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(71) Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors: DONAHUE, Brian, A. ; 8 Brock Street, Brighton, MA 02135 (US). ESSIGMANN, John, M. ; 15 Thatcher Street, Brookline, MA 02146 (US). LIPPARD, Stephen, J. ; 15 Humboldt Street, Cambridge, MA 02138 (US). TONEY, Jeffrey, H. ; 1306 Massachusetts Avenue, Cambridge, MA 02138 (US).

(74) Agents: GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US).

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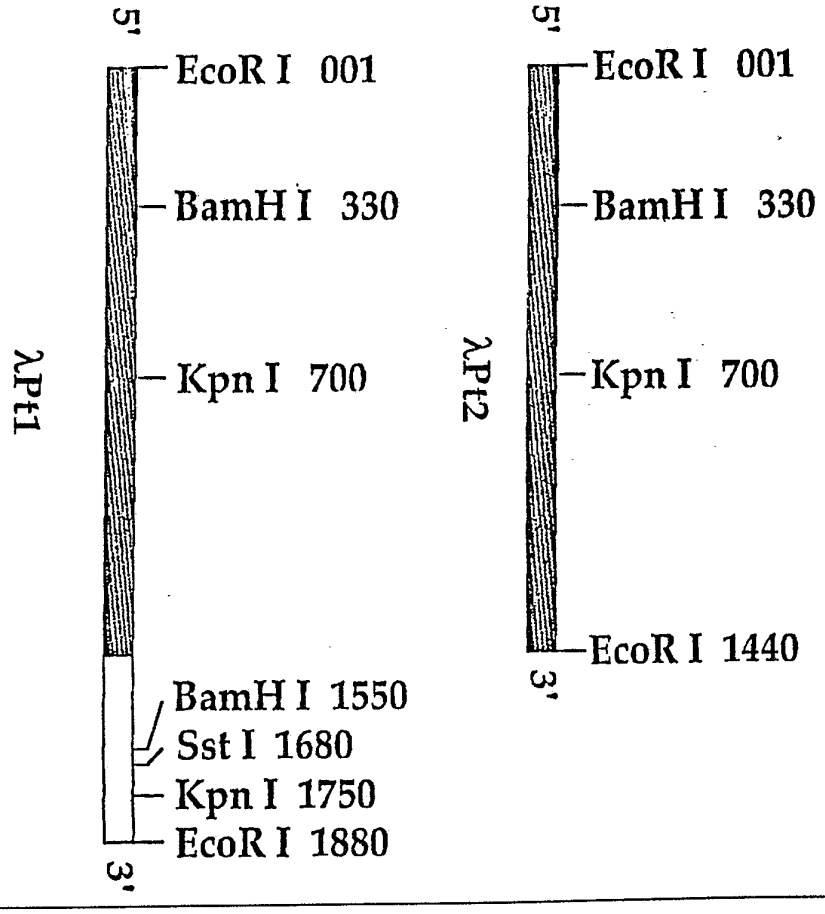
(54) Title: DNA DAMAGE-BINDING FACTOR AND USES THEREFOR

(57) Abstract

DNA damage-binding factor of mammalian origin and DNA encoding such a factor, as well as probes specific for DNA damage-binding factor or DNA encoding it and methods of detecting DNA damage-binding factor in mammalian cells. In particular, a mammalian cellular factor that selectively recognizes and binds DNA damaged or modified by a drug (the anticancer drug, cis-Diammine-dichloroplatinum (II) or cisplatin) has been identified.



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DNA DAMAGE-BINDING FACTOR AND USES THEREFOR

Description

Background

DNA can be damaged by a variety of environmental insults, including antitumor drugs, radiation, carcinogens, mutagens and other genotoxins. Chemical changes in the component nucleotides or of DNA secondary and tertiary structure are all considered herein to be DNA modification or damage.

The mechanism(s) by or conditions under which DNA modification or damage occurs are presently unknown. It would be very helpful to have a better understanding of DNA damage, because DNA damage can lead to mutations and cancer, as well as cell death; the latter is exploited in chemo- and radio-therapeutics. Enhanced ability to repair, or otherwise modify the effects of DNA damage would be helpful because it might serve as the basis for organismal or suborganismal resistance to DNA damaging agents.

Disclosure of the Invention

The present invention relates to DNA damage-binding factor, which has been shown to bind damaged DNA in mammalian cell extracts; to a method of identifying DNA damage-binding factor in mammalian cells; to use of the DNA damage-binding factor and the nucleotide sequence which encodes it; and to methods of preventing or reducing damage to DNA that is the result of DNA processing (replication, recombination and repair) or is caused by contact with or exposure to a chemical compound, physical substance or other damaging agent.

Brief Description of the Drawings

Figure 1 shows binding of cellular factors to platinated DNA at different bound ratios of Pt/nucleotide (r_b).

Figure 2 shows the selectivity of the cellular factor for cisplatin modified DNA.

Figure 3 shows results of an assay which showed that binding to labelled platinated DNA can be competed with unlabelled platinated DNA.

Figure 4 shows results of assessment of the sensitivity of the cellular factor in crude extracts to protease and ribonucleases.

Figure 5 is a photograph of protein replica filters prepared from a human B cell cDNA library constructed in the expression vector lambdagtl1. The top is a representative autoradiogram from a primary screen. The bottom filter is a result of three rounds of successive screening using purified clone lambdaPt1.

Figure 6 is a schematic representation of the restriction maps of phages lambdaPt1 and lambdaPt2 showing their alignment.

Figure 7 is a representation of the lambdaPt1 gene. Figure 7A is a schematic representation of the approximately 1400 bp gene. Figure 7B is a partial nucleotide sequence of the lambda Pt1 gene; the nucleotide sequence is that of 184 nucleotides of strand 1 as represented in Figure 7A. Figure 7C is a partial nucleotide sequence of the lambda Pt1 gene; the nucleotide sequence is that of approximately 250 nucleotides of strand 2 as represented in Figure 7A.

Figure 8 presents results of assays to characterize proteins encoded by lambda Pt1 and lambda Pt2 clones. Figure 8A shows the results of a total protein stain using India ink. Figure 8B shows the results of a Western blot using mouse monoclonal anti-beta-galactosidase antibodies (1:50,000), followed by goat anti-mouse IgG conjugated with alkaline phosphatase (1:7500). Immune complexes were visualized using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. Figure 8C shows the results of a Western blot probed with unmodified 422 bp DNA fragment (1.5×10^5 cpm/ml.). Figure 8D shows the results of a Western blot probed with cis-DDP-modified 422 bp DNA fragment (r_b = 0.04 at 1.5×10^5 cpm ml.).

Crude extract samples resolved were: HeLa nuclear (25 ug total protein) (lane 1); lambdagt11 lysogen (lane 2); solublized pellet (resuspended in SDS-PAGE loading buffer, followed by brief sonication) from lambdaPt1 lysogen (lane 3); soluble fraction from lambdaPt1 lysogen (lane 4); solubilized pellet from lambdaPt2 lysogen (lane 5); and soluble fraction from lambdaPt2 lysogen (lane 6).

Detailed Description of the Invention

The present invention is based on the discovery in extracts of mammalian cells of a factor, referred to herein as DNA damage-binding factor, which binds to damaged DNA. The term damaged DNA includes any DNA whose nucleotide sequence or structure differs from that of the normally occurring nucleotide sequence or structure as a result of an event that occurs during cellular activities in which DNA participates (e.g., DNA replication, recombination, repair), referred to as spontaneous damage, or as a result of contact with or exposure to an agent, such as a chemical compound or a physical agent, referred to as environmental damage. Friedberg, E.C., DNA Repair, Chapter 1, W.H. Freeman & Co., New York (1985). The present invention relates to a method of identifying and isolating DNA damage-binding factors which bind to damaged DNA which is present in mammalian cells as a result of spontaneous damage or environmental damage.

As a result of this discovery, it is now possible to make diagnostic tools, such as nucleotide probes and antibodies, useful for detecting the presence or absence of the DNA damage-binding factor and/or the gene or gene portion by which it is encoded. For example, prenatal screening can include assessment of the occurrence of the factor at lower than normal levels or its absence, which might be indicative of the likelihood of the individual tested to develop cancer during life.

As a result of the present discovery, it is also possible to produce a therapeutic agent useful in protecting against DNA damage or in countering damage that has already occurred. For example, a therapeutic agent protective against the DNA damage caused by chemotherapy or radiotherapy can be administered to an

individual prior to or at the time of such therapy (e.g., in the course of treatment of humans with radiation) or after such treatment has been undergone. In either case, the agent will protect against damage to DNA by creating a DNA damage-refractory phenotype.

A further result of the present discovery is that gene therapy or gene replacement will be available to individuals having less than normal levels of DNA damage-binding factor or lacking the factor. In this case, DNA encoding DNA damage-binding factor can be administered to individuals by means of, for example, genetically-engineered vectors that contain the factor-encoding DNA and regulatory and expression components necessary for its expression. Such recombinant vectors can be used, for example, to infect undifferentiated cells. The resultant cells express the factor, thus overcoming the shortage or lack of DNA damage-binding factor production.

Antibodies prepared against the DNA damage binding factor, or DNA or RNA probes for its DNA, may be useful for classifying the responsiveness of humans or animals to DNA damaging agents. Such antibodies can be used in pre-natal screening for genetic diseases.

Specifically, DNA modified by the antitumor drug cis-diamminedichloroplatinum(II), cis-DDP or cisplatin, was used to identify a factor in mammalian cells that binds to cisplatin-damaged DNA. This factor selectively recognizes double stranded DNA fragments modified by cis-DDP or $[Pt(en)Cl_2]$(en=ethylenediamine). Binding of this factor in crude extracts was observed for as few as 2 platinum adducts per 1,000 nucleotides. Little or no binding occurs to unmodified double stranded DNA or to DNA modified with the clinically ineffective compounds

trans-DDP and [Pt(dien)Cl]Cl (dien=diethylenetriamine). Low levels of binding are observed to single stranded DNA modified by cis-DDP. The apparent molecular weight of the factor in a variety of mammalian cells is approximately 100 kDa as determined by modified Western blotting. As described below, two recombinant phage have been isolated from a human B cell lambdagtll library employing a cis-DDP-modified DNA restriction fragment as a probe. The two clones have insert sizes of 1.88 and 1.44 kb and are aligned at their 5' ends. The polypeptides encoded by the recombinant phage exhibit DNA binding properties similar to those of the cellular factor identified in crude extracts prepared from mammalian cells. Northern analysis using one of the clones revealed an mRNA of 2.8 kb that is conserved in humans and rodents. The methods described herein with specific reference to identification of the factor which binds cisplatin-damaged DNA can be used to identify and characterize other DNA damage-binding factors.

As described below, a mammalian cellular factor that selectively recognizes and binds damaged DNA (i.e., DNA damaged or modified by a drug) has been identified. A factor which is present in extracts from human and hamster cells and which shifts the gel electrophoretic mobility of several DNA restriction fragments modified by chemical means has been identified. Thus, the existence of a factor which specifically recognizes and binds with damaged DNA has been demonstrated. Although the following is described in terms of a factor which binds DNA damaged by a specific drug, it is likely that the factor, or a functional equivalent, has a wider, more generalized role in DNA recognition/processing, since

nature could not have evolved a system specific only for a particular drug or its adducts. That is, it is likely that the factor identified and described herein or a similar factor (i.e., one which has a similar specificity for and ability to bind with damaged DNA) interacts with damaged DNA generated by any means (e.g., spontaneous damage, environmental damage). For example, the factor may be involved in initial recognition of damaged DNA as part of a repair event or, alternatively, it may be a component of cellular response to stress. The cis-DDP binding factor has been shown to be present in human and non-human mammalian cells and it should be emphasized that the cis-DDP binding factor occurs and produces approximately the same band shift in all cell lines tested. It may be ubiquitous to all mammalian cells. A further step taken to elucidate the mechanisms underlying selective recognition and processing of damaged DNA (here, cisplatin damages) is the purification and molecular cloning of this and related factors.

cis-Diamminedichloroplatinum(II) (cis-DDP or cisplatin) is a clinically important antitumor drug used for treatment of several human cancers, especially those of genitourinary origin. Loehrer, P.J. and L.H. Einhorn, Ann. Intern. Med., 100:704-713 (1984). The biological target for cis-DDP is generally accepted to be DNA and considerable information is now available on binding of the drug to DNA. Hacker, M.P. et al., Platinum Coordination Complexes in Cancer Chemotherapy, (Nijhoff: Boston, 1984); Roberts, J.J., A. Thomson, Prog. Nucl. Acids Res. Mol. Biol., 22:71-133 (1979). Covalent coordination of the hydrolysis products of cis-DDP to the bases in DNA can lead to inhibition of DNA synthesis in vitro and in vivo mutagenesis and cell death. Lee, K.W.

and D.S. Martin, Jr., Inorg. Chim. Acta, 17:105-110 (1976); Pinto, A.L., and S.J. Lippard, Proc. Natl. Acad. Sci., USA, 82: 4616-4619 (1985); Harder, H.C., and B. Rosenberg, Int. J. Cancer, 6:207-216 (1970); Howle, J.A. and G.R. Gale, Biochem. Pharmacol, 19:2757-2762 (1970); Burnouf, D. et al., Proc. Natl. Acad. Sci., USA, 84:3758-3762 (1987).

Although prokaryotic DNA repair systems have been identified, comparatively little is known about corresponding factors that process damaged DNA in mammalian cells. Friedberg, E.C., DNA Repair, (W.H. Freeman and Co., New York, 1985). From the information available, however, it appears that mammalian DNA repair enzymes possess damage-specific DNA binding properties, ibid,. pp. 150-152. It therefore seemed reasonable that in mammalian cells there is a damaged DNA binding factor with sufficient generality to recognize cisplatin-modified DNA as an initial step in the repair process. As described, DNA modified by cis-DDP was used as a probe for DNA binding factors present in crude mammalian cellular extracts.

Identification of cellular factors involved in recognition of damaged DNA

The first step towards understanding repair of cisplatin-DNA lesions in mammalian systems was the indentification of cellular factors that might be involved in the recognition of DNA modified by cis-DDP. For this purpose, an electrophoresis gel mobility shift assay, previously used to identify mammalian transcription factors was used. Fried, M.G., and D.M. Crothers, Nucleic Acid Res., 11:141-158 (1983); Singh, H. et al., Nature, 319:154-158 (1986). Specific DNA-binding

factors in a complex mixture of proteins have been identified by this technique through the use of recognition sites containing ^{32}P-labeled DNA fragments in the presence of a large molar excess (10^4-fold) of competitor DNA, such as poly(dI-dC)poly(dI-dC).

As described below, ^{32}P-labeled Pt-modified DNA fragments used to probe crude nuclear and cytosolic mammalian cellular extracts by the electrophoresis gel mobility shift assay have resulted in identification of a factor that selectively binds to cis-DDP-modified double-stranded DNA fragments, but not to DNA modified with the clinically ineffective trans-DDP or [Pt(dien)Cl]Cl (dien = diethylenetriamine) compounds. Treatment of crude extracts with protease or ribonuclease inhibited binding to the cis-DDP-modified DNA probe.

Figure 1 demonstrates the existence of a cellular factor that binds with selectivity to cisplatin-DNA adducts, as detected by the electrophoretic mobility shift assay. The slower migration through the gel of platinated DNA in the presence of the DNA-binding factor allowed it to be readily visualized. The factor was identified in nuclear extracts from human HeLa and Chinese hamster V79 parental and cis-DDP-resistant (adapted to 15 g/mL) cell lines. The selectivity of binding was demonstrated by the correlation between the extent of binding and the extent of DNA modification. A minimum modification level of 0.007 Pt/nucleotide was required to observe binding of the factor to labeled platinated DNA, whereas at a modification level of 0.06 Pt/nucleotide, nearly all labeled DNA was complexed. For probes of higher r_b (ratio of bound Pt per nucleotide) values, two bands are observed in the gel. This result may indicate the binding of two equivalent

cellular factors to those DNA molecules having higher numbers of damaged sites. Probes incubated with nuclear extracts from either V79 parental or resistant cell lines were bound to similar extent, suggesting that this cis-DDP specific factor is present in both cell lines to an approximately equal extent. The expression of this factor, therefore, does not seem to be associated with an acquired resistance to cis-DDP. Similar or identical cis-DDP-specific DNA-binding factors have also been found in nuclear extracts from human B cells and from cytosolic extracts prepared from HeLa cells. As shown in Figure 2, the cellular factor binds selectivley to DNA modified with cis-DDP, but not to DNA modified with either trans-DDP or [Pt(dien)Cl]Cl.

Modified Western blotting was used to identify a factor present in HeLa cells that selectively binds to DNA modified by cis-DDP or [Pt(en)Cl_2]. The size of the binding factor is estimated to be approximately 100 kDa, although species of 28 kDa also bound significantly to the cis-DDP and [Pt(en)Cl_2] modified probes. The latter material may arise from proteolysis of the platinum damaged DNA binding factor. Only double-stranded DNA restriction fragments modified by cis-DDP or [Pt(en)Cl_2] bound selectively to the human cellular factor. A low level of binding of this factor to single stranded DNA modified by cis-DDP was seen, and little or no binding was observed when unmodified single or double stranded DNA restriction fragments were used as probes.
No appreciable binding to the factor, using DNA modified with the clinically ineffective trans-DDP or [Pt(dien)Cl]Cl compounds, was observed, compared with results for unplatinated control DNA. The extent of binding of the factor to DNA depended upon the level of

modification by cis-DDP, with a detection limit of approximately 2 platinum adducts per 1000 nucleotides, (D/N)b = 0.002.

A series of competitive binding experiments was performed to assess the specificity and affinity of the cellular factor for cis-DDP-treated DNA. As shown in Figure 3, the labeled 274 bp fragment of DNA prepared from plasmid pSTR3 and modified at 0.045 pt/nucleotide was very effectively competed by increasing quantities of unlabeled 422 bp fragment derived from M13mp18 and modified at 0.035 Pt/nucleotide. By contrast, unmodified DNA did not compete with the labeled modified DNA for binding of the cellular factor. Competition for binding was complete when a 100-fold excess of unlabeled platinated DNA was added to the binding reaction mixture (Figure 3, lane 10). From the data in lane 8 of Figure 3, the equilibrium constant for binding of the platinated DNA to thecellular factor was estimated to be 3×10^{8}. Muller, R., Methods in Enzymology, 92:589-601 (1983). The same analysis provided an estimate of the concentration of the factor in crude extracts of approximately 4×10^{-9} M. Muller, R., Methods in Enzymology, 92:589-601 (1983). Similar results were obtained when the labeled 274 bp fragment was competed with unlabeled 274 bp fragment modified to the same extent. Binding could be completely competed with a 100-fold excess fo unlabeled modified DNA; however, unmodified 274 bp fragment did not compete for binding of the cellular factor.

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A displacement assay was also performed in which 0.1 ng of labeled cis-DDP-modified DNA (0.035 Pt/nucleotide) was incubated with 7.3 ug of nuclear extract from cis-DDP-resistant cell lines at 37°C for 15 minutes. Unlabeled modified DNA was added to each mixture and incubation was continued for an additional 15 minutes. In contrast to results from the competition assays, results of the displacement assay showed that the cellular factor remained bound to the labeled platinated DNA even when the unlabeled platinated DNA was added at a 1000-fold excess.

Characterization of the cellular factor involved in recognition of damaged DNA

The nature of the cellular factor from crude nuclear extracts was examined by treating them with proteinase K or RNase A prior to incubation with platinated probes. Treatment of extracts with proteinase K completely inhibited binding of the cellular factor to

cis-DDP-modified DNA (Figure 4, lane 3) indicating that the factor contained protein. Similarly, treatment of the extracts with RNase A also completely inhibited binding of the cellular factor (Figure 4, lane 7). This latter result was unexpected and warranted further investigation. Binding studies of the cellular factor to cisplatin-modified DNA revealed inhibition by treatment with RNase T1 and micrococcal nuclease. Binding of the cellular factor is insensitive to treatment with RNase T2 (Figure 4, lane 6). Titration experiments revealed binding to be inhibited by RNase A concentrations as low as 2 but not less than 0.02 ug/mL (Figure 4). In a control experiment, RNase-treated HeLa nuclear extracts were incubated with DNA fragments containing a known octanucleotide-binding domain. Singh, R. et al., Nature, 319:154-158 (1986). Nuclear factors in these extracts that bind to the octanucleotide domain had previously been found to be insensitive to RNase inhibition. HeLa nuclear extracts treated with RNase A that completely inhibited the binding of the cis-DDP-DNA binding factor were found to bind normally to the octanucleotide domain, as determined by the gel mobility shift assay.

Isolation and enzyme mapping analysis of cDNA clones containing DNA modified by cis-DDP

The selective binding of the HeLa cellular factor to DNA modified by cis-DDP suggested that it might be possible to isolate cDNA clones using cis-DDP-modified DNA as a probe. From a primary screen of 360,000 phage plaques, two recombinant phage, lambdaPt1 and lambdaPt2, were isolated from a human B cell expression library probed with a 422 bp DNA fragment modified by cis-DDP at a bound drug/nucleotide ratio $(D/N)_b$ of 0.04. (See

Example 5). E. coli recombinant cells (Y1089) which are E. coli lysogens containing the lambdaPt1 gene have been deposited at the American Type Culture Collection (Rockville, MD) under the terms of the Budapest Treaty and assigned accession number 40498. The restriction maps of the two phages, showing their alignment, are presented in Figure 6. The nucleotide sequence of two segments of the lambdaPt1 gene is shown in Figure 7. In order to demonstrate that these clones encode proteins that specifically bind to DNA modified by cis-DDP, E. coli lysogens were prepared for each clone, as well as for the cloning vector lacking the insert. Crude extracts obtained from induced lysogens were subjected to SDS-PAGE and the resolved proteins were transferred to nitrocellulose filters. Following denaturation and renaturation, according to the method described by Celenza and Carlson, filters were probed with either ^{32}P-labeled unmodified 422 bp restriction fragment or the same DNA probe modified with cis-DDP, [Pt(en)Cl_2], trans-DDP, or [Pt(dien)Cl]$^+$. Celenza, J.L. and M. Carlson, Science, 233:1175-1180 (1986). Parallel filters were treated with a monoclonal antibody raised against β-galactosidase, or stained with India ink to detect total protein. Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York, Section 10.7.1. Only DNA modified by cis-DDP or [Pt(en)Cl_2] bound to the nitrocellulose-immobilized proteins, in accord with results obtained with the HeLa cellular factor. The detection limit of binding of the phage encoded protein to cis-DDP-modified DNA was found to be approximately 2 platinum adducts per 100 nucleotides, $(D/N)_b = 0.02$.

Enzyme mapping analysis of the two recombinant phage lambdaPt1 and lambdaPt2 indicated that they contain nucleotide sequences aligned at their 5' ends (Figure 6) with insert sizes of 1.88 and 1.44 kb, respectively. (See Example 6). Homology between the two clones was confirmed by Southern analysis. The apparent molecular weight of the fusion protein encoded by lambdaPt2 is estimated to be approximately 168 kDa by SDS-PAGE, or approximately 50 kDa for the portion containing the cloned human B cell polypeptide. Two predominant polypeptides that selectively bind to DNA modified by cis-DDP, separated by approximately 4 kDa, are observed in lambdaPt1 lysogens. The slower migrating band corresponds to a molecular weight of approximately 172 kDa. The faster migrating band can be attributed to proteolysis of the phage encoded protein.

Assessment of expression of the lambda Pt2 gene

Expression of the lambdaPt2 gene was assessed by Northern analysis of cytoplasmic RNA prepared from HeLa, murine leukemia L1210 and Chinese hamster V79 cells. (See Example 7). The full length message, conserved in these species, is 2.8 kb. The predicted molecular weight of the full length protein is 100 kDa. This value is similar to the size of the binding factor observed by modified Western analysis of HeLa cytosolic extracts indicating that the clone encodes a portion of this same factor.

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As described below, ^{32}P-labeled Pt-modified DNA fragments used to probe crude nuclear and cytosolic mammalian cellular extracts by the electrophoresis gel mobility shift assay have resulted in identification of a factor that selectively binds to cis-DDP-modified double-stranded DNA fragments, but not to DNA modified with the clinically ineffective trans-DDP or [Pt(dien)Cl]Cl (dien = diethylenetriamine) compounds. Treatment of crude extracts with protease or ribonuclease inhibited binding to the cis-DDP-modified DNA probe.

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A minimum modification level of 0.007 Pt/nucleotide was required to observe binding of the factor to labeled platinated DNA, whereas at a modification level of 0.06 Pt/nucleotide, nearly all labeled DNA was complexed. For probes of higher r_b (ratio of bound Pt per nucleotide) values, two bands are observed in the gel. This result may indicate the binding of two equivalent cellular factors to those DNA molecules having higher numbers of damaged sites. Probes incubated with nuclear extracts from either V79 parental or resistant cell lines were bound to a similar extent, suggesting that this cis-DDP specific factor is present in both cell lines to an approximately equal extent. The expression of this factor, therefore, does not seem to be associated with an acquired resistance to cis-DDP. Similar or identical cis-DDP-specific DNA-binding factors have also been found in nuclear extracts from human B cells and from cytosolic extracts prepared from HeLa cells. As shown in Figure 2, the cellular factor binds selectively to DNA modified with cis-DDP, but not to DNA modified with either trans-DDP or [Pt(dien)Cl]Cl.

A series of competitive binding experiments was performed to assess the specificity and affinity of the cellular factor for cis-DDP-treated DNA. As shown in Figure 3, the labeled 274 bp fragment of DNA prepared from plasmid pSTR3 and modified at 0.045 Pt/nucleotide was very effectively competed by increasing quantities of unlabeled 422 bp fragment derived from M13mp18 and modified at 0.035 Pt/nucleotide. By contrast, unmodified DNA did not compete with the labeled modified DNA for binding of the cellular factor. Competition for binding was complete when a 100-fold excess of unlabeled platinated DNA was added to the binding reaction mixture

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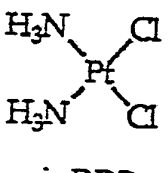
The nature of the cellular factor from crude nuclear extracts was examined by treating them with proteinase K or RNase A prior to incubation with platinated probes. Treatment of extracts with proteinase K completely inhibited binding of the cellular factor to cis-DDP-modified DNA (Figure 4, lane 3) indicating that the factor contained protein. Similarly, treatment of the extracts with RNase A also completely inhibited binding of the cellular factor (Figure 4, lane 7). This latter result was unexpected and warranted further investigation. Binding studies of the cellular factor to cisplatin-modified DNA revealed inhibition by treatment with RNase T1 and micrococcal nuclease. Binding of the cellular factor is insensitive to treatment with RNase T2 (Figure 4, lane 6). Titration experiments revealed binding to be inhibited by RNase A concentrations as low as 2 but not less than 0.2 ug/mL (Figure 4). In a control experiment, RNase-treated HeLa nuclear extracts were incubated with DNA fragments containing a known

octanucleotide-binding domain. Singh, R. et al., Nature, 319:154-158 (1986). Nuclear factors in these extracts that bind to the octanucleotide domain had previously been found to be insensitive to RNase inhibition. HeLa nuclear extracts treated with RNase A that completely inhibited the binding of the cis-DDP-DNA binding factor were found to bind normally to the octanucleotide domain, as determined by the gel mobility shift assay.

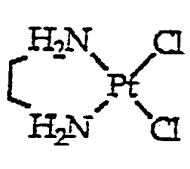
The present invention will now be illustrated by the following examples, which are not to be considered limiting in any way.

EXAMPLE 1 Binding of cellular factors to platinated DNA at different bound ratios of Pt/nucleotide (rb)

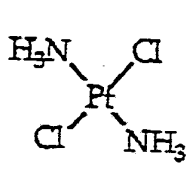
The compounds cis-DDP, trans-DDP, [Pt(dien)Cl]Cl and [Pt(en)Cl_2] were prepared as described. Johnson, G.L., Inorg. Synth., 8:242-244 (1966); Dhara, S.C., Ind. J. Chem., 8:193-194 (1970); Watt, G.W., and W.A. Cude, Inorg. Chem., 7:335-338 (1968); Lippard, S.J. et al., Biochemistry, 22:5165-5168 (1983). The formula of each is as shown below.



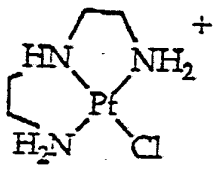
cis-DDP



[Pt(en)Cl_2]



trans-DDP



[Pt(dien)Cl]$^+$

The DNA probe used in these studies was a 422 bp Ava I restriction fragment from M13mp18, purified from low melting agarose gels by phenol extraction, followed by butanol extractions and ethanol precipitation. A portion of the DNA was allowed to react in 1 mM sodium phosphate, 3 mM NaCl, pH 7.4 (buffer B) or 10 mM Tris-HCl pH 7.5, 1 mM Na_2EDTA (TE) at a DNA phosphate concentration of approximately 10^{-8} M, with the appropriate platinum complex at a variety of formal drug/nucleotide ratios, $(D/N)_f$, at 37°C for 12-16 hours in the presence of excess of carrier M13mp18 DNA. Unbound platinum complex was removed by ethanol precipitation of the DNA samples, followed by several washes of the pellet with 70% ethanol. Control, unplatinated DNA was processed identically. Platinum and DNA concentrations were measured by flameless atomic absorption spectroscopy and optical absorption spectrophotometry, respectively. Protein-DNA binding reactions and gel electrophoresis were performed as described with minor modifications in Carthew, R.W. et al., Cell, 43:439-448 (1985).

Platinum-modified and control DNA fragments were labeled using the Klenow fragment of DNA polymerase I and [α-^{32}P]deoxycytidine triphosphate (>5000 Ci/mmole, New England Nuclear) and purified by electrophoresis on native polyacrylamide gels. Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (p. 113, 178). Purity of the labeled DNA fragments was checked by native polyacrylamide gel electrophoresis.

Single stranded DNA was prepared by boiling the unplatinated, radiolabeled 422 bp restriction fragment and then allowing the DNA to reanneal in the presence of a 10-fold molar excess of M13mp18 circular single

stranded DNA (+) strand. The 422 nucleotide (+) strand was then resolved on, and isolated from, a native polyacrylamide gel and platinated as described for the double stranded DNA fragments.

Preparation of Crude Extracts. Mammalian nuclear and cytosolic extracts of HeLa cells and Escherichia coli lysogens were prepared as described in the literature. Stillman, B. and Y. Gluzman, Mol. Cell Biol., 5:2051-2060 (1985) and Singh, H. et al., Cell, 52:415-423 (1988). Extracts used in these studies were derived from either HeLa cells or E. coli strain SG1161 (lon^-) lysogens; the latter was used to reduce proteolytic degradation of the β-galactosidase fusion protein/platinated DNA binding factor.

Modified Western Blots. Extracts were prepared from either IPTG-induced (IPTG=isopropyl-β-D-thiogalacto-pyranoside) lysogens or HeLa cells. Typically, 50 μg total protein per lane were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on an 8% separating gel and transferred onto nitrocellulose (Schleicher & Schueller, BA85, 0.45 μm) as described. Following transfer, filters were processed as described in the literature. Bradford, M.M., Anal. Biochem., 72:248-254 (1976); Laemmli, U.K., Nature, 227:680-685 (1970); Towbin, H. et al., Proc. Natl. Acad. Sci., USA, 76:4350-4354 (1979); Singh, H. et al., Cell, 52:415-423 (1988). To assay for DNA binding, nitrocellulose filter-bound proteins were incubated in binding buffer (30 mM HEPES [N-2-hydroxyethylpiperazine-N-2-ethane-sulfonic acid-NaOH] pH 7.5, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 0.25% nonfat dry milk), using 20 ml per 20 x 20 cm filter, with ^{32}P-labeled DNA fragment ($0.25\text{-}2.0 \times 10^4$ cpm/ml, 10^{-10}-10^{-11} M). Poly(dI-dC)poly(dI-dC) was added

as competitor for non-specific DNA binding proteins at 10 μg/ml or 4 x 10^{-5} M. The incubations were run for 60 minutes at room temperature with gentle agitation. In experimentas using single stranded DNA as a probe, a mixture of 5 μg/ml each of poly(dI-dC)poly(dI-dC) and M13mp18 single stranded (+ strand) DNA was used as competitor. Unbound DNA was then removed by washing the filters twice at 4°C with binding buffer lacking $MgCl_2$ and $MnCl_2$. Protein-DNA complexes were detected by autoradiography with the use of an intensifying screen at -80°C.

Results are shown in Figure 1. End-labeled 422 bp DNA restriction fragments from an AvaI digest of M13mp18 (1-5 x 10^3 cpm, 0.2ng) containing bound cis-DDP levels of 0 (lanes 1-4), 0.007 (lanes 5-8), 0.021 (lanes 9-12), 0.041 (lanes 13-16) and 0.061 (lanes 17-20) were incubated in the absence (lanes 1, 5, 9, 13 and 17) or presence of crude nuclear extract prepared from V79 parental (VP) (lanes 2, 6, 10, 14 and 18), V79 cis-DDP-resistant (VR) (lanes 3, 7, 11, 15 and 19) or HeLa (H) (lanes 4, 8, 12, 16 and 20) cell lines and 6 ug poly(dI-dC)poly(dI-dC) for 15 minutes at 37°C in binding buffer (10 mM Tris HCl pH 7.5, 50 mM NaCl, 0.5 mM Na_2EDTA, 5% glycerol and 1 mM dithiothreitol) in a final volume of 10-50 uL. Nuclear extracts were prepared according to the literature. Hoeffler, W.K., and R.G. Roeder, Cell, 41:955-963 (1985). Protein-DNA complexes were then resolved on a low ionic strength 4% polyacrylamide gel (29 acrylamide:1 bis-acrylamide). Gels were pre-electrophoresed in Tris-glycine buffer (50 mM TrisHCl pH 8.5, 380 mM glycine, 2 mM Na_2EDTA) for two hours at 25 mA. Samples subsequently were electrophoresed for 4 hours at 30 mA. Migration of

unbound DNA is retarded with increasing levels of modification (lanes 1, 5, 9, 13 and 17), owing to increased positive charge and increased structural alterations of the DNA as a result of cis-DDP binding. Sherman, S.E., and S.J. Lippard, Chem. Rev., 87:1153-1181 (1987). Note also that cellular factors from HeLa nuclear extracts bind to unplatinated DNA (lane 4). The binding is reproducible, independent of the oligonucleotide probe, and currently of unknown origin. A second band also appears with the unplatinated DNA probe (lane 1) and probably represents denatured probe DNA.

EXAMPLE 2 Selectivity of the cellular factor for cisplatin modified DNA

The 422 bp DNA fragment (Figure 1) was modified with trans-DDP at r_b levels of 0.013 (lanes 5-8) and 0.064 (lanes 9-12), with [Pt(dien)Cl]Cl at r_b = 0.071 (lanes 13-16) or with cis-DDP at r_b of 0.041 (lanes 17-20). trans-DDP and [Pt(dien)Cl]Cl were prepared as described. Johnson, G.L., Inorg. Synth., 8:242-244 (1966) Dhara, S.C., Ind. J. Chem., 8:193-194 (1970); Watt, G.W., and W.A. Cude, Inorg. Chem., 7:335-338 (1968); Lippard, S.J. et al., Biochemistry, 22:5165-5168 (1983).

Results are shown in Figure 2. Unmodified 422 bp fragment appears in lanes 1-4. DNA fragments were incubated in the absence (lanes 1, 5, 9, 13 and 17) or presence of crude extracts prepared from V79 parental (VP) lanes 2, 6, 10, 14 and 18), V79cis-DDP-resistant (VR) (lanes 3, 7, 11, 15 and 19) or HeLa (H) (lanes 4, 8, 12, 16 and 20) cell lines. Note that an increase in rb for trans-DDP reduced the nonspecific binding of cellular factors in HeLa nuclear extracts (cf. Figure 1). Labeled

material in the wells probably consists of aggregates of labeled probes.

EXAMPLE 3 Binding to labeled platinated DNA can be competed with unlabeled platinated DNA

End-labeled 274 bp fragment (5000 cpm, 0.2 ng) modified with cis-DDP at r_b = 0.045 was incubated in the presence of 7.3 ug nuclear extract from cis-DDP-resistant V79 cells, 6 ug poly(dI-dC)poly(dI-dC) and 0.2-20 ng unlabeled, unmodified 422 bp fragment (Figure 3, lanes 3-6) or 0.2-20 ng unlabeled 422 bp fragment modified with cis-DDP at r_b = 0.035 (Figure 3, lanes 7-10). Unbound 274 bp fragment modified at r_b = 0.045 is shown in Figure 3, lane 1 and binding of the cellular factor to this fragment in the absence of 422 bp competitor DNA is shown in lane 2. End-labeled 422 bp fragment modified with cis-DDP at r_b = 0.035 is shown in Figure 3, lane 11 and binding of the cellular factor to this fragment is shown in Figure 3, lane 12.

EXAMPLE 4 Assessment of effect of protease and ribonucleases on binding of the cellular factor in crude extracts

Binding of the cellular factor in crude extracts was shown to be sensitive to the activity of protease and ribonucleases. Results are shown in Figure 4. Crude nuclear extracts were treated with proteinase K (P) at 100 ug/mL (lane 3), Micrococcal nuclease (M) at 0.075 U/mL (lane 4), RNase T1 (T1) at 0.025 U/mL (lane 5, RNase T2 (T2) at 0.005 U/mL (lane 6) or RNase A at 20 ug/mL (lane 7), 2 ug/mL (lane 8), 0.2 ug/mL (lane 9) or 0.02 µg/ML (lane 10). Extract treated in the absence of enzymes is represented in lane 2. Treatments were at

37°C for 60 minutes. Extracts were subsequently incubated with 5000 cpm (0.2 ng) end-labeled 422 bp fragment modified with cis-DDP at r_b=0.041 and 6 ug poly(dI-dC)poly(dI-dc) at 37°C for 15 minutes prior to gel electrophoresis. Free unlabeled 422 bp platinated fragment appears in lane 1.

EXAMPLE 5 Screening of a human B cell lambdagt11 cDNA library using cis-DDP-modified DNA

Protein replica filters prepared from an unamplified human B cell (RPM 4265) cDNA library (Clontech Laboratories, Inc.) constructed in the expression vector lambdagt11 are shown in Figure 5. The cDNA library was originally prepared by oligo(dT) priming of poly(A)$^+$ RNA. Chan, S.J. et al., Proc. Natl. Acad. Sci., USA, 76:5036-5040 (1979). The library contains approximately 9 X 10^5 independent clones with insert sizes in the range of 0.73 to 4.1 kb and a titer of 3.6 X 10^9 PFU/ml. Screening of the lambdagt11 recombinants plated on E. coli host strain Y1090 was carried out as described by Singh and co-workers. Using cisplatin-modified, ^{32}P-labeled DNA. Singh, H. et al., Cell, 52:415-423 (1988). To screen clones for platinated DNA binding, each filter was incubated for 60 minutes at room temperature in 10 or 25 ml. TNE (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 1 mM Na_2 EDTA, 1 mM DTT) for 100 and 150 mm plates, respectively. The buffer contained ^{32}P-labeled DNA at a final concentration of approximately 3 x 10^4 cpm/ml or approximately 10^{-11} M as well as both sonicated native and denatured calf thymus DNA with an average length of approximately 1 kb at 1.0 and 5.0 μg/ml, respectively. The filters were then washed at room temperature three times for ten minutes eac using TNE,

air dried, and autoradiographed at -80°C with the use of an intensifying screen for 24-48 hours. Putatively positive clones were rescreened for binding to cis-DDP-modified DNA. Secondary screens were carried out on 100 mm plates with plating mixtures of approximately 5 x 10^3 PFU of lambda phase, while tertiary screens used plating mixtures of -100 PFU. Two recombinant phage, lambdaPt1 and lambdaPt2, were purified to homogeneity by using this protocol.

EXAMPLE 6 Restriction enzyme mapping of isolated cDNA clones

Amplified phage stocks prepared from lambdaPt1 and lambdaPt2 were used to isolate lambda recombinant DNA. Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 76-85 (1982). To determine any homologies between the two cDNA clones, the filter was probed with lambdaPt2 cDNA insert labeled with [α-^{32}P]deoxycytidine triphosphate by the Klenow fragment of DNA polymerase I. Maniatis, T. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 113, 178 (1982). Hybridization was carried out with 10% dextran sulfate in 50% formamide for 3 hours at 45°C, and the filters were washed twice with 1 x SSC/0.1% SDS (SSC = 0.15 M NaCl, 15 mM trisodium citrate pH 7.0) at room temperature followed by two additional washes with 0.1 x SSC/0.1% SDS at room temperature. Autoradiography was carried out at -80°C with use of an intensifying screen.

EXAMPLE 7 Assessment of expression of the lambda Pt2 gene

Northern Analysis. Cytoplasmic RNA from human HeLa, hamster V79, and murine L1210 cells was isolated by using a published procedure. Sonenshein, G. et al., J. Exp. Med., 148:301-312 (1978). Twelve micrograms of RNA were loaded in each lane and resolved on a 1% agarose gel containing 6% formaldehyde, 20 mM 3-[N-morpholino]propanesulfonic acid, 5 mM sodium acetate and 1 mM Na_2EDTA. RNA was transferred in 20 x SSC by capillary action to Gene Screen Plus™ (New England Nuclear). The lambdaPt2 DNA insert was labeled with [α-^{32}P]deoxycytidine triphosphate according to literature procedures. Feinberg, A.P. and B. Vogelstein, Anal. Biochem., 132:6-13 (1983). The filter was probed with 10^6 cpm/ml of hybridization mixture (45% formamide, 10% dextran sulfate, 0.1% sodium phosphate, 50 mM Tris-HCl pH 7.5, 5 x Denhardt's solution, 100 μg/ml sheared, denatured salmon sperm DNA and 0.5% sodium dodecyl sulfate) at 42°C. Filters were washed twice using 2 x SSC at 65°C followed by two additional washings with 1 x SSC/0.1% SDS at 65°C. Autoradiography was carried out at -80°C with use of an intensifying screen.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

1. Essentially pure mammalian DNA damage-binding factor.

2. DNA damage-binding factor of Claim 1 which is of human origin.

3. Essentially pure mammalian cell factor which selectively recognizes and binds damaged DNA.

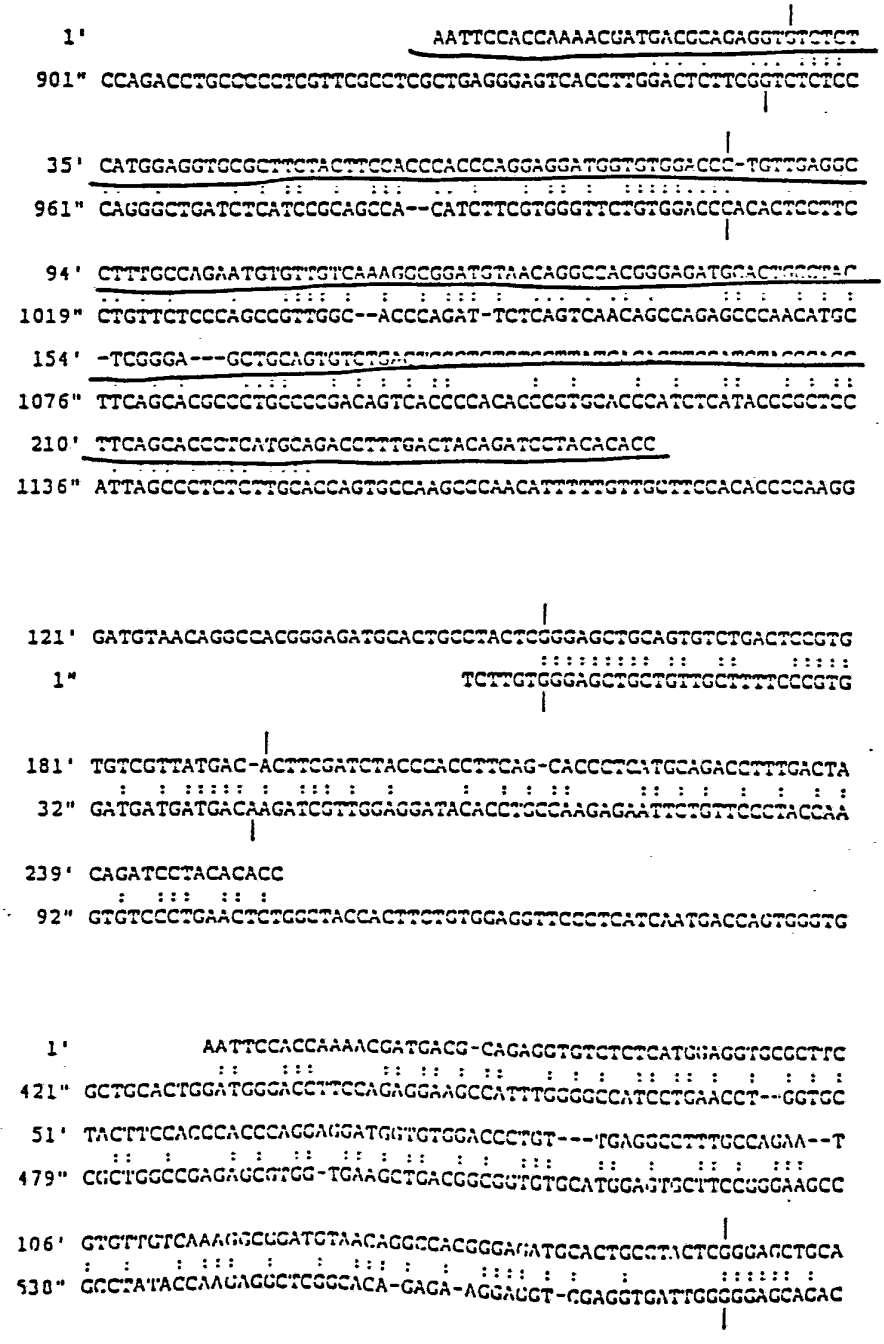
4. Essentially pure mammalian cell factor of Claim 3 which is of human origin and which selectively recognizes and binds DNA damaged by a drug.

5. Essentially pure mammalian cell factor of Claim 4 which selectively recognizes and binds DNA damaged by a drug which is a chemotherapeutic drug.

6. Essentially pure DNA having all or a portion of the nucleotide sequence of the lambda Pt1 gene or all or a portion of the nucleotide sequence of the lambda Pt2 gene.

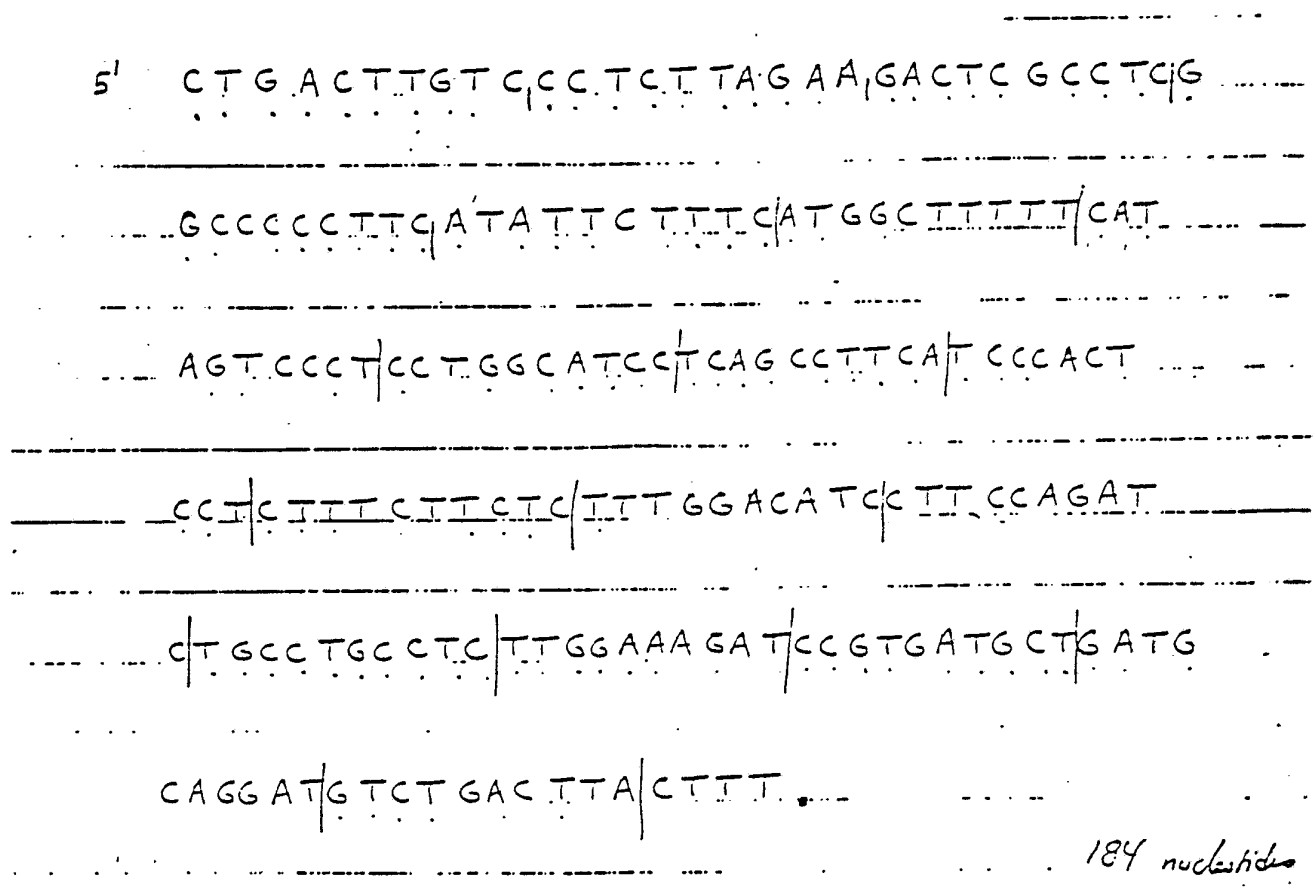
7. Essentially pure DNA having all or a portion of the nucleotide sequence selected from the group consisting of:

a) the following nucleotide sequence:



and

b) the following nucleotide sequence:



8. Isolated DNA having the nucleotide sequence of all or a portion of the lambda Ptl gene present in E. coli recombinant cells deposited at the American Type Culture Collection under accession number 40498.

9. A nucleotide probe capable of hybridizing to all or a portion of DNA encoding mammalian DNA damage-binding factor.

10. A nucleotide probe of Claim 9 capable of hybridizing to all or a portion of DNA encoding DNA damage-binding factor of human origin.

11. A nucleotide probe of Claim 10 capable of hybridizing to all or a portion of DNA encoding DNA damage-binding factor, the DNA damage-binding factor capable of binding to DNA damaged by a drug.

12. An antibody specifically reactive with DNA damage-binding factor of mammalian origin.

13. An antibody of Claim 12 specifically reactive with DNA damage-binding factor of human origin.

14. An antibody of Claim 13 specifically reactive with DNA damage-binding factor capable of binding to DNA damaged by a drug.

15. A method of detecting DNA damage-binding factor in mammalian cells, comprising the steps of:

a. treating mammalian cells in such a manner as to produce a cellular extract containing DNA from the cells;

b. contacting the cellular extract produced in (a) with a nucleotide probe capable of hybridizing with cellular DNA encoding DNA damage-binding factor, under conditions appropriate for hybridizing to occur; and

c. detecting hybridization.

16. A method of Claim 15 in which the mammalian cells are human cells.

17. A method of detecting DNA damage-binding factor in mammalian cells, comprising the steps of:

a. treating mammalian cells in such a manner as to render proteins, and portions thereof, present in the cells available for binding with antibodies specific to the proteins or portions thereof;

b. contacting the product of (a) with antibodies capable of binding with DNA damage-binding factor, under conditions appropriate for binding of antibodies with proteins or portions thereof to which the antibodies are specific; and

c. detecting binding of antibodies and proteins or portions thereof.

18. A method of Claim 17 wherein the mammalian cells are human cells.

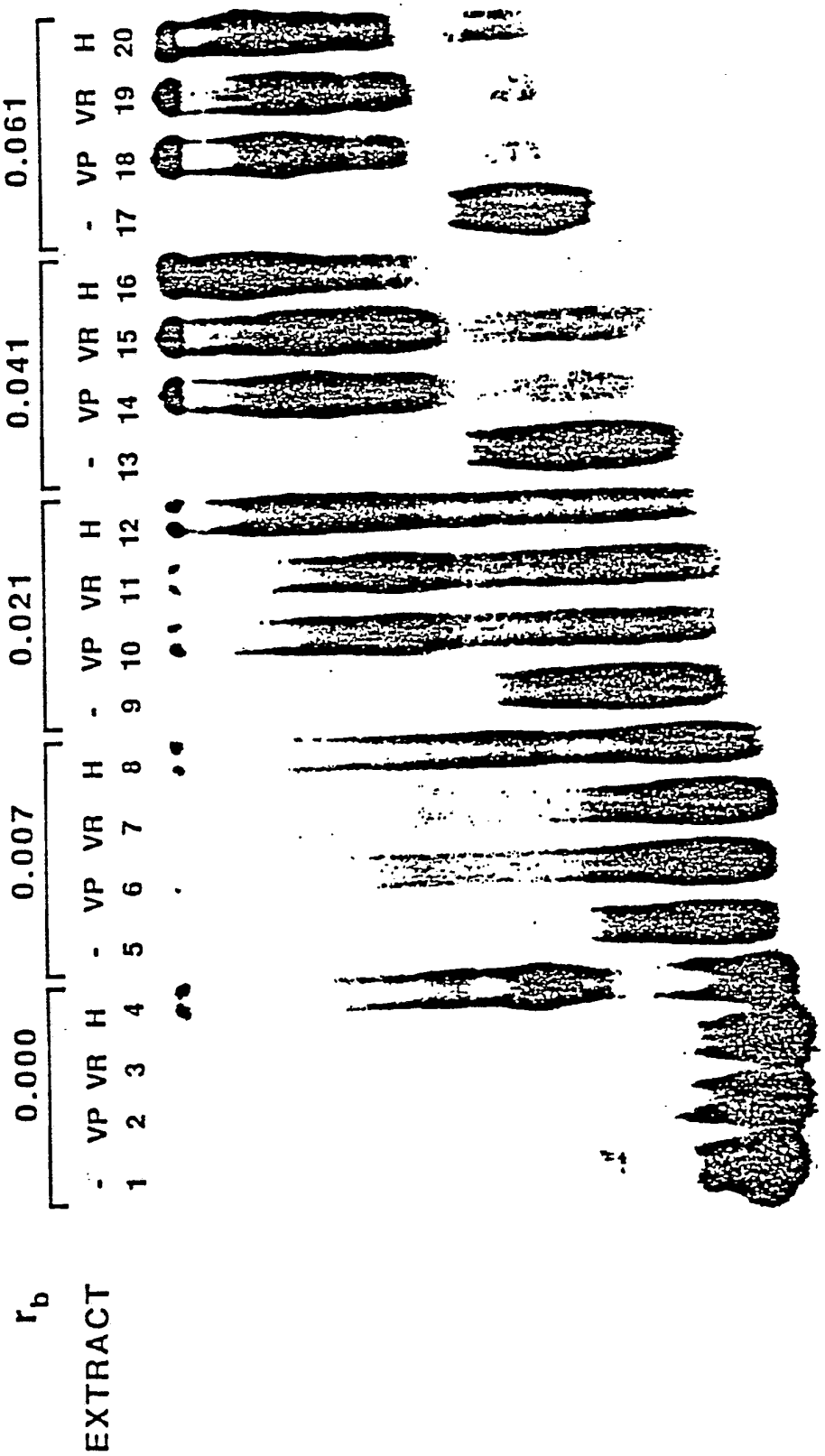

r_b
EXTRACT
0.000
0.007
0.021
0.041
0.061
- VP VR H - VP VR H - VP VR H - VP VR H - VP VR H
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIGURE 1

FIGURE 2

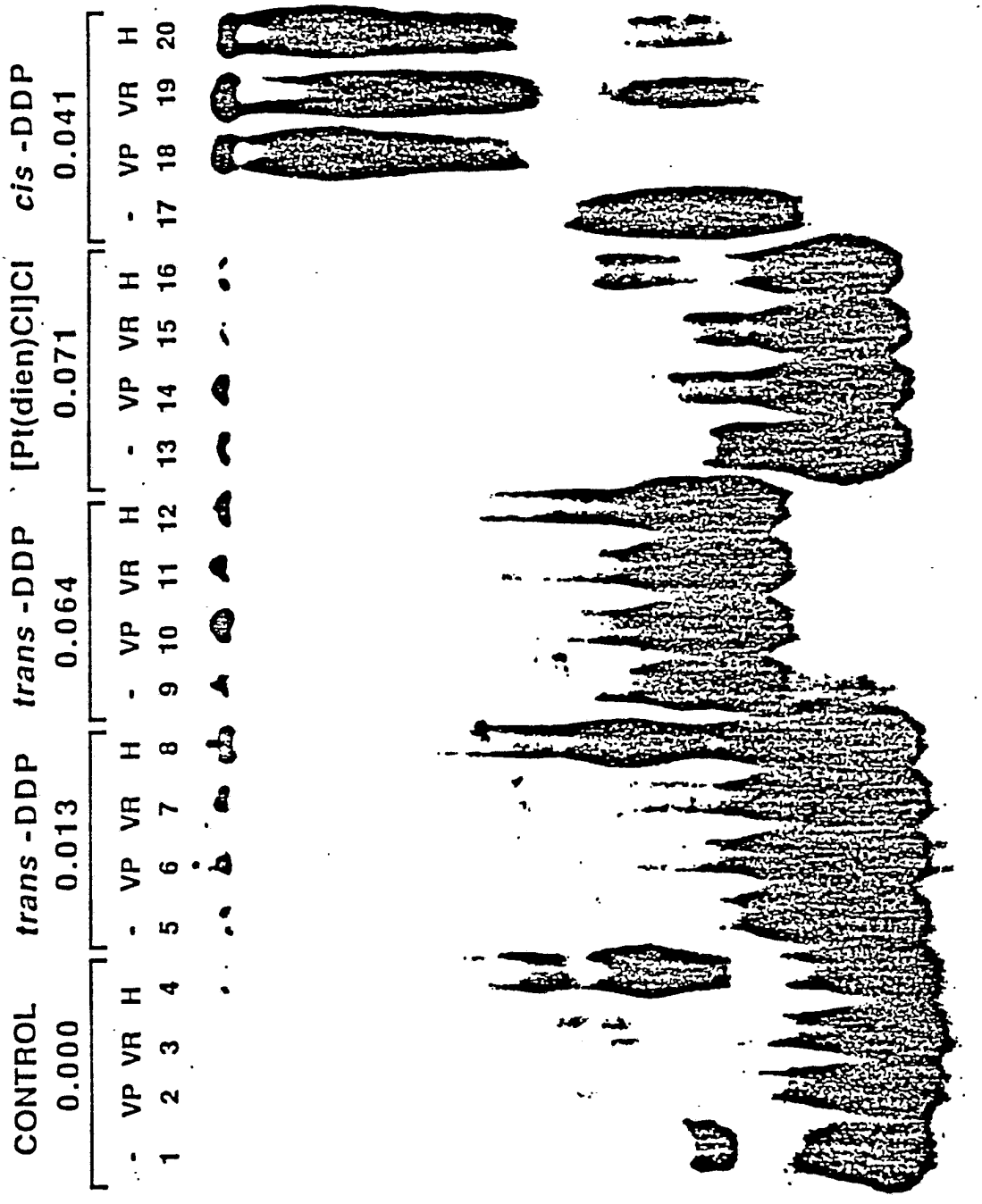
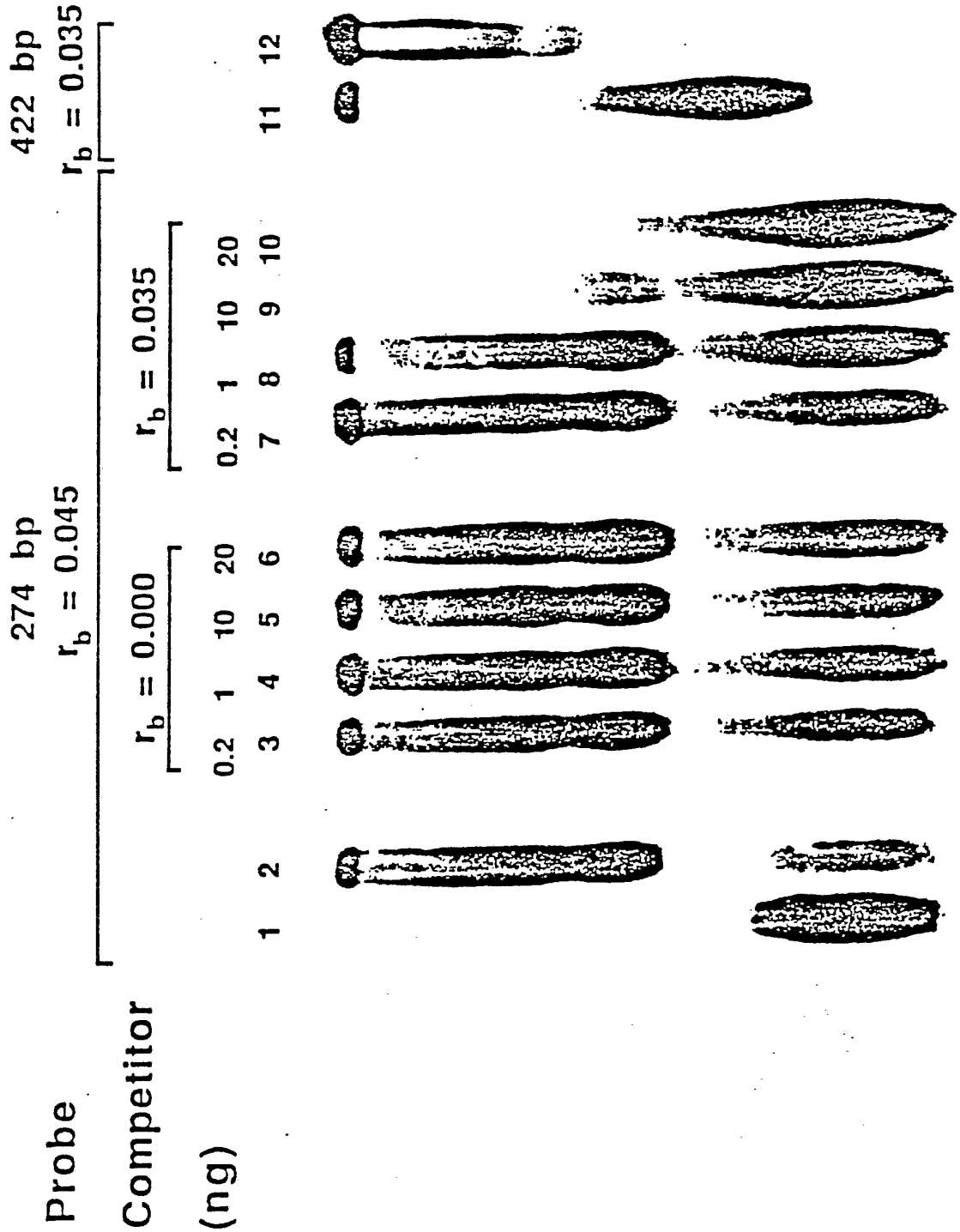

r_b
EXTRACT
CONTROL
0.000
trans -DDP
0.013
trans -DDP
0.064
[Pt(dien)Cl]Cl
0.071
cis -DDP
0.041
- VP VR H - VP VR H - VP VR H - VP VR H
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIGURE 3


422 bp
r_b = 0.035
274 bp
r_b = 0.045
r_b = 0.035
r_b = 0.000
Probe
Competitor
(ng)
0.2 1 10 20
0.2 1 10 20
1 2 3 4 5 6 7 8 9 10 11 12

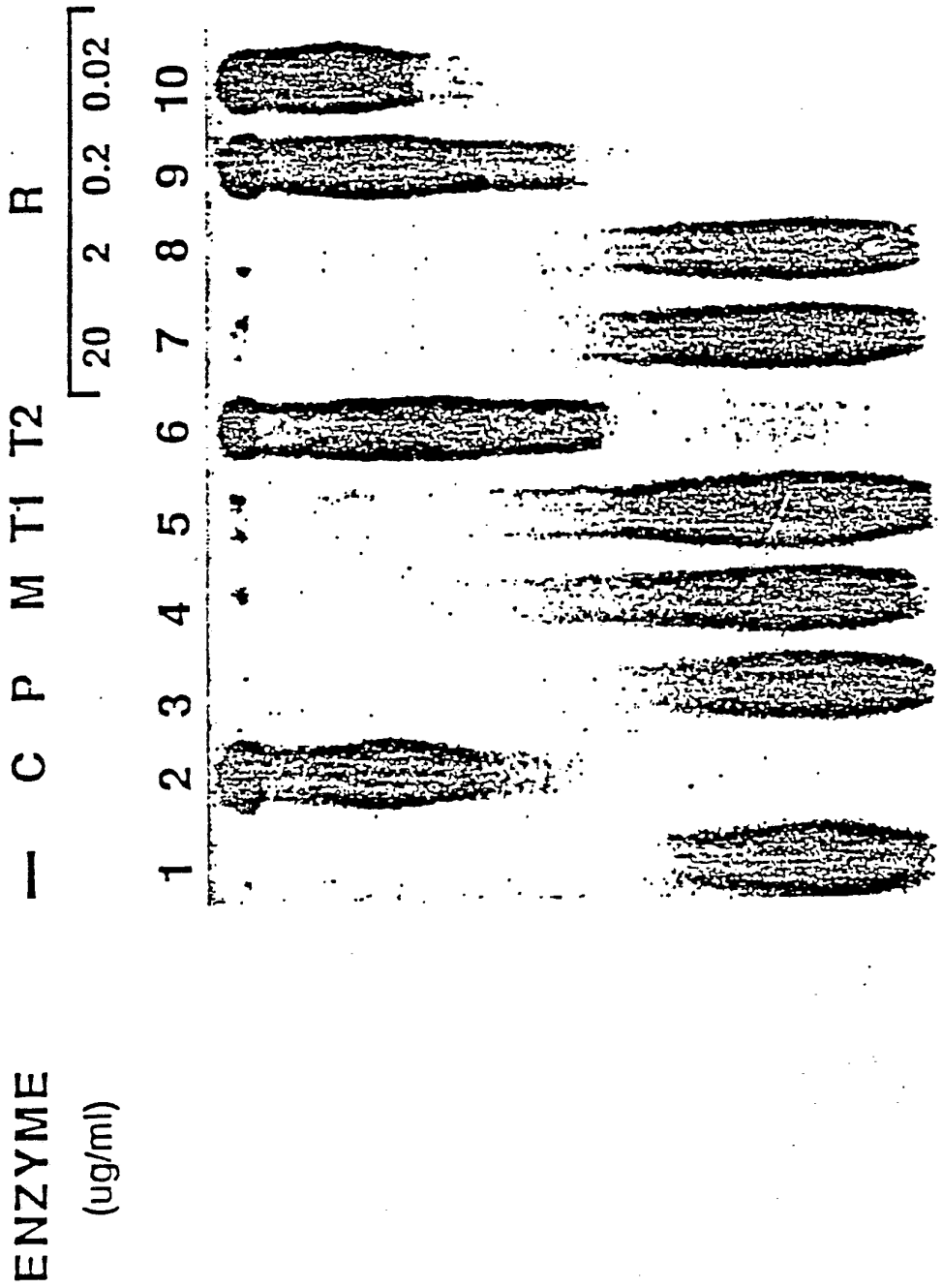
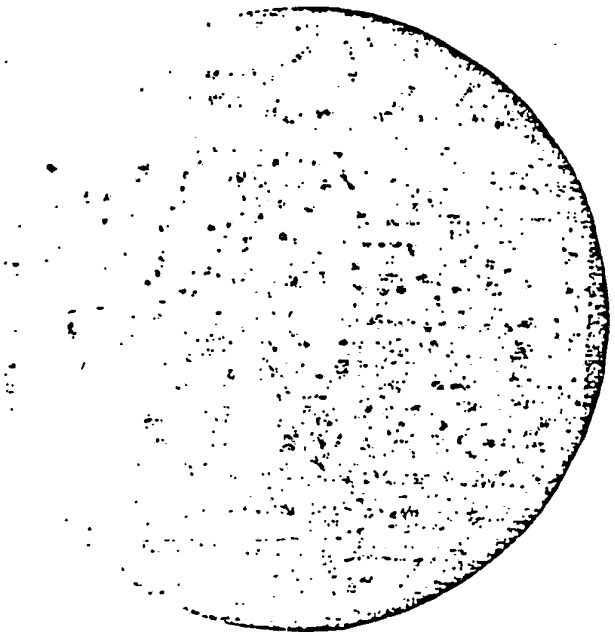
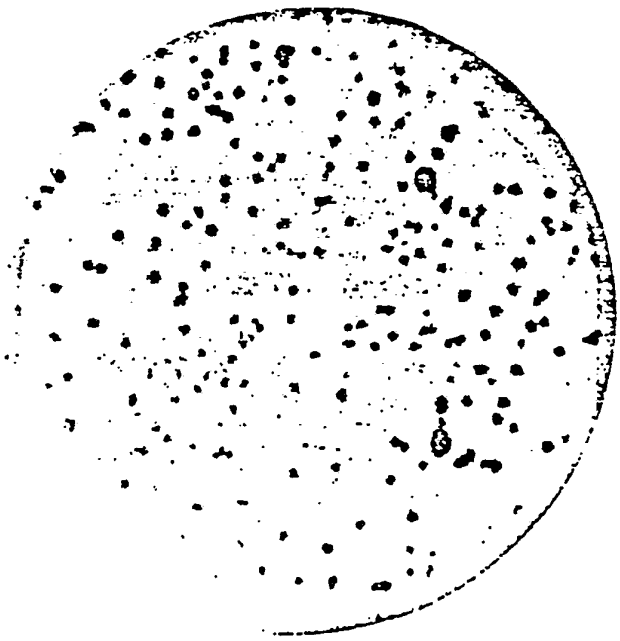
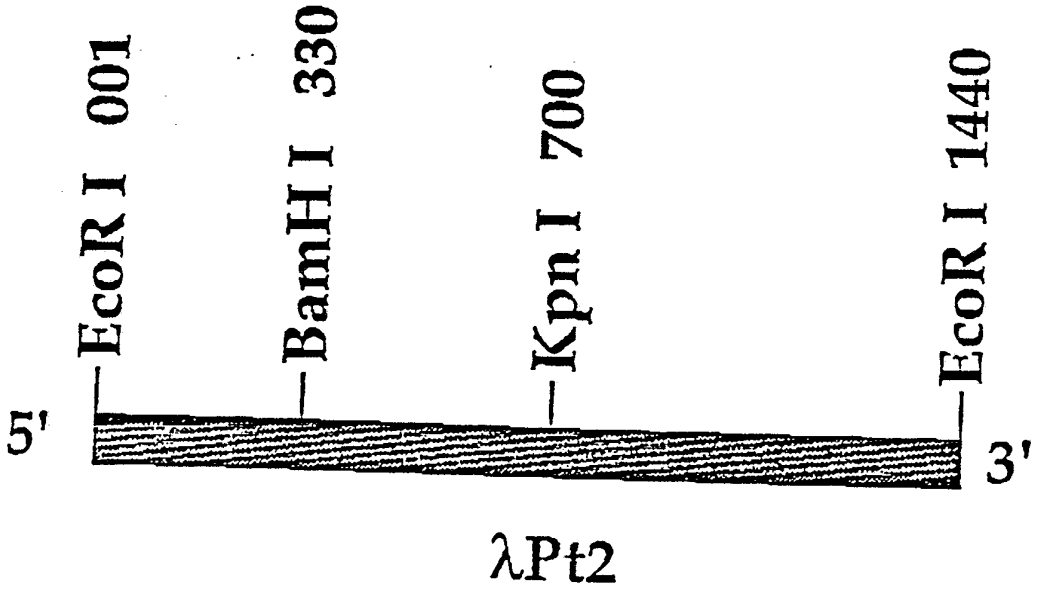


FIGURE 4

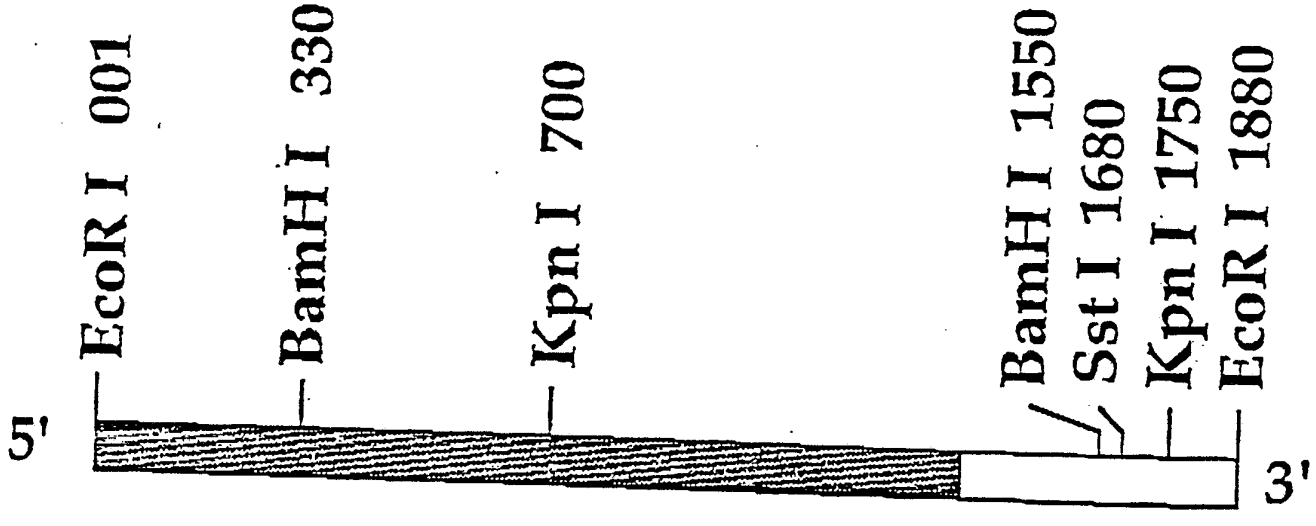
FIGURE 5







λPt2



λPt1

FIGURE 6

FIGURE 7A

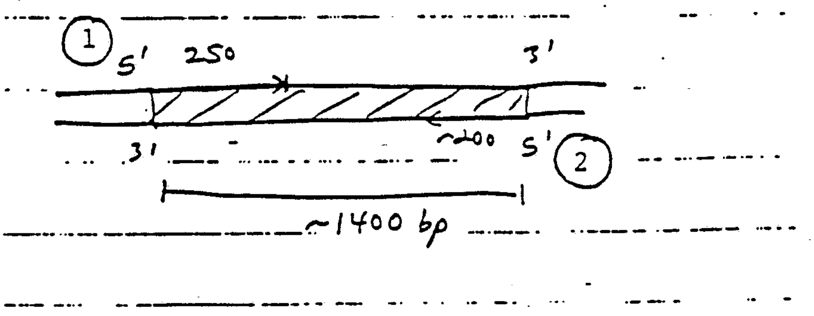
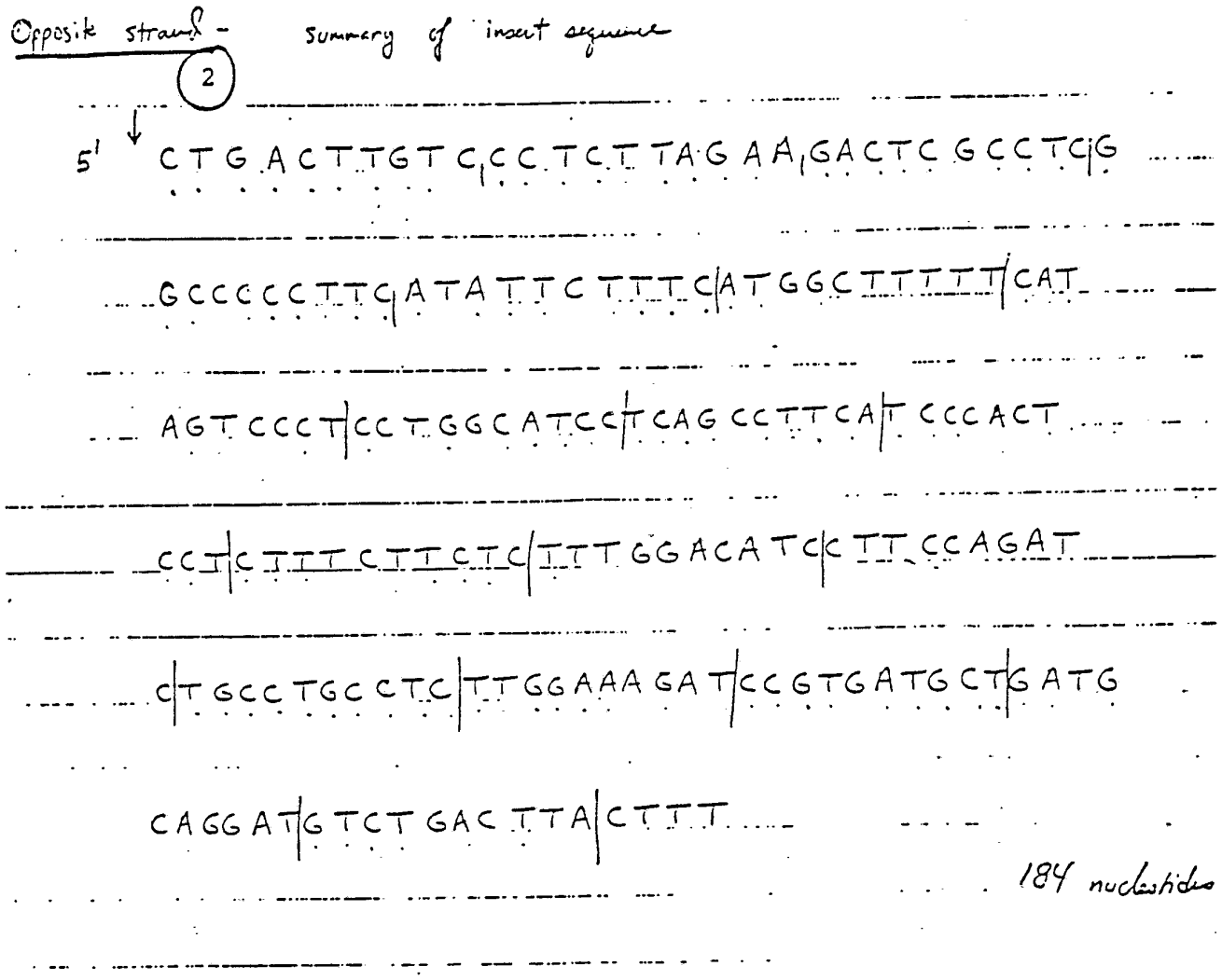


FIGURE 7B

```
>RL;CIPGL   :  Calpain I light chain mRNA - Pig              80,  146
 50.0% identity in 214 NT overlap

   1'                  (1) AATTCCACCAAAACGATGACGCAGAGGTGTCTCT
 901" CCAGACCTGCCCCCTCGTTCGCCTCGCTGAGGGAGTCACCTTGGACTCTTCGGTCTCTCC

  35' CATGGAGGTGCGCTTCTACTTCCACCCACCCAGGAGGATGGTGTGGACCC-TGTTGAGGC
 961" CAGGGCTGATCTCATCCGCAGCCA--CATCTTCGTGGGTTCTGTGGACCCACACTCCTTC

  94' CTTTGCCAGAATGTGTTGTCAAAGGCGGATGTAACAGGCCACGGGAGATGCACTGCCTAC
1019" CTGTTCTCCCAGCCGTTGGC--ACCCAGAT-TCTCAGTCAACAGCCAGAGCCCAACATGC
 154' -TCGGGA---GCTGCAGTGTCTGA[illegible]
1076" TTCAGCACGCCCTGCCCCGACAGTCACCCCACACCCGTGCACCCATCTCATACCCGCTCC
 210' TTCAGCACCCTCATGCAGACCTTTGACTACAGATCCTACACACC
1136" ATTAGCCCTCTCTTGCACCAGTGCCAAGCCCAACATTTTTGTTGCTTCCACACCCCAAGG


 121' GATGTAACAGGCCACGGGAGATGCACTGCCTACTCGGGAGCTGCAGTGTCTGACTCCGTG
   1"                                 TCTTGTGGGAGCTGCTGTTGCTTTTCCCGTG

 181' TGTCGTTATGAC-ACTTCGATCTACCCACCTTCAG-CACCCTCATGCAGACCTTTGACTA
  32" GATGATGATGACAAGATCGTTGGAGGATACACCTGCCAAGAGAATTCTGTTCCCTACCAA

 239' CAGATCCTACACACC
  92" GTGTCCCTGAACTCTGGCTACCACTTCTGTGGAGGTTCCCTCATCAATGACCAGTGGGTG


   1'           AATTCCACCAAAACGATGACG-CAGAGGTGTCTCTCATGGAGGTGCGCTTC
 421" GCTGCACTGGATGGGACCTTCCAGAGGAAGCCATTTGGGGCCATCCTGAACCT--GGTGC

  51' TACTTCCACCCACCCAGGAGGATGGTGTGGACCCTGT---TGAGGCCTTTGCCAGAA--T
 479" CGCTGGCCGAGAGCGTGG-TGAAGCTGACGGCGGTCTGCATGGAGTGCTTCCGGGAAGCC

 106' GTGTTGTCAAAGGCGGATGTAACAGGCCACGGGAGATGCACTGCCTACTCGGGAGCTGCA
 538" GCCTATACCAAGAGGCTCGGCACA-GAGA-AGGAGGT-CGAGGTGATTGGGGGAGCACAC
```

FIGURE 7C


Opposite strand - summary of insert sequence
2
5'
CTGACTTGTCCCTCTTAGAAGACTCGCCTCG
GCCCCCTTCGATATTCTTTCATGGCTTTTTTCAT
AGTCCCTCCTGGCATCCTCAGCCTTCATCCCACT
CCTCTTTCTTCTCTTTGGACATCCTTCCAGAT
CTGCCTGCCTCTTGGAAAGATCCGTGATGCTGATG
CAGGATGTCTGACTTACTTT
184 nucleotides

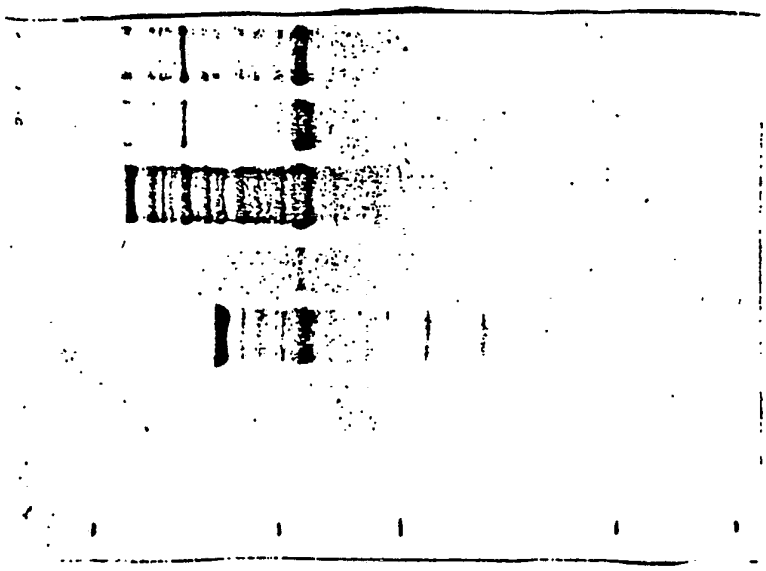


FIGURE 8B

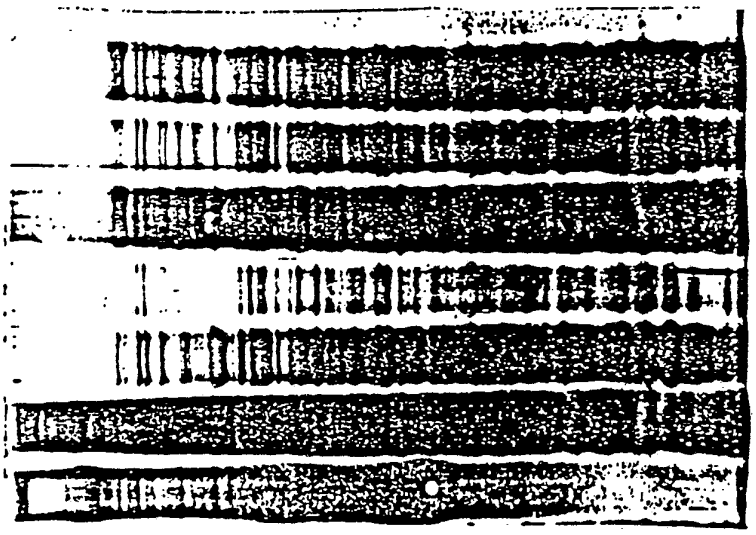


FIGURE 8A

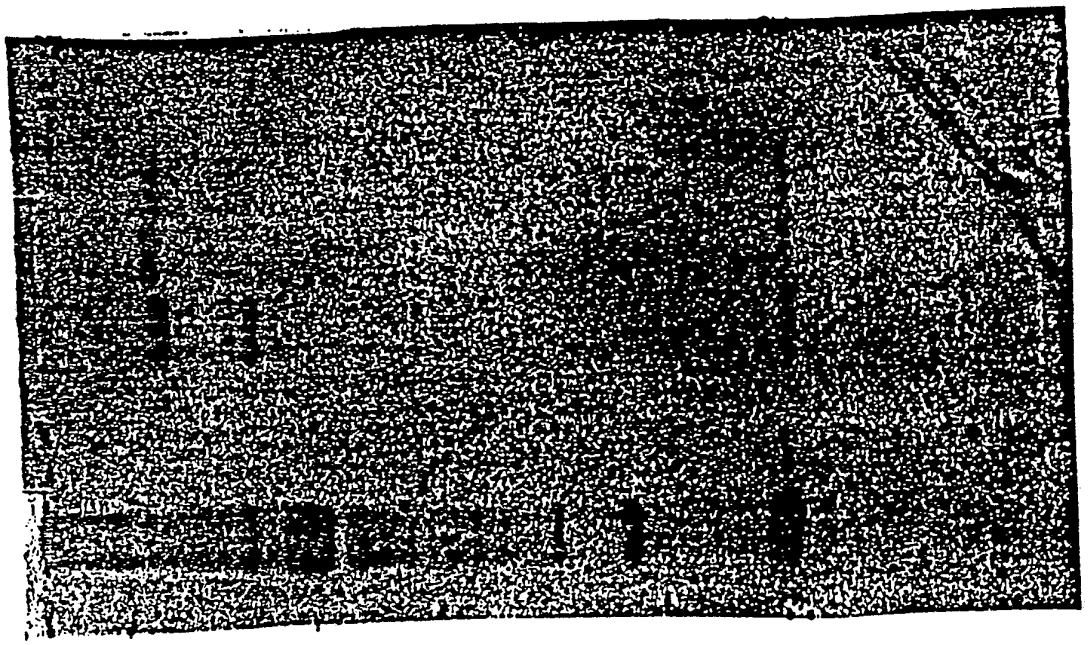


FIGURE 8D

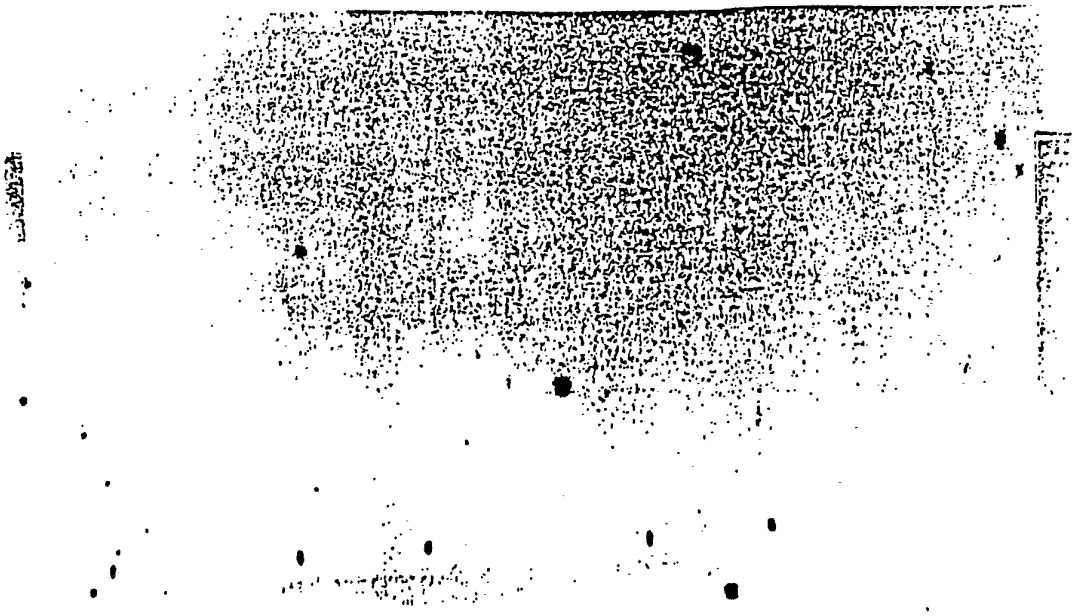


FIGURE 8C

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/04128

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 K 13/00, C 12 Q 1/68

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	C 07 K; C 12 Q

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	The EMBO Journal, Vol. 2, No. 4, 1983, J.J. Toulm et al: "Recognition of chemically damaged DNA by the gene 32 protein from bacteriophage T4", see page 505 - page 510	1 5
	--	
X Y	Nucleic Acids Research, Vol. 13, No. 1, 1985, Josephine A. Carew and Ross S. Feldberg: "Recognition of a cytosine base lesion by a human damage-specific DNA binding protein ", see page 303 - page 315	1-4,9-14 5
	--	
X Y	Bioscience Reports, Vol. 3, 1983, Urs Kuhnlein et al: "Cell lines from xeroderma pigmentosum complementation group A lack a single-stranded-DNA-binding activity ", see page 667 - page 674	1-4,9-14 5
	--	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th January 1990	22 JAN. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Mutation Research, Vol. 145, 1984, M. Germanier et al: "Repair of platinum-DNA lesions in E.coli by a pathway which does not recognize DNA damage caused by MNNG or UV light ", see page 35 - page 41	1-14
A	EP, A1, 0057553 (ATOMIC ENERGY OF CANADA LIMITED) 11 August 1982, see the whole document	1-14

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 89/04128

SA 31827

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 08/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0057553	11/08/82	JP-A- 57144994	07/09/82
		US-A- 4407942	04/10/83
		CA-A- 1161736	07/02/84