MOLECULAR AND CELLULAR BIOLOGY, Jan. 1995, p. 290–297 Vol. 15, No. 1
0270-7306/95/$04.00+0

An *XPG* DNA Repair Defect Causing Mutagen Hypersensitivity in Mouse Leukemia L1210 Cells

JUHANI A. VILPO,† LEENA M. VILPO,† DAVID E. SZYMKOWSKI,‡
ANNE O'DONOVAN, AND RICHARD D. WOOD*

Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Hertfordshire EN6 3LD, United Kingdom

Received 20 June 1994/Returned for modification 24 August 1994/Accepted 28 September 1994

One of the most widely used antitumor drugs is *cis*-diamminedichloroplatinum(II) (cisplatin), and mechanisms of cisplatin resistance have been investigated in numerous model systems. Many studies have used mouse leukemia L1210/0 as a reference wild-type cell line, and cisplatin-resistant subclones have been derived from it. Increased DNA excision repair capacity is thought to play a key role in the acquired cisplatin resistance, and this has influenced development of drugs for clinical trials. We report here that the L1210/0 line is in fact severely deficient in nucleotide excision repair of damaged DNA in vivo and in vitro. L1210/0 cell extracts could be complemented by extracts from repair-defective human xeroderma pigmentosum (XP) or rodent excision repair cross-complementing (ERCC) mutant cells, except for XPG/ERCC5 mutants. Purified XPG protein could restore repair proficiency to L1210/0 extracts. Expression of mouse *XPG* mRNA was similar in all L1210 lines studied, suggesting a point mutation or small alteration of *XPG* in L1210/0 cells. The DNA repair capacity of a cisplatin-resistant subline, L1210/DDP_{10}, is similar to that of type culture collection L1210 cells and to those of other normal mammalian cell lines. Nucleotide excision repair of DNA is thus clearly important in the intrinsic cellular defense against cisplatin. However, in contrast to what is generally believed, enhancement of DNA repair above the normal level in these rodent cells does not appear to be a mechanism of acquired resistance to the drug.

The drug *cis*-diamminedichloroplatinum(II) (cisplatin) has become a major anticancer agent, particularly for the treatment of testicular (40) and ovarian (41) carcinomas. Currently, more than 90% of testicular cancers are cured, a success that is largely the consequence of chemotherapy by cisplatin (40). The cytotoxic effect is thought to be mediated by platinum-DNA adducts that inhibit DNA replication and transcription (10). A major limitation to the clinical efficacy of cisplatin is the intrinsic or acquired resistance of many neoplasms to the drug. As a result, a multitude of studies to investigate the mechanisms of cisplatin resistance have been carried out with human and rodent cells in culture. Acquired resistance has been associated in different cases with changes in drug accumulation, intracellular drug inactivation by enhanced levels of glutathione or metallothionein, and of particular interest for this study, enhanced DNA repair (3, 10, 11, 53).

Removal of cisplatin adducts from DNA is accomplished by only one characterized mechanism, nucleotide excision repair. In mammalian cells, this process involves many proteins, including the xeroderma pigmentosum (*XP*) and excision repair cross-complementing (*ERCC*) gene products (1). If cancer cells can acquire increased resistance to drugs or radiation by increasing their capacity for nucleotide excision repair, it is important to understand the molecular mechanism of the increase. It would also become important to find ways to inhibit the increased repair in tumor cells, in order to make therapies more effective.

The most widely used and cited model system that has provided support for enhanced DNA repair as a means of cellular resistance to platinum compounds has used cisplatin-resistant and -sensitive derivatives of mouse leukemia L1210 cells. A series of sublines developed by Eastman and colleagues provide the most dramatic examples. For instance, the cell line designated L1210/DDP_{10} was developed by growth in increasing drug concentrations over several years and could eventually be maintained in culture medium containing 10 μg of cisplatin per ml (43). The L1210/DDP_{10} line was thus ~100-fold more resistant to cisplatin than the reference L1210/0 line (on the basis of the IC_{50}, the drug concentration at which cell growth is inhibited by 50%). Subsequently it was demonstrated that L1210/DDP_{10} had a much greater rate of removal of cisplatin adducts from cellular DNA than L1210/0 (16) and that L1210/DDP_{10} was better able to reactivate a cisplatin-damaged plasmid vector than L1210/0 (48). Many other studies (6, 7, 9, 17–22, 29, 35, 43, 47) have used L1210/0 and cell lines derived from it in order to evaluate the toxicity of platinum drugs and to investigate mechanisms of resistance. The possibility that enhanced DNA repair can be a mechanism of drug resistance has relied heavily on these studies (reviewed in references 3, 11, and 53).

To investigate the possible role of enhanced DNA repair as a general mechanism for cisplatin resistance, we set out to define the origin of the difference in DNA repair capacity in these L1210 sublines. The ability of cell extracts to repair cisplatin-DNA adducts was analyzed by using techniques that measure nucleotide excision repair of DNA in vitro (23, 24, 26, 51, 52).

MATERIALS AND METHODS

Cell lines. CHO cell lines 43-3B (rodent repair complementation group 1) and 27-1 (group 3) were as described previously (55). The CHO cell lines UV41 (group 4) and UV135 (group 5) were provided by D. Busch (8). The human lymphoblastoid XP-G cell line XPG83 was as described previously (39). The human lymphoblastoid XP cell lines GM2345 (XP-A), GM2252 (XP-B),

* Corresponding author.
† Permanent address: Department of Clinical Chemistry, Tampere University Hospital, Tampere, Finland.
‡ Present address: Roche Research Centre, Welwyn Garden City AL7 3AY, United Kingdom.

GM2246 (XP-C), GM2485 (XP-D), GM8437 (XP-F), and GM2449 (XP-V) as well as a normal line GM1953 were obtained from the National Institute of Medical Sciences Human Mutant Cell Repository (Coriell Institute, Camden, N.J.).

The mouse leukemia cell line L1210/0, originally isolated by Burchenal and coworkers (7), and its cisplatin-resistant derivative L1210/DDP_{10} (43) were kindly provided by A. Eastman. The parental L1210 line (called here L1210/ECACC), deposited in the American Type Culture Collection at about passage 300, was obtained from the European Collection of Animal Cell Cultures (ECACC; Salisbury, England). All cells were confirmed to be free of *Mycoplasma* contamination. Karyotypes of L1210 lines were determined from propidium iodide-stained preparations.

Drug and UV sensitivity. Cells were grown in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum in a humidified atmosphere containing 5% CO_2 at 37°C. Sensitivity to cisplatin (Sigma), mitomycin (Sigma), bleomycin (Calbiochem), and UV irradiation was determined by measuring the inhibition of cell growth. Cells (4×10^5/ml) were incubated in quadruplicate cultures with various concentrations of drug over a 3-day period. Alternatively, the cells were irradiated with UV light (peak wavelength = 254 nm) at a dose rate of 0.25 J/m^2/s and allowed to grow for 3 days, and viable cells were counted with a hemocytometer, employing nigrosine dye exclusion.

DNA damaged with multiple cisplatin or UV lesions. To examine repair of platinated DNA, closed circular M13mp18GG duplex DNA (52) was purified on CsCl gradients and then incubated (15 h, 37°C) with a fresh 15 μM solution of cisplatin as described previously (25). The 7.3-kb cisplatin-treated DNA contained ~5.3 platinum adducts per kbp as determined by atomic absorption spectroscopy of plasmid DNA platinated under identical conditions (25). Plasmid pBluescript KS^+ (3.0 kbp; Stratagene) was irradiated with UV light (450 J/m^2) to give ~4.1 photoproducts per kbp (44).

In vitro excision repair of damaged DNA. Whole-cell extracts were prepared as described previously (56). In some experiments, cell extracts were fractionated on phosphocellulose to give CFII fractions (5, 50). To measure repair synthesis in DNA containing randomly located lesions, 250 ng each of damaged plasmid and control undamaged 3.7-kbp plasmid pHM14 (44) was incubated in reaction mixtures with cell extract protein at 30°C for 3 h (56). DNA was isolated and then linearized with either *Hin*dIII (cisplatin-damaged duplex M13mp18) or *Bam*HI (UV-damaged pBluescript KS^+) and separated by electrophoresis on 1% agarose gels. In experiments with CFII fractions, reaction mixtures included the indicated amount of CFII protein and 300 ng of purified human replication protein A (RPA). After incubation for 60 min at 30°C, 25 ng of proliferating cell nuclear antigen (PCNA) was added, and incubation continued for 10 min before isolation of DNA. To measure repair of a single acetylaminofluorene (AAF)-guanine adduct, repair assays included 100 ng of closed circular duplex M13mp18G DNA instead of the plasmid mixture (51). The DNA was digested with *Bst*NI, and the nine resulting fragments were resolved on a 12% polyacrylamide gel. Dried gels were exposed to X-ray film and quantified with the aid of a PhosphorImager (Molecular Dynamics), a Molecular Dynamics computing densitometer to determine relative synthesis from autoradiographs, and liquid scintillation spectroscopy of plasmid bands excised from dried gels.

Northern (RNA) hybridization analysis. The human *XPG* cDNA insert (~3.7 kbp) was excised from a 9.3-kbp plasmid pVL1392 construct (38) with *Not*I and purified by agarose gel electrophoresis. The purified insert was further cleaved with *Hin*dIII and *Pst*I to give four restriction fragments. The fragments corresponding to ~0.4 kbp near the 5′ end and to ~1.7 kbp near the 3′ end of human *XPG* mRNA were selected as probes. A murine 0.4-kb probe was made by reverse transcription and PCR amplification from mouse poly(A)$^+$ RNA with primers 5′-CAGCARGAACGNATHGCTGC-3′ and 5′-AGAGGTTCCAGGC CNTGCCCAGGGAA-3′. The probes were purified by agarose gel electrophoresis and labeled with ^{32}P by random-primed synthesis (45). Total RNA was isolated by the guanidinium thiocyanate method, and poly(A)$^+$ RNA was isolated by chromatography on oligo(dT)-cellulose. RNA was resolved by electrophoresis through a formaldehyde agarose (1%) gel, capillary transferred to a nylon membrane (Hybond N; Amersham), and hybridized to labelled probe by using standard techniques (45). Internal controls for loading and molecular size were a rat glyceraldehyde 3-phosphate dehydrogenase probe (GAPDH) (42) identifying a 1.3-kb mouse mRNA (2) and mouse ATF1 identifying a 2.5- to 3.0-kb mRNA (33). Marker RNA transcripts (1.6 to 7.4 kb; Boehringer Mannheim) and mouse rRNA (28S and 18S) served as molecular size references in a separate lane.

RESULTS

Cisplatin and UV sensitivity of L1210 cell lines. A growth inhibition assay was used to confirm the reported difference in cisplatin sensitivity between L1210/0 and L1210/DDP_{10} cells. The cisplatin dose-response curve demonstrated that L1210/DDP_{10} cells had an IC_{50} about 100-fold greater than that of L1210/0 cells (Fig. 1A; Table 1). A sample of L1210 cells was also obtained from the American Type Culture Collection via the ECACC cell repository and tested for comparison. These

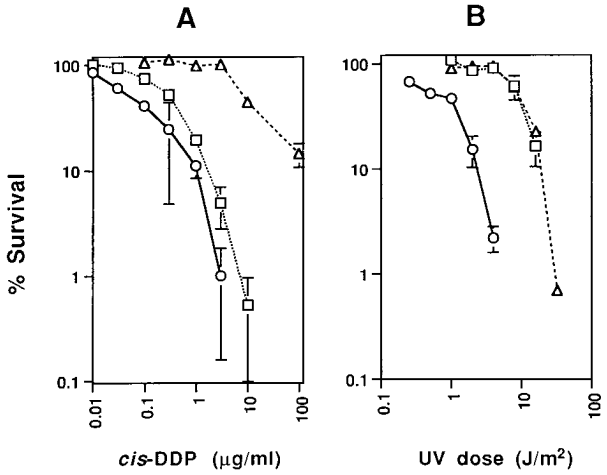

FIG. 1. Cisplatin and UV sensitivity of L1210 cell lines. (A) Cells were grown in suspension in the presence of various cisplatin concentrations for 3 days, and the living cells were then counted. Cells analyzed were L1210/0 (circles), L1210/ECACC (squares), and L1210/DDP_{10} (triangles). Data represent averages ± standard deviations of four replicate cultures. (B) Exponentially growing cells were UV irradiated with various single doses and counted after 3 days in culture. The symbols and statistics are the same as for panel A.

L1210/ECACC cells were found to be significantly more cisplatin resistant (~6- to 7-fold-greater IC_{50}) than the L1210/0 subline. All three cell lines were tested for sensitivity to UV irradiation. No difference between the L1210/ECACC and L1210/DDP_{10} lines was noted (Fig. 1B; Table 1), but these lines tolerated approximately 10 times more UV radiation than the L1210/0 line. The L1210/0 line was also somewhat more sensitive to the chemical agents mitomycin and bleomycin than L1210/DDP_{10} or L1210/ECACC (Table 1). The high resistance of L1210/DDP_{10} to cisplatin is specific to that drug and is not associated with a general increase in resistance to drugs or UV radiation in comparison with the type culture collection cell line L1210/ECACC; this is discussed further below.

DNA repair synthesis in vitro. Since L1210/0 cells appeared to be unusually sensitive to DNA-damaging agents, their DNA repair capacity was investigated. A cell-free system was used to examine the ability of protein extracts from the sensitive and resistant cells to carry out DNA repair synthesis. For the experiments in Fig. 2, closed circular plasmid DNA was either UV irradiated or treated with cisplatin, and an undamaged plasmid of slightly different size served as an internal control. The DNA was incubated with cell extracts in buffer that included [α-^{32}P]dATP. Plasmid DNA recovered from the reaction mixture was linearized with a restriction enzyme and subjected to gel electrophoresis and fluorography for analysis of the incorporation of nucleotides into repair patches (56).

A representative experiment with whole-cell extracts is

TABLE 1. Growth inhibition of L1210 lines by drugs and UV irradiation

Treatment	IC_{50}[a]		
	L1210/0	L1210/ECACC	L1210/DDP_{10}
Cisplatin (μg/ml)	0.050	0.33	9.0
UV irradiation (J/m^2)	0.8	9.8	10.2
Mitomycin (ng/ml)	75	105	300
Bleomycin (μg/ml)	5.2	10.2	9.0

[a] IC_{50}, level of treatment at which growth in 3-day cultures is inhibited by 50%.

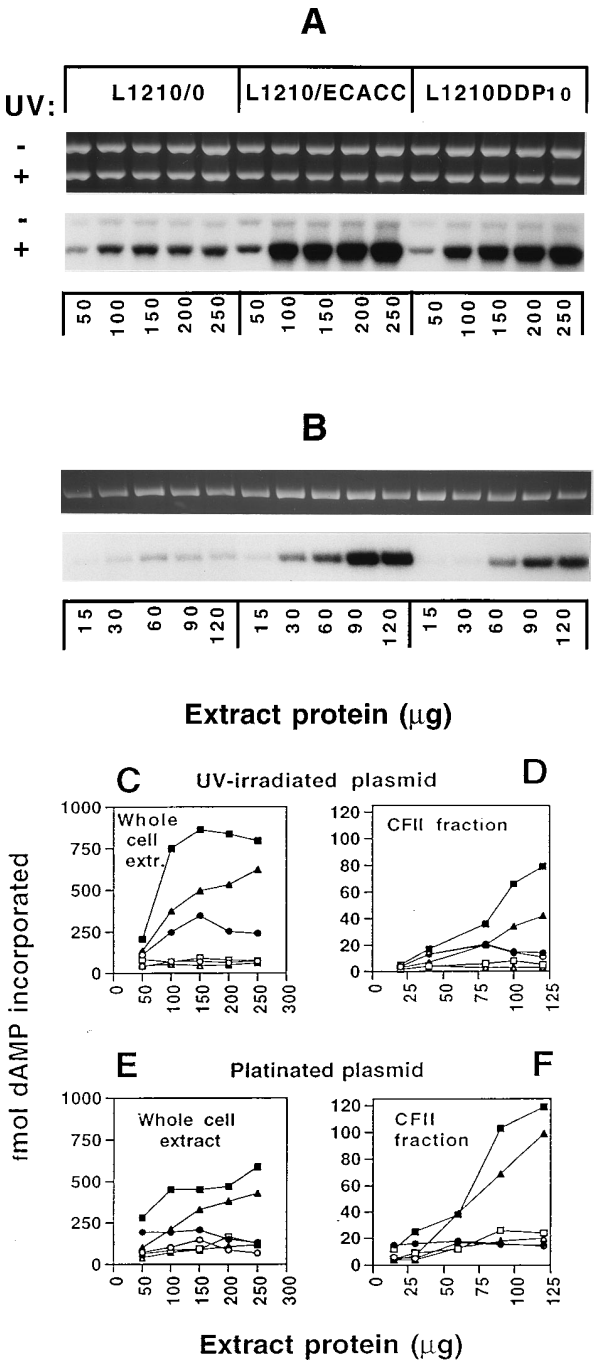

FIG. 2. DNA repair synthesis by extracts from L1210/0, L1210/ECACC, and L1210/DDP_{10} cells. (A) Each reaction mixture included 250 ng each of UV-irradiated pBluescript KS^+ plasmid (UV +) and undamaged pHM14 (UV −) and the indicated amounts of whole-cell extracts. Top, photograph of the ethidium bromide-stained gel showing the linearized plasmid DNA; bottom, autoradiography of the dried agarose gel. (B) DNA repair synthesis in platinated closed circular M13mp18GG duplex DNA (250 ng per reaction) by the indicated amounts of CFII protein fractions. (C) Quantification of data from panel A. (D) DNA repair synthesis in UV-irradiated plasmid carried out by the indicated amounts of CFII fraction protein. (E) DNA repair synthesis in platinated plasmid by the indicated amounts of whole-cell extract protein. (F) Quantification of data from panel B, plus control data. The graphs in panels C to F show femtomoles of dAMP incorporated into UV- or cisplatin-damaged plasmid (closed symbols) and undamaged plasmid (open symbols) after normalization to account for minor variations in DNA recovery between samples. The cell extracts analyzed were L1210/0 (circles), L1210/ECACC (squares), and L1210/DDP_{10} (triangles).

shown in Fig. 2A, and quantitative results are given in subsequent panels. L1210/0 cell extracts were considerably less efficient in repairing UV-irradiated DNA (Fig. 2C) or cisplatin-treated DNA (Fig. 2E) than either L1210/ECACC or L1210/DDP_{10} cell extracts. The repair synthesis performed by extracts from the latter two mouse cell lines was in the same range as that seen with similar amounts of extract protein from repair-proficient normal human cell lines under identical conditions (39). Significantly, the low level of repair of damaged DNA by L1210/0 cell extracts was comparable to that seen with severely nucleotide excision repair-deficient human XP or rodent ERCC cell lines (39). However, with whole-cell extracts from repair-defective cell lines, a low level of spurious synthesis is often seen that does not represent nucleotide excision repair, and this can make results difficult to interpret. To reduce this background, two different approaches were used.

In one approach, the background in randomly damaged DNA was greatly reduced by using fractionated cell extracts and two-stage repair reactions. During the first stage, a phosphocellulose fraction (designated CFII) was supplemented with RPA single-stranded-DNA-binding protein. This mixture is able to carry out the incision reaction in damaged DNA. Specific repair synthesis takes place quickly in a second stage after addition of the DNA polymerase accessory protein PCNA (5, 50). Fractionated cell extracts from the three cell lines were compared by using this protocol. These results clearly showed that CFII fractions from L1210/0 cells were very defective in in vitro nucleotide excision repair of UV-irradiated DNA (Fig. 2D) or cisplatin-treated DNA (Fig. 2B and F).

Another method to reduce background DNA synthesis is to measure repair in the immediate region of the DNA damage. In this approach, a duplex M13 DNA circle was constructed to contain a single AAF modification per molecule on a defined guanine residue (Fig. 3A). This single-lesion substrate was incubated with whole-cell extract to allow repair synthesis to occur. The repair patch of about 30 nucleotides formed after removal of the adduct was analyzed by restriction enzyme digestion of the products and polyacrylamide gel electrophoresis as previously described (25, 51, 52). Figure 3B shows that L1210/0 extracts could not carry out detectable repair of the AAF adduct. L1210/DDP_{10} extracts performed specific repair synthesis in the 31-bp fragment containing the lesion and in the 5′ flanking 68-bp fragment. With both extracts, some nonspecific repair synthesis took place in all fragments in proportion to their size and base composition, and this serves as an index of equal loading of DNA on the gel. The data in Fig. 1 to 3 thus show that L1210/0 cell extracts were unable to repair cisplatin, UV, or AAF adducts in DNA, diagnostic of a deficiency in nucleotide excision repair.

Nature of the repair defect in L1210/0 cells. Having established that there was a nucleotide excision repair defect in the L1210/0 subline, we asked whether the cells could be assigned to a known repair-defective complementation group. Complementation analysis was performed by mixing extract from L1210/0 cells with extracts from defined repair-defective complementation groups (5, 39, 56). Examples of quantitative results from such analyses are shown in Fig. 4A for whole-cell extracts from XP-A, XP-C, ERCC4, and ERCC5 cells. XP-D and XP-F extracts (not shown) were also complemented by L1210/0 extract. Figure 4B shows results with low-background CFII fractions of ERCC1, ERCC3 (XP-B), XP-D (ERCC2), and XP-G extracts. Addition of L1210/0 extract protein could correct the in vitro repair deficiency of all of these extracts, with the significant exceptions of ERCC5 rodent cell extracts (Fig. 4A) and human XP-G cell extracts (Fig. 4B; see also Fig. 5, lanes 7 and 8). Since the *XPG* and *ERCC5* genes are known

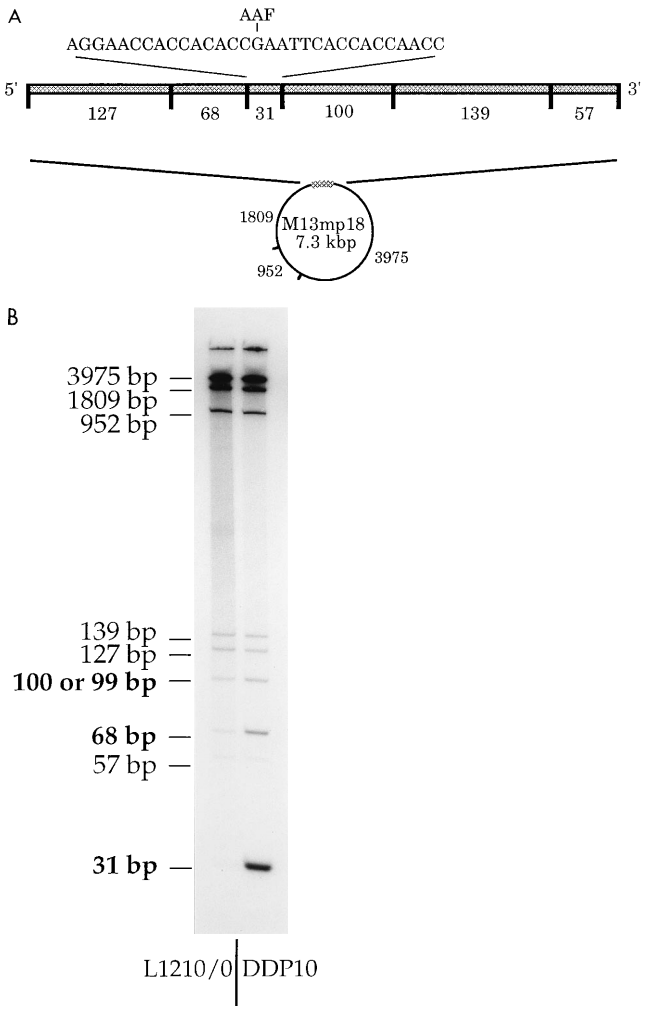

FIG. 3. Repair of a site specifically placed AAF adduct. (A) Construct containing on the minus strand a single AAF-guanine adduct in closed circular duplex M13mp18G DNA. The nucleotide sequence is shown for the minus strand flanking the adduct. The fragment sizes shown (in base pairs) are for *Bst*NI digestion. (B) The single-lesion DNA was incubated with 200 μg of L1210/0 or L1210/DDP_{10} cell extract protein and digested with *Bst*NI. Repair synthesis was detected after gel electrophoresis and autoradiography.

to be equivalent (32, 39, 46, 49), these results strongly suggested that L1210/0 had a defect in XPG/ERCC5.

To test this hypothesis, XPG protein was added to reaction mixtures with L1210/0 cell extracts, either as a partially purified fraction (39) from human HeLa cells (Fig. 5; compare lane 1 with lane 4) or as a purified polypeptide (38) produced from a recombinant baculovirus (Fig. 5; compare lane 10 with lane 11). In both cases, the XPG protein could correct the DNA repair defect of L1210/0 cell extracts but could not correct extracts from repair-deficient cells representing other genetic complementation groups.

Verification of the identity of the L1210 cell lines. The severe repair deficiency of the L1210/0 cell line was unexpected, since this subline has been used as the control in many investigations concerning acquired resistance to platinating drugs. This does, however, explain the high mutagen sensitivity of L1210/0 that has been consistently observed. The repair defect could also be observed in vivo by measuring unscheduled DNA synthesis (incorporation of [*methyl*-^{3}H]thymidine during repair) following irradiation with UV light (15 J/m^2) (13). In a preliminary experiment, the mean numbers (± standard errors) of autoradiographic grains in 50 non-S-phase nuclei were 5.3 ± 1.1 (unirradiated) and 14.1 ± 1.3 (irradiated) for L1210/DDP_{10} cells, whereas L1210/0 cells gave no statistically significant unscheduled DNA synthesis above background (7.7 ± 1.3 grains in unirradiated cells and 6.0 ± 1.4 grains in irradiated cells). To ensure that the L1210 cell lines were related as assumed, their morphologies, chromosome constitution, and DNA fingerprints were analyzed. A lymphoblast-like appearance of all three cell lines was evident. All lines had a mouse karyotype with a wide distribution of chromosome frequencies (Fig. 6A), and numerous aberrations were recorded. Significantly, submetacentric marker chromosomes were observed in L1210/0 and L1210/DDP_{10} that were not present in any of the L1210/ECACC spreads examined, and this is consistent with the derivation of L1210/DDP_{10} from L1210/0. DNA fingerprints obtained by using a minisatellite probe were very similar (although not absolutely identical) for the L1210 cell lines and supported their close relationship. As a group, the L1210 fingerprints were completely different from other human or mouse cell DNA fingerprints as expected (Fig. 6B).

Northern hybridization analysis. To determine whether the XPG defect in L1210/0 was a result of grossly altered expression of the *XPG* gene, blots of poly(A)$^+$ RNA from the three L1210 cell lines were hybridized with *XPG* cDNA probes. Two analyses are shown in Fig. 7, representing the results of probing with a fragment covering the 5′ conserved region (Fig. 7A) and one covering the more 3′ conserved internal region (Fig. 7B) (46). A single mRNA species with an apparent molecular size of ~5.5 kb was observed in both cases. A homospecific probe generated by PCR from mouse poly(A)$^+$ RNA by using primers highly conserved between *Xenopus XPG* and human *XPG* revealed the same bands with very similar intensities (not shown). The *XPG* mRNA in mouse L1210 cells appears to be somewhat larger than the human *XPG* mRNA of 3.9 to 4.6 kb observed by others (32, 46, 49). Unrelated probes (GAPDH and ATF1) were used to normalize for gel loading (Fig. 6), and when these results are taken into account, it is apparent that the levels of expression of *XPG* mRNA are similar in the three mouse cell lines. The expression of XPG was comparatively low in all cases, at least 50-fold less than GAPDH expression.

DISCUSSION

An *XPG* DNA repair defect in cisplatin-sensitive L1210/0 cells. The characteristics of the L1210/0 cell line indicate a severe defect in nucleotide excision repair. Firstly, even though L1210/0 has often been used as a normal control, it is unusually mutagen sensitive. Its UV sensitivity (IC_{50} of about 1 J/m^2) is similar to that of known nucleotide excision repair-deficient XP or ERCC cell lines (54), which typically have an IC_{50} between 0.8 and 2 J/m^2. This mutagen-sensitive phenotype of L1210/0 appears to have existed since its isolation. An IC_{50} for cisplatin of 0.06 μg/ml, reported in 1977 (7), is almost identical to the value obtained in this study (Fig. 1) and to values found by other investigators in the intervening period (19, 29). In a direct comparison, L1210/0 was found to be as sensitive to cisplatin as several repair-defective ERCC cell lines (48). A hypersensitivity to UV irradiation (IC_{50} of 1.5 J/m^2) has also been noted (29). Second, L1210/0 cells have a deficiency in repair synthesis in vitro with DNA containing UV, cisplatin, or AAF lesions, as well as a low level of unscheduled DNA synthesis in vivo.

Complementation of the L1210/0 cell extracts with extracts of cells from known repair-defective complementation groups indicated an XPG/ERCC5 defect in the L1210/0 line. This

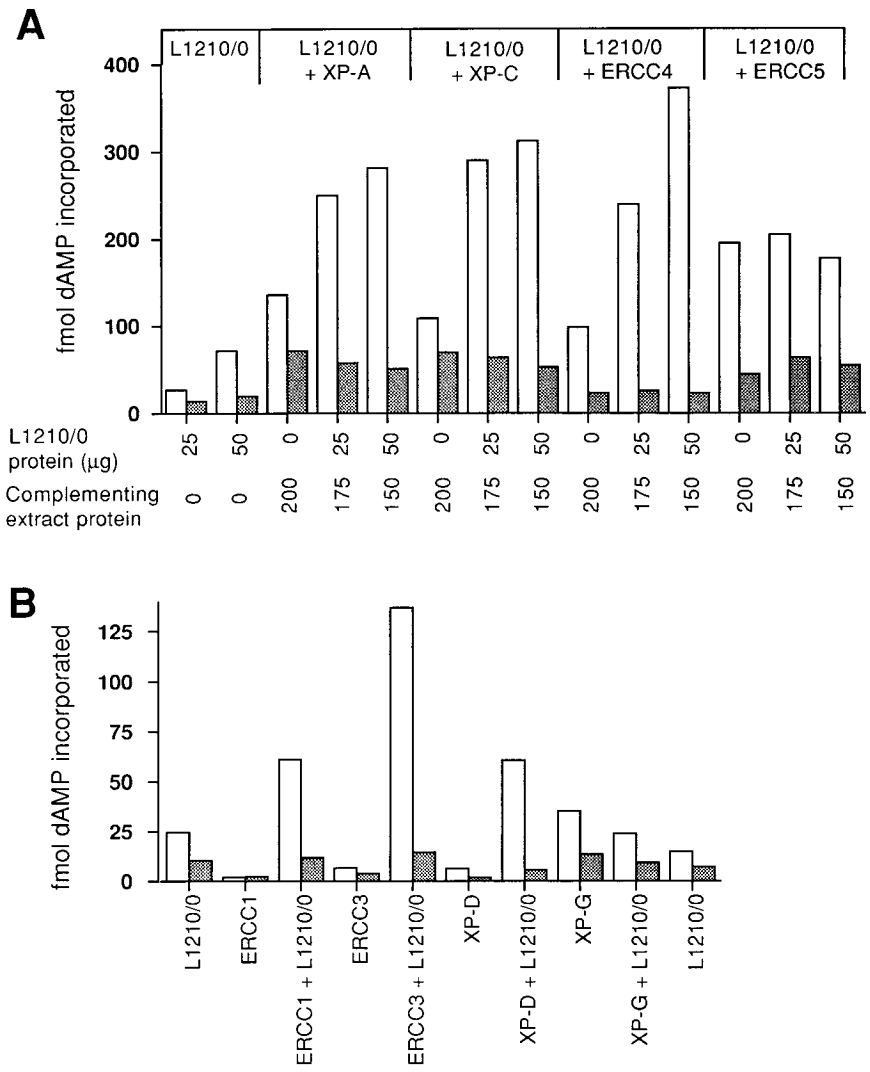

FIG. 4. Complementation of DNA repair synthesis of L1210/0 cells by whole-cell extracts (A) or CFII (B) from XP and ERCC mutants. Graphs show the incorporation of dAMP into UV-damaged plasmid (white bars) and undamaged control plasmid (gray bars). (A) Each standard repair reaction contained a combined total of 200 μg of protein from the indicated whole-cell extracts. The amounts (micrograms) of protein from each extract are indicated. Corresponding reactions with L1210/0 extracts only are also included. (B) Repair reactions with CFII fractions, RPA, and PCNA. Reaction mixture included 60 μg of protein from a CFII fraction when a single extract is indicated or, when mixtures are indicated, 30 μg of protein from each of the two CFIIs.

observation was supported by demonstrating that XPG protein (38, 39) could correct the defect in L1210/0 cells. XPG is a key component in the nucleotide excision repair process and encodes a DNA endonuclease involved in making an incision 3′ to DNA lesions during removal of a damaged oligonucleotide (37). Northern analysis indicated that the sizes and levels of expression of *XPG* mRNA were similar in the three L1210 lines studied, and so the increased UV and cisplatin sensitivity of the L1210/0 line may be due to a relatively small change in the coding sequence of the *XPG* gene. In this respect, L1210/0 resembles all *XPG* mutants examined to date in human or mouse cells in that all retain expression of *XPG* mRNA (32, 36, 46, 49). When the mouse *XPG* cDNA sequence becomes available, it will become practical to locate any relevant sequence alterations.

Origin of L1210/0. How did the repair-defective L1210/0 cell line arise? L1210 cells were originally developed in 1949 as a carcinogen-induced acute lymphoid leukemia in the DBA mouse strain (31). The cells were maintained by serial trans-

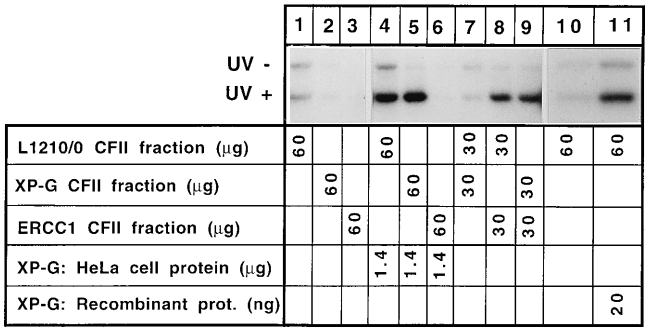

FIG. 5. Complementation of DNA repair synthesis of L1210/0 cells with fractionated extracts and with purified XPG-complementing proteins. Autoradiographs of the DNA repair reactions show the incorporation of dAMP into UV-damaged plasmid (UV +) and undamaged control plasmid (UV −). Reactions contained the indicated amounts of CFII fractions from L1210/0, XPG83 (XP-G), or 43-3B (ERCC1 mutant) cells, with XPG protein where indicated. The reaction mixtures were supplemented with RPA and PCNA proteins as described previously (5, 50).

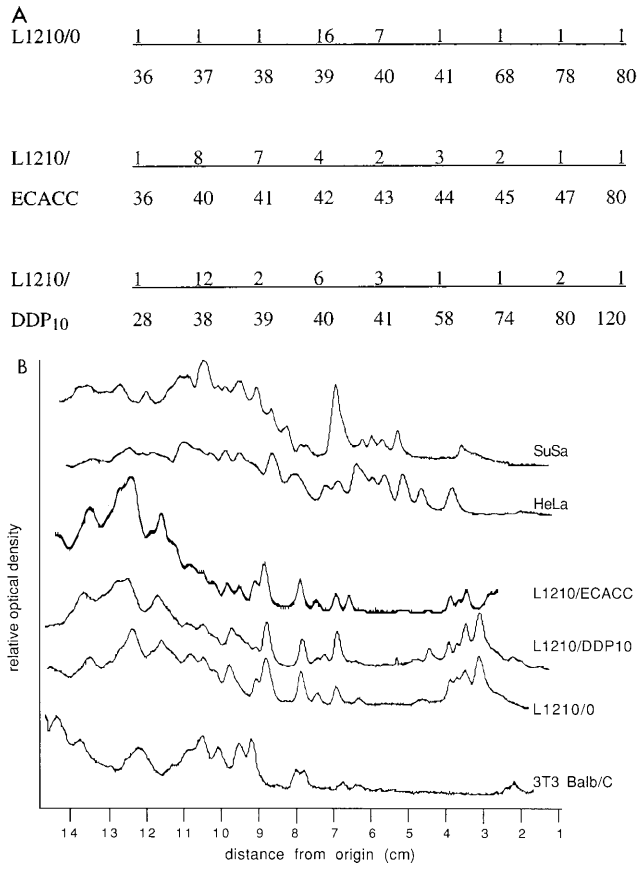

FIG. 6. (A) Chromosome frequency distribution in the L1210 cell lines. For each cell type, 29 to 30 mitotic figures were counted, and the number of spreads shown above the line had the number of chromosomes indicated below the line. (B) Comparison of DNA fingerprint profiles. Ten micrograms of DNA from each cell line was cleaved with *Hin*fI and subjected to electrophoresis on a 0.8% agarose gel. The multilocus probe 33.15 (D7S437) was obtained prelabelled with alkaline phosphatase and detected with the NICE kit (Cellmark Diagnostics, Abingdon, England). A fluorograph of the gel was analyzed by scanning densitometry to give the band profiles shown for the L1210 cell lines, the human HeLa and SuSa cell lines (27), and mouse 3T3 BALB/c cells.

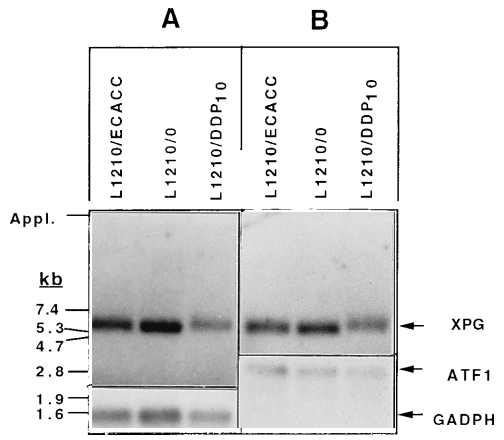

FIG. 7. Expression of *XPG* mRNA in L1210/0, L1210/ECACC, and L1210/DDP_{10} cells. The DNA probes for Northern hybridization were derived from the human *XPG* cDNA. Membrane A was probed with a ~0.4-kbp 5′-end *Hin*dIII fragment, and the separate membrane B was probed with a ~1.7-kbp 3′-end *Pst*I fragment. A second hybridization with the same membranes was performed with rat GAPDH and mouse ATF1 probes, respectively. Ten micrograms of poly(A)$^+$ RNA was used for each lane. Autoradiographic exposures were for 20 h at −80°C for the XPG probes, for 25 min for GAPDH probe, and for 2.5 h for the ATF1 probe. Longer autoradiographic exposures did not provide evidence for alternatively spliced transcripts.

plantation in mice until an in vitro culture system was described in 1966 (34). In 1979, the cells were deposited in the American Type Culture Collection at about the 300th passage. The sensitive subline L1210/0 was first described in 1977 and used as representative of the wild-type form of L1210 cells (6, 7). A cisplatin-resistant subline designated L1210/DDP was selected from L1210/0 (6), and the L1210/DDP_{10} subline used in the present study was developed by further selection of L1210/DDP by growth in the presence of 10 μg of cisplatin per ml (15a, 43). The karyotype data are consistent with this development, since the chromosomal abnormalities of L1210/0 and L1210/DDP_{10} have a greater resemblance to one other than to the reference L1210/ECACC line.

The origin of the L1210/0 line is unique because the *XPG* DNA repair defect apparently developed spontaneously, in contrast to other known excision repair-deficient lines isolated in vitro, for which mutagenizing agents and selection schemes for rare mutants were used (14). During the selection of the L1210/DDP_{10} line, the *XPG* gene has become functional again, perhaps by mutagen-induced reversion of a point mutation as has been described for human XPA cells (12). The repair capacity of L1210/DDP_{10} does not appear to be quite up to the level of normal L1210/ECACC cells (Fig. 2), indicating that XPG in L1210/DDP_{10} may not have reverted to the exact wild-type sequence.

Relationship to previous in vitro results. The in vitro DNA excision repair synthesis assay used here is particularly useful for demonstrating relatively large differences in DNA repair capacities between different cell types and for identifying repair activities (or their absence) by complementation. Because of the importance of a potential relationship between enhanced DNA repair and cisplatin resistance, two previous studies have used cell extracts of L1210/0 to examine this issue. In one analysis, Calsou et al. found no significant difference in repair between L1210/0 and L1210/DDP cell extracts (9). However, a concentration of 30 mM, rather than our standard 70 mM, KCl was used in reaction mixtures. The ionic strength of the reaction mixture is critical in order to observe significant and reproducible differences in repair synthesis between repair-proficient and repair-deficient human cell extracts (56). This is also true for rodent whole-cell extracts, including the mouse cell extracts used here. The best discrimination between L1210/0 and L1210/DDP_{10} extracts is seen in the presence of 50 to 70 mM KCl (Fig. 8). At 30 mM KCl, the spurious background synthesis in whole-cell extracts is disproportionally increased and is more variable. This can obscure the difference between proficient and deficient extracts and may be why the in vitro repair defect of L1210/0 cell extracts was not revealed previously.

In another study, Nichols et al. examined the DNA repair synthesis activity of L1210/0 cell extracts in comparison with extracts from the cisplatin-resistant cell line L1210/DACH (35). L1210/0 extracts were found to have less repair activity than L1210/DACH extracts, but the authors pointed out that the data were difficult to interpret, and the assay was found to be of limited usefulness. The reason for this is now apparent, since L1210/0 is repair deficient and very little (if any) of the repair DNA synthesis observed with whole-cell extracts from L1210/0 reflects true DNA nucleotide excision repair. It is also worth noting that in that study, reaction mixtures contained only 20 to 30 μg of whole-cell extract protein. This amount of

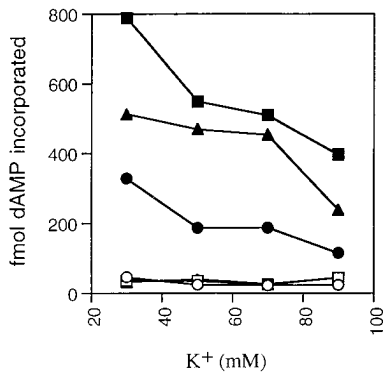

FIG. 8. Dependence of in vitro DNA repair synthesis on KCl concentration in the reaction mixture. Whole-cell extracts (200 μg of protein) of L1210/0 (circles), L1210/ECACC (squares), and L1210/DDP_{10} (triangles) cells were assayed with UV-irradiated plasmid (closed symbols) and unirradiated control plasmid (open symbols) as templates. The reactions were otherwise as for Fig. 2. At salt concentrations higher than those shown, repair synthesis in damaged DNA is severely suppressed, and even in repair-proficient cell extracts, the signal is eliminated at 100 to 120 mM KCl.

protein is usually insufficient to detect significant repair synthesis even with normal cell extracts (Fig. 2). A higher protein concentration (100 to 200 μg per 50-μl reaction) is required to reliably distinguish between the repair-proficient and -deficient whole-cell extracts (Fig. 2).

Implications for studies of acquired cisplatin resistance. In this investigation, a specific defect was found in the DNA nucleotide excision repair pathway of the L1210/0 line, cells that have been used as reference normal controls in many studies investigating the mechanisms of cisplatin resistance (6, 7, 9, 16–22, 29, 35, 43, 47, 48). Because of the DNA excision repair deficiency of the L1210/0 line, these data need to be reinterpreted. Development of new platinum complexes to circumvent drug resistance has relied heavily on the mouse L1210 model. For example, experiments that included L1210/0 were the main impetus for the development of tetraplatin for clinical trials (7, 30). Many platinum complexes have also been identified as promising because, in contrast to cisplatin, they have similar toxicities toward L1210/0 and L1210/DDP cells (6, 17, 19). This may be the first example of a repair-defective line which has been widely used as a control and stimulated selection of drugs for further investigations. The spontaneous acquisition or existence of a repair defect among cell lines might be more common than recognized so far. For instance, we have noticed examples of unusually UV sensitive lines in the literature without any systematic search (e.g., reference 4).

Nucleotide excision repair of DNA adducts is clearly important in determining the intrinsic resistance of cells to cisplatin and can account for the 6.5-fold difference in IC_{50} for cisplatin between the XPG-defective L1210/0 and L1210/ECACC. Repair-proficient Chinese hamster cells show a similar degree of resistance to DNA cross-linking agents in comparison with the known XPG/ERCC5 mutant cell line UV135 (28). Extracts of L1210/ECACC cells repaired cisplatin- or UV-damaged DNA as well as or even better than the cisplatin-resistant L1210/DDP_{10} cells. There is thus no evidence that the L1210/DDP_{10} line has a higher than normal capacity to repair these lesions. This suggests that enhancement of DNA repair (above the level of normal, repair-proficient cells) is not a mechanism of cisplatin resistance in the L1210 system. Nevertheless, the L1210/DDP_{10} line can survive in the presence of approximately 30-fold-higher cisplatin concentrations than can the L1210/ECACC line. Hence, other known features of L1210/DDP_{10} must account for this resistance, including decreased accumulation of cisplatin (43), an increased cellular level of glutathione (43), and increased tolerance of cells to cisplatin-DNA adducts, by an undefined mechanism (16, 22).

The main arguments that have been put forward to suggest a role for increased DNA repair in acquired cisplatin resistance include the increased repair in resistant cell lines, increased levels of DNA polymerases α and β in resistant cells, and a potentiation of the cytotoxic effect of cisplatin by the DNA polymerase inhibitor aphidicolin (reviewed in references 11 and 53). In the widely studied mouse cell system examined here, enhancement of DNA repair above the normal level does not in fact appear to be a mechanism of acquired resistance to cisplatin. Increased DNA repair capacity has been suggested to occur in some other cisplatin-resistant cell lines in addition to the L1210 example (reviewed in references 3, 11, and 53), and it could be of interest to use the approach described here to further investigate those cases. The other two arguments are not compelling, because neither DNA polymerase α nor β appears to play a role in nucleotide excision repair (15) and because aphidicolin inhibits DNA replication as well as repair, which could account for its additional toxic effect. The present results emphasize that DNA repair is important as part of the intrinsic cellular defense against cisplatin damage to the genome but that any role of increased repair in acquired resistance should be reevaluated.

ACKNOWLEDGMENTS

We are grateful to Alan Eastman (Dartmouth Medical School) for providing cell lines and for useful discussions. We thank Sally Ford for chromosome analysis, Louise Bosman for DNA fingerprint analysis, and Debbie Barnes, Stuart Clarkson, Stephen Keyse, Kevin Lee, Abdelilah Aboussekhra, Maureen Biggerstaff, and Mahmud Shivji for donating materials and advice.

This work was supported by the Imperial Cancer Research Fund, with assistance to J.A.V. and L.M.V. from the Maj and Tor Nessling Foundation, the Academy of Sciences of Finland, and the Finnish Foundation for Cancer Research. J.A.V. is a recipient of a U.K. Royal Society/Academy of Sciences of Finland Exchange Visitor Fellowship.

REFERENCES

1. **Aboussekhra, A., and R. D. Wood.** 1994. Repair of ultraviolet light-damaged DNA by mammalian cells and *Saccharomyces cerevisiae*. Curr. Opin. Genet. Dev. **4:**212–220.
2. **Alexander, M., G. Curtis, J. Avruch, and H. M. Goodman.** 1985. Insulin regulation of protein biosynthesis in differentiated 3T3 adipocytes. Regulation of glyceraldehyde-3-phosphate dehydrogenase. J. Biol. Chem. **260:** 11978–11985.
3. **Andrews, P. A., and S. B. Howell.** 1990. Cellular pharmacology of cisplatin—perspectives on mechanisms of acquired-resistance. Cancer Cells **2:**35–43.
4. **Beer, J., E. Budzicka, E. Niepokojczycka, O. Rosiek, I. Szumiel, and M. Walicka.** 1983. Loss of tumorigenicity with simultaneous changes in radiosensitivity and photosensitivity during in vitro growth of L5178Y murine lymphoma cells. Cancer Res. **43:**4736–4742.
5. **Biggerstaff, M., D. E. Szymkowski, and R. D. Wood.** 1993. Co-correction of the ERCC1, ERCC4 and xeroderma pigmentosum group F DNA repair defects *in vitro*. EMBO J. **12:**3685–3692.
6. **Burchenal, J., K. Kalaher, L. Lokys, and G. Gale.** 1978. Studies of cross-resistance, synergistic combinations and blocking of activity of platinum derivatives. Biochimie **60:**961–965.
7. **Burchenal, J. H., K. Kalaher, T. O'Toole, and J. Chisholm.** 1977. Lack of cross-resistance between certain platinum coordination compounds in mouse leukemia. Cancer Res. **37:**3455–3457.
8. **Busch, D., C. Greiner, K. Lewis, R. Ford, G. Adair, and L. Thompson.** 1989. Summary of complementation groups of UV-sensitive CHO cell mutants isolated by large scale screening. Mutagenesis **4:**349–354.
9. **Calsou, P., J.-M. Barret, S. Cros, and B. Salles.** 1993. DNA excision-repair synthesis is enhanced in a murine leukemia L1210 cell line resistant to cisplatin. Eur. J. Biochem. **211:**403–409.
10. **Calvert, H., I. Judson, and W. J. F. van der Vijgh.** 1993. Platinum complexes in cancer medicine—pharmacokinetics and pharmacodynamics in relation to toxicity and therapeutic activity, p. 189–217. *In* P. Workman and M. A.

Graham (ed.), Pharmacokinetics and cancer chemotherapy, vol. 17. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
11. **Chu, G.** 1994. Cellular-responses to cisplatin-the roles of DNA-binding proteins and DNA-repair. J. Biol. Chem. **269:**787–790.
12. **Cleaver, J. E., F. Cortes, L. H. Lutze, W. F. Morgan, A. N. Player, and D. L. Mitchell.** 1987. Unique DNA repair properties of a xeroderma pigmentosum revertant. Mol. Cell. Biol. **7:**3353–3357.
13. **Cleaver, J. E., and G. H. Thomas.** 1981. Measurement of unscheduled DNA synthesis by autoradiography, p. 277–287. *In* E. C. Friedberg and P. C. Hanawalt (ed.), DNA repair. A laboratory manual of research procedures, vol. 1, part B. Marcel Dekker, New York.
14. **Collins, A. R.** 1993. Mutant rodent cell-lines sensitive to ultraviolet-light, ionizing-radiation and cross-linking agents—a comprehensive survey of genetic and biochemical characteristics. Mutat. Res. **293:**99–118.
15. **Coverley, D., M. K. Kenny, D. P. Lane, and R. D. Wood.** 1992. A role for the human single-stranded DNA binding protein HSSB/RPA in an early stage of nucleotide excision repair. Nucleic Acids Res. **20:**3873–3880.
15a. **Eastman, A.** Personal communication.
16. **Eastman, A., and N. Schulte.** 1988. Enhanced DNA repair as a mechanism of resistance to cis-diamminedichloroplatinum(II). Biochemistry **27:**4730–4734.
17. **Farrell, N.** 1991. Structurally novel platinum antitumor compounds, p. 81–91. *In* S. B. Howell (ed.), Platinum and other metal coordination compounds in cancer chemotherapy. Plenum Press, New York.
18. **Farrell, N., L. R. Kelland, J. D. Roberts, and M. V. Beusichem.** 1992. Activation of the *trans* geometry in platinum antitumor complexes: a survey of the cytotoxicity of *trans* complexes containing planar ligands in murine L1210 and human tumor panels and studies on their mechanism of action. Cancer Res. **52:**5065–5072.
19. **Farrell, N., Y. Qu, L. Feng, and B. Van Houten.** 1990. Comparison of chemical reactivity, cytotoxicity, interstrand cross-linking and DNA sequence specificity of bis(platinum) complexes containing monodentate or bidentate coordination spheres with their monomeric analogues. Biochemistry **29:** 9522–9531.
20. **Farrell, N., Y. Qu, and M. P. Hacker.** 1990. Cytotoxicity and antitumor activity of bis(platinum) complexes. A novel class of platinum complexes active in cell lines resistant to both cisplatin and 1,2-diaminocyclohexane complexes. J. Med. Chem. **33:**2179–2184.
21. **Gibbons, G., W. Kaufman, and S. Chaney.** 1991. Role of DNA replication in carrier-ligand-specific resistance to platinum compounds in L1210 cells. Carcinogenesis **12:**2253–2257.
22. **Gibbons, G. R., J. D. Page, S. K. Mauldin, I. Husain, and S. G. Chaney.** 1990. Role of carrier ligand in platinum resistance in L1210 cells. Cancer Res. **50:**6497–6501.
23. **Hansson, J., L. Grossman, T. Lindahl, and R. D. Wood.** 1990. Complementation of the xeroderma pigmentosum DNA repair synthesis defect with *Escherichia coli* UvrABC proteins in a cell-free system. Nucleic Acids Res. **18:**35–40.
24. **Hansson, J., S. M. Keyse, T. Lindahl, and R. D. Wood.** 1991. DNA excision repair in cell extracts from human cell lines exhibiting hypersensitivity to DNA damaging agents. Cancer Res. **51:**3384–3390.
25. **Hansson, J., M. Munn, W. D. Rupp, R. Kahn, and R. D. Wood.** 1989. Localization of DNA repair synthesis by human cell extracts to a short region at the site of a lesion. J. Biol. Chem. **264:**21788–21792.
26. **Hansson, J., and R. D. Wood.** 1989. Repair synthesis by human cell extracts of DNA damaged by *cis*- and *trans*-diamminedichloroplatinum(II). Nucleic Acids Res. **17:**8073–8091.
27. **Hogan, B., M. Fellous, P. Avner, and F. Jacob.** 1977. Isolation of a human teratoma cell line which expresses F9 antigen. Nature (London) **270:**515–518.
28. **Hoy, C., L. Thompson, C. Mooney, and E. Salazar.** 1985. Defective DNA cross-link removal in Chinese hamster cell mutants hypersensitive to bifunctional alkylating agents. Cancer Res. **45:**1737–1743.
29. **Jennerwein, M. M., A. Eastman, and A. R. Khokhar.** 1991. The role of DNA-repair in resistance of L1210 cells to isomeric 1,2-diaminocyclohexane-platinum complexes and ultraviolet-irradiation. Mutat. Res. **254:**89–96.
30. **Kelland, L. R., P. Mistry, G. Abel, S. Y. Loh, C. F. O'Neill, B. A. Murrer, and K. R. Harrap.** 1992. Mechanism-related circumvention of acquired cis-diamminedichloroplatinum(II) resistance using 2 pairs of human ovarian-carcinoma cell-lines by ammine amine platinum(IV) dicarboxylates. Cancer Res. **52:**3857–3864.
31. **Law, L., T. Dunn, P. Boyle, and J. Miller.** 1949. Observation on the effect of a folic-acid antagonist on transplantable lymphoid leukemias in mice. J. Natl. Cancer Inst. **10:**179–192.
32. **MacInnes, M. A., J. A. Dickson, R. R. Hernandez, D. Learmonth, G. Y. Lin, J. S. Mudgett, M. S. Park, S. Schauer, R. J. Reynolds, G. F. Strniste, and J. Y. Yu.** 1993. Human ERCC5 cDNA-cosmid complementation for excision repair and bipartite amino acid domains conserved with RAD proteins of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Mol. Cell. Biol. **13:**6393–6402.
33. **Masson, N., H. Hurst, and K. Lee.** 1993. Identification of proteins that interact with CREB during differentiation of F9 embryonal carcinoma cells. Nucleic Acids Res. **21:**1163–1169.
34. **Moore, G., A. Sandberg, and K. Ulrich.** 1966. Suspension cell culture and in vivo and in vitro chromosome constitution of mouse leukemia L1210. J. Natl. Cancer Inst. **36:**405–421.
35. **Nichols, A. F., W. J. Schmidt, S. G. Chaney, and A. Sancar.** 1992. Limitations of the in vitro repair synthesis assay for probing the role of DNA repair in platinum resistance. Chem. Biol. Interact. **81:**223–231.
36. **Nouspikel, T., and S. G. Clarkson.** 1994. Mutations that disable the DNA repair gene XPG in a xeroderma pigmentosum group G patient. Hum. Mol. Genet. **3:**963–967.
37. **O'Donovan, A., A. A. Davies, J. G. Moggs, S. C. West, and R. D. Wood.** 1994. XPG endonuclease makes the 3′ incision in human DNA nucleotide excision repair. Nature (London) **371:**432–435.
38. **O'Donovan, A., D. Scherly, S. G. Clarkson, and R. D. Wood.** 1994. Isolation of active recombinant XPG protein, a human DNA repair endonuclease. J. Biol. Chem. **269:**15965–15968.
39. **O'Donovan, A., and R. D. Wood.** 1993. Identical defects in DNA repair in xeroderma pigmentosum group G and rodent ERCC group 5. Nature (London) **363:**185–188.
40. **Oliver, T., and G. Mead.** 1993. Testicular cancer. Curr. Opin. Oncol. **5:**559–567.
41. **Ozols, R. F., and R. C. Young.** 1991. Chemotherapy of ovarian cancer. Semin. Oncol. **18:**222–232.
42. **Piechaczyk, M., J. Blanchard, L. Marty, C. Dani, F. Panabieres, S. El Sabouty, P. Fort, and P. Jeanteur.** 1984. Post-transcriptional regulation of glyceraldehyde-3-phosphate-dehydrogenase gene expression in rat tissues. Nucleic Acids Res. **12:**6951–6963.
43. **Richon, V., N. Schulte, and A. Eastman.** 1987. Multiple mechanisms of resistance to cis-diamminedichloroplatinum(II) in murine leukemia L1210 cells. Cancer Res. **47:**2056–2061.
44. **Robins, P., C. J. Jones, M. Biggerstaff, T. Lindahl, and R. D. Wood.** 1991. Complementation of DNA repair in xeroderma pigmentosum group A cell extracts by a protein with affinity for damaged DNA. EMBO J. **10:**3913–3921.
45. **Sambrook, J., E. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
46. **Scherly, D., T. Nouspikel, J. Corlet, C. Ucla, A. Bairoch, and S. G. Clarkson.** 1993. Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast *RAD2*. Nature (London) **363:**182–185.
47. **Sheibani, N., and A. Eastman.** 1990. Analysis of various messenger RNA potentially involved in cisplatin resistance of murine leukemia L1210 cells. Cancer Lett. **52:**179–185.
48. **Sheibani, N., M. M. Jennerwein, and A. Eastman.** 1989. DNA repair in cells sensitive and resistant to *cis*-diamminedichloroplatinum(II): host cell reactivation of damaged plasmid DNA. Biochemistry **28:**3120–3124.
49. **Shiomi, T., Y.-N. Harada, T. Saito, N. Shiomi, Y. Okuno, and M. Yamaizumi.** 1994. An ERCC5 gene with homology to yeast RAD2 is involved in group G xeroderma pigmentosum. Mutat. Res. **314:**167–175.
50. **Shivji, M. K. K., M. K. Kenny, and R. D. Wood.** 1992. Proliferating cell nuclear antigen is required for DNA excision repair. Cell **69:**367–374.
51. **Szymkowski, D. E., C. W. Lawrence, and R. D. Wood.** 1993. Repair by human cell extracts of single (6-4) and cyclobutane thymine-thymine photoproducts in DNA. Proc. Natl. Acad. Sci. USA **90:**9823–9827.
52. **Szymkowski, D. E., K. Yarema, J. E. Essigmann, S. J. Lippard, and R. D. Wood.** 1992. An intrastrand d(GpG) platinum crosslink in duplex M13 DNA is refractory to repair by human cell extracts. Proc. Natl. Acad. Sci. USA **89:** 10772–10776.
53. **Timmer-Bosscha, H., N. Mulder, and E. de Vries.** 1992. Modulation of cis-diamminedichloroplatinum(II) resistance: a review. Br. J. Cancer **66:**227–238.
54. **Weeda, G., J. H. J. Hoeijmakers.** 1993. Genetic analysis of nucleotide excision repair in mammalian cells. Semin. Cancer Biol. **4:**105–117.
55. **Wood, R., and H. J. Burki.** 1982. Repair capability and the cellular age response for killing and mutation induction after UV. Mutat. Res. **95:**505–514.
56. **Wood, R. D., P. Robins, and T. Lindahl.** 1988. Complementation of the xeroderma pigmentosum DNA repair defect in cell-free extracts. Cell **53:** 97–106.