Repair of Damaged Bases

Author: Britt, Anne

Source: The Arabidopsis Book, 2002(1)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0005

The Arabidopsis Book

First published on April 4, 2002: e0005. doi: 10.1199/tab.0005

Repair of Damaged Bases

Anne Britt

Section of Plant Biology, University of California, Davis, CA 95616
Telephone:(530) 752-0699; fax: (530) 752-5410; e-mail: abbritt@ucdavis.edu

I. Introduction

Those of us who focus on DNA repair processes in plants do so with a variety of motivations. There are those labs that have a focus on genetic engineering– we try to understand the mechanism of recombination and mutation with the ultimate goal of controlling these processes. Can we modify the frequency and location of meiotic recombinational events? Can we enhance the efficiency of gene replacement? Can we alter the nature and frequency of mutational events? There are also groups that focus on plant response to environmental stresses. Can plants withstand the toxic effects of predicted increases in environmental UV-B? Will enhanced UV-B result in enhanced mutagenesis? Do general environmental stresses also have a genotoxic effect? Is the plant genome destabilized by environmental stress, and if so, is this a programmed response to environmental change?

The completion of the Arabidopsis genome project has revealed that the suite of repair proteins produced by Arabidopsis is remarkably similar to that of humans. Although there are some critical differences (discussed below), the similarities are impressive. Given this, Arabidopsis may turn out to be an excellent model system for the genetic analysis of many aspects of repair in higher eukaryotes. Gene-specific knockout mutants are a less expensive to isolate in Arabidopsis than in mice. Recent publications, and much unpublished data, also suggests that plants are far more tolerant to persisting DNA damage than mammals. Many mutations that are viable in bacteria or yeast lead to embryonic lethality in mammals. However, at least some of these lethal mutations can be rescued by a second mutation in the signal transduction pathway required for an apoptotic response to DNA damage, indicating that embryonic lethality is a suicidal response to damage, rather than an intrinsic effect of DNA damage itself (Frank et al., 2000; Sekiguchi et al., 2001). The embryonic lethality of repair defective mutants makes it difficult to assess the potential roles of repair genes in later aspects of development, including meiosis.

In contrast, Arabidopsis lacks a p53 homolog and there is as of yet no evidence that plants undergo an apoptotic response to the accumulation of DNA damage. Repair defects that are lethal in mice are viable in plants. For example, knockouts of RAD50 are embryonic lethal in mice; the same knockout produces a viable plant line, enabling us to determine, for the first time in a higher eukaryote, that *RAD50* plays an important role in both meiosis and telomere maintenance (Gallego et al., 2001; Gallego and White, 2001). Arabidopsis may soon emerge as a robust and inexpensive higher eukaryotic model for the genetic analysis of the roles of DNA repair pathways in the repair of various lesions, and for the effects of the persistence of these lesions on organismal development.

In the review below I'll provide an update on the state of our understanding of the mechanisms for the repair of damaged bases in Arabidopsis. This review will not address the repair of double strand breaks, and I direct the reader to recent reviews on that topic (Puchta, 1998; Gorbunova and Levy, 1999). Nor will I cover the study of DNA damage tolerance pathways in plants, a field in which there is only one publication which is not entirely speculative (Garcia et al., 2000). I'll point out some interesting features revealed by the Arabidopsis genome project, the substrate-specificities defined by biochemical analysis of isolated Arabidopsis repair proteins, and the characteristics of various repair-defective mutants. The accession numbers of most of the Arabidopsis repair gene homologs described below can be obtained in Table 5 of the supplementary data to (The Arabidopsis Genome Initiative, 2000), as well as at the Plant DNA Metabolism website at the University of Arizona (http://ag.arizona.edu/dnametab/).

The biological significance of damaged bases.

DNA damage products can be defined as any chemical alteration to DNA that covalently alters its normal structure. Bases can be oxidized, reduced, crosslinked, and alkylated. Each lesion can often be processed via a number of different repair pathways, and different organisms carry a different suite of repair-related genes (Friedberg et al., 1995). Different lesions have different biological consequences depending on the frequency of their induction, their ability to affect or alter the activity of polymerases, and the efficiency and accuracy of their repair.

Lesions that do not affect the ability of RNA polymerase to transcribe DNA, or of DNA polymerase to faithfully replicate it, are harmless in and of themselves (though many of these harmless products decay to form more dangerous ones). "Noncoding" damage products (for example, UV-induced pyrimidine dimers) act as blocks to both RNA and DNA polymerase, and their repair can be critical to the survival of the cell . Other more insidious types of "miscoding" damage products (for example, O^6-methylguanine) do not block DNA polymerase but are often misinterpreted by this enzyme, directly leading to the induction of mutations.

The significance of a DNA damage product also depends on its location in the genome and the developmental state of the cell in which it resides. A pyrimidine dimer in a nontranscribed region, or even a nontranscribed strand, of DNA in a nonreplicating cell has no biological significance. Given the amount of noninformational DNA in the genomes of most higher eukaryotes, and the fact that most cells are nonreplicating, this means that the majority of DNA damage products are innocuous. In contrast, the induction of a dimer on a transcribed strand of a potentially replicating genome is both a toxic and a genotoxic event, and its repair is a very high priority for the cell. The mechanisms by which eukaryotic cells set priorities for the repair of the same kinds of lesions in different types of cells or different parts of the genome have only recently been addressed in yeast and mammals (Hanawalt, 2001). Whether plants share the same mechanisms, or even the same priorities, remains to be seen.

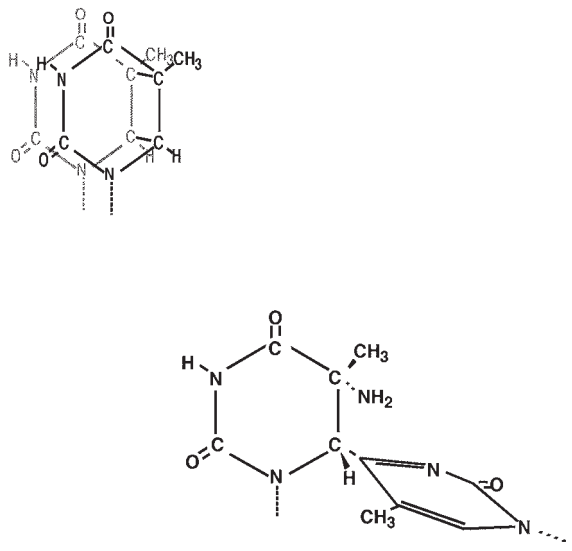

Figure 1: Pyrimidine dimers. The cyclobutane pyrimidine dimer (top) and the pyrimidine [6-4]pyrimidinone dimer (bottom) make up the two major classes of UV-induced DNA damage. Arabidopsis produces both a CPD-specific and a 6-4 specific photolyase.

II. Repair pathways in plants

Direct reversal of damage

Most lesions are repaired through a "remove and replace" strategy. The damaged DNA strand is excised, and the undamaged strand used as a template for repair synthesis. A very few lesions, however, are apparently both commonplace enough and biologically significant enough to merit a specialized repair pathway that directly reverses the damage and restores the damaged site to its original pristine form. The targets for these direct reversal reactions include three types of pyrimidine dimers, and three alkylation products.

UV radiation induces both cyclobutyl pyrimidine dimers and pyrimidine [6-4]pyrimidinone dimers (Figure 1). Arabidopsis, like many other organisms, produces 2 different photolyases (*PHR1*/*UVR2* and *UVR3*) that specifically bind to CPDs and 6-4 products, respectively (Ahmad et al., 1997; Jiang et al., 1997; Landry et al., 1997; Nakajima et al., 1998). Photolyases utilize two chromophores to capture photons in the UV-A to blue wavelengths, and use that captured energy to reverse their cognate damage products via an error-free process (Sancar, 1994). Mutants defective in either of these photolyases show a visible-light dependent UV-sensitive phenotype, with the *uvr3* (6-4 photolyase) deficient mutant being less sensitive than the *uvr2* mutants. This is consistent with the fact that CPDs are more frequently induced (by somewhere between 3 and 10 fold) than 6-4 products (Mitchell and Nairn, 1989). In seedlings, the photoreactivation of CPDs requires exposure to white light prior to UV exposure, as well as during the period of photoreactivation (Chen et al., 1994), and the

UVR2 gene has been shown to be transcriptionally induced by treatment with white or UV-A light (Ahmad et al., 1997). In contrast, the activity catalyzing photoreactivation of 6-4 products (the *UVR3* gene product) was found to be constitutively expressed (Chen et al., 1994). *UVR2* and *UVR3*-dependent photoreactivation of UV-induced growth inhibition occurs in both the root and shoot tips of the Arabidopsis seedling. Surprisingly, no photoreactivation of CPDs in either organellar genome was observed in young seedlings (Chen et al., 1996). More recently, however, the blue-light dependent photoreactivation of the inhibition of organellar genome replication by UV has been observed in the adult tissues of Arabidopsis (Draper and Hays, 2000), suggesting that all three Arabidopsis genomes are subject to photoreactivation in mature plants. The photolyase(s) responsible for this activity have not been identified.

The photolyase family of genes is an ancient one, and all organisms carry homologs that can be roughly sorted into Class I vs. Class II, representing a very early gene duplication event (Todo, 1999). The Arabidopsis genome encodes six predicted or confirmed genes that are closely related to photolyases. Five are of the "Class I" variety (similar to the CPD photolyases found in many bacteria and fungi). The Arabidopsis 6-4 photolyase, like all other characterized 6-4 photolyases, is of this class. A sixth homolog is the "Class II" CPD-specific photolyase. Two of the Class I homologs encode functionally characterized photoreceptors (cryptochromes, *CRY1* and *CRY2*); their C-terminal extensions (relative to bona fide photolyase proteins) mediate several responses to blue light (Cashmore et al., 1999; Yang et al., 2000).

The only other known example of repair of a lesion via direct reversal (rather than excision and resynthesis) is the repair of certain alkylated forms of DNA including O^6-methylguanine (O^6mG), O^4-alkylthymine (O^4mT), and in some cases alkylated phosphate groups by alkyltransferases such as the O^6mG methyltransferase protein of *E. coli* and similar proteins in eukaryotes. This fascinating protein is not a classical enzyme (which would participate in a reaction solely as a catalyst), but rather takes part in a "suicide" reaction in which a methyl group is transferred from the lesion to the protein, but cannot be removed from the protein's acceptor cysteine. The alkyltransferase is thus "consumed" as repair proceeds. Unlike the process of photoreactivation, which is driven by sunlight and therefore quite a bargain in terms of ATP consumed per lesion repaired, the repair of these particular alkylated lesions is extremely costly to the cell, and one can only conclude that the expense must be worth it. Both O^6mG and O^4mT can directly mispair (DNA polymerase often misreads O^6mG as A, and O^4mT as C) and are therefore highly mutagenic. O^6-methylguanine methyltransferases are widespread and the genes are easily identified on the basis of sequence homology; they are found in bacteria, various fungi, and mammals (Samson, 1992; Nakatsu et al., 1993). Surprisingly, no homologs have been identified in any of the plant DNA sequence data, nor has the activity been identified in plant extracts (Frost and Small, 1987; Angelis et al., 1992). Given the presumed importance of this antimutagenic activity (underlined by its metabolic expense) one can only speculate that plants may have found a better way to cope with this class of lesions.

Base excision repair (BER)

Base excision repair initiates with the recognition of a lesion by one of many DNA glycosylases. These enzymes cleave the damaged base from the sugar-phosphate backbone, producing an abasic (apurinic or apyrimidinic, AP) site. Subsequent reactions are catalyzed either by an intrinsic lyase activity of the glycosylase or by an AP endonuclease, resulting in the cleavage of the sugar/phosphate backbone at the AP site. AP endonucleases and AP lyases produce two very different kinds of 5' and 3' ends (Figure 2), as well as a gap. In order for a gap to be repaired, DNA polymerase requires a free 3' hydroxyl end to use as a primer, while DNA ligase requires a 3' hydroxyl group and a 5' phosphate as a substrate. Neither AP lyase activities nor 5' AP endonucleases generate these "conventional" DNA ends. Glycosylases with associated AP lyase activities often generate a 3' end blocked by a fragmented deoxyribose and a 5' phosphate, while AP endonucleases create a 3' OH and a 5' deoxyribose. Several processing steps, following either "long patch" (2-13 nt fill-in) or "short patch" (1 nt) schemes, are required to remove the offending ends and so enable DNA polymerase and DNA ligase to restore the integrity of the DNA (Memisoglu and Samson, 2000).

AP sites arise not only through the actions of glycosylases but also through spontaneous depurination, and must be processed via the pathways illustrated in Figure 2. Similarly, the nicks generated by reactive oxygen species also usually possess unconventional ends and must undergo substantial modification before they can be religated (Caldecott, 2001). Thus, through the actions of AP endonucleases and a variety of repair glycosylases, many disparate types of DNA damage products are processed into a single class of lesions (gapped DNAs) and so funneled into a much smaller set of repair reactions. Knockout mutations in mice affecting the latter steps of these reactions (mutations in the primary AP endonuclease (*APE1)*, *XRCC1*, DNA ligase I, or DNA polymerase beta) are embryonic lethal, while mutations that affect one of the

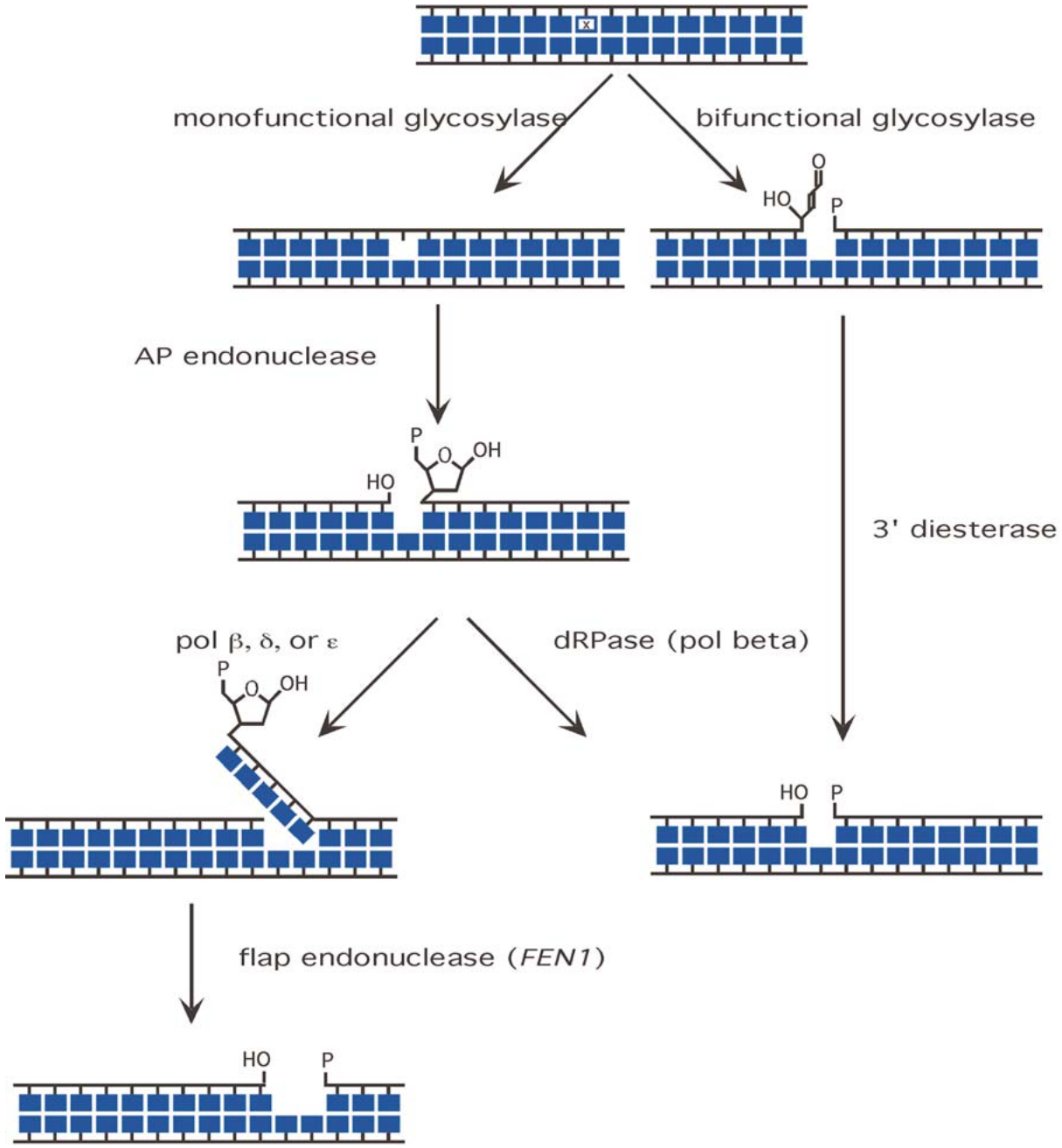

Figure 2: Base excision repair. This process is initiated by any one of a variety of lesion-specific glycosylases. The resulting abasic site is then further processed into a gap, either through the action of the AP lyase activity associated with a bifunctional glycosylase (right) or by an AP endonuclease. Either activity produces a gapped DNA with complex ends which must be further processed to create a 3' OH terminus (to act as a primer for repair synthesis) and a 5' phosphate terminus (to serve as a substrate for ligation).

multiple glycosylases initiating this pathway are not (Engelward et al., 1997; Hang et al., 1997). This suggests that it is the failure to repair a gapped DNA, rather than the failure to repair a particular modified base, that induces lethality in these knockout lines.

Gapped DNAs are potential sources of double strand breaks, as the bases in single stranded DNA are exposed to their aqueous environment and are particularly susceptible to attack by water and other DNA damaging agents. Perhaps in order to prevent the formation of DSBs during repair, the entire process of base excision repair in mammals is highly coordinated, with one enzyme handing off its product to the next enzyme that utilizes this product as a substrate. *XRCC1*, a gene required for radiation resistance in mammalian cell lines, probably plays a key role in coordinating this process.

BER step 1: recognition and excision of the damaged base

Arabidopsis has a remarkably large collection of glycosylases. I would like to be able to say that Arabidopsis has never met a glycosylase it didn't like, but it does not appear to have an obvious homolog of the *MUG* gene. The most common damaged base to arise in any genome is uracil, produced through the spontaneous hydrolysis of cytosine. It is critical that uracil be removed from the genome before DNA replication, as the deaminated C will be misinterpreted by polymerases as a T, resulting in a point mutation. Uracil glycosylase (the product of the *UNG* gene*)* removes U's from DNA. However, when 5 methylcytosine deaminates (and a substantial fraction of the C's in plant DNA are present as 5-meC (Shapiro, 1976)), thymine is produced instead of uracil. Obviously the production of a simple thymine glycosylase is not the answer to this particular problem. The *MUG* (mismatch-specific uracil glycosylase) gene product, related to Ung, specifically recognizes T's that are base paired with G's (as well as U:G pairs) and excises the offending lesion. Like *S. cerevisiae*, but unlike humans or *E. coli,* Arabidopsis has an *UNG* homolog, but not a *MUG.*

Given the fact that both respiration and photosynthesis are occurring simultaneously in plant cells, and that the process of photosynthesis can be perturbed by a variety of environmental stresses, the plant genome is probably particularly subject to oxidative damage. Treatment of cells with hydrogen peroxide or ionizing radiation probably generates the kinds of lesions that are formed under stress *in planta*. In mammalian cells, attack by reactive oxygen species induces a wide variety of damage products. Prominent among these are 8-hydroxyguanine (8oxoG), a miscoding lesion that can base pair with either A or C. Also produced are the noncoding products of imidazole ring opening, Foramidopurine (FaPy)-A and FaPy-G. In *E. coli*, three gene products are dedicated to eliminating 8-hydroxyguanine from the genome. One of these, MutT, is a nucleotide triphosphatase that removes 8-hydroxyGTP from the nucleotide pool. Arabidopsis has a homolog of this gene. The other two genes are glycosylases; one, the *mutM* gene product (also termed *fpg* (FaPy glycosylase)), recognizes 8oxoG:C base pairs and cleaves the glycosidic bond to 8oxoG. The other, the MutY protein, recognizes 8oxoG:A base pairs and cleaves the glycosidic bond to A. The high specificity of the MutM protein for 8oxoG:C base pairs, rather than 8oxoG:A base pairs, ensures that the repair protein does not actively promote 8oxoG-induced mutagenesis. The Arabidopsis genome encodes homologs of both *mutM* and *mutY*. The Arabidopsis *mutM* homolog (*AtMMH*) gene has been shown to produce two different transcripts, formed by alternative splicing (Ohtsubo et al., 1998; Gao and Murphy, 2001). Western analysis detected the presence of only one of the predicted proteins in crude plant extracts. When expressed in vitro, this protein was able to nick 8oxoG:C base pairs, but not 8oxoG:A base pairs .

Mammals also possess functional homologs of the *mutT* and *mutY* genes, but a different protein, Ogg1, acts as the 8oxoG glycosylase. If Arabidopsis is typical of other plants, then plants are unique among the living kingdoms in having functional genes encoding homologs of both *OGG1* and *mutM*. The Arabidopsis OGG1 activity has been particularly well characterized *in vitro* (Dany and Tissier, 2001; Garcia-Ortiz et al., 2001); the recombinant polyhistidine-tagged protein, when expressed in and purified from *E. coli*, displays a specificity for 8-oxoG:C (vs. G:C). Cleavage of the DNA backbone is via the beta elimination mechanism characteristic of Ogg1 (vs. the delta elimination induced by MutM). RT-PCR indicated that both the *AtMMH* and the *AtOGG1* genes were expressed in leaves, flowers, stems, and roots.

A wide variety of other oxidized bases are excised by endonuclease III, a product of the *nth* gene in *E. coli*. The Arabidopsis homolog of this gene has been cloned, its product overexpressed, purified, and its activities characterized (Roldan-Arjona et al., 2000). The protein was able to release damaged bases from substrates treated to induce thymine glycols or urea, suggesting that it had a glycosylase activity that could recognize these substrates.

3-methyladenine glycosylases can be clustered into three unrelated families: those like the *E. coli tag* gene, those like *E. coli's alkA* gene, and those like the human *AAG* gene (Wyatt et al., 1999). Although each of these proteins is a 3-methyladenine glycosylase, all have the

ability to recognize other substrates. The *E. coli tag* gene product is the most specific of the characterized proteins, recognizing only 3-mG and 3-mA. Interestingly, Arabidopsis has *six* homologs of this gene, none of which have yet been characterized. Although it is possible that some of these proteins are simply the same enzymatic activity expressed in different tissues, or transported to different organelles, it is also possible that the different members of this gene family might have taken on different substrate specificities. Certainly no other repair activities appear to have expanded in this way in plants, suggesting that there is little pressure to create and retain genes expressing identical repair activities with different patterns of expression. Arabidopsis, or perhaps plants in general (the maize and rice genome databases also produce significant hits) are alone in expanding this gene family- in fact no *tag* homologs are found in humans or yeast. Yeast (*S. pombe* and *S. cerevisiae*) rely on *alkA* homologs, while mammals (mice, rats, and humans) utilize yet another type of 3-methyladenine glycosylases (termed *Aag*, *ADPG*, and *AAG*, respectively). Arabidopsis, of course, has homologs of both *alkA* and *AAG.*

The *alkA* and *AAG* gene products have very wide substrate ranges; although they are termed 3-methyladenine glycosylases, this lesion is not necessarily the preferred substrate. In addition to a variety of alkylated bases, these enzymes cleave at ethenopurines, 7-ethoxyG, 7-chloroethylG, and hypoxanthine (Dosanjh et al., 1994; Wyatt et al., 1999). AlkA even has significant activity on all four normal bases, and overexpression of this protein has a genotoxic effect in many organisms (Coquerelle et al., 1995; Posnick and Samson, 1999). Arabidopsis possesses two *alkA* homologs, neither of which have been characterized. It also possesses a single *AAG* homolog, termed *AMAG* (Santerre and Britt, 1994). Expression of this protein in *E. coli* has been shown to complement the MMS sensitivity of *tag, alkA* double mutants, and its substrate specificity has been partially characterized (Wyatt et al., 1999). *In situ* RNA hybridization indicates that this gene is most highly expressed in rapidly growing tissues, consistent with a role in clearing noncoding 3-meA and other lesions from the path of both RNA and DNA polymerases (Shi et al., 1997).

BER step 2: nicking of the AP site

The second step in base excision repair, after the cleavage of the damaged base from the sugar phosphate backbone, is the incision of the backbone at the AP site. Many glycosylases are bifunctional, having an intrinsic AP lyase activity that induces this cleavage. The mammalian Ogg1 protein, the *E. coli* Nth protein, and the *E. coli* AlkA protein are all examples of glycosylases that belong to the helix-hairpin-helix superfamily of glycosylases (Nash et al.,

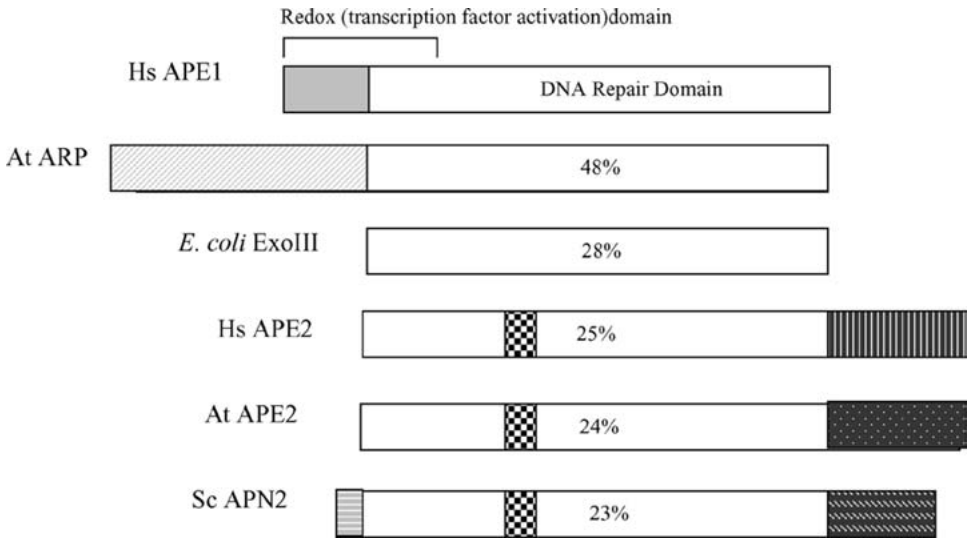

Figure 3: Possible AP endonucleases of Arabidopsis. All of the AP endonuclease-like genes are of the exoIII family; there are no convincing endoIV-like genes. Numbers indicate percent amino acid identity to the exoIII domain of *hsAPE1.* The Arabidopsis Arp protein exhibits, in vitro, the redox activity of the human *Ape1* gene product.

1996) that release their substrate only after producing the gapped product, with a 3' terminal fragmented sugar (illustrated in Figure 2). The Arabidopsis homologs of Ogg1 and Nth have been shown to produce the same product, through the same reaction mechanism (Roldan-Arjona et al., 2000; Dany and Tissier, 2001; Garcia-Ortiz et al., 2001, in press). The Arabidopsis *AtMMH* gene, like its *E. coli* homolog *mutM*, is a bifunctional glycosylase that catalyzes both beta-elimination and the delta elimination of the abasic sugar, resulting in a gapped product with both 5' and 3' phosphate termini (Ohtsubo et al., 1998).

AP endonucleases nick the abasic site generated through spontaneous degradation of DNA or through the actions of monofunctional glycosylases. AP endonucleases fall into two basic classes, those most like *E. coli's* exonuclease III, and those homologous to *E. coli*'s endonuclease IV. The Arabidopsis genome project has revealed the presence of three exoIII-like AP endonuclease homologs (and no endoIV-like proteins). Two are similar to the primary human AP endonuclease, Ape1, in that they have an exoIII domain with a long N terminal extension. However, there is no convincing homology in the amino acid sequences of the N terminal extensions of Ape1 vs. Arp or AtApe1, or between the N-terminal extensions of Arp and AtApe1 (Figure 3). The N terminal extensions of each of these proteins appears to have arisen through three independent gene fusion events.

One of these exoIII related genes, termed *ARP* (Babiychuk et al., 1994) has been expressed in vitro and the protein found to possess both AP endo activity and the ability to activate the human transcription factor AP-1. These activities are shared with the primary human AP endonuclease, encoded by the *APE1* gene. The functional significance of *ARP*'s ability to activate AP-1 is still unclear, given the fact that this transcription factor (a heterodimer of members of the fos and jun family) does not appear to exist in Arabidopsis. It is possible that Arp, like Ape1, is capable of activating a wide variety of transcription factors. However, it is just as possible that Arp is simply a sticky protein, a feature essential to its presumed role in coordinating the handoff of substrates and products during base excision repair.

Humans and yeast (*S. cerevisiae*) each produce a second exoIII-like protein, termed Ape2 and Apn2, respectively, characterized by a C-terminal extension and a highly conserved 10 amino acid insertion within the exoIII domain (Hadi and Wilson, 2000). The function of the fungal and mammalian proteins is currently under investigation (Bennett, 1999; Unk et al., 2000; Tsuchimoto et al., 2001). Arabidopsis possesses a convincing *Ape2* homolog.

BER step 3: restoration of functional 5' and 3' ends

The Arabidopsis genome contains homologs for all of the major players in base excision repair (many glycosylases, *XRCC1*, several AP endonucleases, and the 5' flap endonuclease *FEN1*), with the significant exception of DNA polymerase beta. Pol beta is a small, nonprocessive polymerase with an intrinsic 5' deoxyribose phosphodiesterase activity (dRpase) that enables it to cleave abasic sugars from the 5' ends of DNAs, restoring the simple 5' phosphate group required for the ligation of a nick. Pol beta is a member of eukaryotic polymerase family "X", a family of polymerases related to the nucleotidyl transferases involved in antibiotic resistance rather than to processive polymerases (Holm and Sander, 1995). Although the Arabidopsis Genome Project did not reveal the presence of a pol beta homolog, the sequence of another recently discovered member of family X, pol lambda, is present (Garcia-Diaz et al., 2000). Modeling of the pol lambda amino acid sequence predicts the presence of both the 8 kDal dRPase and the 30 kDal polymerase domains of pol beta, with an additional 230 amino acid N-terminal extension of unknown function. Given the absence of a pol beta homolog, it will be interesting to determine whether pol lambda plays a critical role in the repair of gapped DNAs in plants.

Nucleotide excision repair (NER)

UV radiation induces two major DNA damage products: cyclobutane pyrimidine dimers (CPDs), and pyrimidine[6-4]pyrimidinone dimers (6-4 products). These lesions block the progress of both DNA and RNA polymerases and therefore have a toxic effect even in tissues that are not actively dividing. Because plants are obligatorily exposed to solar radiation it is especially important that they are able to efficiently remove UV-induced DNA damage. Earlier in this chapter we discussed the fact that Arabidopsis produces two distinct photolyases which efficiently remove both CPDs and 6-4 products in the presence of blue light. Arabidopsis also repairs 6-4 products in the absence of light, although this "dark" repair mechanism is less efficient than the photolyase-dependent pathway. In contrast, in Arabidopsis seedlings the light-independent repair of CPDs is too slow to be detected by conventional assays (Britt et al., 1993). This dark repair appears to occur through the nucleotide excision repair (NER) pathway via a mechanism homologous to that found

in other eukaryotes. Bacteria also perform NER, but there are fewer proteins involved and little homology between the bacterial and eukaryotic proteins.

NER has been extensively characterized in yeast and mammals. It is not specific to the repair of dimers, but is an all purpose pathway with a remarkably broad substrate "specificity" (Sancar, 1996). At least 11 genes are required for the recognition of lesions, the dual incision of the damaged strand, and the removal of the damaged oligonucleotide. In bacteria, fungi, and mammals the efficiency of this pathway is greatly enhanced when the damage is present in the transcribed strand of DNA (Friedberg, 1996); whether or not this transcription-coupled repair occurs in plants remains to be determined.

Although the genes required for NER in bacteria and eukaryotes have been well defined, the mechanism through which the remarkably wide variety of substrates is recognized remains unclear. Figure 4 illustrates a current model for the process of global genome repair (as opposed to transcription-coupled repair) in higher eukaryotes. The XPC protein, and a human homolog of yeast's RAD23 (hHR23B) protein are required for the initial recognition of the lesion. These proteins are required only for global genome repair, and play no role in transcription coupled repair. The XPE protein, which is required for expression of the p48 subunit of DDB (damaged DNA binding protein), also plays a role in stimulating global repair of CPDs, but not 6-4 products (Tang et al., 2000). After the initial damage recognition event (mediated by RNA polymerase in transcription-coupled repair), XPA and the basal transcription factor TFIIH then melt the DNA in the vicinity of the damage, and finally the repair endonucleases nick the damaged strand on the 5' (ERCC1/XPF) and 3' (XPG) sides of the lesion. The undamaged strand can then be used as a template for DNA synthesis.

A search of the Arabidopsis genome sequence reveals that higher plants possess obvious homologs of many, though not all, of the eukaryotic genes required for nucleotide excision repair. There are no homologs of the *E. coli* NER excinuclease genes, *uvrA, uvrB,* and *uvrC*, although, interestingly, there is a homolog of the *mfd* gene, a gene required for the coupling of transcription and NER in *E. coli.* The eukaryotic 5' and 3' endonucleases (*XPF+ERCC1* and *XPG*) are present, as are the factors required for initial damage recognition (*XPA, XPC*, two versions of *XPE* (both on chromosome *4*, though at different locations), and several homologs of *RAD23*). Missing are a couple of the components of the TFIIH "repairosome/basal transcription factor", p52 and p35, although homologs of genes encoding those subunits originally identified in mammals as essential for repair (*XPB* and *XPD*) are present.

Mutants defective in the dark repair of 6-4 photoprod-

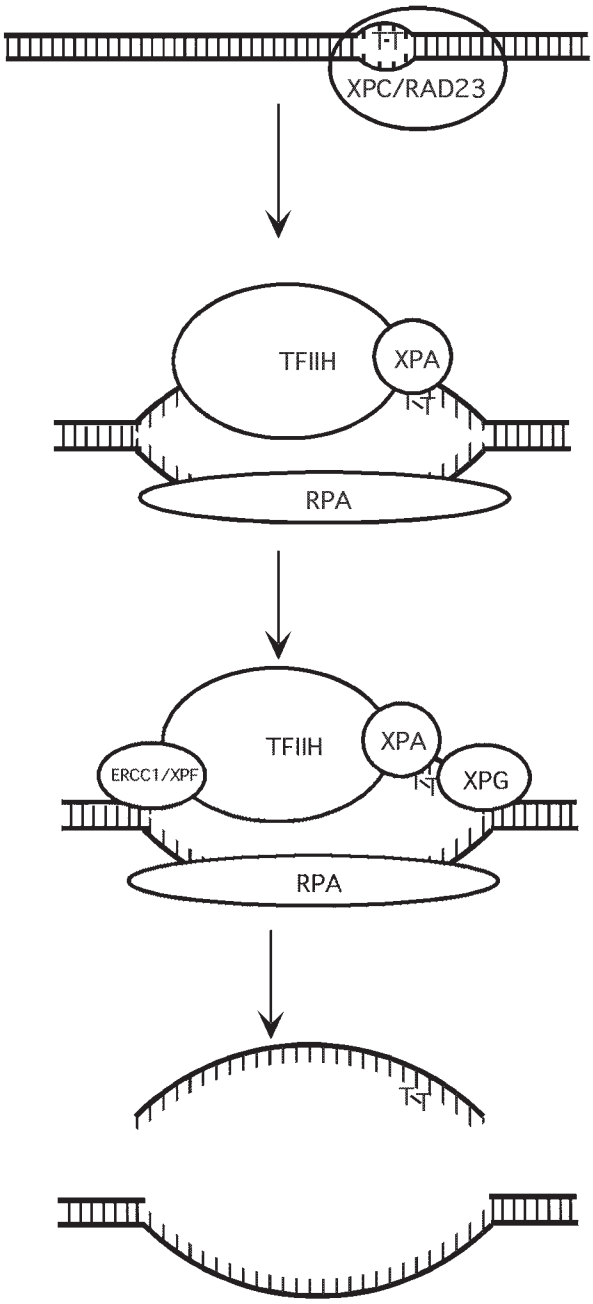

Figure 4: A model for nucleotide excision repair. The global genome repair pathway (GGR), rather than the transcription-coupled repair (TCR) pathway, is illustrated. In GGR initial recognition is established via the XPC/RAD23 complex (XPE also plays a role in the recognition of CPDs). In TCR, primary recognition is via RNA polymerase II. TCR, but not GGR, requires the presence of the CSA and CSB gene products that *may* play a role in pushing the polymerase away from the lesion to provide access for XPA and TFIIH.

ucts have been isolated on the basis of their UV-sensitive phenotype in the absence of photoreactivating light. These represent many complementation groups and the genetics of UV-resistance has certainly not been saturated (Britt et al., 1993; Jenkins et al., 1995; Jiang et al., 1997; Masson et al., 1997; Vonarx et al., 1998). Several of these mutants have been directly assayed for the ability to excise 6-4 products in the dark (Britt et al., 1993; Jiang et al., 1997). One of the first repair-defective mutants isolated, *uvh1* (Harlow et al., 1994), has recently been shown to represent a mutation in the Arabidopsis homolog of a 5' endonuclease component (*XPF* in humans, *RAD1* in *S. cerevisiae*) (Fidantsef et al., 2000; Gallego et al., 2000; Liu et al., 2000). This provides direct evidence that dark repair of 6-4 photoproducts is occurring through an NER-like process.

The *uvh1* mutants are particularly interesting in that they display a sensitivity to gamma radiation that is not observed in other repair defective Arabidopsis lines. The UV-sensitivity of mutant lines can be quantified by observing the effect of UV irradiation on the short-term growth of roots (elongation over 1 or 2 days). This restriction of growth presumably reflects the effect of persisting dimers on transcription, as the majority of root elongation over this short a term is due to expansion of cells in the elongation zone, rather than replication of cells in the root tip meristem. Although high doses of gamma radiation can certainly inhibit short term root growth, none of the mutants characterized as defective in excision repair exhibit enhanced sensitivity to this effect of gamma radiation (Jiang et al., 1997). However, if a completely different assay for gamma sensitivity is employed (an assay for the production of true leaves by seeds irradiated prior to germination (Harlow et al., 1994)), that permits the observation of effects on cell replication, then the *uvh1* mutant displays sensitivity (about 6 fold more sensitive than wild-type) to gamma. This sensitivity to the effects of gamma radiation on the production of new cells, but not the growth of existing cells, suggests that *uvh1* may be required for the repair of a critical lesion (such as a double strand break) that induces cell cycle arrest but does not interfere with transcription. It also tells us that, in Arabidopsis, NER is not essential for clearing transcription-blocking lesions induced by gamma radiation. The *scRAD1* gene product, together with *scRAD10*, forms a repair endonuclease that is active not only at the 5' end of the repair bubble generated during NER, but also on overhanging 3' flaps generated during nonhomologous end joining of double strand breaks (Figure 5). The sensitivity of the meristems of *uvh1* seedlings to the inhibitory effects of gamma radiation on cell division suggests that such 3' flapped structures may be an important intermediate in the repair of double strand breaks in these tissues.

Prioritization of repair in plants?

As described in the introduction to this chapter, the biological significance of a lesion depends on its context. UV-induced pyrimidine dimers block the progress of replicative polymerases. Alternative polymerases exist that can bypass dimers, but many of these are error-prone (Wood, 1999; Lawrence and Maher, 2001). Thus, in replicating cells, it is important that meaningful sequences be cleared of dimers prior to DNA replication. Nonreplicating cells simply need to clear lesions away from the path of oncoming RNA polymerases, which also stall at dimers. Mammals, yeasts, and bacteria have been found to preferentially repair dimers on the transcribed strands of transcriptional units both during BER and NER; this process is termed transcription coupled repair (TCR) as opposed to global genome repair (GGR). Direct evidence for transcription coupled repair in plants is not yet avail-

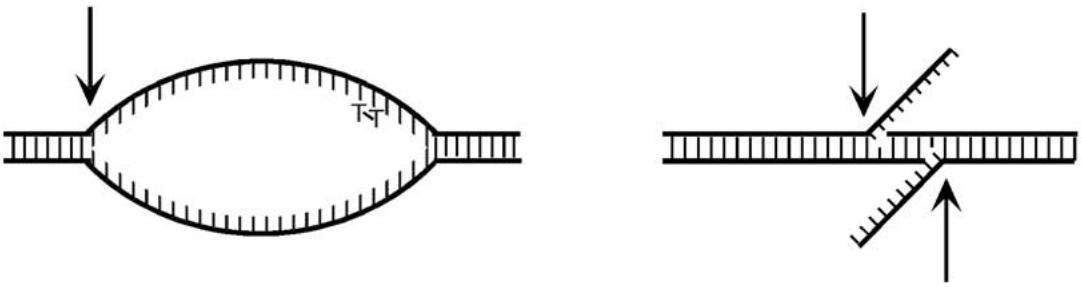

Figure 5: Substrates of the RAD1 repair endonuclease. scRAD1 (and presumably its Arabidopsis homolog, uvh1) is required for both the repair of a variety of bulky lesions via NER and for the trimming of flaps generated either through nonhomologous end joining or ectopic homologous recombination of limited regions of sequence similarity.

able, but given its ubiquitous presence in other living kingdoms it would certainly be predicted to exist in plants. The Arabidopsis genome does contain several homologs of a *CSB* (*scRAD26*) and two recently duplicated versions of *CSA*-like sequences, homologs of genes that are required for TCR, but not GGR, in mammals and yeast. The functionality of these homologs remains to be determined.

Many species regulate the overall level of global genome repair in response to the induction of damage and the replicatory status of the tissue type (Nouspikel and Hanawalt, 2000). The phenomenology of the prioritization of repair in plants has not yet been established. We do not know whether the rate of repair varies with the sequence context or replicatory state of the cell, or whether the expression of genes involved in the repair of damaged bases is enhanced in tissues treated with DNA damaging agents. Plants lack the p53 gene required for both the regulation of basal repair and the induction of repair by DNA damage in humans (Hwang et al., 1999) and thus excision repair must be regulated by a different mechanism in plants.

III. Future directions

The completion of the Arabidopsis genome project will lead to a burst of reverse genetic studies of the functions of the plant homologs of various repair proteins. The classical genetic approach, which can identify truly novel genes required for repair as well as homologs of genes previously identified in other systems, will probably be neglected for a while as the functionality of the most interesting homologs is investigated. Now that repair mutants in genes of known sequence can easily be generated, the attention of the investigators in this field must turn from the mechanics of isolating genes to the development of assays for the functions and significance of these genes. Are the proteins required for life itself? For fertility? For resistance to stress? What are the substrates of these proteins? Do they play a role in reducing mutation? If so, which kinds of mutations?

The field of DNA repair in Arabidopsis is in desperate need of a method for measuring the rate of induction of various types of mutations. The assay for the induction of somatic homologous intrachromosomal recombination (measured as blue somatic spots via the reconstitution of a partially duplicated GUS allele (Swoboda et al., 1994)) is one of the few examples of a successful assay of this type. Recently Ries et al. (Ries et al., 2000) used this construct, in wild-type vs. *uvr2* (CPD photolyase defective) lines to demonstrate that the persistence of CPDs leads to an elevation in the rate of somatic homologous recombination, and that this boost in recombination is dependent on the presence of photosynthetically active radiation. This is an intriguing and novel result that demonstrates the way in which new mutants, and new assays for mutagenesis, will be useful in pinpointing the mechanisms through which genetic stability is maintained in plants.

In order to properly address the biological significance of the various repair pathways, we will also need to become familiar with something akin to the performance of "field trials" with Arabidopsis. This will probably require the formation of alliances with more traditional "whole plant" physiologists. The effects of enhanced UV-B radiation, for example, are extremely sensitive to other environmental factors, particularly near-UV and visible light (Fiscus and Booker, 1995; Rozema et al., 1997). It is easy to determine that a repair-defective is more sensitive than wild-type to a blast of UV-B, but it may be far more difficult to determine whether this difference in sensitivity actually makes a difference in terms of survival, reproductive success, or yield in a natural environment. Such experiments are only beginning to be performed (Fiscus et al., 1999; Ries et al., 2000).

It will also be interesting to characterize the tissue-specificity of expression of the genes required for DNA repair and damage tolerance. One would hope to compare the expression of anti-mutagenic genes in truly somatic vs. potentially germline tissues, and the expression of various lesion-specific enzymes in tissues that are subject to different environmental stresses. Plants differ from animals in two very important ways that are relevant to DNA repair. Plants do not die from cancer, as cancerous tissue cannot metastasize, making plants relatively resistant to the toxic effects of mutagenesis. On the other hand, plant reproductive tissues are derived from meristems that produce the entire plant, and which have gone through many rounds of DNA replication prior to the formation of gametes (Cullis, 1986; Klekowski, 1988), making plants especially sensitive to the potential accumulation of mutations in the germline. Given the differences in their developmental strategies, plants should have different priorities than mammals when it comes to tolerance for mutagenesis, particularly tolerance of a high mutation rate in somatic tissues. This has already been illustrated by the apparent absence of a p53 homolog in plants. p53, sometimes termed "the guardian of the genome" plays a central role in the induction of apoptosis in response to DNA damage (as well as in the regulation of repair). Because tumors are not lethal in plants, there may be no strong evolutionary driving force for the induced suicide of potentially mutated cells in plants.

In summary, a wealth of genetic information now exists for researchers interested in repair processes in plants,

and many interesting questions are lying around waiting to be answered. Some of the tools required to answer these questions already exist, but many more need to be developed and refined. As is true for many fields right now, this is in some ways a bad time to be writing this review, because there is going to be so much more to talk about in just a year or two.

Acknowledgements

The author would like to thank Dr. David Mount and Dr. Jendy Hall, of the University of Arizona, for maintaining the plant DNA metabolism website (http://ag.arizona.edu/dnametab), James Hatteroth of U. C. Davis for providing Figure 3, and the agencies (the NSF, the USDA, the EPA, USDA/BARD, and the University of California Systemwide Program in Biotechnology) which have funded and continue to fund research into all aspects of DNA repair in plants in my laboratory and the labs of my colleagues.

References

Ahmad, M., J. Jarilo, L. Klimczak, L. Landry, T. Peng, R. Last and A. Cashmore. 1997. An enzyme similar to animal type II photolyases mediates photoreactivation in Arabidopsis. *Plant Cell* **9:** 199-207.

Angelis, K., J. Briza, J. Satava, I. Skakal, J. Veleminsky, J. Vlasak, K. Kleibl and G.P. Margison. 1992. Increased resistance to the toxic effects of alkylating agents in tobacco expressing the *E. coli* DNA repair gene *ada*. *Mutat. Res.* **273:** 271-280.

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

Bennett, R.A.O. 1999. The Saccharomyces cerevisiae ETH1 gene, an inducible homolog of exonuclease III that provides resistance to DNA-damaging agents and limits spontaneous mutagenesis. *Molecular and Cellular Biology* **19:** 1800-1809.

Britt, A.B., J.-J. Chen, D. Wykoff and D. Mitchell. 1993. A UV-sensitive mutant of *Arabidopsis* defective in the repair of pyrimidine-pyrimidinone (6-4) dimers. *Science* **261:** 1571-1574.

Caldecott, K.W. 2001. Mammalian DNA single-strand break repair: An X-ra(y)ted affair. *Bioessays* **23:** 447-455.

Cashmore, A.R., J.A. Jarillo, Y.-J. Wu and D. Liu. 1999. Cryptochromes: Blue light receptors for plants and animals. *Science* **284:** 760-765.

Chen, J.-J., C.-Z. Jiang and A.B. Britt. 1996. Little or no repair of cyclobutyl pyrimidine dimers is observed in the organellar genomes of the young Arabidopsis seedling. *Plant Phys.* **111:** 19-25.

Chen, J.-J., D. Mitchell and A.B. Britt. 1994. A light-dependent pathway for the elimination of UV-induced pyrimidine (6-4) pyrimidinone photoproducts in *Arabidopsis thaliana*. *Plant Cell* **6:** 1311-1317.

Coquerelle, T., J. Dosch and B. Kaina. 1995. Overexpression of N-methylpurine-DNA glycosylase in Chinese hamster ovary cells renders them more sensitive to the production of chromosomal aberrations by methylating agents: A case of imbalanced DNA repair. *Mutation Research* **336:** 9-17.

Cullis, C.A. 1986. Phenotypic consequences of environmentally induced changes in plant DNA. *TIG* **2:** 307-309.

Dany, A. and A. Tissier. 2001. A functional *OGG1* homolog from *Arabidopsis thaliana*. *Molec. Gen. Genomics* **265:** 293-301.

Dosanjh, M.K., R. Roy, S. Mitra and B. Singer. 1994. 1,N-6-Ethenoadenine is preferred over 3-methyladenine as substrate by a cloned human N-methylpurine-DNA glycosylase (3-methyladenine-DNA glycosylase). *Biochemistry* **33:** 1624-1628.

Draper, C.K. and J.B. Hays. 2000. Replication of chloroplast, mitochondrial and nuclear DNA during growth of unirradiated and UVB-irradiated Arabidopsis leaves. *Plant Journal* **23:** 255-265.

Engelward, B.P., G. Weeda, M.D. Wyatt, J.L.M. Broekhof, J. De Wit, I. Donker, J.M. Allan, B. Gold, J.H.J. Hoeijmakers and L.D. Samson. 1997. Base excision repair deficient mice lacking the Aag alkyladenine DNA glycosylase. *Proceedings of the National Academy of Sciences of the United States of America* **94:** 13087-13092.

Fidantsef, A., D. Mitchell and A. Britt. 2000. The Arabidopsis *UVH1* gene is a homolog of the yeast repair endonuclease *RAD1*. *Plant Physiol.* **124:** 579-586.

Fiscus, E., P. Philbeck, A. Britt and F. Booker. 1999. Growth of Arabidopsis flavonoid mutants under solar radiation and UV filters. *Envir. & Exper. Bot.* **41:** 231-245.

Fiscus, E.L. and F.L. Booker. 1995. Is increased UV-B a threat to crop photosynthesis and productivity? *Photosyn. Res.* **43:** 81-92.

Frank, K.M., N.E. Sharpless, Y. Gao, J.M. Sekiguchi, D.O. Ferguson, C. Zhu, J.P. Manis, J. Horner, R.A. DePinho and F.W. Alt. 2000. DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. *Molecular Cell* **5:** 993-1002.

Friedberg, E. 1996. Relationships between DNA repair and transcription. *Ann. Rev. Biochem.* **65:** 15-42.

Friedberg, E.C., G.C. Walker and W. Siede. 1995. *DNA repair and mutagenesis*. Washington, D.C, ASM Press.

Frost, B.F. and G. Small. 1987. The apparent lack of repair of O6-methylguanine in nuclear DNA of *Chlamydomonas reinhardtii*. *Mutat. Res.* **181:** 31-36.

Gallego, F., O. Fleck, A. Li, J. Wyrzykowska and B. Tinland. 2000. AtRAD1, a plant homologue of human and yeast nucleotide excision repair endonucleases, is involved in dark repair of UV damages and recombination. *Plant Journal* **21:** 507-518.

Gallego, M.E., M. Jeanneau, F. Granier, D. Bouchez, N. Bechtold and C.I. White. 2001. Disruption of the Arabidopsis RAD50 gene leads to plant sterility and MMS sensitivity. *Plant Journal* **25:** 31-41.

Gallego, M.E. and C.I. White. 2001. RAD50 function is essential for telomere maintenance in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 1711-1716.

Gao, M.-J. and T.M. Murphy. 2001. Alternative forms of formamidopyrimidine-DNA glycosylase from Arabidopsis thaliana. *Photochemistry and Photobiology* **73:** 128-134.

Garcia, V., M. Salanoubat, N. Choisne and A. Tissier. 2000. An ATM homologue from Arabidopsis thaliana: Complete genomic organisation and expression analysis. *Nucleic Acids Research* **28:** 1692-1699.

Garcia-Diaz, M., O. Dominguez, L.A. Lopez-Fernandez, L.T. de Lera, M.L. Saniger, J.F. Ruiz, M. Parraga, M.J. Garcia-Ortiz, T. Kirchhoff, J. del Mazo, A. Bernad and L. Blanco. 2000. DNA polymerase lambda (Pol lambda), a novel eukaryotic DNA polymerase with a potential role in meiosis. *Journal of Molecular Biology* **301:** 851-867.

Garcia-Ortiz, M.-V., R. Ariza and T. Roldan-Arjona. 2001, in press. An *OGG1* ortholog encoding a functional 8-oxoguanine DNA glycosylase/lyase in *Arabidopsis thaliana*. *Plant Molecular Biology* 47:795-804

Gorbunova, V. and A. Levy. 1999. How plants make ends meet: DNA double strand break repair. *Trends in Plant Science* **4:** 263-269.

Hadi, M. and D. Wilson. 2000. Second human protein with homology to the *E. coli* abasic endonuclease exonuclease III. *Environmen. & Molec. Mutagen.* **36:** 312-324.

Hanawalt, P.C. 2001. Controlling the efficiency of excision repair. *Mutation Research* **485:** 3-13.

Hang, B., B. Singer, G.P. Margison and R.H. Elder. 1997. Targeted deletion of alkylpurine-DNA-N-glycosylase in mice eliminates repair of 1,N6-ethenoadenine and hypoxanthine but not of 3,N4-ethenocytosine or 8-oxoguanine. *Proceedings of the National Academy of Sciences of the United States of America* **94:** 12869-12874.

Harlow, G.R., M.E. Jenkins, T.S. Pittalwala and D.W. Mount. 1994. Isolation of Uvh1, an Arabidopsis Mutant Hypersensitive to Ultraviolet Light and Ionizing Radiation. *Plant Cell* **6:** 227-235.

Holm, L. and C. Sander. 1995. DNA polymerase beta belongs to an ancient nucleotyltransferase superfamily. *Trends Biochem.* **20:** 345-347.

Hwang, B.J., J.M. Ford, P.C. Chanawalt and G. Chu. 1999. Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proceedings of the National Academy of Sciences of the United States of America* **96:** 424-428.

Initiative, A.G. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. *Nature* **408:** 796-815.

Jenkins, M.E., G.R. Harlow, Z. Liu, M.A. Shotwell, J. Ma and D.W. Mount. 1995. Radiation-sensitive mutants of *Arabidopsis thaliana*. *Genetics* **140:** 725-732.

Jiang, C.-Z., J. Yee, D. Mitchell and A. Britt. 1997. Photorepair mutants of *Arabidopsis*. *Proc. Natl. Acad. Sci.* **94:** 7441-7445.

Jiang, C.-Z., C.-N. Yen, K. Cronin, D. Mitchell and A. Britt. 1997. UV- and gamma-radiation sensitive mutants of Arabidopsis. *Genetics* **147:** 1401-1409.

Klekowski, E.J. 1988. *Mutation, Developmental Selection, and Plant Evolution*. New York, Columbia University Press.

Landry, L., A. Stapleton, J. Lim, P. Hoffman, J. Hays, V. Walbot and R. Last. 1997. An Arabidopsis photolyase mutant is hypersensitive to UV-B radiation. *Proc. Natl. Acad. Sci.* **94:** 328-332.

Lawrence, C.W. and V.M. Maher. 2001. Mutagenesis in eukaryotes dependent on DNA polymerase zeta and Rev1p. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **356:** 41-46.

Liu, Z.R., G.H. Hossain, M.A. Islas-Osuna, D.L. Mitchell and D.W. Mount. 2000. Repair of UV damage in plants by nucleotide excision repair: Arabidopsis UVH1 DNA repair gene is a homolog of Saccharomyces cerevisiae Rad1. *Plant Journal* **21:** 519-528.

Masson, J., P. King and J. Paszkowski. 1997. Mutants of *Arabidopsis thaliana* hypersensitive to DNA damaging treatments. *Genetics* **146:** 401-407.

Memisoglu, A. and L. Samson. 2000. Base excision repair in yeast and mammals. *Mutation Research* **451:** 39-51.

Mitchell, D.L. and R.S. Nairn. 1989. The biology of the (6-4) photoproduct. *Photochem. Photobiol.* **49:** 805-819.

Nakajima, S., M. Sugiyama, S. Iwai, K. Hitomi, E. Otoshi, S.-T. Kim, C.-Z. Jiang, T. Todo, A. Britt and K. Yamamoto. 1998. Cloning and characterization of a gene (*UVR3*) required for photorepair of 6-4 products in *Arabidopsis thaliana*. *Nucl. Acids Res.* **26:** 638-644

Nakatsu, Y., K. Hattori, H. Hayakawa, K. Shimizu and M. Sekiguchi. 1993. Organization and expression of the human gene for O-6-methylguanine-DNA methyltransferase. *Mutation Research* **293:** 119-132.

Nash, H.M., S.D. Bruner, O.D. Scharer, T. Kawate, T.A. Addona, E. Spooner, W.S. Lane and G.L. Verdine. 1996. Cloning of a yeast 8-oxoguanine DNA glycosylase reveals the existence of a base-excision DNA-repair protein superfamily. *Current Biology* **6:** 968-980.

Nouspikel, T. and P.C. Hanawalt. 2000. Terminally differentiated human neurons repair transcribed genes but display attenuated global DNA repair and modulation of repair gene expression. *Molecular and Cellular Biology.* **20:** 1562-1570.

Ohtsubo, T., O. Matsuda, K. Iba, I. Terashima, M. Sekiguchi and Y. Nakabeppu. 1998. Molecular cloning of AtMMH, an Arabidopsis thaliana ortholog of the Escherichia coli mutM gene, and analysis of functional domains of its product. *Molecular and General Genetics* **259:** 577-590.

Posnick, L.M. and L.D. Samson. 1999. Imbalanced base excision repair increases spontaneous mutation and alkylation sensitivity in Escherichia coli. *Journal of Bacteriology* **181:** 6763-6771.

Puchta, H. 1998. Towards targeted transformation in plants. *Trends in Plant Science* **3:** 77-78.

Ries, G., G. Buchholz, H. Frohnmeyer and B. Hohn. 2000. UV-damage-mediated induction of homologous recombination in Arabidopsis is dependent on photosynthetically active radiation. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 13425-13429.

Ries, G., W. Heller, H. Puchta, H. Sandermann, H.K. Seidlitz and B. Hohn. 2000. Elevated UV-B radiation reduces genome stability in plants. *Nature (London)* **406:** 98-101.

Roldan-Arjona, T., M.-V. Garcia-Ortiz, M. Ruiz-Rubio and R.R. Ariza. 2000. cDNA cloning, expression and functional characterization of an Arabidopsis thaliana homologue of the Escherichia coli DNA repair enzyme endonuclease III. *Plant Molecular Biology* **44:** 43-52.

Rozema, J., J. van de Staaij , L. Björn and M. Caldwell. 1997. UV-B as an environmental factor in plant life: stress and regulation. *Trends Ecol. & Evol.* **12:** 22-28.

Samson, L. 1992. The suicidal DNA repair methyltransferases of microbes. *Mol. Microbiol.* **6:** 825-831.

Sancar, A. 1994. Structure and function of DNA photolyase. *Biochem.* **33:** 2-9.

Sancar, A. 1996. DNA excision repair. *Ann. Rev. Biochem.* **65:** 43-81.

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

Sekiguchi, J., D.O. Ferguson, H.T. Chen, E.M. Yang, J. Earle, K. Frank, S. Whitlow, Y. Gu, Y. Xu, A. Nussenzweig and F.W. Alt. 2001. Genetic interactions between ATM and the nonhomologous end-joining factors in genomic stability and development. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 3243-3248.

Shapiro, H.S. 1976. Distribution of purines and pyrimidines in deoxyribonucleic acids. *Handbook of Biochemistry and Molecular Biology*. Cleveland, CRC Press. 3rd, ed. 241-262.

Shi, L., R. Kent, N. Bence and A.B. Britt. 1997. Developmental expression of a DNA repair gene in Arabidopsis. *Mutation Research* **384:** 145-156.

Swoboda, P., S. Gal, B. Hohn and H. Puchta. 1994. Intrachromosomal homologous recombination in whole plants. *EMBO J.* **13:** 484-489.

Tang, J.Y., B.J. Hwang, J.M. Ford, P.C. Hanawalt and G. Chu. 2000. Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis. *Molecular Cell* **5:** 737-744.

Todo, T. 1999. Functional diversity of the DNA photolyase/blue light receptor family. *Mutation Research* **434:** 89-97.

Tsuchimoto, D., Y. Sakai, K. Sakumi, K. Nishioka, M. Sasaki, T. Fujiwara and Y. Nakabeppu. 2001. Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen. *Nucleic Acids Research* **29:** 2349-2360.

Unk, I., L. Haracska, R.E. Johnson, S. Prakash and L. Prakash. 2000. Apurinic endonuclease activity of yeast Apn2 protein. *Journal of Biological Chemistry* **275:** 22427-22434.

Vonarx, E., H. Mitchell, R. Karthikeyan, I. Chatterjee and B. Kunz. 1998. DNA repair in higher plants. *Mutation Research* **in press:**

Wood, R. 1999. Variants on a theme. *Nature* **399:** 639-640.

Wyatt, M.D., J.M. Allan, A.Y. Lau, T.E. Ellenberger and L.D. Samson. 1999. 3-Methyladenine DNA glycosylases: Structure, function, and biological importance. *Bioessays* **21:** 668-676.

Yang, H.-Q., Y.-J. Wu, R.-H. Tang, D. Liu, Y. Liu and A.R. Cashmore. 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. *Cell* **103:** 815-827.