

## Special article

### Antineoplastic drug resistance and DNA repair

R. K. Burt,<sup>1</sup> M. C. Poirier,<sup>2</sup> C. J. Link Jr<sup>3</sup> & V. A. Bohr<sup>3</sup>

<sup>1</sup>Clinical Oncology Program, <sup>2</sup>Laboratory of Cellular Carcinogenesis and Tumor Promotion, and <sup>3</sup>Laboratory of Molecular Pharmacology, National Cancer Institute, National Institutes of Health, Bethesda, MD, U.S.A.

**Summary.** Important aspects of the DNA repair mechanisms in mammalian, and especially human, cells are reviewed. The DNA repair processes are essential in the maintenance of the integrity of the DNA and in the defense against cancer. It has recently been discovered that the DNA repair efficiency differs in different regions of the genome and that active genes are preferentially repaired. There is mounting evidence that DNA repair processes play a role in the development of drug resistance by tumor cells. We will discuss such data as well as further approaches to clarify the relationship between DNA repair and antineoplastic drug resistance. Specifically, there is an increasing need to investigate the intragenomic heterogeneity of DNA repair and correlate the repair efficiency in specific genes to aspects of drug resistance. We also discuss the therapeutic potential of inhibiting the DNA repair processes and thereby possibly overcoming drug resistance.

#### Introduction

The role of DNA repair in tumor response to treatment is becoming an increasingly active area of research for laboratory scientists and radiation oncologists. Medical oncologists, however, have tended to neglect the role of DNA repair in antineoplastic drug resistance despite evidence that the ultimate target of most chemotherapeutic agents is DNA. Many cancer patients treated with chemotherapeutic agents ultimately die of progressive disease which has become resistant to a multitude of different chemotherapeutic agents. The question of how tumors become resistant to these agents is obviously of major importance. Many mechanisms of drug resistance have been proposed and are under intense laboratory investigation. The role of DNA repair in drug resistance is emerging as a key part of the answer.

Chemotherapeutic drugs may act indirectly or directly on DNA. Indirect damage to DNA can occur by blockage of DNA synthesis by nucleotide analogs such as 5-fluorouracil [1, 2] or cytosine arabinoside [3, 4], generation of free radicals from bleomycin [5] and quinones such as mitomycin C or adriamycin [6–9], and alteration of nucleotide binding proteins such as anthracycline induced stabilization of the DNA topoisomerase II cleavage complex [10]. Drugs which directly damage DNA include alkylating agents [11] and platinum analogs [12]. Alkylating agents, such as the nitrosoureas, mitomycin C, cyclophosphamide, melphalan, chlorambucil and busulfan, form monofunctional or bifunctional covalent bonds between the carbon of an alkyl moiety and the nucleophilic bases of DNA. Although alkylation may occur on any oxygen or

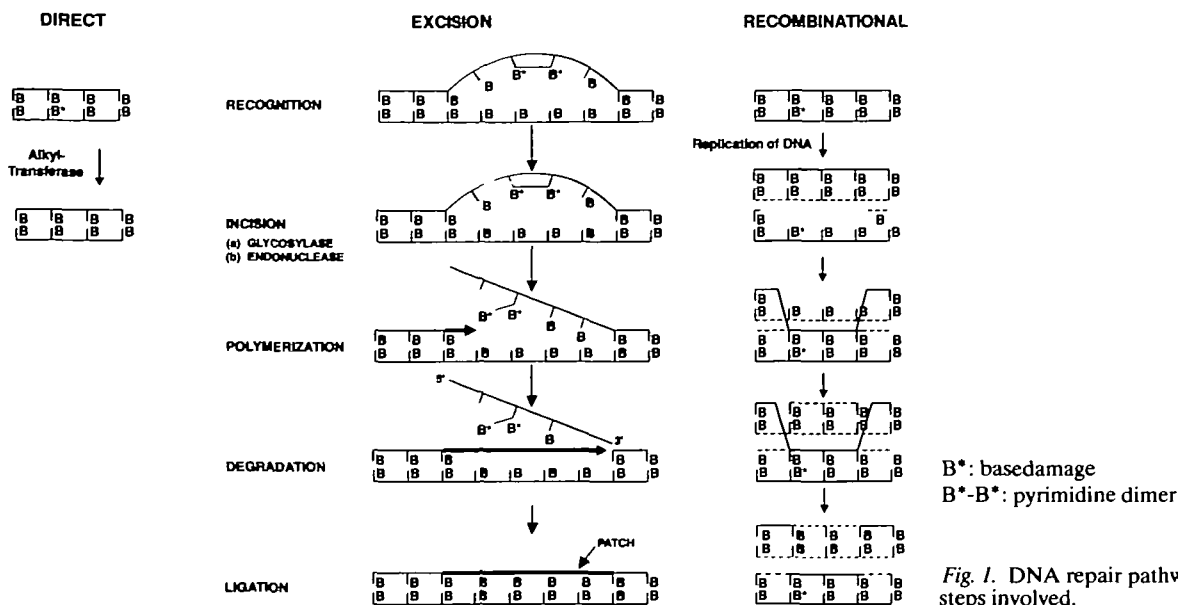
nitrogen in the DNA double helix, the two predominate alkylation sites are the N<sup>7</sup> and O<sup>6</sup> positions of guanine [12, 14]. Cisplatin adducts are bidentate with the platinum moiety bridging bases on the same DNA strand (intrastrand) or bases on opposite strands (interstrand) [15, 16]. The predominate cisplatin adduct is intrastrand between the N<sup>7</sup> position of neighboring guanines [15, 16].

DNA is not only the final target of most antineoplastic drugs but is also damaged by irradiation, ultraviolet light, and numerous other carcinogenic and mutagenic agents [17]. In order to restore the integrity of the genome, organisms have evolved various mechanisms of DNA repair. These protective repair mechanisms may unfortunately also circumvent the intended cytotoxic effect of chemotherapy. Indeed, enhanced efficiency of DNA repair may contribute to the ability of malignant cells to develop cross resistance to a variety of cytotoxic antineoplastic agents.

#### Mechanisms of DNA repair

Most of our present knowledge about DNA repair processes stems from work done in bacteria. Many of these processes have also been identified in mammalian cells, but our models for repair processes are still mainly based on prokaryotic studies.

DNA repair has been classified as direct, base excision, nucleotide excision, and postreplication (Figure 1) [17–19]. An absolute distinction between base excision repair and nucleotide excision repair is not always applicable [17] and therefore we will not attempt to draw any rigorous distinction between these two forms



of excision repair. Direct repair which occurs in both prokaryotes and eukaryotes is simple reversal of the DNA damage without excision and replacement of the modified base or nucleotide. In the case of small alkylating agents, this is accomplished by transfer of the alkyl moiety via an alkyltransferase from the alkylated base to a cysteine residue in the active site of the enzyme [20, 21]. Alkyltransferases are suicide enzymes which are irreversibly inactivated after binding an alkyl group [21]. Therefore, repair of one alkyl adduct stoichiometrically requires consumption of one enzyme molecule.

Excision repair involves recognition and removal of the lesion by a glycosylase which hydrolyzes the glycosidic bond between the damaged base and its deoxyribose sugar [17, 18, 22]. An AP (apurinic, apyrimidinic) endonuclease then cleaves the phosphate internucleotide linkage. DNA repair is completed by an exonuclease that degrades a short segment of DNA from the damaged strand, a polymerase which fills in the gap in the DNA strand using the complementary strand as a template and finally a ligase which reunites the sugar phosphate DNA backbone. In general, damage to DNA by small alkyl groups (such as the chloroethyl groups of the nitrosureas) is reversed by the direct repair pathway. In contrast, cisplatin, bulky alkylating agents like mitomycin C and ultraviolet light induced pyrimidine dimers are repaired by nucleotide excision [17, 18, 22].

Another form of excision repair has been identified in prokaryotes. In *E. coli* the three gene products UVrA, UVrB and UVrC together form the protein complex called ABC excinuclease. Interestingly, this complex recognizes a wide variety of different DNA lesions. The complex hydrolyzes a phosphodiesterase bond on each side of the damaged base, resulting in excision of an oligonucleotide segment containing the

damaged nucleotide [23]. So far a similar protein complex has not been identified in mammalian cells, and microinjection of ABC excinuclease into excision repair deficient mammalian cells does not restore the defect [24].

While direct and excision repair pathways are mechanisms of repairing DNA prior to its replication, postreplication repair (also known as recombinational or daughter strand gap repair) is a mechanism of repairing unexcised and damaged DNA after DNA polymerase replication of the parental strands [17, 18, 25]. In this mechanism, replication of the DNA strands is discontinuous with DNA polymerase skipping over damaged bases. Gaps in the daughter strand can then be filled in by recombination with an undamaged segment from the opposite parental or sister strand. The role, if any, of postreplication repair in reversing chemotherapeutically induced DNA damage is not yet understood.

#### DNA damage inducible genes

In *E. coli* DNA repair enzymes are inducible. There are several types of responses in prokaryotes that are induced by DNA damage. Two distinct types of inducible bacterial DNA repair responses are the adaptive and SOS response [26]. The adaptive response in *E. coli* is induced by alkylating agents which methylate the O<sup>6</sup> position of guanine [26, 27]. Removal of this adduct by the alkyltransferase, O<sup>6</sup> methylguanine methyl transferase (O<sup>6</sup> MGMT), transforms O<sup>6</sup> MGMT into a DNA binding protein which transcriptionally activates the expression of a series of DNA repair genes including the *ada* gene which codes for transcription of more O<sup>6</sup> MGMT [27-29]. Therefore, transcriptional regulation of the O<sup>6</sup> MGMT gene is directly linked to consumption of the gene product.

The SOS response in *E. coli* is induced by a variety of nonspecific DNA damaging agents, including UV light and alkylating agents [30]. The common denominator in the DNA damage which induces this type of repair is the formation of single stranded DNA which upregulates a constitutively expressed protein, rec A [30, 31]. Activated rec A cleaves the repressor protein lex A [32]. Removal of lex A leads to transcription of the SOS operon, a group of more than 20 genes whose transcription is regulated by the same control elements [33]. Three SOS genes (*uvrA*, *uvrB*, and *uvrC*), code for the previously mentioned ABC excinuclease which is involved in nucleotide excision repair [33, 34]. Another SOS gene is *sul A* which codes for a protein that delays the cell cycle in G2 allowing time for DNA repair before the cell enters into mitosis [33, 35, 36]. In addition, the SOS operon also codes for several genes (*rec A*, *rec N*, *ruv*) involved in recombinational or postreplication repair [17, 33].

In contrast to prokaryotes, the regulation of expression of eukaryotic mammalian DNA repair genes is more complex and remains vague. There is no well defined eukaryotic counterpart to the bacterial adaptive and SOS responses. Furthermore, the induction of resistance to alkylating agents in mammalian cells is difficult and at best the cells manifest only a relatively modest 10–15 fold [37–39] resistance. Also, when selected for resistance to one alkylating agent, mammalian cells may or may not display cross resistance to other alkylating agents [37–39]. Mammalian cells do nevertheless develop low level resistance or adaptation to alkylating agents. Mammalian O<sup>6</sup> alkyltransferase [40] excision repair [41] and recombinational activity [42] have been documented to increase after exposure to alkylating agents. In addition, the messenger RNA of several mammalian genes has been shown to increase rapidly following DNA damage [43]. These include *c-fos* [44] metallothionein [45], ubiquitin [46], *gadd* (growth arrest and DNA damage inducible genes) [47] and  $\beta$ -polymerase [48]. Of these genes only *gadd* and  $\beta$ -polymerase are directly involved in DNA repair.  $\beta$ -polymerase is a DNA repair polymerase that fills in the gaps following excision of damaged nucleotides. The incompletely characterized *gadd* genes may be the counterpart to the *E. coli* *sul A* gene. *Gadd* genes thus appear to be involved in the arrest of the cell cycle following DNA damage.

#### *Heterogeneous DNA repair*

The structure of chromatin in mammalian cells is complex and involves a 50,000 fold packaging of cellular DNA in the nucleus. It is therefore not surprising that DNA repair in mammalian cells appears to be non-random. There is evidence that some genes are repaired more efficiently than others. For example, pyrimidine dimers in human cell lines are repaired twice as fast in the actively transcribed dihydrofolate reductase (DHFR) gene as in surrounding hetero-

chromatin [49]. This preferential repair of transcriptionally active genes is even more pronounced in Chinese Hamster ovarian cells in which the DHFR gene is efficiently and completely repaired while damaged heterochromatin is apparently almost not repaired [50]. In a mouse 3T3 fibroblast cell line, pyrimidine dimers in the active *c-abl* protooncogene are 85% repaired within 24 hours while in the transcriptionally silent *c-mos* protooncogene only 22% of damaged DNA is repaired within 24 hours [51]. In CHO cells, it has been shown that transcriptional activation of the metallothionein gene doubles its rate of repair of UV induced pyrimidine dimers [52]. In human cells, the rate of repair of this gene increased both after UV and aflatoxin  $\beta$ 1 induced damage [53]. It appears, therefore, that DNA repair may be directed either by chromatin structure or the transcriptional apparatus itself towards preferential repair of actively transcribed genes. Indeed, preferential DNA repair of active genes appears to be of vital importance for genomic integrity since patients with Cockayne's syndrome who suffer from multiple disabilities including dwarfism and mental retardation have normal overall DNA repair, but deficient preferential gene specific repair after UV damage [54].

DNA repair may not only be gene specific but also strand specific. In both rodents and humans a difference in the rate of nucleotide excision repair of pyrimidine dimers exists between the transcribed and non-transcribed strand of the DHFR gene [55]. Interestingly, in *E. coli* evidence suggests that the difference in the rate of repair between the two DNA strands of the lactose operon exists only when the gene is being actively transcribed [56]. This finding suggests that some gene specific DNA repair is associated with the transcription machinery rather than dependent upon local chromatin accessibility.

Heterogeneity in DNA repair is complicated further by the existence of two compartments of mammalian DNA, nuclear and mitochondrial. Mitochondrial DNA which represents only 0.1–1% of total mammalian DNA [57] is 3 to 500 times more susceptible to damage than nuclear DNA [58–60]. Little is known about the mechanisms of mitochondrial DNA repair. Human mitochondria appear unable to repair DNA damage induced by ultraviolet light [61]. However, repair of mammalian mitochondrial DNA damage induced by chemical alkylation has been reported [62, 63]. Therefore, in studying the association of DNA repair to anti-neoplastic drug resistance, a range of mechanisms must be evaluated, including overall genomic repair, preferential gene repair, strand specific repair and mitochondrial DNA damage and repair.

#### *Human DNA repair genes*

Several human DNA repair genes have been cloned including the alkyltransferase, O<sup>6</sup> methylguanine methyltransferase (O<sup>6</sup> MGMT) [64], the base excision repair

gene Uracil DNA glycosylase [65] and the nucleotide excision repair genes, ERCC-1 [66, 67], ERCC-2 [68], ERCC-3 and ERCC-6 [69]. The human O<sup>6</sup> MGMT gene was cloned by transfecting human DNA into *E. coli* cells deficient in alkyltransferase activity and selecting for colonies resistant to alkylating agents. Human Uracil DNA glycosylase was cloned by screening a human cDNA phage library with monoclonal antibodies to the glycosylase.

Initial attempts to identify human nucleotide excision repair genes involved studies with xeroderma pigmentosum (XP) cell lines. Xeroderma pigmentosum is an inherited genetic disorder characterized by a defect in nucleotide excision repair. Cells from patients with XP are assigned to one of nine different complementation groups according to phenotypic improvement in DNA repair following cell fusion studies. Attempts to characterize the genes responsible for the DNA repair deficit in XP cells by transfection of foreign DNA has been unsuccessful partly because human cells are poor recipients of DNA. Therefore, mutant Chinese Hamster Ovarian (CHO) cell lines sensitive to DNA damage were used to identify human nucleotide excision repair genes. Fusion of different CHO complementation mutants with normal human lymphocytes has resulted in correction of the DNA repair deficit and subsequent identification of the responsible human chromosome or gene. The human genes responsible for reversing the defect in DNA repair in CHO cells were termed ERCC for excision repair complementing Chinese Hamster. A number suffix was then attached to indicate the complementation group from which the gene was obtained. Similarly, a human gene that reverses mutant CHO cell sensitivity to irradiation has been termed XRCC-1 (x-ray complementing Chinese Hamster) and assigned to human chromosome 19 [70].

The evolutionary importance and conservation of DNA repair genes is apparent from the DNA sequence of the human ERCC-1 gene which has homology to both yeast and bacterial nucleotide excision repair genes [71, 72]. Furthermore, transfection of a bacterial direct repair gene (O<sup>6</sup> methylguanine methyl transferase) into human cells confers upon those cells resistance to DNA methylating agents [73, 74].

### Role of DNA repair in drug resistance

Drug resistance in some human cell lines has been associated with changes in DNA repair. Examples are listed in Table 1. Human tumor cells which are deficient in O<sup>6</sup> alkylguanine alkyltransferase are unable to repair methylation of the O<sup>6</sup> position of guanine [75, 76]. These cells are termed Mer (-) (methyl excision repair deficient). Cells that are methyl excision repair proficient are termed Mer (+). Mer (-) cells in comparison to Mer (+) cells are hypersensitive to alkylating agents such as the nitrosoureas [76, 77]. In vitro studies of

Table 1. Antineoplastic drug resistance and DNA repair in human cancer cells.

Author (ref.)	Human cancer cell line	Drug (relative resistance)	Comments
Lai [85]	ovarian	cisplatin (1:20)	2-3 fold increase in DNA repair
Masuda [84]	ovarian	cisplatin (1:3)	3 fold increase in DNA repair
Bedford [83]	bladder/testicular	cisplatin (1:5)	sensitive cells deficient in DNA repair
Gerson [80]	leukemic	nitrosourea	increased alkyltransferase
Lazo [93]	head/neck, squamous	bleomycin (1:4-21)	variable increase in DNA repair
Urade [94]	cervical	bleomycin (1:20)	increased DNA repair
Maynard [78]	melanoma	mtic (1:5-10)	increased alkyltransferase
Torres-garcia [87]	lymphocytes	melphalan	cells from patients resistant to melphalan repaired DNA in 24 hours; cells from patients sensitive to melphalan had no measurable DNA repair
Batist [88]	breast	melphalan (1:3)	enhanced DNA repair

human tumor cell strains have demonstrated a correlation between the level of O<sup>6</sup> alkylguanine transferase and resistance to both 5-(3-methyl-1-triazeno) imidazole-4-carboxamide (MTIC) [78] and chloroethylnitrosoureas [79-82]. In vivo data in patients with chronic myelogenous leukemia has also demonstrated that a correlation exists between the level of O<sup>6</sup> alkylguanine transferase in peripheral blood lymphocytes and cellular resistance to nitrosoureas [80].

DNA nucleotide excision repair is also altered in some drug resistant human cell lines. A human testicular cell line hypersensitive to cisplatin has been shown to be deficient in DNA repair [83]. Human ovarian cells, selected in vitro for resistance to cisplatin, remove platinum adducts more efficiently than their drug sensitive counterparts [84]. In addition, ovarian cell lines have been established from the same patient before and after treatment with cisplatin [85]. Compared to the pretreatment cell line, the post treatment cell line shows 2-3 fold increased drug resistance and a 3-fold increase in DNA repair. Furthermore, expression of the human DNA repair gene ERCC-1 has been shown in a preliminary study to correlate with resistance to cisplatin in human ovarian cancer [86]. In patients with chronic lymphocytic leukemia (CLL) who are resistant to treatment with melphalan, DNA repair in peripheral lymphocytes is completed within 24 hours of treatment [87]. On the other hand, previously untreated CLL pa-

tients demonstrate no DNA repair 24 hours after exposure to melphalan [87]. A breast cancer cell line previously selected for a three fold resistance to melphalan has a rate of DNA repair 2–3 fold higher than in sensitive cells [88]. Therefore, both in vitro and in vivo evidence suggest that human tumor resistance to antineoplastic drugs correlates with efficiency of DNA repair. One aspect that needs attention, however, is that many of the above studies are done at equimolar rather than equitoxic drug levels. They optimally need to be done at both levels.

An association between the level of cisplatin adducts in the DNA of peripheral white blood cells (WBC) and ovarian [89] or testicular tumor [90] response to platinum drug based chemotherapy has been reported. The difference in the number of adducts between responsive and unresponsive tumors may be due to differences in DNA repair. The data suggest that patients with ovarian or testicular cancer who do not respond to treatment form low levels of adducts in WBC or tumor cells. Conversely, patients who do form the highest levels of WBC DNA-adducts have a better chance of responding to treatment. These data also imply that a patient's tumor response to platinum based chemotherapy is a function of innate capacity to repair DNA, a property common to tumor and host, and may be determined by inheritance or as a function of exposure to environmental factors prior to treatment. The tumor response may be predicted during the first few cycles of treatment by measuring peripheral WBC DNA platinum adducts.

The DNA repair polymerase, polymerase  $\beta$ , which fills in the gaps following excision of damaged nucleotides has also been associated with drug resistance [91]. Elevated DNA polymerase  $\beta$  in murine leukemic cells correlates with resistance to cisplatin, melphalan, BCNU, and chlorambucil [91]. Resistance to adriamycin [92] and bleomycin [93, 94] also occurs by means of more efficient DNA repair although the exact enzyme(s) involved is unclear.

The increase in DNA repair is not always sufficient to account for the level of drug resistance [92, 95]. The lack of a direct correlation between DNA repair and drug resistance is probably due to other mechanisms of resistance such as increased rates of drug efflux as determined by levels of P-glycoprotein [96], or by increased drug inactivation by glutathione [97] or metallothionein [98]. Alternatively, DNA repair in human tumor drug resistance has only been studied at the level of overall genome; at this level, gene specific DNA repair changes would not be detected. Further studies of DNA repair in specific genes in sensitive and resistant cells are likely to demonstrate interesting correlations. Drug resistance may relate to changes in gene specific repair, strand specific repair, or mitochondrial DNA repair. These types of repair need to be examined in pleiotropic drug resistant cell lines.

## Inhibitors of DNA repair

Modulation of DNA repair may become a promising method of circumventing drug resistance (Figure 2). In general, significant DNA damage is accompanied by arrest of the cell cycle in G2. Prolongation of the pre-DNA synthesis G2 phase allows more time for DNA repair prior to DNA replication. Methylxanthines such as caffeine prevent cells from arresting in G2 [99, 100] and therefore may limit the time available for DNA repair.

Caffeine also has a myriad of other effects which are not completely understood. For example, in *E. coli*, caffeine has been shown to alter the damage site-specific binding of excision repair enzymes [101]. Whether this also occurs in eukaryotic cells is unknown. Caffeine has also been reported to enhance the cytotoxicity of cyclophosphamide and melphalan in mammalian cells [102, 103]. However, some studies have questioned the enhancement of antineoplastic cytotoxicity by methylxanthines [104].

Individual DNA repair enzymes may also be inhibited. Removal of the alkyl group from the O<sup>6</sup> position of guanine may be blocked by inhibition of O<sup>6</sup> alkyltransferase (O<sup>6</sup> AT) activity. Since O<sup>6</sup> alkyltransferase is a suicide enzyme, agents such as streptozotocin and O<sup>6</sup> methylguanine, that methylate the enzyme's active site, deplete its activity. Streptozotocin is an alkylating agent that methylates guanine resulting in O<sup>6</sup> methylguanine adducts. Repair of each adduct stoichiometrically inactivates one O<sup>6</sup> alkyltransferase enzyme. On the other hand, directly treating cells with O<sup>6</sup> methyl-

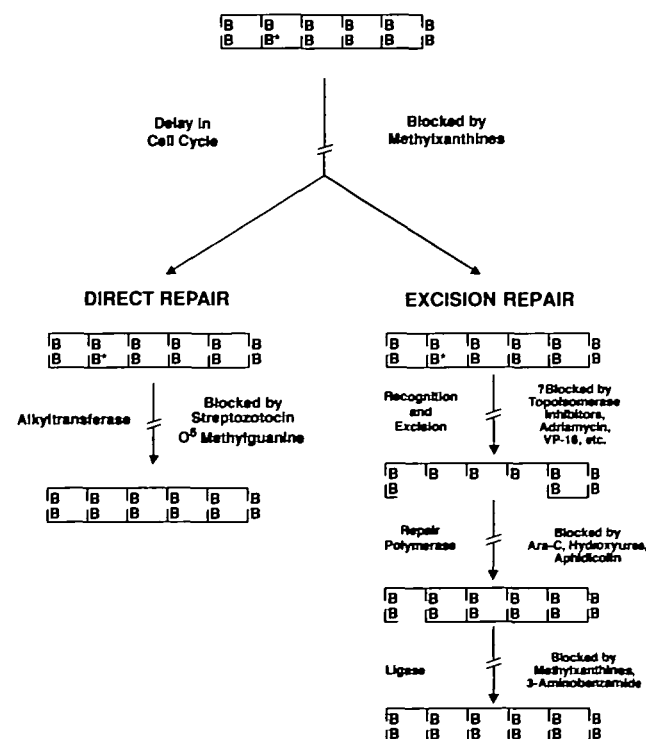


Fig. 2. Possible sites for therapeutical inhibition of DNA repair.

guanine is also effective in reducing O<sup>6</sup> AT activity [105]. In vitro studies of myeloid leukemic cells pretreated with O<sup>6</sup> methylguanine have shown a 87% reduction of O<sup>6</sup> AT activity and a 6.3 fold decrease in the lethal (LD50) dose of nitrosoureas [80]. Furthermore, in vivo human studies have shown that daily treatment with streptozotocin for 3 days reduces O<sup>6</sup> AT activity in peripheral lymphocytes by 26% [106].

The topology of DNA may also be important in DNA repair [107, 108]. DNA topoisomerases are enzymes which are involved in the coiling and relaxation or twisting and untwisting of DNA [109]. Torsional stresses placed upon DNA by replication and transcription, are believed to be relieved by topoisomerase mediated relaxation of torsional twists. Topoisomerase I allows relaxation by cleaving, then religating one strand of the double helix. Topoisomerase II, which is the eukaryotic counterpart to bacterial gyrase, cleaves, relaxes and then religates both strands of DNA. Topoisomerase inhibitors may stabilize the usually transient enzyme DNA cleavage complex. The topoisomerase II DNA cleavage complex is stabilized by amsacrine (mAMSA), adriamycin, etoposide (VP-16) and teniposide (VM-26) [109]. The topoisomerase I DNA cleavage complex is stabilized by camptothecin [110]. Alternatively, novobiocin and merbarone are topoisomerase inhibitors that decrease topoisomerase activity through an unknown mechanism but probably not by stabilization of the cleavage complex [107, 111]. The topoisomerase inhibitors novobiocin, VP-16 and mAMSA have been shown to inhibit repair of DNA damage [112, 113]. The combination of a topoisomerase I inhibitor (camptothecin) and a topoisomerase II inhibitor (merbarone) has also been found inhibit gene specific repair (Stevnsner and Bohr, MS submitted). Interestingly, cytokines such as tumor necrosis factor (TNF), potentiate mAMSA, VP-16, and VM-26 inhibition of topoisomerase II [114]. Therefore TNF and other cytokines may also play a role in inhibition of DNA repair.

Excision repair pathways may also be inhibited by modulation of the repair polymerization step. There are four mammalian DNA polymerases ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) [115]. DNA polymerase  $\gamma$  is found only in mitochondria [115]. DNA polymerase  $\alpha$  and  $\delta$  are mainly involved in DNA replication [115], but can also play a role in DNA repair in some systems [115–117]. The main DNA repair polymerases is thought to be polymerase  $\beta$  [115]. Two antineoplastic agents which may inhibit DNA repair polymerase are hydroxyurea and cytosine arabinoside (ara-c). Hydroxyurea inhibits ribonucleotide reductase [118] which is necessary to maintain an intracellular pool of deoxyribonucleotide triphosphates. Hydroxyurea, therefore, depletes the cell of the building block precursors required to polymerize DNA. Cytosine arabinoside is an analog of deoxycytidine which, when incorporated into DNA, causes chain termination [4]. Although hydroxyurea alone is not generally considered an inhibitor of DNA repair, the

combination of both hydroxyurea and ara-c inhibits repair of cisplatin induced DNA damage in a human colon cell line [119]. Another compound, aphidicolin, can inhibit DNA repair polymerase  $\alpha$  and  $\delta$  [120], and it inhibits repair of cisplatin adducts in a human ovarian cell line [85].

The final step in DNA excision repair pathways is ligation of the newly synthesized oligonucleotide strand to the undamaged original strand by a distinct enzyme, ligase II [121]. The activity of ligase II appears in part to be dependent upon posttranslational modification by ADP ribosylation [121]. ADP ribosylation is a ubiquitous mechanism by which cells regulate protein activity [122]. It has been shown that ADP ribosylation participates in the repair of DNA damage caused by alkylating agents [123]. Many proteins other than ligase II that are likely to be involved in DNA repair such as topoisomerases [124], histones [125] and ERCC-1 [126], may also undergo ADP ribosylation. Although the exact role of poly(ADP)ribosylation in DNA excision repair is unclear, it may be important. Common agents used to inhibit nuclear ADP-ribosylation such as 3-aminobenzamide and methylxanthines increase the toxicity of alkylating agents like dimethyl sulfate [127] and MTIC [128].

Finally both heat and depletion of cellular amines may function by ill defined mechanisms to modulate DNA repair. Polyamines such as putrescine, spermine and spermidine are positively charged at physiological pH and bind with negatively charged DNA [129]. Cellular polyamine levels may be depleted by inhibiting synthesis with  $\alpha$  difluoromethylornithine (DFMO) or methylglyoxal-bis (guanyldrazone) (MGBG) [130]. Polyamine depletion has been documented to increase the cytotoxicity of L-phenylalanine mustard [131] and BCNU [132]. This effect has been speculated to be at least in part due to altered DNA repair [131]. In addition, polyamine depleted cells are deficient in repair of x-ray induced DNA damage [133]. Although the interaction of polyamines and DNA repair pathways is poorly understood, depletion of polyamines causes perturbations of the cell cycle and changes in DNA conformation both of which could affect DNA repair.

Mild hyperthermia (40–43°C) also has been demonstrated to sensitize cells to nitrosoureas [134], cisplatin [135, 136], mitomycin C [135], bleomycins [137] and x-ray irradiation [138–141]. Thermal enhanced sensitivity of cells to bleomycin and x-ray irradiation has been shown to be due to inhibition of DNA repair [137, 142–145]. Heat, however, causes alterations in tumor blood flow and multiple cellular disturbances including alterations in DNA and protein conformation, changes in cytoplasmic membrane fluidity, and depletion of intracellular polyamines any one of which may alter a tumor's sensitivity to chemotherapy [146].

## Acknowledgements

We thank Drs. Snorri Thorgeirsson and Bruce Chabner for helpful comments.

## References

- Heidelberger C, Chaudhuri NK, Danneberg P et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* 1957; 179: 663–6.
- Chabner BA and Collins JM (eds). *Cancer Chemotherapy Principles and Practices*. Fluorinated Pyrimidines. Grem JL, Lippincott Co, Philadelphia. 1990; 180–225.
- Winicov I and Perry RP. Mechanisms of DNA synthesis inhibition by arabinosyl cytosine and arabinosyl adenine. *Nature* 1976; 264: 679–680.
- Chabner BA and Collins JM (eds). *Cancer chemotherapy Principles and Practices*. Cytidine Analogs. Chabner BA. Lippincott Co., Philadelphia. 1990: 341–56.
- Chabner BA and Collins JM (eds). *Cancer Chemotherapy Principles and Practice*. Bleomycin. Chabner BA. Lippincott Co., Philadelphia. 1990: 341–56.
- Lown JW and Chen H-H. Evidence for the generation of free hydroxyl radicals from certain quinone antitumor antibiotics upon reductive activation in solution. *Can J Chem*. 1981; 59: 390–5.
- Kalyanaraman B, Perez-Reyes E, Mason RP. Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anticancer drugs. *Biochim Biophys Acta* 1980; 630: 119–300.
- Doroshov JH. Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones. *Proc Natl Acad Sci USA* 1986; 83: 4514–8.
- Pritsos CA and Sartorelli AC. Generation of reactive oxygen radicals through bioactivation of mitomycin antibiotics. *Cancer Res* 1986; 46: 3528–32.
- Tewey KM, Rowe TC, Yang L et al. Adriamycin induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 1984; 226: 466–8.
- Chabner BA and Collins JM (eds). *Cancer Chemotherapy Principles and Practice*. Alkylating Agents. Colvin M and Chabner BA. Lippincott Co., Philadelphia. 1990: pp. 276–314.
- Chabner BA and Collings JM (eds). *Cancer Chemotherapy Principles and Practice*. Platinum analogs. Reed E and Kohn KW. Lippincott Co. 1990: 465–91.
- Loveless A. Possible relevance of O<sup>6</sup> alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. *Nature* 1969; 223: 206–7.
- Brookes P and Lawley PD. The reaction of mono- and difunctional alkylating agents with nucleic acids. *Biochem J* 1961; 80: 486–503.
- Dijt FJ, Fichtinger-Schepman AMJ, Berends F et al. Formation and repair of cisplatin-induced adducts to DNA in cultured normal and repair-deficient human fibroblasts. *Cancer Res* 1988; 48: 6058–62.
- Sherman SE, Gibson D, Wang A H-J et al. X-ray structure of the major adduct of the anticancer drug cisplatin with DNA: cis [Pt (NH<sub>3</sub>)<sub>2</sub> {d(pGpG)}]. *Science* 1985; 230: 412–7.
- Friedberg EC. *DNA repair*. WH Freeman and Co., NY. 1985: 614 pp.
- Sancar A and Sancar GB. DNA repair enzymes. *Ann Rev Biochem* 1988; 57: 29–67.
- Grossman L. Enzymes involved in the repair of damaged DNA. *Arch Biochem Biophys* 1981; 211: 511–22.
- Lindahl T and Sedgwick B. Regulation and expression of the adaptive response to alkylating agents. *Ann Rev Biochem* 1988; 57: 133–57.
- Demple B, Jacobsson A, Olsson M et al. Repair of alkylated DNA in *Escherichia coli*. Physical properties of O<sup>6</sup>-methylguanine-DNA methyltransferase. *J Biol Chem* 1982; 257: 13776–80.
- Roberts JJ. Cellular responses to carcinogen-induced DNA damage and the role of DNA repair. *Brit Med Bull* 1980; 36: 25–31.
- Van Houten B. Nucleotide Excision Repair in *Escherichia coli*. *Microbiological Reviews* 1990; 54: 18–51.
- Zwetsloot JC, Barbeiro AP, Vermeulen HM et al. Microinjection of *Escherichia coli* UrvA, B, C and D proteins into fibroblasts of xeroderma pigmentosum complementation groups A and C does not result in restoration of UV-induced unscheduled DNA synthesis. *Mutat Res* 1985; 166: 89–98.
- Hanawalt PC, Cooper PK, Ganesan AK et al. DNA repair in bacteria and mammalian cells. *Ann Rev Biochem* 1979; 48: 783–836.
- Walker GC. Inducible DNA repair systems. *Ann Rev Biochem* 1985; 54: 425–57.
- Lindahl T and Sedgwick B. Regulation and expression of the adaptive response to alkylating agents. *Ann Rev Biochem* 1988; 57: 133–57.
- Nakabeppu Y, Sekiguchi M. Regulatory mechanisms for induction of synthesis of repair enzymes in response to alkylating agents: ada protein acts as a transcriptional regulator. *Proc Natl Acad Sci USA* 1986; 83: 6297–301.
- Teo I, Sedgwick B, Kilpatrick MW et al. The intracellular signal for induction of resistance to alkylating agents in *E. coli*. *Cell* 1986; 45: 315–24.
- Little JW, Mount DW. The SOS regulatory system of *Escherichia coli*. *Cell* 1982; 11–22.
- Craig NL, Roberts JW. *E. coli* recA protein-directed cleavage of phage  $\lambda$  repressor requires polynucleotide. *Nature* 1980; 283: 26–30.
- Gudas LJ, Pardee AB. Model for regulation of *Escherichia coli* DNA repair functions. *Proc Natl Acad Sci USA* 1975; 72: 2330–4.
- Walker GC. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol Rev* 1984; 48: 60–93.
- Howard-Flanders P, Boyce RP, Theriot L. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. *Genetics* 1966; 53: 1119–36.
- Cole ST. Characterization of the promoter for the *lex A* regulated *sul A* gene of *Escherichia coli*. *Mol Gen Genet* 1983; 189: 400–4.
- Huisman O, D'ari R. An inducible DNA replication-cell division coupling mechanism in *E. coli*. *Nature* 1981; 290: 797–9.
- Frei E, Cucchi CA, Rosowsky A et al. Alkylating agent resistance: In vitro studies with human cell lines. *Proc Natl Acad Sci USA* 1985; 82: 2158–62.
- Teicher BA, Cucchi CA, Lee JB et al. Alkylating agents: In vitro studies of cross-resistance patterns in human cell lines. *Cancer Res* 1986; 46: 4379–83.
- Teicher BA, Frei E. Development of alkylating agent-resistant human tumor cell lines. *Cancer Chemother Pharmacol* 1988; 21: 292–8.
- Montesano R, Bresil H, Margison GP. Increased excision of O<sup>5</sup>-methylguanine from rat liver DNA after chronic administration of dimethylnitrosamine. *Cancer Res* 1979; 39: 1798–1802.
- Protic M, Roilides E, Levin AS et al. Enhancement of DNA repair capacity of mammalian cells by carcinogen treatment. Somatic cell and Mol Genet 1988; 14: 351–7.
- Wang Y, Maher VM, Liskay RM et al. Carcinogens can induce homologous recombination between duplicated chromosomal sequences in mouse L cells. *Mol Cell Biol* 1988; 8: 196–202.
- Forance AJ, Alamo I, Hollander MC. DNA damage-inducible transcripts in mammalian cells. *Proc Natl Acad Sci USA* 1988; 85: 8800–4.



44. Hollander MC, Forance AJ. Induction of fos RNA by DNA-damaging agents. *Cancer Res* 1989; 49: 1687-92.
45. Fornace AJ, Schalch H, Alamo I. Coordinate induction of metallothioneins I and II in rodent cells by UV irradiation. *Mol Cell Biol* 1988; 8: 4716-20.
46. Fornace AJ, Alamo I, Hollander MC et al. Ubiquitin mRNA is a major stress-induced transcript in mammalian cells. *Nucleic Acids Res* 1989; 17: 1215-30.
47. Fornace AJ, Nebert DW, Hollander MC et al. Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Mol Cell Biol* 1989; 9: 4196-203.
48. Fornace AJ, Zmudzka B, Hollander MC et al. Induction of  $\beta$ -polymerase mRNA by DNA-damaging agents in Chinese hamster ovary cells. *Mol Cell Biol* 1989; 9: 851-3.
49. Mellon I, Bohr VA, Smith CA et al. Preferential DNA repair of an active gene in human cells. *Proc Natl Acad Sci USA* 1986; 83: 8878-82.
50. Bohr VA, Smith CA, Okumoto DS et al. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* 1985; 40: 359-69.
51. Madhani HD, Bohr VA, Hanawalt PC. Differential DNA repair in transcriptionally active and inactive proto-oncogenes: c-abl and c-mos. *Cell* 1986; 45: 417-23.
52. Okumoto DS, Bohr VA. DNA repair in the metallothionein gene increases with transcriptional activation. *Nucl Acids Res* 1987; 15: 10021-30.
53. Leadon SA, Snowden MM. Differential repair of DNA damage in the human metallothionein gene family. *Mol Cell Biol* 1988; 8: 5331-8.
54. Venema J, Mullenders LHF, Natarajan AT, Van Zeeland AA, Mayne LV. The genetic defect in Cockayne Syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. *Proc Natl Acad Sci USA* 1990; 87: 4707-11.
55. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* 1987; 51: 241-9.
56. Mellon I, Hanawalt PC. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* 1989; 342: 95-8.
57. Nass S. The significance of the structural and functional similarities of bacteria and mitochondria. *Int Rev Cytol* 1969; 25: 55-120.
58. Allen JA, Coombs MM. Covalent binding of polycyclic aromatic compounds to mitochondrial and nuclear DNA. *Nature* 1980; 287: 244-5.
59. Niranjana BG, Bhat NK, Avadhani NG. Preferential attack of mitochondrial DNA by aflatoxin B, during hepatocarcinogenesis. *Science* 1981; 215: 73-5.
60. Backer JM, Weinstein IB. Mitochondrial DNA is a major cellular target for a dihydrodiol-epoxide derivative of benzo[a]pyrene. *Science* 1980; 209: 297-9.
61. Clayton DA, Doda JN, Freidberg EC. Absence of pyrimidine dimer repair mechanisms for mitochondrial DNA in mouse and human cells. *Basic Life Sci* 1975; 5: 589-91.
62. Satoh MS, Huh N, Rajewsky MF et al. Enzymatic removal of O<sup>6</sup>-ethylguanine from mitochondrial DNA in rat tissues exposed to N-ethyl-N-nitrosourea in vivo. *J Biol Chem* 1988; 263: 6854-6.
63. Myers KA, Saffhill R, O'Connor PJ. Repair of alkylated purines in the hepatic DNA of mitochondria and nuclei in the rat. *Carcinogenesis* 1988; 9: 285-92.
64. Tano K, Shiota S, Collier J et al. Isolation and structural characterization of a cDNA clone encoding the human DNA repair protein for O<sup>6</sup>-alkylguanine. *Proc Natl Acad Sci USA* 1990; 87: 686-90.
65. Vollberg TM, Siegler KM, Cool BL et al. Isolation and characterization of the human uracil DNA glycosylase gene. *Proc Natl Acad Sci USA* 1989; 86: 8693-7.
66. Westerveld A, Hoeijmakers JHJ, Van Duin M et al. Molecular cloning of a human DNA repair gene. *Nature* 1984; 310: 425-9.
67. Van Duin M, De Wit J, Odijk H et al. Molecular characterization of the human excision repair gene ERCC-1: cDNA cloning and amino acid homology with the yeast DNA repair gene RAD10. *Cell* 1986; 44: 913-23.
68. Weber CA, Salazar EP, Stewart SA et al. Molecular cloning and biological characterization of a human gene, ERCC 2, that corrects the nucleotide excision repair defect in CHO UV5 cells. *Mol Cell Biol* 1988; 8: 1137-46.
69. Hoeijmakers JH, Weeda G, Troelstra C, Van Duin M, Westerveld A, Van Der Eb A, Bootsma D. Molecular genetic dissection of mammalian excision repair. In DNA repair and their biological implications. Lambert M and Laval J (eds). Plenum Publ, NY, 1989; 563-74.
70. Siciliano MJ, Carrano AV, Thompson LH. Assignment of a human DNA-repair gene associated with sister-chromatid exchange to chromosome 19. *Mutat Res* 1986; 174: 303-8.
71. Downes CS. Views of unity and diversity. *Nature* 1988; 332: 208-209.
72. Van Den Tol DJ, Warmerdam P, Odijk H et al. Evolution and denaturation of the mammalian excision repair gene ERCC-1. *Nucleic Acids Res* 1988; 16: 5305-22.
73. Margison GP, Brennan J. Functional expression of the *Escherichia coli* alkyltransferase gene in mammalian cells. *J Cell Sci Suppl* 1987; 6: 83-96.
74. Waldstein EA. Effect of O<sup>6</sup>-methylguanine-DNA methyltransferase gene activity on repair in human cells transformed by a regulatable ada gene. *Charcinogenesis* 1990; 11: 21-6.
75. Yarosh DB, Foote RS, Mitra S et al. Repair of O<sup>6</sup>-methylguanine in DNA by demethylation is lacking in Mer- human tumor cell strains. *Carcinogenesis* 1983; 4: 199-205.
76. Day RS, Ziolkowski CHJ, Scudiero DA et al. Human tumor cell strains defective in the repair of alkylation damage. *Carcinogenesis* 1980; 1: 21-32.
77. Domoradzki J, Pegg AE, Dolan ME et al. Correlation between O<sup>6</sup>-methylguanine-DNA-methyltransferase activity and resistance of human cells to the cytotoxic and mutagenic effect of N-methyl-N'-nitro-N-nitrosoguanidine. *Carcinogenesis* 1984; 5: 1641-7.
78. Maynard K, Parsons PG, Cerny T et al. Relationships among cell survival, O<sup>6</sup>-alkylguanine-DNA alkyltransferase activity, and reactivation of methylated adenovirus 5 and Herpes simplex virus type 1 in human melanoma cell lines. *Cancer Res* 1989; 49: 4813-7.
79. Erickson LC, Laurent G, Sharkey NA et al. DNA cross-linking and mono-adduct repair in nitrosourea-treated human tumour cells. *Nature* 1980; 288: 727-9.
80. Gerson SSL, Trey JE. Modulation of nitrosourea resistance in myeloid leukemias. *Blood* 1988; 71: 1487-94.
81. D'incalci M, Citti L, Taverna P et al. Importance of the DNA repair enzyme O<sup>6</sup>-alkyl guanine alkyltransferase (AT) in cancer chemotherapy. *Cancer Treatment Rev* 1988; 15: 279-92.
82. Bodell WJ, Tokuda K, Ludlum DB. Differences in DNA alkylation products formed in sensitive and resistant human glioma cells treated with N-(2-chloroethyl)-N-nitrosourea. *Cancer Res* 1988; 48: 4489-92.
83. Bedford P, Fichtinger-Schepman AMJ, Shellard SA et al. Differential repair of platinum-DNA adducts in human bladder and testicular tumor continuous cell lines. *Cancer Res* 1988; 48: 3019-24.
84. Masuda H, Ozols RF, Lai G-M et al. Increased DNA repair as a mechanism of acquired resistance to cis-diamminedichloroplatinum (II) in human ovarian cancer cell lines. *Cancer Res* 1988; 48: 5713-6.
85. Lai G-M, Ozols RF, Smyth JF et al. Enhanced DNA repair and resistance to cisplatin in human ovarian cancer. *Biochem Pharmacol* 1988; 37: 4597-600.



86. Reed E, Ormond P, Bohr VA et al. Expression of the human DNA repair gene ERCC-1 relates to cisplatin drug resistance in human ovarian cancer cells. *Amer Ass Can Res (abst 1940)*, 1989.
87. Torres-Garcia SJ, Cousineau L, Caplan S et al. Correlation of resistance to nitrogen mustards in chronic lymphocytic leukemia with enhanced removal of melphalan-induced cross-links. *Biochem Pharm* 1989; 38: 3122-3.
88. Baptist G, Torres-Garcia JM, Demuys J-M et al. Enhanced DNA cross-link removal: The apparent mechanism of resistance in clinically relevant melphalan resistant human breast cancer cell line. *Mol Pharm* 1989; 36: 224-30.
89. Reed E, Ozols RF, Tarone R et al. Platinum-DNA adducts in leukocyte DNA correlate with disease response in ovarian cancer patients receiving platinum-based chemotherapy. *Proc Natl Acad Sci USA* 1987; 84: 5024-8.
90. Reed E, Ozols RF, Tarone R et al. The measurement of cisplatin-DNA adduct levels in testicular cancer patients. *Carcinogenesis* 1988; 9: 1909-11.
91. Kraker AJ, Moore CW. Elevated DNA polymerase  $\beta$  activity in a cis-diamminedichloroplatinum (II) resistant P388 murine leukemia cell line. *Cancer Letters* 1988; 38: 307-14.
92. Deffie AM, Alam T, Seneviratne C et al. Multifactorial resistance to adriamycin: Relationship of DNA repair, glutathione transferase activity, drug efflux, and P-glycoprotein in cloned cell lines of adriamycin-sensitive and resistant P388 leukemia. *Cancer Res* 1988; 48: 3595-602.
93. Lazo JS, Braun ID, Larabee DC et al. Characteristics of bleomycin resistant phenotypes of human cell sublines and circumvention of bleomycin resistance by liblomycin. *Cancer Res* 1989; 49: 185-90.
94. Urade M, Sugi M, Matsuya T. Further characterization of bleomycin resistant HELA cells and analysis of resistance mechanisms. *Jpn J cancer Res* 1988; 79: 491-500.
95. Eastman A, Schulte N. Enhanced DNA repair as a mechanism of resistance to cis-diamminedichloroplatinum (II). *Biochemistry* 1988; 27: 4730-4.
96. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Ann Rev Biochem* 1989; 58: 137-71.
97. Kramer RA, Zakher J, Kim G. Role of the glutathione redox cycle in acquired and de novo multidrug resistance. *Science* 1988; 241: 694-7.
98. Kelley SL, Basu A, Teicher BA et al. Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1988; 241: 1813-5.
99. Fingert HJ, Chang JD, Pardee AB. Cytotoxic, cell cycle, and chromosomal effects of methylxanthines in human tumor cells treated with alkylating agents. *Cancer Res* 1986; 46: 2463-7.
100. Faed MJW, Mourelatos D. Enhancement by caffeine of sister-chromatid exchange frequency in lymphocytes from normal subjects after treatment by mutagens. *Mutat Res* 1978; 49: 437-40.
101. Selby CP, Sancar A. Molecular mechanisms of DNA repair inhibition by caffeine. *Proc Natl Acad Sci* 1990; 87: 3522-5.
102. Mourelatos D, Dozi-Vassiliades J, Kotsis A et al. Enhancement of cytogenetic damage and of antineoplastic effect by caffeine in Erlich ascites tumor cells treated with cyclophosphamide in vivo. *Cancer Res* 1988; 48: 1129-31.
103. Byfield JE, Murnane J, Ward JF et al. Mice, men, mustards and methylated xanthines: The potential role of caffeine and related drugs in the sensitization of human tumours to alkylating agents. *Br J Cancer* 1981; 43: 669-83.
104. Kato H. Induction of sister chromatid exchanges by chemical mutagens and its possible relevance to DNA repair. *Exp Cell Res* 1974 85: 239-47.
105. Gerson SL, Trey JE, Miller K. Potentiation of nitrosourea cytotoxicity in human leukemic cells by inactivation of O<sup>6</sup>-alkylguanine-DNA alkyltransferase. *Cancer Res* 1988; 48: 1521-7.
106. Gerson SL. Modulation of human lymphocyte O<sup>6</sup>-alkylguanine-DNA alkyltransferase by streptozotocin in vivo. *Cancer Res* 1989; 49: 3134-8.
107. Downes CS, Johnson RT. DNA topoisomerases and DNA repair. *Bioessays* 1988; 8: 179-84.
108. Mattern MR, Paone RF, Day RS. Eukaryotic DNA repair is blocked at different steps by inhibitors of DNA topoisomerases and of DNA polymerases  $\alpha$  and  $\beta$ . *Biochim Biophys Acta* 1982; 697: 6-13.
109. Liu LF. DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 1989; 58: 351-75.
110. Hsiang YH, Hertzberg R, Hecht S et al. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985; 260: 14873-8.
111. Drake FH, Hofmann GA, Mong S-M et al. In vitro and intracellular inhibition of topoisomerase II by antitumor agent merbarone. *Cancer Res* 1989; 49: 2578-83.
112. Mattern MR, Scudiero DA. Dependence of mammalian DNA synthesis on DNA supercoiling III. Characterization of the inhibition of replicative and repair-type DNA synthesis by novobicin and nalidixic acid. *Biochim Biophys Acta* 1981; 653: 248-58.
113. Bohr V, Mansbridge J, Hanawalt P. Comparative effects of growth inhibitors on DNA replication, DNA repair, and protein synthesis in human epidermal keratinocytes. *Cancer Res* 1986; 46: 2929-35.
114. Utsugi T, Mattern MR, Mirabelli CK et al. Potentiation of topoisomerase inhibitor-induced DNA strand breakage and cytotoxicity by TNF. *Cancer Res* 1990; 50: 2636-40.
115. Fry M, Loeb LA. In: *Animal cell DNA polymerases*. CRC Press Boca Raton, Florida 1986; 221.
116. Dresler SL, Gowans BJ, Robinson-Hill RM et al. Involvement of DNA polymerase  $\delta$  in DNA repair synthesis in human fibroblasts at late times after ultraviolet irradiation. *Biochemistry* 1988; 27: 6379-83.
117. Nishida C, Reinhard P, Linn S. DNA repair synthesis in human fibroblasts requires DNA polymerase  $\delta$ . *J Biol Chem* 1988; 263: 501-10.
118. Danehower RC. Hydroxyurea. In: *Cancer Chemotherapy Principles and Practice*, Eds. Chabner BA, Collins JM. Lippincott Co., Philadelphia. 1990: 225.
119. Swinnen LJ, Barnes DM, Fisher SG et al. 1- $\beta$ -D-Arabinofuranosylcytosine and hydroxyurea production of cytotoxic synergy with cis-diamminedichloroplatinum (II) and modification of platinum-induced DNA interstrand cross-linking. *Cancer Res* 1989; 49: 1383-9.
120. Lee MYWT, Toomey NL, Wright GE. Differential inhibition of placental DNA polymerases  $\delta$  and  $\alpha$  by BuPdGTP and BuAdATP. *Nucl Acid Res* 1985; 13: 8623-30.
121. Creissen D, Shall S. Regulation of DNA ligase activity by poly (ADP-ribose). *Nature* 1982; 296: 271-2.
122. Shall S. ADP ribosylation of proteins: a ubiquitous cellular control mechanism. *Biochem Soc Trans* 1989; 17: 317-22.
123. Durkacz BW, Omidiji O, Gray DA et al. (ADP-ribose)<sub>n</sub> participates in DNA excision repair. *Nature* 1980; 283: 593-5.
124. Jongstra-Bilen J, Ittel M-E, Niedergang C et al. DNA topoisomerase I from calf thymus is inhibited in vitro by poly (ADP-ribosylation). *Biochemistry* 1983; 136: 391-6.
125. Ogata N, Ueda K, Hayaishi O. ADP-ribosylation of histone H2B. *J Biol Chem* 1980; 255: 7610-5.
126. Hoeijmakers JJ. Characterization of genes and proteins involved in excision repair of human cells. *J Cell Sci Suppl* 1987; 6: 111-25.
127. James MR, Lehmann AR. Role of poly(adenosine diphosphate ribose) in deoxyribonucleic acid repair in human fibroblasts. *Biochemistry* 1982; 21: 4007-13.
128. Hayward IP, Parsons PG. Epigenetic effects of the methylating agent 5-(3-methyl-1-triazeno)imidazole-4-carboxamide in human melanoma cells. *Aust J Exp Biol Med Sci* 1984; 62: 597-606.

129. Bloomfield VA, Wilson RW. Interaction of polyamines with polynucleotides. In: Polyamines in Biology and Medicine. Morris DR and Martin LJ (eds). Marcel Dekker, New York. 1981; 183–206.
130. Pegg AE. Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem J* 1986; 234: 249–62.
131. Ducore JM, McNamara L. Effect of  $\alpha$ -difluoromethylornithine on 1-phenylalanine mustard-induced cytotoxicity and DNA interstrand cross-linking in a human cell line in vitro. *Cancer Res* 1986; 46: 1068–72.
132. Hung DT, Deen DF, Seidenfeld J et al. Sensitization of 9L rat brain gliosarcoma cells to 1,3-bis(2-chloroethyl)-1-nitrosourea by  $\alpha$ -difluoromethylornithine, an ornithine decarboxylase inhibitor. *Cancer Res* 1981; 41: 2783–5.
133. Snyder RD, Lachman PJ. Hyperthermia, polyamine depletion, and inhibition of x-ray-induced DNA strand break repair. *Radiat Res* 1989; 120: 121–8.
134. Mülcahy RT, Gipp JJ, Tanner MA. Sensitization of nitrosourea-resistant Mer<sup>+</sup> human tumor cells to N-(2-chloroethyl)-N-cyclohexyl-N-nitrosourea by mild hyperthermia. *Cancer Res* 1988; 48: 1086–90.
135. Murakami A, Koga S, Maeta M. Thermochemosensitivity: Augmentation by hyperthermia of cytotoxicity of anticancer drugs against human colorectal cancers, measured by the human tumor clonogenic assay. *Oncology* 1988; 45: 236–41.
136. Baba H, Siddik Zh, Strelbel FR et al. Increased therapeutic gain of combined cis-diaminedichloroplatinum (II) and whole body hyperthermia therapy by optimal heat drug scheduling. *Cancer Res* 1989; 49: 7041–4.
137. Kubota Y, Nishimura R, Takai S et al. Effect of hyperthermia on DNA single-stranded breaks induced by bleomycin in HeLa cells. *Gann* 1979; 70: 681–5.
138. Murthy AK, Harris JR, Belli JA. Hyperthermia and radiation response of plateau phase cells. *Radiat Res* 1977; 70: 241–7.
139. Raaphorst GP, Freeman ML, Dewey WC. Radiosensitivity and recovery from radiation damage in cultured CHO cells exposed to hyperthermia at 42.5 or 45.5°C. *Radiat Res* 1979; 79: 390–402.
140. Li GC, Evans RG, Hahn GM. Modification and inhibition of repair of potentially lethal x-ray damage by hyperthermia. *Radiat Res* 1976; 67: 491–501.
141. Raaphorst GP, Feeley MM. Comparison of recovery from potentially lethal damage after exposure to hyperthermia and radiation. *Radiat Res* 1990; 121: 107–10.
142. Raaphorst GP, Azzam EI, Feeley M. Potentially lethal radiation damage repair and its inhibition by hyperthermia in normal hamster cells, mouse cells, and transformed mouse cells. *Radiat Res* 1988; 113: 171–82.
143. McGhie JB, Wold E, Pettersen EO et al. Combined electron radiation and hyperthermia. Repair of DNA strand breaks in NHK 3025 cells irradiated and incubated at 37, 42.5 or 45°C. *Radiat Res* 1983; 96: 31–40.
144. Mills MD, Meyn RE. Effects of hyperthermia on repair of radiation-induced DNA strand breaks. *Radiat Res* 1981; 87: 314–28.
145. Jorritsma JBM, Konings AWT. Inhibition of repair of radiation-induced strand breaks by hyperthermia, and its relationship to cell survival after hyperthermia alone. *Int Radiat Biol* 1983; 43: 505–16.
146. Vaupel P, Kallinowski F. Physiological effects of hyperthermia. In: Recent advances in cancer research: Hyperthermia and the therapy of malignant tumors. Streffer C (eds). Springer-Verlag NY 1987: 71–110.

Received 28 January 1991; accepted 8 February 1991.

*Correspondence to:*

Vilhelm A. Bohr, M. D., Ph. D.  
 Laboratory of Molecular Pharmacology  
 National Cancer Institute  
 Bldg. 37 Rm 5c25, NIH  
 Bethesda, MD 20892  
 U.S.A.