Environmental and Molecular Mutagenesis 36:146–150 (2000)

Adaptation to Alkylation Damage in DNA Measured by the Comet Assay

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The alkylating mutagens N-methyl-N-nitrosourea (MNU) and methyl methanesulfonate (MMS) were studied for their potential to induce DNA strand breaks and abasic (AP) sites in meristematic nuclei of *Vicia faba* root tips by the comet assay. The alkaline unwinding/neutral electrophoresis (A/N) and alkaline unwinding/alkaline electrophoresis (A/A) protocols were used for detection of DNA damage. With the A/N comet assay, less DNA damage was seen after conditioning pretreatment with a low dose prior to a high challenging dose of alkylating mutagens as compared to application of the high dose only, whereas a nearly additive effect was seen when the A/A comet assay was used. Adaptation was even more obvious when AP sites were revealed by the AP-endonuclease activity of exonuclease III. The adaptation observed with the A/N comet assay was abolished by pretreatment with the protein synthesis inhibitor cycloheximide. These data suggest that the comet assay is able to detect on molecular level a phenomenon resembling clastogenic adaptation. Environ. Mol. Mutagen. 36:146–150, 2000. © 2000 Wiley-Liss, Inc.

Key words: single strand breaks; abasic (AP) sites; AP-endonuclease; cycloheximide; cell cycle; *Vicia faba*

INTRODUCTION

Plants possess several protective mechanisms against various stresses, e.g., against genotoxic injury. One of these mechanisms, clastogenic adaptation, which protects plant cells against chromatid aberrations (CA) was first described by Rieger et al. [1982]. Clastogenic adaptation can be described as the reduction of the aberration frequency caused by a high dose of a clastogenic agent (challenge) due to pretreatment (conditioning) with a noneffective low dose of the same agent or another pretreatment that is able to elicit the adaptation. For induction of adaptation to a broad spectrum of clastogenic agents, sublethal heat shock, low doses of some metal salts, and of ionizing irradiation have been successfully applied, provided that de novo protein synthesis was not impaired [for review see Rieger et al., 1990].

Baranczewski et al. [1997] showed that on the molecular level, clastogenic adaptation correlates with accelerated removal of O^6-methylguanine from *Vicia faba* DNA after conditioning pretreatment.

Conflicting results of conditioning pretreatments on the yield of sister chromatid exchanges (SCEs) caused by various mutagens, including MNU, led to the question of whether the adaptation takes place before or even during the processing of primary DNA lesions into various genotoxic end points during progression through the cell cycle [Schubert and Heindorff, 1989].

In this study we show that for *Vicia faba* root tip nuclei, the adaptation to the methylating mutagens MNU and MMS can be measured immediately after the treatment as DNA breaks and abasic (AP) sites by the comet assay. We also show that adaptation on the DNA level is dependent on de novo protein synthesis like clastogenic adaptation but is not dependent on passing through the S phase of cell cycle.

MATERIALS AND METHODS

All chemicals, unless otherwise indicated, were purchased from Sigma (St. Louis, MO).

Abbreviations: AP, abasic site; BER, base excision repair, BrdUrd, bromodeoxyuridine; CA, chromatid aberrations; FdUrd, fluorodeoxyuridine; MMS, methyl methanesulfonate; MNU, N-methyl-N-nitrosourea; ROS, reactive oxygen species; RT, room temperature; SAR, systemic acquired resistance; SCEs, sister chromatid exchanges; ssbs, single strand breaks.

Contract grant sponsor: Deutsche Forschungsgemeinschaft; Contract grant numbers: Schu 951/5–1, 436TSE17/7/98; Contract grant sponsor: Grant Agency of the Czech Republic; Contract grant numbers: 204/97/0154, 203/98/0682, and P2020702

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Received 7 January 2000; provisionally accepted 11 March 2000; and in final form 21 April 2000

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Mutagen Treatment of *Vicia faba* Root Tips

Seeds of *Vicia faba,* karyotype ACB, were germinated at room temperature (RT) in a moist atmosphere. After 4 to 5 days, 0.5-cm long root tips were dissected and kept for 4 hr in Hoagland's Basal Salts (Sigma, H2395) buffered with 0.05% w/v morpholinoethanesulphonic acid (MES) to pH 5.8. This handling did not have any detectable impact on DNA integrity, but suppressed progression of meristematic cells through the cell cycle. The excised root tips were treated with the indicated concentrations of MNU (CAS 684–93-5, Sigma, N4766) or MMS (CAS 66–27-3, Sigma, M4016) in buffered Hoagland solution (pH 5.8), according to the time schedule described in Results, washed, and immediately frozen in liquid nitrogen prior to processing. For each experimental point, five root tips were combined to compensate for slight variations of DNA distribution in comets from individual roots.

Bromodeoxyuridine Labeling of Meristematic Nuclei

In order to detect progression of root cells through the cell cycle by sister chromatid diferentiation, excised root tips and entire seedlings were incubated in 100 μM of bromodeoxyuridine (BrdUrd), 0.1 μM of fluorodeoxyuridine (FdUrd), and 5 μM of uridine for 17 hr followed by incubation in 100 μM of thymidine and 5 μM of uridine for an additional 19 hr. Root tips were then incubated for 2.5 hr in 0.05% colchicine, fixed in 3:1 ethanol:acetic acid, squashed, and stained according to the fluorescence plus Giemsa (FPG)-technique [Schubert et al., 1979].

Preparation of Nuclei for Comet Assay

Root tips were finely chopped in 0.5 ml of phosphate-buffered saline (PBS) with 10 mM of Na_2EDTA using a razor blade. The suspension with released nuclei was filtered through a 20-μm disposable filter (Partec, Münster, Germany) to remove most of the tissue debris. Forty microliters of the filtrate was mixed with 200 μl of liquid 0.5% agarose (Ultra Pure, Cat. No. 15510–019, Life Technologies Ltd., Paisley, UK) in PBS and two separate gels were set by placing a 22 × 22 mm coverslip on top of 80 μl of agarose-nuclear suspension on a microscopic slide (Artikel-Nr.02 1102, Menzel-Gläser, Germany) precoated with 0.5% agarose (Ultra Pure, Cat. No. 15510–019, Life Technologies).

Comet Assay

The protocols used are based on those described by Angelis et al. [1999] for detection of DNA damage at different levels of sensitivity in mammalian and plant cells. For plant cells, the alkaline unwinding/alkaline electrophoresis (A/A) protocol was first used by Koppen and Verschaeve [1996] and the alkaline unwinding/neutral electrophoresis (A/N) protocol by Koppen and Angelis [1998] for the detection of various types of DNA lesions after x-ray irradiation. The described A/A protocol is currently used as a standard comet protocol by several laboratories for both mammalian as well as plant cells.

A/N Protocol

Nuclei embedded in agarose were lysed for 1 hr in 2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris-HCl, 1% Triton X-100, pH 10 on ice. For detection of AP sites, the slides were after the lysis washed two times in exo III buffer (66 mM Tris, 0.66 mM $MgCl_2$, pH 7.5) and then incubated for 40 min at 37°C either with 100 U/ml of exonuclease III (Boehringer Mannheim, Germany) in this buffer or in the buffer alone. After the lysis or enzyme treatment, slides were placed for alkaline unwinding in 0.3 M NaOH, 5 mM Na_2EDTA, pH > 13 for 20 min in the dark, then neutralized for 5 min in 0.4 M Tris (pH 7.5) and equilibrated in TBE buffer (45 mM Tris-borate, 1 mM Na_2EDTA, pH 8) prior to electrophoresis in the same buffer at 30 V (about 1 V/cm across the gels) and 13 mA for 10 min at RT. After electrophoresis, slides were dehydrated in 70% and 100% ethanol, and air-dried.

A/A Protocol

After lysis of embedded nuclei in the same lysis buffer as was used for the A/N protocol, slides were placed in 0.3 M NaOH, 5 mM Na_2EDTA, pH > 13 for 40 min before electrophoresis at 30 V (about 1 V/cm across the gels) and approximately 300 mA for 20 min at RT. After electrophoresis slides were rinsed in water, dehydrated in 70% and 100% ethanol, and air-dried.

Analysis of Comets

Dried slides were stained with 5 μg/ml ethidium bromide in water. The comets were viewed with a Nicon Eclipse 800 epifluorescence microscope (200×) and analyzed with the CometAs module of the Lucia 4.11 image analysis system (LIM, Praha, Czech Republic, www.lim.cz).

The percentage of DNA in tail of comets was used as a measure of DNA damage. For each experimental point mean and standard error (SE) was calculated from median values obtained from duplicated gels of 25 comets each of at least three independent experiments [Lowell et al., 1999]. Significant differences were determined by two-tailed Students' t-test incorporated in the Microsoft Excel 98 program.

RESULTS

Detection of Cell Cycle Arrest in Root Tips

Of 200 metaphases of entire seedlings, 137 showed sister chromatid differentiation, 11 showed late replication patterns, and 52 showed no differentiation. In contrast, excised root tips kept in Hoagland medium without carbohydrate supply showed exclusively nondifferentiated chromatids in metaphases (56) and anaphases (14), indicating that nearly no cell cycle progress occurred during the 36 hr of incubation in salt buffer only.

Detection of DNA Damage

For assessment of any adaptive response at the DNA level we used the same mutagen concentrations and treatment protocol, i.e., 1-hr pretreatment with a low dose followed by 2-hr postincubation and 1-hr treatment with a high challenge dose of alkylating mutagens, as used previously for the studies on clastogenic adaptation [Heindorff et al., 1987]. In the absence of any clastogenicity data for MMS in *Vicia faba*, we used for challenge a concentration of 10 mM of MMS. This concentration has recently been shown to cause approximately the same amount of DNA breakage as 1 mM of MNU in the A/N comet assay [Angelis et al., 1999]. As a conditioning dose, we used 0.5 mM of MMS, a concentration 20 times lower than the challenge dose.

DNA breaks caused by treatment with alkylating agents was measured as DNA breaks using the A/N comet assay. The data obtained are summarized in Tables I and II.

TABLE I. DNA Damage Measured as Percentage DNA in the Tail of Comets from Root Tip Nuclei of *Vicia faba* Under Adaptive and Nonadaptive Conditions After Treatment with MNU

	A/N	A/N with 1 mM cycloheximide pretreatment (2 hr)	A/N + exo III (100 U/ml, 40 min)	A/N + exo III with 1 mM cycloheximide pretreatment	A/A
Control	3.5 ± 0.5	6.4 ± 1.5	11.3 ± 1.6	12.0 ± 2.8	5.8 ± 1.5
0.1 mM MNU	3.9 ± 0.5	7.9 ± 2.9	14.6 ± 4.2	22.5 ± 6.0	17.8 ± 3.1
1 mM MNU	16.4 ± 0.9	14.3 ± 1.4	38.4 ± 4.7	51.8 ± 0.1	61.0 ± 4.8
0.1 + 1 mM MNU	12.1 ± 1.4	14.6 ± 2.6	25.8 ± 5.2	53.4 ± 1.6	66.7 ± 5.9
0.5 mM MMS + 1 mM MNU	9.3 ± 0.1	—	25.5 ± 1.8	—	—

TABLE II. DNA Damage Measured as Percentage DNA in the Tail of Comets from Root Tip Nuclei of *Vicia faba* Under Adaptive and Nonadaptive Conditions After Treatment with MMS

	A/N	A/N with 1 mM cycloheximide pretreatment (2 hr)	A/N + exo III (100 U/ml, 40 min)	A/N + exo III with 1 mM cycloheximide pretreatment	A/A
Control	3.1 ± 0.6	6.4 ± 1.5	13.6 ± 1.3	12.0 ± 2.8	5.9 ± 1.3
0.5 mM MMS	11.5 ± 1.8	11.2 ± 0.9	23.0 ± 2.4	17.0 ± 2.5	19.9 ± 3.4
10 mM MMS	21.5 ± 2.6	24.1 ± 4.7	62.2 ± 10.6	43.9 ± 4.1	85.4 ± 2.2
0.5 + 10 mM MMS	16.8 ± 2.7	24.3 ± 6.2	50.2 ± 3.5	48.4 ± 5.6	88.3 ± 3.4
0.1 mM MNU + 10 mMMS	8.6 ± 2.3	—	46.4 ± 2.5	—	—

Exonuclease III treatment after the lysis of nuclei, for conversion of AP sites into single strand breaks (ssbs), generated more DNA damage under all experimental conditions (Tables I and II). This indicates the presence of AP sites, in addition to ssbs, after the treatment with both mutagens.

When using the A/A comet assay, much more DNA damage was seen after conditioning as well as after challenge treatment with both mutagens than with the A/N comet assay. Other types of DNA damage, particularly alkali-labile sites, are probably converted into DNA ssbs under these conditions (Tables I and II).

After adaptation, the amount of DNA damage, as detected by the A/N comet assay, was reduced by 26% ($P = 0.026$) for MNU (Table I) and by 22% ($P = 0.023$) for MMS (Table II) as compared to treatment with the corresponding challenge doses alone. When pretreatment with MNU was done prior to challenge with MMS and vice versa (cross-adaptation), the observed effect was slightly, although not statistically significant higher.

Adaptation as measured by the A/N comet assay was also observed when exonuclease III was used to convert AP sites into additional DNA breaks. In particular, pretreatment with MNU decreased the amount of DNA migrated in the tail after exonuclease III treatment of nuclei by about 33% ($P = 0.0035$). This suggests that the repair of AP sites also contributes to the phenomenon of adaptation. Indeed, when comparing DNA damage due to DNA breaks and that due to AP sites only (DNA damage induced by exonuclease III in addition to already present damage; for calculation see legend to Fig. 1), adaptation reduced the amount of ssbs by 33% and that of AP sites by 58% in the case of MNU. In the case of MMS, ssbs were reduced by 25% and AP sites by 24% (Fig. 1).

Contrary to the data obtained with the A/N comet assay, DNA damage incurred by conditioning and the subsequent challenge treatment was almost additive for both mutagens, when measured by the A/A comet assay. This indicates that not all types of alkylation damage are subject to adaptation.

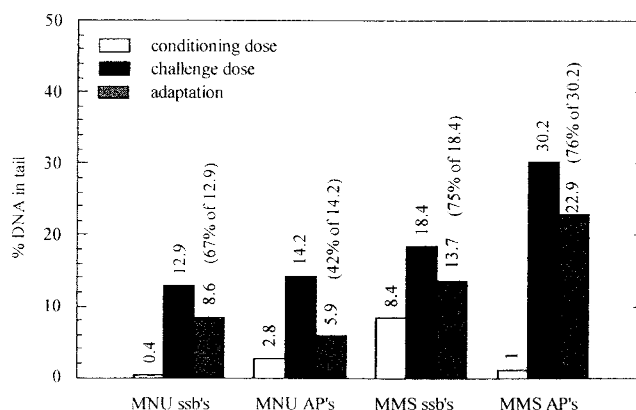

Fig. 1. N-methyl-N-nitrosourea (MNU)- and methyl methanesulfonate (MMS)-mediated true DNA breaks and abasic (AP) sites detected as percent DNA in tail by the alkaline unwinding/neutral electrophoresis (A/N) comet assay. The data were derived from Tables I and II. The proportions of mutagen-induced DNA breaks were calculated by subtracting the control values of DNA in tail of comets from that obtained after mutagen treatments. The proportions of exonuclease III-mediated DNA breaks (AP sites) were calculated by subtracting the values for DNA in tail without exonuclease III treatment (minus the control values) from that obtained after exonuclease III treatment (again minus control values).

Inhibition of Protein Synthesis

Protein synthesis was inhibited by incubation of root tips in 1 μM cycloheximide for 2 hr before conditioning and challenge treatment. Inhibition of protein synthesis abol-

ished the adaptation effect with or without additional exonuclease III treatment (Tables I and II).

DISCUSSION

To find out whether the mechanisms that underlie clastogenic adaptation are prereplicative inducible excision or correction of DNA lesions rather than connected with DNA replication or recombination, we focused our study on detection of DNA breaks and AP sites by comet assay in cells that do not progress through the cell cycle. To detect clastogenic adaptation the treatment schedule (conditioning, intertreatment time, and challenge treatment) takes approximately 4 hr, more than half of the time *Vicia* cells spend in G_1 phase. This is why we used excised *Vicia faba* root tips starved for carbohydrates (van't Hoff, 1985) by incubating them in the salt buffer only. The presence of fewer mitotic cells with only nondifferentiated metaphase and anaphase chromosomes indicated a nearly complete inhibition of DNA replication in excised root tips. Nevertheless we showed earlier that such cells retained the capacity to repair induced DNA damage for at least 24 hr [Koppen and Angelis, 1998].

We used MNU, producing *O*-alkyl and *N*-alkyl lesions by the S_N1 mechanism, and MMS, nearly exclusively producing *N*-alkyl lesions by the S_N2 mechanism to test whether adaptation is detectable by the by the comet assay and whether it differs according to the mutagen used.

In root tip cells, adaptation to DNA damage after MNU and MMS treatment was demonstrated by a reduced amount of DNA in tail as a consequence of a reduced number of true DNA breaks by the comet assay under A/N conditions. Addition of exonuclease III revealed also a reduction in the number of AP sites. In contrast, under A/A conditions when most of the alkylation damage including alkali labile sites was converted into DNA breaks [Angelis et al., 1999], the amount of DNA in the tail measured after conditioning plus challenge treatment was close to the additive value for both treatments. Therefore, it seems that only a subset of MNU- and MMS-induced DNA lesions becomes selectively repaired in pretreated cells, whereas the majority of DNA damage detected by the A/A comet assay remains unaffected.

O^6-methylguanine, the main clastogenic lesion caused by MNU, is efficiently removed from DNA under adaptive conditions [Baranczewski et al., 1997]. O^6-methylguanine represents 8% of alkylation products induced by MNU but only 0.3% of those induced by MMS [Beranek, 1990]. Nevertheless, because we observed similar adaptation and cross-adaptation patterns for both mutagens, *N*-alkylation–mediated ssbs, and possibly also intermediates of base excision repair (BER) were likely detected by the A/N comet assay than those caused by *O*-alkyl lesions. Among *N*-alkyl lesions, a critical one is N^3-methyladenine, which is genotoxic and clastogenic [Kaina, 1998].

N^3-methyladenine, which represents 10%–11% of the alkylation products induced by MMS as well as by MNU [Beranek, 1990], is generally removed from DNA by a specific glycosylase during the first step of BER [Krokan et al., 1997]. The remaining AP site is then cleaved by AP-endonuclease to allow other enzymes involved in BER to fill and seal the gap [Loeb and Preston, 1986]. In plants, N^3-methyladenine glycosylase activity was detected in tobacco by Kraszewska et al. [1998] and the gene itself was cloned from *Arabidopsis* by Santerre and Britt [1994]. Also, the gene encoding the AP endonuclease, *Arp*, has been cloned from *Arabidopsis* [Babiychuk et al., 1994]. In addition to significant sequence homology of plant gene with those isolated from other organisms, *Arabidopsis* AP-endonuclease is also able to activate human transcription factors by reducing cysteine residues in their binding domain [Babiychuk et al., 1994] in a way similar to human AP-endonuclease.

At present, little is known about inducibility of plant AP-endunuclease(s). Therefore, our data showing accelerated removal of AP-sites from DNA of adapted *Vicia faba* root tips represent the first, although indirect, evidence of plant AP-endonuclease(s) induction during the course of adaptation to genotoxic stress. The comet data also show that adaptation can be detected as the removal of certain critical lesions already occurring during challenge treatment. Similar results were obtained by immunodetection of removal of O^6-methylguanine in adapted *Vicia faba* root tips [Baranczewski et al., 1997].

AP-endonuclease in mammalian Chinese hamster ovary (CHO) cells was shown to be inducible by oxidative stress [Grösch and Kaina, 1999], mediated by reactive oxygen species (ROS) [Ramana et al., 1998]. If plant AP-endonuclease(s) prove to be inducible by ROS, it would be tempting to speculate on its role as a part of systemic acquired resistance (SAR) [Levin et al., 1994], which have to be a general defense response of plants largely mediated by oxidative stress and ROS [Reichheld et al., 1999]. In this context, induction of ROS and SAR response has been detected in plants after exposure to genotoxic UV-B [Green and Fluhr, 1995]. Moreover, Levin et al. [1994] suggested that ROS may serve as a diffusable signal for gene activation. Induction of DNA repair as a part of SAR, although speculative at the moment, could explain the broad spectrum of protection by various conditioning pretreatments (e.g., sublethal heat shock, metal salts, low doses of ionizing irradiation) eventually resulting in the phenomenon earlier described as clastogenic adaptation and cross-adaptation [Rieger et al., 1990].

ACKNOWLEDGMENTS

We thank Drs. Geoffrey Margison, Bernd Kaina and Holger Puchta for helpful comments on the manuscript. We also appreciate the technical assistance of Barbara Hildebrand and Marie Martinkova.

Support received from Deutsche Forschungsgemeinschaft Schu 951/5–1 (I.S.), 436TSE17/7/98 (I.S. and K.J.A.), and Grant Agency of the Czech Republic 204/97/0154, 203/98/0682, and P2020702 (K.J.A.).

REFERENCES

Angelis KJ, Dušínská M, Collins AR. 1999. Single cell gel electrophoresis: Detection of DNA damage at different levels of sensitivity. Electrophoresis 20:2133–2138.

Babiychuk E, Kushnir S, Van Montagu M, Inzé D. 1994. The *Arabidopsis thaliana* apurinic endonuclease Arp reduces human transcription factors Fos and Jun. Proc Natl Acad Sci USA 91:3299–3303.

Baranczewski P, Nehls P, Rieger R, Rajewsky MF, Schubert I. 1997. Removal of O^6-methylguanine from plant DNA in vivo is accelerated under conditions of clastogenic adaptation. Environ Mol Mutagen 29:400–405.

Beranek DT. 1990. Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. Mutat Res 231: 11–30.

Green R, Fluhr R. 1995. UV-B–induced PR-1 accumulation is mediated by active oxygen species. Plant Cell 7:203–212.

Grösch S, Kaina B. 1999. Transcriptional activation of apurinic/apyrimidinic endonuclease (Ape, Ref-1) by oxidative stress requires CREB. Biochem Biophys Res Comm 261:859–863.

Heindorff K, Rieger R, Schubert I, Michaelis A, Aurich O. 1987. Clastogenic adaptation of plant cells: Reduction of the yield of clastogen-induced chromatid aberrations by various pretreatment procedures. Mutat Res 181:157–171.

Kaina B. 1998. Critical steps in alkylation-induced aberration formation. Mutat Res 404:119–124.

Koppen G, Verschaeve L. 1996. The alkaline comet test on plant cells: A new genotoxicity test for DNA strand breaks in *Vicia faba* root cells. Mutat Res 360:193–200.

Koppen G, Angelis KJ. 1998. Repair of X-ray induced DNA damage measured by comet assay in root tips of *Vicia faba*. Environ Mol Mutagen 32:281–285.

Kraszewska E, Dobrzańska M, Tudek B. 1998. Tobacco BY-2 cells excise both 3-methyladenine and 7-methylguanine from methylated DNA. Mutat Res 409:91–95.

Krokan HE, Standal R, Slupphaug G. 1997. DNA glycosylases in the base excision repair of DNA. Biochem J 325:1–16.

Levin A, Tenhaken R, Dixon R, Lamb C. 1994. H2O2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell 79:583–593.

Loeb LA, Preston BD. 1986. Mutagenesis by apurinic/apyrimidinic sites. Annu Rev Genet 20:201–230.

Lowell DP, Thomas G, Dubow R. 1999. Issues related to the experimental design and subsequent statistical analysis of in vivo and in vitro comet studies. Teratog Carcinog Mutagen 19:109–119.

Ramana CV, Boldogh I, Izumi T, Mitra S. 1998. Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. Proc Natl Acad Sci USA 95:5061–5066.

Reichheld JP, Vernoux T, Lardon T, Van Montagu M, Inzé D. 1999. Specific checkpoints regulate plant cell cycle progression in response to oxidative stress. Plant J 17:647–656.

Rieger R, Michaelis A, Nicoloff H. 1982. Inducible repair processes in plant meristems? 'Below-additivity effects' of unequally fractionated clastogen concentrations. Biol Zbl 101:125–138.

Rieger R, Michaelis A, Takehisa S. 1990. On adaptive responses of plant meristem cells in vivo: Protection against induction of chromatid aberration. In: Obe G, Nataraja AT, editors. Chromosomal aberrations: basic and applied aspects. Berlin: Springer-Verlag. p 163–179.

Santerre A, Britt A. 1994. Cloning of a 3-methyladenine-DNA glycosylase from *Arabidopsis thaliana*. Proc Natl Acad Sci USA 91:2240–2244.

Schubert I, Heindorff K. 1989. Are SCE frequencies indicative of adaptive response of plant cells? Mutat Res 211:301–306.

Schubert I, Sturelid S, Dübel P, Rieger R. 1979. Intrachromosomal distribution patterns of mutagen-induced SCEs and chromatid aberrations in reconstructed karyotypes of *Vicia faba*. Mutat Res 59:27–38.

Van't Hof J. 1985. Control points within the cell cycle. In: Bryant JA, Francis D, editors. The cell division cycle in plants. Cambridge: Cambridge University Press. p 1–13.

Accepted by—
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