombination. This paper reviews the state of the art with respect to DNA damage processing in the course of aberration formation and the interphase arrangement of homologous chromosome territories as a structural prerequisite for homologous rearrangements in plants.

Spectrum and chromosomal distribution of chromatid-type aberrations

Chromosomal structural aberrations comprise breaks, yielding terminal or intercalary deletions, and rearrangements such as inversions, insertions, symmetric and asymmetric reciprocal exchanges. They represent the consequences of lacking or incomplete repair or of erroneous recombination repair of various types of DNA lesions caused by exogenous or endogenous genotoxic impacts. When induced before replication, aberrations are manifested as chromosome-type and during or after replication as chromatid-type structural changes.

The spectrum of chromatid-type aberrations observed within the first mitosis after their generation, differs between mammals and plants. In plants, isochromatid breaks are the most frequent aberrations, followed by reciprocal translocations, intercalary deletions, duplication deletions and open chromatid breaks, while in mammals duplication deletions and intercalary deletions are very seldom and open chromatid and isochromatid breaks are much more frequently observed than in plants. However, post-treatment with DNA synthesis inhibitors (e.g., hydroxyurea 10^{-2} M, 3 h before fixation) after exposure to S phase-dependent mutagens increases the yield of chro-

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Request reprints from Ingo Schubert
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK)
Corrensstrasse 3, DE–06466 Gatersleben (Germany)
telephone: +49 (0)39482 5239; fax: +49 (0)39482 5137
e-mail: schubert@ipk-gatersleben.de

Dedicated to Guenter Obe on the occasion of his 65th birthday.

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matid-type aberrations 2- to 3-fold and increases the proportion of non-reunion aberrations from 1–5% to 25–35% in plants (Hartley-Asp et al., 1980; Schubert and Rieger, 1987).

The intrachromosomal distribution of aberration breakpoints is not random. Heterochromatic regions, consisting of repetitive DNA sequences, represent "hot spots" of aberration formation (Döbel et al., 1978; Schubert et al., 1986, 1994). Aberration clustering in heterochromatin is more pronounced after exposure to S phase-dependent mutagens causing lesions transformed into breaks via repair or replication and mediating aberrations only when passing through S phase than after exposure to S phase-independent mutagens causing DNA breaks directly (Schubert and Rieger, 1977).

Only a minority of potentially clastogenic lesions result in chromosomal aberrations via erroneous recombination repair

Critical lesions are DNA double-strand breaks (DSB) which are induced either directly or during replication or repair processes at damaged DNA sites and are lethal for proliferating cells if not repaired. Usually, for rearrangements two critical lesions (one per breakpoint) are required (Kihlman et al., 1977; Richardson and Jasin, 2000).

The great majority of potentially clastogenic lesions are repaired correctly. This may occur via reversion of the damage, e.g., photoreactivation of UV-induced pyrimidine dimers or removal of alkyl groups by alkyl-transferase or alkB-like pathways (Begley and Samson, 2003) without generation of discontinuities within the DNA strands. Also excision and mismatch repair pathways, producing DNA discontinuities by an incision step, usually result in a perfect restoration of the pre-damage state. Post-replicative recombination repair that may in part become manifested by mutagen-induced sister chromatid exchange (SCE) represents correct repair (or bypass of lesions) in terms of chromosome structure (Gonzales-Barrera et al., 2003). However, inhibition of complete ligation at sites of recombination may lead to chromosomal aberrations (Lindenhahn and Schubert, 1983).

DSBs induced by restriction endonucleases at endogenous or transgenic target sites may induce chromosomal aberrations in non-plant systems (see for instance Bryant, 1984; Natarajan and Obe, 1984; Obe et al., 1987; Winegar and Preston, 1988; Richardson and Jasin, 2000) in an S phase-independent manner (Obe and Winkel, 1985).

Induction of a DSB within one member of two repeats positioned on heterologous chromosomes in mouse ES cells increases homologous recombination between these repeats at least 1000-fold. A similar DSB-mediated increase in homologous recombination between tandem repeats has been reported for plants (Xiao and Peterson, 2000; Orel et al., 2003). However, at recombinationally repaired DSB loci gene conversion, but no crossing over events that would have led to a translocation, has been observed (Richardson et al., 1998). Only when restrictase-mediated DSBs were induced within the repeats of *both* chromosomes, repair by gene conversion was found in one fifth of the cases accompanied by translocation formation (Richardson and Jasin, 2000). This is in line with more indirect observations on plants.

After treatment of *Vicia faba* meristems with the monofunctional alkylating agent N-methyl-N-nitrosourea (MNU, 10^{-2} M, 1 h), chromatid aberrations were exclusively formed during S phase and appeared in ~30% of metaphases after 12 h recovery (Baranczewski et al., 1997b). More than two thirds of these aberrations occur within heterochromatic regions (~10% of the genome, Baranczewski et al., 1997a). O^6-methylguanine (O^6-MeG), the most efficient preclastogenic lesion generated by MNU treatment (Kaina et al., 1991), is induced in a nearly linear dose-dependent manner during all cell cycle stages and later becomes removed in the same proportions in euchromatic and heterochromatic sequences. About one aberration is formed per ~25 million of the originally induced O^6-MeG residues, as calculated from immuno-slot-blot analyses (Baranczewski et al., 1997a).

Single- but not double-strand breaks (SSB apparently reflect repair intermediates at alkylated sites) were induced by MNU with a linear dose relationship in *Vicia faba* nuclei of all cell cycle stages as measured by the comet assay. Euchromatic and heterochromatic sequences were involved proportionally (Menke et al., 2000).

So-called adaptive conditions (e.g., pre-treatment with a 10-fold lower MNU dose) led to a reduction of the frequency of chromatid aberrations and of O^6-MeG residues induced by challenge treatment, both by >50%, when protein synthesis was not inhibited (Baranczewski et al., 1997b). About the same reduction was found under adaptive conditions for SSBs and abasic sites, both appearing in the course of repair of alkylated sites (Angelis et al., 2000).

These data show that MNU-induced DNA damages (in particular, O^6-MeG but also repair-mediated abasic sites and DNA breaks) are evenly distributed and their great majority is correctly repaired along the entire genome during all cell cycle stages.

Only during S phase, recombinational repair of MNU-induced damage may result in (randomly distributed) SCEs (Schubert and Heindorff, 1989) and, at an up to 1000-fold lower frequency (Lindenhahn and Schubert, 1983), in chromatid aberrations. Therefore, the majority of randomly distributed preclastogenic lesions, induced for instance by MNU, is correctly repaired or by-passed by a hierarchy of processes (dealkylation, [base-]excision repair, recombinational repair). Per >40,000 O^6-MeG residues one SCE and per ~25 million one chromatid aberration is formed. The majority of chromatid aberrations (~70%) are clustered mainly in heterochromatic regions (Baranczewski et al., 1997a). Thus, most of the aberrations induced by S phase-dependent clastogens should be derived from DSBs that may result from (rare) positional coincidence of repair- and replication-mediated DNA discontinuities and are preferentially mis-repaired by reciprocal recombination when broken ends involving homologous repeats interact in *cis* or *trans* (within a chromatid or between sister or non-sister chromatids) (see Fig. 1 and Schubert et al., 1994; Menke et al., 2000).

Using a negatively selectable marker gene combined with a transgenic recognition site for the rare cutting restriction endo-

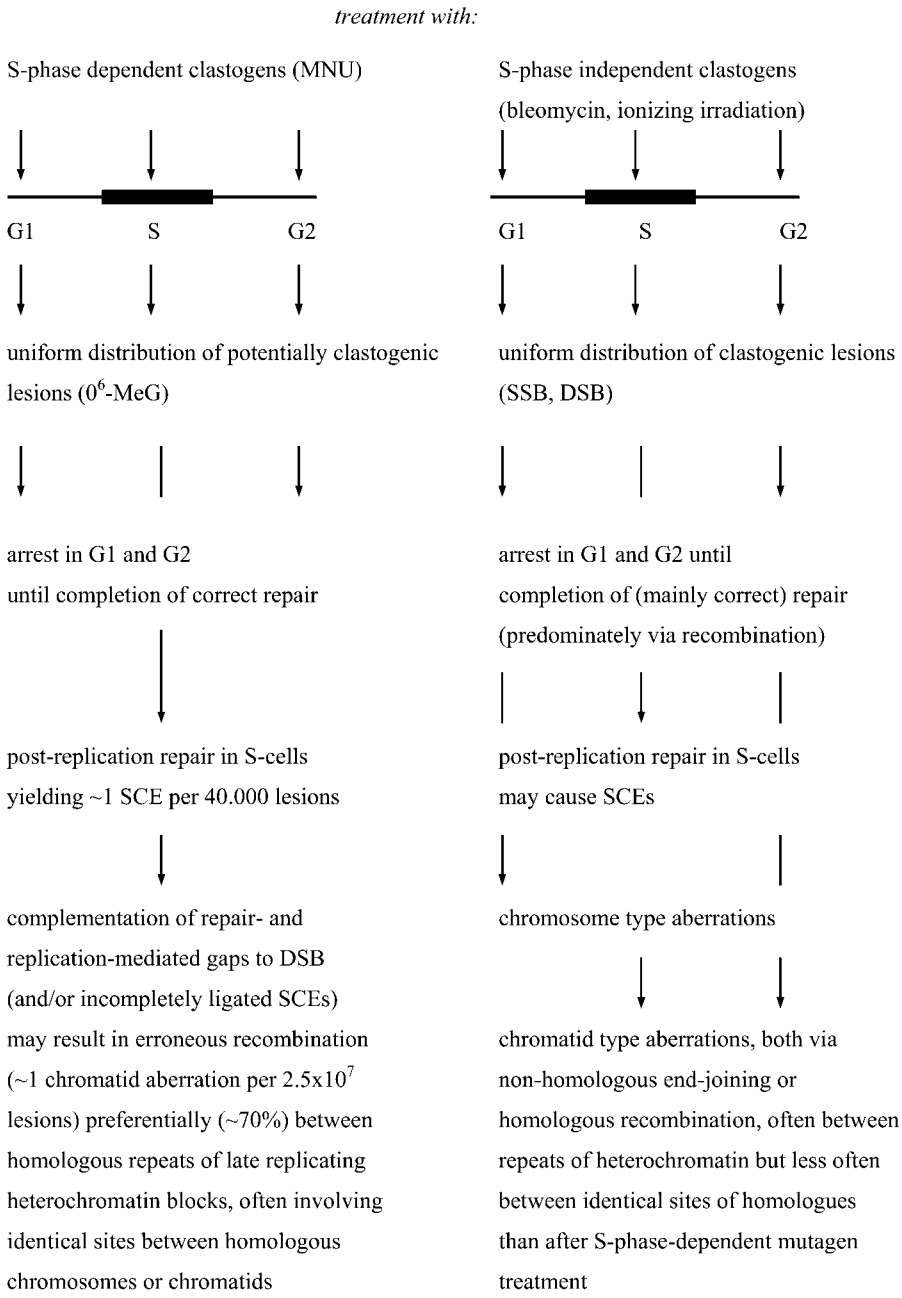

Fig. 1. Scheme of DNA damage processing during aberration formation.

nuclease I-*Sce*I, various mechanisms of recombinational repair of *Sce*I-induced DSBs have been described also for plants (e.g. Puchta, 1999; Kirik et al., 2000; Gisler et al., 2002; Siebert and Puchta, 2002). However, until now these systems did not allow us to quantify the proportions of induced DSBs in relation to those repaired to restore the pre-breakage situation and those potentially resulting in SCEs or different types of chromosomal aberrations. An approach to quantify these proportions is now being established to provide answers as to how DSBs have to be processed to yield SCEs and structural aberrations, respectively, and to compare such data with the calculations derived from experiments with S phase-dependent clastogens and with data obtained from mammalian systems.

Interphase arrangement of chromosome territories appears to be essential for the origin of chromosomal rearrangements

The specific side-by-side arrangement of interphase chromosome territories was tested for all possible heterologous pairs of human chromosomes by chromosome painting after ionizing irradiation and measuring their interchange frequencies. Although in these experiments a non-random central clustering was found for the gene-rich chromosomes 1, 16, 17, 19 and 22, a random spatial arrangement was predominant for the majority of chromosomes (Cornforth et al., 2002). Similarly, irradiation of chicken DT40 lymphocytes (with a central clus-

tering of microchromosomes and a peripheral position of macrochromosomes) yielded a low frequency of translocations between micro- and macrochromosomes and most translocations occurred either between microchromosomes or between macrochromosomes (Grandy et al., 2002). Interestingly, mutagen-induced chromatid translocations in a *Vicia faba* karyotype with individually distinguishable chromosome pairs (2n = 12) revealed a highly significant (~ 8-fold) excess of translocations between homologous chromosomes and a vast majority (up to >90%) of translocation breakpoints at homologous chromosome positions (Rieger et al., 1973). These effects which were more pronounced for S phase-dependent mutagens than for ionizing irradiation, were interpreted as the result of an at least transient/partial association of homologous chromosomes during interphase.

For the first time painting of chromosome territories of a euploid plant has been established in our lab (Lysak et al., 2001, 2003). Using specific sets of BAC contigs that cover entire chromosome arms as probes for FISH, all five chromosomes of the model plant *Arabidopsis thaliana* can now be traced along various cell cycle and developmental stages. Painting of interphase chromosome territories and FISH with individual chromosome-specific sequences (~ 100 kb) in isolated nuclei, flow-sorted according to their DNA content into different cell cycle and developmental fractions, should reveal the potential dynamics of chromosome territory association and the occurrence of somatic homologous pairing in comparison with model simulations for random chromosome arrangement and punctual homologous pairing.

A "Spherical 1 Mb chromatin domain" (SCD) model (Cremer et al., 2001) and a "Random spatial distribution" (RSD) model simulating a random distribution of all *A. thaliana* chromosomes and of ~100 kb chromosome segments, respectively, were computed (in collaboration with Dr. G. Kreth, University of Heidelberg). The frequency of homologous chromosome association was analyzed for chromosome 4 in 2C and 4C nuclei from root and leaf tissues and compared with the punctual homologous pairing of distinct 100-kb segments. The frequency for both phenomena was not identical. Punctual pairing occurred far less frequently than association of homologues but both phenomena occurred with a frequency similar to that predicted by the corresponding computer simulation based on the random models. FISH with individual BAC pairs from different chromosomal positions showed roughly the same frequency of punctual pairing for all tested positions. However, punctual pairing of different loci along a chromosome did not occur simultaneously within the same nucleus indicating that association of homologous territories does not reflect somatic homologous pairing. For chromosomes 1, 3 and 5 we obtained comparable data (Pecinka et al., unpublished results). The at least limited occurrence of homologous pairing might provide a spatial basis for the origin of chromosome rearrangements between homologues. Clustering of aberrations could be reinforced by the tendency of heterochromatic blocks to fuse.

In nearly half of the 4C nuclei, FISH signals for individual BAC pairs (3 or 4 instead of 1 or 2 double signals) indicate that sister chromatids are not permanently cohesed. This supports the assumption that cohesion along the chromosome arms might be essential only shortly after replication for post-replication repair between sister chromatids (Koshland and Guacci, 2000). Later on, cohesion might be required only around centromeres for their bipolar orientation during nuclear division.

In the future, homologue association and punctual pairing during different cell cyle stages of meristematic cells and after mutagen treatment will be studied. Preliminary data have shown that immediately after bleomycin treatment (5 mg/ml, 1 h) of *Arabidopsis* seedlings chromosome territories are frequently (in ~15% of nuclei) disintegrated and dispersed all over the nucleus, while at later recovery times the typical territory structures re-appear. Data obtained with the comet assay showed that bleomycin-induced DSBs increase linearly with dose immediately after treatment and are nearly completely repaired as early as 1 h after treatment (Menke et al., 2001). These data suggest that DSBs may find each other for recombinational repair not only via punctual pairing of homologues, but also randomly due to DSB-mediated dispersion of chromosome territories. The latter may be more typical for treatment with true radiomimetic compounds that lead to less pronounced aberration clustering and fewer translocations between homologous loci than S phase-dependent mutagens (Schubert and Rieger, 1977).

Arabidopsis mutants showing a 20- to 50-fold increase in recombination frequency will be characterized as to the proportion of recombination between sister chromatids versus homologues using transgenic recombination substrates in hemi- or homozygous condition (Barbara Hohn and Jean Molinier, personal communication). The frequency of alignment of homologous chromosome territories and of punctual pairing within nuclei of such transgenic mutants will show whether increased homologous recombination is connected with an increased frequency of homologue association or pairing or is rather due to an intensified activity of damaged homologous chromosome segments to find each other, e.g., by a prolongation of the time span during which the DSBs stay "open".

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