DNA repair mechanisms in plants: crucial sensors and effectors for the maintenance of genome integrity

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Summary

As obligate phototrophs, plants harness energy from sunlight to split water, producing oxygen and reducing power. This lifestyle exposes plants to particularly high levels of genotoxic stress that threatens genomic integrity, leading to mutation, developmental arrest and cell death. Plants, which with algae are the only photosynthetic eukaryotes, have evolved very effective pathways for DNA damage signalling and repair, and this review summarises our current understanding of these processes in the responses of plants to genotoxic stress. We also identify how the use of new and emerging technologies can complement established physiological and ecological studies to progress the application of this knowledge in biotechnology.


I. Introduction

The genome of a living organism is subjected to a wide variety of genotoxic stresses from both endogenous (intracellular) and environmental origin during its lifetime. All of the primary components of DNA – the sugar residues, phosphodiester linkages and the purine and pyrimidine bases that contribute informational content to the genome – can suffer damage. The biological consequences arising from any damage to the genome depend upon the chemical nature of the alteration to the structure of the DNA and can vary from the innocuous to the highly mutagenic. Ultimately, the toxicity or mutagenicity
of the incurred damage to the genome will depend upon the efficiency of the cellular mechanisms that have evolved to sense, recognise and eliminate the damage and the accuracy of the pathways available to the cell to repair the lesion.

The importance to plants of such appropriate and effective responses to potential mutagenic events is emphasised upon examination of a plant’s lifestyle. Plants, unlike most higher eukaryotes, lack a reserve germ line, with gametes arising from undifferentiated meristematic cells on completion of the sporophytic part of the plant life cycle. These progenitor cells have passed through numerous rounds of DNA replication and cell division before gamete formation. This provides the opportunity for mutations to arise in the genome through errors in the process of DNA replication during the cell cycle and long-term exposure of their genome to damaging endogenous and environmental mutagens. The sessile, photosynthetic nature of a plant’s lifestyle also means that plants have had to evolve different strategies to animals in order to minimise the effects of harmful genotoxic environmental agents, such as solar ultraviolet (UV) irradiation, to which they are continually exposed. The observation that mutation rates seen in long-lived plant species, such as trees, are not unexpectedly high indicates that the activities responsible for maintaining the integrity of the genome in plant somatic cells must be very efficient (Hays, 2002). However, there is evidence that in exceptional circumstances – such as in mangrove trees (Rhizophora mangle), where numerous cell divisions over many years separate one generation from the next – that such mutations can accumulate to significant levels in plant meristems (Klekowski & Godfrey, 1989; Britt, 1998).

One of the earliest suggestions that loss of plant cell viability could arise from spontaneous mutations in the genome of cells arising from endogenous (intracellular) sources came from studies on seeds of Crepis tectorum L. (Navashin, 1933). Seeds stored for 6 yr at ambient temperature germinated poorly and produced seedlings that were weak or that had developmental abnormalities, including albinism. The mutant phenotypes arising from seed ageing resembled those produced by high-dose X-ray treatment, including translocations involving two or more chromosomes (Navashin, 1933). The mutation rate was accelerated by increasing the temperature at which the dehydrated seeds were stored (Navashin & Shkvarnikov, 1933). Subsequent studies some 35 years later (Abdallah & Roberts, 1969) demonstrated that chromosome damage appearing during the ageing of seeds, including those of major crop species, was a result of the cumulative effects of temperature, moisture and oxygen levels pertaining over the time period of storage. Accumulation of chromosomal damage and/or an inability to repair such damage during the imbibition period appear to be significant factors contributing to loss of seed viability during storage (Cheah & Osborne, 1978). The relatively easy detection of chromosome breakage or translocations has enabled cytogenetic studies of DNA damage. These gross chromosomal alterations are potentially lethal events but probably occur at low frequency.

Several attempts have been made to quantify other types of damage to the DNA of the eukaryotic genome arising from endogenous sources, but these studies have been focused mainly on animal cells. Cellular metabolites can react with genomic DNA including the intracellular methyl group donor S-adenosyl methionine, which acts on DNA like a weak alkylating agent. Chromosomal proteins offer no significant protection to the DNA in chromatin against cytoxic alkylation of purines by S-adenosyl methionine, although these modifications occur at a rate which is estimated to be ∼20 times lower than the rate of depurination (Lindahl & Barnes, 2000). The level of damage to mammalian DNA arising from these endogenous sources highlights the extent to which the cell continually needs to employ its armoury of DNA repair pathways to maintain the integrity of the genome. Water represents one of the main sources of damage to the genome and DNA is inherently unstable in the aqueous environment. The spontaneous nonenzymatic cleavage of glycosyl bonds in DNA and the hydrolytic deamination of cytosine bases accounts for a considerable amount of damage to the mammalian genome on a daily basis (Table 1). Several thousand oxidative adducts (chemically altered bases) may also accumulate in the DNA of animal cells (Table 1) as a result of oxidation of bases in DNA by biological oxidants such as reactive oxygen species (ROS) (Beckman & Ames, 1997), and the efficient removal of these oxidative products requires effective excision repair pathways to operate in the cell.

The few studies which have attempted to quantify the scale of DNA damage in plant cells have demonstrated the extent to which the plant genome can be damaged by endogenous and exogenous genotoxins. Environmental stresses including drought, high and low temperatures and increased UV-B owing to stratospheric ozone depletion result in significant reductions in both crop quality and productivity largely mediated via the production ROS. In plant cells, ROS are the primary cause of single-strand breaks (SSBs) in the DNA, either directly, through destruction

<table>
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<th>Process</th>
<th>Events per day per cell</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Depurination</td>
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<td>Lindahl &amp; Nyberg (1972)</td>
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<tr>
<td>Cytosine deamination</td>
<td>100–500</td>
<td>Lindahl &amp; Nyberg (1974)</td>
</tr>
<tr>
<td>Alkylation (S-adenosylmethionine)</td>
<td>∼600</td>
<td>Lindahl &amp; Barnes (2000)</td>
</tr>
<tr>
<td>Oxidative adducts</td>
<td>∼150 000</td>
<td>Beckman &amp; Ames (1997)</td>
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</table>

Table 1: Estimates of endogenous DNA damage in animal cells arising from spontaneous hydrolysis, ROS and metabolite induced damage to the genome
Table 2 Estimates of accumulation and repair of apurinic/apyrimidinic (AP) sites in DNA during early germination of maize seeds (from Dandoy et al., 1987)

<table>
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<th>AP sites per 10^6 nucleotides in DNA</th>
<th>Status</th>
<th>AP sites per genome per cell</th>
</tr>
</thead>
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<tr>
<td>38</td>
<td>Quiescent unimbibed seed</td>
<td>9.5 × 10^6</td>
</tr>
<tr>
<td>150</td>
<td>20-h imbibed seed</td>
<td>3.75 × 10^5</td>
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of deoxyribose units, or by covalent modification of bases. Repair of potentially mutagenic modified bases is mediated by excision repair pathways that are themselves an additional secondary source of SSBs. ROS continually arise within plant cells as a result of normal oxidative cellular processes and present a continuous danger to the integrity and viability of the cell even in the absence of external stresses.

The seed may provide an ideal ‘model’ system through which to investigate the effects of a variety of endogenous DNA damaging agents and environmental stresses on genome integrity. From the early stages of development on the mother plant, the seed experiences a range of developmentally programmed and unprogrammed oxidative, water and temperature stresses which are potentially damaging to the genome. In the case of orthodox seed, maturation drying reduces moisture content to 5–15% and production of a new seedling is subsequently initiated by rehydration and germination (Bewley & Black, 1994). In maize seeds, the maturation drying/rehydration cycle results in the appearance of thousands of SSBs in the genome of each cell (Dandoy et al., 1987; Table 2), which can result in cell cycle arrest (Reichheld et al., 1999). Significantly, this study (Dandoy et al., 1987) measured only SSBs arising indirectly from base damage by base excision repair (BER) pathways. Consequently, this figure is an underestimate of the number of SSBs appearing in the genome because it takes no account of those arising directly in the DNA from sugar damage by ROS during imbibition. The increase in apurinic/apyrimidinic sites (AP sites) seen in maize radical tip cells during the first 20 h of imbibition (Table 2) reflects repair of damaged bases either already present in the DNA of radical tip cells in the mature quiescent embryo or which appear in the genome of these cells during early imbibition. These AP sites need to be repaired before the onset of S-phase of the cell cycle, which in these maize embryos does not occur during the 36-h imbibition period studied.

The DNA of living organisms has been subjected to the damaging effects of ionising radiation throughout evolution. Although ionising radiation energy can directly cause both SSBs and DSBs (double-strand breaks, the most lethal form of damage) in DNA, a major source of spontaneous damage arises from radiolysis of water to produce ROS, including the highly damaging hydroxyl radical (Friedberg et al., 1995). Fortunately, a living organism will rarely, if ever, encounter either short bursts (acute) or lengthy (chronic) periods of exposure to high levels of ionising radiation in its lifetime. However, the explosion which occurred in the Number 4 reactor at the Chernobyl nuclear power plant in the Ukraine in April 1986 and the extensive radioactive fallout which followed resulted in the contamination of large tracts of inhabited agricultural land in Belarus, the Ukraine and Russia, and scattered areas beyond these limits. This disaster has provided a fresh impetus and relevance to studies on the effects of ionising radiation in ecosystems. This contaminated region has produced a unique radioecological situation over a large tract of land (over 600 km²) where both habitation and agriculture have been prohibited. Here, nuclear pollution of the environment can be studied and the effects correlated with the genetic hazards to animal and plant life.

The initial fallout exposed plants to radiation doses of over 60 Gy, and pine trees near to the power plant were killed by this dose. In general, woody species appeared to be more susceptible to the killing or damaging effects of radiation than herbaceous species did, but the molecular basis for this difference is unknown. In spite of these initial high levels of radiation, plants have continued to grow since the accident, even in the most contaminated areas. Several investigations into the effects of radiation on plant genome stability and on the ability of plants in this area to survive and adapt to the damaging effects of radiation have been performed (summarised in Kovalchuk et al., 2004). Wheat and rye grown in the exclusion zone exhibited high levels of chromosomal aberrations, indicating significant damage to the genome of these plants from the ionising radiation (Kovalchuk et al., 2000). Seeds of Arabidopsis thaliana that survived high levels of exposure to ionising radiation produced plants that were more resistant to radiomimetic agents or free radical producing agents than plants grown from seed from Arabidopsis exposed to lower radiation levels (Kovalchuk et al., 2004). In addition, Arabidopsis seeds collected from plants grown in the contaminated area 5–6 yr after the explosion produced plants that were more resistant to mutants than plants from the same sites collected 3–4 yr after the explosion, reflecting ongoing adaptation within these populations to survival in the face of continued chronic exposure to the effects of ionising radiation.

A series of elegant experiments utilised a recombination substrate comprised of a disrupted β-glucuronidase (uidA) reporter gene integrated into the Arabidopsis genome to study genome stability (Kovalchuk et al., 1998). These transgenic plants have proved to be useful and sensitive bioindicators of the effects of ionising radiation arising from nuclear fallout and have provided a unique insight into the remarkable adaptability of plants to respond to and survive severe acute and chronic periods of genotoxic insult. Arabidopsis plants growing in the highly contaminated exclusion zone showed adverse effects on seed germination rates and an increasing mutation frequency, which was also observed in the progeny plants from these seeds, reflecting the highly toxic effects of the levels of ionising radiation (up to 6000 Ci km⁻²) in the

polluted soil (Kovalchuk et al., 1998). Chronic exposure to ionising radiation increased both the incidence of strand breaks in the genome of exposed Arabidopsis plants and the frequency of somatic intrachromosomal homologous recombination (HR) events in the genome of transgenic plants, as detected through reconstitution of β-glucuronidase activity on histochemical staining of plants (Kovalchuk et al., 1998). Somewhat surprisingly, HR rates declined in Arabidopsis plants exposed to the highest levels of ionising radiation (above 1000 Ci km$^{-2}$), but this may not reflect a simple decline in overall recombination rates within these plants. This observation could be accounted for by an increase in the level of the alternative, less precise, illegitimate recombination pathway that may result in an inactive β-glucuronidase gene even after a recombination event. Further investigations showed a very low frequency of extrachromosomal HR events in plants from Chernobyl seed collections compared with controls (Kovalchuk et al., 2004). Collectively, these studies suggest that the survival adaptation response to genome damage arising from chronic ionising radiation effects in plants is initially to up-regulate high precision repair via intrachromosomal HR whilst down-regulating extrachromosomal recombination events, thereby preserving genome integrity by restricting potentially harmful genome rearrangements.

II. Photoreactivation

Solar UV radiation reaching the Earth’s surface comprises mainly UV-A (315–400 nm) and UV-B (280–315 nm) wavelengths. A third UV component of the electromagnetic spectrum, UV-C (200–280 nm) fails to penetrate the biosphere because it is strongly absorbed by oxygen and ozone in the atmosphere. The genotoxic effects from solar radiation arise from a combination of UV-A/visible-light-induced photosensitisation reactions and UV-B-induced dimer production between adjacent pyrimidine residues in a DNA strand. Photosensitisation reactions cause oxidative damage to bases in DNA and, to a lesser extent, abasic sites and DNA strand breaks. UV-B photons induce the formation of two major types of pyrimidine dimer, the cyclobutane pyrimidine dimer (CPD) and the pyrimidine–pyrimidone (6–4) photoprotein (6–4PP) (Fig. 1a). The 6–4PP may occasionally convert to the Dewar isomer, which is formed by photoisomerisation of the 6–4PP by light of wavelengths longer than 290 nm. Quantitative estimations of the relative occurrence of the different forms of pyrimidine dimers in plant tissues after exposure to UV-B indicate that CPDs constitute the majority of these dimers being formed at 9 times higher yield than 6–4PPs (Dany et al., 2001).

Exposure of plants to elevated levels of UV-B radiation is detrimental to plant growth, development, morphology and metabolism (Dany et al., 2001). Removal of these pyrimidine dimers is necessary for plant survival (Landry et al., 1997) because their presence in the genome is both mutagenic and cytotoxic owing to their ability to block the progress of DNA and RNA polymerases along the DNA during replication and transcription. However, there is some evidence that plants may tolerate a low level of CPDs in their genome (Britt et al., 1993; Britt & Fiscus, 2003; Schmitz-Hoerner & Weissenbock,
2003). This probably reflects the activity of specialised translesion polymerases which function in the nonmutagenic bypass of UV-B-induced photoproducts persisting in the genome (Hays, 2002; Kozmin et al., 2003). These lesions are removed from DNA in the plant cell by either relatively inefficient excision repair pathways (the so-called ‘dark repair’ pathways which are independent of light and which are described later) or via efficient enzymatic photoreactivation processes which depend upon light of wavelength 350–450 nm (Taylor et al., 1996).

The photoreactivation reaction is catalysed by a DNA photolyase enzyme that binds to UV-damaged DNA and uses a blue light (350–450 nm) photon to power cyclic electron transfer to split the pyrimidine dimer ring structure and restore the bases to their normal undamaged form (Sancar, 2003). In natural ecosystems, light in the visible region of the spectrum always accompanies solar UV-B irradiation; thus the photolyase catalysed reaction constitutes an exquisite light-dependent, error-free mechanism for repair of UV-B-induced DNA damage without the need for any base or nucleotide excision step. Distinct photolyase enzymes repair either the CPD or the 6–4PP lesion, with the enzyme responsible for recognising the 6–4PP also able to recognise and repair the Dewar isomer but at reduced efficiency compared with the 6–4PP (Carell et al., 2001). Almost all organisms, with the possible exception of placental mammals, produce a CPD photolyase, but the presence of a 6–4 photolyase has been detected only in a select number of species, including insects and plants (Table 3; Todo et al., 1996; Nakajima et al., 1998).

The reaction mechanisms by which CPD photolyases and 6–4 photolyases repair the respective pyrimidine dimers are similar. All photolyases contain two cofactors, one of which is always the two-electron-reduced form of FAD (FADH\textsuperscript{2}). The universal photocatalyst in all photolyases (Sancar, 2003). The second chromophore is either 5,10 methenyltetrahydrofolate (MTHF) or 8-hydroxy-7,8-didemethyl-5-deazariboflavin (8-HDF), which, whilst not essential for activity, are far more efficient than FADH\textsuperscript{2} as a photoantenna through which to absorb blue (350–450 nm) photons (Sancar, 2003). Plants appear to contain the reduced pterin MTHF in their CPD photolyase as the second chromophore (Waterworth \textit{et al.}, 2002). In a light-independent reaction, the photolyase binds to a pyrimidine dimer lesion which has ‘flipped out’ of the DNA structure (Berg & Sancar, 1998; Carell \textit{et al.}, 2001) and forms a stable enzyme–substrate complex (Fig. 1b). The folate chromophore of photolyase absorbs a blue light photon and transfers the excitation energy of the photon to FADH\textsuperscript{2}, promoting a one-electron transfer from FADH\textsuperscript{2} to the enzyme-bound pyrimidine dimer complex (Fig. 1b). The covalent linkage between the adjacent pyrimidines of the CPD is split and the two pyrimidine bases return to their original states in the DNA, with the simultaneous transfer of an electron back to FADH\textsuperscript{2} and regeneration of the active two-electron-reduced form FADH\textsuperscript{2} (Sancar, 2003). Thus the overall reaction does not involve either the gain or loss of an electron. A similar mechanism is suggested to operate for 6–4 photolyases, but with the inclusion of a step involving thermal conversion of the 6–4PP to an unstable oxetane intermediate before the photochemical reversion steps (Sancar, 1996).

Levels of UV-B-induced DNA damage and stage of plant development are both factors which influence the pathways employed by plant cells to remove UV-B-induced photoproducts from their genome. Low levels of pyrimidine dimers (< 30 dimers per 10\textsuperscript{6} bases) are removed from alfalfa seedlings via error-free photoreactivation mechanisms, with little contribution from excision repair pathways (Quaite \textit{et al.}, 1994). At higher DNA damage levels, additional repair capacity appears to be needed, and both photoreactivation and excision repair pathways are involved in removal of pyrimidine dimers.

Table 3 \textit{Arabidopsis} NER and photolyase genes and the corresponding ethyl methane sulfonate (EMS) mutants

<table>
<thead>
<tr>
<th>Human</th>
<th>Yeast</th>
<th>Arabidopsis</th>
<th>AGI code</th>
<th>Mutant</th>
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Characterised genes are displayed in bold type. Genes identified by database analysis are taken from Kunz et al. (2005).
dimeric transcripts from the genome. There are also contrasting gene expression patterns for photoreactivation and excision repair genes in different tissues during plant development. Although photoreactivation mechanisms are widely distributed throughout both meristematic and nonproliferating cells in above ground organs in plants, the expression of excision repair pathway transcripts is much higher in proliferating than nonproliferative tissue (Kimura et al., 2004). In Arabidopsis, the highest levels of CPD and 6–4PP photolyase proteins are found in floral tissues, where they may serve to minimise formation of mutagenic lesions in germline DNA because long-term exposure to high UV-B levels may have deleterious effects on the genomes of the cells in plant reproductive tissues (Ries et al., 2000). Repair of UV-B-induced damage to the genome is expected to be more critical in young growing seedlings where DNA replication is associated with elevated levels of cell division. However, young Arabidopsis seedlings contain very low levels of CDP photolyase protein compared with mature leaf tissue indicating an important role for DNA repair in differentiated tissues (Waterworth et al., 2002). In contrast, expression of excision repair genes in wheat and Arabidopsis is higher in proliferating cells compared with developmentally mature cells (Taylor et al., 1996; Waterworth et al., 2002; Kimura et al., 2004).

CPD photolyase gene expression in Arabidopsis tissues is inducible by white light (Ahmad et al., 1997), whereas 6–4PP photolyase gene expression is constitutive (Waterworth et al., 2002). However, white light intensity does affect the rate of removal of both CPD and 6–4PP photoproducts from wheat leaf DNA. Dimer removal rates decrease with decreasing light fluxes (Taylor et al., 1996), with 6–4PP removal affected to a lesser extent than CPD removal, indicating either a decrease in CPD gene transcription or a more rapid turnover of CPD photolyase protein than 6–4PP photolyase under low light conditions or the more effective removal of 6–4PPs by light-independent excision repair pathways. Significantly, the rate of CPD photolyase protein turnover is slow in Arabidopsis (Waterworth et al., 2002), which implies that there will always be a low level of CPD photolyase protein present in tissues from field-grown plants or plants experiencing normal light/dark cycles. Therefore these plants will be capable of photoreactivation upon first exposure to sunlight, of which UV-B is an inevitable component. The white light induction of plant CPD photolyase combined with the CPD protein turnover rate in cells may explain why a delay and initial slow rate of repair of CPDs has been seen in plants exposed to UV-B and white light after prolonged periods in the dark (Chen et al., 1994).

Thinning of the stratospheric ozone layer in the latter part of the 20th century has resulted in elevated solar UV-B irradiation levels reaching the Earth’s surface (Madronich et al., 1998) and stimulated significant research into the biological consequences of these increased UV-B irradiation levels on ecosystems. Tierra del Fuego, at the southern tip of South America, is the only region where a well-developed terrestrial ecosystem is affected by ozone depletion, and this area has been the subject of intensive research into the effects of elevated solar UV-B irradiation on plants in the environment (Ballaré et al., 2001). Using Gunnera magellanica as an indigenous ‘model’ herbaceous plant, these studies demonstrated that steady-state levels of CPDs in leaf tissue were linearly correlated with UV dose at ground level. Increased solar UV-B levels in early spring had a subtle but significant inhibitory effect on leaf growth and morphology in G. magellanica and in other herbaceous species studied but had no effect on growth of woody perennials. This effect of UV-B contrasts with that of γ-irradiation, for which woody species appear more susceptible than herbaceous species (Kovalchuk et al., 2004). These observations indicate distinct species differences in response to enhanced UV-B irradiation levels, and in the case of G. magellanica may reflect the low CPD repair capacity of this species compared to other species studied (Ballaré et al., 2001). These and other studies (Stapleton & Walbot, 1994; Ries et al., 2000; Britt & Fiscus, 2003) have also demonstrated that the production of UV-absorbing molecules such as flavanols and sinapate esters by plant cells in response to elevated UV-B and white light levels is only partially effective in protecting the plant genome against the damaging effects of enhanced solar UV-B irradiation.

Elevated UV-B levels may also be accompanied by global warming in the early part of the 21st century (Moody et al., 1997). Photolyase-mediated repair of UV-B-induced DNA damage has been reported to be sensitive to temperature increases above ambient (Pang & Hays, 1991; Takeuchi et al., 1996), which could have important implications for crop yield and biomass production via detrimental effects on morphology and growth. However, this temperature sensitivity of the CPD photolyase, at least for Arabidopsis, may vary with ecotype because it appears more significant in Columbia than in Landsberg erecta (Pang & Hays, 1991; Waterworth et al., 2002). The residual CPD photolyase activity present in Arabidopsis grown at temperatures up to 37°C would still be sufficient to cope with the anticipated increased levels of UV-B-induced DNA damage sustained under these growth conditions (Waterworth et al., 2002). However, the same may not be true for all species, especially genetically inbred crop species. The long-term effects of increased UV-B irradiation levels accompanying depletion of the stratospheric ozone layer on genome stability within plant populations is difficult to predict with any certainty. However, in addition to photoreactivation and excision repair pathways, evidence is now emerging that HR mechanisms may also contribute to the repair of UV-B-induced DNA damage in Arabidopsis in conditions of long-term exposure to UV-B (Ries et al., 2000). Further research is required to determine whether other species have retained the protective mechanisms against the adverse effects of UV-B irradiation and global warming on genome integrity that have been found in Arabidopsis.
III. Excision repair pathways: BER, NER, mismatch repair

Many forms of DNA damage affect only one of the two strands of the duplex. Highly accurate repair of these DNA lesions is facilitated using the sequence information provided by the complementary strand. This process is initiated by the recognition and excision of the damaged region and completed by repair synthesis and ligation. There are distinct excision repair pathways (termed dark repair pathways in plants) that are highly conserved through evolution.

1. Base excision repair

Metabolic by-products and environmental genotoxins react with cellular DNA to produce SSBs and a variety of different base adducts. Experimentally, base modification is widely exploited in plant mutagenesis, which uses ethyl methane sulfonate (EMS) to produce alkylation products, including O6-ethylguanine. This modified base is recognised as adenine by the DNA replication machinery, causing G–A mutations (Vidal et al., 1995; Greene et al., 2003). Whereas some base adducts are miscoding, others, including 3-methyladenine, block replication and transcription and are highly cytotoxic. A wide range of damaged bases can be eliminated from the genome by BER. This pathway is initiated by removal of the damaged base by a DNA glycosylase enzyme that is specific for the particular base adduct (Caldecott, 2001; Fromme et al., 2004). Several DNA glycosylases have been characterised in plants, including a 3-methyladenine-specific DNA glycosylase (Santerre & Britt, 1994), which showed highest expression in tissues undergoing rapid cell division and growth (Shi et al., 1997). Some glycosylases have a secondary activity, cutting the DNA backbone on the 3′ side of the abasic (AP) site (Fig. 2). For example, repair of the guanine oxidation product 7,8-dihydro-8-oxoguanine (8-oxoG) in Arabidopsis is catalysed by the bifunctional 8-oxoG DNA glycosylase/AP lyase (Dany & Tissier, 2001; Garcia-Ortiz et al., 2001). 8-oxoG is read as thymidine, giving rise to G–T mutations. This damaged base can also be repaired by AtMMH, the Arabidopsis homologue of Escherichia coli MutM, indicating redundancy in plant BER pathways (Ohtsubo et al., 1998; Murphy & Gao, 2001).

DNA glycosylase activity results in release of the damaged base by a DNA glycosylase-endonuclease and the AP site is cleaved by AP endonuclease. POLβ removes the deoxyribose sugar if necessary and fills in the gap. In mammals the single-stranded gap is sealed by the XRCC1-LIG3 complex. This pathway may be active in plant cells, although there is no LIG3 homologue. (b) Long-patch repair. After base removal and APE nicking, the replicative DNA polymerase δ/ε complex fills the gap and displaces several nucleotides adjacent to the AP site. Endonuclease activity removes the overhang and the nick is re-joined by LIG1. Adapted from Sancar et al. (2004).
In cases where the damaged base is removed by a bifunctional DNA glycosylase/AP lyase, the subsequent 5′ incision by APE results in the release of the deoxyribose moiety. In mammalian cells, the resulting gap can be filled by short-patch repair mediated by the POLβ-XRCC1-LIG3α pathway. POLβ has intrinsic deoxyribose (dRP) lyase activity, and this enzyme can also remove the sugar left by a monofunctional DNA glycosylase, and the DNA polymerase activity fills the single nucleotide gap (Caldecott, 2001). The short-patch repair appears to be less well conserved, and there are no clear plant homologues of DNA polymerase β (POLβ) or of DNA ligase III. However, rice DNA polymerase λ, which shares a similar N-terminal domain with human Polλ, has in vitro dRP-lyase activity, indicating a potential POLβ-like role in BER (Uchiyama et al., 2004). Interestingly, an Arabidopsis XRCC1-like protein lacks the domains responsible in mammalian XRCC1 for interaction with POLβ and LIG3α but retains a conserved BRCT domain which may mediate interaction with poly(ADP-ribose)polymerase (PARP) (Doucet-Chabeaud et al., 2001; Taylor et al., 2002; Uchiyama et al., 2004). There are at least two PARP activities in plants and, whilst the functions of these proteins remain uncertain, they may have a role in DNA damage sensing in both excision and recombinational repair pathways (Amor et al., 1998; Babiychuk et al., 1998; Babiychuk et al., 2001).

2. Nucleotide excision repair
In contrast to BER, NER can act on a wide range of substrates as this pathway detects modifications indirectly by conformational changes to the DNA duplex rather than relying on the recognition of specific DNA damage products. NER targets the damaged strand and removes a 24–32 base oligonucleotide containing the damaged product (Fig. 3). DNA synthesis and ligation completes the repair process (Sancar et al., 2004). This highly conserved DNA repair pathway is also found in plants and was initially characterised by EMS mutagenesis. These experiments defined a number of uvr and uvh complementation groups that showed hypersensitivity to UV-C, some of which were also hypersensitive to γ-irradiation (Jenkins et al., 1995; Jiang et al., 1997). In addition to the NER genes identified in these genetic screens, many putative homologues of the evolutionarily conserved NER components can be found in plant genome databases (Table 3; The Arabidopsis Genome Initiative, 2000; Kunz et al., 2005).

![Fig. 3 Pathway for NER in human cells. In global genomic repair, DNA damage is recognised through binding by the XPC/RAD23 complex which recruits the TFIIH transcription factor. Transcription-coupled repair (not shown) occurs where RNA polymerase stalls at the site of DNA damage and is independent of XPC. Helicase activity of the TFIIH components XPD and XPB unwinds the damaged region and the endonucleases XPG and XPF/ERCC1 allow the release of the single-stranded oligonucleotide. Repair synthesis is catalysed by the POLβ/ε complex and completed by LIG1-mediated ligation. Adapted from Sancar et al. (2004).]
NER is catalysed by six multiprotein complexes, and the initial DNA damage detection step can occur by two different processes dependent on the transcriptional activity of the damaged region. Global or general genomic repair (Fig. 3) is initiated by damage recognition mediated by the xeroderma pigmentosum group C (XPC)/hHR23B complex (Thoma & Vasquez, 2003). In contrast, transcription-coupled repair (TCR) is independent of XPC and is initiated when RNA polymerase II stalls at the site of DNA damage (Mu & Sancar, 1997). TCR in mammals requires the Cockayne syndrome (CS) CSB and CSA proteins, which may help remove the stalled RNA polymerase complex and facilitate DNA repair (Svejstrup, 2002; de Waard et al., 2004).

In general genomic NER in humans, the XPC/hHR23 complex is stabilised by hCEN2, and Arabidopsis plants deficient in AtCEN2 show decreased repair of UV-C-induced DNA damage in vitro (Molnier et al., 2004). XPC/hHR23B binding recruits TFIIH, which functions in the cell as an RNA polymerase II transcription factor in addition to its role in repair. A rice hHR23B homologue was first identified through its interaction with the transcription factor VP1 (Schultz & Quatrano, 1997). The TFIIH transcription factor complex contains the helicases XPB and XPD which unwind the DNA, allowing binding of XPG with the concomitant release of XPC (Fig. 3). Unlike most organisms, Arabidopsis has two isoforms of XBP with similar expression patterns, and both genes are able to complement repair defects in the corresponding Saccharomyces cerevisiae mutant (Costa et al., 2001; Morgante et al., 2005). Despite this apparent redundancy, atxbp1 mutant plants show hypersensitivity to alkylating agents and to ROS, and display delayed development, especially during germination and early seedling growth (Costa et al., 2001). In contrast with Arabidopsis XPB, human XBP and yeast RAD25 are single-copy genes and null mutations are lethal, indicating the essential role of the TFIIH transcription factor in development. The comparatively mild phenotype of atxbp1 mutants may suggest that AtXPB2 partially compensates for the lack of AtXPB1 activity or that the truncated AtXPB1 transcript expressed in the mutant lines may encode a protein with residual activity (Costa et al., 2001). The 5′–3′ TFIIH helicase component, XPD, is encoded by a single-copy gene in Arabidopsis and null mutants are lethal (Liu et al., 2003). However, a point mutation in AtXPD in the ush6–1 Arabidopsis line resulted in reduced growth, yellowing leaves and a slight decrease in the rate of repair of UV-induced 6–4 photoproducts (Liu et al., 2003).

After unwinding of the DNA by the helicase action of XPD and XPB, the RPA/XPA complex is recruited (Tapias et al., 2004). This is followed by endonuclease activity of XPG, which nicks the damaged DNA strand 3′ to the lesion (Fig. 3), and an Arabidopsis xpg mutant, ush3–1, which shows hypersensitivity to UV-C consistent with this role of AtXPG in NER (Liu et al., 2001). The 5′ incision is catalysed by the XPF-ERCC1 nuclease complex, allowing the release of the damaged DNA stretch as a 24–32 nucleotide oligomer. Arabidopsis XPF is named using the Saccharomyces cerevisiae nomenclature as AtRAD1, and the full-length gene was shown to be partially functional in yeast rad1 mutants (Gallego et al., 2000). Arabidopsis ush1–1 mutants are deficient in AtRAD1 and display a small reduction in CPD repair and hypersensitivity to DNA damage (Fidantsef et al., 2000; Gallego et al., 2000; Liu et al., 2000). A Lily (Lilium longiflorum) ERCC1 homologue was able to complement the MMC hypersensitivity of ERCC1-deficient Chinese hamster ovary cells (Xu et al., 1998). Mouse ERCC1 mutants are embryonic lethal, whereas an Arabidopsis uth7–1 mutant containing a premature stop codon in the AtERCC1 gene is viable, although hypersensitive to UV-C and γ-irradiation (Heffner et al., 2003).

RAD1/ERCC1 has additional roles in the removal of DNA ‘flap’ structures formed during recombination. Differences in recombination frequencies between inverted chromosomal repeats in aterc1 and wild-type plants showed that AtERCC1 also has a role in the major HR pathways responsible for gene conversion in plants (Dubest et al., 2004). The gap generated by the endonuclease activities of XPG and the AtRAD1/ERCC1 complexes is filled by the replicative DNA polymerase complex of Polβ/ε, PCNA, RFC and RPA and the phosphodiester backbone rejoined by DNA ligase – probably AtLIG1 (Taylor et al., 1998).

### 3. Mismatch repair

During genome replication, the replicative DNA polymerases accurately select their deoxynucleotide substrates and use their associated proof-reading functions to remove misincorporated nucleotides. Together, these checking mechanisms realise a fidelity level of one incorrect base incorporated per 10^5–10^6 base pairs in replicating DNA. However, this level of accuracy is still insufficient for effective genome integrity maintenance, and a mismatch repair (MMR) mechanism which is highly conserved between species subsequently removes the vast majority (99.9%) of the errors remaining after polymerase proofreading to reduce the error rate to one misincorporated base per 10^5–10^10 nucleotides in the nascent DNA chain (Leonard et al., 2003). MMR may also have an important role in recognising mismatches at sites of recombination between DNA sequences, thereby reducing the rate of occurrence of recombination events which might lead to inappropriate chromosome rearrangements or interspecies hybridisation (Wu et al., 2003).

The eukaryotic homologues of the evolutionarily conserved prokaryotic MutS proteins involved in MMR are the MSH protein subunits. Their role in MMR is to recognise the rare sporadic mismatches in replicating DNA and to prevent the establishment of mutations in the genome by discriminating between the existing correct nucleotide in the template strand and the incorrect nucleotide inserted into the replicating DNA strand (de Wind & Hays, 2001). In plants, heterodimeric proteins involving MSH2, MSH3, MSH6 and MSH7 are responsible for executing this initial stage of MMR. MSH2, 3 and 6 homologues are found in all eukaryotes but MSH7 is unique.
to plants. *Arabidopsis* also contains a homologue of the yeast MSH1 protein which is absent from mammalian cells. AtMSH1 appears to be targeted to mitochondria but its role in MMR or mitochondrial genome stability has yet to be established (Hays, 2002). In *Arabidopsis*, the heterodimeric proteins AtMSH2.MSH6 (termed AtMutSα) and AtMSH2.MSH7 (AtMutSγ) recognise mismatched bases (Fig. 4) but differ in specificity of which mismatches are recognised (Wu et al., 2003). AtMSH2.MSH3 (AtMutSβ) recognises regions of DNA with extrahelical loops but not mispairs, similar to the human orthologue (Culligan & Hays, 2000). Use of *Arabidopsis Atmsh2* lines in which AtMSH2 is inactivated has demonstrated a critical requirement for efficient MMR in germline cells but a less critical role for MMR in differentiated somatic cells (Hoffman et al., 2004). *Arabidopsis* has two other proteins resembling MSH homologues MSH4 and MSH5 (Hays, 2002). The *Arabidopsis* MSH4 homologue (AtMSH4) has been shown to function at an early stage in meiotic recombination (Higgins et al., 2004), but a role for either AtMSH4 or AtMSH5 in MMR has yet to be established.

In the final step of MMR in animal cells, an MLH1.PMS2 heterodimer plays a key role in discriminating between the template and nascent DNA strands, and then specifically promotes the excision of a stretch of nascent strand DNA containing the misincorporated nucleotide, with the excision process terminating just beyond the mismatched base on this strand. Finally, a high-fidelity DNA polymerase fills in the stretch of gapped DNA and a DNA ligase, probably DNA ligase 1 in plants (Taylor et al., 1998), rejoins the DNA ends (Fig. 4). *Arabidopsis* contains orthologues of MLH1, PMS2 and also a further MLH orthologue, MLH3 (Hays, 2002), and these proteins may play a role in the coupling of mismatch recognition to nascent strand identification and excision steps in MMR in the plant cell (Fig. 4).

**IV. DNA double-strand break repair**

There are two fundamental mechanisms for recombination which are required for the repair of DNA DSBs. HR uses an identical or very similar DNA sequence as a template for the repair of a DSB. Illegitimate recombination, also termed nonhomologous end joining (NHEJ) recombines DNA largely independent of the sequence. These pathways are responsible for balancing genome stability against the generation of genetic diversity.

1. **Homologous recombination**

HR is important in DNA repair in somatic plant cells and also occurs during meiosis. There are three models for HR, which are largely based on work done in yeast (reviewed in Aylon & Kupiec, 2004; Krogh & Symington, 2004).

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**Fig. 4** Simplified overview of DNA mismatch repair in *Arabidopsis*. Initial recognition of the mismatch lesion by the appropriate ATMSH heterodimer(s) is followed by a step in which an enzyme complex, possibly involving AtMLH1. PMS2, discriminates between the nascent and template DNA strands, promoting unwinding of the DNA helix, excision of the nascent strand to a point beyond the mismatch lesion and culminating in resynthesis and ligation steps to fill in the gap in the nascent DNA strand.
DNA double-strand break repair (DSBR) model  Meiotic recombination is best described by the double-strand break repair (DSBR) model (Fig. 5b). DSBR is initiated by a DSB and mediates Holliday junction formation, crossing over between aligned homologous chromosomes rather than sister chromatids. Initially, the ends of the duplex at the DSB are extensively resected to produce long 3' tails that invade the homologous chromosome. DNA synthesis is primed from the invading DNA double-strand break repair (DSBR) model

Fig. 5 Pathways for double-strand break (DSB) repair in plants. (a) Nonhomologous end joining is catalysed by a complex of KU70 and KU80 which binds the exposed DNA ends. The DSB may then be processed by the MRE11/RAD50/NBS1 (MRN) complex before ligation by the LIG4/XRCC4. Other pathways of illegitimate recombination are likely to be present in plant cells. Homologous recombination (HR) is initiated by extensive resection to form long 3' tails which are coated with RPA. Formation of a RAD51 filament promotes homology search and strand invasion. In meiotic cells, HR occurs largely by the DSBR model (b), resulting in Holliday junction formation and crossing over. In somatic plant cells, HR predominantly occurs by synthesis-dependent single-strand annealing (c), in which DNA synthesis using a homologous template results in sequence overlap across the region of the break allowing re-annealing and break repair. Adapted from Krogh & Symington (2004).
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strand using the homologous chromosome as a template. Ligation of the invading strand with the other side of the DSB results in the formation of a joint molecule containing two Holliday junctions which can be resolved with or without crossing over (Fig. 5b). Recombination between sister chromatids is inhibited in meiotic cells, promoting the genetic mixing between maternal and paternal homologous chromosomes. Meiotic recombination in plants has been the subject of recent reviews (Bhatt et al., 2001; Caryl et al., 2003; Jones et al., 2003; Schuermann et al., 2005).

Synthesis-dependent strand annealing (SDSA) A second model for HR, termed synthesis dependent strand annealing (SDSA) plays an important role in DSB repair in somatic cells (Aylon & Kupiec, 2004; Puchta, 2005). This pathway can use a sister chromatid, homologous chromosome or ectopic region of homology in the genome as a recombination substrate. As with the DSBR model, SDSA is initiated by a 3′ resection forming a long single-stranded DNA tail that invades a homologous duplex and primes DNA synthesis. In contrast to the DSBR model, the newly synthesised DNA then re-anneals with the other side of the DSB, repairing the break and avoiding the formation of the joint molecule (Fig. 5c). SDSA reduces the likelihood of Holliday junction formation and crossing over, which would be highly mutagenic if this occurred between ectopic regions of homology (Paques & Haber, 1999). However, Holliday junction formation can occur during SDSA and crossing over would not be mutagenic if restricted to sister chromatids. An attractive mechanism proposes that crossing over only occurs between regions of extensive homology, thus reducing the chances of chromosomal translocations arising from ectopic recombination (Inbar & Kupiec, 1999; Inbar et al., 2000). Both SDSA and DSBR-mediated HR may operate in somatic and meiotic cells, although the relative frequencies may differ between the different cell types.

Single-strand annealing (SSA) A third mechanism of HR may occur between tandemly repeated sequences, where homologous regions exposed during resection anneal by single-strand annealing (SSA) and the intervening sequence is deleted. This pathway may be of particular importance on regions of the genome containing repeated gene arrays.

HR pathways in plants There is evidence for all three HR pathways operating in plants and growing evidence that much of the molecular machinery is common between the different HR mechanisms (Puchta, 2005; Schuermann et al., 2005). Recombination frequencies vary greatly, depending on the origin of the donor and recipient sequences. In plant somatic cells, HR-mediated DSB repair using allelic sequences occurs very rarely, accounting for around 1 in 10 000 repair events (Gisler et al., 2002). Similar frequencies were observed for the repair of a DSB using ectopic homologous sequences, indicating that, at least in cells in G1 phase of the cell cycle, HR plays a very minor role in DSB repair in plants (Puchta, 1999). However, in situations where homologous sequences are readily available, for example if a break occurs between tandem repeats, a third of DSBs are repaired by SSA and around 7% are repaired by SDSA (Siebert & Puchta, 2002; Orel et al., 2003). But, despite the close proximity of homologous sequences, the majority of repair was catalysed by the NHEJ pathway. The frequency of recombination between sister chromatids is harder to measure and it is still unclear how significant this pathway is in DSB repair in plants (Schuermann et al., 2005). DNA damage, including that induced by UV-B irradiation and bleomycin treatment, significantly increases recombination frequencies. Increased recombination may result from a combination of factors, including activation of the HR pathway by signalling pathways on detection of DNA damage, the formation of HR-repair substrates and an indirect effect of chromatin remodelling around the sites of DNA damage, making the recombination substrate more accessible to the HR machinery (Ries et al., 2000; Molinier et al., 2004). Similar explanations may account for the observed increases in somatic HR found in DNA repair mutants including atcen2 (CPD-photolyase), atrad50 and atmre11.

Molecular mechanism of HR Meiotic and somatic recombination pathways share similarities at the molecular level (Caryl et al., 2003; Schuermann et al., 2005). In yeast and mammals, HR is catalysed by RPA and the RAD52 epistasis group (RAD51, RAD52, RAD54 and the MRN complex of RAD50, MRE11 and yeast XRS2/human NBS1). RAD51 is similar to the bacterial recombinase RecA and binds the 3′ ssDNA tails, catalysing strand invasion. Arabidopsis HR null mutants, including AtRAD51, AtRAD50 and AtMRE11, are completely sterile owing to severe meiotic defects; meiocytes of these mutant lines show extensive chromosome fragmentation, resulting in nonviable gametes (Li et al., 2004; Puizina et al., 2004; Bleuyard et al., 2005). These mutants also fail to align homologous chromosomes (synapsis) during the early stages of meiosis.

Arabidopsis and mammalian HR also involves the RAD51-like proteins RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 and the regulatory proteins BRCA1 and BRCA2 (Mclverraith et al., 2000; Bleuyard et al., 2005). Interestingly, Arabidopsis knockouts in the RAD51 paralogues display hypersensitivity to the DNA cross-linking reagent mitomycin C (MMC) but little or no hypersensitivity to γ-radiation (Bleuyard et al., 2005). Hypersensitivity to interstrand DNA cross-links (ICLs) suggests a role for HR in the repair of this form of DNA damage, as was found in yeast (Grossmann et al., 2001). The mild sensitivity to γ-radiation may suggest that HR has a minor role in DSB repair in plants. The DSB hypersensitivity found in Arabidopsis rad50 and mre11 knockouts may reflect the roles of these genes in NHEJ (Galleco et al., 2001; Bundock & Hooykaas, 2002).
2. Nonhomologous end joining

NHEJ is the major pathway for DSB repair in most higher eukaryote cells. The initial recognition of the DSB may be mediated by a complex of Ku70 and Ku80. Mutation of Arabidopsis KU genes results in plants that are hypersensitive to γ-irradiation, bleomycin (which induces strand breaks in DNA) and methyl methane sulphonate (MMS, a DNA alkylating agent), consistent with the role of these genes in DSB repair (Riha et al., 2002; West et al., 2002). Ku proteins have additional roles in eukaryotes in maintaining telomeres, providing an interesting link between DNA-damage-related DSBs and naturally occurring chromosome ends (Riha et al., 2002; Gallego et al., 2003a). The DNA ends may then be processed (possibly by the MRN complex) to make them suitable substrates for DNA ligase. Processing can involve the alignment of DNA ends at microhomologies of one or more bases and trimming of overhanging DNA 'flaps'. Ligation is then catalysed by a complex of DNA ligase IV and XRCC4 (West et al., 2000). Although Arabidopsis NHEJ mutants show clear hypersensitivity to DSB-inducing agents, plants are still able to grow in the presence of relatively high concentrations of bleomycin, suggesting that alternative repair pathways are active in Arabidopsis. This supposition is supported by several lines of evidence, including Ku70-independent NHEJ between chromosomes in atku70 and attert double mutants and the rapid repair of DSBs in Arabidopsis NHEJ mutants assayed by single-cell electrophoresis (Angelis, West & Bray, unpublished results; Heacock et al., 2004). Further analysis of chromosome fusions in atku70.attert double and atku70.attert.mre11 triple mutants indicated that Ku-independent end joining showed greater use of microhomologies, whereas pathways independent of both Mre11 and Ku were associated with the insertion of a filler sequence (Heacock et al., 2004). Deletions and insertions of filler sequences are often observed during plant illegitimate recombination, and the insertions may be copied from ectopic sites in the genome or from extrachromosomal DNA during plant transgene integration into the host genome (Kirik et al., 2000). The insertion of filler DNA suggests a mechanism for illegitimate end joining involving a synthesis-dependent annealing-like reaction, although given the differences in requirements for homology this pathway presumably differs mechanistically from SDSA-mediated HR (Puchta, 2005). NHEJ in atku70.attert.mre11 triple mutants indicates the presence of novel recombination pathway(s) which may be responsible for the rapid DSB repair observed in NHEJ mutant backgrounds (Heacock et al., 2004). Interestingly, Agrobacterium-mediated transformation frequencies of NHEJ mutants show little or no difference to wild-type plants, indicating that Ku-dependent NHEJ is not essential for transgene integration, in marked contrast to the situation in yeast (van Attkum et al., 2001; Friesner & Britt, 2003; Gallego et al., 2003b; van Attkum et al., 2003).

V. Molecular responses to genotoxic stress: DNA damage sensing and signalling

DNA damage sensing is an essential requirement to minimise the deleterious effects of genotoxins on cellular growth. Sensing and signalling mechanisms promote genomic integrity by delaying passage through the cell cycle to allow time for repair and activation of repair pathways. In the absence of this signalling, DNA replication and mitosis (or meiosis) occurs unchecked and exacerbates the DNA damage. For example, DNA replication can convert SSBs into DSBs, and nuclear division in the presence of a DSB leads to loss of chromosome fragments and aneuploid daughter cells (Sancar et al., 2004).

Gamma irradiation of Arabidopsis plants results in an increase in the numbers of cells in G2, suggesting the presence of a DNA damage responsive G2/M checkpoint in plants (Preuss & Britt, 2003; Culligan et al., 2004). This G2 arrest appears to be a controlled event rather than a direct effect of DNA damage because mutants can be isolated that overcome this checkpoint, allowing entry into mitosis and radioresistant growth (Preuss & Britt, 2003). In yeast and mammals, the phosphoinositotol-3 kinase-like kinases (PI3KK), which have protein rather than lipid substrates, play important roles in signalling DNA damage within the cell (Durocher & Jackson, 2001). These include ATM (ataxia telangiectasia-mutated, Mec1 in S. cerevisiae), ATR (ATM-Rad3-related) and the mammalian DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Plant homologues of ATM and ATR play important roles in the response to DNA damage and programmed DSBs induced during meiosis.

Whereas ATM responds primarily to damage causing DSBs, ATR mediates responses to a wider range of genotoxins, especially those that interfere with DNA replication. Consistent with this, Arabidopsis atr mutants show only mild hypersensitivity to γ-irradiation, but severe growth defects in the presence of hydroxyurea, which inhibits DNA replication by reducing the availability of dNTPs through inactivation of ribonucleotide reductase (Culligan et al., 2004). This contrasts with mammals, in which mutations in ATR are lethal (Cortez et al., 2001). In addition, aphidicolin, an inhibitor of DNA polymerases δ and ε, used at concentrations that disrupt rather than inhibit DNA replication, causes G2 arrest in wild-type but not in atratr mutant plants (Culligan et al., 2004). Some overlap in function exists between Arabidopsis ATM and ATR because double mutants are completely sterile, whereas atr mutants are normal and atm mutants have reduced fertility (Culligan et al., 2004).

The sensing mechanisms that activate ATR integrate a wide range of insults to genomic DNA into a single damage response. The DNA damage signal resulting in ATR activation in mammals is ssDNA, which is present at collapsed replication forks, in recombination intermediates and is formed during excision repair pathways. Recognition of ssDNA is mediated by RPA, ATR and ATRIP (Zou & Elledge, 2003).
In contrast to ATR, activation of ATM occurs largely in response to DSBs, and in mammals requires the MRN complex (Falck et al., 2005; Lee & Paull, 2005). Arabidopsis plants deficient in ATM are hypersensitive to γ-radiation and display extensive chromosome fragmentation during meiosis, leading to reduced fertility (Garcia et al., 2003). Arabidopsis atm mutants fail to induce transcription of DNA repair genes following γ-irradiation, consistent with the role of ATM in DNA damage signalling in plants. This ATM-mediated signalling is rapid in Arabidopsis, resulting in transcriptional induction of AtRAD51 within 30 min. Mammalian ATM phosphorylates a number of proteins involved in DNA repair and cell cycle control, including p53, NBS1, the cell cycle checkpoint proteins CHK1 and CHK2 and the histone H2A variant termed H2AX (Shiloh, 2003). Phosphorylation of Arabidopsis H2AX occurs within 20 min of irradiation and is largely dependent on ATM during M-phase and ATR during S-phase (Friesner et al., 2005). Phosphorylated histone H2A has proven to be a powerful marker for DSBs in situ in yeast and mammalian systems, and the application of this approach to plants will enhance our understanding of repair processes.

The combined and partially overlapping activities of ATM and ATR in plants results in the phosphorylation of target proteins and activation of checkpoint and DNA repair processes. Downstream targets of DNA damage signalling include the transcriptional activation of genes involved in DNA repair and the cell cycle (Chen et al., 2003; Garcia et al., 2003). Putative substrates of Arabidopsis PI3KKs have been characterised recently and include the checkpoint complex AtRAD9, AtRAD1-like and AtHUS1 (the 9−1 complex) and the DSB-inducible AtRAD17 checkpoint protein (Heitzberg et al., 2004). RAD17 forms an RFC-like complex which loads the 9−1 complex onto chromatin. This complex forms a ‘sliding clamp’ analogous to PCNA and may function to recruit further repair factors and initiate signalling pathways (Bermudez et al., 2003).

**VI. Conclusions and future prospects**

The ability of plants to perceive and respond to environmental stresses which compromise genomic integrity is crucial, not only to the survival of the individual plant but also to future generations. The identification and characterisation of the molecular components that detect specific DNA damage products and the elucidation of how these signals trigger responses that lead to cell cycle arrest and promote DNA repair will reveal the molecular mechanisms by which intracellular receptors give rise to appropriate DNA damage responses. Many similarities have emerged in the comparative study of plant and animal responses to genome damage. However, several key components integral to the animal signalling pathway appear to be absent from plants, indicating that fundamental differences exist between distantly related phyla in the recognition and signalling of DNA damage.

An intriguing recent discovery relevant to DNA repair has arisen from investigations on Arabidopsis plants homozygous for recessive mutant alleles of the organ fusion gene HOT-HEAD (HTH). This study has presented evidence for the existence of a pathway in Arabidopsis for the non-Mendelian inheritance of sequence information maintained outside the plant genome (Lolle et al., 2005). The pathway utilises a type of ‘memory’ mediated possibly via an ancestral stable RNA-sequence cache similar to that which permits RNA silencing effects. Most crucial is the observation that the extragenomic information in this RNA cache can persist over several plant generations. Thus the RNA components of this cache represent a library of useful allelic sequences containing genetic information that has proved to be functionally important for survival of the plant. Specifically this ‘genetic memory’ within the RNA cache can be used by the plant cell to restore deleterious mutations arising in key genes back to their ancestral functional wild-type sequence, even in the absence of a wild-type allele of the gene in the nuclear genome. This landmark discovery thus represents an as yet ill-defined, but exquisitely unique, DNA repair mechanism distinct from all pathways known to date and could also be present in other organisms in addition to plants. The ramifications of this exciting discovery for DNA repair processes are numerous and significant, and elucidation of the detailed mechanism of this pathway and how and when it is used by plants is eagerly awaited.

The proteomics era has brought an increasing awareness that most proteins exert their function by way of protein–protein interactions and that enzymes are often held in tightly controlled regions of the cell by such interactions. The control of subcellular localisation of proteins and their interaction with specific protein partners in vivo are important parameters that provide clues to their function and regulation. It is now becoming clear that eukaryotes employ extensive and dynamic multiprotein complexes to perform DNA replication, repair and recombination. Additionally, there is substantial evidence that the distribution of DNA recognition and protein–protein interaction domains amongst the putative components of DNA replication and repair complexes in plants is clearly distinct from that in mammals. Integration of approaches based on functional genomics and proteomics can complement established whole-plant physiological and ecological studies to accelerate characterisation of components of intracellular signal transduction pathways that mediate the plant’s response to DNA damage. A fundamental knowledge of plant DNA damage sensing, signalling and repair pathways is essential for a more directed approach to the improvement of crop species to tolerate environmental genotoxins. Furthermore, an understanding of these pathways will help elucidate the mechanism by which transgenes insert into the host plant genome. Such knowledge will impact on biotechnology because it may lead to the development of more controlled methodologies, leading to site-specific T-DNA insertion during plant transformations. Arabidopsis plants mutated in a
range of key DNA repair genes are now becoming available and their use combined with major advances in protein analytical technologies will hasten progress towards these aims. The complex organisation and compartmentalisation of plant cells makes it possible that the molecular behaviour of proteins in vitro may be different to that in planta. Therefore it is essential to demonstrate that those proteins identified through in vitro studies on plant DNA repair pathways as interacting proteins can associate with their putative partners in planta. The presence of such higher order complexes in planta will give compelling support for the significance of these protein–protein interactions in genome maintenance functions. The use of new approaches such as fluorescence resonance energy transfer (FRET) imaging microscopy will advance our knowledge on the direct interactions of such candidate partner proteins in planta. FRET can be used to monitor protein–protein interactions in vivo in real-time using host proteins tagged with two spectrally overlapping chromophores such as the GFP variants cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). This approach will not only establish whether the specific direct protein–protein interactions identified through in vitro studies occur in planta but should also provide a unique insight into the extent of DNA repair protein complex formation in planta in real-time in response to changes in developmental status and genome integrity in cells. Through the use of such approaches it will then be possible to establish any novel interactions of key repair proteins with specific protein partners. This will permit a more comprehensive understanding of how the complex interrelated network of signalling and effector molecules serves to maintain the integrity of the plant genome in the face of a continuous assault from both endogenous and environmental sources.

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