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DNA DAMAGE AND REPAIR IN PLANTS

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ABSTRACT

The biological impact of any DNA damaging agent is a combined function of the chemical nature of the induced lesions and the efficiency and accuracy of their repair. Although much has been learned from microbes and mammals about both the repair of DNA damage and the biological effects of the persistence of these lesions, much remains to be learned about the mechanism and tissue-specificity of repair in plants. This review focuses on recent work on the induction and repair of DNA damage in higher plants, with special emphasis on UV-induced DNA damage products.

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INTRODUCTION

DNA is constantly subject to chemical modification; even under the best of circumstances, purine bases are lost from the mammalian genome at a rate of several thousand bases per cell per day (62). Alkylating agents, essential for a variety of biosynthetic processes, can turn a legitimate base into either a mutagenic, miscoding deviant, or a lethal, noncoding lesion. Hydrolytic deamination can directly change one base into another. Fresh air (oxygen) and sunshine (UV) are undoubtedly the two major genotoxic agents for most organisms, and plants are obliged to be exposed to both of these mutagens. For this reason, plants, like all living things, have mechanisms that enable them to tolerate or repair the DNA damage they inevitably experience.

DNA damage has both genotoxic and cytotoxic effects. The study of the induction of DNA damage and its repair in humans has been of interest largely because of the demonstrable role of mutagenesis in carcinogenesis and a postulated role of DNA damage in aging. The contribution of DNA damaging agents to genetic load in animals has also been of interest, particularly in the study of radiation-induced mutagenesis (85). While carcinogenesis is not particularly relevant to most agronomically important plants, it is possible that DNA damage may play a significant role in the "aging" of seeds stocks and perennial crops. Although DNA damage is often thought of primarily in regard to its mutagenic effects, the persistence of damaged bases also has a significant growth-inhibitory influence. Many DNA damage products act as blocks to the progress of both DNA and RNA polymerases. Accumulated damage will not only preclude cell division but will eventually kill even terminally differentiated, nonreplicating tissues such as a mature leaf. For this reason DNA repair mechanisms are required in all living plant tissues to alleviate the toxic effects of the accumulation of DNA damage on plant metabolism.

The role of DNA damage and repair in the creation of genetic diversity is also of interest. Some DNA damage "tolerance" pathways, which enable the cell to replicate in spite of the persistence of damage, are actually responsible for the mutagenic effects of many DNA damaging agents. Because the genetic variation created in part through point mutation and recombination are prerequisites of both natural and artificial selection, understanding the mechanisms of genetic change is relevant to both theoretical evolution and genetic engineering. Although the two major damage tolerance mechanisms, lesion bypass and recombinational "repair," have been clearly established as mutagenic events in microbes, it remains to be determined whether these pathways exist in higher plants.

DNA damage can be broadly classified into three types of lesions: mismatched bases, double-strand breaks, and chemically modified bases. Each of these classes of lesions is corrected via distinct repair pathways. Reviews of

DNA repair often focus on the last class of lesions, but the repair pathways for the first two classes of damage are particularly interesting to the plant geneticist. Although no work has been published on any aspect of mismatch repair in plants, there have been some very interesting recent developments pertaining to the repair of double-strand breaks. Our knowledge of general DNA repair pathways in plants lags far behind our knowledge of these pathways in bacteria, yeast, and mammals. For an in-depth review of DNA damage products and their repair in these organisms, I refer the reader to the recently published textbook *DNA Repair and Mutagenesis* (31).

DNA DAMAGE PRODUCTS

Assessing the biological significance of any single type of lesion is complex, requiring knowledge of the frequency at which it occurs, the immediate effects of its persistence (whether the lesion can mispair directly, or whether it acts as a block to replication and/or transcription), and the number and efficiency of repair and tolerance pathways that pertain to the lesion. Repair and tolerance pathways are addressed below. In this section I review some of the most common naturally occurring DNA damage products and their immediate biological effects.

As is the case with many of the repair mechanisms I discuss, the information on the induction of damage was derived from the study of microbes and mammals. The relative importance of individual lesion types may be quite different in plants. It is possible that some genotoxic, unique plant metabolites [i.e. psoralens or metabolized xenobiotics (90a)] occasionally find their way into the plant nucleus or perhaps reach an organellar genome. It is unknown whether plant metabolites have a significant effect on the stability of plant DNA. In addition, the DNA present in seeds, like that present in bacterial or fungal spores, experiences a very different chemical environment from that enjoyed by the DNA in the nucleus of actively metabolizing cells (84). The type and extent of damage that occurs during seed storage is an important and still developing area of research. Finally, the distribution of naturally occurring DNA damage products changes not only from one organism to the next but also from one tissue to the next. To the epidermal cells of a plant, the damage induced by UV radiation is as inevitable as hydrolytic damage induced by the water present in the cell's nucleus. Even subtle changes produced under controlled laboratory conditions can substantially alter the type and frequency of spontaneous mutations (121). For this reason, the reader should understand that the relative importance of the various DNA damage products discussed below (and this is not an all-inclusive list) and, in fact, the repair pathways discussed later, should *always* be regarded within the context of the organism in question, the tissue studied, and the nature of its environment.

Hydrolytic Damage

For an excellent review of the chemical stability of DNA (both in vivo and, interestingly, from ancient sources) I direct the reader to a recent article (61). The DNA of living cells is subject to a variety of hydrolytic reactions, the most common being the hydrolysis of the glycosylic bond between purine bases and the DNA backbone. Although the overall rate of depurination is quite slow, in organisms with a large genome, such as humans or maize, spontaneous hydrolysis would be expected to induce the loss of several thousand purine bases per day per cell. If an abasic site were to persist, it would block DNA replication and transcription and it would be lethal in replicating cells. There is also some evidence that AP (apurinic) sites are potentially mutagenic as a result of occasional lesion bypass events during DNA replication (34). Generally, abasic sites are rapidly recognized and repaired. As a result, spontaneously generated AP sites do not play a significant role in spontaneous mutagenesis in microbes or mammals, and this is likely to be true for plants as well.

A second type of hydrolysis reaction appears to be responsible for the majority of spontaneous point mutations in human cells. Both cytosine and 5-methylcytosine are subject to hydrolytic deamination, resulting in the formaion of uracil and thymine, respectively. These two deamination products, both of which base pair with adenine and so are potentially mutagenic, do in fact differ widely in their mutagenicity. Uracil is rapidly recognized as an inappropriate base in DNA, and it is excised by uracil glycosylase in both plants and animals. Thymine, however, cannot be recognized as a DNA damage product and so is highly mutagenic, producing C:G to T:A transition mutations. A study of disease-related point mutations in the p53 tumor suppressor gene revealed that 43% were C to T transitions at 5-methyl CpG dinucleotides (101). This strongly suggests that the deamination product of 5-methylcytosine may be the most important single cause of spontaneous point mutations in the mammalian cell. The tumor DNA analyzed did not include skin cancers; the spectrum of mutations induced in tissues exposed to sunlight is different from that listed above and is discussed in the section UV-Induced Damage.

Given that plants methylate cytosine not only at CpG dinucleotides but also at CpNpG trinucleotides (37) and potentially other sites as well, and the fact that a much higher percentage of the angiosperm genome, compared with the human genome, is made up of 5-methylcytosine (approximately 10% vs less than 1%) (115), it is likely that 5-methylcytosine to thymine transitions are also a frequent spontaneous mutation event in plants. The underrepresentation of CpG vs GpC nucleotides in the plant genome supports the notion that these sequences are unstable (114).

Although the exact structure and hydration state of DNA in dried seeds is unknown (84), one would expect that seeds, when stored for long periods of time in a desiccated state in which no DNA repair occurs, would progressively accumulate AP sites and other spontaneously generated lesions. At some point the amount of damage incurred by aging seeds may exceed the repair capacity of the germinating seedling. Although desiccated seeds accumulate hydrolytic damage at a much slower rate than fully hydrated cells (21), long-term seed storage has been correlated with a delay in replicative DNA synthesis, the limited synthesis of low molecular weight, untranslated RNAs, and an increase in unscheduled (repair) DNA synthesis (83). These phenomena are consistent with a requirement for a period of genomic repair before cell division can occur in germinating seeds. At least some of the beneficial effects of "osmo-priming," a procedure involving partial hydration of seed designed to enhance early and uniform germination, may result from DNA repair activities during the priming period (4).

Alkylation Damage

Ethylmethane sulfonate (EMS), an ethylating agent, is a commonly employed artificial mutagen in plant genetics. Even in the absence of exogenously applied alkylating agents, all cells experience a biologically significant level of spontaneous DNA methylation (98b). The majority of the bonds in all four bases are susceptible to methylation, to widely varying extents, and some of the methylation products, if left unrepaired, are premutagenic and/or lethal. Most methylation damage occurs at purine bases. The most frequently generated alkylation product, 7-methyladenine, base pairs normally and is regarded as neither mutagenic nor toxic. In contrast, 3-methyladenine cannot serve as a template for DNA synthesis and therefore acts as a block to DNA replication. DNA damage products that cannot successfully base pair with any base are frequently termed "noninformational lesions" and are regarded as potentially lethal events. A third lesion, O^6-methylguanine, base pairs efficiently with thymine and therefore is a very potent premutagenic lesion. A survey of the published sequences of eight mutant alleles generated by EMS treatment of *Arabidopsis* seeds indicated that all eight were G to A transition mutants, consistent with the hypothesis that O^6-alkylguanine is the major mutagenic lesion induced by EMS in this tissue (26, 78, 82)—though see also Reference 81 for an exception to this conclusion. Because methylated bases (particularly 7-methylguanine and 3-methyladenine) are generated by endogenous methylating agents, plants, animals, and microbes have developed specialized repair pathways to reverse or excise methylation damage.

The level of genome methylation is subject to environmental perturbation. Many microbes have developed an elaborate regulatory system, termed "the adaptive response," that enables them to enhance their capacity to repair

methylation damage upon exposure to a lower "challenge" dose of alkylating agents (113). The chemical nature of the significant environmental and endogenous alkylating agents is still a subject of speculation (134), although certain bacteria, fungi, and algae have been shown to produce potent inducers of the adaptive response. It is possible that plants, especially plant roots, may experience a wide range of variation in the rate of alkylation damage and therefore may also have developed an adaptive response to this class of DNA damaging agents.

Oxidative Damage

A wide variety of oxidative damage products are induced in DNA by hydroxyl radicals, superoxide, and nitric oxide (23). Some of these damaged bases, including thymine glycol and its degradation product, urea, act as blocks to DNA synthesis but are not particularly mutagenic. Oxidation products of cytosine undergo an enhanced rate of deamination (via the hydrolytic reaction discussed above) to form mutagenic uracil derivatives. Perhaps the most significant premutagenic oxidized base is 8-hydroxyguanine, which base pairs with equal facility to A and C. In addition, the nucleotide 8-hydroxydGTP can be used as a substrate for DNA synthesis by DNA polymerase. Both human and *E. coli* cells produce an enzyme that specifically degrades this deoxynucleotide triphosphate to its monophosphate form, thereby preventing its incorporation into DNA (64, 75). Because bases are easily oxidized in vitro during standard DNA purification procedures, and because some oxidation products are inherently unstable, it is difficult to determine the spontaneous rate at which certain oxidized bases arise, persist, or are repaired in the genome. It should also be noted that the bases in an intact double helix are shielded from attack by hydroxyl radicals to a large degree by their stacked, interior conformation. For this reason, a relatively large fraction of oxidation damage occurs at the sugar phosphate backbone, leading to single-stranded breaks. Such nicks are generally repaired in an efficient and error-free fashion. Because the double helix is more likely to "breathe" (become transiently single-stranded) near a nick, the bases located near a nick are substantially more accessible to hydroxyl radical attack.

The major sources of activated oxygen in the cell are almost certainly the organelles; reactions in both the chloroplast (11) and the mitochondrion (138) frequently misdirect electrons to oxygen, generating superoxide. The plastid possesses a number of enzymatic and nonenzymatic defenses against superoxide, peroxide, singlet oxygen, and hydroxyl radicals (11), designed to capture free radicals before they can interact with critical cellular components such as the photosynthetic apparatus or the genome. These defenses can be overwhelmed during periods of stress when NADP, the electron acceptor for reduced ferredoxin, becomes limiting (3). Under these "photoinhibitory" (63)

conditions the production of activated oxygen species may exceed the chloroplast's extensive scavenging capacity. In addition, because hydrogen peroxide can diffuse rapidly across the lipid bilayer, no cellular compartment is completely isolated from the reactive oxidative species produced during either respiration or the light reactions of photosynthesis.

Significant extracellular sources of activated oxygen might include air pollutants such as ozone (50, 68) or perhaps radicals produced by neighboring cells during the hypersensitive response (57). Very high levels of UV-B radiation can also induce oxidative damage in DNA (40); however, it is not clear whether the amount of oxidative damage induced by the relatively low levels of UV-B radiation in solar radiation is significant in comparison with the baseline level of oxidative damage produced by normally functioning organelles. It is important to note, however, that screens for UV-sensitive *Arabidopsis* mutants (13, 22, 41, 49) have employed unnaturally intense, brief doses of UV. A screen performed in this manner may also yield mutants specifically defective in the repair of oxidative damage.

Damage Induced by Ionizing Radiation

Ionizing radiation differs from UV radiation in its complete lack of target specificity. The probability of any component of the cell directly interacting with ionizing radiation depends simply on the mass fraction it makes up of the cell. For this reason, the most frequent primary target of ionizing radiation in actively metabolizing plant cells is water, and the majority of DNA damage induced by ionizing radiation probably results from interaction of DNA with hydroxyl radicals (139). Direct absorption of radiation by the sugar phosphate backbone can also generate a nick; the sensitization of the opposing, unnicked strand may result in an increased yield of double-strand breaks. Ionizing radiation is often used to generate chromosomal breaks, inversions, duplications, and translocations in plant stocks, but it should be noted that point mutations may also be generated by this type of mutagen as a result of oxidative damage to bases. A survey of mutations induced in irradiated *Arabidopsis* seeds suggests that ionizing radiation is a fairly reliable source of chromosomal rearrangements; of nine alleles analyzed at the Southern blot level, only one (125), a fast neutron-induced mutation at *GA1*, was found to have a "point-like" mutation (116, 140, 141).

UV-Induced Damage

The cyclobutane pyrimidine dimer (CPD) and the pyrimidine (6-4) pyrimidinone dimer (the 6-4 photoproduct) make up approximately 75% and 25%, respectively, of the UV-induced DNA damage products (72). The action spectrum for the induction of pyrimidine dimers in purified DNA follows the absorbance spectrum of DNA; dimers are induced most efficiently by radia-

tion at approximately 260 nm, i.e. by radiation in the UV-C range. Although no biologically significant UV-C ($\lambda < 280$ nm) radiation is present at the earth's surface, the small amount of UV-B (280–320 nm) and much greater flux of UV-A (320–400 nm) present in sunlight are the single most important sources of epidermal DNA damage in plants or animals. The contribution of longer (UV-A) wavelengths of the solar UV spectrum to the overall load of dimers in both human and plant tissues is further enhanced by its greater ability to penetrate through the outermost layers of cells (95). Plants are thought to produce natural sunscreens, which selectively absorb photons in the UV-B and UV-A range, and flavonoid pigments are generally regarded as UV-absorbing agents. Evidence suggests that plants defective in the synthesis of anthocyanins are shielded from the growth-inhibiting effects of UV-B (59) and from the induction of DNA damage (123). Yet, some of the UV-protective effects observed in the chalcone isomerase-defective *tt5* mutant of *Arabidopsis* may result from its defect in the synthesis of sinapic acid esters, some of which are highly UV absorbent (17, 59).

The biological effects of pyrimidine dimers have been extensively studied in microbes and mammals. Like some of the DNA damage products discussed above, pyrimidine dimers have been shown to inhibit the progress of microbial and mammalian DNA polymerases and are not directly mutagenic. Mammalian RNA polymerase II has been shown to "stall" at both CPDs and 6-4 photoproducts (74, 92). Thus, in the absence of repair, a single pyrimidine dimer is sufficient to completely eliminate expression of a transcriptional unit. In addition, evidence suggests that the stalled mammalian RNA polymerase II remains bound to the site of the obstruction (27). Thus persisting lesions may actually reduce the overall concentration of free RNA polymerase, in addition to eliminating transcription of the gene in which they are located. Every pyrimidine dimer acts as a block to transcription and replication, while only a small fraction of dimers results in a mutation. For this reason, the inhibitory effects of UV on transcription and replication in plant epidermal tissues are probably more significant (in terms of plant growth) than its mutagenic effects are.

DNA REPAIR PATHWAYS

Direct Reversal of Damage

PHOTOREACTIVATION In some organisms the biological effects of UV radiation are significantly reduced by subsequent exposure to light in the blue or UV-A range of the spectrum, a phenomenon known as photoreactivation. The photoreactivating effects of visible light usually reflect the actions of photolyase enzymes. This class of enzyme binds specifically to cyclobutane pyrimidine dimers and, upon absorption of a photon of the appropriate wavelength

(350–450 nm), directly reverses the damage in an error-free manner. Microbial photolyases carry two prosthetic groups. One chromophore (either methenyltetrahydrofolate or 8-hydroxy-5-deazaflavin) absorbs the photoreactivating light and transfers the energy to the other chromophore, a fully reduced flavin adenine dinucleotide (FAD). The excited $FADH^{-}$ then transfers an electron to the dimer, inducing its reversal (107). Once photolyase has bound to a cyclobutane dimer, the efficiency of photoreactivation is extremely high; approximately one dimer is split for every blue-light photon absorbed. Microbial photolyase genes have been cloned from a variety of bacteria and fungi, and their sequences display obvious homologies (143).

Evidence for the biological effects of photoreactivation in plants is complicated by the obvious detrimental effects of growing plants in the dark. This problem can be partially alleviated by the use of appropriate controls and of filters that absorb the shorter wavelengths required for photoreactivation (450 nm and under) while transmitting photons of longer photosynthetically active wavelengths. Photoreactivation results in the reversal of several UV-induced phenomena in plants, including mutagenesis, chromosome rearrangements (47), inhibition of growth, induction of flavonoid pigments (7), and unscheduled synthesis of DNA (48). Light-enhanced repair of dimers from total cellular DNA has been documented in tobacco, *Haplopappus gracilis* (132), ginkgo (133), *Chlamydomonas* (120), *Arabidopsis* (19, 87), and wheat (130), and the action spectrum for reversal of CPDs by partially purified maize and *Arabidopsis* photolyases has been shown to be similar to that of *E. coli*, a methenyl tetrahydrofolate-type photolyase (46, 87).

The cyclobutane dimer photolyase activities of higher plants are known to be regulated by visible light. The CPD photolyase activity of the common bean is induced twofold by a brief exposure to red light; this effect is partially reversed by subsequent exposure to far red light, suggesting that the induction is phytochrome mediated (55). Similarly, the light-dependent repair of CPDs in *Arabidopsis* requires exposure to visible light prior to as well as after UV irradiation (19). Thus the repair capacity of the plant depends on the quality and timing, as well as quantity, of light in its environment. The influence of the environment on the steady-state level of pyrimidine dimers, the rate of induction of dimers, and the rate of photoreactivation of dimers has been illustrated in recent work on alfalfa (127). Researchers found that seedlings grown in an essentially UV-free environment had the same steady-state levels of cyclobutane dimers (approximately 6 dimers/megabase) as seedlings grown under unfiltered sunlight. In addition, a given dose of UV was found to induce twofold more dimers in the seedlings grown under artificial light, and these seedlings also had a lower rate of photoreactivation of CPDs than the identical strain grown under natural light. Thus both the UV transparency and the repair capacity of higher plants is altered in response to the ambient levels of UV and

visible radiation. Similar effects have been observed in experiments that directly measure the effects of enhanced UV-B on yield (14, 30, 71).

A putative plant photolyase was cloned from wild mustard (6) by probing a cDNA library with a degenerate oligonucleotide specific to a conserved region of the microbial cyclobutane dimer photolyases. The clone, labeled SA-*phr1*, displays significant stretches of similarity to previously cloned microbial photolyases. Moreover, the cDNA hybridizes to an mRNA that is strongly regulated by light; seedlings grown in the dark express low levels of the mRNA, whereas light-grown seedlings express the mRNA at high levels. The protein encoded by this cDNA was expressed in *E. coli* and found to bind, like the *E. coli* photolyase, both FAD and methenyltetrahydrofolate (65). The *E. coli*–expressed mustard protein did not, however, display any photolyase activity; it neither enhanced the UV resistance of a photolyase-defective host strain nor did it split thymine dimers in vitro. For this reason, the authors concluded that the SA-*phr1* clone represents a blue light photoreceptor rather than a photolyase. This conclusion gains support because a constitutively expressed *Arabidopsis* gene (*HY4*) known to be involved in the blue light response was also found to have a region of substantial homology to the microbial photolyases (1). The *HY4* gene product, when expressed in *E. coli*, also binds both FAD and methenyltetrahydrofolate but fails to exhibit any photoreactivating activity (65).

Although the failure to find enzymatic activity in a heterologously expressed gene product is not definitive proof that a protein would lack photoreactivating activity if expressed *in planta*, it should be noted that the gene was cloned on the basis of its homology to microbial photolyases. A second class of "metazoan" photolyases, currently cloned from fish, insects, and marsupials, apparently has little sequence similarity to the more thoroughly studied microbial enzymes (146). It is conceivable that the plant photolyase is more closely related to the metazoan proteins or perhaps represents yet another class of photolyases.

In contrast with findings on microbes and mammals, experimental evidence suggests that *Arabidopsis* may have a light-dependent pathway for the repair of pyrimidine (6-4) pyrimidinone photoproducts (19). Unlike its CPD-specific photolyase activity, this repair pathway does not require induction by prior exposure to visible light. It also does not require the *UVR1* gene product (13), which is essential for dark repair of 6-4 photoproducts. Thus *Arabidopsis* has the ability to photoreactivate both of the major UV-induced DNA damage products. This ability probably extends to other plants; exposure to visible light greatly enhances the rate of removal of 6-4 photoproducts from the DNA of wheat seedlings (130). Although photoreactivation of 6-4 photoproducts has not been observed in microbial or most animals tested, a 6-4 photoproduct-specific photolyase activity has been partially characterized in extracts of

Drosophila larvae (53, 131). If 6-4 photolyase activity exists in organisms as distantly related as plants and insects, it is important to determine whether the activity is universal. The discovery of the 6-4 photolyase is particularly significant in that the biological effects of photoreactivation have previously been ascribed to the alleviation of the toxic effects of cyclobutane dimers alone.

LACK OF EVIDENCE FOR O^6-METHYLGUANINE METHYLTRANSFERASE As described above, O^6-alkylguanine base pairs directly with thymine and therefore is directly mutagenic. For this reason, most organisms produce a protein, O^6-methylguanine methyltransferase (MGMT), that removes the methyl group from the lesion, transferring it to a serine residue on the protein itself. Because no mechanism exists for the demethylation of this protein, this "enzyme" is permanently inactivated by the reaction and is sometimes termed a "suicide" DNA methyltransferase. MGMT has been identified in and cloned from bacteria (24, 91, 112), yeast (142), and mammals (43, 103, 129). No evidence for its existence in plants has been established. A careful search for the activity was performed in *Chlamydomonas,* with negative results (32). One of the two *E. coli* copies of MGMT, the *ada* gene, was recently transformed into tobacco callus, and resistance to the growth-inhibitory effects of methylating agents was enhanced in the transformed callus (2). Activity in plants grown from the callus was poor, however (135), making it difficult to determine whether the expression of the *ada* gene had an antimutator effect. It is difficult, if not impossible, to provide definitive proof that an enzymatic activity does not exist in a particular organism. The presence of MGMT in yeast was in doubt until the gene was cloned and sequenced (142). In fact, the existence of a bona fide photolyase in placental mammals is still a matter of some debate (60, 102).

Excision Repair

In contrast with photoreactivation, dark repair pathways do not directly reverse DNA damage but instead replace the damaged DNA with new, undamaged nucleotides. These "excision repair" pathways fall into two major categories: base excision repair and nucleotide excision repair.

BASE EXCISION REPAIR Base excision repair involves the removal of a single damaged base through the action of one of many lesion-specific glycosylases, which leaves the DNA sugar-phosphate backbone intact. The resulting abasic sites are then recognized by an apurinic/apyrimidinic (AP) endonuclease or AP lyase, which nicks the backbone of the DNA at the AP site (105). The nicked DNA is then restored to its original sequence through the combined actions of exonucleases, a repair polymerase, and DNA ligase. Recent evidence has suggested that the repair polymerase itself, polβ, possesses the ability to excise

the 5′ deoxyribose phosphate residue that is generated by the combined actions of DNA glycosylases and class II AP endonucleases (66).

URACIL GLYCOSYLASE As described above, uracil accumulates in the genome at a rate of approximately 100 lesions per cell per day (for a genome size of 3×10^9 bp). Because this lesion is directly mutagenic, all living things probably produce a uracil glycosylase. The crystal structure of uracil glycosylase from nonplant sources has recently been solved and suggests that the protein actually binds to a uracil base that has swiveled out to the exterior of the double helix (76, 109). Although a gene corresponding to this protein has not yet been identified in plants, the activity has been purified from several plant sources (10, 128). There is some evidence that this activity is downregulated by as much as 20-fold in fully differentiated cells (38).

3-METHYLADENINE GLYCOSYLASE 3-Methyladenine is a noncoding lesion that, like uracil, occurs spontaneously at a significant rate. 3-Methyladenine glycosylases have been identified in bacteria, yeast, mammals, and *Arabidopsis* and vary in their substrate-specificity. *E. coli* expresses two 3-methyladenine glycosylases. The product of the *tag* gene is highly specific for 3-methyladenine, whereas the product of the *alkA* gene has a broad substrate specificity, cleaving the N-glycosylic bond at 7-methylguanine, 3-methylguanine, O2-methylthymine, and O2-methylcytosine, as well as 3-methyladenine (29, 51). The biological effects of an *alkA*- mutation can be suppressed by the artificial overexpression of the *tagA* gene (145), which suggests that these additional substrates do not play an important role in the lethality induced by methylating agents. The *tag* and *alkA* genes share no significant homology. All of the cloned higher eukaryote 3-methyladenine glycosylases, including one from *Arabidopsis* (108), have been isolated via complementation of the MMS-sensitivity of the *E. coli* double mutant (8, 18; summarized in 28, 80). Although the mammalian genes have a high degree of homology with one another, the overall transkingdom homology is fairly weak.

UV-ENDONUCLEASES Glycosylases and endonucleases specific for cyclobutane dimers have been observed in bacteria and bacteriophage and have been useful as diagnostic agents for the assay of UV-induced damage (31). True eukaryotic UV-endonucleases that recognize both cyclobutane dimers and 6-4 photoproducts and that generate an incision immediately 5′ to the lesion were recently identified in *Saccharomyces pombe* and *Nuerospora crassa* (12, 144). Several groups have described the partial characterization of endonucleolytic activities obtained from plant extracts that exhibit some specificity for UV-irradiated DNA (25, 77, 136). Some of these activities are particularly intriguing in that they do not appear to recognize CPDs, which suggests that the recognition site may be the 6-4 photoproduct. In only one case (the endonuclease SP purified

from spinach) has a plant UV-specific endonuclease been substantially purified and characterized; this enzyme was suggested to be a single-stranded endonuclease, which apparently recognizes a single-stranded region that is induced by 6-4 photoproducts but not by CPDs (124).

NUCLEOTIDE EXCISION REPAIR (NER) NER differs from base excision repair in two ways: The spectrum of DNA damage products recognized by the repair complex is remarkably wide, and the repair complex initiates removal of the damage by generating nicks on the damaged strand. These nicks occur at a specific distance both 5′ and 3′ of the lesion, which is then excised as an oligonucleotide through the action of a helicase. The excision repair complex will, with varying efficiencies, cleave almost any abnormality in DNA structure–-from very small, nondistorting lesions (such as O^6-methylguanine or abasic sites) to very bulky adducts (thymine-psoralen adducts or pyrimidine dimers). It is not likely that the cell produces a specific repair protein for every possible lesion, and nucleotide excision repair may exist, in part, to cope with the unexpected. As discussed above, placental mammals are generally thought to lack photolyase, and in mammalian cells NER is apparently the sole pathway for the repair of bulky adducts (106). It should be kept in mind, however, that most mammalian repair studies, for obvious reasons, are performed in tissue culture rather than in actual skin. It is possible, and even reasonable, that only those tissues that are normally exposed to sunlight express a specific repair pathway for UV-induced damage. Pyrimidine dimers may indeed represent an "unexpected" class of lesions to most types of cultured cells.

Light-independent ("dark") repair of CPDs, which might represent either NER or base excision repair, has been observed in several plant species. Early studies, previously reviewed by McLennan (67), involved the use of a germicidal lamp (UV-C, 254 nm) to irradiate cell suspension cultures or protoplasts (for uniformity of UV penetration) producing high concentrations of CPDs. The disappearance of dimers from the nuclear fraction was measured by hydrolyzing the nuclear DNA and assaying, via thin layer chromatography, the fraction of total thymidine bases that were present as dimers. The appearance of excised dimers in the cytosol, indicative of excision repair, was followed using similar techniques. The rate of dark repair of CPDs was found to vary widely between plant species, with high rates of repair demonstrated for carrot suspension cultures (44) and protoplasts of carrot, *Haplopappus*, petunia, and tobacco (45), whereas excision repair of CPDs was undetectable in cultured soybean cells (100). It should be stressed, however, that photoreactivation is generally a more rapid and efficient pathway for the excision of UV-induced dimers and probably provides the bulk of the protection against UV-induced DNA damage. Excision repair may, however, be essential for the repair of minor, nondimer, UV-induced photoproducts.

Recently, more sensitive techniques have been developed for the detection of UV-induced damage, including the use of lesion-specific antibodies (73), the T4 endonuclease/alkaline sucrose gradient assay (33, 87), and an exquisitely sensitive gel-electrophoresis-based method involving the extraction of intact DNA, followed by cleavage of the DNA at CPDs and the quantitative assay of various size classes of single-stranded DNA sizes to arrive at an average frequency of dimers (96). These technical advances have enabled investigators to use relatively low doses of UV to study repair in intact plants. Dark repair rates for CPDs have been assayed in 5-day-old *Arabidopsis* seedlings, where no significant repair of CPDs was detectable in 24 h, although repair of 6-4 photoproducts was efficient (13). In contrast, rapid dark repair of CPDs was observed in alfalfa (97), and an intermediate level of repair was detected in wheat seedlings (130). While these plants may actually differ in their inherent capacity for dark repair, this disparity might also result from the differing experimental conditions employed. It has recently been demonstrated that excision repair in the alfalfa seedling, while efficient and easily detectable at high levels of initial UV damage, is undetectable at lower initial damage levels (97). Extremely high doses of UV can also inhibit repair in plant tissues (44). Thus, while laboratory studies are essential for the determination of the biochemical basis of repair, caution must be used in extrapolating these results to make predictions concerning UV resistance in the field, where growth conditions, the plant tissues employed, and the levels of DNA damage induced by sunlight can radically affect both the extent of damage and the rate of repair.

Double-Strand Break Repair

Double-strand breaks (DSBs) are generated in plant DNA through a variety of mechanisms: spontaneous oxidative damage to the genome, treatment with ionizing radiation, the formation of a dicentric chromosome, cleavage with artificially introduced nucleases, and (perhaps) excision of transposable elements. To a plant molecular biologist, the most important source of DSBs is probably the recombinant DNA with which the researcher hopes to transform the plant cell. Because the DNA sequences near the ends of these breaks are rapidly degraded, DSBs generally expand into gaps that cannot simply be religated to restore the original sequence. Unlike recombination-proficient yeast cells, which will virtually always repair DSBs via homologous recombination, the cells of higher plants behave very much like those of most mammalian tissues; DSBs are simply rejoined, end to end, in what appears to be a random fashion. This end-to-end joining process is sometimes termed “illegitimate recombination.” Analysis of repaired DSBs generated by ionizing radiation (116), T-DNA insertion (35, 36, 117), and transposable element excision [most recently (111)] indicates that a bias does exist toward the formation of

joint molecules at regions of homology. However, this homology is extremely limited (2–5 bases) and is probably simply the result of enhanced stability of the joint for ligation, rather than the sort of extensive homology search associated with homologous recombination. In addition, DNA junctions are often characterized by multiple recombination events, such as an inversion of substantial portions of the target site (116), and novel sequences that may represent template switching by a repair polymerase (104), the transient formation of a covalently closed hairpin loop (20), or addition of nontemplate nucleotides. The natural propensity of mammalian and higher plant cells to incorporate exogenous DNAs into random rather than homologous sites [the fraction of events from homologous recombination among all integration events is approximately $1/10^{-4}$ (90, 93)] is particularly vexing to the molecular biologist, because true gene replacement is very infrequent in these systems (with the exception, for unknown reasons, of mouse embryonic stem cells).

Very little is known about the genes required for illegitimate recombination in either plants or animals. X-ray-sensitive mutant animal cell lines exist that are defective in the repair of DSBs. Some of the genes that complement these defects have been cloned [reviewed in (31)]. *scid* mice, which are severely immunodeficient as a result a defect in V(D)J recombination, are also X-ray sensitive and fail to incorporate exogenous DNAs (42). Mutants of *Arabidopsis* specifically sensitive to the growth-inhibitory effects of ionizing radiation have also been isolated (22), and some UV-sensitive *Arabidopsis* mutants also display sensitivity to ionizing radiation (49). Although these mutants have not been directly assayed for the ability to repair DSBs, recent evidence has shown that a subclass of these mutants are defective in the stable incorporation of T-DNA into their genome (122). This result is particularly significant for two reasons. First, it suggests that the mutants are indeed defective in end-to-end joining. Second, it provides the first direct evidence for the role of host enzymes in T-DNA transformation. The availability of plant mutants defective in illegitimate recombination will enable us to better understand and perhaps modify this process.

REPAIR OF THE ORGANELLAR GENOMES

Any proteins present in the organelle are either synthesized there or are specifically transported into the organelle. For this reason, the presence of a repair activity in the nucleus does not imply that the activity is present in the organelle; the presence or absence of organellar repair activities has to be established independently. For example, Chinese hamster ovary cells express only a subset of their repair activities in their mitochondria; methylated purines and interstrand crosslinks are removed efficiently, but dimer and intrastrand crosslinks are not (56). This suggests that some types of base excision repair

function in mitochondria, but the more general nucleotide excision repair mechanism functions only in the nucleus. Neither the mitochondrial nor the plastid genomes encode any DNA repair proteins. Do organisms possess multiple, nuclearly encoded sets of certain repair genes, one for each genetic compartment, or are some repair proteins targeted to more than one compartment? *S. cerevisiae* has five copies of the *MSH* gene required for mismatch repair; one of the gene products is targeted to the mitochondrion (99). In contrast, *S. cerevisiae's PHR1* photolyase appears to photoreactivate both the nuclear and mitochondrial genomes, and its 5′ end can direct the transport of a *lacZ′* fusion protein to the mitochondrion (147). Similarly, the nuclearly encoded human uracil glycosylase is directed to both the mitochondrion and the nucleus (119). It is conceivable that some plant repair proteins might possess a unique targeting signal that facilitates their transport into all three compartments.

Chlamydomonas is known to photoreactivate both its nuclear and plastid genomes. The *phr1* mutant has been shown to be defective in the photoreactivation of the nuclear genome but not the plastid genome. This suggests that *Chlamydomonas* produces two distinct photolyases. Unfortunately, no studies, to my knowledge, have been published documenting repair of any kind in any higher plant organellar genome. A homologue of the *E. coli recA* gene has been cloned from *Arabidopsis* (16), and it encodes at its amino terminus a conserved recognition site for the stromal processing protease. Southern blot analysis using this cDNA as a probe suggests that there is more than one copy of this gene encoded by the *Arabidopsis* nucleus (9). This chloroplast *recA* homologue may play a role in recombinational "repair" (see below). Several other *Arabidopsis* cDNAs, cloned on the basis of their ability to partially complement the UV-sensitive and recombination-defective phenotype of *E. coli* repair-defective mutants, also appear to possess chloroplast-targeting sequences (88, 89).

Sequence analysis of the two plant organellar genomes suggests that they evolve by different mechanisms and at different paces (86). It will be interesting to determine whether some of these differences can be ascribed to differences in the mode and efficiency of their DNA repair pathways.

DNA Damage Tolerance Pathways

The excision repair pathways described above can all be divided into two steps: First the damaged base is removed, and then the undamaged strand is used as a template to fill the resulting gap. These repair pathways are essentially error free. If, however, a cell undergoes DNA replication before repair is complete, a "noninformational" DNA damage product, such as a pyrimidine dimer, will act as a block to DNA replication. DNA polymerase will normally reinitiate synthesis 3′ to the lesion, but a gap remains in the newly synthesized

daughter strand at the site opposite the DNA damage product. The resulting incompletely synthesized chromosome will, as a result, no longer act as a substrate for excision repair because the sister strand is no longer available as a template. Although one would expect the persistence of such a lesion to be lethal, a variety of organisms have been shown to undergo repeated rounds of DNA synthesis and cell division in spite of the continued presence of noninformational lesions. At least two independent pathways permitting the completion of replication of damaged chromosomes exist; these are dimer bypass and recombinational "repair." These pathways are sometimes collectively termed "postreplication repair" but are better thought of as "damage tolerance pathways" because they do not involve DNA repair but instead help the cell to survive despite persisting damage.

Dimer Bypass

Although noninformational lesions normally act as blocks to DNA replication, some organisms produce a modified polymerase that is capable of performing translesion synthesis. For example, the *E. coli umuC,D* gene products are thought to bind to DNA polymerase and relax its normally stringent requirements for the stable insertion of a new base, thereby enabling it to perform translesion synthesis (98a). The altered polymerase generally installs adenine residues across from noninformational DNA damage products. As a result, UV-induced thymine dimers are not mutagenic, but cytosine-containing dimers are. Similarly, because UV radiation induces primarily pyrimidine dimers and because the *umuC,D* gene products are required for translesion synthesis, strains with defects in these genes display an enhanced sensitivity to the lethal effects of UV while completely lacking a mutagenic response to this DNA damaging agent (52). Translesion synthesis permits DNA replication (and therefore enhanced survival) at the expense of accuracy. Because of their inherent potential for generating mutations, the *umuC,D* gene products are expressed only when the cell has been exposed to a substantial dose of DNA damaging agents (5). Similarly, the *REV3* gene of *S. cerevisiae* produces a nonessential, mutagenic polymerase with a specialized ability to synthesize DNA using damaged templates (118). Humans may produce a modified polymerase with a similar tendency to install A's at pyrimidine dimers: sunlight-induced mutations in humans occur mainly at dipyrimidines and are primarily C to T or CC to TT transversions (148).

Whether mutagenesis in plants occurs as a result of lesion bypass remains to be seen. UV radiation is an excellent source of noninformational DNA damage products, and the spectrum of mutations induced by UV could provide insights into the means by which plants tolerate the persistence of DNA damage. Unfortunately, few UV-induced mutations have been generated, and to my knowledge none has been sequenced. Because the plant's germline is

shielded from UV during virtually all stages of growth, studies of UV-induced mutations in higher plants have been limited to the mutagenic effects of UV irradiation of pollen. Mutagenesis of pollen has the advantage of enabling the investigator to observe the induction of mutations such as large deletions that might otherwise be nontransmissable as a result of selection during the post-meiotic mitoses and growth of the pollen tube. In fact, UV-induced mutations in maize pollen were generally found to be nontransmissable or to have reduced transmission beyond the first generation, which indicates that UV-induced lesions result in large deletions rather than point mutations (79). This finding suggests that translesion synthesis (which induces point mutations) rarely occurs during repair in pollen or during the early stages of embryonic development and that UV-induced DNA damage results in chromosome breaks and/or recombination. However, one must bear in mind that large chromosomal deletions, which result in the simultaneous loss of many genes, are simply easier to score as mutations than are single base changes, the majority of which fail to affect gene function. It is also possible that dimer bypass is preferentially employed in somatic cell lines (where mutagenesis is relatively inconsequential) but is not expressed during the critical last stage of pollen development, when mutations can no longer be eliminated through diplontic selection (54). Because of its potential role in the creation of genetic diversity (as well as in UV tolerance), more research on translesion synthesis is needed in both plants and animals.

Recombinational Repair

In contrast with lesion bypass, recombinational "repair" fills the daughter-strand gap by transferring a preexisting complementary strand from a homologous region of DNA to the site opposite the damage. As in the dimer bypass mechanism, the lesion is left unrepaired, but the cell manages to get through another round of replication, and the damaged base is now available as a substrate for excision repair. When the complementary strand is obtained from the newly replicated sister chromatid, the resulting "repair" is error free. If the information is obtained from the homologous chromosome, or perhaps from a similar DNA sequence elsewhere in the genome, there is a possibility that a change will be generated in the gene's sequence either via gene conversion or through the formation of deletions, duplications, and translocations. While UV irradiation has been shown to induce chromosomal rearrangements in plants (79), including homologous intrachromosomal recombination events (94), it remains to be seen whether the filling of daughter-strand gaps via homologous recombination is a significant UV tolerance mechanism in plants. UV radiation has been shown to induce previously quiescent transposable elements (137); it is possible that this effect is the result of chromosomal rearrange-

ments or other repair-related activities. Conversely, some UV-induced mutations may result from the activation of transposable element activities.

Other Damage Tolerance Mechanisms

The two pathways described above permit the cell to replicate in spite of the persistence of dimers but do not reduce the deleterious effects of DNA damage on transcription. One of the most interesting recent developments in the field of DNA repair is the discovery that the template strand employed for transcription is repaired more rapidly than the untranscribed strand or untranscribed regions (39). In fact, the relationship between repair and transcription is particularly intimate–-not only are some repair proteins physically coupled to RNA polymerase, but a subset of those proteins, notably the TFIIH complex, actually act independently both as transcription factors and as repair complexes (110). By selectively removing damage from actively transcribed units, targeted repair substantially reduces the toxic effect of UV. Although preferential repair of transcribed strands has been shown to exist in mammals (70), yeast (126), and *E. coli* (69), this phenomenon has not yet been investigated in plants.

Lesions opposite a daughter-strand gap are particularly problematic because the damage cannot be repaired via excision repair. If the cell is unfortunate enough to not only replicate its damaged DNA but to also undergo cell division, then the information at the site of the lesion is permanently lost because no sister chromatid is available to take part in recombinational repair. For this reason, some organisms are capable of detecting genome damage and will delay cell division until the integrity of the genome is restored. Yeasts (*S. cerevisiae, S. pombe*) damaged in G1 or S phase will cease further DNA synthesis, while G2 cells will delay mitosis (15). Cells defective in genes required for the G2 "checkpoint" will proceed with cell division in spite of the presence of gapped DNA and will therefore exhibit an increase in sensitivity to both the toxic and mutagenic effects of DNA damaging agents. Similar "checkpoint" responses to DNA damage have been observed in other fungi and in mammals (58).

Several labs are currently in the process of isolating *Arabidopsis* mutants that are hypersensitive to the growth-inhibiting effects of DNA damaging agents (13, 22, 41, 49). Unfortunately, few of these mutants have been characterized in terms of their repair capabilities. Although many of these UV-sensitive mutants will have demonstrable defects in repair, undoubtedly some fraction will display normal rates of repair. This second class of mutants is a particularly interesting one, as it may include mutants defective in damage tolerance. Thus a screen for UV-sensitivity might yield mutants defective in mutagenesis, recombination, transcription, and cell cycle control.

SUMMARY

Plants are now known to possess many of the same repair pathways as other eukaryotes; UV-induced pyrimidine dimers (both 6-4 photoproducts and cyclobutane dimers) can be removed via photoreactivation or through excision repair, and certain lesion-specific glycosylases have been shown to exist in higher plants. What may be more important is that researchers have proven that the currently available assays for repair can be applied to plants. The DNA of higher plants can be radiolabeled in vivo and can be extracted in the very intact state required for the assay of DNA damage (and its repair) induced at a very low frequency. The feasibility of employing both classical and molecular genetic approaches to DNA repair has been established in plants; at least one *Arabidopsis* mutant defective in the repair of UV-induced lesions has been isolated, and at least two radiation-sensitive mutants appear to be defective in the rejoining of double-strand breaks. Several repair-related genes have been cloned from *Arabidopsis* either via complementation of repair-defective mutants from other species or by probing for the presence of homologues to known repair genes.

Many repair-related issues remain unexplored. Although photoreactivation is undoubtedly the plant kingdom's major line of defense against UV-induced damage, the molecular nature of the two plant photolyase genes is unknown. Virtually nothing is known about organellar repair or organellar damage tolerance pathways, although the identification of an *Arabidopsis* plastid *recA* homologue should shed some light on this process. Nothing is known, in any plant species, about the mismatch repair process. We have yet to identify any DNA damage tolerance pathways in plants. Our understanding of the molecular mechanisms of both illegitimate and homologous recombination is still in its infancy. Many of these questions could be easily addressed with currently available technologies. A wide range of useful tools have been developed by researchers working on microbes and animals. These include lesion-specific antibodies, repair-defective mutants, and a multitude of cloned repair genes from a wide range of species. All of these tools can be directly applied to the study of repair and repair-related processes in plants.

The study of DNA repair and DNA damage tolerance processes in plants touches on a surprisingly wide range of subjects, including not only the effects of DNA damaging agents on plant growth and mutagenesis but also transcription, cell cycle control, and both homologous and illegitimate recombination. It also has applications beyond mutagenesis; an understanding of DNA transactions in plants is essential if we hope to progress beyond the relatively crude and haphazard level of "genetic engineering" currently available to both basic and applied plant geneticists.

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