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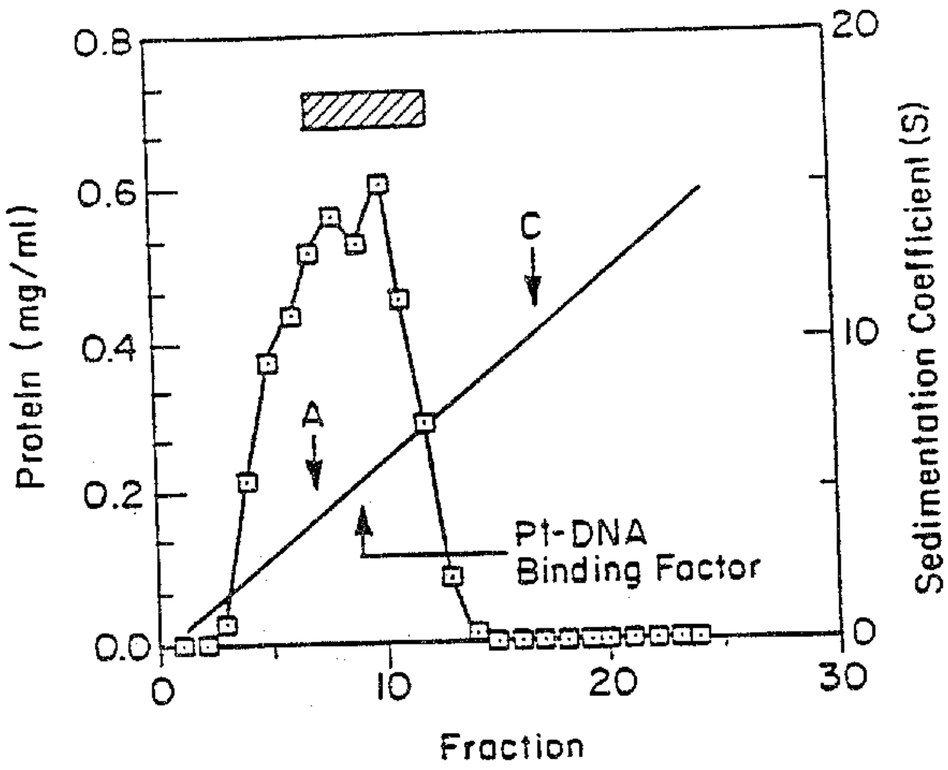
(54) **PROTEINE RECONNAISSANT SPECIFIQUEMENT UNE STRUCTURE D'ADN ET SON UTILISATION**

(54) **DNA STRUCTURE-SPECIFIC RECOGNITION PROTEIN AND USES THEREFOR**



(57) Protéine de reconnaissance spécifique à la structure de l'ADN d'origine eukaryotique et ADN codant ce facteur, ainsi que des sondes spécifiques à la protéine de reconnaissance spécifique à la structure de l'ADN ou à l'ADN la codant, et méthodes de détection de la protéine de reconnaissance spécifique à la structure de l'ADN dans les cellules eukaryotiques. On a notamment identifié un facteur cellulaire de mammifère qui reconnaît sélectivement l'ADN endommagé ou modifié par un médicament (le médicament anticancéreux cis-diaminedichloroplatinum (II) ou cisplatine).

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

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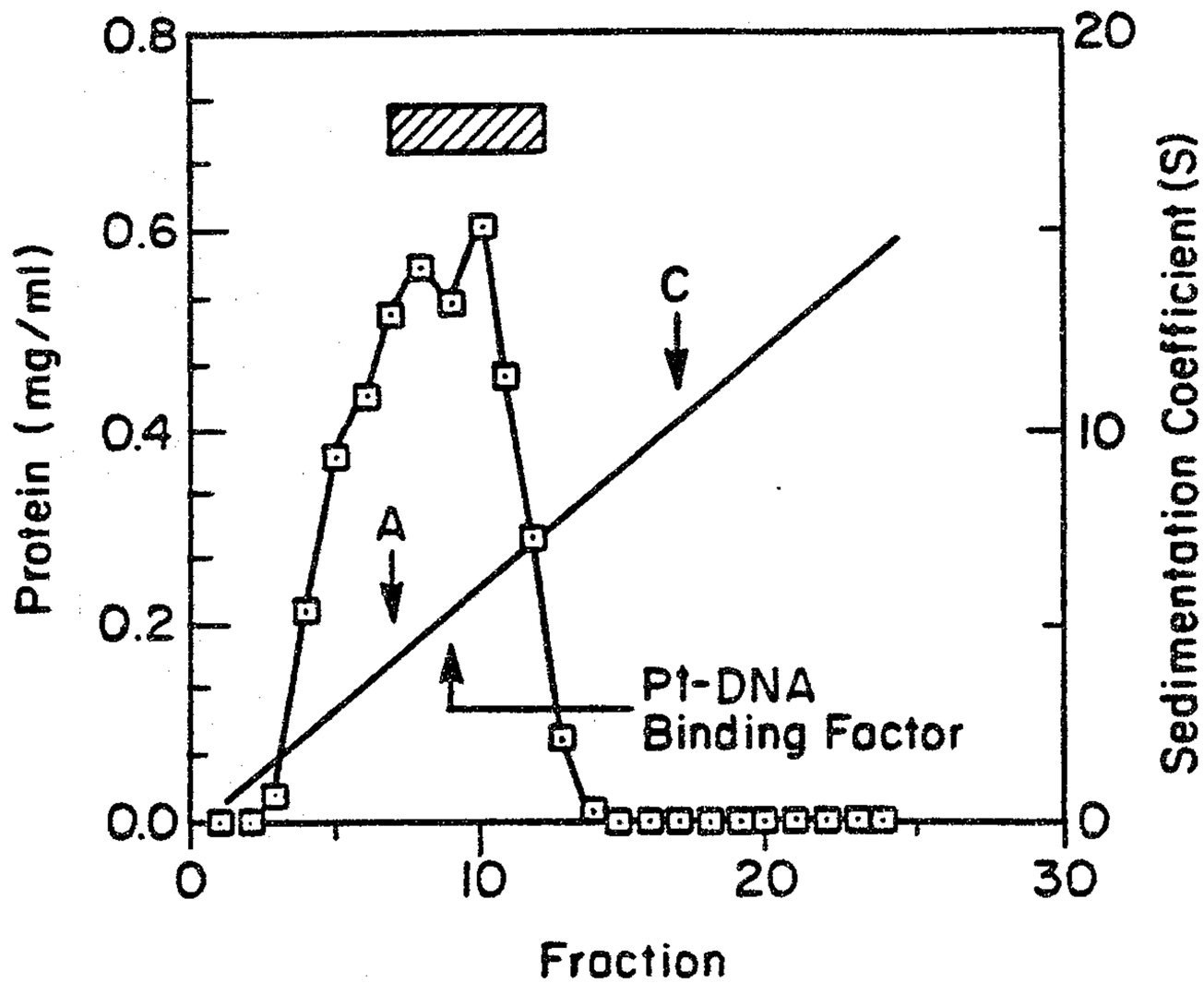

(57) Abstract

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

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DNA STRUCTURE-SPECIFIC RECOGNITION PROTEIN AND USES THEREFOR

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 which arise from such external causes are all considered herein to be DNA modification or damage. In addition, it is recognized that certain chemical and/or structural modifications in DNA may occur naturally, and may play a role in, for example, DNA replication, expression, or the coordinate regulation of specific genes. It has been proposed that some types of DNA modification or damage arising from external sources are similar to, or even mimic, certain types of natural DNA chemical and/or structural modification.

The mechanism(s) by or conditions under which DNA modification or damage occurs are presently unknown or poorly understood. 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. A better understanding of DNA chemical and structural modifications, including DNA damage, would also be helpful in that it might serve as the basis for developing an enhanced ability to repair or otherwise modify the effects of such damage, leading in turn to improved organismal or suborganismal resistance to DNA damaging agents.

Summary of the Invention

The present invention relates in one aspect to a DNA damage-binding factor, referred to herein as a DNA structure-specific recognition protein or SSRP; it has

previously been referred to as a DNA damage recognition protein or DRP. The SSRP has been shown to bind selectively to damaged DNA in mammalian cell extracts.

In another aspect, the invention described herein relates to nucleotide sequences which encode SSRP. In still another aspect, it relates to a method of identifying SSRP in eukaryotic cells. Other aspects of the present invention relate to use of SSRP, amino acid sequences encoding SSRP and antibodies which bind to the structure-specific recognition protein described herein.

Furthermore, this invention relates to methods of preventing or reducing damage to DNA that is the result of DNA processing (e.g., replication, recombination and repair) or is caused by contact with or exposure to a chemical compound, physical substance or other damaging agent which produces a particular, recognizable type of DNA structural damage.

The DNA structure-specific recognition protein of the present invention binds selectively to double-stranded (ds) DNA which has been structurally modified as a result of exposure to a chemical agent, such as a therapeutic agent administered for cancer therapy. Specifically, SSRP of the present invention binds selectively to ds DNA containing at least one 1,2-intrastrand dinucleotide adduct. SSRP has been shown to bind selectively to a damaged DNA fragment, by which is meant a ds DNA fragment which contains a 1,2-intrastrand dinucleotide adduct of a therapeutically active platinum compound, such as *cis*-diamminedichloroplatinum (II) (*cis*-DDP or cisplatin). As a result of selective binding of the SSRP to cisplatin-damaged DNA, a (damaged DNA fragment):(protein) complex is formed. The electrophoretic mobility of this complex is retarded, relative to the mobility of the damaged DNA fragment alone (i.e., not having SSRP bound thereto). Therefore, the complex can be electrophoretically resolved from the damaged DNA fragment alone.

cis-DDP SSRP of the present invention has been shown to bind selectively to damaged ds DNA containing the 1,2-intrastrand d(GPG) and d(ApG) dinucleotide adducts formed by *cis*-DDP. This binding is selective in that the SSRP does not significantly bind to single-stranded (ss) DNA, or to ds DNA lacking a 1,2-intrastrand dinucleotide adduct such as the d(ApG) and d(GpG) adducts formed by cisplatin.

The present invention also encompasses a generally applicable method of identifying other DNA structure-specific recognition proteins in eukaryotic cells, particularly those encoded by DNA which hybridizes to the DNA encoding the *cis*-DDP SSRP described and claimed herein. That is, this method can be used to identify other proteins having *cis*-DDP SSRP activity, encoded by DNA which comprises at least a region of sequence homologous to the *cis*-DDP SSRP gene. The present invention encompasses SSRPs identified by this method.

Brief Description of the Drawings

Figure 1 is an illustration of the nucleotide sequence of synthetic duplex oligonucleotides containing specific platinum adducts. The 22-base oligonucleotides containing specific platinum adducts and designated as Top strands are shown 5′ → 3′ with their complementary bottom strands.

Figure 2 is a graphic illustration of the sedimentation of the cellular SSRP through a sucrose density gradient. □, protein concentration (mg/mL); A, C, and -, sedimentation coefficient size markers (A, albumin (M_r of 67 000 daltons); C, catalase (M_r of 232 000 daltons)). The hatched box indicates the sedimentation region corresponding to *cis*-DDP-DNA binding activity (as determined by EMSA study of the fractions).

Figure 3 is a schematic representation of the restriction endonuclease maps of phages λPt1 and λPt2 showing the 5' alignment of their cDNA inserts.

Figure 4 is a schematic illustration showing the relationship among human cDNA clones encoding SSRP.

Figure 5 (A-F) is a composite nucleotide sequence of the human gene for structure-specific recognition protein, shown together with the predicted amino acid sequence of the encoded protein.

Figure 6 which appears on the same drawing sheet as Figure 4, is a schematic illustration, prepared from the predicted amino acid sequence of the human SSRP gene showing various domains of the human structure-specific recognition protein. HMG or HMG-box; domain having a high degree of sequence homology to high mobility group 1 protein.

Figure 7 is a schematic illustration showing the relationship between *Drosophila melanogaster* cDNA clones DM 3002 and DM 1001.

Figure 8 (A-F) is a composite nucleotide sequence of the *D. melanogaster* gene for structure-specific recognition protein, shown together with the predicted amino acid sequence of the encoded protein.

Figure 9 which appears on the same drawing sheet as Figure 7, is a schematic illustration, prepared from the predicted amino acid sequences of the human and the *D. melanogaster* (Dmel) SSRP genes, showing various domains of the structure-specific recognition protein homologs.

Figure 10 which appears on the same drawing sheet as Figure 1, is a schematic illustration of the positions of restriction endonuclease sites in the λyPt clone.

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Figure 11 is the nucleotide sequence of the λyPt clone, which includes a fractional sequence for the yeast structure-specific recognition protein (ySSRP) gene.

Figure 12 is the predicted amino acid sequence of fractional yeast structure-specific recognition protein (fySSRP), encoded by λyPt.

Detailed Description of the Invention

The present invention is based on the discovery in extracts of eukaryotic cells of a DNA structure-specific recognition protein (SSRP), which recognizes and selectively binds to a structural motif present in damaged DNA. SSRP was originally defined by its characteristic of selectively binding to DNA damaged by therapeutically active platinum compounds and thus it was previously referred to as a DNA damage-recognition protein (DRP), and specifically as a *cis*-DDP DRP.

The term "structural motif" is intended to encompass any type of nucleic acid secondary structure or tertiary structure which differs in a detectable manner from ordinary helical duplex DNA. Structural motifs can be sequence-dependent or sequence-independent. Thus, cruciform DNA, kinked DNA, overwound, partially unwound or underwound helical DNA, different helical forms of DNA (e.g., A or Z helices), junctions between different helical forms, modified bases (e.g., thymine dimers, methylated guanosine or cytosine residues), and combinations thereof, are all examples of DNA structural motifs. See generally, W. Saenger, *Principles of Nucleic Acid Structure*, Springer Advanced Texts in Chemistry, C. Cantor, series ed., Springer-Verlag New York, Inc., New York (1984).

Structural motifs can be generated during the course of normal or aberrant cellular activities in which DNA participates, such as DNA replication, recombination, or repair. Certain structural motifs comprise DNA damage or lesions; others are thought to be associated with the control of cellular processes. Structural motifs generally classified as DNA damage can be produced by drugs which interact with nucleic acids to form detectable

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lesions such as base- or sugar-drug adducts, or intercalations. DNA damage-associated structural motifs can also be produced spontaneously, e.g., by exposure to or contact with an environmental damage-causing agent. Such an agent can be a chemical compound or a physical agent (e.g., UV radiation). Friedberg, E.C., DNA Repair, Chapter 1, W.H. Freeman & Co., New York (1985).

A DNA structural motif of particular interest comprises a 1,2-intrastrand dinucleotide adduct. This type of structural motif or lesion is known to be formed as a result of the interaction of therapeutically effective platinum compounds which are used for the treatment of cancer (e.g., *cis*-DDP or cisplatin) with DNA. As described more fully below, it has been suggested that the structural motif or lesion produced by therapeutically active platinum drugs interacts with the cellular machinery for DNA repair. Therefore, a factor, such as a protein, which is capable of selectively recognizing this structural motif (i.e., a platinated DNA motif comprising a region of DNA damage or a lesion, specifically a 1,2-intrastrand dinucleotide adduct of cisplatin), is a valuable tool for developing an understanding of the mechanisms underlying susceptability and/or resistance to cancer and to particular cancer therapeutics. Accordingly, the platinated 1,2-intrastrand dinucleotide adduct DNA structural motif has been employed as a model system for the method of the invention described herein. It will be understood that the present method of identification and isolation of structure-specific recognition proteins (SSRPs) can also be used to identify and isolate SSRPs which recognize other DNA structural motifs; its utility is not confined to the 1,2-intrastrand dinucleotide adduct of a therapeutically effective platinum compound.

The present invention relates to a method of identifying and isolating DNA structure specific

recognition proteins (SSRPs) which bind selectively to particular DNA structural motifs present in mamalian cells as a result of spontaneous damage or environmental damage. It relates to SSRPs identified according to this method, and to antibodies reactive with these SSRPs. It relates further to DNA and RNA and to nucleic acid probes encoding SSRPs identified according to the method described herein. The method of the present invention will now be described in the context of its use to identify and characterize a DNA structure-specific recognition protein which selectively binds cisplatin-modified DNA. *cis*-DDP SSRP was identified and characterized in mammalian and other eukaryotic cells, as described more fully in the Examples which follow. Isolation and cloning of a human cDNA encoding SSRP of the present invention is also described herein. Other aspects of the present invention comprising the use of SSRP as well as of nucleotide sequences encoding it and antibodies reactive with it, for therapeutic, diagnostic and prophylactic purposes are also discussed below.

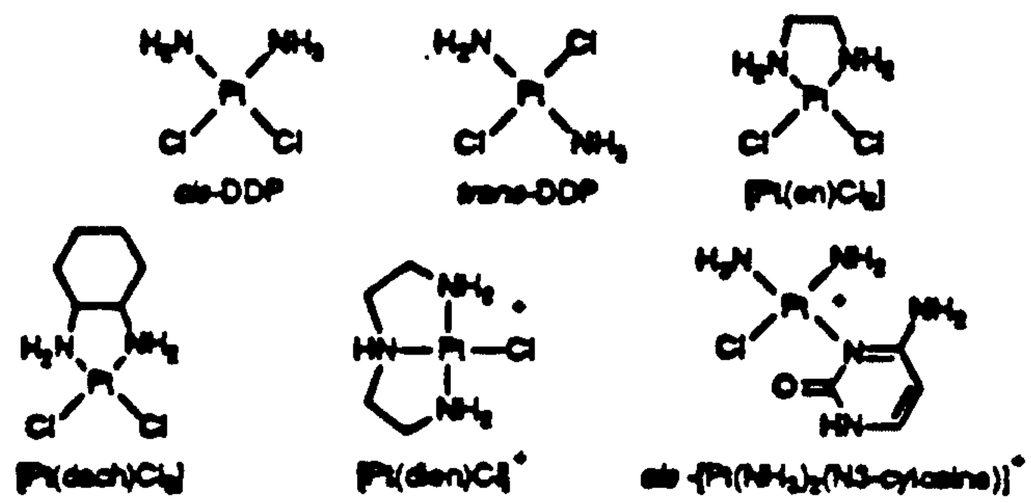
Platinated DNA structural motifs

cis-Diamminedichloroplatinum(II) (*cis*-DDP or cisplatin) is a clinically important antitumor drug used mainly to combat ovarian and testicular malignancies. Loehrer, P.J. and L.H. Einhorn, Ann. Intern. Med., **100**:704-713 (1984). The major cellular target for *cis*-DDP is generally accepted to be DNA, although it is not yet certain whether antitumor efficacy is a consequence of impaired replication or transcription. Sorenson, S.M. and A. Eastman, Cancer Res. **48**:4484-4488 and 6703-6707 (1988). 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* and cause mutagenesis. Lee, K.W. and D.S. Martin, Jr., Inorg. Chim. Acta, **17**:105-

110 (1976); Lim, M.C. and R.B. Martini, J. Inorg. Nucl. Chem. 38:119-1914 (1984); 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).

trans-Diamminedichloroplatinum(II), the geometic isomer of *cis*-DDP in which the amine and chloride moieties are in mutually *trans* positions, is ineffective as a chemotherapeutic agent. Connors, T.A. et al., Chem.-Biol. Interact. 5:415-424 (1972). *trans*-DDP will block replication at doses equitoxic to those of *cis*-DDP. It has been postulated that differential repair may be responsible for the chemotherapeutic effectiveness of *cis*-DDP compared to *trans*-DDP. Ciccarelli, R.B. et al., Biochemistry 24:7533-7540 (1985). The *trans*-DDP reaction products with DNA include monofunctional adducts, intrastrand cross-links, interstrand cross-links, and protein-DNA cross-links. Pinto A.L. and S.J. Lippard, Proc. Natl. Acad. Sci. USA 82:4616-4619 (1985); Eastman, A. and M.A. Barry, Biochemistry 26:3303-3307 (1987). *trans*-DDP cannot form intrastrand cross-links between adjacent nucleotides, and this observation has led to the suggestion that the d(GpG) and d(ApG) adducts formed uniquely by *cis*-DDP are responsible for its antitumor activity. Cardonna, J.P. and S. J. Lippard, Adv. Chem. Ser. 209:14-16 (1983); and Pinto, A.L. and S.J. Lippard, Biochem. Biophys. Acta 780:167-180 (1985). This hypothesis is supported by the observation that most chemotherapeutically effective platinum compounds have chloride moieties in *cis* positions and are believed to form a spectrum of DNA adducts similar to those of *cis*-DDP, i.e., 1,2-intrastrand cross-links. Lippard, S.J. et al., Biochemistry 22:5165-5168 (1983).

The chemical formulae for *cis*- and *trans*-DDP, and for several clinically related platinum compounds are as follows:



cis-DDP binds to DNA in a bidentate manner, forming mainly 1,2-intrastrand d(GpG) and d(ApG) crosslinks that kink the strand of the helix bearing the platinated adduct, and possibly concurrently form a localized single stranded region of the opposite strand which would be detectable by antinucleoside antibodies. Sherman, S.E., and S.J. Lippard, Chem. Rev., 87:1153-1181 (1987); Rice, J.A. et at., Proc. Natl. Acad. Sci., USA. 85:4158-4161 (1988); Sundquist, W.I. et al., Biochemistry, 25:1520-1524 (1986). The 1,2- intrastrand d(GpG) adduct of *cis*-DDP produces a bend in the helix of DNA by 32-34° directed toward the major groove (Rice, J. A., Crothers, D.M., Pinto, A.L. & Lippard, S.J. (1988) Proc. Natl. Acad. Sci. U.S.A. 85:4158-4161; Bellon, D.F. & Lippard, S.J. (1990) Biophys. Chem. 35: 179-188). Initially, it was thought that either this kink or the postulated local region of ss DNA opposite to the platinum adduct could comprise a recognizable structural motif.

The 1,3-intrastrand d(GpTpG) adduct of *cis*-DDP also bends the helix by 34°, concurrently unwinding the DNA strand opposite to the adduct to a much greater degree than in the 1,2-intrastrand adducts produced by this compound. Moreover, it is not known if this bend is

directed toward the major groove of the DNA helix. It is possible that the helix bend produced by this platinum adduct is more flexible than the helix kink produced by the 1,2-intrastrand adducts of *cis*-DDP. Bellon, S.F. & Lippard, S.J. (1990) Biophys. Chem. **35**:179-188. It should be noted that cyclobutane-type pyrimidine dimers formed by UV irradiation also have been suggested to bend the DNA helix by 30°. Husain, I., Griffith, J., & Sancar, A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**:2558-2562. This bend is probably in the direction of the major groove. Pearlman, D.A., Holbrook, S.R., Pirkle, D.H. & Kim, S. (1985) Science **227**:1304-1308.

The other platinum compounds illustrated above form interstrand platinated DNA adducts (e.g., *trans*-DDP) or monofunctional adducts (e.g., {Pt(dien)Cl}Cl or $\{Pt(NH_3)_2(N3\text{-cytosine})\}$).

The above-illustrated platinum compounds were employed to investigate the nature of the structural motif produced by therapeutically active platinum compounds and selectively recognized by SSRP. It was possible to determine whether the motif recognized by the *cis*-DDP SSRP described below comprised a particular helix kink or bend, a local region of DNA unwinding, the platinum atom itself, or a combination of these elements.

Method of identifying SSRP in cell extracts

DNA modified by the antitumor drug *cis*-diammine-dichloroplatinum(II) (*cis*-DDP or cisplatin) was used to identify a factor present in crude extracts of mammalian cells which binds to cisplatin-damaged DNA. This factor, referred to as *cis*-DDP DNA structure-specific recognition protein (*cis*-DDP SSRP) binds selectively to double stranded DNA fragments modified by *cis*-DDP, $\{Pt(en)Cl_2\}$ ("en" refers to ethylenediamine) or $\{Pt(dach)Cl_2\}$ ("dach" refers to 1,2-diaminocyclohexane), but not to DNA modified

with either *trans*-DDP or {Pt(dien)Cl}Cl ("dien" refers to diethylenetriamine). It is important to note that the latter two platinum compounds are clinically ineffective and are unable to form 1,2-intrastrand dinucleotide adducts, whereas the first three compounds are capable of forming this type of DNA structural motif. The major DNA adducts of *cis*-DDP or cisplatin are d(GpG) and d(ApG) 1,2-intrastrand cross-links, which represent 65% and 25% of all such adducts, respectively. Thus, SSRP described herein binds specficially to these intrastrand d(GpG) and d(ApG) adducts.

It is likely that SSRP (or a similar factor) also binds to DNA which has been damaged by other means, such as other genotoxic agents, which result in the formation of motifs comprising intrastrand cross-links and/or the introduction of platinum into the DNA. SSRP may recognize a strutural motif common to certain platinum-DNA adducts and to other types of DNA damage. It is also possible that it recognizes sequences which form tertiary DNA structural domains or motifs comprising sites of specific protein-DNA interactions.

It is of interest to note that although prokaryotic DNA repair systems have been identified, comparatively little is known about corresponding factors that process damaged DNA in eukaryotic cells. Friedberg, E.C., DNA Repair, (W.H. Freeman and Co., New York (1985). From the information available, however, it appears that mamalian DNA repair enzymes possess damage-specific DNA binding properties, ibid., pp. 150-152. In other words, repair enzymes and possibly other components of the cellular DNA repair machinery bind selectively to DNA structural motifs associated with DNA damage or lesions. The studies described herein were initially designed to investigate the hypothesis that in eukaryotic cells there is a structure-specific DNA binding factor or recognition protein with sufficient generality to recognize cisplatin-

modified DNA as an initial step in the DNA-lesion repair process.

These studies culminated in the discovery of a eukaryotic cellular factor (SSRP) in mammalian cells, both human and non-human, which selectively recognizes and binds a DNA structural motif associated with DNA damage. It follows that the factor described herein, alone or in conjunction with other cellular constituents, could be of general importance in the initial stages of processing of eukaryotic DNA which has been damaged by a genotoxic agent, such as cisplatin, and may belong to a wider class of cellular damage- or structure-specific recognition proteins. The *cis*-DDP SSRP has been shown to be present at least in human (i.e., HeLa) and non-human (i.e., hamster V79) mammalian cells and it should be emphasized that the *cis*-DDP binding factor occurs and produces approximately the same electrophoretic band shift in all cell lines tested. *cis*-DDP SSRP may be ubiquitous to all eukaryotic cells.

Thus, the existence of at least one factor which specifically recognizes and binds to a damaged DNA structural motif has been demonstrated. It is important to note that the factor selectively recognizes a DNA structural motif produced by the interaction of an antitumor drug with DNA. Little or no binding of the cellular *cis*-DDP SSRP to unmodified (unplatinated) DNA occurs. Cellular *cis*-DDP SSRP binding to DNA fragments containing the above platinum adducts could be observed using damaged DNA fragments having as few as two platinated DNA lesions per 1,000 nucleotides. Low levels of binding to singled stranded DNA modified by *cis*-DDP were also observed.

Although SSRP is described herein in the context of its ability to bind DNA damaged by an exogenous agent (a specific anticancer drug, cisplatin) it is likely that it, or a functional equivalent thereof, has a wider, more

generalized role in DNA recognition and processing. This conclusion is based upon the fact that nature could not have evolved a system specific only for a particular drug or its adducts. That is, it is likely that the SSRP identified and described herein or a similar factor (i.e., one which has a similar specificity for and ability to bind to damaged DNA) interacts with DNA damaged by other means (e.g., spontaneous damage, environmental damage).

Turning now to the method by which SSRP was identified, cellular extracts were assessed for the presence of the *cis*-DDP SSRP by a method comprising two independent, mutually corroborative techniques. One of these was a modified Western blot analysis (also known as Southwestern blotting) wherein electrophoretically resolved, blotted cellular proteins were renatured *in situ* (i.e., on the blot surface) and assessed for the ability to bind to a ^{32}P-labelled, damaged DNA fragment (e.g., comprising at least one cisplatin-DNA adduct). A protein identified as cellular *cis*-DDP SSRP by its ability to form a (damaged DNA fragment):(protein) complex on the blot surface was observed to have an apparent molecular weight of approximately 100 000 daltons; these results are described more fully in the Examples which follow.

The other technique relied upon in the present method of identifying SSRPs was electrophoretic gel mobility shift assay (EMSA, also known as bandshift analysis). Initially, cell extracts were incubated in the presence of a ^{32}P-labelled, damaged DNA fragment (e.g., comprising at least one cisplatin-DNA adduct) and subjected to electrophoretic resolution, whereupon a (damaged DNA fragment):(protein) complex formed in solution was detectably resolved from the soluble, damaged DNA fragment alone. This analysis for the presence of SSRP was further refined by EMSA studies wherein chemically synthesized oligonucleotide probes containing predefined chemical DNA

adducts were used to characterize the structual features of platinated DNA which comprise the motif recognized by the cellular SSRP. These studies demonstrated that the 1,2-intrastrand d(GpG) and d(ApG) aducts formed by *cis*-DDP were specifically recognized by the *cis*-DDP SSRP.

A competitive EMSA technique also allowed the determination of the dissociation constant (which is the reciprocal of the binding constant to platinum-damaged DNA) and other properties of the cisplatin SSRP. With this technique, it was demonstrated that the dissociation constant for in-solution formation of a (damaged DNA fragment):(protein) complex is in the range of (1-20) x 10^{-10} M, and that the protein described herein as cellular *cis*-DDP SSRP has an apparent molecular weight of about 91 000 daltons.

It should be emphasized that the method of identifying SSRPs, while described herein with specific reference to the identification of at least one factor which selectively binds cisplatin-damaged DNA, can be used to identify and characterize other DNA structure-specific recognition proteins. For example, the present method can be used to identify other DNA SSRPs which hybridize to a particular probe, such as a *cis*-DDP-modified DNA restriction fragment, which has been previously shown to identify a factor which binds a particular type of damaged DNA (e.g., cisplatin-damaged DNA). If lower stringency conditions are used, for example, the probes described herein can be used to identify other DNA SSRPs (possibly also including factors which bind DNA damaged through the action of another chemical agent or radiation).

Both of the above techniques are described more fully below, particularly in the Examples. The similarity of the molecular weights of the cellular proteins identified by these two independent techniques supports the conclusion that, in each case, the same SSRP is observed.

Further support is derived from the fact that the two have the same binding specificities for DNA modified with different platinum compounds. The cloning and characterization of human, *Drosophila melanogaster* and *Saccharomyces cerevisiae* cDNAs encoding a protein having the characteristics of the cellular SSRP is also described below.

I. Electrophoretic Mobility Shift Analysis

A gel electrophoretic mobility shift assay (EMSA) was used in conjunction with radiolabelled DNA restriction fragments or chemically synthesized oligonucleotide probes containing specific, predefined platinum-DNA adducts, to characterize the structural features of platinated DNA which are specifically recognized by the structure specific DNA recognition protein (SSRP) described herein. EMSA, also known as bandshift analysis, was originally described as useful for characterizing mammalian transcriptional control factors. Fried, M. and D.M. Crothers, Nucleic Acids Res. **9**:6505-6525 (1981); 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 (e.g. 10^4-fold) of competitor DNA, such as poly(dI-dC)•poly(dI-dC).

Briefly, the studies described in Examples A-K resulted in identification and characterization of a cellular protein that selectively recognizes a DNA structural motif produced by the interaction of particular platinum compounds with DNA. In particular, this work has elucidated several key properties of a cellular protein that binds selectively to DNA modified with the antitumor drug *cis*-DDP. The platinum damage- or structure-specific recognition protein may be part of a DNA repair complex or

it may be a cellular constituent that responds to structural elements that occur or arise naturally in the genome. For present purposes, it is not important to distinguish between these two possibilities. However, it should be emphasized that since it is unlikely that biological systems would evolve a protein to complex with cisplatin adducts specifically, *cis*-DDP SSRP probably recognizes a naturally-occurring structural motif common both to certain platinum-DNA adducts and to other types of DNA damage, or possibly to sequences which form tertiary DNA structural domains that are the sites of specific protein-DNA interactions.

The results of EMSA studies described in Example A demonstrate the existence of a cellular factor that binds with selectivity to cisplatin-DNA adducts. The slower migration through the gel of platinated DNA associated with (i.e., complexed with) 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 μg/mL *cis*-DDP) cell lines. Selectivity of binding was demonstrated by the positive 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.

Cisplatin-damaged DNA fragments incubated with nuclear extracts from either V79 parental or resistant cell lines were bound to a similar extent, suggesting that its expression is not associated with an acquired

resistance to *cis*-DDP. The results also revealed the presence of a factor causing approximately the same magnitude of band shift in cell extracts obtained from two dissimilar species, supporting the postulate that a similar (e.g., highly conserved) factor was being observed in both species. The *cis*-DDP specific DNA-binding factor has also been found in nuclear extracts from human B cells and from cytosolic extracts prepared from HeLa cells.

A preliminary study of the selectivity of the cellular DNA binding factor for *cis*-DDP DNA adducts is described in Example B. The results of this study showed that the cellular factor bound selectively to DNA modified with *cis*-DDP, but not to DNA modified with either *trans*-DDP or {Pt(dien)Cl}Cl.

The nature of the structural motif selectively recognized by SSRP was further elucidated in a more refined EMSA selectivity study, discussed in Example G. These results demonstrated that the cellular SSRP binds selectively to DNA modified with *cis*-DDP, $\{Pt(en)Cl_2\}$, and $\{Pt(dach)Cl_2\}$, but not to DNA modified with either *trans*-DDP or {Pt(dien(Cl)}Cl. It is important to note that the latter two platinum compounds are unable to link adjacent nucleotides in DNA, whereas the former three are known to form 1,2-intrastrand d(ApG) and d(GpG) adducts. These results directly support the conclusion that SSRP selectively recognizes a DNA structural motif comprising a 1,2-intrastrand dinucleotide adduct.

A preliminary competitive binding experiment, described in Example C, was performed to assess the specificity and affinity of the cellular factor for *cis*-DDP-treated DNA. The results showed that binding of the cellular factor to a radiolabelled, *cis*-DDP-modified 274 bp restriction fragment of DNA prepared from the plasmid pSTR3 was effectively competed by increasing quantities of an unlabelled, *cis*-DDP-modified 422 bp restriction

fragment derived from M13mp18 DNA. Binding could be completely competed with a 100-fold excess of unlabeled modified DNA; however, unmodified 274 bp fragment did not compete for binding of the cellular factor.

From the data obtained, the equilibrium constant for binding of the platinated DNA to the cellular factor was initially estimated to be 3 x 10^8 M^{-1}. Müller, 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 x 10^{-9} M. Ibid. Similar results were obtained when the labeled 274 bp fragment was competed with unlabeled 274 bp fragment modified to the same extent.

The results of a subsequent competition study, discussed in Example F, demonstrated that the true value of the dissociation constant of the cellular factor identified as SSRP for its ligand, a particular DNA structural motif produced as a result of cis-DDP DNA adduct formation, lies in the range (1-20) x 10^{-10} M.

A displacement assay was also performed in which 0.1 ng of radiolabelled, *cis*-DDP-modified DNA (0.035 Pt/nucleotide) was incubated with 7.3 μg of nuclear extract from *cis*-DDP-resistant cell lines at 37°C for 15 minutes. Subsequently, varying concentrations of unlabelled, modified DNA were added to the mixtures and incubation was continued for an additional 15 minutes. In contrast to the results from the above competition assays, results of the displacement assay showed that the cellular factor remained bound to the labelled, platinated DNA even in the presence of a 1000-fold excess of unlabelled, platinated DNA.

The competitive EMSA approach was also successfully employed for a concurrent analysis of the specificity and affinity of the cellular structure-specific recognition protein for *cis*-DDP-treated DNA. In this study, discussed

in detail in Example H, synthetic DNA fragments containing predetermined types of platinum-DNA adducts were prepared from the oligonucleotides depicted in Figure 1. These fragments were radiolabelled and used in EMSA binding reactions in conjunction with an unlabelled competitor DNA fragment, comprising the 422 bp restriction fragment described in Example A, either untreated or treated with *cis*-DDP. The results of this competitive analysis revealed that SSRP binds selectively to DNA modified with the antitumor drug *cis*-DDP and that it is specific for the 1,2-intrastrand d(GpG) and d(ApG) adducts formed by *cis*-DDP. In contrast, SSRP does not recognize the 1,3-intrastrand d(GpTpG) adducts formed by *cis*- and *trans*-DDP, nor does it recognize a monofunctional adduct formed by $\{Pt(NH_3)_2(N3\text{-cytosine})^{2+}$ at the N7 position of deoxyguanosine. As noted previously, the *cis* 1,3-intrastrand d(GpTpG) adduct unwinds the DNA helix to a much greater extent than the 1,2-intrastrand d(GpG) and d(ApG) adducts of this drug. This 1,3-intrastrand cross-linked adduct may therefore unwind the helix too much for SSRP recognition. Furthermore, the possibility that an amino acid residue of SSRP interacts directly with the platinum atom is unlikely since the protein does not bind to DNA modified with structurally distinct (e.g., interstrand or monofunctional) DNA adducts having a platinum atom as a common element.

The above-described studies did not conclusively exclude the possibility that the cellular factor observed to bind selectively to platinated DNA might actually recognize a single-stranded domain adjacent the platinum-DNA adducts. Recognition of ss DNA was affirmatively excluded by a competitive EMSA study (Example I) in which nuclear extracts from HeLa cells were presented with unlabelled, ss M13mp18 DNA in addition to the putative platinated DNA ligand, represented by the above

radiolabelled, platinated 274 bp double-stranded restriction fragment. The ss M13mp18 DNA did not compete for binding of the cellular factor, indicating the absence of a ss DNA binding factor.

As noted previously, cyclobutane-type pyrimidine dimers formed by UV irradiation also have been suggested to bend the DNA helix by 30°, probably in the direction of the major groove. Recently, Chu and Chang reported the presence of a factor in nuclear extracts prepared from HeLa cells that binds specifically to DNA damage induced by UV irradiation. Chu, G. and E. Chang, Science 242:564-567 (1988). A study was initiated to test the logical hypothesis that SSRP and the factor described by Chu and Chang recognizes a common structural motif: a helical bend or kink of about 30° in the direction of the major groove.

The results of this EMSA study, which relied upon differential competition between *cis*-DDP modified and UV-damaged DNA fragments, are set forth in Example J. The results of this comparison, reported in Donahue, B.A. *et al*. (1990), Biochemistry **29**:5872-5880, demonstrate that the DNA binding factor described herein as *cis*-DDP SSRP does not recognize DNA lesions induced by UV light. Therefore, the structural motif recognized by *cis*-DDP SSRP does not correspond to the type of lesion produced by the irradiation of DNA with UV light.

The conclusion can be drawn from the above EMSA studies that the cellular *cis*-DDP SSRP does not specifically recognize 30-34° kinks in the helix, nor does it simply respond to the presence of ss DNA formed opposite the cisplatin lesion, as evidenced by the failure of ss DNA to compete with platinum-modified DNA for binding. The protein may, however, recognize a particular combination of directed helix axis bending and local unwinding at the site of platination in 1,2-intrastrand *cis*-DDP-DNA cross-links.

II. Modified Western Blotting Analysis

In an alternative approach to the EMSA technique described above, modified Western (i.e., Southwestern) blotting was used to identify a factor, present in HeLa cells, which selectively binds to DNA modified by *cis*-DDP or {$Pt(en)Cl_2$}. This technique is described more fully in Example L. Southwestern blotting analysis allowed a determination of the apparent size of the cellular protein having the ability to form (damaged DNA fragment):(protein) complexes with platinum-modified DNA fragments. SSRP was observed to have an electrophoretic mobility corresponding to a molecular mass of approximately 100 000 daltons for a globular protein. Only double-stranded DNA restriction fragments modified by *cis*-DDP or {$Pt(en)Cl_2$} bound selectively to the human cellular SSRP. A low level of SSRP binding to single stranded (ss) DNA modified by *cis*-DDP was observed, and little or no detectable binding was seen when unmodified single or double stranded DNA restriction fragments were used as probes for the blotted proteins. 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.

It should also be noted that a molecular species of about M_r = 28 000 daltons also bound a significant amount of the *cis*-DDP and {$Pt(en)Cl_2$} modified DNA fragments with which the Southwestern blots were probed. Initially, it was thought that this factor arose through proteolytic degradation of the cellular SSRP. Results of subsequent investigations suggest that this factor is, or is related to, the known protein HMG-1. Southwestern blotting studies also demonstrated that extent of (damaged DNA fragment):(protein) complex formation depended upon the level of DNA modification by *cis*-DDP. In addition, the

Southwestern blotting system described herein was found to have a detection limit for SSRP of approximately 2 platinum adducts per 1000 nucleotides, also expressed as an r_b level of 0.002. This technique was also used, as described below, for screening a human cDNA expression library for the presence of transcripts corresponding to polypeptides having SSRP activity.

Further Characterisation of the Cellular SSRP

The chemical nature of the cellular factor observed in HeLa cells was also assessed, by treating cytosolic extracts with either proteinase K or RNases, as described in Example D. Pretreatment of crude extracts with proteinase K resulted in loss of binding activity, confirming that the factor is a protein. Pretreatment of crude extracts with RNase A also resulted in a loss of activity, however, this sensitivity disappeared after partial purification of the *cis*-DDP-DNA binding factor by ammonium sulfate fractionation and ion exchange chromatography as described below.

A study was carried out, as described in Example E, with the object of assessing the possible requirements of (damaged DNA fragment):(protein) complex formation as observed in EMSA studies with the cellular SSRP for certain metal ions or cofactors. No specific cofactor dependencies were revealed, however SSRP binding was observed to be inhibited by the presence, during the EMSA incubation step, of metal ions that have an affinity for sulfur donor ligands. This suggests that thiol moieties present in the protein may be involved at or near the site(s) of SSRP-DNA structural motif interaction.

The cellular protein identified as SSRP based upon its ability, observed in EMSA studies, to form (damaged DNA fragment):(protein) complexes with a soluble DNA fragment containing at least one 1,2-intrastrand

dinucleotide adduct, was partially purified and subjected to preliminary characterization by sucrose gradient sedimentation as discussed in Example K. Fractions obtained from the sucrose gradient were assessed in paralell by SDS-PAGE and EMSA. These results, summarized in Figure 2, indicated that the protein having SSRP activity has a sedimentation coefficient of 5.6*S*, corresponding to an apparent molecular weight of 91 000 daltons for a globular protein.

Thus, as described herein, DNA structure specific recognition factor, which has been shown to be a protein, has been identified in mammalian cells, using two independent, corroborative approaches. The DNA structure specific recognition protein has been shown to bind selectively to DNA modified with cisplatin and to bind specifically to intrastrand d(GpG) and d(ApG) DNA adducts formed by *cis*-DDP. The protein may be involved in initial recognition of damaged DNA as part of a repair event. Alternatively, it may be part of the cellular response to stress, may be involved in maintaining the tertiary structure of DNA, or may initiate or suppress a DNA-directed function at a specific structural motif. It should be emphasized that *cis*-DDP SSRP occurs and produces approximately the same band shift in all cell lines tested; hence, it may be ubiquitous to all eukaryotic cells. The apparent molecular mass of SSRP as observed in the two techniques employed for identification of the factor are 91 000 daltons and 100 000 daltons (by EMSA and Southwestern blotting analysis, respectively). Further anaylsis, using known techniques, is expected to demonstrate conclusively whether the 100 000 dalton and the 91 000 dalton proteins identified by the two methods are, in fact, the same protein or are two members of a family of functionally related SSRPs. In either case, SSRP can be used to produce substances, as described

herein, useful in the treatment (prevention, reduction) of DNA damage by genotoxic agents, such as anticancer drugs.

Cloning of SSRP from a cDNA Expression Library by a Modified Western Blot Screening Procedure

The above-described selective binding of the HeLa cellular factor to DNA modified by *cis*-DDP suggested that it might be possible to isolate cDNA clones encoding the factor using *cis*-DDP-modified DNA as a probe. This approach proved fruitful: from a primary screen of 360,000 phage plaques, two recombinant phage, λPt1 and λPt2, were isolated from a human B cell expression library based upon the results of a Southwestern blot screening assay. This Southwestern blot screening assay is described below in Example H; it was based upon the use of a radiolabelled 422 bp DNA restriction fragment modified by *cis*-DDP to an r_b level of 0.040 (discussed in Example A).

E. coli lysogens (Y1089) containing the recombinant λPt1 gene were deposited on September 22, 1988 at the American Type Tissue Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA, under the terms of the Budapest Treaty and assigned accession number 40498. Restriction maps of the γPt1 and γPt2 inserts are presented in Figure 3. The two clones have insert sizes of 1.44 and 1.88 kb (for λPt2 and λPt1, respectively) and are aligned at their 5′ ends (see also Example O). A consequence of the method by which these clones were isolated (i.e., a functional assay which depended upon the presence of polypeptides capable of binding the selected ligand, a cisplatin-damaged DNA fragment), the shorter clone, λPt2, serves to more precisely delimit the polypeptide sequence responsible for *cis*-DDP SSRP binding activity.

B

The polypeptides encoded by the recombinant phage have been assessed by Southwestern blotting analysis, described in Example N. A comparative study demonstrated that the recombinant polypeptides exhibit DNA binding properties similar to those of the cellular factor identified by Southwestern blotting studies of crude extracts prepared from mammalian cells.

Expression of the Cellular Gene Encoding λPt2

Northern blot analysis of cytoplasmic RNA was carried out using clone λPt2 as a hybridization probe (Example P) for the presence of RNAs encoding cellular SSRP. An initial study revealed the presence of a 2.8 kb mRNA which is conserved at least between humans and rodents. The predicted molecular mass of the protein encoded by this mRNA transcript is 100 000 daltons, a size which correlates well with the results, discussed above, of modified Western blot analysis. See also, Toney, J.H., *et al*. (1989), Proc. Nat. Acad. Sci. USA **86**:8328-8332.

Further studies revealed an expression pattern for the SSRP gene which is consistent with a function that is critical to a variety of tissues. Its presence does not correlate with the tissue-specific antitumor activity of cisplatin, however, nor with drug sensitivity in a series of resistant cell lines. Moreover, expression of the encoded message was not inducible in HeLa cells treated with a range of drug concentrations.

The Full-Length cDNA Sequence of Human SSRP was obtained by Screening cDNA Libraries with Clone λPt2

As noted previously, λPt2, the shorter of the two clones obtained initially by using a functional screen (based upon protein binding to cisplatin-modified DNA), served to define the region of SSRP responsible for DNA structual motif binding activity. As discussed below in

Examples Q, R and S, the two clones obtained from Southwestern blot screening of a human cDNA expression library were in turn successfully employed as hybridization probes for the presence of additional SSRP sequences in several human cDNA libraries. The results of Southern blotting studies of the additional clones isolated in this manner are summarized in Figure 4. Sequencing studies, described in Example S, allowed the construction of a predicted amino acid sequence of the human DNA structure specific recognition protein, and revealed the presence of several distinct regions. The full length nucleic acid sequence, together with the predicted amino acid sequence encoded therein, are shown in Figure 5. Several functional domains observed in the encoded protein are schematically illustrated in Figure 6. The polypeptide encoded by λPt2 extends from residues 149-627 of the full length protein, and includes the acidic domain, Basic I, and the HMG box.

The latter domain comprises a region having interesting homologies to other proteins that recognize altered DNA structures, and thus is considered to be the domain of SSRP most likely to contain the site which selectively recognizes and binds to the 1,2-intrastrand dinucleotide structural motif produced by the interaction of *cis*-DDP with DNA. Proteins found to have sequence homology to SSRP include the high mobility group (HMG) proteins 1 and 2. Eink, L. and Bustin, M. (1985) Exp. Cell Res. **156**:295-310; Bustin, M., Lehn, D.A. and Landsman, D. (1990) Biochim. Biophys. Acta **1049**:231-243; van Holde, K.E., in Chromatin, Springer-Verlag, NY (1988). Homology is also observed with the HMG-box domain in human upstream binding factor (hUBF), which activates transcription of RNA polymerase I. Jantzen, H.M., Admon, A., Bell, S. and Tijan, R. (1990) Nature **344**:830-836. Other recently identified HMG-box proteins include sex-determining region Y (SRY) (Sinclair, A.H., Berta, P.,

Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J., Foster, J.W., Frischauf, A.-M., Lovell-Badge, R. and Goodfellow, P.N. (1990) Nature 346:240-244; Gubbay, J., Collignon, J., Koopman, P., Capel, B., Economou, A., Munsterberg, A., Vivian, N., Goddfellow, P. and Lovell-Badge, R. (1990) Nature 346:245-250), mitochondrial transcription factor II (Parisi, M.A. and Clayton, D.A. (1991) Science 25:965-968), lymphoid enhancer binding factor I (Lef-1) (Travis, A., Amsterdam, A., Belanger, C. and Grosschedl, R. (1991) Genes & Dev. 5:880-894), a T-cell specific transcription factor (TCF-1α) (Waterman, M.L., Fischer, W.H. and Jones, K.A. (1991) Genes & Dev. 5:656-669), and the yeast autonomously replicating sequence factor ABF2 (Diffley, J.F.X. and Stillman, B. (1991) Proc. Nat. Acad. Sci. USA 88:7864-7868). A particularly interesting report is that of Shirakata, M., Hüppi, K., Usuda, S., Okazaki, K., Yoshida, K. and Sakano, H. (1991) Mol. Cell. Biol. 11:4528-4536, wherein the cloning of a mouse cDNA encoding an expression product capable of binding to V(D)J recombination signal sequence (RSS) probes is disclosed. The sequence of the protein encoded by this murine cDNA is 95.5% homologous to that of the human SSRP; presumptively, it is the murine homolog of SSRP as described herein.

An additional factor which supports the idea that the HMG-box contains the cisplatin-DNA adduct structure specific recognition site is that HMG-1 binds strongly and specifically to cisplatin-modified oligonucleotides. Furthermore, Scovell, W.M. (1989) J. Macromol. Sci.-Chem. A26:455-480 and Hayes, J.J. and Scovell, W.M. (1991) Biochim. Biophys. Acta 1088:413-418 have concluded that cisplatin forms covalent cross-links between DNA and the proteins HMG-1 and -2. The biological relevance of this emerging family of HMG-box proteins, and of SSRP in particular, is discussed more fully below.

Evolutionary Conservation of the Eukaryotic SSRP gene

A Southern blot study was carried out with the object of determining the extent of evolutionary conservation of the DNA structure specific recognition protein described herein. For this purpose, a "zoo" blot comprising electrophoretically resolved DNA from a large number of species (generously donated by Dr. Paula Fracasso, in the laboratory of Professor David E. Housman, MIT) was probed with the 1.44 kb human cDNA clone, λPt2. Homologous sequences were observed in DNA derived from chimpanzee, monkey, elephant, pig, dog, rabbit, mouse, opossum, chicken, fish, and the fruitfly, *Drosophila melanogaster*. Conversely, no hybridization was observed to DNA prepared from the nematode *Caenorhabditis elegans*, yeast, the parasite *Giardia* (which retains both prokaryotic and eukaryotic characteristics), or the prokaryotic organisms *Pseudomonas* and *Streptomyces*.

Identification and Characterization of a Full-length *Drosophila melanogaster* SSRP cDNA Sequence

The studies presented herein demonstrated clearly that *cis*-DDP SSRP was evolutionarily conserved at least among mammalian species, such as humans and rodents (J.H. Toney, *et al.*, Proc. Natl. Acad. Sci. USA **86**:8328 (1989); Shirakata, M., Hüppi, K., Usuda, S., Okazaki, K., Yoshida, K. and Sakano, H. (1991) Mol. Cell. Biol. **11**:4528-4536), and that homologs exist in several other vertebrate species (see preceeding section). The presence of an SSRP homolog in the invertebrate fruit fly, *Drosophila melanogaster*, was of particular interest. Since regions of proteins that remain intact through evolutionary distance are generally critical for functional activity, the cloning of homologs from lower species often sheds light on the cellular role of the protein. For this reason, a low stringency screen of a *Drosophila* head cDNA

library was conducted by using the original human cDNA clone λPt2 as a probe (described below in Example T). From the pool of ten clones originally isolated, two cDNA clones were chosen for further study (see Example U). Sequence analysis of these clones, denoted DM 3002 and DM 1001, revealed a significant region of overlap, shown in Figure 7. Within these cDNAs is contained all of the coding sequence of the *Drosophila* protein. These findings are discussed more fully below in Example V; the full length sequence of the *Drosophila* nucleic acid and the predicted amino acid sequence of the protein encoded therein are shown in Figure 8.

The human DNA structure-specific recognition protein and its *Drosophila* counterpart share extensive homology at both the DNA and protein level. Both proteins contain a high percentage of charged amino acids that are concentrated within a few domains (illustrated in Figure 9). Sequence analysis revealed that both proteins can potentially undergo a high degree of post-translational modification, with several phosphorylaton and one glycosylation site conserved between species. As noted previously in connection with the human protein, both the human and the *Drosophila* homologs of SSRP share homology with high mobility group proteins 1 and 2, with hUBF (a transcription factor containing an HMG-box domain) and with the transcriptional activator nucleolin. With great interest, it was observed that the structure of *cis*-DDP structure-specific recognition protein has also been conserved through evolution: Figure 9 shows that all charged domains and the HMG-box are located in the same relative positions in the human and the fly. These domains in the carboxy terminal half of the protein are clearly critical for the function of this structure-recognition factor, but it is important to note that extensive homology also exists in the less well understood amino terminal portion. As discussed more fully below,

the dramatically high level of evolutionary conservation of this protein strongly supports the idea that it must provide a crucial intracellular function.

Identification and Characterization of a *Saccharomyces cerevisiae* protein having *cis*-DDP SSRP-like Activity; Isolation of a cDNA Sequence Encoding Same

The yeast, *S. cerevisiae*, provides an excellent lower eukaryotic model system, especially for studies involving molecular genetic techniques to dissect the possible *in vivo* functions of SSRP. As discussed briefly above, a Southern blotting approach failed to reveal the presence of a yeast gene homologous to the human SSRP gene sequence encoded by clone λPt2. However, EMSA and Southwestern blotting investigations revealed the existence of at least one yeast cellular protein having *cis*-DDP SSRP-like activity. As discussed in Example Y, a Pt-DNA binding factor has now been purified from yeast whole cell extracts (YWCE); this has yielded samples enriched in SSRP specific activity, as assessed by EMSA or bandshift analysis.

A Southwestern blot analysis of pooled bandshift active fractions from an S-Sepharose* column corroborated that some active proteins appear to be enriched, relative to YWCE. In the first peak of bandshift activity, both a 42 000 and a 40 000 dalton protein are present. In the second peak of activity, these two proteins are also enriched, as well an 82 300 dalton protein and two smaller proteins of approximately 30 000 and 25 000 daltons.

Bandshift activity that did not bind to a DEAE-sepharose column yields a similar modified Western blot banding pattern as the second peak of banshift-active proteins. It should be noted that, at present, it is difficult to correlate bandshift activity with Southwestern blotting results. However, it seems quite possible that several proteins are responsible for the

* trade-mark

observed bandshift activity. The small size of the known yeast proteins containing HMG-box domains, namely ABF2 (20 000 daltons) and NHP6 (11 400 daltons) has resulted in these proteins running off of the gels. (Kolodrubetz D. and A. Burgem (1990) Journal of Biological Chemistry 265(6):3234-3239; Diffley J.F.X. and S.B. (1991) Proceedings National Academy of Science, USA 88:7864-7868). Thus, the proteins that are observed in Southwestern blots may be known proteins, or may be entirely novel. It is important to note that, in studies geared toward assessing the specificity of these proteins for platinated DNA structural motifs, it has been shown that the yeast proteins possess a binding specificity pattern similar to that found in HeLa extracts (see above). Therefore, SSRPs present in yeast and humans may have similar biological relevance.

Accordingly, a yeast genomic expression library was screened for the presence of expressed polypeptides capable of binding to a radiolabelled, platinated DNA fragment in the same manner as the above-discussed screening procedure which resulted in the isolation of the human cDNA clones λPt1 and λPt2 from a human B cell expression library. This approach was successful: it resulted in the isolation of a single clone, λyPt, encoding a polypeptide having *cis*-DDP SSRP-like activity. A schematic illustration of clone λyPt is shown in Figure 10. The cloning and sequencing of this gene are described more fully below in Example AA; the yeast nucleic acid sequence and the predicted protein sequence encoded therein are shown in Figures 11 and 12, respectively.

Northern blot analysis of total yeast RNA, using radiolabelled λyPt as a probe, demonstrated that the cloned DNA encodes a transcribed gene, resulting in a 2.1 kB mRNA. A translated protein of ~78 kDa might possibly result from a mRNA of this size, thus the ySSRP is presumed at present to be the 82 000 dalton protein

observed in Southwestern blots. It is important to note that since the open-reading frame contained within the λyPt sequence (discussed below) is 1.63 kB, approximately 0.5 kB of sequence is missing from the 5′ end of the gene.

A homology search with the partial or fractional ySSRP sequence encoded by clone λyPt resulted in the identification of regions of homology with numerous glutamine rich proteins. Interestingly, the polyglutamine region of transcription factor Sp1 is required for protein-protein interactions. Courey, A.J., D.A. Holtzman et al. (1989) Cell **59**:827-836. A search limited to the non-glutamine rich portion of ySSRP, residues 282-510, yields a much more limited set of proteins. Almost all of these proteins belong to the recently discovered and rapidly growing class of proteins which contain the HMG-box domain. The highest degree of similarity is found to the yeast protein ABF2. ABF2 is contains two HMG-boxes and is highly related (37% identical, 65% similar) to ySSRP over 151 of its 183 amino acids. ABF2 binds to ARS1 domains that do not demonstrate consensus DNA sequences. Based on this fact, it has been suggested that ABF2 recognizes DNA structural features. Diffley, J.F.X. (1991) Proc. Nat. Acad. Sci. USA **88**:7864-7868). Thus, like ABF2, ySSRP may also be recognizing DNA structures.

Sequence homology of ySSRP to the predicted amino acid sequence of the human SSRP is rather low, with only 12.7% identity and 38% similarity found with an optimal alignment. Moreover, alignment with the *D. melangaster* SSRP reveals the same level of homology (14.5% identical, 38% similar) to the yeast protein. Yeast ySSRP, like human SSRP, does contain HMG-box domains towards its carboxy terminus. Thus, this region is probably important for DNA structural motif recognition. The high glutamine content of the remainder of the ySSRP sequence suggests that it may be important in protein-protein interactions,

or in protein oligomerization. This hypothesis may be enlarged to the human SSRP.

Functional Significance of SSRP

At present, the precise nature of the *in vivo* role of *cis*-DDP SSRP is unknown; however, mounting circumstantial evidence has been presented that it may play a significant part in the initiation or control of cellular processes responsive to specific DNA structural motifs. Thus, one possible role is to recognize sites of DNA damage as a signaling event for DNA repair. A current model for recognition of DNA damage by the *E. coli* ABC excision system is that UvrA forms a complex with UvrB, either in solution or after it has bound to DNA at a site of damage. Orren, D.K. & Sancar, A. (1989) Proc. Natl. Acad. Sci. U.S.A. 86: 5237-5241. UvrA then dissociates from DNA, and UvrB, in conjunction with UvrC, excises an oligonucleotide encompassing the damage. The resulting gap is then filled in with the correct nucleotides by DNA polymerase. It is reasonable to surmise, then, that if this model of the *E. coli* excision repair system is valid and if it can be extrapolated to eukaryotic DNA excision repair, SSRP may function in a manner analogous to UvrA.

Regardless of whether this proposed *in vivo* role for SSRP is ultimately substantiated, the fact remains that *cis*-DDP SSRP has been demonstrated to posess the highly interesting and significant ability to bind selectively to a DNA structural motif produced by the DNA adducts of chemotherapeutically active platinum drugs, but not the adducts of two clinically ineffective platinum compounds. Moreover, the specific adducts recognized by SSRP (1,2-intrastrand dinucleotide adducts) comprise 90% of all cisplatin-DNA structures formed *in vivo*. These facts strongly support the conclusion that SSRP plays an important role in cellular recognition of, and response

to, the presence of certain DNA structural motifs including those associated with DNA damage or lesions.

It thus is reasonable to propose that if SSRP is a component of a repair complex, it will facilitate the antitumor effectiveness of cisplatin. For example, if tumor cells were deficient, relative to nontumor cells, in their ability to repair platinum-damaged DNA, the platinum drug would be selectively lethal to tumor cells, whereas repair-proficient surrounding cells would remove platinum adducts from their DNA and hence survive. This model, however, does not account for the anticancer utility of certain platinum drugs, such as $\{Pt(NH_3)_2(N3\text{-cytosine})\}^{+2}$, although it has been proposed that the latter compound could act through a different mechanism than *cis*-DDP.

Alternatively, SSRP may not be involved in DNA repair at all. It may actually impede DNA repair by binding to the 1,2-intrastrand d(GpG) and d(ApG) adducts of *cis*-DDP, thereby shielding these adducts from the DNA repair machinery. Donahue, B.A., Augot, M., Bellon, S.F., Treiber, D.K., Toney, J.H., Lippard, S.J. and Essigmann, J.M. (1990) Biochemistry **29**:5872-5880. This proposed *in vivo* role for SSRP is consistent with its observed pattern of gene expression in different tissues, and in several cancer cell lines, including cisplatin-resistant cell lines.

Still another possibility is that the normal role of SSRP is to regulate the function of genes implicated in the emergence of malignancies, or conversely in the maintenance of normal phenotypes. Platinum adducts, by providing DNA structural motifs which mimic those of the natural regulatory sequences of such genes, would displace SSRP from its normal DNA binding sites, thereby effectively sequestering the protein. Donahue, B.A., Augot, M., Bellon, S.F., Treiber, D.K., Toney, J.H., Lippard, S.J. and Essigmann, J.M. (1990) Biochemistry

29:5872-5880; Scovell, W.M. (1989) J. Macromol. Sci.-Chem. A26:455-480. It follows that, if tumor cells had lost the ability to compensate for this effect, *cis*-DDP would selectively compromise the welfare of tumor cells.

As discussed previously, SSRP as described herein is a protein that recognizes a DNA structural motif comprising the 1,2-intrastrand dinucleotide adducts which are the predominant drug-DNA adducts formed as a result of the interaction of *cis*-DDP with DNA. These intrastrand d(GpG) and d(ApG) cross-links unwind the DNA duplex by 13° and cause a 34° bend in the direction of the major groove. Churchill, M.E.A. and Travers, A.A. (1991) TIBS 16:92-97; Bellon, S.F. and Lippard, S.J. (1990) Biophys. Chem. 35:179-188; Rice, J.A., Crothers, D.M., Pinto, A.L. and Lippard, S.J. (1988) Proc. Nat. Acad. Sci. USA 85:4158-4161. Important clues for identifying the type of protein that might interact with such an altered structure are provided by the striking homology of the human SSRP to HMG-1, which is known to bind cruciform DNA (Bianchi, M.E., Beltrame, M. and Paonessa, G. (1989) Science 243:1056-1058), and the near identity, at the protein sequence level, of the human SSRP disclosed herein and a mouse protein which has been reported to bind to signal sequences for V(D)J recombination. Shirakata, M., Hüppi, K., Usuda, S., Okazaki, K., Yoshida, K. and Sakano, H. (1991) Mol. Cell. Biol. 11:4528-4536. The common DNA structural element recognized by SSRP and HMG-1, while not yet defined, most likely mimics the unwinding and bending known to occur in cisplatin-modified DNA. Taken together, the observed properties of SSRP raise the possibility that HMG-1, the family of HMG-box proteins, and recombination functions may be involved in the molecular mechanism of the effective antitumor drug, cisplatin.

Homology between SSRP as described herein and HMG-1 and -2 is particularly interesting because the latter proteins can also specifically recognize structural

distortions to DNA such as B-Z junctions and cruciforms (H. Hamada and M. Bustin, Biochemistry, 24:1428 (1985); Bianchi, M.E., *et al*. Science 243:1056 (1989)). They too are evolutionarily conserved, with homologs known in human (L. Wen, *et al*., Nucl. Acids Res., 17:1197 (1989)), bovine (B. Pentecost and G.H. Dixon, Biosci. Rep., 4:49 (1984); D.J. Kaplan and C.H. Duncan, Nucl. Acids Res., 16:10375 (1988), porcine (K. Tsuda, *et al*., Biochemistry, 27:6159 (1988)), rodent (G. Paonessa, *et al*., Nucl. Acids Res., 15:9077 (1987); K.-L.D., Lee, *et al*., Nucl. Acids Res., 15:5051 (1987)), fish (B.T. Pentecost, *et al*., Nucl. Acids Res., 13:4871 (1985)), yeast (D. Kolodrubetz and A. Burgum, J. Biol. Chem., 265:3234 (1990), maize (K.D. Grasser and G. Feix, Nucl. Acids Res., 19:2573 (1991)), and protazoa (S.Y. Roth, *et al*., Nucl. Acids Res., 15:8112 (1987); T. Hayashi, *et al*., J. Biochem., 105:577 (1989)). Many studies support a role for HMG-1 and -2 in DNA processing, particularly in transcriptional regulation. They influence transcription of RNA polymerase II and III by altering the DNase I footprint of the major late transcription factor, presumably by conferring a structure to the binding site which optimized the process (D.J. Tremethick and P.L. Molloy, J. Bio. Chem., 261:6986 (1986); F. Watt and P.L. Molloy, Nucl. Acids Res., 16:1471 (1988)). HMG-1 has also been shown to modify DNA structures, such as B-Z junctions and cruciforms, in *in vitro* transcription assays, thereby permitting transcription to proceed past these structural blocks (S. Waga, *et al*., Biochem. and Biophys. Res. Comm., 153:334 (1988); S. Waga, *et al*., J. Biol. Chem., 265:19424 (1990)). Other work has suggested that HMG-1 and -2 can act as general class II transcription factors, and may be tightly associated with or identical to transcription factor IIB (J. Singh and G.H. Dixon, Biochemistry, 29:6295 (1990)).

These studies, taken together, suggest that HMG-1 and -2 act to facilitate transcription by binding to specific DNA conformations to create or preserve structures necessary for transciption initiation. A salient feature of the cDNA clones identified as encoding SSRP is that each includes the region of nucleotide sequence identified as an HMG box domain. HMG box domains are emerging as an important recognition element of proteins for DNA. Deletion analysis of HMG-box family members hUBF (Jantzen, H.M., Admon, A., Bell, S. and Tijan, R. (1990) Nature 344:830-836) and TCF-1α (Waterman, M.L., Fischer, W.H. and Jones, K.A. (1991) Genes & Dev. 5:656-669) has demonstrated that a single HMG-box domain is sufficient for the specific interactions of these proteins with DNA. It is important to note, however, that in spite of the emergence of several proteins identified as HMG-box family members, a consensus sequence has not yet emerged for the HMG box domain. Lack of a clearly defined consensus sequence among the HMG-box domains in a variety of proteins may indicate either that such proteins recognize different DNA structures, or that they do not share a common mode of DNA recognition. Whereas mutations in the sequences of target recognition sites in DNA alter binding of the HMG-box proteins, such changes could also modify the shape of the recognition site, reducing its protein affinity. The suggestion (Diffley, J.F.X. and Stillman, B. (1991) Proc. Nat. Acad. Sci. USA 88:7864-7868) that HMG-box proteins recognize DNA structure rather than sequence is strongly supported by the observations reported herein, that SSRP binds selectively to cisplatin-modified DNA fragments, but not to unmodified fragments having the same sequence.

Other properties of HMG-1 are fully consistent with its role in binding to altered DNA structures. For example, HMG-1 suppresses nucleosome core particle formation (Waga, S., Mizuno, S. and Yoshida, M. (1989)

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Biochim. Biophys. Acta 1007:209-214), and it can selectively unwind negatively supercoiled DNA, thereby protecting it from relaxation by *E. coli* topoisomerase I and preventing the formation of higher order secondary structure (Sheflin, L.G. and Spaulding, S.W. (1989) Biochem. 28:5658-5664). It binds preferentially to A-T rich regions (Reeves, R. and Nissen, M.S. (1990) J. Biol. Sciences 265:8573-8582), single stranded DNA (Isackson, P.J., Fishback, J.L., Bidney, D.L. and Reeck, G.R. (1979) J. Biol. Chem. 254:5569-5572), B-Z junctions (Hamada, H. and Bustin, M. (1985) Biochem. 24:1428-1433), and to cruciform structures (Bianchi, M.E., Beltrame, M. and Paonessa, G. (1989) Science 243:1056-1058). Moreover, studies of plasmid DNA containing a number of structural domains suggest that HMG-1 can differentiate among various DNA conformations (Hamada, H. and Bustin, M. (1985) Biochem. 24:1428-1433).

Of particular interest are several studies which suggest that HMG-1 and -2 act by binding to specific structural elements in DNA upstream from actively transcribed genes to preserve conformations necessary for the binding of sequence-specific transcription factors. Tremethick, D.J. and Molloy, P.L. (1986) J. Biol. Chem. 261:6986-6992; Tremethick, D.J. and Molloy, P.L. (1988) Nucl. Acids Res. 16:1471-1486; Watt, F. and Molloy, P.L. (1988) Nucl. Acids Res. 16:1471-1486; Waga, S., Mizuno, S. and Yoshida, M. (1988) Biochem. Biophys. Res. Comm. 153:334-339; Singh, J. and Dixon, G.H. (1990) Biochem. 29:6295-6302. In particular, HMG-1 removes the transcriptional block caused by cruciforms in supercoiled DNA. Waga, S., Mizuno. S. and Yoshida, M. (1990) J. Biol. Chem. 265:19424-19428. Eukaryotic DNA contains palindromic sequences that form cruciform structures, which may in turn have elements in common with the 1,2-intrastrand d(ApG) and d(GpG) adducts formed by cisplatin modified DNA.

Additional insights into the possible *in vivo* role of *cis*-DDP SSRP are provided by the recent characterization of a mouse cDNA clone isolated by screening an expression library with oligonucleotides containing recombination signal sequences (RSS). Shirakata, M., Hüppi, K., Usuda, S., Okazaki, K., Yoshida, K. and Sakano, H. (1991) Mol. Cell. Biol. 11:4528-4536. RSS sequences are signals for somatic DNA recombination to generate antibody diversity through V(D)J joining. The predicted amino acid sequence of this mouse protein is 95.5% identical with that of the human SSRP described herein. Therefore, it is presumed to be encoded by the mouse homolog of the human and *Drosophila* SSRP genes as disclosed herein. Interestingly, V(D)J recombination is postulated to proceed *via* stem-loop structures formed by RSS sequences (Max, E.E., Seidman, J.G. and Leder, P. (1979) Proc. Nat. Acad. Sci. USA 76:3450-3454; Sakano, H., Hüppi, K., Heinrich, G., and Tonegawa, S. (1979) Nature 280:88-94; Early, P., Huang, H., Davis, M., Calame, K. and Hood, L. (1980) Cell 19:981-992; Tonegawa, S. (1983) Nature 302:575-581), although this model has been challenged (Hesse, J.E., Lieber, M.R., Mizuuchi, K. and Gellert, M. (1989) Genes & Dev. 3:1053-1061). The similarity among stem-loop DNA, cruciforms recognized by HMG-1, and the bent, unwound cisplatin-DNA 1,2-intrastrand cross-link structural motif is intriging and supports the postulate that binding of the mouse HMG-box protein reported by Shirakata *et al*. to RSS involves shape as well as sequence recognition.

When the present invention is viewed in the context of the foregoing remarks, it will be apparent that SSRP, and possibly other HMG-box proteins, may be diverted or sequestered from their normal regulatory intracellular roles by the presence of cisplatin-DNA adducts, and that somatic DNA recombination and transcription are specific cellular functions likely to be affected by the platinum anticancer drug family. Understanding the shape

recognition elements of these proteins may provide a basis for the design of future generations of rationally designed chemotherapeutic agents.

Use of SSRP for diagnostic, therapeutic and prophylactic purposes

As a result of the discovery embodied in this invention, new diagnostic tools are available, including, for example, nucleotide probes and antibodies which are useful for detecting the presence or absence of SSRP and/or of the gene or portion thereof which encodes SSRP. Antibodies prepared against the SSRP, or DNA or RNA probes which bind to DNA encoding the SSRP, may be useful for classifying the responsiveness of humans or animals to DNA damaging agents. Antibodies against the DNA structure-specific regognition factor described herein have been generated by injecting a fusion protein (β-galactosidase-λPt2) into rabbits, in whom specific polyclonal antibodies were subsequently produced. These antibodies have been shown by Western blot analysis to bind the λPt2 fusion protein.

These diagnostic tools can be used, for example, in prenatal screening. Thus, prenatal genetic screening for known genetic defects or genetic characteristics associated with particular diseases can now include assessment of the absence of SSRP, or of its occurence at altered (e.g., lowered) levels. Absence or abnormal (e.g., subnormal) expression of the SSRP is putatively indicative of the likelihood that the individual tested will develop cancer during life.

The invention described herein also makes possible the production of a therapeutic agent useful in protecting an individual against DNA damage, or in countering DNA damage that has already occurred. For example, a therapeutic agent protective against the DNA structural or

chemical damage caused by chemotherapy or radiotherapy can be administered to an individual prior to therapy, at the time of therapy (e.g., in the course of treatment of humans with radiation or with the anticancer drug cisplatin), or after such treatment has been undergone. The agent will protect against damage to DNA by creating a DNA damage-refractory phenotype.

A further result of the present invention is that gene therapy or gene replacement will be available to individuals lacking SSRP or having less than normal expression levels of the factor. In such a case, DNA encoding SSRP 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 *in situ* in the individual. The resultant cells express the encoded factor (SSRP), thereby overcoming the shortage or lack of natural DNA structure-specific recognition protein production in the individual.

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

Example A:
Electrophoretic Mobility Shift Analysis (EMSA) of the DNA binding Characteristics of the Cellular *cis*-DDP Structure-Specific Recognition Protein (*cis*-DDP SSRP)

Materials. Restriction endonucleases and polynucleotide kinase were purchased from New England Biolabs. The Klenow fragment of *E. coli* polymerase I and bacteriophage T4 DNA ligase (Boehringer Mannheim Biochemicals), proteinase K and RNase A (Sigma), (hexamethyldecyl)trimethylammonium bromide (CTAB) (Fluka), and poly(dI-dC)•poly(dI-dC) (Pharmacia) were obtained from commercial sources as indicated. The cell lines used were HeLa (kindly provided by M. Chow, MIT), *cis*-DDP-resistant HeLa, Chinese hamster V79, and *cis*-DDP-resistant V79 cells (kindly provided by S.L. Bruhn, MIT; *cis*-DDP resistant V79 cells were adapted to 15 μg/mL cisplatin, making them about 30-fold more resistant than parental cells), and human B cells (RPMI 4265; kindly provided by H. Singh, MIT).

Cell Extracts. Cytosolic, nuclear and whole-cell extracts were prepared according to published procedures. Stillman, B.W. and Y. Gluzman, Mol. Cell. Biol. **5**:2051-2060 (1985); Dignam *et al.*, Nucleic Acids Res. **13**:1475-1489 (1983); and Wood *et al.*, Cell **53**:97-106 (1983), respectively. Protein concentrations were determined by the method of Bradford. Bradford, Anal. Biochem. **72**:248-254 (1976).

Platinum-Modified damaged DNA fragments. *cis*-DDP, *trans*-DDP, [Pt(en)Cl_2], and [Pt(dien)Cl]Cl were prepared as described (Johnson, G.L. Inorg. Synth. **8**:242-244 (1966); Dhara, S.C., Indian J. Chem. **8**:193-

194 (1970); Watt, G.W. and W.A. Cude, Inorg. Chem. 7: 335-338 (1968); Lippard *et al.*, Biochemistry 22:5165-5168 (1983). Restriction fragments, a 274 bp ClaI-SmaI fragment generated from pSTR3 (see Couto *et al.*, J. Bacteriol. 171:4170-4177 (1989)) and a 422 bp AvaI fragment generated from bacteriophage M13mp18 DNA, were purified on low melting point agarose electrophoresis gels followed by phenol extraction (Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982)) or butanol extraction in the presence of CTAB (Langridge *et al.*, Anal. Biochem. 103:264-271 (1980)).

Restriction fragments were suspended in 1 mM sodium phosphate buffer, pH 7.4, containing 3 mM NaCl (buffer B) or in TE at a DNA nucleotide concentration of about 10^{-4} M. A portion of the DNA was allowed to react with the appropriate platinum complex at a variety of formal drug/nucleotide ratios at 37°C for 12-24 hours. An identical volume of buffer B or TE was added to control, unmodified DNA and incubated in parallel with the modified DNA fragment. Unbound platinum was removed by ethanol precipitation of the Pt-modified DNA restriction fragments, followed by several washes of the pellet with 80% ethanol. DNA concentrations were determined by UV spectroscopy with the relation 1 OD_{260} = 50 μg/mL. Bound levels of Pt to DNA were measured on a Varian AA-1475 atomic absorption spectrometer equipped with a GTA-95 graphite furnace. DNA fragments were radiolabelled with [α-^{32}P]dCTP (>5000 Ci/mmol, New England Nuclear) by the Klenow fragment of DNA polymerase I. Labeled, damaged DNA fragments were purified on native polyacrylamide gels as described in Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring

Harbor, N.Y. 1982) and resuspended in TE to 5000 cpm/μL prior to use in EMSA or other studies.

Electrophoretic Gel Mobility Shift Assay (EMSA or bandshift analysis). Studies of (damaged DNA frgament):(protein) complexes formed as a result of binding of SSRP to radiolabelled, platinated DNA fragments with the use of gel electrophoresis was carried out as described by Carthew *et al.*, Cell 43:439-448 (1985) with minor modifications. End-radiolabeled DNA restriction fragments [(1-5) x 10^3 cpm; ~0.2 ng] that were either unmodified or modified with the various platinum compounds as indicated below were incubated in the presence of crude extracts, typically 5-10 μg of protein, and 6 μg of competitor 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 DTT) in a final volume of 10-50 μL.

(Damaged DNA fragment):(protein) complexes were thereafter resolved from uncomplexed DNA fragments on a 4% polyacrylamide gel [29:1 acrylamide:N,N'-methylene-bis(acrylamide)]. Gels were preelectrophoresed in Tris-glycine buffer (50 mM Tris-HCl, pH 8.5, 380 mM glycine, 2 mM Na_2EDTA) for >1 hour at 25 mA. Samples were then electrophoresed for about 4 hours at 30 mA. Gels were dried and autoradiographed overnight at -20°C with an intensifying screen.

The results of this study showed the binding of a cellular protein to a damaged DNA fragment comprising a radiolabelled, *cis*-DDP modified 422 bp AvaI restriction fragment of M13mp18 DNA. Radiolabelled DNA fragments (1-5 x 10^3 cpm; 0.2 ng) contained bound *cis*-DDP levels as follows: lanes 1-4, r_b of 0; lanes 5-8, r_b of 0.007; lanes 9-12, r_b of 0.021; lanes 13-16, r_b of 0.041; and lanes 17-20, r_b of 0.061. These radiolabelled,

platinum damaged DNA fragments were incubated in the absence (-; lanes 1, 5, 9, 13 and 17) or presence of crude nuclear extract prepared from V79 parental cells (VP; lanes 2, 6, 10, 14 and 18), *cis*-DDP-resistant V79 cells (VR; lanes 3, 7, 11, 15 and 19) or HeLa cells (H; lanes 4, 8, 12, 16 and 20). It was seen from the resulting autoradiograph that migration of the DNA fragment alone was 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). It was also seen that that cellular factors present in HeLa nuclear extract bind to unplatinated DNA (lane 4). This binding was reproducible, independent of the oligonucleotide probe, and currently of unknown origin. A second band also appeared with the unplatinated DNA probe (lane 1) and probably represents denatured probe DNA.

In pertinent part, the EMSA results showed the presence of a cellular structure-specific DNA recognition protein (SSRP) which binds selectivity to cisplatin-modified DNA. This DNA binding protein formed a (damaged DNA fragment): (protein) complex having a retarded electrophoretic migration relative to that of the damaged DNA fragment alone (e.g., lanes 1, 5, 9, 13 and 17), allowing the complex to be visualized in nuclear extracts from human HeLa and Chinese hamster V79 parental and *cis*-DDP-resistant cell lines.

Selectivity for platinated DNA was demonstrated by the correlation between the extent of binding and the level of DNA platination. An estimated minimum modification level of about 0.007 Pt/nucleotide was required to observe binding of the protein to labeled modified DNA, whereas, at a modification level of 0.06 Pt/nucleotide, nearly all labeled DNA was complexed. For probes of

higher r_b, two bands were observed in the gel (lanes 18-20), possibly indicating the binding of two protein molecules to those DNA fragments having higher numbers of damaged sites. In other experiments, *cis*-DDP-specific SSRPs were found in cytosolic extracts and whole-cell extracts prepared from HeLa cells and in nuclear extracts from human B cells. Cytosolic and whole-cell extracts from this latter source were not examined. It has not yet been conclusively established that the protein observed in cytosolic extracts is the same as that found in nuclear extracts. However, as described below, both proteins have similar specificities of binding to DNAs modified with various platinum compounds. Furthermore, both proteins are precipitated with 40-65% ammonium sulfate.

It should also be noted that the *cis*-DDP SSRP appears to be present at the same levels in platinum-sensitive and platinum-resistant cell lines. Platinated DNA fragments incubated with nuclear extracts from either V79 parental or *cis*-DDP-resistant cell lines were bound to similar extent. Similar results were obtained with parental and approximately 50-fold *cis*-DDP-resistant HeLa cell extracts (data not shown). Hence, in these cell lines the level of SSRP present does not seem to be related to acquired cellular resistance to *cis*-DDP.

Example B:
EMSA Study of the Selectivity Characteristics of the Cellular SSRP for *cis*-DDP

An EMSA study was carried out with the object of assessing the ability of the SSRP disclosed in Example A to discriminate among different platinated DNA adducts. Here, the 422 bp AvaI DNA restriction fragment described in Example A was modified with

various platinum compounds and incubated in the absence (lanes 1, 5, 9, 13 and 17) or presence of crude extracts prepared from V79 parental cells (VP; lanes 2, 6, 10, 14 and 18), V79 *cis*-DDP-resistant cells (VR; lanes 3, 7, 11, 15 and 19) or HeLa cells (H; lanes 4, 8, 12, 16 and 20), all as described above in Example A.

SSRP was observed to form complexes only with DNA fragments containing adducts of platinum drugs which are capable of forming 1,2-intrastrand dinucleotide adducts.

Example C:
Competitive EMSA Study of the Cellular SSRP

A competition study was carried out wherein protein-DNA fragment binding reactions were incubated in the presence of escalating concentrations of unlabelled DNA fragments containing or lacking sites of platinum modification. More specifically, a preparation of end-labeled 274 bp ClaI-SmaI restriction fragment generated from pSTR3 as described above in Example A (5000 cpm; 0.2 ng) was modified with *cis*-DDP at r_b = 0.045. Labelled DNA fragments were incubated in the presence of 7.3 μg nuclear extract from *cis*-DDP-resistant V79 cells, nonspecific competitor DNA, and competitors as follows: lanes 3-6 = 0.2, 1, 10 and 20 ng unlabeled, unmodified 422 bp AvaI restriction fragment of M13mp18; lanes 7-10 = 0.2, 1, 10 and 20 ng unlabeled 422 bp fragment modified with *cis*-DDP at an r_b level of 0.035. It was shown that the *cis*-DDP modified fragments were effective competitors, and that the corresponding unmodified fragments were ineffective.

Example D:
EMSA Study of the Sensitivity the Cellular SSRP to Protease and Ribonucleases

A sensitivity study was designed to investigate the effects of incubation in the presence of protease or ribonuclease on the ability of the cellular SSRP to form (damaged DNA fragment):(protein) complexes. The results obtained by EMSA analysis demonstrated that the cellular factor in crude extracts was sensitive to the activity of protease and ribonucleases. Crude nuclear extracts were pretreated at 37°C for 60 minutes in the presence or absence of enzymes. The pretreated extracts were then incubated with 5000 cpm (0.2 ng) end-labeled 422 bp AvaI restriction fragment, modified with *cis*-DDP at an r_b level of 0.041 as described in Example A. Electrophoretically resolved samples included: lane 1, free unlabeled 422 bp platinated fragment; and lane 2, extract pretreated in the absence of lytic enzymes. The remaining lanes were as follows: lane 3 (P), proteinase K at 100 μg/mL; lane 4 (M), micrococcal nuclease at 0.075 U/mL; lane 5 (T1), RNase T1 at 0.025 U/mL; lane 6 (T2), RNase T2 at 0.005 U/mL; lanes 7-10 (R), RNase A at 20 μg/mL, 2 μg/mL, 0.2 μg/mL, and 0.02 μg/mL.

In subsequent studies, cell extracts and partially purified SSRP (described below) were incubated in the presence of proteinase K at 100 μg/mL or RNase A at 20 μg/mL for 1 hour at 37°C in 10 mM Tris-HCl, pH 7.4, containing 1 mM Na_2EDTA, then subjected to EMSA analysis as described in Example A. The results of this study showed that pretreatment of crude extracts with proteinase K resulted in loss of binding activity, confirming that the observed factor (SSRP) is a protein. Pretreatment of crude extracts with RNase A also resulted in loss of activity, but this sensitivity disappeared after partial purification of the *cis*-DDP SSRP factor by ammonium sulfate fractionation and ion exchange chromatography as hereinafter described.

Example E:
EMSA Investigation of Possible Requirements of the Cellular *cis*-DDP SSRP for cofactors and metal ions

The gel mobility shift assay was also used to assess the possible cofactor and metal ion requirements for binding of SSRP to *cis*-DDP-modified DNA. The factor in crude cellular extracts required neither ATP nor divalent cations such as Mg^{2+} and was insensitive to EDTA at concentrations up to 100 mM. Binding activity was sensitive, however, to some metal ions. (Damaged DNA fragment):(protein) complex formation was completely inhibited in the presence of 5 mM $ZnCl_2$, $MnCl_2$, $CoCl_2$, or $CdCl_2$ and by 1 mM $HgCl_2$. The protein bound to platinated DNA at both 37 and 0°C, but heat treatment of the extracts (42°C for 15 minutes) prior to the EMSA incubation step (see Example A) resulted in complete loss of activity. SSRP binding activity was also inhibited at high salt concenrations, such as 500 mM KCl.

Example F:
Competitive Electrophoretic Mobility Shift Analysis of the Cellular SSRP

Competition Assays. Competition assays were performed by adding various amounts of unlabeled competitor DNA to the binding reactions of the gel mobility shift assay before the 15-min incubation step described in Example A. Competitor DNA was either a restriction fragment as described above, or M13mp18RF (replicative form) DNA that was either unmodified or modified with *cis*-DDP or UV light.

Determination of the Binding Constant of *cis*-DDP SSRP. The binding constant of the protein for platinated DNA was estimated as described by Müller, R., Methods Enzymol. 9:589-601 (1983). A competition

assay was performed in which radiolabeled 274-bp fragment modified with *cis*-DDP at an r_b level of 0.036 (see Example A) was incubated in the presence of increasing amounts of unlabeled 274-bp fragment modified with *cis*-DDP to the same extent. Binding reactions were done in triplicate for each level of competitor DNA. The amount of labeled platinated DNA bound to the protein was estimated by scintillation counting of the free and bound labeled DNA excised from dried gels.

Cellular SSRP binding to the labeled 274-bp fragment platinated at 0.036 Pt/nucleotide was effectively competed by increasing quantities of unlabeled fragment modified to the same extent (lanes 6-20). By contrast, unplatinated DNA did not compete with the labelled platinated DNA for binding of the cellular factor. Competition for binding was complete when a 100-fold excess of unlabelled platinated DNA was added to the binding reaction (lanes 18-20). Binding of SSRP to labeled, platinated DNA was inhibited by 50% in the presence of a 3-fold excess of unlabeled platinated DNA.

From these results, the affinity constant of the *cis*-DDP SSRP could be estimated. It was assumed that bands 1-3 observed in the autoradiograph represented one, two, and three bound protein molecules, repectively. DNA in the well of each lane was also assumed to contain bound protein. From these data, the extent of inhibition of binding due to the competitor DNA could be calculated. The affinity constant was determined from the equation derived by Müller, R., Methods Enzymol. 9:589-601 (1983):

$$K = {}^{8}/_{3}([I_t]-[T_t])$$

where [I_t] represents the concentration of unlabeled platinated DNA that results in 50% inhibition of binding and [T_t] represents the concentration of labeled platinated DNA. The dissociation constant (K_d) is the reciprocal of the binding constant (K). From the results of this competition study, K_d was estimated to be about 1 x 10^{-10}M. This estimate, which is a lower limit, was made by assuming one binding site for each molecule of DNA. Bands 2 and 3, however, suggest that more than one protein can bind per molecule of DNA. Both the radiolabeled and unlabelled competitor DNA fragments contained an average of 20 platinum adducts. Since the *cis*-DDP SSRP binds only to the 1,2-d(GpG) and -d(ApG) adducts formed by cisplatin (see Example F), comprising 90% of all platinum adducts of this drug, it was assumed that each molecule of competitor DNA contained about 18 potential binding sites. When the concentrations of unlabelled and labelled binding sites were used in the above equation, the upper limit of the dissociation constant was calculated to be 2 x 10^{-9} M. The true value of the dissociation constant, therefore, lies in the range of (1-20) x 10^{-10}M. Of course, competition assays with purified protein and probes containing single, site-specific platinum adducts can be used to determine the dissociation constant more accurately.

Example G:
EMSA Study of the Selectivity Characteristics of the Cellular SSRP for *cis*-DDP DNA Adducts

A more refined EMSA study was carried out to follow up on the results discussed in Example B. The 422 bp AvaI DNA restriction fragment of M13mp18 described in Example A was modified with various therapeutically active platinum compounds. HeLa

extracts were prepared as described in Example A. Labelled, damaged DNA fragments were incubated in the absence of cell extract (-; lanes 1, 4, 7, 10, 13 and 16), in the presence of HeLa cytosolic extract (S; lanes 2, 5, 8, 11, 14 and 17), or in the presence of HeLa nuclear extract (N; lanes 3, 6, 9, 12, 15 and 18). Samples were incubated and electrophoretically resolved as described previously.

Results obtained by gel mobility shift analysis demonstrated that the cellular SSRP binds selectively to DNA modified with *cis*-DDP, $[Pt(en)Cl_2]$, and $[Pt(dach)Cl_2]$, but not to DNA modified with either *trans*-DDP or [Pt(dien(Cl)]Cl. The latter two platinum compounds are unable to link adjacent nucleotides in DNA, whereas the former three are known to form 1,2-intrastrand d(ApG) and d(GpG) adducts.

Example H:
Further EMSA Study of the Platinated DNA Structural Motif Recognized by the Cellular *cis*-DDP SSRP

Construction of Oligonucleotides Containing Specific Platinum-DNA Adducts. Oligonucleotides 22 bases in length containing single 1,2-intrastrand d(GpG) or d(ApG) or 1,3-intrastrand d(GpTpG) adducts of *cis*-DDP, the 1,3-intrastrand d(GpTpG) adduct of *trans*-DDP, or the monofunctional N7-d(G) adduct of $[Pt(NH_3)_2(N3\text{-cytosine})]^{2+}$ were prepared as previously reported. Rice *et al.*, Proc. Natl. Acad. Sci. USA **85**:4158-4161 (1988). These oligonucleotides are designated as "Top" strands. Unmodified Top strands were also constructed as controls. Complementary oligonucleotides designated as "Bottom" strands were constructed such that, when annealed to the adducted single-stranded fragments, they formed duplexes containing two-base 3′-overhangs at both ends. These

synthetic, double-stranded oligonucleotides containing predefined types of platinum adducts are shown in Figure 1.

The Bottom oligonucleotides were 5'-end labeled with [γ-^{32}P]ATP (<3000 Ci/mmol, New England Nuclear) by polynucleotide kinase and purified from unincorporated ATP on a Nensorb-20* column (New England Nuclear). Adducted and control Top oligonucleotides were 5'-end phosphorylated with nonradioactive ATP and also purified on Nensorb-20 columns.

Top and Bottom strands were mixed at a mole ratio of 4:3, heated at 90°C, and then cooled slowly to 4°C to allow the two strands to anneal. High-concentration T4 DNA ligase (10,000 units/mL) was added, and the samples were incubated overnight at 13°C. Double-stranded oligonucleotides of 44, 66, 88 and 110 bp in length were then purified from native polyacrylamide gels according to the method of Maniatis (supra). These synthetic duplex oligonucleotides containing predefined, specifically placed platinated DNA structural motifs (shown in Figure 1) were used as damaged DNA fragments to investigate SSRP binding specificity in the competitive EMSA studies.

Substantial nonspecific binding to these oligonucleotides was observed, as evidenced by the presence of slower migrating bands seen in the cases where the oligonucleotides were not modified with platinum. Specific binding was observed, however, to DNA fragments containing the 1,2-intrastand d(GpG) and d(ApG) cross-linked adducts of *cis*-DDP. SSRP bound to oligonucleotides 88 or 110 bp in length, but not to those that were 44 or 66 bp long. This probe size limitation presumably reflects a minimum requirement for a flanking nucleic acid domain in order for protein binding to occur. Binding was not observed with

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randomly modified DNA fragments at r_b values of less than 0.007, suggesting that a minimum level of modification is required for binding of the DRP in crude extracts. The band representing specific binding to platinated oligonucleotides of 110 bp could be competed away with an about 340-fold excess of unlabeled M13mp18RF DNA modified with *cis*-DDP at a bound drug to nucleotide level of 0.041 but not with unlabeled unplatinated M13mp18 DNA at the same approximately 340-fold excess. No specific binding occurred in cases where the DNA probes contained the d(GpTpG) 1,3-intrastrand cross-linked adducts of *cis*-DDP and *trans*-DDP or the monofunctional d(G)-N7 adduct of $\{Pt(NH_3)_2(N3\text{-cytosine})\}Cl$. Thus, the results of this study further support the postulate that SSRP recognizes a structural motif comprising a 1,2-intrastrand dinucleotide adduct.

Example I:
EMSA Studies Revealed that the Cellular *cis*-DDP SSRP does not simply respond to ss DNA

As noted previously in the Detailed Description, the 1,2-intrastrand d(GpG) and d(ApG) DNA adducts of *cis*-DDP bend the helix in the direction of the major groove, and are thought to produce a local region of ss DNA opposite to the site of the platinum lesion. In fact, such a ss motif could be detected by antinucleoside antibodies (reported by Sundquist *et al.*, Biochemistry **25**;1520-1524 (1986)). This observation suggested that SSRP might recognize a single-stranded domain, rather than a structural motif (e.g., a helix kink) produced by the platinated DNA adduct itself.

This possibility was excluded by a competitive EMSA study in which nuclear extracts from HeLa cells

were incubated in the presence of 5000 cpm (0.2 ng) of the 274 bp ds restriction fragment described in Example A, modified with *cis*-DDP at 0.040 Pt/nucleotide. Single stranded DNA was prepared by boiling the unplatinated, radiolabeled 422 bp restriction fragment disclosed in Example A, and then allowing the DNA to reanneal in the presence of a 10-fold molar excess of M13mp18 circular ss 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. Escalating concentrations (0.2-100 ng) of this unlabeled ss M12mp18 DNA was added to EMSA samples as a competitor. Single-stranded DNA was not observed to compete with the *cis*-DDP modified ds DNA fragment for binding to SSRP, a result which bolsters the suggestion that SSRP does not simply respond to ss domains.

Example J:
EMSA Studies Also Showed that the Cellular *cis*-DDP SSRP does not bind to UV-induced DNA lesions

A factor has been reported in nuclear extracts prepared from HeLa cells that binds specifically to DNA damage induced by UV irradiation. Chu, G. and E. Chang, Science 242:564-567 (1988). Accordingly, UV-damaged DNA fragments were prepared and employed in a competitive EMSA study to determine whether the factor reported by Chu and Chang is related to SSRP (see also Example F). The 422-bp DNA fragment derived from AvaI digestion of M13mp18 (Example A) was purified by electrophoresis through a low-melting agarose gel followed by butanol extraction in the presence of CTAB. DNA fragments were labeled with [α-^{32}P]dCTP and purified as described above. The labeled DNA fragments were then irradiated with a General Electric 15-W germicidal

lamp (maximum output at 254 nm) calibrated with a UVX digital radiometer at a flux of 5 J/(m^2-s) and a final dose of 1500 J/m^2.

Competition reactions included the end-labeled, Pt-modified (r_b of 0.038) 422 bp fragment, 10 μg of HeLa nuclear extract, and escalating levels (0.1-10 ng) of unlabelled competitor M13mp18 DNA modified with either *cis*-DDP at an r_b of 0.041, or with UV light as described in the preceeding paragraph. In a second series of competition reactions, end-labelled, UV-modified 422 bp fragment was used.

The results of this study revealed that SSRP binding was not competed by a 1000-fold excess of M13mp18RF DNA treated with UV at 1500 J/m^2, which corresponds to a calculated level (Spivak *et al.*, Mutat. Res. **193**:97-108 (1988)) of about 5.7 cyclobutane dimers per kilobase. Conversely, the binding of a factor found only in nuclear extracts to labelled DNA modified with UV light at 1500 J/m^2 could be competed with a 1000-fold excess of unlabeled, UV-irradiated M13mp18 DNA, but not with a 1000-fold excess of DNA platinated with *cis*-DDP. These results bolster the conclusion that *cis*-DDP SSRP is not the factor described by Chu and Chang as capable of recognizing UV-induced DNA lesions.

Example K:
Partial Purification and Characterization of the Cellular *cis*-DDP SSRP

Purification of Cellular *cis*-DDP SSRP. Saturated ammonium sulfate was added dropwise to HeLa crude cytosolic extracts to a final concentration of 40%. The mixture was stirred on ice for 30 minutes and centrifuged at 11,000 rpm in a Sorvall SM24 rotor for 30 minutes. Proteins present in the supernatant were

precipitated with ammonium sulfate added as above to a final concentration of 65%. The 40-65% fraction (i.e., the second precipitate) was resuspended in buffer H (25 mM HEPES, pH 7.5, 150 mM KCl, 0.1 mM Na_2EDTA, 1 mM DTT, and 10% glycerol) and dialyzed extensively against the same buffer.

Sucrose Gradient Ultracentrifugation.

Essentially, the method of Johns, P. and D.R. Stanworth, J. Immunol. Methods 10:231-252 (1976) was followed. A portion of the 40-65% fraction representing 1 mg of protein was centrifuged through a 0-15% linear sucrose gradient for 18 hours at 43,600 rpm ($\omega t^2 = 1.34 \times 10^{12}$, 170 000g) in a Beckman SW 50.1 rotor. Fractions were removed from the top of the gradient and dialyzed extensively against buffer H. Each fraction was subsequently assayed for *cis*-DDP-DNA binding activity by EMSA, in the manner described in Example A (i.e., using the end-labelled, *cis*-DDP modified 422 bp AvaI restriction fragment of M13mp18). Protein standards were centrifuged in parallel as molecular weight markers. Fractions from this gradient were precipitated with methanol/chloroform (3:1 and resuspended in SDS loading dye (0.3 M Tris base, pH 9.0, 50% glycerol, 5% SDS, 5% 2-mercaptoethanol, 0.0025% brophenol blue). The fractions were then electrophoresed through a 12% SDS-polyacrylamide gel, and the gel was stained with Coomassie blue R-250 to detect protein.

Figure 2 presents the results of this study to determine the size of the cellular *cis*-DDP SSRP by sucrose gradient sedimentation. The profile of the gradient is shown; EMSA study of the fractions revealed that SSRP was located in fractions 7-12, with the peak of activity in fraction 9. From these data, the sedimentation coefficient of SSRP was calculated to be

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5.6S, which corresponds to an apparent molecular weight of 91 000 daltons for a globular protein. It will be seen from the Examples which follow that this result is in agreement with assessments of the molecular weight of SSRP based upon modified Western blot analysis.

Example L:
Modified Western (Southwestern) Blotting Technique for Detecting the Presence of SSRP

Preparation of Crude Extracts. Eukaryotic nuclear and cytosolic extracts of HeLa cells were prepared as described in Example A. *Escherichia coli* strain SG1161 (lon$^-$) lysogens were prepared as described in the literature. Singh, H. *et al.*, Cell, 52:415-423 (1988). This strain of *E. coli* was chosen to reduce proteolytic degradation of the expressed fusion protein (comprising β-galactosidase and at least a portion of SSRP).

Radiolabelled and platinum-modified DNA fragments used for modified Western Blotting studies were prepared as described in Example A.

Southwestern Blot Procedure. Extracts were prepared from either IPTG-induced (IPTG refers to isopropyl-β-D-thiogalactopyranoside) lysogens or HeLa cells. Typically, 50 μg total protein per lane were separated by sodium doedecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on an 8% separating gel and transferred onto nitrocellulose (Schleicher & Schuell, BA85, 0.45 μm) according to conventional techniques. Following transfer, filters were processed as described in the literature. 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-hydroxyethyl-piperazine-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 (0.25-2.0 x 10^4 cpm/mL, 10^{-10} to 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 an experiment 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 $MnCl_2$. Thereafter, (damaged DNA fragment):(protein) complexes present on the blot surface were detected by autoradiography with the use of an intensifying screen at -80°C.

This procedure was used successfully to visualize HeLa cellular SSRP and recombinant fusion proteins having SSRP activity. The cellular protein was observed to have electrophoretic migration properties consistent with a globular protein of about 100 000 daltons. These studies are more fully described below.

Example M:
Southwestern Blot Screening Procedures for Detection of Recombinant Expression Products having SSRP Activity

Protein replica filters were prepared from an unamplified human B cell (RPMI 4265) cDNA library (Clontech Laboratories, Inc.) constructed in the expression vector λgt11. 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 plaque forming units (PFU)/mL. Screening of the λgt11 recombinants plated on *E. coli* host strain Y1090 was carried out as described in Singh, H. *et al.*, Cell, 52:415-423 (1988), using cisplatin-modified, ^{32}P-labeled DNA 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_2EDTA, 1 mM DTT) for 100 and 150 mm plates, respectively. The buffer contained ^{32}P-labeled platinated DNA at a final concentration of approximately 3 x 10^4 cpm/mL (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 per wash 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 phage, while tertiary screens used plating mixtures of about 100 PFU. This protocol was employed successfully to purify two recombinant phage, λPt1 and λPt2, to homogeneity.

Example N:
Southwestern Blot Study of Cellular and Recombinant Proteins having SSRP Activity

In order to demonstrate that the clones isolated in Example M encode proteins which 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. As a control, HeLa extract was also prepared and included in the analysis.

Crude extracts obtained from induced lysogens were subjected to SDS-PAGE and the resolved proteins were transferred to nitrocellulose filters. Four filters were prepared, comprising the following samples: lane 1, HeLa cytosolic extract; lane 2, bacterial lysogen crude extract from the λgt11 vector (lacking insert); lane 3, bacterial lysogen crude extract from λPt2; and lane 4, bacterial lysogen crude extract from λPt1. Following denaturation and renaturation according to the method of Celenza, J.L. and M. Carlson, Science, 233:1175-1180 (1986), the four filters were probed and developed as follows: A, India Ink stain to visualize total proteins; B, a monoclonal antibody raised against β-galactosidase, followed by immunoglobulin-specific detection according to the Western Blotting method of Ausubel, F.M. *et al.*, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, New York, Section 10.7.1.; C, ^{32}P-labeled, unmodified 422 bp AvaI restriction fragment of M13mp18 (Example A); and D, the same DNA fragment modified with *cis*-DDP.

Thus, filters C and D depicted the results of Southwestern blotting studies. These investigations showed the presence of two predominant polypeptides in λPt1 lysogens having β-galactosidase immunoreactivity, which selectively bind to DNA fragments modified by *cis*-DDP and not to the corresponding unmodified DNA fragments. These bands are separated by approximately 4 kDa. 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.

In subsequent studies, filter-bound, electrophoretically resolved proteins were also probed with DNA fragments modified with [Pt(en)Cl$_2$], *trans*-DDP, or

[Pt(dien)Cl]$^+$. These studies revealed that bacterial induced lysogens from λPt2 and λPt1 bound only to DNA modified by *cis*-DDP or [Pt(en)Cl_2], in accord with results obtained with the HeLa cellular SSRP. The detection limit of this modified Western (Southwestern) Blot technique for binding of the phage-encoded proteins to *cis*-DDP-modified DNA was found to be approximately 2 platinum adducts per 100 nucleotides, corresponding to an r_b level of 0.02.

Example O:
Restriction Enzyme Mapping of the Isolated cDNA Clones, λPt1 and λPt2

Amplified phage stocks prepared from λPt1 and λPt2 were used to isolate recombinant DNA. Maniatis, T. *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 76-85 (1982). Each clone was digested with a variety of restriction enzymes (obtained from International Biotechnologies and Bethesda Research Laboratories). After electrophoretic separation, DNA fragments were transferred to a nitrocellulose filter. Id., pp. 383-386. To determine any homologies between the two cDNA clones, the filter was probed with λPt2 cDNA insert labelled with [α-^{32}P]deoxy-cytidine triphosphate by the Klenow fragment of DNA polymerase I. Id., pp. 113, 178. 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 (wherein SSC is 0.15 M NaCl, 15 mM trisodium citrate pH 7.0, and SDS is sodium doedecyl sulfate) 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.

The results of these studies are summarized in Figure 3. Enzyme mapping analysis of the two recombinant phage λPt1 and λPt2 indicated that they contain nucleotide sequences aligned at their 5′ ends, with insert sizes of 1.44 and 1.88 kb, respectively. Southern blotting analysis confirmed homology between the two clones. The apparent molecular weight of the portion of the fusion protein encoded by λPt2 which represents the cloned human B cell polypeptide is estimated to be approximately 50 kDa. This polypeptide represents at least a portion of a cellular protein having *cis*-DDP SSRP activity.

Example P
Expression Studies of the Cellular Protein encoded by the λPt1 and λPt2 Sequences

Northern Blotting Technique. Cytoplasmic RNA from human HeLa, hamster V79, and murine leukemia L1210 cells were 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™ brand blotting paper (New England Nuclear). The λPt2 DNA insert was labeled with [α-^{32}P] deoxycytidine triphosphate according to a known technique. Feinberg, A.P. and B. Vogelstein, Anal. Biochem., **132**:6-13 (1983). The filter was probed with 10^6 cpm/mL of this probe in hybridization mixture (45% formamide, 10% dextran sulfate, 0.1% sodium phosphate, 50 mM Tris-HCl pH 7.5, 5x Denhardt's solution, 100 µg/mL sheared, denatured salmon sperm DNA and 0.5% sodium doedecyl sulfate) at 42°C. Thereafter, 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.

Preliminary Northern analysis of the expression of the λPt2 gene demonstrated the presence of a conserved cytoplasmic RNA species of 2.8 kb in HeLa, murine leukemia L1210 and Chinese hamster V79 cells. The predicted molecular weight of the full length cellular protein encoded by this mRNA is 100 000 daltons. It will be noted that this mass is similar to that of the binding factor identified as SSRP, as observed by Southwestern blot analysis of HeLa cytosolic extracts. This correlation supports the inference that the clone λPt2 encodes a portion of this same factor.

In a subsequent study, the following Northern blotting technique was employed to further characterize expression patterns of the SSRP gene:

Northern Analysis. RNA was isolated by using standard procedures (Sambrook, J., *et al.*, Molecular Cloning: A Laboratory Manual (1989). Typically, 12 μg of RNA were used for electrophoretic analysis in 1% agarose gels containing 6% formaldehyde, 20 mM MOPS, 5 mM NaOAc, and 1 mM EDTA. Gels were denatured for 15 minutes in 50 mM NaOH, 100 mM NaCl, neutralized in 100 mM Tris (pH 7.5), and transferred to GeneScreenPlus™ (New England Nuclear) by capillary action in 10X SSC. Filters were rinsed in 2X SSC and baked in a vacuum oven for two hours at 80°C. Pre-hybridization for four hours and hybridization for 16 hours with $1x10^6$ cpm of labelled λPt2 DNA per ml of hybridization fluid was carried out at 42°C in 30-40% formamide, 10% dextran sulfate, 0.1% $NaPP_i$, 50 mM Tris (pH 7.5), 5X Denhardt's, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. Filters were washed at 55°C with 2X SSC,

0.1% SDS twice, and in 1X SSC, 0.1% SDS twice for 30 minutes each and exposed to X-ray film.

In order to determine the tissue specificity of SSRP gene expression, total RNA was isolated from baboon brain, heart, ileum, jejunum, kidney, liver, muscle, and spleen tissue and subjected to Northern analysis. The results of this survey revealed that the 2.8 kb SSRP message is expressed in all tissues examined. Rehybridization probing of the filter with a fragment of human β-actin allowed normalization for RNA loading levels, and showed that the relative levels of SSRP expression were similar each of the tissues analyzed, except for brain tissue, in which it is higher.

Because of the exceptional success of cisplatin in treating testicular cancer, a more detailed analysis of expression was carried out in a series of testicular carcinoma cell lines. Several bladder cancer cell lines (Masters, J.R.W. Cancer Res. 46:3630-3636 (1986)) were studied concurrently because cisplatin is less active against this type of cancer. SSRP is expressed in all of the bladder and testicular cell lines examined; no general trends were apparent. These data indicate that the intracellular level of SSRP mRNA does not correlate with the antitumor activity of cisplatin for a particular tissue type.

Since the protein described herein specifically recognizes DNA adducts of active antitumor platinum complexes, its possible role in acquired resistance of cells to cisplatin was also investigated. A Northern blot analysis in which the λPt2 clone was used to probe cytoplasmic RNA levels in a series of cisplatin resistant human, mouse, and hamster cell lines was carried out. Data obtained from this study indicate that the level of SSRP expression does not correlate with resistance in these cell lines.

In order to study whether expression of the cisplatin-DNA SSRP could be induced in cells treated with the drug, cytoplasmic RNA was isolated from HeLa cells which had been exposed to a range of concentrations of cisplatin. The 2.8 kb mRNA SSRP gene transcript was not inducible by a wide range of cisplatin concentrations over the course of 48 hours.

Example Q
Use of Clones λPt1 and λPt2 to Obtain the Full Length human cDNA Sequence Encoding SSRP

Labelling of Probes for Hybridization. The λPt2 clone (reported in Toney, J.H. *et al.*, Proc. Natl. Acad. Sci., USA 86:8328-8332 (1989)) was used as a probe for hybridization and library screening. λPt2 was radiolabelled by random oligonucleotide priming as described in Feinberg, A.P. and Vogelstein, B. Anal. Biochem 132:6-13 (1983). Typically, 50-100 ng of DNA in low melting point agarose was boiled, primed with $pd(N)_6$ oligonucleotides (Pharmacia), and labelled with α-[^{32}P]dCTP by *Escherichia coli* DNA polymerase I (Klenow fragment). Labelled fragments were purified by spin dialysis over Spehadex G-50* columns and the extent of incorporation of radioactivity was monitored by scintillation counting.

Library Screening. For the primary screen of each cDNA library, $5x10^6$ recombinant phage were plated on *E. coli* host strain Y1088. Duplicate replica nitrocellulose filters were prepared and then denatured (0.5 M NaOH, 1.5 M NaCl), neutralized (1 M Tris (pH 7.4), 1.5 M NaCl), and rinsed with 2X SSC (20X SSC: 3 M NaCl, 0.3 M $Na_3C_6H_5O_7$). After baking for two hours at 80°C in a vacuum oven the filters were pre-incubated at 42°C for four hours with hybridization fluid (50% formamide, 1M NaCl, 50 mM Tris (pH 7.5), 0.5% SDS, 10%

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dextran sulfate, 1X Denhardt's solution, and 1 mg/ml denatured salmon sperm DNA). Probe was then added at a concentration of $1x10^6$ cpm of labeled DNA per ml of hybridization fluid and the incubation was continued for an additional 16 hours. The filters were washed once at room temperature in 2X SSC/0.1% SDS, twice at 65°C in 2X SSC/0.1% SDS, and twice at 65°C in 0.1X SSC/0.1% SDS for fifteen minutes each. The filters were air dried briefly and analyzed by autoradiography. Multiple rounds of screening were used to isolate plaque pure bacteriophage clones. Single plaques were amplified in liquid culture for DNA preparation and further analysis.

In this manner, overlapping cDNA clones spanning the entire coding sequence of the human SSRP gene were identified and isolated from human embryonic kidney (HEK) fetal muscle (M), and basal ganglia (BG) cDNA libraries. These clones were subjected to Southern blot and sequencing analyses as described below.

Example R
Southern Blotting Studies of Overlapping cDNAs Encoding Human SSRP

Southern Analysis. High molecular weight genomic DNA was prepared by slowly dripping cells into lysis buffer (10 mM Tris (pH 8.0), 100 mM NaCl, 1 mM EDTA, 1% SDS), followed by overnight digestion with proteinase K (100 μg/ml), multiple phenol and chloroform extractions, and resuspension in TE (50 mM Tris (pH 7.5), 10 mM EDTA). For each sample, 10 μg of DNA was digested to completion and the fragments separated by electrophoresis in 0.8% agarose gels. Gels were denatured for 45 minutes (0.5 M NaOH, 1.5 M NaCl), neutralized for 60 minutes (1 M Tris (pH 7.4), 1.5 M NaCl) and the DNA immobilized on Zetabind™ membrane

(Cuno) by capillary transfer for 16 hours in 10X SSC. After rinsing the filter with 2X SSC, it was baked in a vacuum oven at 80°C for two hours. Following pretreatment at 65°C for one hour (0.5X SSC, 0.5% SDS) the filters were hybridized and washed as described above for library screening, and then analyzed by autoradiography.

A schematic representation showing the relationship between human cDNA clones encoding SSRP is presented in Figure 4. Clones λPt1 and λPt2 were isolated from a human B cell library as discussed previously. Clone HEK 402 was isolated from a human embryonic kidney library, and contains the complete SSRP cDNA sequence and polyadenylation signal. Clone M 801 was isolated from a fetal muscle library, and lacks the 3′ end of the gene but contains 147 bases of additional 5′ untranslated sequence. Clone BG 801 was isolated from a basal ganglia cDNA library and also lacks the 3′ end of the gene, but served to confirm the sequence of its 5′ end. All cDNA clones were completely sequenced in both directions as described in the following Example, and were found to be identical in overlapping regions.

Example 8
Sequencing of Human cDNAs Encoding SSRP and Characterization Thereof

Subcloning. Purified phage DNA was digested with EcoRI to release the cDNA inserts. The EcoRI fragments were isolated from low melting point agarose gels using GENECLEAN™ (Bio 101) and ligated into the EcoRI site of plasmid pBluescript* SKII+. After transformation of competent E. coli XL-1 cells, single colonies were isolated and amplified in liquid culture. DNA was purified by using Quiagen* affinity chromatography.

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Sequence Determination and Analysis. Sequence determination was performed on double-stranded plasmid DNA by using the chain termination method (Sanger, F., *et al.*, Proc. Natl. Acad. Sci., USA 74:5463-5467 (1977)) and Sequenase*T7 DNA polymerase (United States Biochemical). Sequence analysis employed software from Genetics Computer Group (GCG) at the University of Wisconsin (Devereaux, J., *et al.*, Nucl. Acids. Res. 12:387-395 (1984)). Homology searches were made by using the BLAST Network Service at the National Center for Biotechnology Information (Altschul, S.F. *et al.*, J. Mol. Biol. 215:403-410 (1990)).

By using the sequence information from these clones, a composite human sequence representing 2839 bases of DNA was generated. This sequence is shown in Figure 5; it contains a continuous open reading frame of 2130 bases beginning at position 275. The sequence surrounding the methionine start codon conforms well with the initiation sites of other vertebrate cDNAs (Kozak, M. Nucl. Acids. Res. 15:8125-8132 (1987)) and is conserved in homologs isolated from mouse (Shirakata, M. *et al.*, Molecular and Cellular Biology 11:4528-4536 (1991)) and *Drosophila melanogaster* (Bruhn, S., *et al.*, Prog. Inorg. Chem. 38:477-516 (1990)). A consensus polyadenylation signal AATAAA is present within the 435 bases of 3′ untranslated sequence beginning at position 2800.

The sequence predicts a 710 amino acid protein of molecular weight 81,068 Daltons, also shown in Figure 5. The amino acid composition reveals a strikingly high percentage of charged residues (36%). Further analysis of the protein sequence indicated the presence of several highly charged domains, illustrated in Figure 6. There is an acidic domain, aa 440-496, which contains 26 negatively charged and 4 positively charged amino acids. Two basic domains, denoted Basic I and

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Basic II, are located at aa 512-534 and aa 623-640, respectively. At the carboxyl terminus of the protein, aa 661-709, there is another highly charged series of amino acids containing 14 negative and 9 positive residues. Analysis of the hydropathy profile shows the entire region from aa 400 to the carboxyl terminus of the protein to be highly hydrophilic (not shown).

A search of protein data bases with the predicted amino acid sequence revealed some interesting homologies. SSRP showed the greatest homology to high mobility group (HMG) 1 and 2 proteins from several species, (Eink, L. and Bustin, M. Exp. Cell Res. **156**:295-310 (1985); Bustin, M., *et al.*, Biochim. Biophys. Acta **1049**:231-243 (1990)) and to a transcription factor containing HMG-box domains, hUBF (Jantzen, H.M., *et al.*, Nature **344**:830-836 (1990)). The location of the HMG box is indicated in Figure 6. Optimal alignment of human cisplatin-DNA SSRP with human HMG1 revealing 47% identity in the regions compared. Homology was also found between SSRP and other HMG-box proteins which have been recently reported. See Jantzen, H.M., *et al.*, Nature **344**:830-836 (1990); Sinclair, A.H., *et al.*, Nature **346**:240-244 (1990); Gubbay, J., *et al.*, Nature **346**:245-250 (1990); Parisi, M.A. & Clayton, D.A. Science **25**:965-968 (1991); Travis, A., *et al.*, Genes & Dev. **5**:880-894 (1991); Waterman, M.L. *et al.*, Genes & Dev. **5**:656-669 (1991); Diffley, J.F. *et al.*, Proc. Natl. Acad. Sci. USA **88**:7864-7868 (1991). It is important to note, however, that no obvious consensus HMG-box sequence emerges from such a comparison. In addition, the acidic region of SSRP has limited homology to nucleolin, (Srivastava, M., *et al.*, FEBS Lett. **250**:99-105 (1989)) which is involved in transcriptional control of rRNA genes.

The human map position of the SSRP was also determined, using a panel of human chromosome-specific

human-rodent hybrids. Initial experiments placed the gene on chromosome 11. Further refinement with a series of hybrid cell lines containing only small defined segments of human chromosome 11 on a rodent genomic background (Glaser, T. Ph.D. dissertation, Massachusetts Institute of Technology (1989)) localized the position of the clone to 11q12. Placement of the sequence on the long arm of human chromosome 11 is particularly interesting because the murine homolog to SSRP has been mapped to mouse chromosome 2 (Shirakata, M., *et al.*, Molecular and Cellular Biology 11:4528-4536 (1991)). Previously, a syntonic relationship had been demonstrated only for mouse chromosome 2 and human chromosome 11p (Nadeau, J.H., *et al.*, Mamm. Genome 1:S461-S515 (1991)).

Example T
Use of the human cDNA Clone λPt2 to Obtain the Full Length *Drosophila melanogaster* homolog of human SSRP

In view of the expression pattern and evolutionary conservation of the SSRP gene, indicating a protein with an *in vivo* role important for normal biological functions, at least one SSRP homolog from a lower species was desired in order to further delineate conserved domains likely to be critical for SSRP function. Accordingly, a *D. melanogaster* head cDNA library was screened using the human cDNA clone λPt2 (radiolabelled as described in Example O), under low stringency conditions according to the following procedure:

Library Screening. For the primary screen of the *Drosophila* head cDNA library (N. Itoh, *et al.*, Proc. Natl. Acad. Sci. USA 83:4081 (1986)), $5x10^6$ recombinant phage were plated on *E. coli* host strain Y1088. Duplicate replica nitrocellulose filters were prepared

and subsequently denatured (0.5 M NaOH, 1.5 M NaCl), neutralized (1 M Tris (pH 7.4), 1.5 M NaCl), and rinsed with 2X SSC (20 X SSC: 3 M NaCl, 0.3 M $Na_3C_6H_5O_7$). Baking for two hours at 80°C in a vacuum oven was followed by pre-incubation at 42°C for 4 hours with hybridization fluid (30% formamide, 1M NaCl, 50 mM Tris (pH 7.5), 0.5% SDS, 10% Dextran Sulfate, 1X Denhardt's, and 1 mg/ml denatured salmon sperm DNA). Labelled λPt2 probe was added to a final concentration of $1x10^6$ cpm of labelled DNA per ml of hybridization fluid and incubation continued for 16 hours. The filters were washed once at room temperature in 2X SSC/0.1% SDS, twice at 55°C in 2X SSC/0.1% SDS, and twice at 55°C in 1X SSC/0.1% SDS for fifteen minutes each. After the washing was completed the filters were air dried briefly and analyzed by autoradiography. Plaque pure bacteriophage clones were isolated by multiple rounds of screening. Single plaques were amplified in liquid culture for DNA preparation and further analysis.

Ten *Drosophila* cDNA clones were identified, with varying degrees of hybridization to the human cDNA. These bacteriophage clones were isolated and purified through successive rounds of screening. Two of these, denoted DM 3002 and DM 1001, were chosen for further study based on their strong hybridization to the human clone and their large size relative to other clones. Restriction and sequence analyses of these clones is described in the Examples which follow.

Example U

Southern Blotting Analysis of Overlapping cDNAs Encoding *Drosophila* SSRP

Southern Analysis. DNA from each species (human and fly) was digested to completion with EcoRI and the fragments were separated by electrophoresis in 0.8%

agarose gels. The gel was then denatured for 45 minutes (0.5 M NaOH, 1.5 M NaCl), neutralized for 60 minutes (1 M Tris (pH 7.4), 1.5 M NaCl) and the DNA transferred to Zetabind™ membrane (Cuno) by capillary action for 16 hours in 10X SSC. After rinsing the filter with 2X SSC, it was baked in a vacuum oven at 80°C for 2 hours. Following pretreatment at 65°C for one hour (0.5X SSC, 0.5% SDS), the filters were hybridized and washed as described above for library screening.

EcoRI digestion of the bacteriophage clones, DM 3002 and DM 1001, with EcoRI released a 2.3 kb insert from DM 3002, and two fragments of size 1.4 and 1.8 kb from clone DM 1001. These three fragments were gel purified, subcloned individually and subjected to sequence analysis (described below), as well as restriction endonuclease mapping. Sequence analysis of the three subcloned fragments confirmed that there was significant overlap between DM 3002 and the 1.8 kb EcoRI fragment of DM 1001. Northern analysis of the two EcoRI fragments of DM 1001 indicated that the 1.4 kb fragment recognized two head-specific RNA species of 3.5 and 1.6 kb. However, rehybridization of this blot with the 1.8 kb EcoRI fragment revealed that these RNA species were not recognized by this portion of the clone, indicating that clone DM 1001 was a chimera. Therefore, the 1.4 kb EcoRI fragment was not considered further. Figure 15 shows the alignment of clones DM 3002 and the 1.8 kb EcoRI fragment of DM 1001.

Example V
Sequencing of *Drosophila* cDNAs Encoding SSRP and Characterization Thereof

Subcloning and sequencing of the *D. melanogaster* cDNA sequences was carried out essentially as described

above in Example S. Clones DM 3002 and 1001 were sequenced completely in both directions; as noted above, significant overlap between DM 3002 and the 1.8 kb EcoRI fragment of DM 1001 was observed.

The sequences of clones DM 3002 and the 1.8 kb fragment of DM 1001 were combined to create a composite sequence of 2384 bases, shown in Figure 8. Interestingly, there are large open reading frames in both directions from bases 123-2291 and from bases 2300-600. The larger of the two open reading frames predicts a 723 amino acid protein of molecular weight 81 524 daltons, the sequence of which is also shown in Figure 8. This sequence shows extensive homology to the human structure specific recognition protein, the cDNA of which was used as a probe. For this reason, the 81 kD protein was assumed to be the correct reading frame. The AUG codon at position 123 of this open reading frame is believed to be the true start site, both because there is an in-frame stop codon upstream from this site and because the start site is the same as for the human protein. No consensus polyadenylation signal is seen within the 93 bases of 5′ untranslated sequence. It seems clear, however, that the complete coding sequence of the *Drosophila* homolog of human *cis*-DDP SSRP is contained within the clones sequenced.

The homology at the nucleotide level between the human and *Drosophila* cDNAs is 54%, and this similarity is confined mainly to the coding regions of the sequences. The homology in the 5′ and 3′ untranslated regions is 32% and 37%, respectively, whereas the predicted amino acid sequences of the two species' SSRPs share 53% identity and 72% similarity at the amino acid level over their entire length. Moreover, the sizes of the two SSRPs are quite comparable, and both contain a large number of charged amino acids (36% for the human protein and 38% for the *Drosophila*

protein). However, the *Drosophila* protein is more acidic than the human protein with an isoelectric point of 5.40. Both proteins have their charged residues concentrated within small discrete regions, and these domains are conserved, depicted schematically in Figure 9.

A search of the PROSITE database revealed one potential glycosylation site and several potential phosphorylation sites which are conserved between these proteins. An asparagine residue which fits the consensus for glycosylation (R.D. Marshall, Ann. Rev. Biochem., **41**:673 (1972)) is at position 567 in the *Drosophila* protein and at position 559 in the human protein. At position 324 in both proteins there is a conserved threonine residue with the two required amino terminal basic residues which is potentially phosphorylated by cyclic AMP-dependent protein kinase (J.R. Feramisco, *et al.*, J. Bio. Chem., **255**:4240 (1980); D.B. Glass, *et al.*, Bio. Chem., **261**:2987 (1986)). Also conserved are five sites consisting of a serine residue with an amino acid at the +3 position which fits the consensus sequence for phophorylation by casein kinase II (O. Marin, *et al.*, Eur. J. Biochem., **160**:230 (1986); E.A. Kuenzel, *et al.*, J. Bio. Chem., **262**:9136 (1987)). These serines are at positions 80 and 399 in both proteins, and at positions 443, 472 and 670 in the *Drosophila* protein, equivalent to positions 444, 474, and 672 in the human protein. Protein kinase C requires a basic amino acid two positions away from the phosphorylated serine or threonine residue on the carboxy terminal side of the protein (A. Kishimoto, *et al.*, J. Bio. Chem., **260**:12492 (1985); J.R. Woodgett, *et al.*, Eur. J. Biochem., **161**:177 (1986)). There are seven such sites conserved between these proteins at positions 37, 111, 141, 209, 344, and 385 in both

proteins and at position 636 in the *Drosophila* protein, equivalent to position 627 in the human protein.

Using the BLAST Network Service at the National Center for Biotechnology Information (S.F. Altschul, J. Mol. Biol., 215:403 (1990)), a nonredundant search of protein databases with the predicted *Drosophila* amino acid sequence revealed homologies consistent with the human protein. The DNA structure-specific recognition protein showed homology to HMG-1 and -2 proteins from several species, and to a transcription factor protein (hUBF) which contains an HMG box. As was found for the human protein sequence, the highly charged domains of the protein proved to be homologous to highly charged domains of other proteins, especially the transcriptional regulator nucleolin.

Computer analysis for the presence of potential structural domains was also carried out. For both the human protein and its *Drosophila* homolog, Chou and Fasman analysis of hydropathy (P.Y. Chou and G.D. Fasman, Biochem., 13:211 (1974); (P.Y. Chou, and G.D. Fasman, Ann. Rev. Biochem., 47:251 (1978) predicts the entire carboxy terminal half of the proteins, from aa 400 to the end, to be highly hydrophilic. No major regions of amphiphilicity are apparent in either protein. Comparison of secondary structural predictions for the human protein and its *Drosophila* homolog reveal a number of regions that appear to be helical in both proteins when analyzed either with the method of Chou and Fasman (P.Y. Chou and G.D. Fasman, Biochem., 13:211 (1974); (P.Y. Chou and G.D. Fasman, Ann. Rev. Biochem., 47:251 (1978) or with the method of Robson and Garnier (B. Robson and E. Suzuki, J. Mol. Biol. 107:327 (1976); (J. Garnier, *et al.*, J. Mol. Biol. 120:97 (1978)). Specifically, these regions surround approximately aa75-105, 150-165, 290-300, 405-425, 450-465, 480-495, 525-540, 580-620, and 675-690.

Example W
***In situ* Hybridization Studies of the *Drosophila* SSRP Gene**

In situ Hybridization to Polytene Chromosomes.

Polytene chromosomes were prepared from the salivary glands of third instar larvae as described previously (M. Ashburner, Drosophila: A Laboratory Manual pp. 37-47 (1989)). Nick translation of plasmid DNA containing clone DM 3002 with biotinylated-16-dUTP (ENZO Diagnostics), detection with Streptavidin-biotinylated peroxidase (Detek-1-HRP, ENZO Diagnostics), and hybridization steps were all performed with standard techniques (M. Ashburner, Drosophila: A Laboratory Manual pp. 37-47 (1989)).

The results of this study placed the *Drosophila* clone on the right arm of chromosome 2, in band 60A 1-4. Deficiencies in this region, specifically from 59D4-5; 50A1-2 and 59D8-11; 60A7 produce maternal effect mutations that are female steriles (T. Schupbach and E. Weischaus, Genetics, **121**:101 (1989)). Interestingly, the *egalitarian* gene which also maps to the region, is required for oocyte differentiation (P.F. Lasko and M. Ashburner, Genes and Dev., **4**:905 (1990)). Other mutants which map to the region include *abbreviated* and *forkoid*, which affect bristle formation, and *lanceloated*, which elongates the wing (Diaz-Benjumea and A. Garcia-Bellido, Roux's Arch. Dev. Biol., **198**:336 (1990)). The *Drosophila* guanine nucleotide-binding protein $G_o\alpha$, also maps to position 60A on polytene chromosomes (F. Quan, *et al.*, Proc. Natl. Acad. Sci. USA **86**:4321 (1989)). Recently, a member of the transforming growth factor-β family, denoted the 60A gene, has also been mapped to this region. K.A. Wharton, *et al.*, submitted for publication.

Example Y
EMSA and Modified Western Blotting Studies of Yeast Cell Extracts

Purification of Pt-DNA mobility shift activity. Yeast cells (BJ 296) were grown to late log phase (O.D. 0.8) in 6 L YPD and harvested by centrifugation for 15 minutes at 5000 g. The yield was 60 G wet cells. The pellet was washed once with water and resuspended in 180 mL TM (50 mM Tris-HCl (pH 8) 12.5 mM $MgCl_2$, 1 mM EDTA, 1mM DTT and 20% glycerol) buffer containing 0.1 M KCl, 100 mM PMSF, 10mg/ml leupeptin and 1mg/mL pepstatin. After two passages through a french press at 24,000 psi the lysate was cleared of debris by centrifugation at 14,000 g for 5 minutes. Ammonium sulfate (15.2 g, 25% saturation) was added to the supernatant (190 mL) and the solution was stirred for 30 minutes on ice. Following centrifugation at 14,000 g for 10 minutes, ammonium sulfate (34.2 g, 55% saturation) was added. The precipitated proteins were collected by centrifugation, dissolved in TM buffer 0.1 M KCl, 1 mM PMSF and desalted on a Biorad P-6 size-exclusion column. The resulting solution was loaded onto either a DEAE-sepharose or a S-sepharose column. The columns were washed with TM buffer, 0.1 M KCl and eluted with a TM buffer 0.1 to 1.0 M KCl gradient. Bandshift active fractions from these columns were diluted to 0.1 M KCl with TM buffer, loaded onto a heparin fast-flow* column (BioRad) and eluted with a 0.1-1.0 M KCl gradient.

Bandshift assay (EMSA). 15 µL aliquots of selected fractions from the column chromatography were mixed in solutions containing 10 mM Tris-HCl 10 mM NaCl, 0.5 mM EDTA, 1mM DTT and 20% glycerol, 0.2 µg/mL poly(dIdC) and 1000 cpm of a [^{32}P] end-labeled 123 bp DNA fragment. For platinated samples, the ratio of

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cis-DDP/nucleotide was 0.021. The reactions were incubated at 25°C for 15 minutes, loaded onto 8% TBE polyacrylamide gels and electrophoresed at 4°C. Dried gels were exposed to Kodax X-AR*film. Bandshift activity was quantified using a Molecular Dynamics phosphor-imager.

Modified Western Analysis. Proteins were resolved on SDS-polyacrylamide gels and electroblotted to nitrocellulose filters. The filters were treated with blotto (50 mM Tris HCI pH 7.5, 50 mM NaC1, 1 mM EDTA, 5% nonfat dry milk powder) for 1 hour, washed twice for 10 minutes with TNE 50 (10 mM Tris pH 7.5, 50 mM NaC1, mM EDTA, 1 mM DTT) and denatured (50 mM Tris HC1 pH 8.0, 7 M guanidine HC1, 1 mM EDTA, 50 mM DTT, and 5% (v/v) blotto) for 1 hour. Following overnight renaturation (50 mM Tris HC1 pH 8.0, 50 mM NaC1, 1 mM EDTA, 2 mM DTT, 0.1% Nonidet*P-40 and 5% (v/v) blotto) the filters were washed with 5% dry milk in 20 mM HEPES pH 7.5. The filters were incubated for two hours in 30 mM HEPES, 10 mM $MgCl_2$, 0.25% dry milk, 20 µg/mL poly(dIdC)•poly(dIdC) and 1 x 10^6 cpm/mL [^{32}P] end-labeled probe. Excess probe was removed by washing the filters twice for 10 minutes at 4°C with a 30 mM HEPES, 0.25% dry milk solution.

In an initial experiment to determine conditions required to purify the yeast SSRP protein(s), $(NH_4)_2SO_4$ was added to 25%, 40% and 60% saturation. The proteins precipitating at the various $(NH_4)_2SO_4$ concentrations were analyzed by modified Western (i.e., Southwestern) blotting, and corroborated by EMSA. As assessed by Southwestern blot, an 82 000 dalton protein is present in the yeast whole cell extract, was well as in the 0-25%, 25-40% and 40-60% $(NH_4)_2SO_4$ saturation fractions. This band is apparently absent from the supernatant. In addition, a rather large protein, ~190 kDa, came

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down in the clearing spin, preceding the $(NH_4)_2SO_4$ precipitations. A parallel blot was probed with unmodified DNA; no DNA binding was observed on it. EMSA analysis of the same samples showed that the 40-60% fraction apparently contains the highest mobility-shift activity, but activity is also present in the 25-40% fraction and the supernatant.

Further purification of the bandshift activity was achieved with S-sepharose* chromatography. In one preparation, the 25-60% proteins were redissolved, desalted by dialysis or gel filtration, loaded onto a S-sepharose column, and eluted with a 0.1-1.0 M KCl gradient. It was found that bandshift activity elutes in two peaks with a complex pattern of shifted probe. Samples of the fractions representing the peaks of activity by EMSA were pooled and subjected to modified Western blotting. This study showed an enrichment of two proteins having electrophoretic mobilities consistent with masses of 42 000 and 40 000 daltons.

Example Z
EMSA and Modified Western Blotting Studies of the Polypeptide Encoded by Yeast SSRP Clone λyPt

Fusion Protein Preparation. Stable lysogens of λyPt and λgt11 were prepared in Y1090 E. coli cells. Lysogens were grown in LB at 32°C to OD 0.5 when the temperature was shifted to 42°C for 20 minutes. The β-galactosidase fusion protein was induced by adding to IPTG (10 mM). Two methods were used to harvest total protein: Method A, cells were harvested 1 hour after IPTG induction, by centrifugation and resuspended in 0.01 volumes of TM buffer containing 100 mM PMSF and flash frozen in liquid nitrogen; Method B, 2.0 mL aliquots were harvested by centrifugation at 10 minutes intervals following the IPTG treatment, resuspended in

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SDS-PAGE loading buffer, placed in boiling water for 5 minutes and stored at -80°C. Cell debris was removed by centrifugation from samples prepared by either method immediately prior to SDS-polyacrylamide gel electrophoresis.

The fusion protein produced by the lysogen of λyPt in Y1090 is capable of binding *cis*-DDP modified DNA on a modified Western blot (using essentially the same procedure as discussed in the preceeding Example). The fusion protein was observed to have an electrophoretic mobility consistent with a protein of 180 000 daltons. Since the β-galactosidase portion of this polypeptide accounts for 113 000 daltons, the remaining 63 000 daltons is the expression product of the cloned gene. It should be noted that this fusion protein has proven to have uncertain stability (i.e., Southwestern blotting reveals the presence of multiple reactive bands, presumably arising from proteolysis).

Example AA
Subcloning, Sequencing and Sequence Characterization of Yeast SSRP Clone λyPt

Subcloning and DNA Sequencing. The 1.7, 1.1 and 0.6 kB EcoRI fragments from λyPt were ligated into the EcoRI site of pBluescript*IISK+ yielding plasmids pSB1, pSB2 and pSB3, respectively. Plasmid DNA was alkaline denatured for the sequencing reactions. Double-stranded λyPt DNA was prepared for sequencing by SacI digestion and treatment with T7 gene 6 exonuclease to produce a single-stranded DNA template. Sequencing was performed by the dideoxy chain termination method using sequenase*T7 DNA polymerase (US Biochemical Corp.). Sequence fragments were assembled using the GCG program. (Devereux, Haberli, *et al.*, (1984) Nucleic Acids Research **12**(1):387-395. Nonredundant searches or

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protein and DNA sequence databases were performed with the BLAST network service provided by the National Center for Biotechnology Information (NCBI).

Complete sequencing of λyPt was achieved by sequencing the three subcloned EcoRI fragments identified above. Since EcoRI digestions of λyPt DNA releases three fragments, the cloned DNA apparently contains two internal EcoRI sites (See Figure 10; further details are given below). The yeast genomic DNA contained in λyPt was found to total 3292 bases the sequence of which is shown in Figure 11. An open reading frame, contiguous with the reading frame for the β-galactosidase gene of lamda phage, is found in the λyPt DNA sequence. This reading frame extends from bases 1 to 1626 and is shown in Figure 12. The hexanucleotide polyadenylation signal, AATAAA, found at nucleotides 1632-1637, is present in approximately 50% of *S. cerevisiae* genes (Hyman, L., S.H. Seiler *et al.*, (1991) Mol. Cell. Biol. 11(4):2004-2012).

Translation of the open reading frame found in clone λyPt yields an amino acid sequence of 534 residues. This peptide sequence is herein referred to as fySSRP, for fractional yeast structure specific recognition protein. Examination of the amino acid sequence of fySSRP reveals a striking feature: there are eight runs of five or more glutamines, of which the longest is fifteen. In all, there are 110 glutamine residues, or one fifth of the total. Fifty asparagine residues account for another 9.2% of the amino acids.

Example BB
Expression of the Yeast SSRP Gene

Probe Preparation. The 0.6, 1.1 or 1.7 Kb EcoRI fragments from pSB1, pSB2 or pSB3 were used as templates for probe preparation. Approximately 0.2 μg

of DNA in low melting point agarose was boiled with 0.1 μg $d(N)_6$ oligonucleotides (New England Biolabs), and labeled with α-[^{32}P]dCTP by *E. coli* DNA polymerase I (Klenow fragment). Reactions were stopped by extraction with phenol/chloroform.

Northern Analysis. Total yeast RNA was prepared by the published procedure. (Kohrer, K. and H. Domdey (1991) Guide to Yeast Genetics San Diego, Academic Press Inc. 398-405). RNA MW markers (BRL Inc.) and 10 μg total yeast RNA were subjected to electrophoretic analysis in 0.8% agarose gels containing 6% formaldehyde, 20 mM MOPS, 5 mM NaOAc, and 1 mM EDTA. Gels were denatured for 15 mins. in 50 mM NaOH, 100 mM NaCl, neutralized in 100 mM Tris (pH 7.5), and transferred to nitrocellulose by capillary action in 20X SSC. Filters were baked for two hrs. at 80°C. The filter was prehybridized (50% formamide, 0.1% $NaPO_4$, 50 mM Tris (pH 7.5), 5X Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA) for four hours at 42°C and hybridized overnight in prehybridization solution containing 10% dextran sulfate and with $1x10^6$ cpm/mL of labeled DNA probe. Filters were washed at 55°C twice for 30 mins. with 2X SSC, 0.1% SDS twice, and in 1X SSC, 0.1% SDS and exposed to X-ray film.

Northern blotting analysis established that ySSRP is encoded by a 2.1 kb mRNA species.

Example CC
Southern Blotting Studies of Clone λypt

Southern Analysis. Typically, 10 μg of genomic yeast DNA or lamda DNA were treated with restriction enzymes and the fragments resolved by electrophoresis on 0.8% agarose gels. Gels were treated with 0.2 N HCl for 10 min., denatured for 20 minutes (0.5 M NaOH, 1.5 M NaCl), and neutralized for 40 minutes with two

changes of 1 M Tris pH 7.5, 1.5 M NaCl. The DNA was transferred to nitrocellulose filters (Schleicher and Schuell) by capillary transfer overnight with 20X SSC (i.e., 3 M NaCl, 0.3 M sodium citrate). The filters were baked for 2 hours at 80°C, prehybridized (50% formamide, 5X SSC, 1X Denhardt's solution 1mg/mL denatured calf thymus DNA) for 8 hours at 42°C and hybridized (50% formamide, 5X SSC, 1X Denhardt's solution 1mg/mL denatured calf thymus DNA, 10% dextran sulfate, 1 x 10^6 cpm/mL probe (see the preceeding Example)) overnight. Thereafter, filters were washed twice for 15 minutes with 2X SSC, 0.1% SDS and twice for 15 minutes with 0.5X SSC, 0.1% SDS. Results were visualized by autoradiography.

Southern analysis of λyPt and yeast genomic DNA digested with EcoRI and probed with the 0.6 kB fragment revealed that a 0.6 kB piece is present in both digests. Therefore, the 0.6 kB piece is located in the middle of the cloned DNA. The 0.6 and 1.1 kB EcoRI fragments were oriented to each other by sequencing λyPt DNA. The orientation of the 1.7 kB EcoRI fragment was determined by Southern analysis of yeast genomic DNA digested with PstI and EcoRV, probed with the 0.6 kB EcoRI fragment. A 2.3 kB piece hybridized on this blot, locating the EcoRV restriction site in the 1.7 kB fragment towards the 3′ end of the clone. In the other possible orientation, with the EcoRV site closer to the 5′ end, a 1.2 kB fragment would have been released by DNA digested with PstI and EcoRV.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: US 07/539,906
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Synthetic oligonucleotide

(ix) FEATURE:
(A) NAME/KEY: misc_difference
(B) LOCATION: replace(11..12)
(D) OTHER INFORMATION: /label= Pt-DNA
/note= "cis-(Pt(NH3)3) 1,2-d(GpG) intrastrand
Platinated DNA Structural Motif"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TCTCCTTCTT GGTTCTCTTC TC 22

(2) INFORMATION FOR SEQ ID NO:2:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
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/note= "cis-(Pt(NH3)2) 1,2-d(ApG) intrastrand
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Synthetic oligonucleotide

(ix) FEATURE:
(A) NAME/KEY: misc_difference
(B) LOCATION: replace(11..13)
(D) OTHER INFORMATION: /label= Pt-DNA
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Platinated DNA Structural Motif"

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(2) INFORMATION FOR SEQ ID NO:4:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Synthetic oligonucleotide

(ix) FEATURE:
(A) NAME/KEY: misc_difference
(B) LOCATION: replace(11..13)
(D) OTHER INFORMATION: /label= Pt-DNA
/note= "trans-{Pt(NH3)2} 1,3-d(GpTpG) intrastrand Platinated DNA Structural Motif"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCTCCTTCTT GTGTCTCTTC TC 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Synthetic oligonucleotide

(ix) FEATURE:
(A) NAME/KEY: misc_difference
(B) LOCATION: replace(12)
(D) OTHER INFORMATION: /label= Pt-DNA
/note= "cis-{Pt(NH3)2(N3-cytosine)} dG monofunctional Platinated DNA Structural Motif"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCTCCTTCTT CGTTCTCTTC TC 22

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2839 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

2125673

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: human SSRP - composite of six overlapping cDNA clones

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 11q12

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 275..2404

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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GAATTCCGTA CGGCTTCCGG TGGCGGGACG CGGGGCCGCG CACGCGGGAA AAGCTTCCCC      60

GGTGTCCCCC CATCCCCCTC CCCGCGCCCC CCCCGCGTCC CCCCAGCGCG CCCACCTCTC     120

GCGCCGGGGC CCTCGCGAGG CCGCAGCCTG AGGAGATTCC CAACCTGCTG AGCATCCGCA     180

CACCCACTCA GGAGTTGGGG CCCAGCTCCC AGTTTACTTG GTTTCCCTTG TGCAGCCTGG     240

GGCTCTGCCC AGGCCACCAC AGGCAGGGGT CGAC ATG GCA GAG ACA CTG GAG          292
                                      Met Ala Glu Thr Leu Glu
                                        1               5

TTC AAC GAC GTC TAT CAG GAG GTG AAA GGT TCC ATG AAT GAT GGT CGA        340
Phe Asn Asp Val Tyr Gln Glu Val Lys Gly Ser Met Asn Asp Gly Arg
            10                  15                  20

CTG AGG TTG AGC CGT CAG GGC ATC ATC TTC AAG AAT AGC AAG ACA GGC        388
Leu Arg Leu Ser Arg Gln Gly Ile Ile Phe Lys Asn Ser Lys Thr Gly
        25                  30                  35

AAA GTG GAC AAC ATC CAG GCT GGG GAG TTA ACA GAA GGT ATC TGG CGC        436
Lys Val Asp Asn Ile Gln Ala Gly Glu Leu Thr Glu Gly Ile Trp Arg
    40                  45                  50

CGT GTT GCT CTG GGC CAT GGA CTT AAA CTG CTT ACA AAG AAT GGC CAT        484
Arg Val Ala Leu Gly His Gly Leu Lys Leu Leu Thr Lys Asn Gly His
55                  60                  65                  70

GTC TAC AAG TAT GAT GGC TTC CGA GAA TCG GAG TTT GAG AAA CTC TCT        532
Val Tyr Lys Tyr Asp Gly Phe Arg Glu Ser Glu Phe Glu Lys Leu Ser
                75                  80                  85

GAT TTC TTC AAA ACT CAC TAT CGC CTT GAG CTA ATG GAG AAG GAC CTT        580
Asp Phe Phe Lys Thr His Tyr Arg Leu Glu Leu Met Glu Lys Asp Leu
            90                  95                  100

TGT GTG AAG GGC TGG AAC TGG GGG ACA GTG AAA TTT GGT GGG CAG CTG        628
Cys Val Lys Gly Trp Asn Trp Gly Thr Val Lys Phe Gly Gly Gln Leu
        105                 110                 115

CTT TCC TTT GAC ATT GGT GAC CAG CCA GTC TTT GAG ATA CCC CTC AGC        676
Leu Ser Phe Asp Ile Gly Asp Gln Pro Val Phe Glu Ile Pro Leu Ser
    120                 125                 130

AAT GTG TCC CAG TGC ACC ACA GGC AAG AAT GAG GTG ACA CTG GAA TTC        724
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Asn Val Ser Gln Cys Thr Thr Gly Lys Asn Glu Val Thr Leu Glu Phe
135                 140                 145                 150

CAC CAA AAC GAT GAC GCA GAG GTG TCT CTC ATG GAG GTG CGC TTC TAC      772
His Gln Asn Asp Asp Ala Glu Val Ser Leu Met Glu Val Arg Phe Tyr
                155                 160                 165

GTC CCA CCC ACC CAG GAG GAT GGT GTG GAC CCT GTT GAG GCC TTT GCC      820
Val Pro Pro Thr Gln Glu Asp Gly Val Asp Pro Val Glu Ala Phe Ala
            170                 175                 180

CAG AAT GTG TTG TCA AAG GCG GAT GTA ATC CAG GCC ACG GGA GAT GCC      868
Gln Asn Val Leu Ser Lys Ala Asp Val Ile Gln Ala Thr Gly Asp Ala
        185                 190                 195

ATC TGC ATC TTC CGG GAG CTG CAG TGT CTG ACT CCT CGT GGT CGT TAT      916
Ile Cys Ile Phe Arg Glu Leu Gln Cys Leu Thr Pro Arg Gly Arg Tyr
    200                 205                 210

GAC ATT CGG ATC TAC CCC ACC TTT CTG CAC CTG CAT GGC AAG ACC TTT      964
Asp Ile Arg Ile Tyr Pro Thr Phe Leu His Leu His Gly Lys Thr Phe
215                 220                 225                 230

GAC TAC AAG ATC CCC TAC ACC ACA GTA CTG CGT CTG TTT TTG TTA CCC      1012
Asp Tyr Lys Ile Pro Tyr Thr Thr Val Leu Arg Leu Phe Leu Leu Pro
                235                 240                 245

CAC AAG GAC CAG CGC CAG ATG TTC TTT GTG ATC AGC CTG GAT CCC CCA      1060
His Lys Asp Gln Arg Gln Met Phe Phe Val Ile Ser Leu Asp Pro Pro
            250                 255                 260

ATC AAG CAA GGC CAA ACT CGC TAC CAC TTC CTG ATC CTC CTC TTC TCC      1108
Ile Lys Gln Gly Gln Thr Arg Tyr His Phe Leu Ile Leu Leu Phe Ser
        265                 270                 275

AAG GAC GAG GAC ATT TCG TTG ACT CTG AAC ATG AAC GAG GAA GAA GTG      1156
Lys Asp Glu Asp Ile Ser Leu Thr Leu Asn Met Asn Glu Glu Glu Val
    280                 285                 290

GAG AAG CGC TTT GAG GGT CGG CTC ACC AAG AAC ATG TCA GGA TCC CTC      1204
Glu Lys Arg Phe Glu Gly Arg Leu Thr Lys Asn Met Ser Gly Ser Leu
295                 300                 305                 310

TAT GAG ATG GTC AGC CGG GTC ATG AAA GCA CTG GTA AAC CGC AAG ATC      1252
Tyr Glu Met Val Ser Arg Val Met Lys Ala Leu Val Asn Arg Lys Ile
                315                 320                 325

ACA GTG CCA GGC AAC TTC CAA GGG CAC TCA GGG GCC CAG TGC ATT ACC      1300
Thr Val Pro Gly Asn Phe Gln Gly His Ser Gly Ala Gln Cys Ile Thr
            330                 335                 340

TGT TCC TAC AAG GCA AGC TCA GGA CTG CTC TAC CCG CTG GAG CGG GGC      1348
Cys Ser Tyr Lys Ala Ser Ser Gly Leu Leu Tyr Pro Leu Glu Arg Gly
        345                 350                 355

TTC ATC TAC GTC CAC AAG CCA CCT GTG CAC ATC CGC TTC GAT GAG ATC      1396
Phe Ile Tyr Val His Lys Pro Pro Val His Ile Arg Phe Asp Glu Ile
    360                 365                 370

TCC TTT GTC AAC TTT GCT CGT GGT ACC ACT ACT ACT CGT TCC TTT GAC      1444
Ser Phe Val Asn Phe Ala Arg Gly Thr Thr Thr Thr Arg Ser Phe Asp
375                 380                 385                 390
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2125673


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TTT GAA ATT GAG ACC AAG CAG GGC ACT CAG TAT ACC TTC AGC AGC ATT          1492
Phe Glu Ile Glu Thr Lys Gln Gly Thr Gln Tyr Thr Phe Ser Ser Ile
                395                 400                 405

GAG AGG GAG GAG TAC GGG AAA CTG TTT GAT TTT GTC AAC GCG AAA AAG          1540
Glu Arg Glu Glu Tyr Gly Lys Leu Phe Asp Phe Val Asn Ala Lys Lys
            410                 415                 420

CTC AAC ATC AAA AAC CGA GGA TTG AAA GAG GGC ATG AAC CCA AGC TAC          1588
Leu Asn Ile Lys Asn Arg Gly Leu Lys Glu Gly Met Asn Pro Ser Tyr
        425                 430                 435

GAT GAA TAT GCT GAC TCT GAT GAG GAC CAG CAT GAT GCC TAC TTG GAG          1636
Asp Glu Tyr Ala Asp Ser Asp Glu Asp Gln His Asp Ala Tyr Leu Glu
    440                 445                 450

AGG ATG AAG GAG GAA GGC AAG ATC CGG GAG GAG AAT GCC AAT GAC AGC          1684
Arg Met Lys Glu Glu Gly Lys Ile Arg Glu Glu Asn Ala Asn Asp Ser
455                 460                 465                 470

AGC GAT GAC TCA GGA GAA GAA ACC GAT GAG TCA TTC AAC CCA GGT GAA          1732
Ser Asp Asp Ser Gly Glu Glu Thr Asp Glu Ser Phe Asn Pro Gly Glu
                475                 480                 485

GAG GAG GAA GAT GTG GCA GAG GAG TTT GAC AGC AAC GCC TCT GCC AGC          1780
Glu Glu Glu Asp Val Ala Glu Glu Phe Asp Ser Asn Ala Ser Ala Ser
            490                 495                 500

TCC TCC AGT AAT GAG GGT GAC AGT GAC CGG GAT GAG AAG AAG CGG AAA          1828
Ser Ser Ser Asn Glu Gly Asp Ser Asp Arg Asp Glu Lys Lys Arg Lys
        505                 510                 515

CAG CTC AAA AAG GCC AAG ATG GCC AAG GAC CGC AAG AGC CGC AAG AAG          1876
Gln Leu Lys Lys Ala Lys Met Ala Lys Asp Arg Lys Ser Arg Lys Lys
    520                 525                 530

CCT GTG GAG GTG AAG AAG GGC AAA GAC CCC AAT GCC CCC AAG AGG CCC          1924
Pro Val Glu Val Lys Lys Gly Lys Asp Pro Asn Ala Pro Lys Arg Pro
535                 540                 545                 550

ATG TCT GCA TAC ATG CTG TGG CTC AAT GCC AGC CGA GAG AAG ATC AAG          1972
Met Ser Ala Tyr Met Leu Trp Leu Asn Ala Ser Arg Glu Lys Ile Lys
                555                 560                 565

TCA GAC CAT CCT GGC ATC AGC ATC ACG GAT CTT TCC AAG AAG GCA GGC          2020
Ser Asp His Pro Gly Ile Ser Ile Thr Asp Leu Ser Lys Lys Ala Gly
            570                 575                 580

GAG ATC TGG AAG GGA ATG TCC AAA GAG AAG AAA GAG GAG TGG GAT CGC          2068
Glu Ile Trp Lys Gly Met Ser Lys Glu Lys Lys Glu Glu Trp Asp Arg
        585                 590                 595

AAG GCT GAG GAT GCC AGG AGG GAC TAT GAA AAA GCC ATG AAA GAA TAT          2116
Lys Ala Glu Asp Ala Arg Arg Asp Tyr Glu Lys Ala Met Lys Glu Tyr
    600                 605                 610

GAA GGG GGC CGA GGC GAG TCT TCT AAG AGG GAC AAG TCA AAG AAG AAG          2164
Glu Gly Gly Arg Gly Glu Ser Ser Lys Arg Asp Lys Ser Lys Lys Lys
615                 620                 625                 630

AAG AAA GTA AAG GTA AAG ATG GAA AAG AAA TCC ACG CCC TCT AGG GGC          2212
Lys Lys Val Lys Val Lys Met Glu Lys Lys Ser Thr Pro Ser Arg Gly
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635 640 645

TCA TCA TCC AAG TCG TCC TCA AGG CAG CTA AGC GAG AGC TTC AAG AGC 2260
Ser Ser Ser Lys Ser Ser Ser Arg Gln Leu Ser Glu Ser Phe Lys Ser
650 655 660

AAA GAG TTT GTG TCT AGT GAT GAG AGC TCT TCG GGA GAG AAC AAG AGC 2308
Lys Glu Phe Val Ser Ser Asp Glu Ser Ser Ser Gly Glu Asn Lys Ser
665 670 675

AAA AAG AAG AGG AGG AGG AGC GAG GAC TCT GAA GAA GAA GAA CTA GCC 2356
Lys Lys Lys Arg Arg Arg Ser Glu Asp Ser Glu Glu Glu Glu Leu Ala
680 685 690

AGT ACT CCC CCC AGC TCA GAG GAC TCA GCG TCA GGA TCC GAT GAG TAGAAACGGA 2411
Ser Thr Pro Pro Ser Ser Glu Asp Ser Ala Ser Gly Ser Asp Glu
695 700 705 710

GGAAGGTTCT CTTTGCGCTT GCCTTCTCAC ACCCCCCGAC TCCCCACCCA TATTTTGGTA 2471

CCAGTTTCTC CTCATGAAAT GCAGTCCCTG GATTCTGTGC CATCTGAACA TGCTCTCCTG 2531

TTGGTGTGTA TGTCACTAGG GCAGTGGGGA GACGTCTTAA CTCTGCTGCT TCCCAAGGAT 2591

GGCTGTTTAT AATTTGGGGA GAGATAGGGT GGGAGGCAGG GCAATGCAGG ATCCAAATCC 2651

TCATCTTACT TTCCCGACCT TAAGGATGTA GCTGCTGCTT GTCCTGTTCA AGTTGCTGGA 2711

GCAGGGGTCA TGTGAGGCCA GGCCTGTAGC TCCTACCTGG GGCCTATTTC TACTTTCATT 2771

TTGTATTTCT GGTCTGTGAA AATGATTTAA TAAAGGGAAC TGACTTTGGA AACCAAAAAA 2831

AGGAATTC 2839

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 709 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: human SSRP (predicted)

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 440..496
(D) OTHER INFORMATION: /label= Acidic

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 512..534
(D) OTHER INFORMATION: /label= Basic I

(ix) FEATURE:

(A) NAME/KEY: Domain
(B) LOCATION: 539..614
(D) OTHER INFORMATION: /label= HMG-box

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 623..640
(D) OTHER INFORMATION: /label= Basic II

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 661..709
(D) OTHER INFORMATION: /label= Mixed Charge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ala Glu Thr Leu Glu Phe Asn Asp Val Tyr Gln Glu Val Lys Gly
1 5 10 15

Ser Met Asn Asp Gly Arg Leu Arg Leu Ser Arg Gln Gly Ile Ile Phe
20 25 30

Lys Asn Ser Lys Thr Gly Lys Val Asp Asn Ile Gln Ala Gly Glu Leu
35 40 45

Thr Glu Gly Ile Trp Arg Arg Val Ala Leu Gly His Gly Leu Lys Leu
50 55 60

Leu Thr Lys Asn Gly His Val Tyr Lys Tyr Asp Gly Phe Arg Glu Ser
65 70 75 80

Glu Phe Glu Lys Leu Ser Asp Phe Phe Lys Thr His Tyr Arg Leu Glu
85 90 95

Leu Met Glu Lys Asp Leu Cys Val Lys Gly Trp Asn Trp Gly Thr Val
100 105 110

Lys Phe Gly Gly Gln Leu Leu Ser Phe Asp Ile Gly Asp Gln Pro Val
115 120 125

Phe Glu Ile Pro Leu Ser Asn Val Ser Gln Cys Thr Thr Gly Lys Asn
130 135 140

Glu Val Thr Leu Glu Phe His Gln Asn Asp Asp Ala Glu Val Ser Leu
145 150 155 160

Met Glu Val Arg Phe Tyr Val Pro Pro Thr Gln Glu Asp Gly Val Asp
165 170 175

Pro Val Glu Ala Phe Ala Gln Asn Val Leu Ser Lys Ala Asp Val Ile
180 185 190

Gln Ala Thr Gly Asp Ala Ile Cys Ile Phe Arg Glu Leu Gln Cys Leu
195 200 205

Thr Pro Arg Gly Arg Tyr Asp Ile Arg Ile Tyr Pro Thr Phe Leu His
210 215 220

Leu His Gly Lys Thr Phe Asp Tyr Lys Ile Pro Tyr Thr Thr Val Leu
225 230 235 240

Arg Leu Phe Leu Leu Pro His Lys Asp Gln Arg Gln Met Phe Phe Val
245 250 255

Ile Ser Leu Asp Pro Pro Ile Lys Gln Gly Gln Thr Arg Tyr His Phe
260 265 270

Leu Ile Leu Leu Phe Ser Lys Asp Glu Asp Ile Ser Leu Thr Leu Asn
275 280 285

Met Asn Glu Glu Glu Val Glu Lys Arg Phe Glu Gly Arg Leu Thr Lys
290 295 300

Asn Met Ser Gly Ser Leu Tyr Glu Met Val Ser Arg Val Met Lys Ala
305 310 315 320

Leu Val Asn Arg Lys Ile Thr Val Pro Gly Asn Phe Gln Gly His Ser
325 330 335

Gly Ala Gln Cys Ile Thr Cys Ser Tyr Lys Ala Ser Ser Gly Leu Leu
340 345 350

Tyr Pro Leu Glu Arg Gly Phe Ile Tyr Val His Lys Pro Pro Val His
355 360 365

Ile Arg Phe Asp Glu Ile Ser Phe Val Asn Phe Ala Arg Gly Thr Thr
370 375 380

Thr Thr Arg Ser Phe Asp Phe Glu Ile Glu Thr Lys Gln Gly Thr Gln
385 390 395 400

Tyr Thr Phe Ser Ser Ile Glu Arg Glu Glu Tyr Gly Lys Leu Phe Asp
405 410 415

Phe Val Asn Ala Lys Lys Leu Asn Ile Lys Asn Arg Gly Leu Lys Glu
420 425 430

Gly Met Asn Pro Ser Tyr Asp Glu Tyr Ala Asp Ser Asp Glu Asp Gln
435 440 445

His Asp Ala Tyr Leu Glu Arg Met Lys Glu Glu Gly Lys Ile Arg Glu
450 455 460

Glu Asn Ala Asn Asp Ser Ser Asp Asp Ser Gly Glu Glu Thr Asp Glu
465 470 475 480

Ser Phe Asn Pro Gly Glu Glu Glu Glu Asp Val Ala Glu Glu Phe Asp
485 490 495

Ser Asn Ala Ser Ala Ser Ser Ser Ser Asn Glu Gly Asp Ser Asp Arg
500 505 510

Asp Glu Lys Lys Arg Lys Gln Leu Lys Lys Ala Lys Met Ala Lys Asp
515 520 525

Arg Lys Ser Arg Lys Lys Pro Val Glu Val Lys Lys Gly Lys Asp Pro
530 535 540

Asn Ala Pro Lys Arg Pro Met Ser Ala Tyr Met Leu Trp Leu Asn Ala
545 550 555 560

Ser Arg Glu Lys Ile Lys Ser Asp His Pro Gly Ile Ser Ile Thr Asp
565 570 575

Leu Ser Lys Lys Ala Gly Glu Ile Trp Lys Gly Met Ser Lys Glu Lys
580 585 590

Lys Glu Glu Trp Asp Arg Lys Ala Glu Asp Ala Arg Arg Asp Tyr Glu
595 600 605

Lys Ala Met Lys Glu Tyr Glu Gly Gly Arg Gly Glu Ser Ser Lys Arg
610 615 620

Asp Lys Ser Lys Lys Lys Lys Lys Val Lys Val Lys Met Glu Lys Lys
625 630 635 640

Ser Thr Pro Ser Arg Gly Ser Ser Ser Lys Ser Ser Ser Arg Gln Leu
645 650 655

Ser Glu Ser Phe Lys Ser Lys Glu Phe Val Ser Ser Asp Glu Ser Ser
660 665 670

Ser Gly Glu Asn Lys Ser Lys Lys Lys Arg Arg Arg Ser Glu Asp Ser
675 680 685

Glu Glu Glu Glu Leu Ala Ser Thr Pro Pro Ser Ser Glu Asp Ser Ala
690 695 700

Ser Gly Ser Asp Glu
705

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1898 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Human B cell
(B) CLONE: lambda-Pt1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAATTCCACC AAAACGATGA CGCAGAGGTG TCTCTCATGG AGGTGCGCTT CTACGTCCCA 60

CCCACCCAGG AGGATGGTGT GGACCCTGTT GAGGCCTTTG CCCAGAATGT GTTGTCAAAG 120

GCGGATGTAA TCCAGGCCAC GGGAGATGCC ATCTGCATCT TCCGGGAGCT GCAGTGTCTG 180

ACTCCTCGTG GTCGTTATGA CATTCGGATC TACCCCACCT TTCTGCACCT GCATGGCAAG 240

ACCTTTGACT ACAAGATCCC CTACACCACA GTACTGCGTC TGTTTTTGTT ACCCCACAAG 300

GACCAGCGCC AGATGTTCTT TGTGATCAGC CTGGATCCCC CAATCAAGCA AGGCCAAACT 360

CGCTACCACT TCCTGATCCT CCTCTTCTCC AAGGACGAGG ACATTTCGTT GACTCTGAAC 420

ATGAACGAGG AAGAAGTGGA GAAGCGCTTT GAGGGTCGGC TCACCAAGAA CATGTCAGGA 480

TCCCTCTATG AGATGGTCAG CCGGGTCATG AAAGCACTGG TAAACCGCAA GATCACAGTG 540

CCAGGCAACT TCCAAGGGCA CTCAGGGGCC CAGTGCATTA CCTGTTCCTA CAAGGCAAGC 600

TCAGGACTGC TCTACCCGCT GGAGCGGGGC TTCATCTACG TCCACAAGCC ACCTGTGCAC 660

ATCCGCTTCG ATGAGATCTC CTTTGTCAAC TTTGCTCGTG GTACCACTAC TACTCGTTCC 720

TTTGACTTTG AAATTGAGAC CAAGCAGGGC ACTCAGTATA CCTTCAGCAG CATTGAGAGG 780

GAGGAGTACG GGAAACTGTT TGATTTTGTC AACGCGAAAA AGCTCAACAT CAAAAACCGA 840

GGATTGAAAG AGGGCATGAA CCCAAGCTAC GATGAATATG CTGACTCTGA TGAGGACCAG 900

CATGATGCCT ACTTGGAGAG GATGAAGGAG GAAGGCAAGA TCCGGGAGGA GAATGCCAAT 960

GACAGCAGCG ATGACTCAGG AGAAGAAACC GATGAGTCAT TCAACCCAGG TGAAGAGGAG 1020

GAAGATGTGG CAGAGGAGTT TGACAGCAAC GCCTCTGCCA GCTCCTCCAG TAATGAGGGT 1080

GACAGTGACC GGGATGAGAA GAAGCGGAAA CAGCTCAAAA AGGCCAAGAT GGCCAAGGAC 1140

CGCAAGAGCC GCAAGAAGCC TGTGGAGGTG AAGAAGGGCA AAGACCCCAA TGCCCCCAAG 1200

AGGCCCATGT CTGCATACAT GCTGTGGCTC AATGCCAGCC GAGAGAAGAT CAAGTCAGAC 1260

CATCCTGGCA TCAGCATCAC GGATCTTTCC AAGAAGGCAG GCGAGATCTG GAAGGGAATG 1320

TCCAAAGAGA AGAAAGAGGA GTGGGATCGC AAGGCTGAGG ATGCCAGGAG GGACTATGAA 1380

AAAGCCATGA AAGAATATGA AGGGGGCCGA GGCGAGTCTT CTAAGAGGGA CAAGTCAAAG 1440

AAGAAGAAGA AAGTAAAGGT AAAGATGGAA AAGAAATCCA CGCCCTCTAG GGGCTCATCA 1500

TCCAAGTCGT CCTCAAGGCA GCTAAGCGAG AGCTTCAAGA GCAAAGAGTT TGTGTCTAGT 1560

GATGAGAGCT CTTCGGGAGA GAACAAGAGC AAAAAGAAGA GGAGGAGGAG CGAGGACTCT 1620

GAAGAAGAAG AACTAGCCAG TACTCCCCCC AGCTCAGAGG ACTCAGCGTC AGGATCCGAT 1680

GAGTAGAAAC GGAGGAAGGT TCTCTTTGCG CTTGCCTTCT CACACCCCCC GACTCCCCAC 1740

CCATATTTTG GTACCAGTTT CTCCTCATGA AATGCAGTCC CTGGATTCTG TGCCATCTGA 1800

ACATGCTCTC CTGTTGGTGT GTATGTCACT AGGGCAGTGG GGAGACGTCT TAACTCTGCT 1860

GCTTCCCAAG GATGGCTGTT TATAATTTGG GGAGAGAT 1898

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1444 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Human B cell
(B) CLONE: lambda Pt2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAATTCCACC AAAACGATGA CGCAGAGGTG TCTCTCATGG AGGTGCGCTT CTACGTCCCA 60

CCCACCCAGG AGGATGGTGT GGACCCTGTT GAGGCCTTTG CCCAGAATGT GTTGTCAAAG 120

GCGGATGTAA TCCAGGCCAC GGGAGATGCC ATCTGCATCT TCCGGGAGCT GCAGTGTCTG 180

ACTCCTCGTG GTCGTTATGA CATTCGGATC TACCCCACCT TTCTGCACCT GCATGGCAAG 240

ACCTTTGACT ACAAGATCCC CTACACCACA GTACTGCGTC TGTTTTTGTT ACCCCACAAG 300

GACCAGCGCC AGATGTTCTT TGTGATCAGC CTGGATCCCC CAATCAAGCA AGGCCAAACT 360

CGCTACCACT TCCTGATCCT CCTCTTCTCC AAGGACGAGG ACATTTCGTT GACTCTGAAC 420

ATGAACGAGG AAGAAGTGGA GAAGCGCTTT GAGGGTCGGC TCACCAAGAA CATGTCAGGA 480

TCCCTCTATG AGATGGTCAG CCGGGTCATG AAAGCACTGG TAAACCGCAA GATCACAGTG 540

CCAGGCAACT TCCAAGGGCA CTCAGGGGCC CAGTGCATTA CCTGTTCCTA CAAGGCAAGC 600

TCAGGACTGC TCTACCCGCT GGAGCGGGGC TTCATCTACG TCCACAAGCC ACCTGTGCAC 660

ATCCGCTTCG ATGAGATCTC CTTTGTCAAC TTTGCTCGTG GTACCACTAC TACTCGTTCC 720

TTTGACTTTG AAATTGAGAC CAAGCAGGGC ACTCAGTATA CCTTCAGCAG CATTGAGAGG 780

GAGGAGTACG GGAAACTGTT TGATTTTGTC AACGCGAAAA AGCTCAACAT CAAAAACCGA 840

GGATTGAAAG AGGGCATGAA CCCAAGCTAC GATGAATATG CTGACTCTGA TGAGGACCAG 900

CATGATGCCT ACTTGGAGAG GATGAAGGAG GAAGGCAAGA TCCGGGAGGA GAATGCCAAT 960

GACAGCAGCG ATGACTCAGG AGAAGAAACC GATGAGTCAT TCAACCCAGG TGAAGAGGAG 1020

GAAGATGTGG CAGAGGAGTT TGACAGCAAC GCCTCTGCCA GCTCCTCCAG TAATGAGGGT 1080

GACAGTGACC GGGATGAGAA GAAGCGGAAA CAGCTCAAAA AGGCCAAGAT GGCCAAGGAC 1140

CGCAAGAGCC GCAAGAAGCC TGTGGAGGTG AAGAAGGGCA AAGACCCCAA TGCCCCCAAG 1200

AGGCCCATGT CTGCATACAT GCTGTGGCTC AATGCCAGCC GAGAGAAGAT CAAGTCAGAC 1260

CATCCTGGCA TCAGCATCAC GGATCTTTCC AAGAAGGCAG GCGAGATCTG GAAGGGAATG 1320

TCCAAAGAGA AGAAAGAGGA GTGGGATCGC AAGGCTGAGG ATGCCAGGAG GGACTATGAA 1380

AAAGCCATGA AAGAATATGA AGGGGGCCGA GGCGAGTCTT CTAAGAGGGA CAAGTCAAAG 1440

AAGA 1444

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2384 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Drosophila melanogaster

(vii) IMMEDIATE SOURCE:
(B) CLONE: Drosophila SSRP - composite sequence

(viii) POSITION IN GENOME:
(A) CHROMOSOME/SEGMENT: 2
(B) MAP POSITION: 60A 1-4

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 123..2291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
GAATTCCGCG CGCAGTGTTG TTTTGTGTCT GCCGGAATTA TTGTAAATTG GTGACAATTT      60

CGCAAGGCGG CGTAATACAT AGTTGATCTA TTATCTTGTT ACTGGAGAGG AAGAAGTGCA     120

GG ATG ACA GAC TCT CTG GAG TAC AAC GAC ATA AAC GCC GAA GTG CGC         167
   Met Thr Asp Ser Leu Glu Tyr Asn Asp Ile Asn Ala Glu Val Arg
     1               5                  10                  15

GGA GTC TTG TGT TCC GGA CGC CTA AAG ATG ACC GAG CAG AAC ATC ATC       215
Gly Val Leu Cys Ser Gly Arg Leu Lys Met Thr Glu Gln Asn Ile Ile
                 20                  25                  30

TTC AAG AAC ACC AAG ACC GGC AAG GTG GAG CAG ATC TCG GCA GAG GAC       263
Phe Lys Asn Thr Lys Thr Gly Lys Val Glu Gln Ile Ser Ala Glu Asp
             35                  40                  45

ATA GAC CTG ATC AAT TCG CAG AAG TTC GTG GGC ACC TGG GGA CTG AGG       311
Ile Asp Leu Ile Asn Ser Gln Lys Phe Val Gly Thr Trp Gly Leu Arg
         50                  55                  60

GTG TTC ACC AAA GGC GGC GTG CTC CAC CGC TTC ACC GGA TTC CGC GAC       359
Val Phe Thr Lys Gly Gly Val Leu His Arg Phe Thr Gly Phe Arg Asp
     65                  70                  75

AGC GAG CAC GAG AAG CTG GGC AAG TTT ATC AAG GCT GCC TAC TCG CAG       407
Ser Glu His Glu Lys Leu Gly Lys Phe Ile Lys Ala Ala Tyr Ser Gln
 80                  85                  90                  95

GAG ATG GTC GAG AAG GAG ATG TGC GTC AAG GGC TGG AAC TGG GGC ACC       455
Glu Met Val Glu Lys Glu Met Cys Val Lys Gly Trp Asn Trp Gly Thr
                100                 105                 110

GCC CGC TTC ATG GGC TCC GTC CTG AGC TTC GAC AAG GAG TCG AAG ACC       503
Ala Arg Phe Met Gly Ser Val Leu Ser Phe Asp Lys Glu Ser Lys Thr
            115                 120                 125

ATC TTC GAG GTG CCG CTG TCG CAC GTT TCG CAG TGC GTG ACC GGC AAG       551
Ile Phe Glu Val Pro Leu Ser His Val Ser Gln Cys Val Thr Gly Lys
        130                 135                 140

AAC GAG GTC ACC CTG GAG TTC CAC CAA AAC GAC GAT GCG CCC GTG GGT       599
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Asn Glu Val Thr Leu Glu Phe His Gln Asn Asp Asp Ala Pro Val Gly
145                 150                 155

CTA CTG GAG ATG CGG TTC CAC ATA CCC GCC GTG GAG TCG GCC GAG GAG      647
Leu Leu Glu Met Arg Phe His Ile Pro Ala Val Glu Ser Ala Glu Glu
160                 165                 170                 175

GAT CCG GTA GAC AAG TTC CAC CAG AAC GTA ATG AGC AAG GCC TCG GTC      695
Asp Pro Val Asp Lys Phe His Gln Asn Val Met Ser Lys Ala Ser Val
                180                 185                 190

ATC TCG GCT TCG GGC GAG TCC ATC GCC ATT TTC AGA GAG ATC CAG ATC      743
Ile Ser Ala Ser Gly Glu Ser Ile Ala Ile Phe Arg Glu Ile Gln Ile
            195                 200                 205

CTC ACG CCT CGC GGT CGC TAT GAC ATC AAG ATC TTC TCG ACC TTC TTC      791
Leu Thr Pro Arg Gly Arg Tyr Asp Ile Lys Ile Phe Ser Thr Phe Phe
        210                 215                 220

CAG CTG CAC GGC AAG ACG TTC GAC TAC AAG ATT CCC ATG GAC TCG GTG      839
Gln Leu His Gly Lys Thr Phe Asp Tyr Lys Ile Pro Met Asp Ser Val
225                 230                 235

CTG CGG CTC TTC ATG CTG CCC CAC AAA GAC AGT CGA CAG ATG TTC TTT      887
Leu Arg Leu Phe Met Leu Pro His Lys Asp Ser Arg Gln Met Phe Phe
240                 245                 250                 255

GTG CTC TCC TTG GAT CCG CCC ATC AAG CAG GGA CAA ACG CGT TAC CAC      935
Val Leu Ser Leu Asp Pro Pro Ile Lys Gln Gly Gln Thr Arg Tyr His
                260                 265                 270

TAC CTG GTC CTG CTG TTT GCT CCC GAT GAG GAG ACC ACC ATT GAG CTG      983
Tyr Leu Val Leu Leu Phe Ala Pro Asp Glu Glu Thr Thr Ile Glu Leu
            275                 280                 285

CCA TTC TCG GAA GCC GAG TTG CGA GAC AAG TAC GAG GGC AAG CTG GAG     1031
Pro Phe Ser Glu Ala Glu Leu Arg Asp Lys Tyr Glu Gly Lys Leu Glu
        290                 295                 300

AAA GAG ATC TCC GGG CCG GTG TAC GAG GTG ATG GGC AAA GTG ATG AAG     1079
Lys Glu Ile Ser Gly Pro Val Tyr Glu Val Met Gly Lys Val Met Lys
305                 310                 315

GTG CTG ATC GGT CGA AAA ATT ACC GGA CCC GGT AAC TTT ATC GGA CAC     1127
Val Leu Ile Gly Arg Lys Ile Thr Gly Pro Gly Asn Phe Ile Gly His
320                 325                 330                 335

TCT GGC ACG GCT GCA GTG GGC TGC TCG TTC AAG GCT GCA GCT GGA TAT     1175
Ser Gly Thr Ala Ala Val Gly Cys Ser Phe Lys Ala Ala Ala Gly Tyr
                340                 345                 350

CTG TAT CCC CTG GAG CGA GGA TTC ATC TAT ATC CAC AAG CCA CCG CTG     1223
Leu Tyr Pro Leu Glu Arg Gly Phe Ile Tyr Ile His Lys Pro Pro Leu
            355                 360                 365

CAT ATC CGC TTT GAG GAG ATT AGT TCT GTG AAC TTT GCC CGC AGC GGC     1271
His Ile Arg Phe Glu Glu Ile Ser Ser Val Asn Phe Ala Arg Ser Gly
        370                 375                 380

GGA TCC ACG CGA TCT TTC GAC TTC GAA GTG ACG CTC AAG AAC GGA ACT     1319
Gly Ser Thr Arg Ser Phe Asp Phe Glu Val Thr Leu Lys Asn Gly Thr
385                 390                 395
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GTT CAC ATC TTC TCC TCC ATC GAG AAG GAG GAG TAT GCC AAG CTC TTC          1367
Val His Ile Phe Ser Ser Ile Glu Lys Glu Glu Tyr Ala Lys Leu Phe
400                 405                 410                 415

GAC TAC ATC ACA CAG AAG AAG TTG CAT GTC AGC AAC ATG GGC AAG GAC          1415
Asp Tyr Ile Thr Gln Lys Lys Leu His Val Ser Asn Met Gly Lys Asp
                420                 425                 430

AAG AGC GGC TAC AAG GAC GTG GAC TTT GGT GAT TCG GAC AAC GAG AAC          1463
Lys Ser Gly Tyr Lys Asp Val Asp Phe Gly Asp Ser Asp Asn Glu Asn
            435                 440                 445

GAA CCA GAT GCC TAT CTG GCT CGC CTC AAG GCT GAG GCG AGG GAA AAG          1511
Glu Pro Asp Ala Tyr Leu Ala Arg Leu Lys Ala Glu Ala Arg Glu Lys
        450                 455                 460

GAG GAG GAC GAC GAC GAT GGC GAC TCG GAT GAA GAG TCC ACG GAT GAG          1559
Glu Glu Asp Asp Asp Asp Gly Asp Ser Asp Glu Glu Ser Thr Asp Glu
    465                 470                 475

GAC TTC AAG CCC AAC GAG AAC GAG TCC GAT GTG GCC GAG GAG TAT GAC          1607
Asp Phe Lys Pro Asn Glu Asn Glu Ser Asp Val Ala Glu Glu Tyr Asp
480                 485                 490                 495

AGC AAC GTG GAG AGT GAT TCG GAC GAT GAC AGC GAT GCT AGT GGC GGC          1655
Ser Asn Val Glu Ser Asp Ser Asp Asp Asp Ser Asp Ala Ser Gly Gly
                500                 505                 510

GGA GGC GAC AGC GAC GGC GCC AAG AAA AAG AAG GAG AAG AAG TCC GAG          1703
Gly Gly Asp Ser Asp Gly Ala Lys Lys Lys Lys Glu Lys Lys Ser Glu
            515                 520                 525

AAG AAA GAG AAA AAG GAG AAA AAA CAC AAG GAG AAG GAG AGA ACA AAG          1751
Lys Lys Glu Lys Lys Glu Lys Lys His Lys Glu Lys Glu Arg Thr Lys
        530                 535                 540

AAA CCC TCC AAG AAG AAG AAG GAC TCT GGC AAA CCC AAG CGC GCC ACC          1799
Lys Pro Ser Lys Lys Lys Lys Asp Ser Gly Lys Pro Lys Arg Ala Thr
    545                 550                 555

ACC GCT TTC ATG CTC TGG CTG AAC GAC ACG CGC GAG AGC ATC AAG AGG          1847
Thr Ala Phe Met Leu Trp Leu Asn Asp Thr Arg Glu Ser Ile Lys Arg
560                 565                 570                 575

GAA AAT CCG GGC ATA AAG GTT ACC GAG ATC GCC AAG AAG GGC GGC GAG          1895
Glu Asn Pro Gly Ile Lys Val Thr Glu Ile Ala Lys Lys Gly Gly Glu
                580                 585                 590

ATG TGG AAG GAG CTG AAG GAC AAG TCC AAG TGG GAG GAT GCG GCG GCC          1943
Met Trp Lys Glu Leu Lys Asp Lys Ser Lys Trp Glu Asp Ala Ala Ala
            595                 600                 605

AAG GAC AAG CAG CGC TAC CAC GAC GAG ATG CGC AAC TAC AAG CCT GAA          1991
Lys Asp Lys Gln Arg Tyr His Asp Glu Met Arg Asn Tyr Lys Pro Glu
        610                 615                 620

GCG GGC GGT GAC AGC GAC AAC GAG AAG GGT GGA AAG TCC TCC AAG AAG          2039
Ala Gly Gly Asp Ser Asp Asn Glu Lys Gly Gly Lys Ser Ser Lys Lys
    625                 630                 635

CGC AAG ACG GAG CCT TCT CCA TCC AAG AAG GCG AAT ACC TCG GGC AGC          2087
Arg Lys Thr Glu Pro Ser Pro Ser Lys Lys Ala Asn Thr Ser Gly Ser
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640                 645                 650                 655

GGC TTC AAG AGC AAG GAG TAC ATT TCG GAC GAC GAC TCC ACC AGC TCC      2135
Gly Phe Lys Ser Lys Glu Tyr Ile Ser Asp Asp Asp Ser Thr Ser Ser
                660                 665                 670

GAC GAC GAG AAG GAC AAC GAG CCT GCC AAG AAG AAG AGC AAG CCC CCA      2183
Asp Asp Glu Lys Asp Asn Glu Pro Ala Lys Lys Lys Ser Lys Pro Pro
            675                 680                 685

TCC GAC GGC GAT GCC AAG AAG AAA AAG GCC AAG AGC GAG AGC GAA CCG      2231
Ser Asp Gly Asp Ala Lys Lys Lys Lys Ala Lys Ser Glu Ser Glu Pro
        690                 695                 700

GAG GAG AGC GAG GAG GAC AGC AAT GCC AGC GAT GAG GAT GAG GAA GAT      2279
Glu Glu Ser Glu Glu Asp Ser Asn Ala Ser Asp Glu Asp Glu Glu Asp
    705                 710                 715

GAG GCC AGT GAT TAGGGCCATA AACACAACAA ATCAATTCCA TAAACACACA          2331
Glu Ala Ser Asp
720

CCACGCTCCT CACACACCCA TGTCCCAAAT CTAGTTTACA TTCGCCGGAA TTC           2384
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 723 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Drosophila melanogaster

(vii) IMMEDIATE SOURCE:
(B) CLONE: Drosophila SSRP (predicted)

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 458..507
(D) OTHER INFORMATION: /label= Acidic

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 518..547
(D) OTHER INFORMATION: /label= Basic I

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 547..620
(D) OTHER INFORMATION: /label= HMG-box

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 632..649
(D) OTHER INFORMATION: /label= Basic II

(ix) FEATURE:

(A) NAME/KEY: Domain
(B) LOCATION: 657..723
(D) OTHER INFORMATION: /label= Mixed Charge

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Thr Asp Ser Leu Glu Tyr Asn Asp Ile Asn Ala Glu Val Arg Gly
1 5 10 15

Val Leu Cys Ser Gly Arg Leu Lys Met Thr Glu Gln Asn Ile Ile Phe
20 25 30

Lys Asn Thr Lys Thr Gly Lys Val Glu Gln Ile Ser Ala Glu Asp Ile
35 40 45

Asp Leu Ile Asn Ser Gln Lys Phe Val Gly Thr Trp Gly Leu Arg Val
50 55 60

Phe Thr Lys Gly Gly Val Leu His Arg Phe Thr Gly Phe Arg Asp Ser
65 70 75 80

Glu His Glu Lys Leu Gly Lys Phe Ile Lys Ala Ala Tyr Ser Gln Glu
85 90 95

Met Val Glu Lys Glu Met Cys Val Lys Gly Trp Asn Trp Gly Thr Ala
100 105 110

Arg Phe Met Gly Ser Val Leu Ser Phe Asp Lys Glu Ser Lys Thr Ile
115 120 125

Phe Glu Val Pro Leu Ser His Val Ser Gln Cys Val Thr Gly Lys Asn
130 135 140

Glu Val Thr Leu Glu Phe His Gln Asn Asp Asp Ala Pro Val Gly Leu
145 150 155 160

Leu Glu Met Arg Phe His Ile Pro Ala Val Glu Ser Ala Glu Glu Asp
165 170 175

Pro Val Asp Lys Phe His Gln Asn Val Met Ser Lys Ala Ser Val Ile
180 185 190

Ser Ala Ser Gly Glu Ser Ile Ala Ile Phe Arg Glu Ile Gln Ile Leu
195 200 205

Thr Pro Arg Gly Arg Tyr Asp Ile Lys Ile Phe Ser Thr Phe Phe Gln
210 215 220

Leu His Gly Lys Thr Phe Asp Tyr Lys Ile Pro Met Asp Ser Val Leu
225 230 235 240

Arg Leu Phe Met Leu Pro His Lys Asp Ser Arg Gln Met Phe Phe Val
245 250 255

Leu Ser Leu Asp Pro Pro Ile Lys Gln Gly Gln Thr Arg Tyr His Tyr
260 265 270

Leu Val Leu Leu Phe Ala Pro Asp Glu Glu Thr Thr Ile Glu Leu Pro
275 280 285

Phe Ser Glu Ala Glu Leu Arg Asp Lys Tyr Glu Gly Lys Leu Glu Lys

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290 295 300

Glu Ile Ser Gly Pro Val Tyr Glu Val Met Gly Lys Val Met Lys Val
305 310 315 320

Leu Ile Gly Arg Lys Ile Thr Gly Pro Gly Asn Phe Ile Gly His Ser
325 330 335

Gly Thr Ala Ala Val Gly Cys Ser Phe Lys Ala Ala Ala Gly Tyr Leu
340 345 350

Tyr Pro Leu Glu Arg Gly Phe Ile Tyr Ile His Lys Pro Pro Leu His
355 360 365

Ile Arg Phe Glu Glu Ile Ser Ser Val Asn Phe Ala Arg Ser Gly Gly
370 375 380

Ser Thr Arg Ser Phe Asp Phe Glu Val Thr Leu Lys Asn Gly Thr Val
385 390 395 400

His Ile Phe Ser Ser Ile Glu Lys Glu Glu Tyr Ala Lys Leu Phe Asp
405 410 415

Tyr Ile Thr Gln Lys Lys Leu His Val Ser Asn Met Gly Lys Asp Lys
420 425 430

Ser Gly Tyr Lys Asp Val Asp Phe Gly Asp Ser Asp Asn Glu Asn Glu
435 440 445

Pro Asp Ala Tyr Leu Ala Arg Leu Lys Ala Glu Ala Arg Glu Lys Glu
450 455 460

Glu Asp Asp Asp Asp Gly Asp Ser Asp Glu Glu Ser Thr Asp Glu Asp
465 470 475 480

Phe Lys Pro Asn Glu Asn Glu Ser Asp Val Ala Glu Glu Tyr Asp Ser
485 490 495

Asn Val Glu Ser Asp Ser Asp Asp Asp Ser Asp Ala Ser Gly Gly Gly
500 505 510

Gly Asp Ser Asp Gly Ala Lys Lys Lys Lys Glu Lys Lys Ser Glu Lys
515 520 525

Lys Glu Lys Lys Glu Lys Lys His Lys Glu Lys Glu Arg Thr Lys Lys
530 535 540

Pro Ser Lys Lys Lys Lys Asp Ser Gly Lys Pro Lys Arg Ala Thr Thr
545 550 555 560

Ala Phe Met Leu Trp Leu Asn Asp Thr Arg Glu Ser Ile Lys Arg Glu
565 570 575

Asn Pro Gly Ile Lys Val Thr Glu Ile Ala Lys Lys Gly Gly Glu Met
580 585 590

Trp Lys Glu Leu Lys Asp Lys Ser Lys Trp Glu Asp Ala Ala Ala Lys
595 600 605

Asp Lys Gln Arg Tyr His Asp Glu Met Arg Asn Tyr Lys Pro Glu Ala
610 615 620

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Gly Gly Asp Ser Asp Asn Glu Lys Gly Gly Lys Ser Ser Lys Lys Arg
625                 630                 635                 640

Lys Thr Glu Pro Ser Pro Ser Lys Lys Ala Asn Thr Ser Gly Ser Gly
                645                 650                 655

Phe Lys Ser Lys Glu Tyr Ile Ser Asp Asp Asp Ser Thr Ser Ser Asp
            660                 665                 670

Asp Glu Lys Asp Asn Glu Pro Ala Lys Lys Lys Ser Lys Pro Pro Ser
        675                 680                 685

Asp Gly Asp Ala Lys Lys Lys Lys Ala Lys Ser Glu Ser Glu Pro Glu
    690                 695                 700

Glu Ser Glu Glu Asp Ser Asn Ala Ser Asp Glu Asp Glu Glu Asp Glu
705                 710                 715                 720

Ala Ser Asp
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3292 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(vii) IMMEDIATE SOURCE:
(B) CLONE: lambda yPt

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1626

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
GAA TTC GGG TTT CAA GCC CAG CCT CAA CAA CAA CAA CAG CAG CAG CAG          48
Glu Phe Gly Phe Gln Ala Gln Pro Gln Gln Gln Gln Gln Gln Gln Gln
 1               5                  10                  15

CAA CAA CAG CAA CAA CAA CAA GCG CCT TAT CAA GGT CAC TTC CAG CAG          96
Gln Gln Gln Gln Gln Gln Gln Ala Pro Tyr Gln Gly His Phe Gln Gln
            20                  25                  30

TCG CCT CAA CAA CAA CAG CAA AAT GTT TAT TTT CCA CTA CCT CCA CAA         144
Ser Pro Gln Gln Gln Gln Gln Asn Val Tyr Phe Pro Leu Pro Pro Gln
        35                  40                  45

TCT TTG ACG CAA CCT ACT TCG CAG TCG CAA CAA CAA CAA CAA CAG TAT         192
Ser Leu Thr Gln Pro Thr Ser Gln Ser Gln Gln Gln Gln Gln Gln Tyr
    50                  55                  60

GCT AAT TCG AAC TCA AAT TCA AAC AAC AAT GTT AAT GTT AAC GCG CTA         240
Ala Asn Ser Asn Ser Asn Ser Asn Asn Asn Val Asn Val Asn Ala Leu
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65                  70                  75                  80

CCT CAG GAT TTC GGT TAC ATG CAA CAA ACC GGA TCG GGC CAA AAC TAT        288
Pro Gln Asp Phe Gly Tyr Met Gln Gln Thr Gly Ser Gly Gln Asn Tyr
                85                  90                  95

CCG ACG ATC AAT CAA CAA CAA TTT TCC GAG TTT TAC AAC TCC TTT TTA        336
Pro Thr Ile Asn Gln Gln Gln Phe Ser Glu Phe Tyr Asn Ser Phe Leu
            100                 105                 110

AGT CAT TTA ACT CAA AAA CAG ACA AAC CCT TCT GTC ACG GGT ACA GGC        384
Ser His Leu Thr Gln Lys Gln Thr Asn Pro Ser Val Thr Gly Thr Gly
        115                 120                 125

GCG TCT AGT AAC AAC AAC AGT AAC AAC AAC AAT GTT AGT AGC GGC AAT        432
Ala Ser Ser Asn Asn Asn Ser Asn Asn Asn Asn Val Ser Ser Gly Asn
    130                 135                 140

AAC AGC ACT AGC AGT AAT CCT ACC CAG CTG GCA GCC TCC CAA TTA AAC        480
Asn Ser Thr Ser Ser Asn Pro Thr Gln Leu Ala Ala Ser Gln Leu Asn
145                 150                 155                 160

CCT GCC ACG GCT ACT ACG GCC GCC GCA AAC AAT GCT GCT GGC CCG GCT        528
Pro Ala Thr Ala Thr Thr Ala Ala Ala Asn Asn Ala Ala Gly Pro Ala
                165                 170                 175

TCG TAC TTG TCT CAG CTC CCA CAG GTG CAG AGA TAC TAC CCG AAC AAC        576
Ser Tyr Leu Ser Gln Leu Pro Gln Val Gln Arg Tyr Tyr Pro Asn Asn
            180                 185                 190

ATG AAC GCT CTG TCT AGT CTT TTG GAC CCT TCC TCT GCA GGA AAT GCT        624
Met Asn Ala Leu Ser Ser Leu Leu Asp Pro Ser Ser Ala Gly Asn Ala
        195                 200                 205

GCA GGA AAT GCC AAC ACC GCT ACT CAT CCT GGT TTG TTA CCA CCC AAT        672
Ala Gly Asn Ala Asn Thr Ala Thr His Pro Gly Leu Leu Pro Pro Asn
    210                 215                 220

CTG CAA CCT CAA TTG ACT CAC CAC CAG CAG CAG ATG CAG CAA CAG CTG        720
Leu Gln Pro Gln Leu Thr His His Gln Gln Gln Met Gln Gln Gln Leu
225                 230                 235                 240

CAA TTA CAA CAA CAA CAG CAG TTG CAG CAA CAG CAG CAG CTA CAA CAG        768
Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln
                245                 250                 255

CAA CAC CAG TTG CAA CAA CAA CAA CAA CTT CAA CAA CAA CAT CAT CAT        816
Gln His Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln Gln His His His
            260                 265                 270

CTA CAA CAG CAA CAG CAG CAA CAA CAG CAT CCA GTG GTG AAG AAA TTA        864
Leu Gln Gln Gln Gln Gln Gln Gln Gln His Pro Val Val Lys Lys Leu
        275                 280                 285

TCT TCC ACT CAA AGC AGA ATT GAG AGA AGA AAA CAA CTG AAA AAG CAA        912
Ser Ser Thr Gln Ser Arg Ile Glu Arg Arg Lys Gln Leu Lys Lys Gln
    290                 295                 300

GGC CCA AAG AGA CCT TCT TCC GCT TAT TTC CTG TTT TCT ATG TCC ATA        960
Gly Pro Lys Arg Pro Ser Ser Ala Tyr Phe Leu Phe Ser Met Ser Ile
305                 310                 315                 320
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AGA AAT GAG TTG CTT CAA CAA TTC CCT GAA GCA AAG GTC CCC GAA TTG 1008
Arg Asn Glu Leu Leu Gln Gln Phe Pro Glu Ala Lys Val Pro Glu Leu
325 330 335

TCT AAA TTG GCT TCT GCA AGG TGG AAA GAG TTA ACG GAT GAT CAA AAA 1056
Ser Lys Leu Ala Ser Ala Arg Trp Lys Glu Leu Thr Asp Asp Gln Lys
340 345 350

AAA CCA TTC TAC GAA GAA TTC AGA ACC AAC TGG GAG AAG TAC AGA GTT 1104
Lys Pro Phe Tyr Glu Glu Phe Arg Thr Asn Trp Glu Lys Tyr Arg Val
355 360 365

GTG AGA GAT GCT TAC GAA AAG ACT TTG CCC CCA AAG AGA CCC TCT GGT 1152
Val Arg Asp Ala Tyr Glu Lys Thr Leu Pro Pro Lys Arg Pro Ser Gly
370 375 380

CCC TTT ATT CAG TTC ACC CAG GAG ATT AGA CCT ACC GTC GTC AAG GAA 1200
Pro Phe Ile Gln Phe Thr Gln Glu Ile Arg Pro Thr Val Val Lys Glu
385 390 395 400

AAT CCT GAT AAA GGT TTA ATC GAA ATT ACC AAG ATA ATC GGT GAA AGA 1248
Asn Pro Asp Lys Gly Leu Ile Glu Ile Thr Lys Ile Ile Gly Glu Arg
405 410 415

TGG CGC GAG TTA GAC CCC TGC CAA AAG GCG GAA TAC ACT GAA ACT TAC 1296
Trp Arg Glu Leu Asp Pro Cys Gln Lys Ala Glu Tyr Thr Glu Thr Tyr
420 425 430

AAG AAA AGA TTA AAG GAA TGG GAA AGT TGT TAT CCC GAC GAA AAT GAT 1344
Lys Lys Arg Leu Lys Glu Trp Glu Ser Cys Tyr Pro Asp Glu Asn Asp
435 440 445

CCA AAC GGT AAC CCA ACC GGT CAC TCA CAT AAG GCC ATG AAC ATG AAT 1392
Pro Asn Gly Asn Pro Thr Gly His Ser His Lys Ala Met Asn Met Asn
450 455 460

TTG AAT ATG GAC ACT AAA ATC ATG GAG AAC CAA GAC AGT ATC GAG CAC 1440
Leu Asn Met Asp Thr Lys Ile Met Glu Asn Gln Asp Ser Ile Glu His
465 470 475 480

ATA ACC GCA AAT GCC ATC GAC TCA GTT ACC GGA AGC AAC AGT AAC AGT 1488
Ile Thr Ala Asn Ala Ile Asp Ser Val Thr Gly Ser Asn Ser Asn Ser
485 490 495

ACC ACC CCA AAT ACG CCC GTT TCT CCT CCG ATT TCA TTA CAG CAG CAG 1536
Thr Thr Pro Asn Thr Pro Val Ser Pro Pro Ile Ser Leu Gln Gln Gln
500 505 510

CCG CTC CAA CAA CAA CAA CAA CAG CAG CAA CAA CAA CAA CAC ATG TTA 1584
Pro Leu Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln His Met Leu
515 520 525

TTG GCT GAC CCC ACT ACA AAT GGT TCG ATC ATA AAA AAT GAA 1626
Leu Ala Asp Pro Thr Thr Asn Gly Ser Ile Ile Lys Asn Glu
530 535 540

TAACAAATAA ACAACTTTAG TTTTCCACTG TAACATTATC CGACGCAAAC AACGAGAATA 1686

AGGAATTCGA ATTCCTTTTT CAACATTTGT TTAATATTGT ACTACTCTAT TTCCTATTAC 1746

TACAAATTTT ACTTTATTTA ATAATAATTT TTCTTTCCCT TTTTCTAACT TCAGTCTATA 1806

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TGTATTTGCC TGTATACATA TACGCATGTG TGTAGTCTTC CCTCCTTCTT GTTTTTGTAA 1866

TATACTTAAG CCAAATTCAA GTTTGCCTCT GATGCTGTGC GAGCTCAACT GACGAGCGTG 1926

ATGAAGCCAA AAAAATTAAT TGATTTCGCC CAGATCGAAC TGGGGATCTG CTGCGTGTTA 1986

AGCAGATCCA TAGCGACTAG ACCACGAAAC CTATTAATCT GTAAAATTGA TCATTTTAAA 2046

GTGGCATAGT TGTACGATAC ACAAGGGCGA CTTATCAACT TACACATAAA TATGTTTGAA 2106

ACATGTCAGA AACACTCGTT ACAAAGCAGA CAAAATTTAT TACATCAAAC GATACCCTGC 2166

CTAGACAAAC CAGTTAAACG TTGTAAATAC CTGGACAACT AGTTTAGTTC CGAGATTCTG 2226

CGCTTCCATT GAGTCTTATG ACTGTTTCTC AGTTTTCATG TCATCTTTTG ACGCCGCATG 2286

GGATAATGTG TACTAATAAC ATAAATACTA GTCAATAGAT GATATTACGA TTCCATCCAC 2346

AAAGGTGAGG TGCTAGTCAC CACCTAAGGA TATTAGATTG TCAAGATGCC CGCTATTACT 2406

GGAGCCCTTA GTATAACGGA TATTTTCAGG ATAGCAGACT TACTTCTCCA AGTGTAAGGG 2466

AACACCGAAT CTAAAGTAGC TACTGCTCCT CCATTCCGTG TATATAATCT TGCTTTTTTT 2526

TAGGAAAATA CTAATACTCG CATATATTGG TTATTATCAT TACTTGGACA CTGTCTGTTC 2586

TATCGCTTCA TTTGTAATAT GCGTATTGCC CTTCTTATTA ATTGGCTAAT ATTTCACCTG 2646

CAACATAGGT CCCTGTTGAT TAACGTGTTT ATCCATTTCA ATCATGAGAA ATGTTTCTTC 2706

TGTTTTCCAA TGCCTGGCCG AGCTGGTAAT ATATATATAT ATATGTACAT AATACTTTAT 2766

TAGATATATT GTTGATGATT AGTAGACAAG TGGTACTACC AACCGAGAAT AAAAGCTGGT 2826

CTTCTTATAT AATATGAGTA TGGTATAAAT AGCAGTCACC GATATCATTG GTTACCCAAA 2886

GTGACAATTC ATGTCTTTCA TAGATATAAA TCGTAAGCTA AAATTGAATT AAAAGATCTT 2946

TAATTTAGCT GCCCTGCTAA TCTGAAGTCA CATATCATTC CTCATTCTGG ATCACTCACA 3006

ACATTTATTG TCTAATAACT TATGTAATCA CTATAGTCAC TGGTGTGAAC AATGTGAGCA 3066

ATAATAAACC ACTGTATTAC CATATACAAA TGCATATGTT TAGCCACATA AGTTTAATTT 3126

ATATTTCTTA TTTTCCACAC GATATCCCCA CTATCAATGA CATAGATGAT ATTTTCTCCA 3186

CTGGAACAAC CTGAATACAA CAATATATTA TTTGTTCAAG TACCGCTTCA GAAATTAAAT 3246

ACTCTGTAAT TTTGACCCCT TCTAGCACCA TATGTACCCC GAATTC 3292

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

2125673

(vii) IMMEDIATE SOURCE:
(B) CLONE: fractional yeast SSRP (fySSRP) (predicted)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Glu Phe Gly Phe Gln Ala Gln Pro Gln Gln Gln Gln Gln Gln Gln
1 5 10 15

Gln Gln Gln Gln Gln Gln Gln Ala Pro Tyr Gln Gly His Phe Gln Gln
20 25 30

Ser Pro Gln Gln Gln Gln Gln Asn Val Tyr Phe Pro Leu Pro Pro Gln
35 40 45

Ser Leu Thr Gln Pro Thr Ser Gln Ser Gln Gln Gln Gln Gln Gln Tyr
50 55 60

Ala Asn Ser Asn Ser Asn Ser Asn Ser Asn Asn Asn Val Asn Val Asn Ala Leu
65 70 75 80

Pro Gln Asp Phe Gly Tyr Met Gln Gln Thr Gly Ser Gly Gln Asn Tyr
85 90 95

Pro Thr Ile Asn Gln Gln Gln Phe Ser Glu Phe Tyr Asn Ser Phe Leu
100 105 110

Ser His Leu Thr Gln Lys Gln Thr Asn Pro Ser Val Thr Gly Thr Gly
115 120 125

Ala Ser Ser Asn Asn Asn Ser Asn Asn Asn Asn Asn Val Ser Ser Gly Asn
130 135 140

Asn Ser Thr Ser Ser Asn Pro Thr Gln Leu Ala Ala Ser Gln Leu Asn
145 150 155 160

Pro Ala Thr Ala Thr Thr Ala Ala Ala Asn Asn Ala Ala Gly Pro Ala
165 170 175

Ser Tyr Leu Ser Gln Leu Pro Gln Val Gln Arg Tyr Tyr Pro Asn Asn
180 185 190

Met Asn Ala Leu Ser Ser Leu Leu Asp Pro Ser Ser Ala Gly Asn Ala
195 200 205

Ala Gly Asn Ala Asn Thr Ala Thr His Pro Gly Leu Leu Pro Pro Asn
210 215 220

Leu Gln Pro Gln Leu Thr His His Gln Gln Gln Met Gln Gln Gln Leu
225 230 235 240

Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln
245 250 255

Gln His Gln Leu Gln Gln Gln Gln Gln Leu Gln Gln Gln His His His
260 265 270

Leu Gln Gln Gln Gln Gln Gln Gln Gln His Pro Val Val Lys Lys Leu
275 280 285

Ser Ser Thr Gln Ser Arg Ile Glu Arg Arg Lys Gln Leu Lys Lys Gln
290 295 300

Gly Pro Lys Arg Pro Ser Ser Ala Tyr Phe Leu Phe Ser Met Ser Ile
305 310 315 320

Arg Asn Glu Leu Leu Gln Gln Phe Pro Glu Ala Lys Val Pro Glu Leu
325 330 335

Ser Lys Leu Ala Ser Ala Arg Trp Lys Glu Leu Thr Asp Asp Gln Lys
340 345 350

Lys Pro Phe Tyr Glu Glu Phe Arg Thr Asn Trp Glu Lys Tyr Arg Val
355 360 365

Val Arg Asp Ala Tyr Glu Lys Thr Leu Pro Pro Lys Arg Pro Ser Gly
370 375 380

Pro Phe Ile Gln Phe Thr Gln Glu Ile Arg Pro Thr Val Val Lys Glu
385 390 395 400

Asn Pro Asp Lys Gly Leu Ile Glu Ile Thr Lys Ile Ile Gly Glu Arg
405 410 415

Trp Arg Glu Leu Asp Pro Ala Lys Lys Ala Glu Tyr Thr Glu Thr Tyr
420 425 430

Lys Lys Arg Leu Lys Glu Trp Glu Ser Cys Tyr Pro Asp Glu Asn Asp
435 440 445

Pro Asn Gly Asn Pro Thr Gly His Ser His Lys Ala Met Asn Met Asn
450 455 460

Leu Asn Met Asp Thr Lys Ile Met Glu Asn Gln Asp Ser Ile Glu His
465 470 475 480

Ile Thr Ala Asn Ala Ile Asp Ser Val Thr Gly Ser Asn Ser Asn Ser
485 490 495

Thr Asn Pro Asn Thr Pro Val Ser Pro Pro Ile Ser Leu Gln Gln Gln
500 505 510

Pro Leu Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln His Met Leu
515 520 525

Leu Ala Asp Pro Thr Thr Asn Gly Ser Ile Ile Lys Asn Glu
530 535 540

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A nucleic acid sequence essentially free of proteins and other cellular components, said nucleic acid sequence encoding a eukaryotic DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage,

said nucleic acid sequence being of human origin, and comprising a sequence selected from the group consisting of:

(a) SEQ ID NO:6;

(b) a sequence that hybridizes specifically to SEQ ID No. 6;

(c) SEQ ID NO:8;

(d) a sequence that hybridizes specifically to SEQ ID NO:8;

(e) SEQ ID NO:9;

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(f) a sequence that hybridizes specifically to SEQ ID NO:9;

(g) the HMG box domain of SEQ ID NO:6; and

(h) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:6.

2. A nucleic acid sequence essentially free of proteins and other cellular components, said nucleic acid sequence encoding a eukaryotic DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage,

said nucleic acid sequence being of Drosophila melanogaster origin, and comprising a sequence selected from the group consisting of:

(a) SEQ ID NO:10;

(b) a sequence that hybridizes specifically to SEQ ID NO:10;

(c) the HMG box domain of SEQ ID NO:10; and

(d) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:10.

3. A nucleic acid sequence essentially free of proteins and other cellular components, said nucleic acid sequence encoding a eukaryotic DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-int.rastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-int:rastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage,

said nucleic acid sequence being of Saccharomyces cerevesiae origin, and comprising a sequence selected from the group consisting of:

(a) SEQ ID NO:12;

(b) a sequence that hybridizes specifically to SEQ ID NO:12;

(c) an HMG box domain of SEQ ID NO:12; and

(d) a sequence that hybridizes specifically to a HMG box domain of SEQ ID NO:12.

4. A nucleotide probe that hybridizes specifically to a nucleotide sequence encoding a human DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a doublestranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

said nucleotide sequence being selected from the group consisting of:

(a) SEQ ID NO:6;

(b) a sequence that hybridizes specifically to SEQ ID NO:6;

(c) SEQ ID NO:8;

(d) a sequence that hybridizes specifically to SEQ ID NO:8;

(e) SEQ ID NO:9;

(f) a sequence that hybridizes specifically to SEQ ID NO:9;

(g) the HMG box domain of SEQ ID NO:6; and

(h) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:6.

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5. A nucleotide probe that hybridizes specifically to a nucleotide sequence encoding a Drosophila melanogaster DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

said nucleotide sequence being selected from the group consisting of:

(a) SEQ ID NO:10;

(b) a sequence that hybridizes specifically to SEQ ID NO:10;

(c) the HMG box domain of SEQ ID NO:10; and

(d) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:10.

6. A nucleotide probe that hybridizes specifically to a nucleotide sequence encoding a Saccharomyces cerevesiae DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

said nucleotide sequence being selected from the group consisting of:

(a) SEQ ID NO:12;

(b) a sequence that hybridizes specifically to SEQ ID NO:12;

(c) an HMG box domain of SEQ ID NO:12; and

(d) a sequence that hybridizes specifically to a HMG box domain of SEQ ID NO:12.

7. A recombinant protein expressed from DNA of eukaryotic origin, said protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the nucleic acid sequence of said DNA comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:6;

(b) a sequence that hybridizes specifically to SEQ ID NO:6;

(c) SEQ ID NO:8;

(d) a sequence that hybridizes specifically to SEQ ID NO:8;

(e) SEQ ID NO:9;

(f) a sequence that hybridizes specifically to SEQ ID NO:9

(g) the HMG box domain of SEQ ID NO:6; and

(h) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:6.

8. A recombinant protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

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(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the amino acid sequence of said protein comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:7; and

(b) the HMG box domain of SEQ ID NO:7.

9. A polyclonal antibody selectively reactive with the protein of claim 7 or 8.

10. A recombinant protein expressed from DNA of eukaryotic origin, said protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the nucleic acid sequence of said DNA comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:10;

(b) a sequence that hybridizes specifically to SEQ ID NO:10;

(c) the HMG box domain of SEQ ID NO:10; and

(d) a sequence that hybridizes specifically to the HMG box domain of SEQ ID NO:10.

11. A recombinant protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the amino acid sequence of said protein comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:11; and

(b) the HMG box domain of SEQ ID NO:11.

12. A polyclonal antibody selectively reactive with the protein of claim 10 or 11.

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13. A recombinant protein expressed from DNA of eukaryotic origin, said protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the nucleic acid sequence of said DNA comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:12;

(b) a sequence that hybridizes specifically to SEQ ID NO:12;

(c) an HMG box domain of SEQ ID NO:12; and

(d) a sequence that hybridizes specifically to an HMG box domain of SEQ ID NO:12.

14. A recombinant protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG)

dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage;

wherein the amino acid sequence of said protein comprises a sequence selected from the group consisting of:

(a) SEQ ID NO:13; and

(b) an HMG box domain of SEQ ID NO:13.

15. A polyclonal antibody selectively reactive with the protein of claim 13 or 14.

16. A method of detecting, in eukaryotic cells, nucleic acid encoding a DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment having at least one region of DNA damage comprising

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a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage,

said method comprising the steps of:

(a) treating eukaryotic cells in such a manner as to produce an extract containing nucleic acid from the cells;

(b) contacting the extract with a nucleotide probe that hybridizes to cDNA having a sequence selected from the group consisting of the sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9 and the HMG box domain of SEQ ID NO:6; and

(c) detecting hybridization of the probe to cellular DNA encoding said structure specific recognition protein.

17. A method according to claim 16 wherein the eukaryotic cells are human cells.

18. A method of detecting, in eukaryotic cells, a DNA structure specific recognition protein having the following properties:

(i) selective binding to a double-stranded DNA fragment having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, to form a (damaged DNA fragment):(protein) complex which has a retarded electrophoretic mobility relative to the mobility of the damaged DNA fragment alone; and

(ii) when affixed to a solid support, said protein binds selectively to a double-stranded DNA fragment

having at least one region of DNA damage comprising a 1,2-intrastrand d(ApG) or d(GpG) dinucleotide adduct, and not to a double-stranded DNA fragment lacking said region of DNA damage,

said method comprising the steps of:

(a) treating eukaryotic cells in such a manner as to render proteins and portions thereof present in the cells available for binding to polyclonal antibodies specific to said proteins or portions thereof;

b) contacting the product of (a) under conditions appropriate for the binding of polyclonal antibodies to selected proteins or portions thereof for which the polyclonal antibodies have specificity, with a polyclonal antibody that binds selectively to a recombinant protein, the amino acid sequence of which comprises a sequence selected from the group consisting of SEQ ID NO:7, the HMG box domain of SEQ ID NO:7, and an amino acid sequence encoded by any of: the nucleic acid sequence of SEQ ID NO:6, a nucleic acid sequence that hybridizes specifically to that of SEQ ID NO:6, the nucleic acid sequence of SEQ ID NO:8, a nucleic acid sequence that hybridizes specifically to that of SEQ ID NO:8, the nucleic acid sequence of SEQ ID NO:9, a nucleic acid sequence that hybridizes specifically to that of SEQ ID NO:9, the nucleic acid sequence of the HMG box domain of SEQ ID NO:6, and a

nucleic acid sequence that hybridizes specifically to that of SEQ ID NO:6; and

(c) detecting binding of the antibody to cellular DNA structure specific recognition protein.

19. A method according to claim 18 wherein the eukaryotic cells are human cells.

20. RNA transcribed from the DNA of claim 1, 2 or 3.

21. An expression vector comprising the DNA of claim 1, 2 or 3.

22. A host cell transformed with the expression vector of claim 21.

cis-d(GpG)	*cis*-{Pt(NH$_3$)2} TCTCCTTCTTGGTTCTCTTCTC ACAGAGGAAGAACCAAGAGAAG
cis-d(ApG)	*cis*-{Pt(NH$_3$)2} TCTCCTTCTTAGTTCTCTTCTC AGAGAGGAAGAATCAAGAGAAG
cis-d(GpTpG)	*cis*-{Pt(NH$_3$)2} TCTCCTTCTTGTGTCTCTTCTC AGAGAGGAAGAACACAGAGAAG
trans-d(GpTpG)	*trans*-{Pt(NH$_3$)2} TCTCCTTCTTGTGTCTCTTCTC AGAGAGGAAGAACACAGAGAAG
N7-d(G)	*cis*-{Pt(NH$_3$)2(N3-cytosine)} TCTCCTTCTTCGTTCTCTTCTC AGAGAGGAAGAAGCAAGAGAAG

FIG. 1

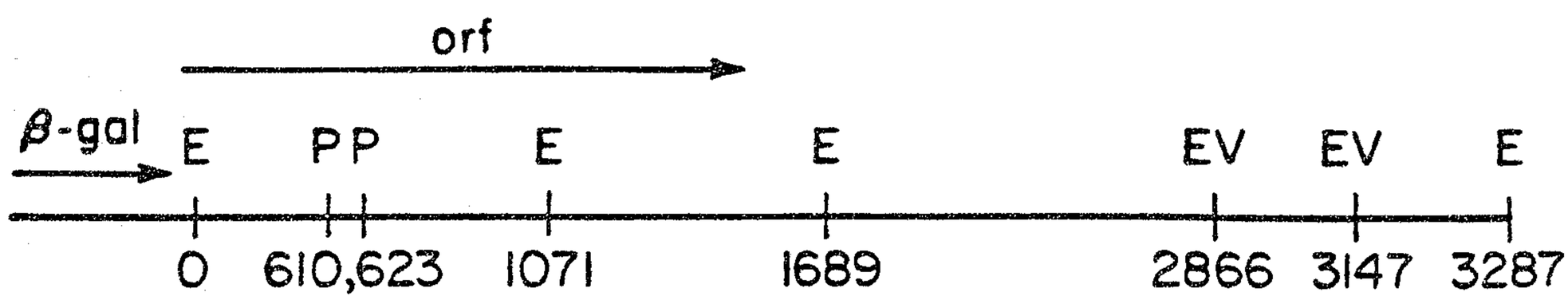

FIG. 10

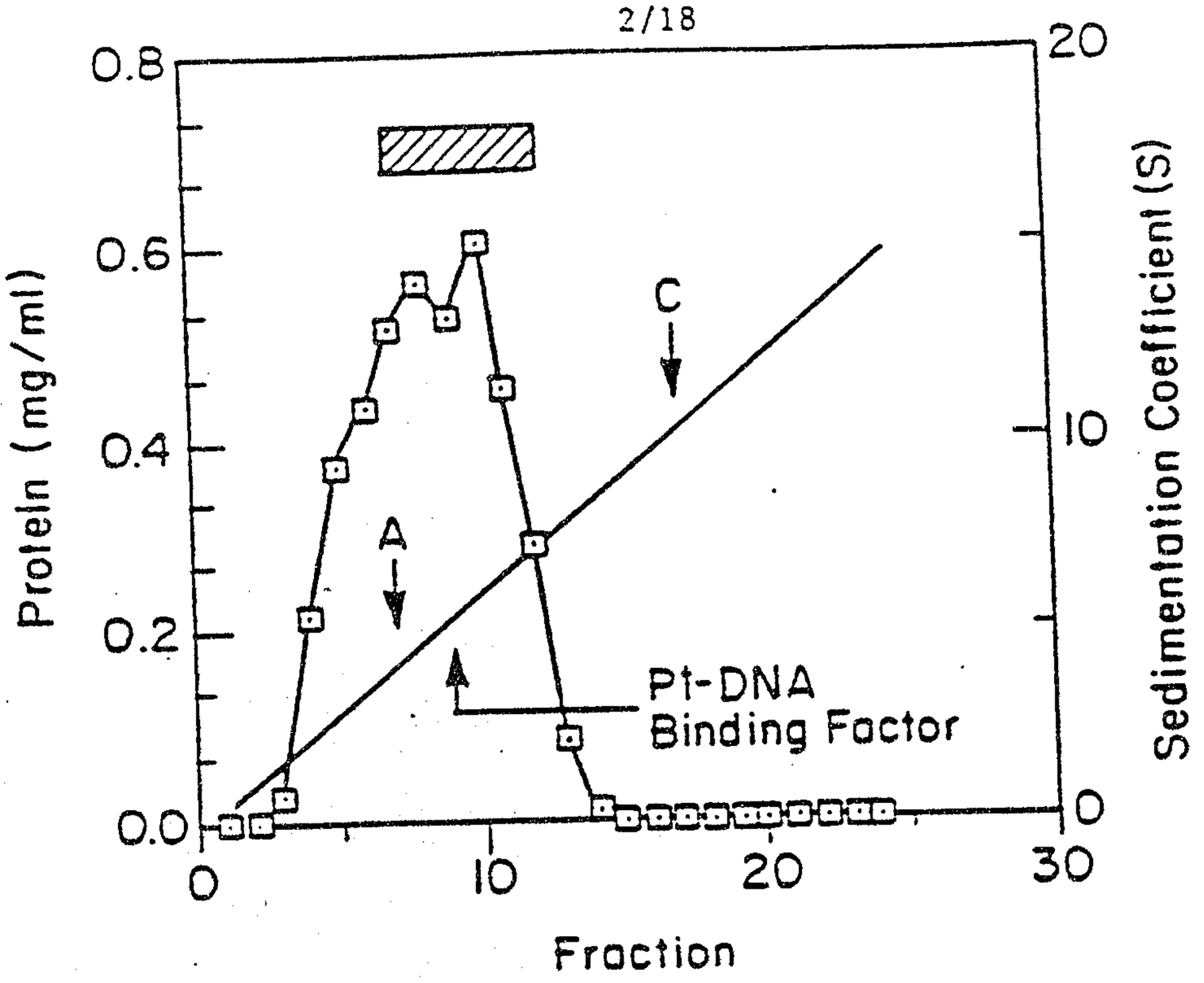
0.8
0.6
0.4
0.2
0.0
Protein (mg/ml)
0
10
20
30
Fraction
20
10
0
Sedimentation Coefficient (S)
A
C
Pt-DNA
Binding Factor

FIG. 2

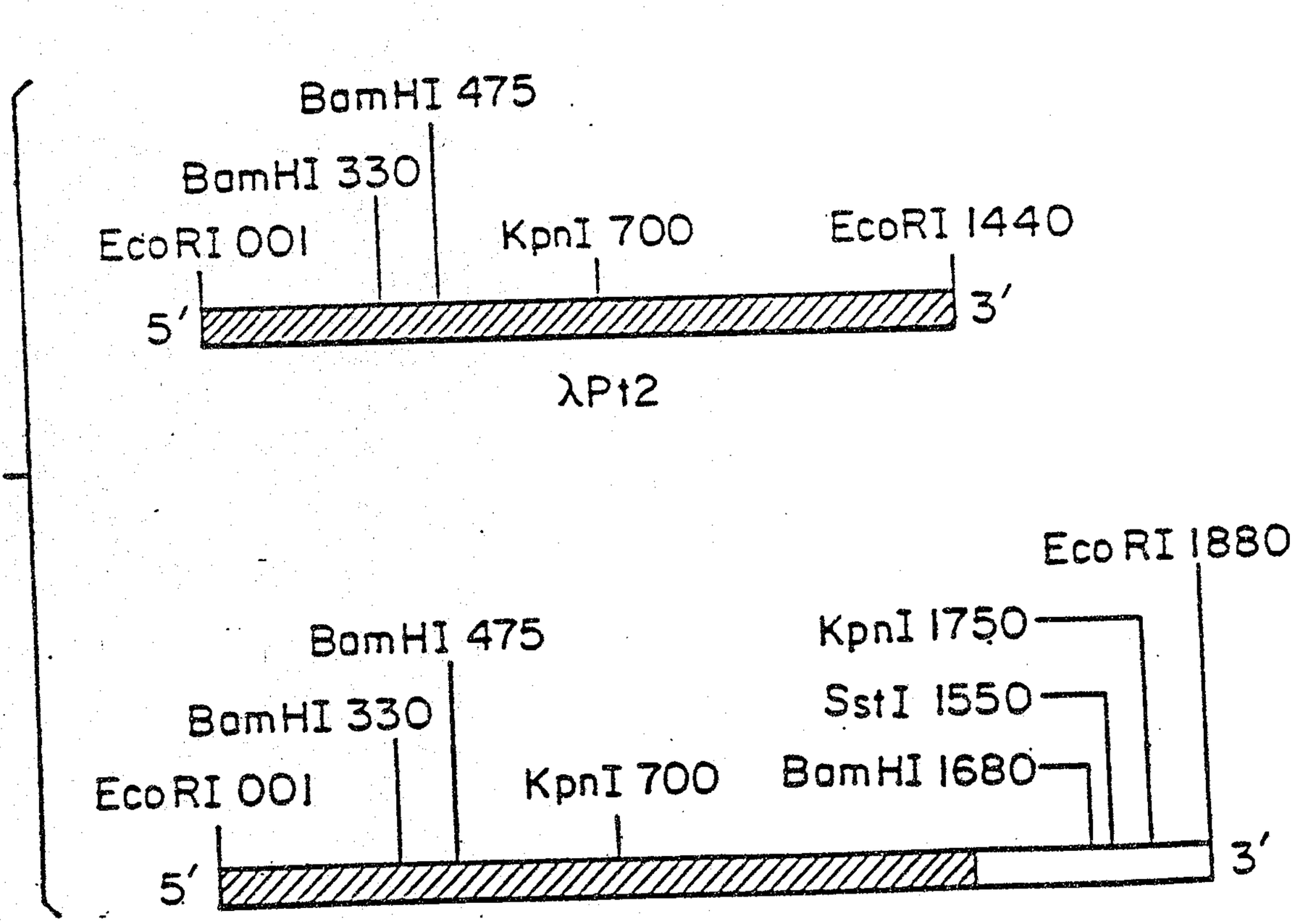
BamHI 475
BamHI 330
EcoRI 001
KpnI 700
EcoRI 1440
5'
3'
λP12
EcoRI 1880
KpnI 1750
SstI 1550
BamHI 1680
BamHI 475
BamHI 330
EcoRI 001
KpnI 700
5'
3'
λPt1

FIG. 3

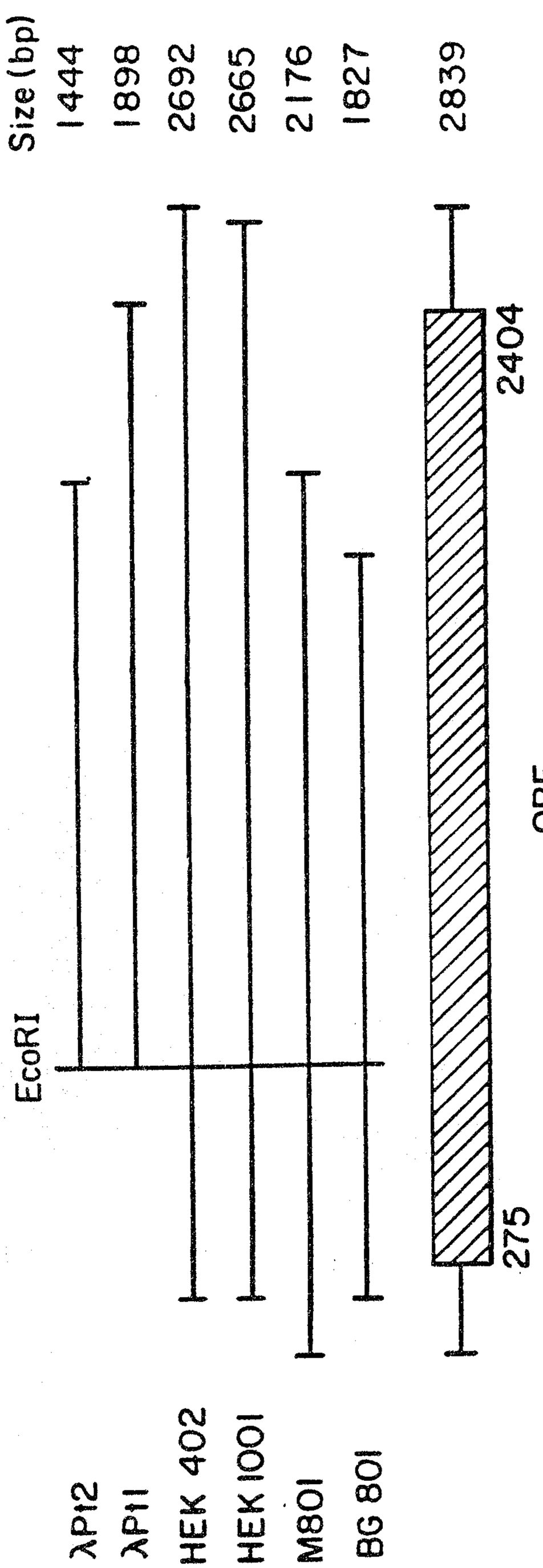
Size (bp)
1444
1898
2692
2665
2176
1827
2839
2404
EcoRI
275
ORF
λP12
λP11
HEK 402
HEK 1001
M801
BG 801

FIG. 4

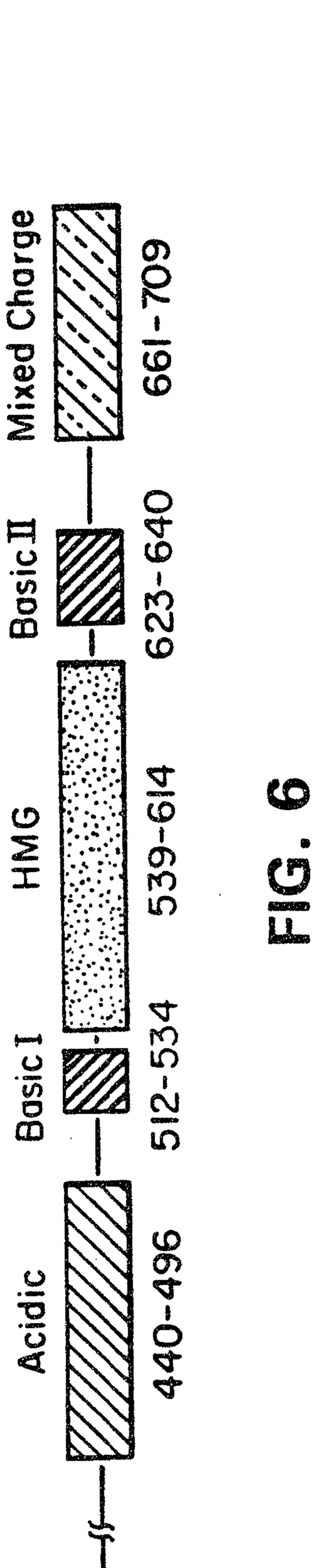
Basic II
Mixed Charge
623-640
661-709
Acidic
Basic I
HMG
440-496
512-534
539-614

FIG. 6

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GAATTCCGTA CGGCTTCCGG TGGCGGGACG CGGGGCCGCG CACGCGGGAA AAGCTTCCCC      60

GGTGTCCCCC CATCCCCCTC CCCGCGCCCC CCCCGCGTCC CCCCAGCGCG CCCACCTCTC     120

GCGCCGGGGC CCTCGCGAGG CCGCAGCCTG AGGAGATTCC CAACCTGCTG AGCATCCGCA     180

CACCCACTCA GGAGTTGGGG CCCAGCTCCC AGTTTACTTG GTTTCCCTTG TGCAGCCTGG     240

GGCTCTGCCC AGGCCACCAC AGGCAGGGGT CGAC ATG GCA GAG ACA CTG GAG          292
                                      Met Ala Glu Thr Leu Glu
                                        1               5

TTC AAC GAC GTC TAT CAG GAG GTG AAA GGT TCC ATG AAT GAT GGT CGA        340
Phe Asn Asp Val Tyr Gln Glu Val Lys Gly Ser Met Asn Asp Gly Arg
            10                  15                  20

CTG AGG TTG AGC CGT CAG GGC ATC ATC TTC AAG AAT AGC AAG ACA GGC        388
Leu Arg Leu Ser Arg Gln Gly Ile Ile Phe Lys Asn Ser Lys Thr Gly
        25                  30                  35

AAA GTG GAC AAC ATC CAG GCT GGG GAG TTA ACA GAA GGT ATC TGG CGC        436
Lys Val Asp Asn Ile Gln Ala Gly Glu Leu Thr Glu Gly Ile Trp Arg
    40                  45                  50

CGT GTT GCT CTG GGC CAT GGA CTT AAA CTG CTT ACA AAG AAT GGC CAT        484
Arg Val Ala Leu Gly His Gly Leu Lys Leu Leu Thr Lys Asn Gly His
55                  60                  65                  70

GTC TAC AAG TAT GAT GGC TTC CGA GAA TCG GAG TTT GAG AAA CTC TCT        532
Val Tyr Lys Tyr Asp Gly Phe Arg Glu Ser Glu Phe Glu Lys Leu Ser
                75                  80                  85

GAT TTC TTC AAA ACT CAC TAT CGC CTT GAG CTA ATG GAG AAG GAC CTT        580
Asp Phe Phe Lys Thr His Tyr Arg Leu Glu Leu Met Glu Lys Asp Leu
            90                  95                  100
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FIG. 5A

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TGT GTG AAG GGC TGG AAC TGG GGG ACA GTG AAA TTT GGT GGG CAG CTG          628
Cys Val Lys Gly Trp Asn Trp Gly Thr Val Lys Phe Gly Gly Gln Leu
        105                 110                 115

CTT TCC TTT GAC ATT GGT GAC CAG CCA GTC TTT GAG ATA CCC CTC AGC          676
Leu Ser Phe Asp Ile Gly Asp Gln Pro Val Phe Glu Ile Pro Leu Ser
    120                 125                 130

AAT GTG TCC CAG TGC ACC ACA GGC AAG AAT GAG GTG ACA CTG GAA TTC          724
Asn Val Ser Gln Cys Thr Thr Gly Lys Asn Glu Val Thr Leu Glu Phe
135                 140                 145                 150

CAC CAA AAC GAT GAC GCA GAG GTG TCT CTC ATG GAG GTG CGC TTC TAC          772
His Gln Asn Asp Asp Ala Glu Val Ser Leu Met Glu Val Arg Phe Tyr
                155                 160                 165

GTC CCA CCC ACC CAG GAG GAT GGT GTG GAC CCT GTT GAG GCC TTT GCC          820
Val Pro Pro Thr Gln Glu Asp Gly Val Asp Pro Val Glu Ala Phe Ala
            170                 175                 180

CAG AAT GTG TTG TCA AAG GCG GAT GTA ATC CAG GCC ACG GGA GAT GCC          868
Gln Asn Val Leu Ser Lys Ala Asp Val Ile Gln Ala Thr Gly Asp Ala
        185                 190                 195

ATC TGC ATC TTC CGG GAG CTG CAG TGT CTG ACT CCT CGT GGT CGT TAT          916
Ile Cys Ile Phe Arg Glu Leu Gln Cys Leu Thr Pro Arg Gly Arg Tyr
    200                 205                 210

GAC ATT CGG ATC TAC CCC ACC TTT CTG CAC CTG CAT GGC AAG ACC TTT          964
Asp Ile Arg Ile Tyr Pro Thr Phe Leu His Leu His Gly Lys Thr Phe
215                 220                 225                 230

GAC TAC AAG ATC CCC TAC ACC ACA GTA CTG CGT CTG TTT TTG TTA CCC         1012
Asp Tyr Lys Ile Pro Tyr Thr Thr Val Leu Arg Leu Phe Leu Leu Pro
                235                 240                 245
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FIG. 5B

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CAC AAG GAC CAG CGC CAG ATG TTC TTT GTG ATC AGC CTG GAT CCC CCA      1060
His Lys Asp Gln Arg Gln Met Phe Phe Val Ile Ser Leu Asp Pro Pro
            250                 255                 260

ATC AAG CAA GGC CAA ACT CGC TAC CAC TTC CTG ATC CTC CTC TTC TCC      1108
Ile Lys Gln Gly Gln Thr Arg Tyr His Phe Leu Ile Leu Leu Phe Ser
        265                 270                 275

AAG GAC GAG GAC ATT TCG TTG ACT CTG AAC ATG AAC GAG GAA GAA GTG      1156
Lys Asp Glu Asp Ile Ser Leu Thr Leu Asn Met Asn Glu Glu Glu Val
    280                 285                 290

GAG AAG CGC TTT GAG GGT CGG CTC ACC AAG AAC ATG TCA GGA TCC CTC      1204
Glu Lys Arg Phe Glu Gly Arg Leu Thr Lys Asn Met Ser Gly Ser Leu
295                 300                 305                 310

TAT GAG ATG GTC AGC CGG GTC ATG AAA GCA CTG GTA AAC CGC AAG ATC      1252
Tyr Glu Met Val Ser Arg Val Met Lys Ala Leu Val Asn Arg Lys Ile
                315                 320                 325

ACA GTG CCA GGC AAC TTC CAA GGG CAC TCA GGG GCC CAG TGC ATT ACC      1300
Thr Val Pro Gly Asn Phe Gln Gly His Ser Gly Ala Gln Cys Ile Thr
            330                 335                 340

TGT TCC TAC AAG GCA AGC TCA GGA CTG CTC TAC CCG CTG GAG CGG GGC      1348
Cys Ser Tyr Lys Ala Ser Ser Gly Leu Leu Tyr Pro Leu Glu Arg Gly
        345                 350                 355

TTC ATC TAC GTC CAC AAG CCA CCT GTG CAC ATC CGC TTC GAT GAG ATC      1396
Phe Ile Tyr Val His Lys Pro Pro Val His Ile Arg Phe Asp Glu Ile
    360                 365                 370

TCC TTT GTC AAC TTT GCT CGT GGT ACC ACT ACT ACT CGT TCC TTT GAC      1444
Ser Phe Val Asn Phe Ala Arg Gly Thr Thr Thr Thr Arg Ser Phe Asp
375                 380                 385                 390
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FIG. 5C

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TTT GAA ATT GAG ACC AAG CAG GGC ACT CAG TAT ACC TTC AGC AGC ATT      1492
Phe Glu Ile Glu Thr Lys Gln Gly Thr Gln Tyr Thr Phe Ser Ser Ile
                395                 400                 405

GAG AGG GAG GAG TAC GGG AAA CTG TTT GAT TTT GTC AAC GCG AAA AAG      1540
Glu Arg Glu Glu Tyr Gly Lys Leu Phe Asp Phe Val Asn Ala Lys Lys
            410                 415                 420

CTC AAC ATC AAA AAC CGA GGA TTG AAA GAG GGC ATG AAC CCA AGC TAC      1588
Leu Asn Ile Lys Asn Arg Gly Leu Lys Glu Gly Met Asn Pro Ser Tyr
        425                 430                 435

GAT GAA TAT GCT GAC TCT GAT GAG GAC CAG CAT GAT GCC TAC TTG GAG      1636
Asp Glu Tyr Ala Asp Ser Asp Glu Asp Gln His Asp Ala Tyr Leu Glu
    440                 445                 450

AGG ATG AAG GAG GAA GGC AAG ATC CGG GAG GAG AAT GCC AAT GAC AGC      1684
Arg Met Lys Glu Glu Gly Lys Ile Arg Glu Glu Asn Ala Asn Asp Ser
455                 460                 465                 470

AGC GAT GAC TCA GGA GAA GAA ACC GAT GAG TCA TTC AAC CCA GGT GAA      1732
Ser Asp Asp Ser Gly Glu Glu Thr Asp Glu Ser Phe Asn Pro Gly Glu
                475                 480                 485

GAG GAG GAA GAT GTG GCA GAG GAG TTT GAC AGC AAC GCC TCT GCC AGC      1780
Glu Glu Glu Asp Val Ala Glu Glu Phe Asp Ser Asn Ala Ser Ala Ser
            490                 495                 500

TCC TCC AGT AAT GAG GGT GAC AGT GAC CGG GAT GAG AAG AAG CGG AAA      1828
Ser Ser Ser Asn Glu Gly Asp Ser Asp Arg Asp Glu Lys Lys Arg Lys
        505                 510                 515

CAG CTC AAA AAG GCC AAG ATG GCC AAG GAC CGC AAG AGC CGC AAG AAG      1876
Gln Leu Lys Lys Ala Lys Met Ala Lys Asp Arg Lys Ser Arg Lys Lys
    520                 525                 530
```

FIG. 5D

FIG. 5E

```
CCT GTG GAG GTG AAG AAG GGC AAA GAC CCC AAT GCC CCC AAG AGG CCC      1924
Pro Val Glu Val Lys Lys Gly Lys Asp Pro Asn Ala Pro Lys Arg Pro
535                 540                 545                 550

ATG TCT GCA TAC ATG CTG TGG CTC AAT GCC AGC CGA GAG AAG ATC AAG      1972
Met Ser Ala Tyr Met Leu Trp Leu Asn Ala Ser Arg Glu Lys Ile Lys
                555                 560                 565

TCA GAC CAT CCT GGC ATC AGC ATC ACG GAT CTT TCC AAG AAG GCA GGC      2020
Ser Asp His Pro Gly Ile Ser Ile Thr Asp Leu Ser Lys Lys Ala Gly
            570                 575                 580

GAG ATC TGG AAG GGA ATG TCC AAA GAG AAG AAA GAG GAG TGG GAT CGC      2068
Glu Ile Trp Lys Gly Met Ser Lys Glu Lys Lys Glu Glu Trp Asp Arg
        585                 590                 595

AAG GCT GAG GAT GCC AGG AGG GAC TAT GAA AAA GCC ATG AAA GAA TAT      2116
Lys Ala Glu Asp Ala Arg Arg Asp Tyr Glu Lys Ala Met Lys Glu Tyr
    600                 605                 610

GAA GGG GGC CGA GGC GAG TCT TCT AAG AGG GAC AAG TCA AAG AAG AAG      2164
Glu Gly Gly Arg Gly Glu Ser Ser Lys Arg Asp Lys Ser Lys Lys Lys
615                 620                 625                 630

AAG AAA GTA AAG GTA AAG ATG GAA AAG AAA TCC ACG CCC TCT AGG GGC      2212
Lys Lys Val Lys Val Lys Met Glu Lys Lys Ser Thr Pro Ser Arg Gly
                635                 640                 645

TCA TCA TCC AAG TCG TCC TCA AGG CAG CTA AGC GAG AGC TTC AAG AGC      2260
Ser Ser Ser Lys Ser Ser Ser Arg Gln Leu Ser Glu Ser Phe Lys Ser
            650                 655                 660

AAA GAG TTT GTG TCT AGT GAT GAG AGC TCT TCG GGA GAG AAC AAG AGC      2308
Lys Glu Phe Val Ser Ser Asp Glu Ser Ser Ser Gly Glu Asn Lys Ser
        665                 670                 675
```

```
AAA AAG AAG AGG AGG AGG AGC GAG GAC TCT GAA GAA GAA GAA CTA GCC      2356
Lys Lys Lys Arg Arg Arg Ser Glu Asp Ser Glu Glu Glu Glu Leu Ala
    680                 685                 690

AGT ACT CCC CCC AGC TCA GAG GAC TCA GCG TCA GGA TCC GAT GAG TAGAAACGGA   2411
Ser Thr Pro Pro Ser Ser Glu Asp Ser Ala Ser Gly Ser Asp Glu
695                 700                 705                 710

GGAAGGTTCT CTTTGCGCTT GCCTTCTCAC ACCCCCCGAC TCCCCACCCA TATTTTGGTA    2471

CCAGTTTCTC CTCATGAAAT GCAGTCCCTG GATTCTGTGC CATCTGAACA TGCTCTCCTG    2531

TTGGTGTGTA TGTCACTAGG GCAGTGGGGA GACGTCTTAA CTCTGCTGCT TCCCAAGGAT    2591

GGCTGTTTAT AATTTGGGGA GAGATAGGGT GGGAGGCAGG GCAATGCAGG ATCCAAATCC    2651

TCATCTTACT TTCCCGACCT TAAGGATGTA GCTGCTGCTT GTCCTGTTCA AGTTGCTGGA    2711

GCAGGGGTCA TGTGAGGCCA GGCCTGTAGC TCCTACCTGG GGCCTATTTC TACTTTCATT    2771

TTGTATTTCT GGTCTGTGAA AATGATTTAA TAAAGGGAAC TGACTTTGGA AACCAAAAAA    2831

AGGAATTC                                                            2839
```

FIG. 5F

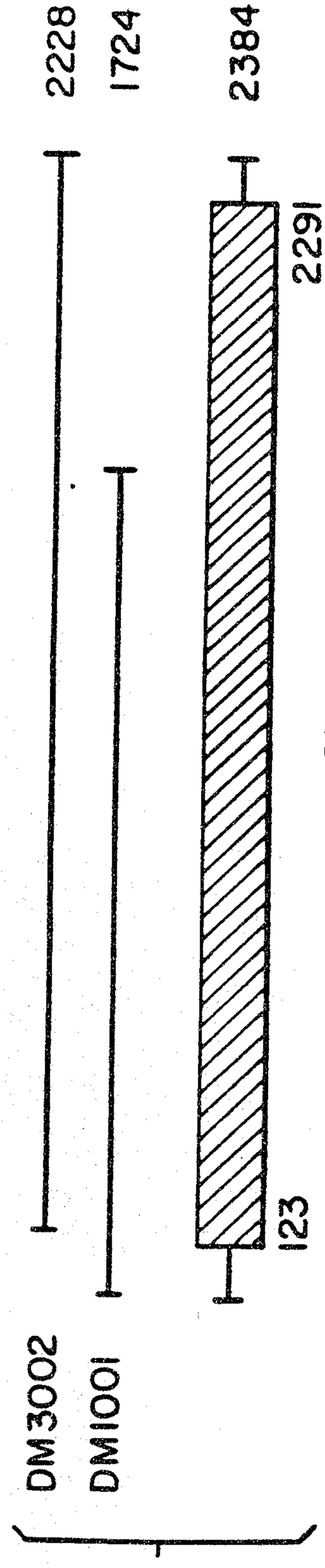

DM3002
DM1001
ORF
2228
1724
2384
2291
123

FIG. 7

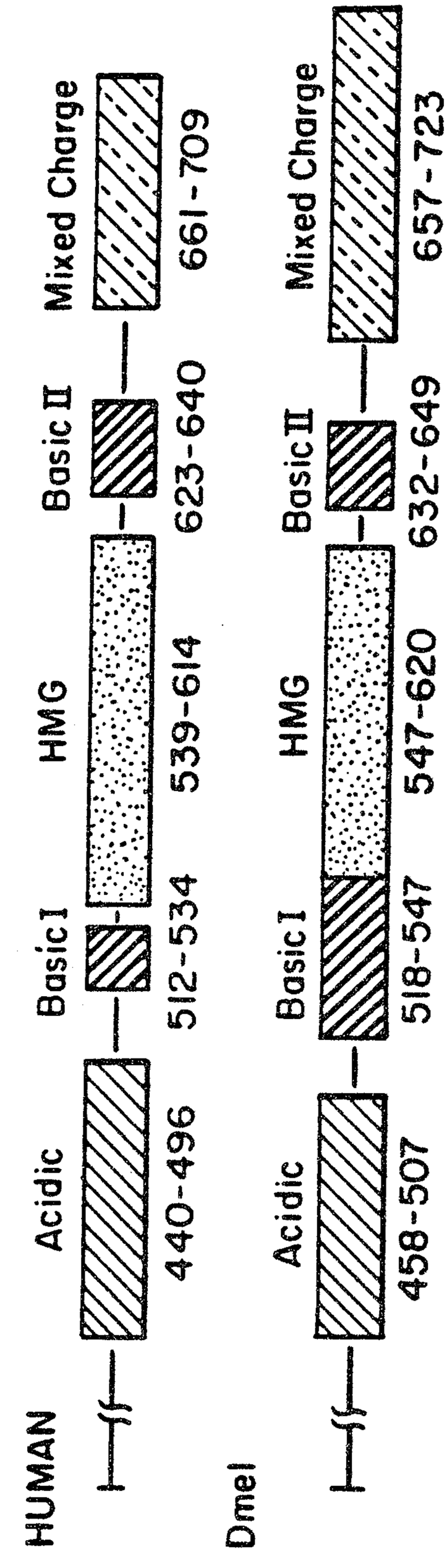

HUMAN
Dmel
HMG
Acidic
Basic I
Basic II
Mixed Charge
440-496
458-507
512-534
518-547
539-614
547-620
623-640
632-649
661-706
657-723

FIG. 9

FIG. 8A

```
GAATTCCGCG CGCAGTGTTG TTTTGTGTCT GCCGGAATTA TTGTAAATTG GTGACAATTT      60

CGCAAGGCGG CGTAATACAT AGTTGATCTA TTATCTTGTT ACTGGAGAGG AAGAAGTGCA     120

GG ATG ACA GAC TCT CTG GAG TAC AAC GAC ATA AAC GCC GAA GTG CGC         167
   Met Thr Asp Ser Leu Glu Tyr Asn Asp Ile Asn Ala Glu Val Arg
     1               5                  10                  15

GGA GTC TTG TGT TCC GGA CGC CTA AAG ATG ACC GAG CAG AAC ATC ATC       215
Gly Val Leu Cys Ser Gly Arg Leu Lys Met Thr Glu Gln Asn Ile Ile
                 20                  25                  30

TTC AAG AAC ACC AAG ACC GGC AAG GTG GAG CAG ATC TCG GCA GAG GAC       263
Phe Lys Asn Thr Lys Thr Gly Lys Val Glu Gln Ile Ser Ala Glu Asp
             35                  40                  45

ATA GAC CTG ATC AAT TCG CAG AAG TTC GTG GGC ACC TGG GGA CTG AGG       311
Ile Asp Leu Ile Asn Ser Gln Lys Phe Val Gly Thr Trp Gly Leu Arg
         50                  55                  60

GTG TTC ACC AAA GGC GGC GTG CTC CAC CGC TTC ACC GGA TTC CGC GAC       359
Val Phe Thr Lys Gly Gly Val Leu His Arg Phe Thr Gly Phe Arg Asp
     65                  70                  75

AGC GAG CAC GAG AAG CTG GGC AAG TTT ATC AAG GCT GCC TAC TCG CAG       407
Ser Glu His Glu Lys Leu Gly Lys Phe Ile Lys Ala Ala Tyr Ser Gln
 80                  85                  90                  95

GAG ATG GTC GAG AAG GAG ATG TGC GTC AAG GGC TGG AAC TGG GGC ACC       455
Glu Met Val Glu Lys Glu Met Cys Val Lys Gly Trp Asn Trp Gly Thr
                100                 105                 110

GCC CGC TTC ATG GGC TCC GTC CTG AGC TTC GAC AAG GAG TCG AAG ACC       503
Ala Arg Phe Met Gly Ser Val Leu Ser Phe Asp Lys Glu Ser Lys Thr
            115                 120                 125
```

```
ATC TTC GAG GTG CCG CTG TCG CAC GTT TCG CAG TGC GTG ACC GGC AAG          551
Ile Phe Glu Val Pro Leu Ser His Val Ser Gln Cys Val Thr Gly Lys
        130                 135                 140

AAC GAG GTC ACC CTG GAG TTC CAC CAA AAC GAC GAT GCG CCC GTG GGT          599
Asn Glu Val Thr Leu Glu Phe His Gln Asn Asp Asp Ala Pro Val Gly
    145                 150                 155

CTA CTG GAG ATG CGG TTC CAC ATA CCC GCC GTG GAG TCG GCC GAG GAG          647
Leu Leu Glu Met Arg Phe His Ile Pro Ala Val Glu Ser Ala Glu Glu
160                 165                 170                 175

GAT CCG GTA GAC AAG TTC CAC CAG AAC GTA ATG AGC AAG GCC TCG GTC          695
Asp Pro Val Asp Lys Phe His Gln Asn Val Met Ser Lys Ala Ser Val
                180                 185                 190

ATC TCG GCT TCG GGC GAG TCC ATC GCC ATT TTC AGA GAG ATC CAG ATC          743
Ile Ser Ala Ser Gly Glu Ser Ile Ala Ile Phe Arg Glu Ile Gln Ile
            195                 200                 205

CTC ACG CCT CGC GGT CGC TAT GAC ATC AAG ATC TTC TCG ACC TTC TTC          791
Leu Thr Pro Arg Gly Arg Tyr Asp Ile Lys Ile Phe Ser Thr Phe Phe
        210                 215                 220

CAG CTG CAC GGC AAG ACG TTC GAC TAC AAG ATT CCC ATG GAC TCG GTG          839
Gln Leu His Gly Lys Thr Phe Asp Tyr Lys Ile Pro Met Asp Ser Val
    225                 230                 235

CTG CGG CTC TTC ATG CTG CCC CAC AAA GAC AGT CGA CAG ATG TTC TTT          887
Leu Arg Leu Phe Met Leu Pro His Lys Asp Ser Arg Gln Met Phe Phe
240                 245                 250                 255

GTG CTC TCC TTG GAT CCG CCC ATC AAG CAG GGA CAA ACG CGT TAC CAC          935
Val Leu Ser Leu Asp Pro Pro Ile Lys Gln Gly Gln Thr Arg Tyr His
                260                 265                 270
```

FIG. 8B

SUBSTITUTE SHEET

2123673

```
TAC CTG GTC CTG CTG TTT GCT CCC GAT GAG GAG ACC ACC ATT GAG CTG          983
Tyr Leu Val Leu Leu Phe Ala Pro Asp Glu Glu Thr Thr Ile Glu Leu
                275                 280                 285

CCA TTC TCG GAA GCC GAG TTG CGA GAC AAG TAC GAG GGC AAG CTG GAG         1031
Pro Phe Ser Glu Ala Glu Leu Arg Asp Lys Tyr Glu Gly Lys Leu Glu
        290                 295                 300

AAA GAG ATC TCC GGG CCG GTG TAC GAG GTG ATG GGC AAA GTG ATG AAG         1079
Lys Glu Ile Ser Gly Pro Val Tyr Glu Val Met Gly Lys Val Met Lys
    305                 310                 315

GTG CTG ATC GGT CGA AAA ATT ACC GGA CCC GGT AAC TTT ATC GGA CAC         1127
Val Leu Ile Gly Arg Lys Ile Thr Gly Pro Gly Asn Phe Ile Gly His
320                 325                 330                 335

TCT GGC ACG GCT GCA GTG GGC TGC TCG TTC AAG GCT GCA GCT GGA TAT         1175
Ser Gly Thr Ala Ala Val Gly Cys Ser Phe Lys Ala Ala Ala Gly Tyr
                340                 345                 350

CTG TAT CCC CTG GAG CGA GGA TTC ATC TAT ATC CAC AAG CCA CCG CTG         1223
Leu Tyr Pro Leu Glu Arg Gly Phe Ile Tyr Ile His Lys Pro Pro Leu
            355                 360                 365

CAT ATC CGC TTT GAG GAG ATT AGT TCT GTG AAC TTT GCC CGC AGC GGC         1271
His Ile Arg Phe Glu Glu Ile Ser Ser Val Asn Phe Ala Arg Ser Gly
        370                 375                 380

GGA TCC ACG CGA TCT TTC GAC TTC GAA GTG ACG CTC AAG AAC GGA ACT         1319
Gly Ser Thr Arg Ser Phe Asp Phe Glu Val Thr Leu Lys Asn Gly Thr
    385                 390                 395

GTT CAC ATC TTC TCC TCC ATC GAG AAG GAG GAG TAT GCC AAG CTC TTC         1367
Val His Ile Phe Ser Ser Ile Glu Lys Glu Glu Tyr Ala Lys Leu Phe
400                 405                 410                 415
```

FIG. 8C

SUBSTITUTE SHEET

```
GAC TAC ATC ACA CAG AAG AAG TTG CAT GTC AGC AAC ATG GGC AAG GAC      1415
Asp Tyr Ile Thr Gln Lys Lys Leu His Val Ser Asn Met Gly Lys Asp
                420                 425                 430

AAG AGC GGC TAC AAG GAC GTG GAC TTT GGT GAT TCG GAC AAC GAG AAC      1463
Lys Ser Gly Tyr Lys Asp Val Asp Phe Gly Asp Ser Asp Asn Glu Asn
            435                 440                 445

GAA CCA GAT GCC TAT CTG GCT CGC CTC AAG GCT GAG GCG AGG GAA AAG      1511
Glu Pro Asp Ala Tyr Leu Ala Arg Leu Lys Ala Glu Ala Arg Glu Lys
        450                 455                 460

GAG GAG GAC GAC GAC GAT GGC GAC TCG GAT GAA GAG TCC ACG GAT GAG      1559
Glu Glu Asp Asp Asp Asp Gly Asp Ser Asp Glu Glu Ser Thr Asp Glu
    465                 470                 475

GAC TTC AAG CCC AAC GAG AAC GAG TCC GAT GTG GCC GAG GAG TAT GAC      1607
Asp Phe Lys Pro Asn Glu Asn Glu Ser Asp Val Ala Glu Glu Tyr Asp
480                 485                 490                 495

AGC AAC GTG GAG AGT GAT TCG GAC GAT GAC AGC GAT GCT AGT GGC GGC      1655
Ser Asn Val Glu Ser Asp Ser Asp Asp Asp Ser Asp Ala Ser Gly Gly
                500                 505                 510

GGA GGC GAC AGC GAC GGC GCC AAG AAA AAG AAG GAG AAG AAG TCC GAG      1703
Gly Gly Asp Ser Asp Gly Ala Lys Lys Lys Lys Glu Lys Lys Ser Glu
            515                 520                 525

AAG AAA GAG AAA AAG GAG AAA AAA CAC AAG GAG AAG GAG AGA ACA AAG      1751
Lys Lys Glu Lys Lys Glu Lys Lys His Lys Glu Lys Glu Arg Thr Lys
        530                 535                 540

AAA CCC TCC AAG AAG AAG AAG GAC TCT GGC AAA CCC AAG CGC GCC ACC      1799
Lys Pro Ser Lys Lys Lys Lys Asp Ser Gly Lys Pro Lys Arg Ala Thr
    545                 550                 555
```

FIG. 8D

SUBSTITUTE SHEET

```
ACC GCT TTC ATG CTC TGG CTG AAC GAC ACG CGC GAG AGC ATC AAG AGG      1847
Thr Ala Phe Met Leu Trp Leu Asn Asp Thr Arg Glu Ser Ile Lys Arg
560                 565                 570                 575

GAA AAT CCG GGC ATA AAG GTT ACC GAG ATC GCC AAG AAG GGC GGC GAG      1895
Glu Asn Pro Gly Ile Lys Val Thr Glu Ile Ala Lys Lys Gly Gly Glu
                    580                 585                 590

ATG TGG AAG GAG CTG AAG GAC AAG TCC AAG TGG GAG GAT GCG GCG GCC      1943
Met Trp Lys Glu Leu Lys Asp Lys Ser Lys Trp Glu Asp Ala Ala Ala
            595                 600                 605

AAG GAC AAG CAG CGC TAC CAC GAC GAG ATG CGC AAC TAC AAG CCT GAA      1991
Lys Asp Lys Gln Arg Tyr His Asp Glu Met Arg Asn Tyr Lys Pro Glu
        610                 615                 620

GCG GGC GGT GAC AGC GAC AAC GAG AAG GGT GGA AAG TCC TCC AAG AAG      2039
Ala Gly Gly Asp Ser Asp Asn Glu Lys Gly Gly Lys Ser Ser Lys Lys
    625                 630                 635

CGC AAG ACG GAG CCT TCT CCA TCC AAG AAG GCG AAT ACC TCG GGC AGC      2087
Arg Lys Thr Glu Pro Ser Pro Ser Lys Lys Ala Asn Thr Ser Gly Ser
640                 645                 650                 655

GGC TTC AAG AGC AAG GAG TAC ATT TCG GAC GAC GAC TCC ACC AGC TCC      2135
Gly Phe Lys Ser Lys Glu Tyr Ile Ser Asp Asp Asp Ser Thr Ser Ser
                660                 665                 670

GAC GAC GAG AAG GAC AAC GAG CCT GCC AAG AAG AAG AGC AAG CCC CCA      2183
Asp Asp Glu Lys Asp Asn Glu Pro Ala Lys Lys Lys Ser Lys Pro Pro
            675                 680                 685

TCC GAC GGC GAT GCC AAG AAG AAA AAG GCC AAG AGC GAG AGC GAA CCG      2231
Ser Asp Gly Asp Ala Lys Lys Lys Lys Ala Lys Ser Glu Ser Glu Pro
        690                 695                 700
```

FIG. 8E

```
GAG GAG AGC GAG GAG GAC AGC AAT GCC AGC GAT GAG GAT GAG GAA GAT      2279
Glu Glu Ser Glu Glu Asp Ser Asn Ala Ser Asp Glu Asp Glu Glu Asp
    705                 710                 715

GAG GCC AGT GAT TAGGGCCATA AACACAACAA ATCAATTCCA TAAACACACA          2331
Glu Ala Ser Asp
720

CCACGCTCCT CACACACCCA TGTCCCAAAT CTAGTTTACA TTCGCCGGAA TTC           2384
```

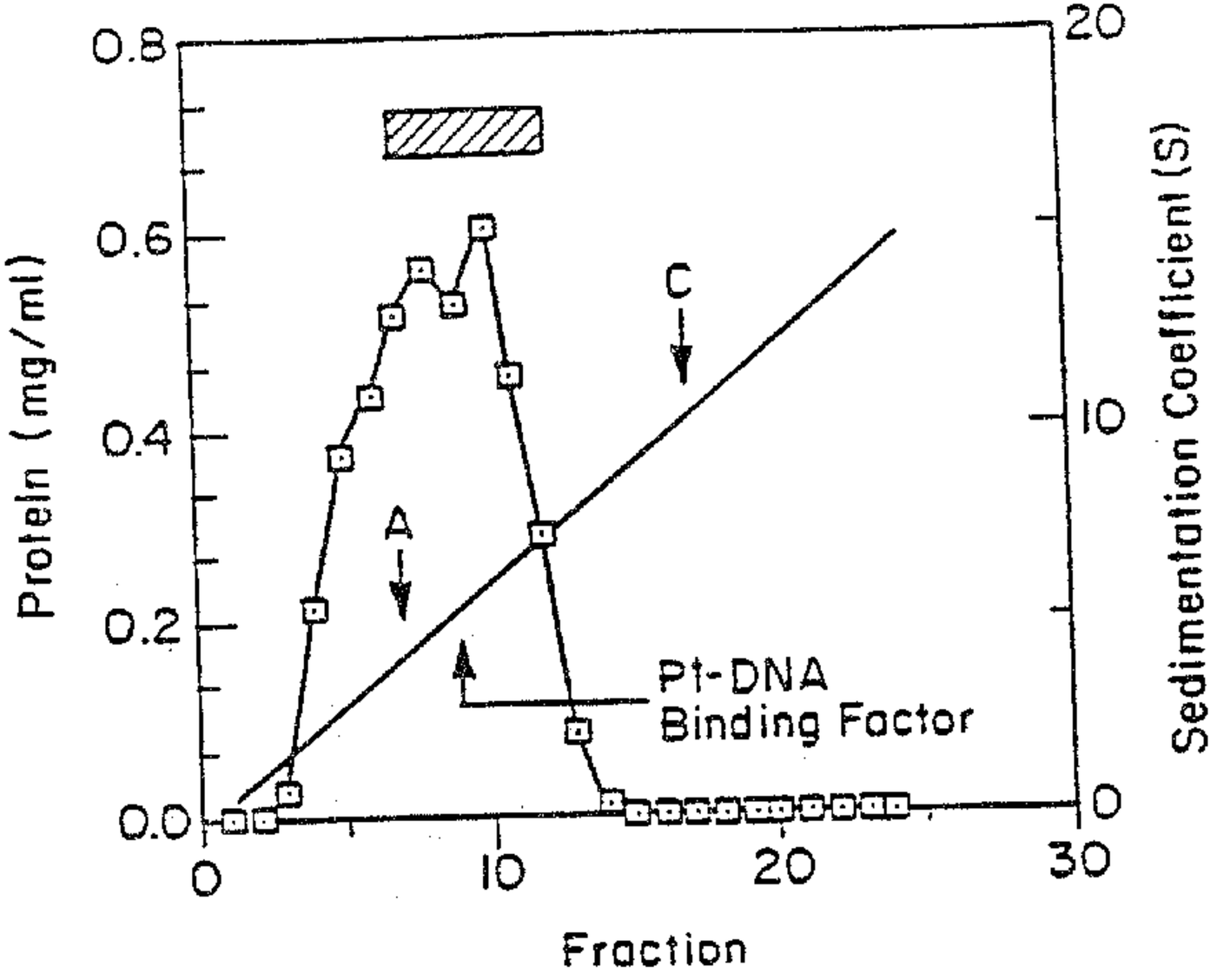
FIG. 8F

```
      123456789012345678901234567890123456789012345678901234567890123456789012345
    1 GAATTCGGGTTTCAAGCCCAGCCTCAACAACAACAACAGCAGCAGCAGCAACAACAGCAACAACAACAAGCGCCT
   76 TATCAAGGTCACTTCCAGCAGTCGCCTCAACAACAACAGCAAAATGTTTATTTTCCACTACCTCCACAATCTTTG
  151 ACGCAACCTACTTCGCAGTCGCAACAACAACAACAACAGTATGCTAATTCGAACTCAAATTCAAACAACAATGTT
  226 AATGTTAACGCGCTACCTCAGGATTTCGGTTACATGCAACAAACCGGATCGGGCCAAAACTATCCGACGATCAAT
  301 CAACAACAATTTTCCGAGTTTTACAACTCCTTTTTAAGTCATTTAACTCAAAAACAGACAAACCCTTCTGTCACG
  376 GGTACAGGCGCGTCTAGTAACAACAACAGTAACAACAACAATGTTAGTAGCGGCAATAACAGCACTAGCAGTAAT
  451 CCTACCCAGCTGGCAGCCTCCCAATTAAACCCTGCCACGGCTACTACGGCCGCCGCAAACAATGCTGCTGGCCCG
  526 GCTTCGTACTTGTCTCAGCTCCCACAGGTGCAGAGATACTACCCGAACAACATGAACGCTCTGTCTAGTCTTTTG
  601 GACCCTTCCTCTGCAGGAAATGCTGCAGGAAATGCCAACACCGCTACTCATCCTGGTTTGTTACCACCCAATCTG
  676 CAACCTCAATTGACTCACCACCAGCAGCAGATGCAGCAACAGCTGCAATTACAACAACAACAGCAGTTGCAGCAA
  751 CAGCAGCAGCTACAACAGCAACACCAGTTGCAACAACAACAACAACTTCAACAACAACATCATCATCTACAACAG
  826 CAACAGCAGCAACAACAGCATCCAGTGGTGAAGAAATTATCTTCCACTCAAAGCAGAATTGAGAGAAGAAAACAA
  901 CTGAAAAAGCAAGGCCCAAAGAGACCTTCTTCCGCTTATTTCCTGTTTTCTATGTCCATAAGAAATGAGTTGCTT
  976 CAACAATTCCCTGAAGCAAAGGTCCCCGAATTGTCTAAATTGGCTTCTGCAAGGTGGAAAGAGTTAACGGATGAT
 1051 CAAAAAAAACCATTCTACGAAGAATTCAGAACCAACTGGGAGAAGTACAGAGTTGTGAGAGATGCTTACGAAAAG
 1126 ACTTTGCCCCCAAAGAGACCCTCTGGTCCCTTTATTCAGTTCACCCAGGAGATTAGACCTACCGTCGTCAAGGAA
 1201 AATCCTGATAAAGGTTTAATCGAAATTACCAAGATAATCGGTGAAAGATGGCGCGAGTTAGACCCTGCCAAAAAG
 1276 GCGGAATACACTGAAACTTACAAGAAAAGATTAAAGGAATGGGAAAGTTGTTATCCCGACGAAAATGATCCAAAC
 1351 GGTAACCCAACCGGTCACTCACATAAGGCCATGAACATGAATTTGAATATGGACACTAAAATCATGGAGAACCAA
 1426 GACAGTATCGAGCACATAACCGCAAATGCCATCGACTCAGTTACCGGAAGCAACAGTAACAGTACCAACCCAAAT
 1501 ACGCCCGTTTCTCCTCCGATTTCATTACAGCAGCAGCCGCTCCAACAACAACAACAACAGCAGCAACAACAACAA
 1576 CACATGTTATTGGCTGACCCCACTACAAATGGTTCGATCATAAAAAATGAATAACAAATAAACAACTTTAGTTTT
 1651 CCACTGTAACATTATCCGACGCAAACAACGAGAATAAGGAATTCGAATTCCTTTTTCAACATTTGTTTTATATTG
 1726 TACTACTCTATTTCCTATTACTACAAATTTTACTTTATTTAATAATAATTTTTCTTTCCCTTTTTCTAACTTCAG
 1801 TCTATATGTATTTGCCTGTATACATATACGCATGTGTGTAGTCTTCCCTCCTTCTTGTTTTTGTAATATACTTAA
 1876 GCCAAATTCAAGTTTGCCTCTGATGCTGTGCGAGCTCAACTGACGAGCGTGATGAAGCCAAAAAAATTAATTGAT
 1951 TTCGCCCAGATCGAACTGGGGACTTGCTGCGTGTTAAGCAGATCCATAGCGACTAGACCACGAAACCTATTAATC
 2026 TGTAAAATTGATCATTTTAAAGTGGCATAGTTGTACGATACACAAGGGCGACTTATCAACTTACACATAAATATG
 2101 TTTGAAACATGTCAGAAACACTCGTTACAAAGCAGACAAAATTTATTACATCAAACGATACCCTGCCTAGACAAA
 2176 CCAGTTAAACGTTGTAAATACCTGGACAACTAGTTTAGTTCCGAGATTCTGCGCTTCCATTGAGTCTTATGACTG
 2251 TTTCTCAGTTTTCATGTCATCTTTTGACGCCGCATGGGATAATGTGTACTAATAACATAAATACTAGTCAATAGA
 2326 TGATATTACGATTCCATCCACAAAGGTGAGGTGCTAGTCACCACCTAAGGATATTAGATTGTCAAGATGCCCGCT
 2401 ATTACTGGAGCCCTTAGTATAACGGATATTTTCAGGATAGCAGACTTACTTCTCCAAGTGTAAGGGAACACCGAA
 2476 TCTAAAGTAGCTACTGCTCCTCCATTCCGTGTATATAATCTTGCTTTTTTTTTAGGAAAATACTAATACTCGCATA
 2551 TATTGGTTATTATCATTACTTGGACACTGTCTGTTCTATCGCTTCATTTGTAATATGCGTATTGCCCTTCTTATT
 2626 AATTGGCTAATATTTCACCTGCAACATAGGTCCCTGTTGATTAACGTGTTTATCCATTTCAATCATGAGAAATGT
 2701 TTCTTCTGTTTTCCAATGCCTGGCCGAGCTGGTAATATATATATATATATGTACATAATACTTTATTAGATATAT
 2776 TGTTGATGATTAGTAGACAAGTGGTACTACCAACCGAGAATAAAAGCTGGTCTTCTTATATAATATGAGTATGGT
 2851 ATAAATAGCAGTCACCGATATCATTGGTTACCCAAAGTGACAATTCATGTCTTTCATAGATATAAATCGTAAGCT
 2926 AAAATTGAATTAAAAGATCTTTAATTTAGCTGCCCTGCTAATCTGAAGTCACATATCATTCCTCATTCTGGATCA
 3001 CTCACAACATTTATTGTCTAATTACTTATGTAATCACTATAGTCACTGGTGTGAACAATGTGAGCAATAATAAAC
 3076 CACTGTATTACCATATACAAATGCATATGTTTAGCCACATAAGTTTAATTTATATTTCTTATTTTCCACACGATA
 3151 TCCCCACTATCAATGACATAGATGATATTTTCTCCACTGGAACAACCTGAATACAACAATATATTATTTGTTCAA
 3226 GTACCGCTTCAGAAATTAAATACTCTGTAATTTTGACCCCTTCTAGCACCATATGTACCCCGAATTC
```

FIG. 11

```
    12345678901234567890123456789012345678901234567890
51  EFGFQAQPQQQQQQQQQQQQQQQAPYQGHFQQSPQQQQQNVYFPLPPQSL
101 TQPTSQSQQQQQQYANSNSNSNNNVNVNALPQDFGYMQQTGSGQNYPTIN
151 QQQFSEFYNSFLSHLTQKQTNPSVTGTGASSNNNSNNNNVSSGNNSTSSN
201 PTQLAASQLNPATATTAAANNAAGPASYLSQLPQVQRYYPNNMNALSSLL
251 DPSSAGNAAGNANTATHPGLLPPNLQPQLTHHQQQMQQQLQLQQQQQLQQ
301 QQQLQQQHQLQQQQQLQQQHHHLQQQQQQQQHPVVKKLSSTQSRIERRKQ
351 LKKQGPKRPSSAYFLFSMSIRNELLQQFPEAKVPELSKLASARWKELTDD
401 QKKPFYEEFRTNWEKYRVVRDAYEKTLPPKRPSGPFIQFTQEIRPTVVKE
451 NPDKGLIEITKIIGERWRELDPAKKAEYTETYKKRLKEWESCYPDENDPN
501 GNPTGHSHKAMNMNLNMDTKIMENQDSIEHITANAIDSVTGSNSNSTNPN
551 TPVSPPISLQQQPLQQQQQQQQQQQHMLLADPTTNGSIIKNE*
```

FIG. 12


0.8
0.6
0.4
0.2
0.0
Protein (mg/ml)
0
10
20
30
Fraction
20
10
0
Sedimentation Coefficient (S)
A
C
Pt-DNA
Binding Factor