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DNA Repair Functions in Heterologous Cells

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ABSTRACT: Our genetic information is constantly challenged by exposure to endogenous and exogenous DNA-damaging agents, by DNA polymerase errors, and thereby inherent instability of the DNA molecule itself. The integrity of our genetic information is maintained by numerous DNA repair pathways, and the importance of these pathways is underscored by their remarkable structural and functional conservation across the evolutionary spectrum. Because of the highly conserved nature of DNA repair, the enzymes involved in this crucial function are often able to function in heterologous cells; as an example, the *E. coli* Ada DNA repair methyltransferase functions efficiently in yeast, in cultured rodent and human cells, in transgenic mice, and in *ex vivo*-modified mouse bone marrow cells. The heterologous expression of DNA repair functions has not only been used as a powerful cloning strategy, but also for the exploration of the biological and biochemical features of numerous enzymes involved in DNA repair pathways. In this review we highlight examples where the expression of DNA repair enzymes in heterologous cells was used to address fundamental questions about DNA repair processes in many different organisms.

KEY WORDS: photolyase, DNA repair methyltransferase, base excision repair, mismatch repair, functional suppression, dominant-negative phenotype.

I. INTRODUCTION

DNA damage is inevitable. Damage is continually generated in the genome of all organisms owing to the inherent chemical instability of nucleic acids under physiological conditions and owing to the chemical reactivity of DNA with endogenous and exogenous chemicals.[124] In addition, ionizing and ultraviolet (UV) radiation constitute a natural, ever-present environmental source of physical damage to nucleic acids, in particular to DNA. DNA alterations also emanate from the rare errors made by DNA polymerases as they duplicate the genome prior to cell division. Some variation in DNA sequence is required for organisms to evolve, but too much variation destabilizes the organism. The level of damage that DNA unavoidably suffers appears to be potentially destabilizing, because, in order to achieve genomic stability, all organisms employ an array of sophisticated mechanisms to repair DNA damage.[66] Many of these mechanisms must have arisen early in the evolutionary process because they display remarkable structural and functional homology across the evolutionary spectrum, from unicellular prokaryotes to *Homo sapiens.* The exquisite conservation of these mechanisms underscores their importance for the survival of each species.

The structural and functional conservation of DNA repair mechanisms was revealed during the last decade, with the cloning and characterization of a large number of DNA repair genes from numerous organisms. DNA repair deficiencies generally confer sensitiv-

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ity to the cytotoxic effects of particular DNA-damaging agents, furnishing an obvious strategy for the cloning of DNA repair genes by their phenotypic suppression of DNA repair-deficient mutants. It soon became clear that one could also exploit the highly conserved features of DNA repair proteins to clone their genes. Structural homologies were exploited to clone new DNA repair genes based on a knowledge of their probable amino acid sequence. Functional homologies were exploited by replacing DNA repair functions from one organism with those from another. In this way, DNA repair genes could be cloned based on their ability to suppress DNA damage sensitivity in heterologous cells. The first example of a DNA repair protein functioning in heterologous cells dates back over 20 years, to the experiments of Tanaka et al.[212,213] They introduced the purified bacteriophage T4 UV-endonuclease protein (as it was then called) into permeabilized Xeroderma Pigmentosum (XP) skin cells, that is, human cells that are unable to initiate nucleotide excision repair (NER) of UV-induced DNA damage. Introduction of the bacteriophage DNA repair enzyme restored the ability of these human cells to repair UV-damaged DNA. It was not until 10 years later that the cloned T4 UV-endonuclease gene was stably introduced into XP cells, producing cells that could resist the *in vivo* cytotoxic effects of UV light .[122,223]

Thus, interspecies functional complementation made it possible to clone similar DNA repair genes from several different organisms, by their ability to functionally complement a DNA repair deficiency in one particular organism, often *E. coli*. It is now clear that many DNA repair genes can function very efficiently in such heterologous cells. Heterologous expression across the evolutionary spectrum works most effectively with DNA repair proteins that are not required to complex with other proteins in order to function. However, proteins that must form complexes in order to participate in DNA repair may operate in closely related heterologous cells, provided the domains required for protein-protein interaction are sufficiently conserved. In some instances, expression of a DNA repair protein in heterologous cells can actually disrupt the endogenous pathway, generating a DNA repair deficiency. In this review, we give many examples where the expression of DNA repair genes in heterologous cells has been an important and powerful tool in unmasking interesting biological phenomena. To review this particular aspect of DNA repair we must also incorporate a brief general review of some aspects of DNA repair; for a much more detailed general review we recommend the excellent text *DNA Repair and Mutagenesis*, by Friedberg, Walker, and Siede (1995). Inasmuch as the cloning of the human nucleotide excision repair genes (cloned, for the most part, by their heterologous expression in rodent cells) has been the subject of numerous excellent reviews in the last few years,[19,20,37,85,131,184,214] our review concentrates on the direct reversal and base excision repair (BER) pathways and briefly addresses the DNA mismatch repair pathway.

II. PHOTOLYASES

The direct reversal of DNA damage restores proper DNA structure without requiring the excision of damaged bases or nucleotides, followed by DNA resynthesis and ligation (Figure 1). Photolyases, also known as photoreactivating (PR) enzymes, utilize visible light energy to catalyze the monomerization of *cis-syn* cyclobutane pyrimidine dimers (CPDs) and (6–4) pyrimidone photoproducts (Figure 2); both of these lesions are produced in DNA exposed to UV light.[66,104,128,185,188,218,220] In addition to their role in light-dependent repair of UV-damaged DNA, photolyases from

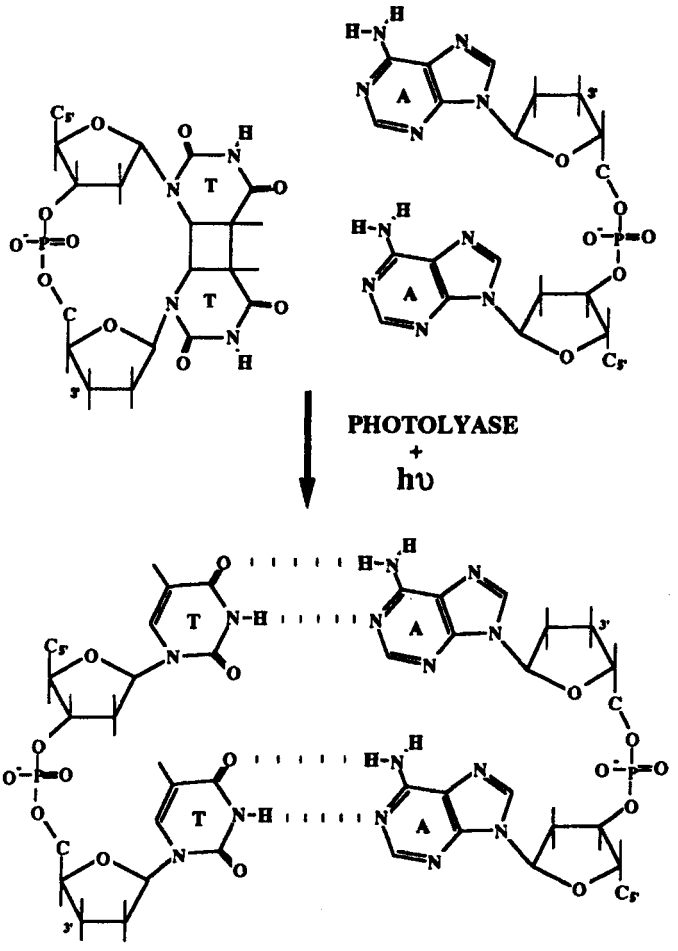

FIGURE 1. The reversal of cyclobutane pyrimidine dimers (CPDs) by photolyase. Photolyase specifically recognizes CPDs in double-stranded DNA, binds, absorbs light, and uses the captured light energy to catalyze CPD monomerization to generate two adjacent, undamaged pyrimidines, in this case two thymines.

some species have been shown to facilitate the repair of UV-damaged DNA by NER.[84,190,245,246] Most of the known photolyases specifically repair CPDs, but a recently discovered insect photolyase specifically repairs (6–4) photoproducts (Figure 2). For the purposes of this review, unless otherwise indicated, photolyase refers to the CPD photolyases, and Table 1 summarizes the features of the various photolyases discussed in this section.

Photolyases catalyze the direct reversal of CPDs by generating two adjacent undamaged pyrimidines. These enzymes require light-absorbing cofactors in order to function, and they contain two noncovalently linked chromophores. One chromophore captures a photon of light and then transfers the excitation energy to the other chromophore, which in turn initiates a series of electron transfers that result in monomerization of the cyclobutane pyrimidine dimer.[66,104,185,188]

Photoreactivation of UV-induced CPDs has long been known to operate in *Escherichia coli* and in the budding yeast *Saccharomyces cerevisiae*. The photolyase genes from both organisms have been cloned

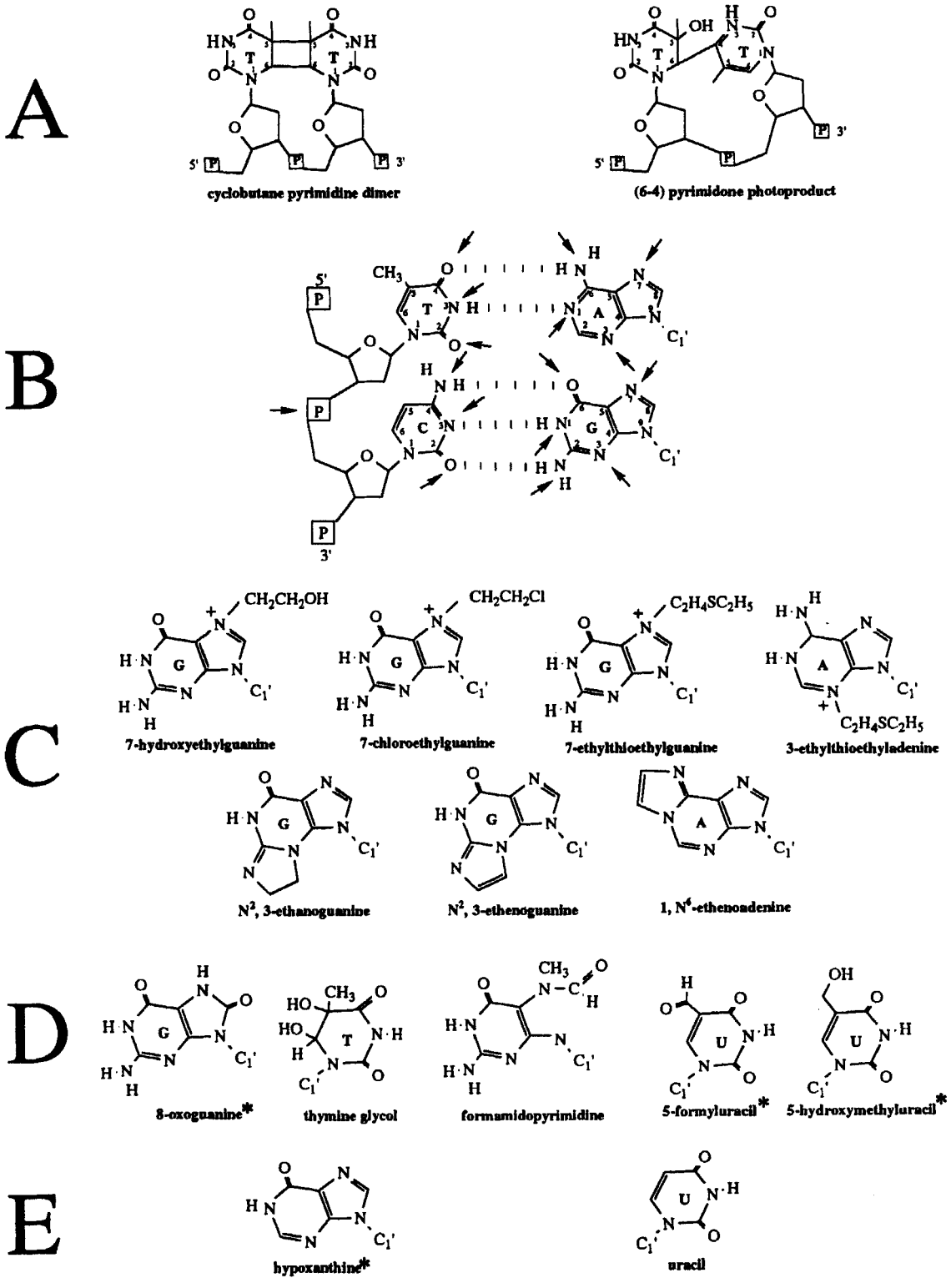

FIGURE 2. Chemical structures of DNA lesions. (A) Lesions produced by ultraviolet light. (B) Arrows indicate positions on DNA modified by simple alkylating agents. (C) Additional *in vitro* substrates for 3MeA DNA glycosylases. (D) Lesions produced by oxidative damage. Asterisks indicate bases that are substrates for 3MeA DNA glycosylase. (E) Deamination products of normal bases in DNA.

Table 1: **Summary of cloned photolyses from various species**

	Species	Common name	Chromophores	Class based on sequence similarity	Expression confers UV resistance in *E. coli*
CPD Photolyase	*E. coli*		$FADH^-$/MTHF	Class I	+
	S. cerevisiae		$FADH^-$/MTHF	Class I	+
	Streptomyces griseus		$FADH^-$/8-HDF	Class I	+
	Anacystis nidulans		$FADH^-$/8-HDF	Class I	+
	Sinabis alba	mustard	$FADH^-$/MTHF	Class I (blue-light photoreceptor)	-
	Arabidopsis thaliana		$FADH^-$/MTHF	Class I (blue-light photoreceptor)	-
	Carrassius auratus	goldfish		Class II	+
	Drosophila melanogaster	fruitfly		Class II	+
	Oryzias latipes	killifish		Class II	+
	Potorous tridactylis	rat kangaroo		Class II	+
	Monodelphis domestica	South American opossum		Class II	+
	Methanobacterium thermoautotrophicum			Class II	not determined
6-4 photolyase	*D. melanogaster*	fruitfly		Class I (to a lesser extent Class II)	+
	Human			Class I (to a lesser extent Class II)	not determined

(the *E. coli phr* gene and the *S. cerevisiae PHR1* gene); photolyase-deficient mutants of both cell types are unable to utilize light energy to stimulate DNA repair. Consequently, these mutants are sensitive to the cytotoxic effects of UV light.[7,187,195] Photolyase genes were cloned from a number of organisms based on their amino acid sequence homology with known PR enzymes, for example, the *Streptomyces griseus*, *Anacystis nidulans*, and *Sinapis alba* (mustard) photolyase genes were cloned based on their similarity to the *E. coli* and *S. cerevisiae* enzymes.[107,211] Clearly, this approach only allows the identification of photolyases with similar structures. Despite the demonstration in 1985 that the UV-sensitive phenotype of *E. coli phr–* cells can be reversed by expression of the *S. cerevisiae PHR1* gene[189] and vice versa,[116] it was not until recently that such interspecies functional complementation was exploited for the identification of what turned out to be a new class of DNA photolyases. Functional complementation of *E. coli phr–* mutants captured photolyase genes from *Carassius auratus* (goldfish),[248] *Drosophila melanogaster* (fruitfly),[249] and *Oryzias latipes* (killifish).[249] However, it turned out that although the amino acid sequence of the photolyases from these three organisms resembled each other, they did not resemble any of the previously characterized photolyases. Clearly, cloning strategies using sequence conservation alone would never have uncovered this new class of PR enzymes. The sequence similarity of the goldfish, fruitfly, and killifish photolyases was used in turn to clone homologous genes from *Patters tridactylis* (rat kangaroo),[249] *Monodelphis domestica* (South American Opossum),[101] and *Methanobacterium thermoautotrophicum* (an archaebacterium).[249] Although the amino acid sequence of the rat kangaroo and South American opossum photolyases bears no similarity to that of *E. coli*, the expression of both these genes in *E. coli phr–* cells, like that of their homologs, reverses the UV-sensitive phenotype.[101,249]

The expression of heterologous photolyases in *E. coli* revealed that photolyases with completely different structures could suppress the UV sensitivity of *phr⁻* mutants; the only requirement for suppression was that they catalyze the same reaction. Indeed, expression of heterologous photolyases in *E. coli* has revealed interesting features about the chromophore requirements of photolyases. The mechanisms by which photolyase cofactors capture and utilize visible light to initiate the cleavage of cyclobutane pyrimidine dimers fall into two distinct classes, known as the folate class and the deazaflavin class.[188] All photolyases contain flavin adenine dinucleotide ($FADH^-$) as one cofactor; the second cofactor is either the folate methylenetetrahydrofolate (MTHF) or the deazaflavin 8-hydroxy-5-deazariboflavin (8-HDF). A photon of light is captured by the folate or deazaflavin chromophores and the excitation energy is then transferred to $FADH^-$ that initiates the series of electron transfers that ultimately result in monomerization of the cyclobutane pyrimidine dimer.[66,104,185,188] The presence of the folate and the deazaflavin chromophores can be distinguished by their action/absorption spectra. *E. coli* and *S. cerevisiae* photolyases fall into the folate class, whereas *A. nidulans* and *S. griseus* fall into the deazaflavin class.[93,134] However, expression of the *A. nidulans* and *S. griseus* photolyases in *E. coli phr⁻* mutants efficiently restores photoreactivation[107,211] despite the fact that they normally use different chromophores in their natural environment. Moreover, analysis of the absorption/action spectra of the *A. nidulans* enzyme when expressed in *E. coli* suggests that it uses cofactors similar to the *E. coli* enzyme, that is, $FADH^-$ and MTHF.[211] Thus, heterologous expression revealed that the activity of the *A. nidulans* photolyase

may not strictly depend on using 8-HDF as a cofactor, and that this enzyme may be able to interchange 8-HDF and MTHF. Similarly, the deazaflavin class photolyase from *Halobacterium halobium* functionally complements *E. coli phr*⁻ mutants, presumably in the same manner as the *A. nidulans* enzyme.[210]

In contrast, it has been reported that putative photolyase genes from the plants *Arabidopsis thaliana* and *Sinapis alba* were unable to produce functional photolyase in *E. coli phr*⁻ mutants despite a high degree of amino acid sequence similarity to the *E. coli* PHR1 enzyme and despite the association of the $FADH^-$ and MTHF chromophores.[134] These heterologous expression experiments lend weight to the possibility that, in their natural environment, the plant photolyase-like proteins may primarily act as blue-light photoreceptors that initiate signal transduction in plants via a novel mechanism, namely, electron transfer. Indeed, the *A. thaliana* gene was originally identified as being required for blue-light responsiveness with respect to hypocotyl growth,[1] and it was only later presumed to be a putative photolyase because of its structural similarity to this class of enzyme.

Heterologous expression experiments indicate that although *Schizosaccharomyces pombe* cells lack endogenous photolyase activity, they do not appear to lack the chromophores necessary for photolyase function. This was deduced from the observation that expression of the *S. cerevisiae PHR1* gene in *S. pombe* cells produces active photolyase and confers considerable UV-resistance.[250] Presumably, the photolyase cofactors are present because they are normally used for other cellular functions.

In addition to their role in providing light-dependent resistance to UV damage, photolyases from *E. coli* and *S. cerevisiae* provide resistance to UV in the absence of light. This so-called dark repair by photolyases is independent of photoreactivating light but dependent on a functional NER pathway.[84,190,245,246] The *E. coli* photolyase binds CPDs in the absence of light but cannot catalyze their direct reversal. However, photolyase binding appears to facilitate CPD repair by the *E. coli* NER machinery via specific protein-protein interactions.[186] Although heterologous expression of *S. cerevisiae PHR1* in *phr*⁻ *E. coli* provides resistance to UV damage in the presence of light; in the absence of light, the cells actually become more sensitive to UV light.[190] This sensitization is also dependent on a functional NER pathway and presumably reflects the fact that the yeast photolyase cannot interact productively with the *E. coli* NER proteins. Thus, expression of *S. cerevisiae PHR1* enzyme in *phr*⁻ *E. coli* inhibits, rather than facilitates the excision of CPDs.

Finally, a *Drosophila melanogaster* photolyase was recently discovered that can repair pyrimidine pyrimidone (6–4) lesions, where two adjacent pyrimidines are covalently linked between the 6 position of the 5′ pyrimidine ring and the 4 position of the 3′ pyrimidine ring; not surprisingly, this lesion introduces a major distortion in the double helical structure of DNA (Figure 2). A *Drosophila* cDNA encoding the (6–4) photolyase was successfully cloned by its heterologous expression in *E. coli* in a rather ingenious way.[219] *E. coli* were engineered to overexpress a CPD photolyase such that the majority of UV-induced toxicity was due to unrepaired (6–4) lesions, which are produced much less frequently than CPDs. Thus, a selection for insect cDNAs that rescued these bacteria from UV-induced cell death was biased toward the isolation of a cDNA that produced (6–4) photolyase activity. Interestingly, the amino acid sequence of the *D. melanogaster* (6–4) photolyase bears extensive similarity to the class I photolyases (the microbial photolyases and plant blue-light

photoreceptors) and is less similar to the class II photolyases (photolyases from higher eukaryotes) (Table 1). Further, the regions thought to be involved in $FADH^-$ binding to the *E. coli* CPD photolyase are well conserved in the (6–4) photolyase, suggesting that this enzyme may receive and convert light energy by a mechanism similar to that employed by the CPD photolyases and blue-light photoreceptors. The sequence of the *D. melanogaster* (6–4) photolyase was in turn used to identify a human cDNA encoding a homologous protein.[219]

III. DNA REPAIR METHYLTRANSFERASES

DNA repair methyltransferases (MTases), like the photolyases, directly reverse DNA damage, but in this case they reverse DNA alkylation damage. The MTases, as their name suggests, transfer methyl groups (as well as larger alkyl groups) from certain oxygens in DNA to an active site cysteine residue in the MTase protein itself. More specifically, methyl groups can be transferred from the O^6 position of guanine (O^6MeG), from the O^4 position of thymine (O^4MeT) and from methylphosphotriesters (MePTs) on the sugar-phosphate backbone (Figure 3). These methyl transfer reactions are quite extraordinary because they irreversibly inactivate the MTase; the DNA repair MTases have therefore been termed suicide enzymes.[66,126,159,177] DNA repair MTases were first discovered in *E. coli*, and genetic and biochemical analysis of *O*-alkyl repair in *E. coli* led to the following conclusions:[127,129,162] O^6MeG and O^4MeT DNA lesions cause transition mutations because they have the potential to mispair with thymine and guanine (respectively) during DNA replication. The methyl groups from these bases can be transferred to an active site cysteine in either of two MTases, namely, Ada and Ogt. Ada, but not Ogt, has a second active site cysteine for the transfer of methyl groups from MePT lesions, and methylation at this active site converts the Ada protein into a potent transcriptional activator of four genes, namely, *ada*, *alkA*, *alkB*, and *aidB*. In other words the repair of alkylated DNA by the Ada protein acts both as a sensor of DNA alkylation damage and as an activator for the expression of its own gene, the *alkA* 3-methyladenine (3MeA) DNA glycosylase gene (see later) and two other genes whose precise functions are not yet understood. The induced transcription of these four genes by the Ada protein confers tremendous alkylation resistance on *E. coli* and was called the adaptive response to alkylating agents.[127,178]

The dual role of the Ada MTase in repair and in regulating genes whose products protect *E. coli* against alkylation-induced cytotoxicity made it hard to determine whether O^6MeG/O^4MeT can be cytotoxic as well as mutagenic. However, because Ogt only repairs O^6MeG and O^4MeT and is not known to regulate any genes, characterization of *ogt* mutants revealed that O^6MeG and/or O^4MeT can indeed cause *E. coli* cell death.[169] Perhaps this is not unexpected, because some fraction of the transition mutations will inevitably inactivate essential genes. However, lethal mutations probably do not account for all of the cytotoxicity induced by these lesions, and other ways that O-alkylated bases cause cell death are just beginning to be understood. Some lesions may inhibit DNA replication, causing cell death;[53] however, it is obvious that not all O^6MeGs and O^4MeTs permanently inhibit replication, because base mispairing at some of these lesions produces viable cells bearing transition mutations. Another pathway for O^6MeG/O^4MeT-mediated cell death appears to involve the postreplicative DNA mismatch repair pathway that is initiated in *E. coli* by MutS, MutH, and MutL proteins (see below).[23,95,98,99]

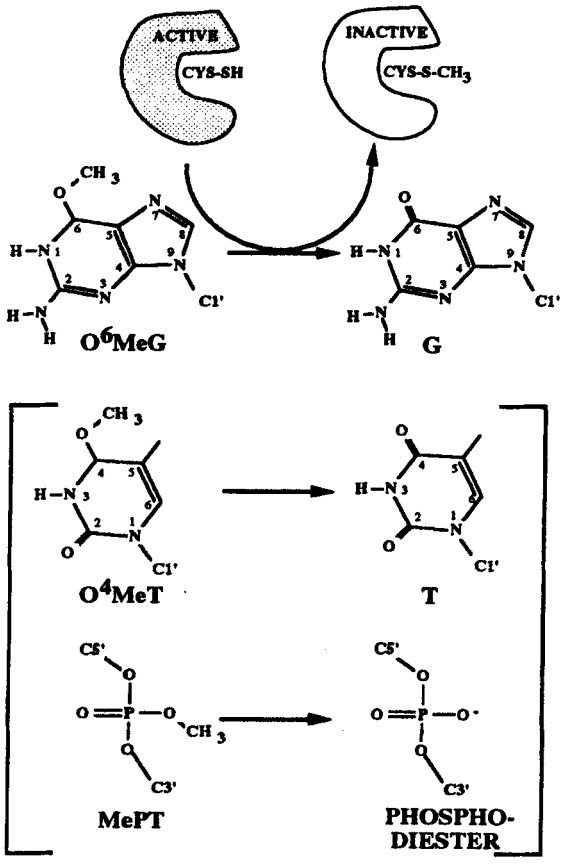

FIGURE 3. The transfer of methyl groups from DNA by DNA repair MTases. O^6MeG, O^4MeT, and MePT (S-siastereomer only) lesions in double-stranded DNA are recognized by MTases and the aberrant methyl group is transferred to a cysteine residue in the active site of the MTase protein. Methyl transfer inactiviates the MTase and so these DNA repair proteins have been called suicide enzymes.

Futile rounds of mismatch repair on the newly synthesized DNA strand are believed to occur at some O^6MeG (and perhaps O^4MeT) lesions that have gone through the replication fork; the repair may be futile because pairing of O^6MeG/O^4MeT with any base produces an apparent mismatch. Futile rounds of unproductive mismatch repair presumably prevents cells from completing a proper cell cycle. Indeed, it was shown recently that the *E. coli* and human mismatch binding proteins (MutS and the hMutSα heterodimer, respectively) bind O^6MeG containing duplex DNA, supporting the proposed role of mismatch repair in alkylation-induced killing.[54,168]

Heterologous expression of a DNA repair MTase was used to determine whether O^6MeG and O^4MeT have biological consequences in human and rodent cells. The ability of these lesions to cause mutation in *E. coli* depends on how they are perceived by the *E. coli* DNA replication machinery. It therefore follows that the mutagenicity of O^6MeG and O^4MeT in other organisms must depend on how the DNA replication complex in those organisms reacts to this kind of DNA template damage. In the 1980s, isogenic

cell lines with and without MTase activity were unavailable, and the human MTase had not yet been cloned. The generation of isogenic mammalian cells lines differing only in their expression of a DNA repair MTase was therefore first accomplished by introducing the *E. coli ada* gene into cultured human and rodent cells lacking endogenous MTase activity.[25–27,88,100,180,227,232] (MTase-deficient mammalian cell lines are referred to as Mer⁻ or Mex⁻.) The *ada* gene was cloned into various mammalian expression vectors to achieve high-level expression in Mer⁻ HeLa, CHO, and V79 cells. The resulting phenotype was striking. Acquisition of Ada MTase activity conferred tremendous resistance to the mutagenic, cytotoxic, and chromosome-damaging effects of a variety of different alkylating agents, indicating that the O-alkyl lesions repaired by Ada do indeed produce biological consequences in mammalian cells. As mentioned above, Ada has two active sites, one for O^6MeG/O^4MeT repair and a second for MePT repair. Although MePT repair is required for Ada's gene regulatory function, it was not clear whether unrepaired MePTs could contribute to alkylation-induced cytotoxicity. Further heterologous expression experiments answered this question for mammalian cells. The expression of active Ada protein fragments, containing one or other active site, demonstrated that the repair of MePTs conferred no alkylation resistance, whereas the repair of O^6MeG/O^4MeT conferred full resistance to alkylation-induced mutation, cell death, and chromosome damage.[25,77] Support for this conclusion came from the fact that expression of the *E. coli* Ogt MTase (which only repairs O^6MeG/O^4MeT) in murine cells conferred resistance to alkylation-induced killing and mutation.[226] Moreover, when expression of the cloned human *MGMT* MTase cDNA was later achieved in Mer⁻ mammalian cells, it was found to confer the same alkylation-resistant phenotype as Ada and Ogt;[82,238,239] because the mammalian MTases do not repair MePTs [110] and because they are extremely inefficient at repairing O^4MeTs,[253] it seems likely that O^6MeG is primarily responsible for mutation, cell death, and chromosome damage in alkylated mammalian cells.

The striking observation that an *E. coli* DNA alkylation repair protein could rescue mammalian cells from alkylation-induced cell death led to the idea that repair proteins from mammals (or other organisms) might be able to rescue *E. coli* cells from alkylation-induced cell death. This turned out to be a powerful cloning strategy, and a large number of DNA alkylation repair genes have now been cloned (Table 2). Among these are the human MTase cDNA (*MGMT*), a *B. subtilis* MTase gene (*dat1*), the *S. typhimurium* MTase gene (ada_{ST}), and the *S. cerevisiae* MTase gene (*MGT1*), each cloned by their ability to rescue MTase-deficient *ada⁻ ogt⁻ E. coli* from alkylation-induced cell death.[76,108,215,240] Similarly, the heterologous expression of human MTase in *mgt1⁻ S. cerevisiae* protects the yeast cells from alkylation-induced mutation and cell death. [241,242] In addition, the human MTase reduced the *S. cerevisiae* spontaneous mutation rate, suggesting that endogenous metabolites alkylate the yeast genome to produce O-alkylated bases capable of causing mutation and capable of being repaired by the human MTase.[241,242] The identity of these endogenous metabolites remains to be established.

Heterologous expression experiments have revealed that the affinity of a DNA repair protein for its substrates may have a profound biological effect, and that a poor affinity can lead to rather unexpected biological consequences. *In vitro* biochemical characterization showed that various MTases have very different affinities for O^6MeG and O^4MeT, but perhaps the most striking observation was that the rate constant of the mam-

Table 2: **DNA repair genes functionally expressed in *E. coli* host**

Protein	Source	*E. coli* host genotype	Phenotype conferred by heterologous expression	Reference
CPD Photolyase	*Saccharomyces cerevisiae*	*phr*	UV resistance	(189)
	*Carassius auratus**	*phr*	UV resistance	(248)
	*Drosophila melanogaster**	*phr*	UV resistance	(249)
	*Oryzias latipes**	*phr*	UV resistance	(249)
	Monodelphis domestica	*phr*	UV resistance	(249)
	Patters tridactylis	*phr*	UV resistance	(101)
	Methanobacterium thermoautotrophicum	*phr*	UV resistance	
	Halobacterium halobium	*phr*	UV resistance	(210)
	Anacystis nidulans	*phr*	UV resistance	(211)
(6-4) Photolyase	*Drosophila melanogaster**	*phr* overexpressing	UV resistance	(220)
DNA Methyltransferase	Human*	*ada ogt*	Alkylation resistance	(215)
	*Bacillus subtilis**	*ada*	Alkylation resistance	(108)
	*S. typhimurium**	*ada ogt*	Alkylation resistance	(76)
	*S. cerevisiae**	*ada ogt*	Alkylation resistance	(240)
	mouse	*ada ogt*	Alkylation resistance	(103)
3-methyladenine DNA glycosylase	*S. cerevisiae**	*alkA tag*	Alkylation resistance	(8, 33)
	Human*	*alkA tag*	Alkylation resistance	(30, 155, 179, 215)
	rat*	*alkA tag*	Alkylation resistance	(154)
	*Arabidopsis thaliana**	*alkA tag*	Alkylation resistance	(192)
	*Schizosaccharomyces pombe**	*alkA tag*	Alkylation resistance	(141)
	B. subtilis	*alkA tag*	Alkylation resistance	(148)
	mouse	*alkA tag*	Alkylation resistance	(61)

Table 2: continued

8-oxoguanine DNA glycosylase	*S. cerevisiae* *	*fpg mutY*	Reduced spontaneous mutagenesis	(224)
	L. lactis	*fpg*	Reduced spontaneous mutagenesis	(58)
	D. melanogaster ribosomal protein S3	*fpg*	Reduced spontaneous mutagenesis	
	L. lactis	*mutY*	Reduced spontaneous mutagenesis	(58)
	human*	*fpg mutY*	Reduced spontaneous mutagenesis	(224)
8-oxo-dGTPase	human	*mutT*	Reduced spontaneous mutagenesis	(174)
dUTPase	human*	*dut*^{ts} *xthA*	Viable at restrictive temperature	(140)
Uracil DNA glycosylase				
	human	*ung*	Reduced spontaneous mutagenesis Restricted for growth of phage with uracil-containing DNA	(156)
AP endonuclease	*S. cervisiae*	*xthA nfo*	Alkylation, H_2O_2, tBH and bleomycin resistance	(166)
	D. melanogaster	*xthA nfo*	Alkylation, H_2O_2, tBH, MMC and bleomycin resistance	(72)

Table 2: continued

	human	*xthA nfo*	Alkylation and γ-ray resistance Reduced spontaneous mutation	(32, 49, 171)
β polymerase	rat	*polA*	Alkylation resistance Growth on rich media	(205, 206)
DNA ligase	human	*cdc9^{ts}* (DNA ligase I mutant)	Growth at restrictive temperature	(109)
MutS homologues	*S. pneumoniae*	*wildtype*	Increased spontaneous mutation	(163)
	human	*wildtype*	Increased spontaneous mutation	(64)

*= Heterologous expression in *E. coli* was a successful cloning strategy

malian MTase for the removal of O^4MeT is up to several thousandfold lower than that for O^6MeG.[71,253] It is well established that O^6MeG drives G:C to A:T, and that O^4MeT drives A:T to G:C transition mutations in *E. coli*.[129,162] Thus, the ability of a MTase to prevent alkylating agents from inducing these two specific mutational events in *E. coli* should reflect their ability to repair O^6MeG and O^4MeT *in vivo*. Five different DNA repair MTases were individually expressed in *ada⁻ ogt⁻ E. coli*, and their ability to prevent alkylation-induced G:C to A:T and A:T to G:C mutations measured.[181] It was possible to specifically monitor each kind of transition mutation using two *E. coli* strains containing different *lacZ–* alleles, one that only reverts to Lac$^+$ via a G:C to A:T transition, and the other via an A:T to G:C transition.[45] The *E. coli* Ada and Ogt MTases, the *S. cerevisiae* MGT1 MTase, and the human and mouse MGMT MTases were each able to prevent alkylation-induced G:C to A:T mutations, reflecting their efficient repair of O^6MeG *in vivo*. However, only Ada, Ogt, and MGT1 (i.e., the microbial MTases) could prevent alkylation-induced A:T to G:C mutations, presumably because they efficiently repair O^4MeT *in vivo*. Most unexpectedly, both of the mammalian MTases actually sensitized *E. coli* cells to the induction of A:T to G:C transition mutations by alkylating agents. In other words, expressing MTases with poor affinity for O^4MeT was actually more detrimental than expressing no MTase at all. We hypothesized that the mammalian MTases bind O^4MeT lesions and block their repair by another DNA repair pathway, namely, the NER pathway; that is, MGMT expression effectively creates a NER-deficient phenotype for O^4MeT repair. Several lines of experimental evidence strongly support this hypothesis.[86,105,173,181,182,225] These experiments point to the importance of considering how different DNA repair pathways overlap and interact with each other, and point to the importance of obtaining both *in vitro* and *in vivo* data to deduce the relative roles of DNA repair pathways in protecting cells. In these studies, heterologous expression was a powerful tool for addressing the biological relevance of the biochemical differences seen among the DNA repair MTases. Expressing the five proteins in *E. coli* took advantage of bacterial genetics to provide a way to compare MTase function *in vivo* in a single biological setting.

The functional complementation of *ada⁻ ogt⁻ E. coli* by the human MGMT MTase has also been exploited to explore structure function relationships.[36,43] MGMT not only prevents alkylation-induced G:C to A:T mutations in MTase-deficient *E. coli*, it also protects against alkylation-induced cell killing.[215] Seventeen amino acid residues in the MGMT protein were specifically mutated, and the mutant proteins expressed in *ada⁻ ogt⁻ E. coli*. Thirteen of the amino acid changes completely abolished MTase activity as measured biochemically *in vitro*. However, nine of these mutants were able to confer an alkylation-resistant phenotype to these bacterial cells, indicating that the mutant proteins were in fact active *in vivo*. These results indicate that none of the nine residues are absolutely essential for MTase function. Two of the four mutant proteins that did not provide *in vivo* alkylation resistance were shown to be stably expressed in *E. coli*, indicating that the two mutated residues, Y114E and C145A, are truly required for MTase function; indeed, C145 represents the active site cysteine residue. Without this sensitive biological assay employing the expression of human MGMT in *E. coli*, spurious conclusions might have been drawn about the relative importance of these 13 amino acid residues for human MGMT MTase structure function relationships.

That the human MGMT protein can confer an alkylation-resistant phenotype on *E.*

coli was also exploited by Christians and Loeb[36] to isolate mutated but functional versions of this human DNA repair protein. Their aim was to create new versions of MGMT that are better able to provide resistance to the cytotoxic and mutagenic effects of alkylating agents. After selecting for alkylation resistance in *ada⁻ ogt⁻ E. coli* containing randomly mutated MGMT cDNAs, they found mutant MGMT sequences that provide greater alkylation resistance than the wild-type MGMT cDNA, increasing the D_{37} for a simple alkylating agent by fourfold and reducing mutagenesis by 3- to 5-fold. Their goal is to create a "super MTase" that might be useful for a gene therapy approach to protecting cancer patients undergoing alkylating agent chemotherapy.

Several recent studies tried to determine whether the expression of MTase in bone marrow could provide a useful level of extra resistance after exposure to the alkylating agents that are commonly used for the chemotherapeutic treatment of cancer patients. It was previously shown that bone marrow cells (in both mice and humans) express extremely low levels of DNA MTase activity, providing a plausible explanation for the fact that this tissue is extremely sensitive to alkylating agents.[67] Indeed, the extreme alkylation sensitivity of human bone marrow is dose limiting for the treatment of cancer patients with chemotherapeutic alkylating agents, such as the chloronitrosoureas.[194] The heterologous expression of *E. coli* and human MTases in mouse hematopoietic cells was achieved by the *ex vivo* introduction of MTase genes or cDNAs (cloned into retroviral expression vectors) into bone marrow cells.[2,79,137,147,230] The benefits of expressing the Ada MTase in mouse bone marrow has not yet been reported. However, it is quite clear that increasing the expression of the human MGMT MTase in this tissue provides significant protection against the myelosuppressive effects of chloronitrosoureas and, consequently, protects against the lethal effects of these agents.[137,147] A similar protection of human bone marrow may enable the clinical use of higher and more effective doses of chemotherapeutic alkylating agents and may protect this tissue from secondary cancers induced by the alkylating agents. Indeed, the success of the heterologous expression of human MGMT in mouse bone marrow has led to the initiation of Phase I clinical trials to express MGMT in human bone marrow of cancer patients undergoing CNU chemotherapy.

Heterologous expression of the microbial MTases in mammalian bone marrow may prove to be particularly effective. The mammalian MGMT MTases are sensitive to inhibition by O^6-benzylguanine (O^6BG); MGMT readily transfers the benzyl group to its active site cysteine, being thereby inactivated.[158] In fact, O^6BG is currently in clinical trials as an agent for decreasing the resistance of tumor cells to CNUs by inhibiting MTase activity. Surprisingly, the microbial MTases are extremely resistant to O^6BG inhibition[158] and so their expression could protect bone marrow against CNUs during O^6BG administration for sensitizing tumor cells to CNUs. These experiments are currently underway in mice and may ultimately lead to a clinically beneficial use of the heterologous expression of DNA repair proteins.

Bacterial and human MTases have been expressed in transgenic mice.[55–57,150,151,165,229,251,252] The Ada protein was expressed from the zinc-inducible metallothionein promoter, and up to an eightfold increase in MTase levels was observed in the livers of the transgenic animals.[150,151] Increased DNA repair by the Ada MTase rendered these animals resistant to the induction of liver tumors after exposure to nitrosamines. Increased MTase expression has also been targeted to other tissues. The human MGMT MTase was specifically overexpressed in mouse liver and brain from

the transferrin promoter[229] and in thymus, spleen, muscle, and colon from the CD2 promoter.[55–57,251] It has not yet been reported whether increased MGMT in liver and brain protects against alkylation-induced tumorigenesis, but it is quite clear that MGMT expression in the thymus and colon prevents alkylation-induced thymic lymphomas and reduces the induction of putative preneoplastic lesions (aberrant crypt foci) in the colon.[55,252] These data underscore the important role that DNA repair plays in preventing a normal cell from being transformed into a cancer cell after exposure to exogenous DNA-damaging agents. The role that various DNA repair pathways play in protecting against the induction of mutations and cancer by endogenous DNA-damaging agents will ultimately be addressed by studying animals with DNA repair deficiencies, in addition to studying animals with increased DNA repair capabilities. Indeed, mutant mice (and people) lacking a DNA mismatch repair pathway are known to suffer increased incidence of certain kinds of tumors in the absence of any obvious exposure to exogenous DNA-damaging agents.[4,47,64,121] As the number of transgenic mice and human diseases with well-defined mutations in known DNA repair pathways increases, it will become possible to use mammalian genetics to establish which DNA repair pathways are the most influential for protecting against tumorigenesis.

IV. BASE EXCISION REPAIR

Base excision repair (BER) involves the removal of an abnormal or damaged base from double-stranded DNA, generation of a DNA strand break in the vicinity of the resulting abasic site, exonucleolytic digestion at this break site to remove a small number of nucleotides, and the restoration of normal, undamaged, double-stranded DNA by DNA polymerase and DNA ligase (recently reviewed in References 50, 66, 144, 176, and 183). BER is initiated by a DNA glycosylase cleaving the *N*-glycosylic bond linking the unwanted base to deoxyribose in the sugar-phosphate DNA backbone. There exists a wide variety of DNA glycosylases, and the specificity of BER is dictated by the specificity of the initiating DNA glycosylase. The sugar-phosphate backbone at abasic sites is cleaved either by an apurinic/apyrimidinic (AP) endonuclease that cleaves by hydrolysis to leave an undamaged 3′-hydroxyl terminus and a 5′-deoxyribose-5-phosphate end or by an AP lyase that cleaves by β-elimination to leave an undamaged 5′-phosphate terminus and a 3′-α,β-unsaturated aldehyde (Figure 4). A variety of AP endonucleases and AP lyases exists in many organisms. AP lyase activity is always associated with a DNA glycosylase activity, although not all DNA glycosylases have associated AP lyase activity.[48,66] Several different DNA polymerases exist in *E. coli* and in mammalian cells. In *E. coli*, DNA polymerases I is believed to participate in BER,[66] and it was recently shown in mammalian cells that DNA polymerase β is responsible for virtually all BER synthesis.[202] Finally, DNA ligases operate to seal the 3′-hydroxyl end of the newly synthesized DNA patch after it reaches the 5′-phosphate end of the adjacent strand; there is just one major DNA ligase in *E. coli*, but several different DNA ligases in mammalian cells.[66]

Although BER is a multistep pathway, there does not appear to be a requirement for the formation of a BER multienzyme complex, as each enzymatic step can be carried out in the absence of other proteins, at least *in vitro*. [50] The functional independence of the BER enzymes makes them ideal candidates for the functional complementation of BER defects in heterologous cells. In this section we review studies that have exploited the expression of DNA glycosylases, AP endonucleases, DNA polymerases, and DNA ligases in heterologous cells.

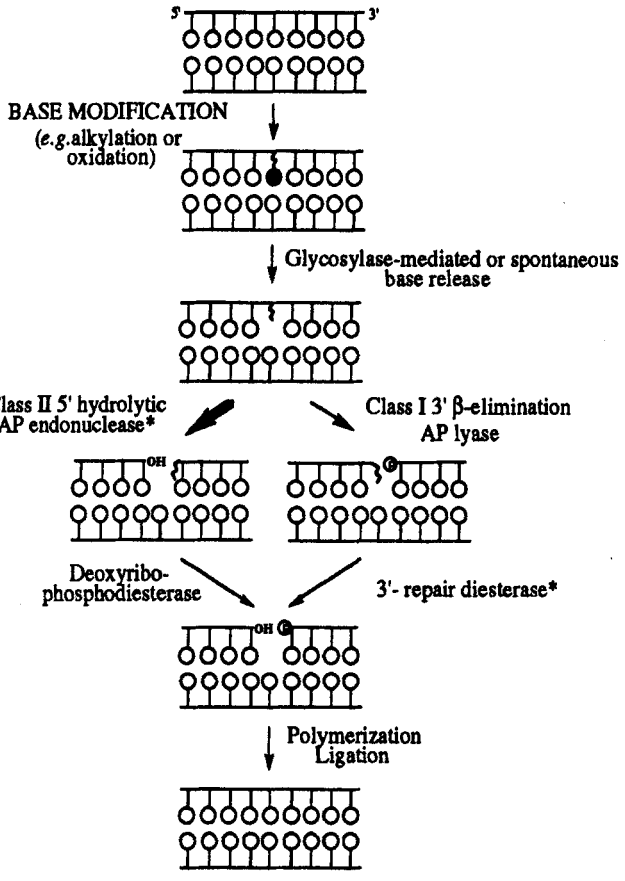

FIGURE 4. The base excision repair (BER) pathway. BER is initiated by cleavage of the glycosylic bond either spontaneously or by the activity of DNA glycosylases resulting in an abasic site. The sugar-phosphate backbone at abasic sites is cleaved either by an apurinic/apyrimidinic (AP) endonuclease that cleaves by hydrolysis to leave an undamaged 3′-hydroxyl terminus and a 5′-deoxyribose-5-phosphate end or by an AP lyase that cleaves by β-elimination to leave an undamaged 5′-phosphate terminus and a 3′-α,β-unsaturated aldehyde. Further processing of both these products by either deoxyribophosphodiesterase or 3′-repair diesterase enables repair synthesis to occur. Finally, DNA ligases operate to seal the 3′-hydroxyl end of the newly synthesized DNA patch after it reaches the 5′-phosphate end of the adjacent strand.

*3′-repair diesterase activity is associated with several AP endonucleases

A. DNA Repair Glycosylases

3-Methyladenine (3MeA) DNA glycosylases were first discovered in *E. coli* and have been best studied in this organism.[119,120,125,170,217] Two *E. coli* 3MeA DNA glycosylases have been well characterized. They were originally differentiated biochemically as 20- and 27-kDa proteins[149,175,217] and later differentiated genetically as being encoded by the constitutively expressed *tag* gene and the alkylation-inducible *alkA* gene, respectively.[149,203] As described previously, the methylated Ada MTase acts as a power-

ful transcriptional activator for the *alkA* gene so that cells experiencing DNA alkylation damage adapt to express high levels of the AlkA 3MeA DNA glycosylase.[63,97] 3MeA is thought to block DNA replication in *E. coli,* thus causing cell death, as scored by lack of colony-forming ability.[17,117] The action of Tag and AlkA thus rescues bacteria from alkylation-induced cytotoxicity. Recently, it became clear that AlkA has a much broader substrate specificity than Tag. Both AlkA and Tag can remove 3-methylguanine (3MeG), 3-ethyladenine, and 3-ethylthioethyladenine from alkylated DNA, in addition to 3MeA.[12,13,149,170,175,217] However, AlkA, but not Tag, has also been shown to remove the following bases from DNA: 7-methylguanine, 7-methyladenine, 7-ethylguanine, 7-ethyladenine, 3-ethyl-guanine, 7-ethylthioethylguanine, O^2-methylthymine, O^2-methylcytosine, 5-formyluracil, 5-hydroxymethyluracil, and 1, N^6-ethenoadenine[11,75,138,170,217] In addition, AlkA is known to remove 7-chloroethylguanine, 7-hydroxyethylguanine, N^2,3-ethanoguanine, N^2,3-ethenoguanine, and hypoxanthine, but Tag has not yet been tested for these substrates.[29,73,136,193] As will become evident below, it appears that the eukaryotic 3MeA DNA glycosylases are more similar to AlkA than Tag, in that they display a similarly broad substrate specificity.

Because DNA glycosylases do not have to complex with other proteins in order to function, it seemed plausible that the expression of any 3MeA DNA glycosylase in *alkA⁻ tag⁻ E. coli* would relieve their extreme alkylation-sensitive phenotype. This turned out to be a reasonable supposition, and the functional complementation of *alkA⁻ tag⁻ E. coli* was used to clone a number of eukaryotic 3MeA DNA glycosylase genes (Table 2). These include the *S. cerevisiae MAG* gene,[8,33] the human *AAG/MPG/ANPG* cDNA,[30,155,179,215] the rat *Apdg* cDNA, the *Arabidopsis thaliana ATMAG* cDNA,[192] and the *Schizosaccharomyces pombe Mag1* cDNA.[141] The *Bacillus subtilis alkA* gene and the mouse *Aag* 3MeA DNA glycosylase cDNA (each cloned by other means) were also shown to rescue the alkylation-sensitive phenotype of *alkA⁻ tag⁻ E. coli.*[60,148]

Several eukaryotic 3MeA DNA glycosylases were cloned by virtue of their ability to rescue *alkA⁻ tag⁻ E. coli* from killing by the methylating agent methyl methanesulfonate (MMS). Whereas most of the clones conferred substantial MMS-resistance, it is worth mentioning that for the human *AAG* and *S. pombe Mag1* cDNAs, MMS-resistance conferred by the original cDNA clones was exceedingly modest.[141,179] However, this modest phenotype was still enough to allow isolation of the clones from a library of many thousands of cDNAs, underscoring the power of this approach for cloning new DNA repair genes.

Although all the aforementioned eukaryotic 3MeA DNA glycosylases confer resistance to methylating agents, some also confer resistance to other kinds of alkylating agents, but with some interesting differences. Compared with wild type, *alkA⁻ tag⁻ E. coli* are extremely sensitive to methylating, ethylating, and propylating agents, and from this we infer that together these glycosylases remove all three kinds of damage.[141,155,198] The *S. pombe* glycosylase also protects against all three agents;[141] however, the mouse glycosylase only protects against methylating agents, and the human and *S. cerevisiae* glycosylases only protect against methylating and propylating agents.[61,141,198] It is hard to envision how a DNA glycosylase could recognize methyl and propyl lesions but fail to recognize ethyl lesions. However, because all the glycosylases were compared in the same *E. coli* strain, it seems likely that these results reflect real differences in *in vivo* substrate specificities between these enzymes. A structural comparison of each active site, which so far has only been solved

for the AlkA 3MeA DNA glycosylase,[115,244] may ultimately explain these differences.

The *S. cerevisiae* and human 3MeA DNA glycosylases were both shown to protect *alkA⁻ tag⁻ E. coli* from killing by chloroethylnitrosourea (CNU), a compound, as mentioned previously, commonly used for cancer chemotherapy.[132,135] These heterologous expression experiments were the first to compare the CNU sensitivity of isogenic cells differing only in glycosylase expression, providing direct evidence that BER, along with MTase, could participate in the removal of CNU-induced cytotoxic DNA damage.[14,21,62,70] These conclusions, drawn from expressing the eukaryotic glycosylases in *E. coli*, were confirmed recently. Mouse embryonic stem cells bearing homozygous null *Aag* alleles were generated, and comparison with wild-type ES cells shows that BER, initiated by the *Aag* glycosylase, provides mammalian cells with substantial resistance to CNU-induced cell killing and chromosome damage.[60] It therefore appears that at least two DNA repair pathways can protect against CNU-induced cytotoxicity, namely, BER and DNA repair MTase.[14,21,62,70]

Prior to the isolation of *Aag* null mouse cells, several groups tested the effects of overexpressing the Tag, AlkA, rat, and human 3MeA DNA glycosylases in the heterologous Chinese hamster V79 and CHO mammalian cells (Table 3). These studies produced contradictory results and conclusions: overexpression sometimes protected cells against the toxic effects of alkylating agents,[74,106] sometimes had no effect,[22,87] and sometimes even sensitized the cells.[42] The possible reasons for these seemingly contradictory results are worthy of discussion. Unlike photolyase and MTase, which directly reverse DNA damage, the action of a DNA glycosylase actually produces another form of DNA damage, namely, an abasic site. Further, the DNA glycosylase catalyzes just one of the five steps required for the completion of BER, and the rate-limiting step may differ between cell types depending on the relative levels of the BER enzymes. Thus, overexpression of 3MeA DNA glycosylase in cells where the endogenous glycosylase is limiting may be expected to provide alkylation resistance, but where the endogenous glycosylase is not limiting, extra glycosylase activity may not add extra protection. Moreover, in cell lines where AP endonuclease is limiting, the generation of excess abasic sites by an overexpressed glycosylase, may actually sensitize cells to alkylating agents. Finally, DNA glycosylase overexpression in cells where neither glycosylase nor AP endonuclease is limiting may simply have no effect on alkylation resistance. Because of these complexities, the recently developed *Aag* null mouse cells now provide a powerful tool (analogous to *alkA⁻ tag⁻ E. coli*) for exploring the roles of 3MeA DNA glycosylases as they function *in vivo* in mammalian cells.

These studies all relate to the role of 3MeA DNA glycosylases in the repair of DNA damage induced by exogenous agents. However, it appears that some 3MeA DNA glycosylases also repair DNA damage produced by endogenous agents.[242] This was revealed by dramatic increases in spontaneous mutation rates when BER enzymes become grossly imbalanced by overexpressing the MAG glycosylase in yeast cells lacking the major AP endonuclease, namely, APN1.[91,92] Previous experiments showed that *apn1* mutants display an increased spontaneous mutation rate compared with wild-type cells, presumably due to replication past unrepaired abasic sites.[167] However, this increase in spontaneous mutation is greatly magnified by the expression of high levels of the MAG glycosylase, presumably because MAG removes DNA bases that were damaged by endogenous metabolites.[242] The endogenous DNA damage recognized by

Table 3: **DNA repair genes functionally expressed in eukaryotic cells**

Protein	Source, gene or cDNA	Host species	Host genotype or phenotype	Phenotype conferred by heterologous expression	Reference
CPD Photolyase	*E. coli phr*	*S. cerevisiae*	*phr*	UV resistance	(116)
DNA MTase	*E. coli ada*	human	Mer$^-$	Alkylation resistance	(88, 180, 227)
	E. coli ada	hamster	Mer$^-$	Alkylation resistance	(25-27, 77, 100, 232)
	Human *MGMT*	*S. cerevisiae*	*mgt1*	Alkylation resistance	(241, 242)
	E. coli ada	mouse	*wildtype*	Alkylation resistance	(79, 90)
	human *MGMT*	mouse	*wildtype*	Alkylation resistance	(2, 147, 230)
	human *MGMT*	hamster	*wildtype*, NER$^-$	Alkylation resistance	(10, 94, 238, 239)
3-methyladenine DNA glycosylase	*E. coli tag*	hamster	*wildtype*	Alkylation resistance	(106)
	E. coli alkA	hamster	*wildtype*	Alkylation resistance	(74)
	rat *APDG*	hamster	*wildtype*	Alkylation resistance	(74)
	human *AAG/MPG*	hamster	*wildtype*	No effect	(22, 87)
	human *AAG/MPG*	hamster	*wildtype*	Increased alkylation-induced aberrations	(42)
8-oxoguanine DNA glycosylase	*E. coli fpg/mutM*	hamster	*wildtype*	Aziridine resistance	(46, 68)
Thymine glycol DNA glycosylase	*E. coli nth*	hamster	γ-ray sensitive (xrs7)	H_2O_2 resistance, bleomycin sensitivity	(80)
Pyrimidine dimer DNA glycosylase	T4 phage *denV*	human	UV sensitive (XPA ,C and E)	UV resistance	(41, 123, 223)
		hamster	UV sensitive (UV5)	UV resistance	(221)
		mouse	*wildtype*	none	(114)
		D. melanogaster	UV sensitive	UV resistance	(5)
AP endonuclease	human *HAP1/APE*	*S. cerevisiae*	*apn1*	Alkylation resistance Reduced spontaneous mutation	(233)

MAG remains to be determined and clearly could be one of several different lesions, based on the extraordinarily diverse *in vitro* substrate specificity of this class of enzyme. The heterologous expression of other DNA glycosylases in this *apn1* strain should provide a sensitive biological test of whether those enzymes also act on endogenously produced DNA damage and may identify enzymes that are important for limiting spontaneous mutation. Indeed, preliminary evidence indicates that expression of the human AAG glycosylase also drives up spontaneous mutation rates in APN1-deficient *S. cerevisiae*, although not nearly as dramatically as MAG.[69] In these experiments it is presumed that glycosylases affect spontaneous mutation by removing damaged bases from the genome. Although this remains the most likely explanation, it is formally possible that the glycosylases actually remove normal bases (at low frequency) and that it is this anomalous repair activity that affects spontaneous mutation. In either case, it is important to identify any activity that influences spontaneous mutation rates, and glycosylases may fall into this group, either because they remove mutagenic lesions or because they produce mutagenic abasic sites.

Reactive oxygen species represent another source of DNA damage from endogenous and exogenous sources.[3] Exogenous sources include ionizing radiation, UV, H_2O_2, 4-nitroquinoline oxide (4NQO), and bleomycin.[66] Endogenous reactive oxygen species are produced as byproducts of aerobic metabolism and from nitric oxide that is produced by some cells for signal transduction, and by activated macrophages as part of the cellular immune response.[130,196,201] Exposure of DNA to active oxygen is therefore inevitable, and so it is not surprising that numerous pathways have evolved, either to prevent oxidative damage from occurring or to repair oxidative damage once it has occurred. Some of the enzymes involved in repairing DNA damaged by reactive oxygen species have been expressed in heterologous cells, and these are discussed below. Reactive oxygen species induce numerous types of damage to the bases and to the sugar-phosphate backbone of DNA.[51,228] 8-oxoguanine (8-oxoG) and thymine glycols (TGs) represent a major fraction of oxidative DNA damage, and DNA glycosylases have been identified that act at base pairs containing these lesions.[66,142] Such glycosylases have been expressed in heterologous systems.

Fpg/MutM 8-oxoG DNA glycosylase activity was initially found in *E. coli*, but its discovery was somewhat circuitous. The enzyme was first identified and purified as a DNA glycosylase that releases formamidopyrimidine (FaPy) lesions from DNA.[15,34,35] FaPy lesions represent an imidazole ring-opened form of 7-alkylguanine (similar FaPys can be induced by γ-rays) that block DNA replication *in vitro* Figure 2).[201] The FaPy DNA glycosylase gene, *fpg*, was later cloned[18] and used to generate *fpg* mutants, but, surprisingly, these mutants displayed no apparent alkylation or γ-ray-sensitive phenotype.[16] Meanwhile, another study led to the identification of the *fpg* gene by completely different means; the defective gene in the *E. coli mutM* mutator strain that displayed elevated spontaneous G:C to T:A transversions was traced to the *fpg* gene.[28] Because G:C to T:A transversions were known to be induced by 8-oxoG (237) and because an 8-oxoG repair activity had previously been identified in *E. coli*,[38] several groups combined their materials and expertise to demonstrate that the *fpg/mutM* gene in fact encodes an 8-oxoG DNA glycosylase whose action prevents spontaneous G:C to T:A transversions.[143,216]

The functional suppression of the *E. coli fpg/mutM* mutator phenotype by heterologous genes was used successfully to isolate a *fpg/mutM* homolog from *S. cerevisiae*

named the *OGG1* gene.[224] The *S. cerevisiae OGG1* gene was also cloned recently by reverse genetics.[153] It seems highly likely that mammalian homologs could also be cloned in similar ways. Indeed, even if mammalian homologs are identified by reverse genetics or by virtue of their amino acid sequence homology, heterologous expression in *E. coli* can be used to confirm their functional homology. Thus, an open reading frame encoding a putative Fpg/MutM glycosylase homolog was identified in the *Lactococcus lactis* genome, and its heterologous expression suppressed the G:C to T:A mutator phenotype in *fpg/mutM E. coli.*[58] Whether these *S. cerevisiae* and *L. lactis* enzymes act to modulate spontaneous mutation in their normal environment remains to be determined.

Expression of the *E. coli fpg/mutM* gene in mammalian cells has been shown to confer resistant phenotypes. γ-rays are thought to induce both 8-oxoG and FaPy DNA lesions (among many others); Fpg/MutM expression did not provide resistance to γ-ray-induced cytotoxicity (which one would expect from FaPy repair), but it did protect against mutation (which one would expect from 8-oxoG repair).[118] However, *fpg* expression in mammalian cells did protect against both the cytotoxic and mutagenic effects of aziridine, a compound thought to induce primarily FaPy DNA lesions, indicating that the Fpg/MutM glycosylase is capable of FaPy repair in mammalian cells.[46,68] Taken together, these heterologous expression experiments suggest that γ-rays may not induce significant levels of FaPy DNA damage and that they do induce significant levels of 8-oxoG.

Surprisingly, biochemical characterization of the human and mouse 3MeA DNA glycosylases (AAG and Aag, respectively) demonstrated that they can release 8-oxoG from oxidized DNA, at least *in vitro*. To determine whether their *in vitro* activity has any *in vivo* relevance, it was shown that their expression could partially suppress the *fpg/mutM* mutator phenotype,[9] although the suppression was modest. The possibility that the mammalian DNA glycosylases influence spontaneous (or induced) G:C to T:A transversions in mammalian cells is currently being tested by expressing them in the recently developed *Aag* null mouse cell lines.[60]

An extremely interesting study in *D. melanogaster* recently uncovered the surprising fact that the S3 ribosomal protein, as well as associating with ribosomes, localizes to the nuclear matrix *in vivo* and exhibits AP-lyase activity *in vitro*.[234] Because all the authentic AP-lyases known to exist in both prokaryotes and eukaryotes are also DNA glycosylases, the *D. melanogaster* S3 protein was tested for DNA glycosylase activity. The S3 ribosomal/AP-lyase protein can release both FaPy and 8oxoG lesions from DNA *in vitro*. Moreover, expression of the *D. melanogaster* S3 protein in *E. coli fpg/mutM* mutants completely suppressed the G:C to T:A mutator phenotype.[243] These results demonstrate that the DNA repair activity of this ribosomal protein is robust enough to exert an *in vivo* biological effect, and they strengthen the notion that the *D. melanogaster* S3 ribosomal protein has the potential to participate in two very different processes, namely, protein synthesis and DNA repair.

The *E. coli* MutY DNA glycosylase and MutT 8oxo-dGTPase enzymes collaborate with FaPy/MutM to provide an elegant defense network for preventing the accumulation of 8oxoG in the bacterial genome.[142] Should FaPy/MutM fail to remove 8oxoG from the genome, this lesion will encounter the replication machinery, whereupon it may direct the insertion of either C or A. If C is incorporated, the 8oxoG:C base pair remains susceptible to repair by FaPy/MutM. However, if A is incorporated opposite 8oxoG, the oxidized guanine lesion becomes refractory to removal by FaPy/MutM, presumably because such repair would drive G:C to T:A

transversions. Instead, the A in the 8oxoG:A base pair is subject to removal by the MutY glycosylase, and BER is presumably initiated by MutY until a MutM repairable 8oxoG:C base pair is formed. [142] As one would predict, *mutY E. coli* suffer elevated G:C to T:A transversions. MutT prevents the introduction of 8oxoG into DNA from the nucleotide precursor pool by hydrolyzing 8oxo-dGTP to 8oxo-dGMP plus PPi.[133] In other words, MutT eradicates these oxidized nucleotides from the precursor pool to reduce the incorporation of 8oxoG opposite A or C during DNA replication. *mutT E. coli* suffer an enormous increase in spontaneous A:T to C:G transversion mutations.[247] The FaPy/MutM-MutY-MutT defense network appears to be crucial to aerobically growing *E. coli* because mutants with defects in all three of these genes have a 250-fold increase in spontaneous mutation.[208]

Human homologs of both MutY and MutT enzyme activities have been identified.[139,174] A MutY-like activity was identified in human cell extracts,[139] and a human cDNA that encodes a putative protein with extensive sequence homology to the *E. coli* MutY protein was recently found by random cDNA sequencing.[200] However, it has not yet been established whether the cloned cDNA suppresses the mutator phenotype of *mutY E. coli* mutants, and it has not been established whether this cDNA encodes the previously identified human enzyme activity. A MutT-like activity was purified to homogeneity from human cells and its cDNA cloned by reverse genetics; the predicted amino acid sequence had only very short regions similar to *E. coli* MutT (and so was unlikely to have been cloned by sequence homology); nevertheless, expression of the *hMTH* (**h**uman **MutT H**omolog) cDNA in *mutT E. coli* partially reversed its mutator phenotype.[174]

The thymine glycol (TG) DNA glycosylases repair a family of oxidized thymines produced by the reaction of active oxygen species with DNA. The TGs are not thought to be particularly mutagenic,[6] but they have been shown to block DNA replication *in vitro*.[40,51,83] The *E. coli* Endonuclease III enzyme (encoded by the *nth* gene) acts as both a TG glycosylase and an AP-lyase and efficiently initiates BER at these replication-blocking lesions.[24] In addition, Nth cleaves at a number of other oxidized and fragmented pyrimidines.[78,80,81] Expression of the *E. coli nth* gene in γ-ray-sensitive Chinese hamster ovary cells conferred resistance to H_2O_2, as one might expect, but conferred sensitivity to bleomycin.[80] In addition to oxidizing thymines, bleomycin also produces a large number of DNA single strand breaks; Nth-related bleomycin sensitivity was thought to be caused by DNA double strand breaks created by the release of oxidized thymines followed by the generation of a strand break in close proximity to another strand break on the opposite strand.

Pyrimidine Dimer (CPD) DNA glycosylase from T4 phage. UV light induces the formation of CPDs (cyclobutane pyrimidines) in DNA, and these CPDs can be either mutagenic or cytotoxic. The bacteriophage T4 *denV* gene encodes a CPD DNA glycosylase with an associated AP lyase activity. This enzyme hydrolyzes the glycosylic bond of the 5′ pyrimidine, then cleaves the resulting abasic site by β-elimination, leaving an abasic 3′-OH terminus and the CPD attached to the sugar at the phosphorylated 5′ terminus at the strand break. Further processing of both termini would be required for BER to proceed to completion. In fact, the T4 CPD DNA glycosylase furnished the first known example of heterologous DNA repair, but these experiments were carried out in the early 1960s before any DNA repair mechanism had been described.[66] In short, the T4 bacteriophage genome was found to produce an activity that could act *in trans* (in *E. coli*) to rescue UV-irradiated T2 bacteriophage; this activity was later localized to the T4 *denV*

gene, and, to our knowledge, this represents the first known demonstration that a DNA repair protein encoded by a gene from one organism (T4) could act on the damaged genome of another (T2).

So far, CPD DNA glycosylases have been identified in the bacteriophage T4 and in the bacterium *Micrococcus luteus*.[65,204] The *M. luteus* CPD DNA glycosylase gene was recently cloned, but, surprisingly, the enzyme turned out to bear no amino acid sequence homology to the T4 enzyme.[160] It appears that nucleotide excision repair (NER), rather than BER, is primarily responsible for CPD repair in most organisms, indeed significant CPD DNA glycosylase activity has not been detected for any organism other than T4 and *M. luteus*. In fact, even for *M. luteus*, CPD DNA glycosylase mutants only exert a UV-sensitive phenotype in a NER-deficient background.[152]

Relevant to this review is the fact that the heterologous expression of the T4 CPD DNA glycosylase in several different UV-sensitive eukaryotic cell types confers significant resistance to UV exposure. One study demonstrated that *denV* expression in strains of *Drosophila melanogaster* that cannot initiate the repair of UV-induced DNA damage confers considerable UV-resistance by restoring the repair of CPDs.[5] Further, *denV* expression in UV-sensitive rodent and human cell lines (CHO UV5, XP groups A, C, and E), known to be deficient in CPD repair, enabled the cells to remove CPDs and to survive better after UV-irradiation.[122,221–223] These heterologous expression experiments confirmed that the NER-deficient mammalian cell lines were only deficient in the initiation of NER (i.e., incision at the site of DNA damage), but were capable of carrying out the subsequent steps of gap formation, gap filling, and DNA ligation. In contrast, *denV* expression in normal murine fibroblasts did not enhance their resistance to UV-induced cytotoxicity, despite an increase in CPD removal.[114] Although the reasons for this remain unclear, it is important to point out that the mouse fibroblasts were neither NER deficient nor UV sensitive, suggesting that in this case the rate of CPD repair was not rate-limiting for survival after UV exposure.

Uracil DNA glycosylase (UDG), UDG inhibitors, and dUTPase. Uracil and thymine display similar base pairing properties, that is, they both pair with adenine. It was therefore not immediately obvious what selective pressures dictated that DNA contain thymine and RNA contain uracil. However, it is now clear that the deamination of cytosine to form uracil occurs at a physiologically significant rate,[124] and this presents a mutational threat. Cytosine deamination in the genome produces a G:U base pair that, if left unrepaired, would drive a G:C to A:T transition. It is quite clear that, at least in *E. coli*, uracil DNA glycosylase (UDG), encoded by *ung*, plays a significant role in preventing spontaneous G:C to A:T mutation. Deamination of cytosine in RNA molecules would not, for the most part, result in an inherited change in the genome. In addition to cytosine deamination, uracil can infiltrate DNA from dUTP in the nucleotide precursor pools; a major role of the dUTPase enzyme is to maintain low dUTP levels so that the incorporation of uracil instead of thymine in DNA is kept to a minimum. Uracils that creep into DNA from the precursor pool to form A:U base pairs are also removed by the UDG; in other words, UDG can remove uracil from both G:U and A:U base pairs. Together, dUTPase and UDG strive to maintain a uracil-free genome for most organisms.

Although most organisms go to great lengths to minimize the amount of uracil in DNA, very high levels may be tolerated under certain circumstances. *E. coli* can tolerate a 90% replacement of thymines by uracil (although growth is inhibited), and *Bacillus*

subtilis PBS2 bacteriophage contain uracil in their genomes.[59,209] However, genomes harboring high levels of uracil can only survive in the absence of UDG initiated BER (e.g., in *E. coli ung* mutants) because otherwise the genome becomes irreversibly fragmented due to the incessant action of UDG followed by cleavage at the AP sites. Not surprisingly, the *B. subtilis* PBS2 bacteriophage encodes a small protein that irreversibly binds and inhibits UDG; the inhibitor gene was cloned by virtue of its ability to make wild-type *E. coli* permissive for the growth of uracil containing single-stranded M13 bacteriophage.[231] The inhibitor protein was found to inhibit UDGs from *B. subtilis*, *E. coli*, *M. luteus*, *S. cerevisiae*, and human cells,[96] suggesting that these enzymes are probably very highly conserved both structurally and functionally.

A detailed knowledge of the biochemistry and genetics of how *E. coli* controls the incorporation and removal of uracil in DNA has been exploited for the cloning and characterization of human cDNAs and enzymes presumed to be involved in limiting the presence of uracil in the human genome. dUTPase-deficient *E. coli* are not viable (and neither are dUTPase-deficient *S. cerevisiae*), and this severe phenotype was taken advantage of to clone a human dUTPase cDNA.[140] Heterologous expression and functional complementation of *E. coli* harboring a temperature-sensitive mutant dUTPase allele was used to isolate a human dUTPase cDNA. It was hoped that these experiments would lead to further characterization of the essential role of dUTPase in DNA replication and to the possible development of dUTPase inhibitors for use in chemotherapy.

The human UDG was purified and its cDNA cloned based on N-terminal amino acid sequence information.[157] The UDG cDNA was used recently to express the human DNA repair glycosylase in *E. coli ung–* mutants in order to further characterize this important enzyme. Specifically, the expression of human UDG suppressed the mutator phenotype of *ung–* bacteria and rendered them nonpermissive for the growth of uracil containing M13 bacteriophage.[156] These results indicate that, like the *E. coli* UDG, the human enzyme can remove uracil from G:U as well as A:U base pairs. This group recognized that simple assays such as these, in *E. coli*, would enable the rapid characterization and evaluation of mutant forms of the human UDG enzyme, allowing the localization of functional domains. Indeed, site-specifically mutated forms of the human UDG were created that broadened the substrate specificity of the enzyme such that normal thymine and cytosine, as well as uracil bases, were removed from DNA.[102] Expression of the mutant human enzymes in wild-type and *ung– E. coli* resulted in increases in spontaneous mutation of up to 100-fold. These results suggest that single amino acid substitutions in DNA repair glycosylases could produce enzymes that confer a mutator phenotype. It remains to be determined whether these types of mutations occur *in vivo* and whether UDG mutation could contribute to cancer.

B. AP Endonucleases

Class I and class II AP endonucleases cleave the DNA backbone at either the 3′ or the 5′ side of a baseless sugar residue, respectively (Figure 4). Class I enzymes (which are intrinsic to some, but not all, DNA glycosylases) cleave via β-elimination and have been renamed AP lyases. Class II enzymes cleave hydrolytically and have retained the name AP endonuclease. Cleavage by either class of enzymes produces a DNA terminus that requires further processing in order for DNA repair synthesis and DNA ligation to complete BER, as indicated in Figure 1; note that the 3′-diesterase activity that is required following AP lyase cleavage is intrinsic to all class II AP endonucleases,

Table 4: **Properties of AP endonuclease from *E. coli***

	Exonuclease III ***xthA***	**Endonuclease IV** ***nfoIV***
Enzymatic Activities	Class II AP endonuclease 3'-diesterase 3' to 5'-exonuclease RNase H	Class II AP endonuclease 3'-diesterase
Sensitivity of null cells *		
very sensitive	MMS, mitomycin C, H_2O_2	tBH, bleomycin
moderately sensitive	tBH	MMS, mitomycin C
slightly sensitive	UV	
not sensitive	bleomycin, γ-rays	UV, γ -rays, H_2O_2

* see reference (44)

although to varying degrees;[48,52] as will become clear below, the 3′ diesterase activity is also important for the repair of other types of damaged 3′ termini produced by the direct strand-breaking effects of agents like H_2O_2 and bleomycin.

The *xthA*-encoded exonuclease III and *nfo*-encoded endonuclease IV enzymes of *E. coli* turn out to be class II hydrolytic AP endonucleases, both of which display 3′-diesterase activity (Table 4). In addition, exonuclease III but not endonuclease IV has 3′ to 5′ exonuclease and RNaseH activity. Although exonuclease III and endonuclease IV have largely overlapping enzymatic activities, the phenotypes of *xthA*$^-$ and *nfo*$^-$ single and double mutants show surprising differences (Table 4); for example, *xthA*$^-$ are sensitive to H_2O_2, whereas *nfo* are not. Interestingly, *xth*$^-$ *nfo*$^-$ double mutants are much more sensitive to H_2O_2 than the *xthA*$^-$ single mutant, indicating that Endo IV can play a role in preventing H_2O_2-mediated toxicity.[44]

Another notable difference is that *nfo*$^-$ but not *xthA*$^-$ cells are very sensitive to bleomycin-induced killing. It is not certain what biochemical difference between the two enzymes accounts for these phenotypic differences. They may reflect different abilities of the two enzymes to repair particular types of damaged 3′ termini produced by H_2O_2 and bleomycin, that is, exonuclease III may be better able to repair shattered 3′ termini produced by H_2O_2 than endonuclease IV, and the reverse may be true for bleomycin-induced DNA damage. The phenotypic differences between *xthA*$^-$ and *nfo*$^-$ mutants proved useful for the characterization of heterologously expressed eukaryotic AP endonucleases; indeed, exonuclease III and endonuclease IV are now known to represent two broad classes of highly conserved AP endonucleases.[48,66]

The *S. cerevisiae APN1* gene encodes the most abundant AP endonuclease in this organism. The yeast enzyme turns out be

much more similar to *E. coli* endonuclease IV than to exonuclease III.[161] *APN1* expression rescues *xthA⁻ nfo⁻ E. coli* from the cytotoxic effects of oxidizing agents, bleomycin, and the alkylating agent MMS.[166] In addition, Apn1 reversed the sensitivity of *nfo⁻* mutants to *tert*-butyl hydroperoxide (tBH) and bleomycin, demonstrating that the amino acid sequence similarity between Apn1 and endonuclease IV also extends to functional similarity. In agreement with these observations, the *S. cerevisiae apn1* null mutants are sensitive to oxidizing agents, bleomycin, and MMS.[167]

A number of animal AP endonucleases have been cloned and characterized and, in contrast to the *S. cerevisiae* enzyme, they all turn out to be more similar to exonuclease III than to endonuclease IV.[49,171,172,191,197] Thus, the *Drosophila* (Rrp1), bovine (BAP1), murine (APEX), and human (APE/HAP1) AP endonucleases, in addition to being very similar to each other, are similar to the *E. coli xthA* gene product. Heterologous expression studies indicate that the human APE/HAP1 enzyme protects *xthA⁻nfo⁻ E. coli* mutants very well against alkylating agents and γ-rays, but less well against H_2O_2.[32,49,171] Indeed, the APE/HAP1 enzyme turns out to have limited 3′-diesterase activity, and thus one would not expect this enzyme to be proficient in the repair of the damaged 3′ ends produced by H_2O_2.[31,235] That 3′-diesterase activity is important for the repair of H_2O_2-induced DNA damage is supported by the fact that expression of a mutant Nfo protein that has lost 3′-diesterase but not AP endonuclease function is unable to reverse the H_2O_2 sensitivity of *xthA⁻ nfo⁻ E. coli*;[89] note that this mutant can still confer resistance to MMS-induced cell death, indicating that only the AP endonuclease function is required to confer alkylation resistance. Expression of *Drosophila* AP endonuclease (*Rrp1*) in *xthA⁻ nfo⁻ E. coli* effectively reverses the sensitivity to MMS, tBH, H_2O_2, bleomycin, and mitomycin C (MMC), suggesting that, unlike the human enzyme, *Drosophila* AP endonuclease is able to catalyze all the reactions necessary to repair damage produced by each of these agents.[72]

Finally, expression of the APE/HAP1 human AP endonuclease activity in *E. coli xthA⁻nfo⁻* and yeast *apn1* mutant strains can reverse their mutator phenotype.[171,233] Because APE/HAP1 has a powerful AP endonuclease activity but a weak 3′-diesterase activity, these experiments indicate that endogenously generated AP sites, rather than endogenously generated strand breaks, are likely to be responsible for the elevated spontaneous mutation rates in these cells. Whether an AP endonuclease deficiency in human cells leads to a mutator phenotype remains to be determined.

C. DNA Polymerases and Ligases

Having considered the DNA glycosylase and AP endonuclease steps of BER, we now turn to the DNA repair synthesis and ligation steps. Unlike base removal and strand cleavage, the DNA polymerase and ligase functions are not unique to BER, because they are also integral to DNA replication. *E. coli* has three distinct DNA polymerases, namely, DNA polymerases I, II, and III. DNA polymerase I (Pol I) is believed to be the major activity responsible for DNA repair synthesis, but it also participates in the joining of Okazaki fragments during DNA replication. Pol III is the major replicative enzyme, and the role of Pol II is unclear.[66,112] Mammalian cells have at least five polymerases (Pol α, β, γ, δ, and ε), and of these Pol β is now known to be responsible for virtually all of mammalian BER DNA synthesis;[199,202] in fact, Pol β turns out to perform an essential function, because a homozygous pol β null mutation is embryonic lethal in mice.

The rat Pol β enzyme can functionally substitute for Pol I when expressed in *E. coli polA⁻* mutants, and this functional comple-

mentation has been used imaginatively to explore Pol β structure/function relationships.[39,205] One particular *E. coli polA* strain is MMS sensitive (because of a defect in DNA repair synthesis) and is also unable to efficiently form colonies on rich growth media because of a defect in the joining of Okazaki fragments.[205,236] Expression of the rat Pol β enzyme functionally complemented both defects, showing that this mammalian polymerase can fully substitute for *E. coli* Pol I. [205,206] Because the DNA replicative and repair functions of Pol I can be monitored separately (and easily) in *E. coli*, it was possible to identify mutant Pol β enzymes defective in one or other function using this powerful functional complementation approach. Thus, the replicative and repair DNA synthetic roles of Pol β can be genetically separated and therefore must have different biochemical and structural requirements. Further, some Pol β mutants were found to exert a dominant negative phenotype in *E. coli*; the dominant negative mutants prevented wild-type Pol I (during co-expression in *E. coli*) from correcting both the replication and the repair defect.[207] In addition, the Pol β dominant mutants created a mutator phenotype. Further, the same dominant negative mutant polymerases also interfere with BER when expressed in *S. cerevisiae*,[39] but whether they interfere with BER in mammalian cells is not yet known. These heterologous expression studies furnished a powerful way to identify functional domains of an essential mammalian DNA polymerase; it clearly would have been much more difficult to identify such informative Pol β mutants using mammalian cell systems.

Finally, a similar approach was used to locate the active site region of the human DNA ligase I protein and to identify specific amino acid residues required for the formation of the ligase-adenylate catalytic intermediate.[109] Human DNA ligase I is a large protein (919 amino acids). The smallest fragment that could still function as a DNA ligase was identified by its ability to functionally complement the growth defect of an *E. coli* temperature-sensitive DNA ligase mutant. Site-directed mutagenesis of this subfragment, and analysis of the ability of the mutants to function in *E. coli* (along with biochemical analysis), was used to locate the precise active site region of this important human enzyme and to identify the amino acids absolutely required for ligase function.

V. MISMATCH REPAIR

DNA mismatch repair (MMR) has received tremendous attention in the last few years, because defects in MMR were linked to a human cancer-prone disease that predisposes people to human nonpolyposis colorectal cancer (HNPCC), as well as to certain other cancers.[64,121] The DNA mismatches that are substrates for this repair pathway can arise in several ways, but the most common is via nucleotide misincorporation during DNA replication; many of these misincorporations may be corrected by 3′-5′ exonucleolytic editing functions, but the mismatches that escape such proofreading become subject to the MMR pathway.[111,113,145,146,164] *E. coli* MMR, initiated by the MutS, MutL, and MutH proteins, has become the prototype after which all other models for MMR pathways are patterned. The *E. coli* MutS protein recognizes and binds mismatched bases in newly replicated DNA. The MutH protein recognizes and binds GATC Dam methylase target sequences, which are hemimethylated in the newly replicated DNA. The MutL protein forms a bridge between the bound MutS and MutH whereupon MutH is stimulated to cleave the GATC sequence in the unmethylated, newly synthesized strand. This initiates an excision repair process that removes all the DNA from the nick at the GATC site to beyond the mismatched base

pair; similar to BER and NER, the resulting gap is filled by DNA repair synthesis and sealed by ligase.

It is quite clear that the interactions between MutS, MutL, and MutH are essential for the initiation of MMR in *E. coli*, and it is this feature that has produced some interesting results from the heterologous expression of other MMR proteins in *E. coli*. Heterologous expression studies indicate that the binding of MutS and its homologs from other organisms appears to proceed in the absence of the other MMR repair proteins. This conclusion was drawn from the observation that expression of the *S. pneumoniae* and human MutS homologs in wild-type *E. coli* actually produces a mutator phenotype; presumably, the MutS homologs bind to mismatched base pairs but are unable to interact productively with MutL and MutH.[64,163] Binding of the MutS homolog proteins to the mismatches blocks the binding of endogenous MutS protein, thus preventing the proper repair of DNA replication errors, and creating a dominant mutator phenotype. In retrospect, the human (and other) MutS homologs could have been cloned by screening for cDNAs that produce a mutator phenotype in *E. coli*.

In addition to mammalian MTases (with respect to O^4MeT repair) and *S. cerevisiae* PHR1 (with respect to dark repair of UV-damaged DNA) expression of MutS homologs is the third example of a common phenomenon. That is, in certain instances heterologous expression of DNA repair proteins in not only fails to functionally complement a DNA repair defect, but actually disrupts normal repair, producing a deleterious effect on the host. This may be caused by the foreign protein binding to DNA damage accompanied by its inability to efficiently interact with the rest of the repair machinery necessary for the completion of DNA repair. In an extreme case, a foreign protein may bind to DNA damage and simply shield it from endogenous DNA repair pathways. Thus, when a lesion is subject to repair by more than one DNA repair pathway, the *in vivo* repair rate may not simply default to the most efficient pathway, since proteins with low turnover rates may effectively block the access of more efficient proteins.

The ability of MutS homologs to disrupt an endogenous *E. coli* DNA repair pathway raises the possibility that, in addition to functional complementation of *E. coli* DNA repair mutants, dominant negative phenotypes in wild type *E. coli* could be exploited for the identification of new mammalian DNA repair proteins. Indeed, while the functional complementation approach works well with proteins that can operate in isolation, the dominant negative approach should identify proteins that normally operate in the context of a multiprotein complex, but which fail to behave properly because its *E. coli* partners are too diverged. The identification of eukaryotic proteins that produce DNA repair deficiencies in *E. coli* could produce a new crop of DNA repair genes.

VI. CONCLUDING REMARKS

Functional complementation by the heterologous expression eukaryotic genes in prokaryotic cells, and prokaryotic genes in eukaryotic cells, has been highly successful for a wide variety of genes, and particularly successful for DNA repair genes. Perhaps the reasons for this lie in the fact that the genetic material of virtually all organisms has exactly the same fundamental structure, namely, double-stranded DNA. The DNA may be packaged differently in prokaryotes and eukaryotes, but at the heart of chromatin is the same duplex DNA molecule. Further, for the most part, the types of damage that DNA suffers is structurally identical, whether it occurs in a bacteriophage genome or in a human genome. It is therefore not surprising that DNA repair proteins that can recognize specific types of DNA damage should be able to recognize that damage in almost any genome; in other words, it should not be

surprising that DNA repair genes from one organism can function effectively in another organism.

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