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# Direct Cellular Responses to Platinum-Induced DNA Damage

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## 1. Introduction

Since the serendipitous discovery of the biological activity of cisplatin,[1] the drug has been applied extensively in cancer chemotherapy.[2] Platinum-based drugs such as cisplatin, carboplatin, and oxaliplatin (Figure 1) are widely used against various solid tumors including genitourinary, colorectal, and non-small cell lung cancers.[3] Cisplatin is particularly effective in the treatment of testicular cancer with a cure rate of over 90% and nearly 100% when tumors are discovered early.[4] The clinical use of cisplatin, however, is restricted by dose-limiting side effects including nephrotoxicity, emetogenesis, and neurotoxicity.[2] Moreover, many tumor cells display inherent or acquired resistance to platinum-based drugs, which further limits their utility.[5] For over three decades, continuous efforts have been made to alleviate these limitations with a primary focus on the development of new platinum drugs. Over 3000 platinum compounds have been synthesized and tested for their biological activity.[6] Of these, however, fewer than 30 compounds have entered clinical trials.[7] Attempts to develop new anticancer platinum drugs have encountered difficulties in overcoming the drawbacks of cisplatin in actual clinical tests. At present, only four platinum drugs are registered as marketed drugs (cisplatin, carboplatin, oxaliplatin, and nedaplatin) and only one compound (oxaliplatin) has been approved by the FDA (for colorectal cancer) since the release of cisplatin and carboplatin (Figure 1).[5,8,9]

A better understanding of the cellular responses to platinum compounds would both aid in the design of novel platinum-based anticancer agents and suggest strategies for improving the effectiveness of cancer therapy with the existing drugs. Although platinum compounds bind to a variety of cellular targets, many of them a source of toxicity

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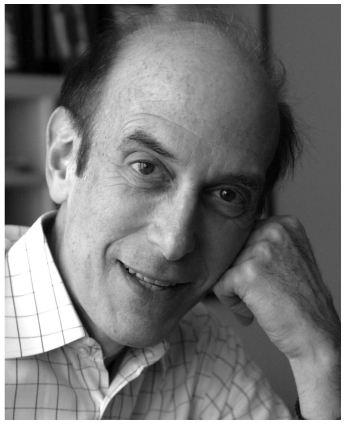

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and undesired side effects,[10] DNA binding is the main biological event that triggers the anticancer properties of the platinum drugs.[11] The mechanism of action by which cisplatin manifests its selective toxicity to tumor cells is complex and includes cellular uptake and transport of the drug to the nucleus, formation of DNA adducts in chromatin, and recognition by damage-response proteins.[11] Subsequent signal transduction pathways activated by this interaction between platinum−DNA damage and other nuclear proteins lead to cell-cycle arrest, attempts to repair the DNA lesions, and apoptosis or necrosis. The results of these processes decide the fate of treated cells.[12] Knowledge of the precise mechanisms by which cisplatin triggers these actions is still incomplete. In particular, there is a considerable gap in our understanding of the mechanisms by which the platinum drugs enter cells and how platinum−DNA damage initiates various cellular signaling pathways.

The present review focuses on cellular processes that lead to formation of platinum−DNA adducts and the early events that subsequently transpire. The initiation of downstream signaling pathways primarily occurs through platinum−DNA adduct recognition by a number of cellular proteins.[13] Proteins that encounter platinum−DNA lesions can be divided into two classes. One class comprises proteins that selectively recognize severely distorted DNA generated by formation of platinum−DNA cross-links. They include DNA-damage recognition proteins. The other category of proteins, which are involved in DNA packaging or DNA-dependent functions, are in frequent contact with the duplex. These proteins, such as histones and DNA and RNA polymerases, inevitably encounter platinum−DNA adducts. Here we review recent information about how platinum complexes enter cells and discuss the interactions of cellular proteins with platinum−DNA adducts as well as the effects that these adducts have on proteins that are involved in various DNA-related processes. The topics discussed are chosen to offer a useful guide for understanding how platinum−DNA damage provokes subsequent cellular pathways, the ultimate goal being to provide a rational basis for the development of better therapeutic strategies with platinum-based agents.[14,15] Other aspects of cellular processes that mediate cisplatin cytotoxicity or confer drug resistance are reviewed elsewhere.[16−18]

## *2. How Do Platinum Drugs Enter the Cancer Cell?*

The conveyance of platinum compounds across the cancer cell membrane is the first step toward successful therapy. Subsequently, activation and translocation of the platinum complex to the nucleus must occur for DNA binding to ensue. In a sense, these sequential steps of drug entry and target modification resemble the substrate binding and activation components of an enzyme reaction mechanism. If either is slow or can be inhibited, activity will be compromised. The first step, entry into the cell, has the potential to target platinum drugs to cancer cells or, if that strategy is not possible, at least to specific tissues where the tumor resides. Either possibility would be valuable in reducing dose-limiting side effects.

### 2.1. Passive Diffusion as a Cell Entry Mechanism

For many years it had been taken for granted that *cis*-[Pt-$(NH_3)_2Cl_2$] enters cells largely by passive diffusion, based on early experiments and knowledge of the aquation chemistry of the compound. Early studies revealed the platinum concentration to be the rate-limiting factor for drug accumulation inside cells, and the uptake was not saturable.[19−21] In addition, cellular entry of cisplatin was not inhibited by its structural analogues.[22] In aqueous solution, cisplatin undergoes stepwise aquation reactions in which the chloride ions are replaced by water ligands with retention of the cis configuration (Figure 2).[23] The loss of chloride ions results in formation of cationic mono- and diaqua complexes. Upon administration to the bloodstream as an intravenous injection, cisplatin maintains a relatively stable neutral state, because of the high concentration of chloride ion (∼100 mM), until the drug enters the cell. Inside the cell; however, the lower ambient chloride ion concentration (∼4−12 mM) facilitates cisplatin aquation to form the cationic aqua complexes (Figure 2). Thermodynamic analysis with data obtained for a model platinum(II) complex,

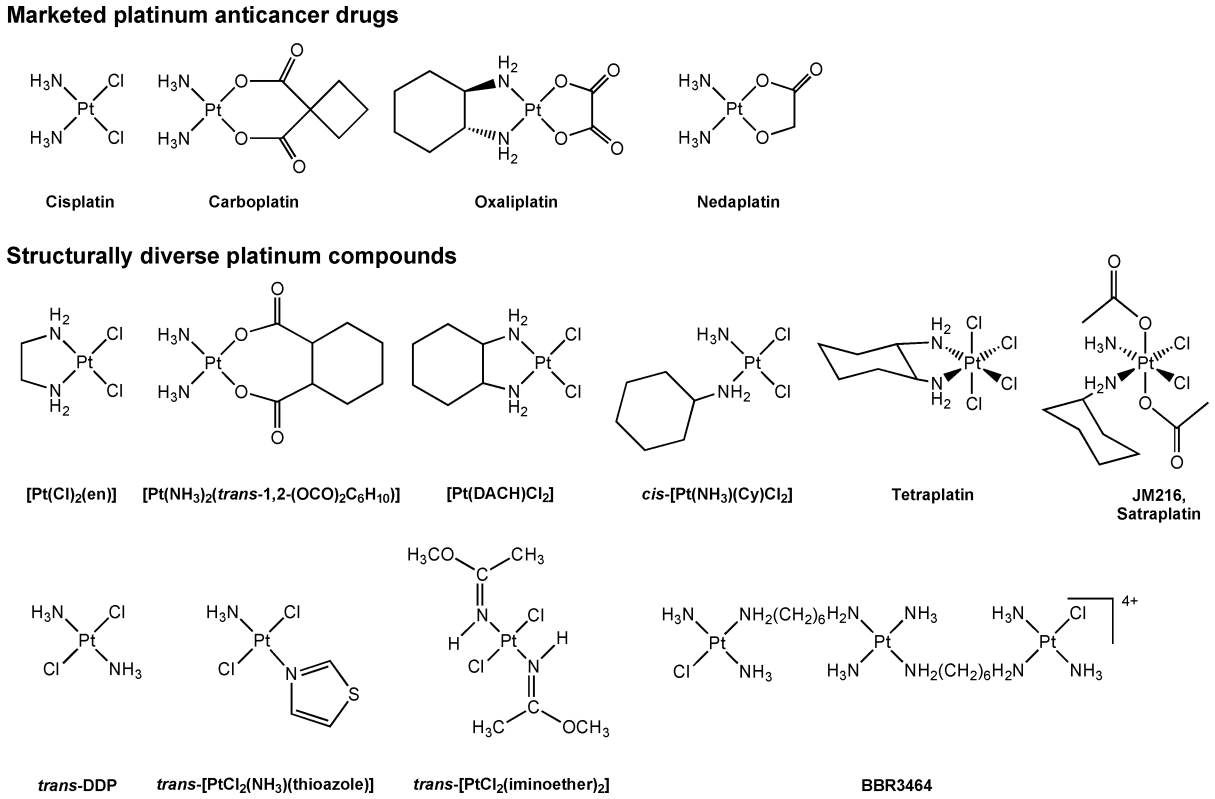


**Figure 1.** Chemical structures of platinum compounds including marketed platinum anticancer drugs.

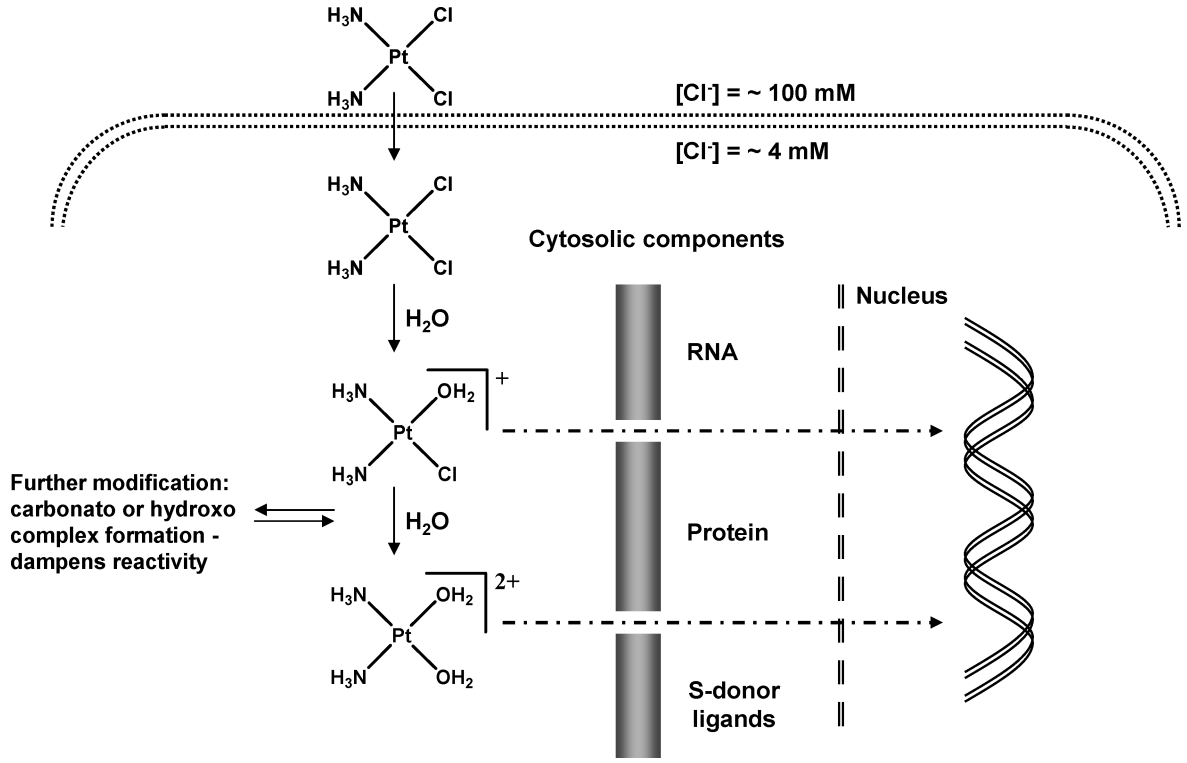


**Figure 2.** Aquation chemistry of cisplatin in the cell.

$[Pt(en)Cl_2]$, suggested that ~42% of the total platinum is maintained in these aquated species.[24] Cationic molecules with no hydrocarbon component are rarely able to diffuse through the lipid bilayer that constitutes biological membranes. The aqua derivatives of cisplatin, therefore, may not readily diffuse back out of the cell before binding to intracellular targets, most notably DNA. This behavior, conversion of cisplatin to a form trapped within the cell, may be a significant contributor to the potent cytotoxicity of the drug following its passive diffusion into cells without prior chemical modification (see also section 3.1).

## 2.2. Facilitated Cellular Uptake and Efflux of Platinum Complexes

Evidence for a role of active transporters in the uptake and efflux of cisplatin and other platinum compounds has

**Table 1. Sensitivity of Cells Expressing OCTs to Structurally Diverse Platinum Complexes[a]**

| platinum complexes[b] | MDCK-MOCK ($\mu$M)[c] | MDCK-hOCT1 ($\mu$M)[c] | sensitization factor[d] | HEK-MOCK ($\mu$M)[c] | HEK-hOCT2 ($\mu$M)[c] | sensitization factor[d] |
|---|---|---|---|---|---|---|
| cisplatin | 6.3 ± 0.74 | 3.6 ± 0.30 | 1.7 | 2.6 ± 0.52 | 1.2 ± 0.54 | 2.1 |
| carboplatin | 260 ± 86 | 230 ± 86 | 1.1 | 110 ± 46 | 62 ± 46 | 1.8 |
| $[Pt(NH_3)_2(trans\text{-}1,2\text{-}(OCO)_2C_6H_{10})]$ | 21 ± 29 | 11 ± 27 | 2.0 | 19 ± 5.7 | 9.9 ± 2.8 | 1.9 |
| $[Pt(en)Cl_2]$ | 33 ± 12 | 10 ± 48 | 3.3 | 6.6 ± 1.5 | 1.1 ± 0.42 | 6.0 |
| $cis\text{-}[Pt(NH_3)(Cy)Cl_2]$ | 1.4 ± 0.15 | 0.16 ± 0.030 | 9.0 | 0.22 ± 0.043 | 0.020 ± 0.0065 | 11 |
| oxaliplatin | 11 ± 37 | 0.48 ± 0.19 | 22 | 4.1 ± 1.69 | 0.11 ± 0.020 | 37 |
| [Pt(*S*,*S*-DACH)oxalate] | 30 ± 14 | 1.4 ± 1.2 | 21 | 9.0 ± 1.7 | 0.27 ± 0.062 | 33 |
| $[Pt(R,R\text{-DACH})Cl_2]$ | 15 ± 3.2 | 0.65 ± 0.26 | 23 | 2.1 ± 0.28 | 0.074 ± 0.026 | 28 |
| $[Pt(S,S\text{-DACH})Cl_2]$ | 16 ± 3.7 | 0.57 ± 0.18 | 28 | 4.5 ± 0.71 | 0.14 ± 0.041 | 33 |

[a] Values are taken from ref 42. [b] Chemical structures of platinum complexes are provided in Figure 1. [c] The $IC_{50}$ values ($\mu$M) of all the complexes are expressed as mean ± SD of six experiments. [d] The sensitization factor is defined as the ratio of the mean $IC_{50}$ value in the MOCK cells to that in the OCT-transfected cells.

been available in the literature for some time.[22] For example, multiple studies demonstrated that reactive aldehydes inhibit cisplatin accumulation in cells, possibly by modifying membrane proteins.[25,26] Recently, a series of experiments have indicated a direct link between copper transporters and the uptake and efflux of platinum compounds.[27] The first clue came when cisplatin resistance was observed following transfection of a copper-transporting P-type ATPase (ATP7B), a key player in copper homeostasis, into human epidermoid carcinoma cells, which enhanced the efflux of the drug.[28] A direct connection between the presence of a copper transporter and cisplatin uptake, however, was discovered in a transposon mutagenesis experiment in yeast.[29] Yeast cells lacking the copper uptake protein Ctr1 displayed increased resistance to cisplatin and decreased accumulation of the drug. The same results were also obtained in mouse embryonic fibroblast cells, and a later study confirmed that Ctr1 mediates the uptake of other platinum drugs including cisplatin analogues.[30] Additional studies with ATP7B as well as ATP7A, another copper transport protein, suggested that these proteins modulate cisplatin levels in cells, presumably by provoking drug efflux.[31−34] The roles of copper transporters in the uptake and efflux of platinum-based anticancer drugs have recently been reviewed.[35] Proteins managing copper homeostasis may participate in regulating the sensitivity of cells to platinum-based drugs, probably by controlling cellular platinum levels.[36−39] A recent study, however, reported that greater cellular accumulation of cisplatin by increased expression of human Ctr1 did not lead to the increased extent of cisplatin−DNA damage.[36] The importance of copper transporters in promoting the anti-tumor activity of platinum compounds is therefore uncertain at this point.

In addition to copper transporters, the facilitated delivery of platinum compounds into cells has also been associated with the presence of organic cation transporters, or OCTs. In human renal proximal tubules cisplatin uptake was mediated by OCT2, but not OCT1, and an OCT substrate suppressed cisplatin-induced apoptosis.[40] The accumulation of cisplatin was also greater in HEK293 cells stably expressing rat OCT2 than in mock-transfected cells.[41] Both studies suggest OCT2 as the critical transporter for cisplatin nephrotoxicity and that OCT2-specific antagonists may provide an important modality for managing nephrotoxicity in clinical applications. A very recent study examined the roles of human OCTs in tumor-specific activities of various platinum complexes by using colon cancer cell lines.[42] Human OCT1 and 2 clearly increased accumulation and cytotoxicity of oxaliplatin but not cisplatin or carboplatin. Only oxaliplatin is active against colorectal tumors.[43] This study was further expanded to investigate several structural analogues for their ability to enter and kill cells expressing human OCTs (Table 1). The nature of the nonleaving group coordinated to platinum, such as the DACH moiety in oxaliplatin, is a key determinant for selective uptake of these platinum complexes by OCTs. In addition, since the *R*,*R* and *S*,*S* isomers of oxaliplatin and $[Pt(DACH)Cl_2]$ are equally taken up by the cells and have the same cytotoxicity, their differential anticancer properties must lie elsewhere than in OCT-mediated drug uptake. Only the *R*,*R* isomer is clinically effective.

With the availability of this information, it has become clear that platinum drugs enter the cell through multiple routes including both passive and active mechanisms. In addition, each of these cell-entry pathways is likely to influence differently the cellular accumulation of a platinum compound, depending on its chemical composition and structure. Additional work is needed to understand fully how the cellular levels of platinum are managed by passive diffusion, copper homeostasis proteins, OCTs, and other as yet unidentified transporters.

## *3. Modifying Cellular DNA by Platinum-Based Anticancer Drugs*

Inside the cell, the activated platinum drug reacts with various cellular components, including DNA as the main biological target responsible for anticancer activity. The details by which platinum complexes reach their biological targets, however, are not yet fully revealed. The inorganic chemistry of the drug in the cellular context and the routes to DNA in the nucleus must be addressed to understand the antitumor activity of platinum-based drugs. Various platinum agents have unique structural and kinetic properties for DNA binding and display different DNA-adduct profiles. Understanding the nature of these platinum adducts is important for elucidating how these adducts are recognized and processed by cellular proteins.

### 3.1. Inorganic Chemistry of Platinum Drugs Prior to DNA Modification

Once the platinum drug has entered the cell, either as a cation or as the neutral species, aquation and reactions with cellular components will occur. Reactive cellular components include proteins, RNA, DNA, membrane phospholipids, microfilaments, and thiol-containing molecules such as glutathione. The detailed nature of these interactions is not known because of the complexity of the intracellular milieu and the lack of methodologies to investigate these processes.

$^{195}$Pt NMR spectroscopy would be a valuable tool in this regard, but it is too insensitive at sublethal platinum concentrations.[44] The use of $^{195}$Pt-radiolabeled cisplatin in conjunction with HPLC analysis of cellular components would be another useful approach, but the short, 4-day half-life of this isotope makes such experiments challenging.[45−49] As a consequence, much of our information is inferred from studies of the reactions of platinum complexes with aqueous buffer and medium components in vitro.[44,50]

Multiple investigations have identified different forms of platinum complexes in aqueous solution. Following cisplatin aquation, the resulting mono- and diaqua complexes can lose a proton to form hydroxo species.[51] Moreover, several early studies revealed formation of hydroxo-bridged platinum(II) multimers.[24,52] Aqua diammineplatinum(II) complexes are considered to be the most reactive and hydroxo-bridged species the least reactive. Most recently, a series of papers has appeared revealing that carbonate ion may also participate in this chemistry. Under physiological conditions, carbonate, which is an important component in blood and the cytosol, reacts with cisplatin to form carbonate or bicarbonate complexes.[53] These cisplatin−carbonato species have been reported to modify DNA in vitro and form mostly monofunctional adducts.[54,55] Although the authors of these studies proposed that cisplatin binding to DNA most likely takes place through its carbonato forms, it is not clear how such cisplatin−carbonate complexes would convert into biologically potent bifunctional cross-links, which are the major DNA adducts present in cellular DNA obtained from cisplatin-treated patients.[56] Platinum carbonato complexes formed in serum might also modulate the uptake of the drug into cells (section 2).

In order to modify nuclear DNA, cisplatin must traverse a number of cytosolic components and enter the nucleus (Figure 2). Because of the high nucleotide concentration in the cell nucleus, cisplatin, once in the nucleus, will react primarily with DNA. The reaction of cisplatin with other cellular components is postulated to be under kinetic rather than thermodynamic control, a consequence of slow ligand exchange at platinum. This hypothesis explains the fact that cisplatin binds to DNA in the nucleus instead of reacting solely with S-donor ligands such as glutathione and methionine, which form stable platinum complexes.[57] Moreover, platinum migration from S-donor ligands to guanine bases can also occur.[16] The monoaqua cisplatin derivative, $[Pt(NH_3)Cl(OH_2)]^+$ ($t_{1/2}$ of formation, ∼2 h), readily modifies DNA through binding to the N$^7$ atom of a guanine or adenine base to form a monofunctional adduct ($t_{1/2} \approx 0.1$ h).[11,58] The second chloride ligand is aquated with a half-life of ∼2 h, and eventually a bifunctional adduct (intra- or interstrand cross-link) is formed.[44]

Controlling cisplatin aquation and transporting activated cisplatin to biological targets are among the key elements that must be appreciated to comprehend the mechanism of the drug.[57] Irrespective of the details of the chemistry that converts the platinum drugs into their DNA-modifying forms, however, there is extensive evidence that a defined family of DNA adducts forms in which the platinum atom and its two cis N-donor ligands cross-link nucleobases on DNA.[11]

## 3.2. DNA: A Primary Target for Platinum Drugs

The evidence that DNA is the primary target of cisplatin among many potential cellular possibilities has been extensively discussed.[11] Cisplatin-treated bacteria show phenotypes that are characteristic of those evoked by DNA-damaging agents.[59] More convincing proof came from experiments with DNA repair-deficient cells,[60,61] which are universally more sensitive to cisplatin. In addition, the levels of platinum atoms bound to proteins and RNA are too low to exhibit significant inhibitory effects on the targets.[62] Cisplatin modification levels on cellular DNA can be determined by using antibodies raised against various cisplatin−DNA adducts, and these antibodies can also be utilized to define the nature of cisplatin cross-links on DNA.[56,63−65] A significant correlation is usually found between platinum−DNA adduct levels and the sensitivity of treated cells to the drug.[66,67] Although we do not cover this work here, there is evidence that platinum reacts with a number of specific cellular components such as glutathione, as reviewed elsewhere.[5] The significance of these interactions to the antitumor mechanism is unknown, but they are likely at least to affect the general patient toxicity profile.

## 3.3. Nature of Platinum–DNA Adducts

Analysis of purified DNA treated with cisplatin or DNA isolated from cisplatin-treated patients demonstrates the presence of approximately 65% 1,2-d(GpG), 25% 1,2-d(ApG), and 5−10% 1,3-d(GpNpG) intrastrand cross-links as major components.[13] A small percentage of interstrand cross-links and monofunctional adducts are also present. *trans*-Diamminedichloroplatinum(II), *trans*-DDP, a clinically ineffective isomer of cisplatin (Figure 1), is unable to form 1,2-intrastrand cross-links, owing to stereochemical constraints. Carboplatin and oxaliplatin contain different leaving groups than the chloride ions of cisplatin and therefore exhibit different kinetics for DNA binding; they also generate disparate adduct profiles from that of cisplatin.[9] Among various platinum−DNA adducts, intrastrand cross-links have been the focal point of interest in the field of platinum-based anticancer drugs and are therefore the main subject of the present review. To a lesser extent, however, minor but highly detrimental interstrand cross-links have also drawn attention.

Formation of cisplatin adducts significantly alters the structure of the target DNA. Early biochemical studies demonstrated unwinding and bending as well as destabilization of the duplex induced by cisplatin lesions.[68,69] The structural details of platinum−DNA adducts were subsequently elucidated for a number of specific adducts.[11,70] Structures of duplex DNA containing a 1,2- or 1,3-intrastrand cross-link are illustrated in Figure 3.[71−73] The major platinum adducts, the 1,2-intrastrand cross-links, unwind the DNA duplex in the vicinity of the site of platination, bending it toward the major groove and generating a widened and shallow minor groove. On the other hand, the interstrand DNA cross-link formed by cisplatin bends the helix toward the minor groove in which the platinum moiety is now located (Figure 3).[73] Although these platinum adducts display some degree of structural similarity, arising from coordination to the N$^7$ position of the guanine base, it is clear that each distorts duplex DNA in a distinctive manner. Moreover, the structures of DNA adducts formed by platinum drugs with nonleaving groups other than the ammine ligands of cisplatin display additional variations.[9] These nuances may convey distinctive recognition and processing by cellular proteins, which possibly translate also different roles in mediating the cytotoxicity and anticancer properties of the compounds. Much work remains to be done to test this idea.

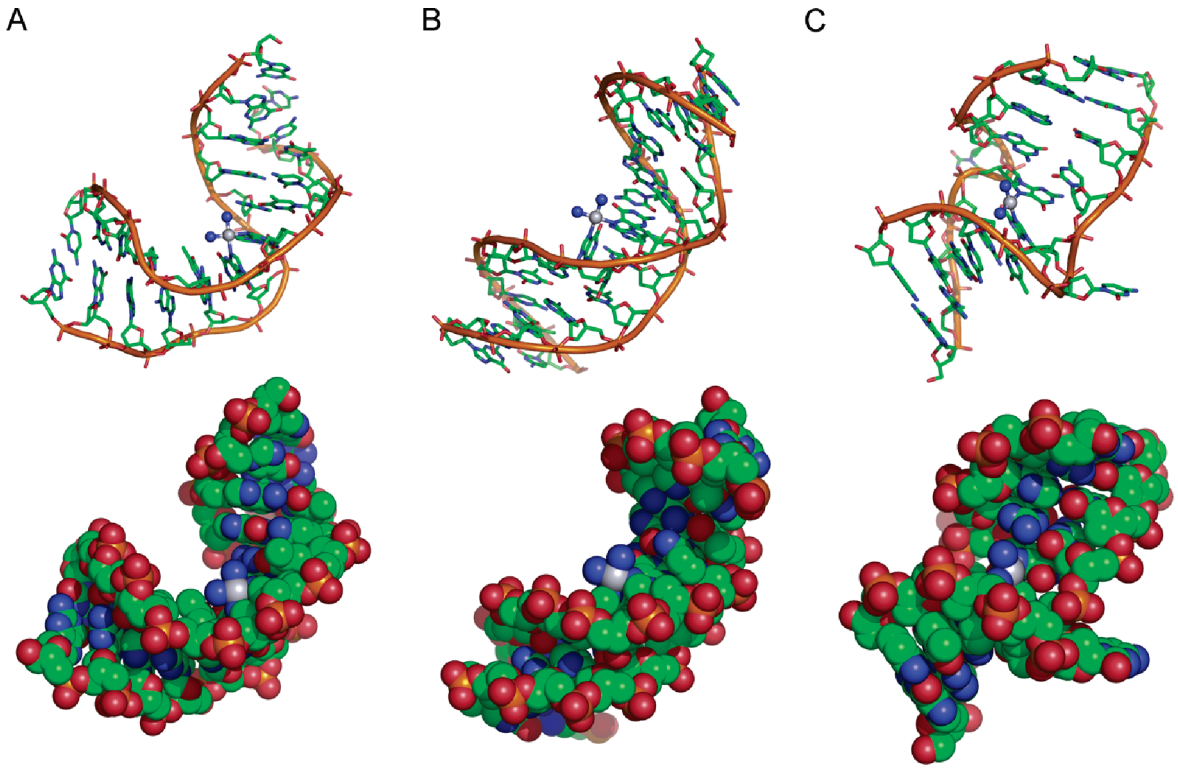


**Figure 3.** Platinum-DNA adduct structures. Duplex DNA containing (A) cisplatin 1,2-d(GpG),[71] (B) 1,3-d(GpTpG) intrastrand,[72] and (C) interstrand[73] cross-links, generated by PyMol. The DNA sequences are d(CCTCTG*G*TCTCC)·d(GGAGACCAGAGG), d(CTCTAG*-TG*CTCAC)·d(GTGAGCACTAGAG), and d(CCTCG*CTCTC)·d(GAGAG*CGAGG) for the cisplatin 1,2-d(GpG), 1,3-d(GpTpG) intrastrand, and interstrand cross-links, respectively. Guanine residues cross-linked by cisplatin at the $N^7$ position are indicated by asterisks.

## *4. Effects of Platinum–DNA Adducts on the Structure and Function of Proteins That Interact Directly with the Genome*

The presence of a kinetically inert bond between a platinum compound and DNA, especially if cross-linking to nearby bases is involved, has several consequences on the structure of protein−DNA complexes and the ability of the duplex to function as a template for replication and transcription. These early events are the trigger points for all subsequent response pathways in the cell, from attempts to repair the damage to the ultimate steps leading to cell death, the desired consequence in cancer treatment. In the present section we describe recent work on the structure of a platinated nucleosome and review the effects of platinum adducts on replication and transcription.

### 4.1. Effects of a Site-Specific Platinum Cross-Link on the Nucleosome Structure

In a eukaryotic nucleus, DNA is wound around basic, positively charged histone proteins forming nucleosomes, which are further compacted into chromatin. Alteration of chromatin properties significantly affects various DNA metabolic processes, such as replication, transcription, and repair. Platinum drugs modify cellular DNA in chromatin in vivo. The binding of a platinum compound to chromosomal DNA as well as processing of the adduct in the cell are expected to be different from similar events involving free DNA. For example, nucleotide excision repair (NER) of nucleosomal DNA containing a site-specific platinum lesion is significantly less efficient than that of free DNA containing the same platinum lesion in cell extracts, as discussed in more detail in section 5.1.[74,75] Moreover, the repair efficiency of damaged nucleosomes depends on the post-translational modification of histone proteins.[75]

#### *4.1.1. Platination of Chromosomal DNA*

The effects of the histone octamer core proteins on the reactivity of platinum compounds with DNA have been studied by using isolated nucleosomes.[45] The majority of the cisplatin adducts appeared to involve only the DNA, and the results closely resemble those for platination of the DNA alone. A parallel study with the inactive isomer of cisplatin, *trans*-diamminedichloroplatinum(II), however, revealed a significantly larger number of DNA−DNA and protein−DNA cross-links. Experiments with polysomes revealed the linker DNA of chromatin to be a preferred target for platinum compounds,[76−78] although this effect was diminished at higher concentrations.[78] The level of cisplatin adducts on DNA depends on the amount and nature of post-translational modification of the histones. An increase in cisplatin-adduct levels occurs in human cancer cell lines following treatment with arginine butyrate, which inhibits histone deacetylases, affording hyperacetylation of histone proteins and promoting chromatin unfolding.[79] Platinum drugs clearly bind more favorably to an open form of chromatin. Structural changes in chromatin by transcription activation[80] or protein binding[81] also modulate cisplatin binding to DNA in human cells.

#### *4.1.2. Effects of Platinum on the Nucleosome Structure*

The influence of cisplatin modification on chromatin structure has been investigated both in vivo and in vitro. In early studies, chicken erythrocyte nuclei and nucleosome core particles were treated with cisplatin and the resulting chromatin or nucleosomes were isolated and digested by micrococcal nuclease[82] and DNase I.[83] The digestion profiles

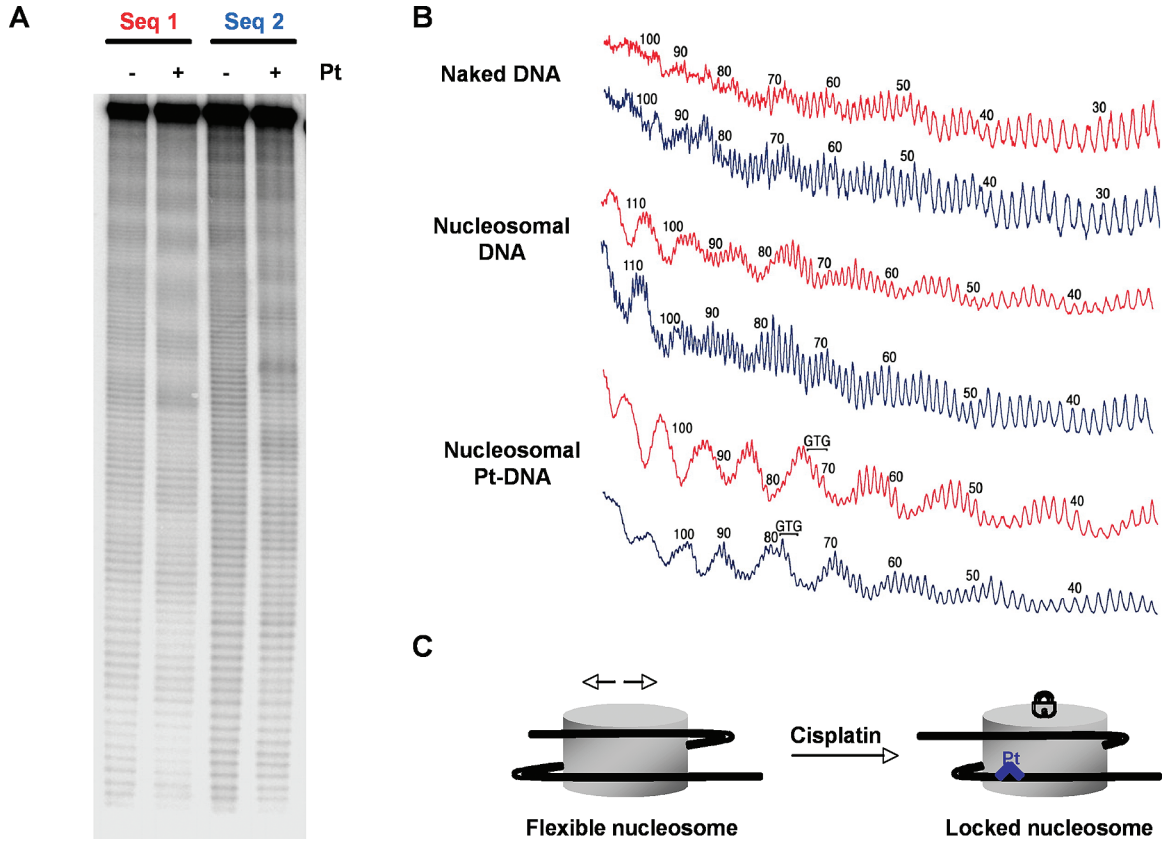


**Figure 4.** Hydroxyl-radical footprinting analysis of cisplatin-modified nucleosomes. (A) A platinated DNA strand was radiolabeled at the 5′ end and hydroxyl radical cleavage products were separated on a denaturing polyacrylamide gel depicted in the figure. (B) Hydroxyl radical cleavage patterns of DNA and nucleosomes. Two DNA sequences (Seq 1, red; Seq 2, blue) were used in the experiment. The d(GpTpG) intrastrand cross-links are denoted. Reprinted with permission from ref 85. Copyright 2004 The American Society for Biochemistry and Molecular Biology. (C) Consequences of cisplatin modification of a nucleosome.

indicated that cisplatin binding does not significantly alter the DNA structure of the nucleosome core particle but rather affects the higher order structure of chromatin. This finding is supported by a later observation that chromatin remodeling and transcription factor binding are severely impaired by cisplatin modification.[84] Cisplatin treatment of HeLa cells itself induces post-translational modification of histones H3 (phosphorylation) and H4 (hyperacetylation),[85] modifications that modulate chromatin structure. It is unclear at this point whether these modifications are direct cellular responses to cisplatin binding to chromatin or indirect results from one or more downstream cellular pathways following cisplatin treatment. In either case, alteration of histone proteins involved in chromatin in response to platinum drugs is a topic of considerable interest and worthy of extensive future exploration.

Recently, hydroxyl-radical footprinting and exonuclease III digestion were employed to analyze the structure of a nucleosome containing a site-specific cisplatin 1,3-intrastrand d(GpTpG) cross-link.[86] Hydroxyl-radical footprinting of a nucleosome shows a distinctive DNA cleavage pattern with an approximately 10-nucleotide periodicity (Figure 4A). This pattern is more evident in cisplatin-modified nucleosomes than in unmodified nucleosomes (Figure 4B), indicating that the platinum cross-link decides the specific rotational setting of the DNA wrapped around the histone octamer. In addition, only platinated nucleosomal DNA displays clear stop sites following exonuclease III digestion. These data reveal that a cisplatin intrastrand cross-link forces the translational positioning of the DNA into a specific arrangement with respect to the core histone proteins. It will be interesting to study the effects of additional intrastrand as well as interstrand cross-links on nucleosome structure, since they may bring about further structural nuances. Enhanced phasing of the nucleosome by cisplatin lesions may explain the effects of the drug on the higher order structure of chromatin.

## 4.2. Effects of Platinum Adducts on DNA Polymerases

The inhibition of DNA synthesis by cisplatin was discovered early and believed to contribute to the cytotoxicity of cisplatin.[87] DNA replication by partially purified human DNA polymerases $\alpha$ and $\beta$ is inhibited by cisplatin treatment of the DNA template.[88] Cisplatin-induced inhibition of DNA replication also occurs in African green monkey CV-1 cells transfected with SV40 chromosomal DNA.[89] More detailed studies of the abilities of different platinum adducts to block a variety of DNA polymerases ensued.[90,91] Most bifunctional adducts, intra- and interstrand cross-links, effectively inhibit DNA polymerases, whereas monofunctional adducts seem not to block the polymerases as effectively. T4 and T7 DNA polymerases, DNA polymerase I, and DNA polymerase III are blocked by platinum adducts, bypassing the lesion only ~10% of the time.[90] Despite the evident inhibition of DNA synthesis by cisplatin based on these reports, murine leukemia L1210 cells treated with cisplatin progress through the S phase of the cell cycle and are arrested only in the G2 phase.[92] DNA replication continues even in the cells that do not divide. Furthermore, a study with Chinese hamster ovary cell lines both proficient and deficient for DNA nucleotide excision repair demonstrated that the inhibition of DNA

synthesis depends only on the concentration of cisplatin and not on the sensitivity of the cell line to the drug.[93] Only the level of cells arrested in the G2 phase correlates with cell line sensitivity to cisplatin. It is therefore likely that direct inhibition of DNA replication by cisplatin−DNA damage is not the key biological event that confers the unique properties of this anticancer agent.[94]

Mammalian cells have the ability to synthesize DNA while ignoring various chemical lesions. The process, called translesion synthesis (TLS), demands specialized DNA polymerases, which are less stringent than the major replicative DNA polymerases and can accommodate damaged bases.[95] In eukaryotes, the Y-family DNA polymerases ($\eta$, $\iota$, $\kappa$, and Rev 1) and DNA polymerase $\zeta$, a member of the B family, replicate across DNA lesions.[96] Translesion synthesis through cisplatin−DNA adducts has been an interesting aspect of DNA synthesis in cisplatin-treated cells because of its correlation to drug sensitivity.[97] Cisplatin-resistant cells exhibit more TLS than drug-sensitive cells.[98−101] Specialized DNA polymerases are overexpressed in many cancer cells[102] and have a role in the cellular tolerance to cisplatin DNA damage.[103] The process also has a critical role in conveying the mutagenic properties of cisplatin because of the nature of TLS, which carries out both error-prone and error-free DNA synthesis.[96] The mutagenicity of cisplatin is closely related to the evolution of resistance of cell lines against the drug. In particular, the reduced ability to replicate cisplatin-damaged DNA decreases the rate at which the cells become resistant to cisplatin. For example, suppression of human DNA polymerase involved in TLS, such as polymerase Rev 1[104] or $\zeta$,[105,106] increases the sensitivity of cells to cisplatin and reduces the rate of appearance of cisplatin resistance.

DNA polymerases that bypass cisplatin adducts in vitro include DNA polymerase $\beta$, $\mu$, and $\eta$, whereas polymerases $\alpha$, $\iota$, $\kappa$, and $\lambda$ are unable to perform TLS past platinum adducts.[107−110] Each DNA polymerase displays a distinct specificity in its lesion-bypass properties, including bypass ability, fidelity, and extension ability. For example, DNA polymerase $\eta$ bypasses platinum adducts most efficiently in error-free TLS, as proved both in vivo and in vitro.[101,111] Polymerase $\mu$ is the most error-prone enzyme, mediating mainly frame-shift mutations.[112] Currently, the identities of DNA polymerases that are responsible for TLS past platinum adducts in vivo are not clear. Moreover, two DNA polymerases often work together to complete TLS.[96] Although polymerase $\zeta$ is unable to bypass certain DNA lesions including those by platinum agents, the enzyme has the ability to extend TLS once nucleotides are inserted opposite DNA adducts by other polymerases.[96,105] Little is known about the TLS past platinum interstrand cross-links, although a single DNA polymerase is not likely to be able to bypass this lesion. It has been suggested, however, that TLS may occur during the repair of interstrand cross-links.[113]

The immediate cellular responses to a stalled replication fork at the site of platinum−DNA lesion are still undefined. Recent studies indicate that proliferating cell nuclear antigen (PCNA) plays a key role for the TLS process by recruiting TLS DNA polymerases to the site of stalled replication forks.[95] It is proposed that, following replication fork blockage, Rad18 binds to exposed single-stranded DNA at the fork and together with Rad6 mediates mono-ubiquitylation of PCNA. Mono-ubiquitylated PCNA then physically interacts with a TLS DNA polymerase to recruit and replace it with the stalled replicative DNA polymerase. The efficiency and fidelity of TLS depends on the nature of the adduct and the recruited polymerase. Oxaliplatin has different properties from cisplatin for replication bypass of its platinated DNA both in vivo and in vitro, presumably due to the sterically bulky *R,R*-1,2-diaminocyclohexane (DACH) carrier ligand (Figure 1).[70] This behavior of oxaliplatin is thought to contribute to its distinct anticancer activity compared to that of cisplatin.

### 4.3. Effects of Platinum Adducts on RNA Polymerases

Early in vitro studies reported the ability of cisplatin adducts to inhibit transcription elongation by various RNA polymerases including wheat germ RNA polymerase II (Pol II) and *E. coli* T7 and SP6 RNA polymerases.[114,115] Similar to the inhibition of DNA synthesis, RNA polymerases are strongly blocked by bifunctional adducts and not by monofunctional adducts. Direct transcription inhibition by cisplatin and *trans*-DDP is observed in human and hamster cell lines that are transfected with a plasmid containing a reporter gene and premodified by platinum compounds.[84,116] A higher level of *trans*-DDP adducts is required to inhibit transcription to the same degree as cisplatin adducts. Accumulated data indicate a close relation between transcription inhibition by cisplatin and the ability of a platinum compound to kill cells.

RNA polymerases are believed to encounter platinum lesions at a relatively early stage in the DNA damage-response process. Approximately 100 copies of RNA polymerase I are constantly transcribing the rRNA gene in the cell.[117] Although the inhibition of RNA polymerase I by platinum adducts has not been directly studied, it is speculated that platinum damage can block this polymerase.[118] RNA polymerase II transcribes most eukaryotic genes and is one of the most abundant proteins, with ~300 000 copies in a single cell.[119] A photobleaching experiment revealed that 25% of this enzyme is persistently associated with cellular DNA to generate mRNA.[120,121] RNA polymerase II has been a major focus of the experiments designed to investigate cellular responses to DNA damage including those by platinum drugs because of its dual roles in the process. Arrested polymerase at the site of the platinum lesion not only functions as a damage recognition factor, triggering transcription-coupled repair (TCR),[122] but also mediates programmed cell death.[123]

Our knowledge of cisplatin adduct-induced blockage of RNA polymerase II has been greatly advanced over the past several years. DNA probes containing a site-specific platinum lesion are employed in transcription assays in vitro with human cell extracts or partially purified human transcription factors.[124,125] Platinum 1,2-(GpG) and 1,3-(GpTpG) intrastrand cross-links strongly block the elongation complex. A study with T7 RNA polymerase revealed that polymerase action is inhibited at multiple sites in the vicinity of the platinum lesion, the nature of which can be altered by the concentration of NTPs and types of platinum adducts.[126] The elongation complex is able to proceed into the site of platinum damage, where the polymerase inserts an incorrect nucleotide UTP, rather than a correct nucleotide CTP, opposite a cisplatin 1,2-(GpG) cross-link. The fate of stalled RNA polymerase II at a platinum lesion has also been closely examined, which can provide useful insight into the mechanism of TCR. Solid-phase in vitro transcription experiments have been employed in multiple studies.[125,127,128] Stalled polymerases are fairly stable but can be released from DNA in an ATP-dependent manner by cellular release factors

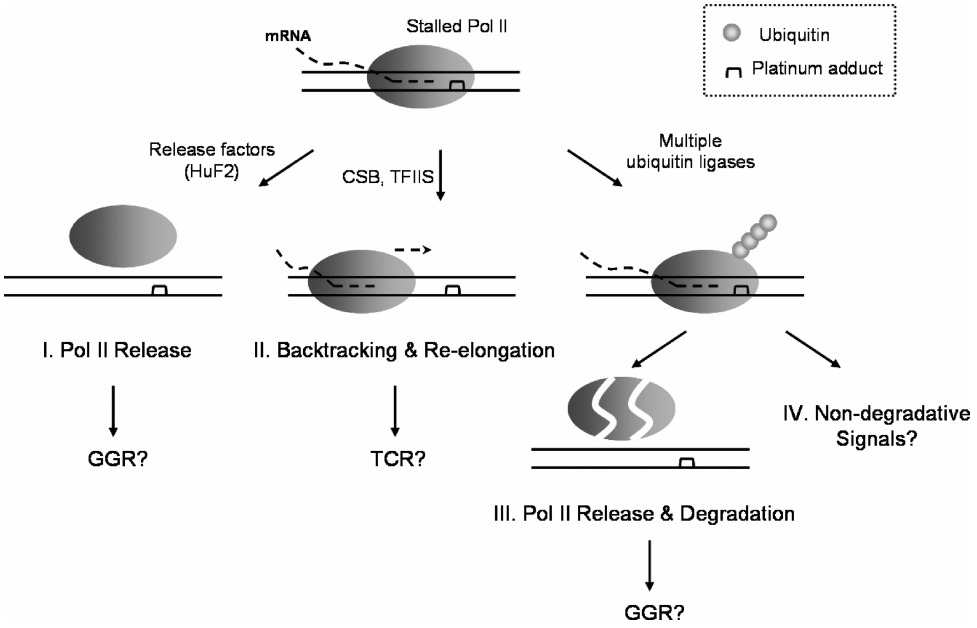


**Figure 5.** Schematic representation of transcription inhibition by platinum lesions and consequent outcomes. Stalled RNA polymerase II (Pol II) triggers transcription-coupled repair (TCR) and global genomic repair (GGR) through multiple pathways.

including human release factor 2 (HuF2).[118,125,129] A differently designed in vitro experiment indicated that a considerable level of stalled polymerase II proteins can remain strongly associated with damaged DNA in cell extracts.[127] This result was supported by a cell fractionation experiment using cisplatin-treated HeLa cells, which demonstrated an increased amount of chromatin-associated polymerase II proteins following DNA damage. These polymerases are able to backtrack from the damage sites, cleave the transcripts, and re-elongate. Various cellular proteins, including CSB and TFIIS, are thought to mediate this process (Figure 5).[125,130] Nucleotide excision repair (NER) can occur in vitro at the DNA damage site with polymerase remaining on the DNA and recruiting repair factors.[128,129]

In mammalian cells, cisplatin treatment facilitates RNA polymerase II degradation following ubiquitylation of the protein.[127,131,132] In vitro transcription experiments with a cisplatin-damaged plasmid also demonstrate ubiquitylation of polymerase II in a transcription-dependent manner.[133] Although ubiquitylation-mediated polymerase degradation is required for DNA damage repair in yeast,[134] the role of this process in human cells is unclear. Recent experiments both in cell extracts and living cells suggest that polyubiquitylation of polymerase II following cisplatin treatment can occur through Lys-6, Lys-48, Lys-63, and possibly other lysines of ubiquitin.[127,135] Ubiquitylation may trigger nondegradative signals or affect the properties of stalled polymerase in addition to its degradative roles.[136] RNA polymerase II degradation is prevented by the proteosomal inhibitor MG132 with a subsequent increase in the relative amount of ubiquitylated polymerase. Fractionation of polymerase II from cells co-treated with MG132 and cisplatin indicates that this additional ubiquitylated polymerase is mostly unbound or only loosely associated with chromatin.[127] Only a fraction of ubiquitylated polymerase II dissociates from damage sites and is destroyed rapidly by proteosomes (Figure 5).

## *5. Repair of Platinum-Damaged DNA*

Following platinum-induced DNA modification, cellular repair systems act to recognize the damage and continuously function until the fate of drug-treated cells is decided. Knowledge of these repair mechanisms involving platinum-damaged DNA provides essential clues to understanding the cellular responses to platinum-based anticancer drugs and for improving the efficacy of therapies.

### 5.1. Nucleotide Excision Repair

NER is a primary process for repairing platinum-damaged DNA. Bacterial and mammalian cells deficient in NER are more sensitive to platinum compounds.[11,137] For example, *xeroderma pigmentosum* (XP) cell lines lacking one or more components of NER are 5- to 10-fold more sensitive to cisplatin than normal cells,[138] and extracts obtained from these cell lines exhibit no repair activity toward cisplatin-modified DNA.[139,140] A cisplatin-resistant tumor cell line displays over 2-fold higher levels of genes producing NER proteins such as XPC, XPA, and ERCC1,[141] with a concomitant higher repair activity of their cell extracts[142] compared to those from wild-type cells. Moreover, enhanced expression of XPC and ERCC1 mRNA is observed in ovarian cancer tissues obtained from patients clinically resistant to cisplatin or carboplatin.[143] It is suggested that the exceptional sensitivity of testicular tumors to cisplatin is a consequence of lower levels of several repair proteins, such as XPA, ERCC1, and XPF, in these cells.[144,145] Recently, the enhanced sensitivity to cisplatin of human cancer cells was observed when ERCC1 was suppressed by small interfering RNA (siRNA).[146,147]

The molecular mechanism by which NER removes platinum intrastrand cross-links from DNA has been extensively studied (Figure 6).[148] During the early stage of NER, platinum lesions are recognized by different mechanisms for two subpathways of NER, transcription-coupled repair (TCR) and global genomic repair (GGR). Stalled RNA polymerase II acts as a damage recognition flag to initiate TCR as discussed above.[122] Cockayne syndrome (CS) proteins, CSA and CSB, participate in this process, although their exact roles are unknown.[128,149] For GGR, damage recognition is initiated by XPC-HR23B.[150,151] After initial recognition of DNA damage, TCR and GGR are thought to follow similar

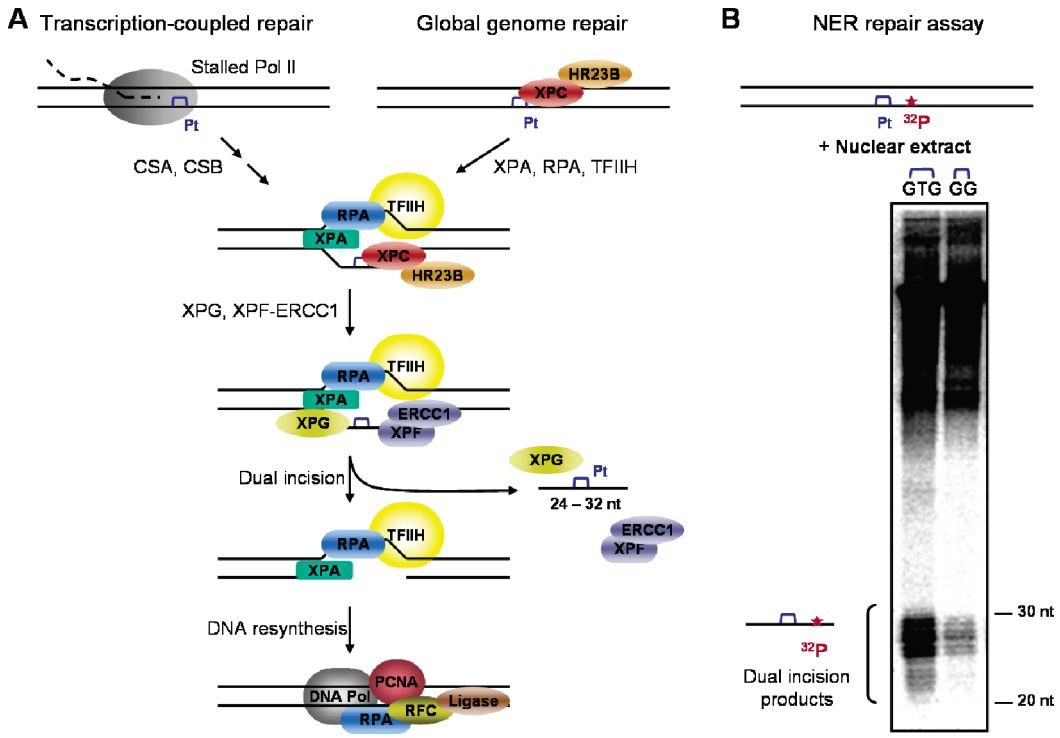


**Figure 6.** (A) Steps in the mechanism of nucleotide excision repair of platinum lesions. (B) Primary data of NER dual incision analysis. The 199 base-pair DNA probe contains a site-specific platinum cross-link and an internal label with $^{32}P$ close to the d(GpG) or d(GpTpG) cross-link. The repair efficiency is calculated from the ratio of the dual incision signal to total probe signal. Reprinted with permission from ref 75. Copyright 2003 American Chemical Society.

paths since the NER proteins required for both are the same except for XPC-HR23B. In particular, after damage recognition, TFIIH, XPA, and RPA are the next set of proteins to assemble on the DNA. Although the exact binding order of these proteins is controversial, they may be cooperatively recruited to the damage site.[151,152] In a subsequent step, XPB and XPD helicases, components of TFIIH, unwind the DNA in a process that requires ATP. XPC-HR23B is released when endonuclease XPG binds to this unfolded DNA. The structure-specific endonuclease XPF-ERCC1 is finally recruited to the NER complex, and dual incision occurs to remove platinated oligonucleotides. Excised oligonucleotides (24−32 nucleotides in length), containing a platinum lesion, and dual incision factors are then released from the DNA. Small oligonucleotides are degraded in the nucleus,[153] and the excised platinated oligomer is most likely processed in a similar manner. The fate of the platinum is unknown and would be very difficult to track in cells. RPA remains associated with the incised DNA and possibly recruits DNA resynthesis factors such as PCNA and replication factor C (RFC) to fill in the gap (Figure 6A).[151]

Recently, several groups have investigated the dynamic behavior of the NER factors XPF-ERCC1,[154] TFIIH,[155] and RPA and PCNA[156] in living cells. The data consistently indicate that each component of NER diffuses freely and participates in repair processes randomly rather than assembling as an intact repair holo complex. Most notably, dynamic targeting of RPA and PCNA to sites of cisplatin−DNA damage was examined in Rat-1 and U2OS cells expressing GFP fused to these proteins.[156] Cisplatin treatment readily induces relocalization of PCNA and RPA into discrete foci, whereas platinum-DNA lesions are relatively dispersed throughout the nucleus. PCNA and RPA levels recruited to repair foci are proportional to the platinum adduct levels. Proteins at repair foci are highly immobile and turn over only on the order of minutes.

The repair of different DNA adducts generated by cisplatin has been investigated in cell-free extracts as well as reconstituted NER systems. Figure 6B shows typical gel electrophoresis data obtained from an excision repair assay. In vitro studies revealed that cisplatin 1,3-(GpNpG) intrastrand cross-links are more efficiently repaired by NER than 1,2-intrastrand cross-links.[157,158] The cisplatin interstrand cross-link, however, is not repaired in the same fashion.[158] The NER of other platinum compounds containing DNA lesions has also been evaluated, and the results differ from those for cisplatin adducts. Intrastrand DNA adducts generated by cisplatin, oxaliplatin, and JM216 (Figure 1) are repaired similarly by NER in vitro,[159] suggesting that the carrier ligand does not affect the repair efficiency for these compounds. Although monofunctional adducts of cisplatin and $[Pt(dien)Cl]^+$ are not substrates of NER, several biologically active trans compounds, such as *trans*-$[PtCl_2(NH_3)$(thioazole)][160] and *trans*-$[PtCl_2$(iminoether)$_2]$,[161] form monofunctional adducts that are successfully removed by the NER system. Monofunctional adducts of these compounds are presumed to cause a local conformational distortion at the site of DNA damage similar to that of cisplatin intrastrand cross-links. The efficient repair of DNA intrastrand cross-links generated by a trinuclear platinum complex has also been reported.[162] As with cisplatin, however, cell-free extracts did not promote the repair of DNA interstrand cross-links formed by this complex. Nucleotide excision repair of platinum lesions located on nucleosomal DNA was also investigated by reconstituting a mononucleosome with recombinant histone proteins and a site-specifically modified cisplatin−DNA probe.[75] The nucleosome inhibits excision repair of 1,3-(GpTpG) intrastrand cross-links to about 10%

of the level obtained with free DNA. In the same study, the platinated mononucleosome was also reconstituted with native histone octamers, which retain their post-translational modifications such as acetylation and phosphorylation. Platinum lesions on native nucleosomes were repaired ~2-fold more efficiently than those on recombinant nucleosomes. The state of chromatin structure, which affects various DNA metabolic processes such as replication, transcription, and repair, closely regulates repair of platinum damage.[74]

### 5.2. Mismatch Repair

Some studies indicate that the mismatch repair (MMR) process closely correlates with cisplatin resistance.[163−165] Cisplatin-resistant cell lines, with either intrinsic or acquired resistance to the drug, are often defective in MMR.[166,167] Cancer or mouse model cell lines deficient in MMR are several times more resistant to cisplatin than corresponding MMR proficient cells.[100] On the other hand, a variety of other investigations failed to establish a clear correlation between MMR deficiency and cisplatin resistance.[18] The MMR process is most likely only one of several pathways linked to cisplatin action, and the influence of MMR on platinum cytotoxicity will vary depending on experimental conditions. The MMR system eliminates base−base mismatches as well as deletion and insertion mutations.[168] In eukaryotic cells, hMutSα (MSH2−MSH6 heterodimer) initiates MMR by binding to single mismatches and small insertion/deletion loops and hMutS$\beta$ (MSH3−MSH6 heterodimer) starts MMR through recognition of insertion/deletion loops of different sizes. Following damage recognition by hMutSα, hMutLα (MLH1-PMS2 heterodimer) and PCNA are recruited to the site of DNA mismatch to carry on the repair. Several exonucleases and helicases, the replication machinery, and DNA ligase I are subsequently recruited to degrade the error-containing strand and fill in the gap.

MMR proteins are probably also engaged in active attempts to repair newly synthesized DNA opposite platinum adducts generated by translesion synthesis (TLS) past the platinum lesions, a process discussed above. It is proposed that this process can bring about a futile cycle of attempted repair of cisplatin damage, which may lead to cell death.[13] Methylating compounds that serve as anticancer agents activate cell cycle arrest by a similar mechanism.[169] Repeated unsuccessful repair of methylated DNA mispairs, obtained by TLS past adducts such as O6-methylguanine, trigger the ataxia telangiectasia and Rad 3 related (ATR) kinase-mediated cell cycle checkpoint. Upon replication bypass, both cisplatin and DNA methylation damage may share a common signaling pathway. In addition to their ability to repair DNA, MMR proteins also mediate DNA damage-induced apoptosis as part of the cellular response to endogenous and exogenous stress.[170] The activation of JUN and c-Abl kinases appears to be involved in MMR-induced apoptosis by cisplatin treatment.[171,172] Phosphorylation of p53 in response to cisplatin damage is also affected by the MMR protein hMLH1.[173] These cellular pathways triggered by MMR proteins are independent from the repair process because certain mutations in hMutS homologs cause mismatch repair deficiencies but do not interfere with the signaling functions of MMR proteins.[174,175]

Direct interactions of MMR components, especially MutS proteins, with cisplatin-DNA adducts have been studied in vitro. The bacterial MMR protein MutS[176] and its eukaryotic homologues hMutSα[177] and hMSH2 (a component of hMutSα heterodimer)[178] specifically bind to the major cisplatin adduct, a 1,2-intrastrand cross-link. Interestingly, hMSH2 and MutS preferentially recognize cisplatin-modified DNA over oxaliplatin-modified DNA. Defects in MMR do not affect cellular resistance to oxaliplatin,[100] suggesting that the interaction of MMR proteins with DNA adducts is important for mediating MMR functions in response to DNA damage. In recent studies, the binding interactions of MutS[179] and hMutSα[180] to duplex DNA containing cisplatin lesions were investigated with a variety of mismatches opposite a cisplatin 1,2-(GpG) intrastrand cross-link. These so-called cisplatin compound lesions, formed by misincorporation of a base opposite the sites of platinum adducts, are better substrates for MutS binding, the affinities of which are changed by the nature of the mismatches.

### 5.3. DNA Recombination

A role for recombinational repair in protecting cells from cisplatin treatment has been established in experiments using *E. coli*.[17,181] Many recombination-deficient strains show enhanced sensitivity to cisplatin compared to wild-type cells. Recombinational repair (RR) is independent from NER, since cells containing double mutations in both NER and recombination proteins are more sensitive to cisplatin than cells with either mutation alone.[181] Spontaneous and cisplatin-induced recombinational processes are also observed in *E. coli*.[182] Impaired recombination DNA repair in yeast[183] and prostate cancer cells[184] enhances sensitivity to cisplatin. In mammalian cells, disruption of homologous recombination repair (HR) increases cisplatin sensitivity whereas a knockout of the nonhomologous endjoining (NHEJ) does not affect cell sensitivity to the drug.[185]

DNA recombination has been more closely associated with the cellular repair of DNA interstrand rather than intrastrand cross-links. Comprehensive studies with prokaryotic as well as eukaryotic systems demonstrated that NER, DNA recombination, and TLS are required to repair interstrand cross-links, as recently reviewed.[186] For example, chicken DT40 cells deficient in Rev3, the catalytic subunit of a TLS polymerase, or Fanconi anemia complementation groups (FANC), a key protein for homologous recombination, showed the enhanced sensitivity to cisplatin compared to wild-type cells.[187] At present, the manner by which RR proteins specifically recognize platinum adducts is unclear. Moreover, the several repair proteins that bind specifically to platinum intrastrand cross-links do not interact with interstrand cross-links.[179,188] It was recently proved that collapsed replication forks induced by strong obstacles on DNA, possibly including platinum adducts, recruit recombination proteins to restore synthesis.[189]

## *6. Proteins Binding to Platinum-Modified DNA*

Platinum modification distorts the structure of duplex DNA in a distinctive manner. A variety of cellular proteins specifically recognize these uniquely altered structural forms of DNA. These proteins include those involved in repair processes, proteins containing HMG domains, and many others. The interaction of the proteins with platinum-damaged DNA plays a key role in early cellular responses to platinum drugs. Continuous efforts have been made to identify such proteins and characterize their interaction with cisplatin adducts. Although the subject has been reviewed previ-

**Table 2. Key Human Proteins That Bind to Cisplatin-Modified DNA**

| protein | function | $K_d$ | specificity | notes | refs |
|---|---|---|---|---|---|
| XPC | NER: damage recognition protein | 3 nM | 9-fold | interacts with XPA | 152,199,200 |
| XPA | NER: damage recognition protein | 0.4-2 $\mu$M | <3-fold | interact with RPA and XPC | 201,202 |
| RPA | NER: damage recognition protein | 25-79 nM | 4-15-fold | interacts with XPA | 188,203−205 |
| hMSH2 | MMR: damage recognition protein | ~67 nM | 5-fold | | 178 |
| hMutSα | MMR: damage recognition protein | ~25 nM | >10-fold | high specificity for compound lesions | 177,180,210 |
| Ku80 | DNA-PK: DNA-binding subunit | 0.11 nM | nd | interacts with PARP-1 | 216,217 |
| HMGB1 | non-histone chromatin protein and extracellular signaling protein | 0.3−370 nM | 10-100-fold | interacts with p53, TBP, and MutSa | 242,244,248,249, 252,254 |
| SSRP1 | chromatin modulator | >0.3 $\mu$M | >50-fold | component of FACT | 257 |
| hUBF | rRNA transcription factor | 60 pM | nd | | 267,271 |
| tsHMG | testis-specific HMG protein | 24 nM | 230-fold | | 265 |
| TBP | transcription initiation factor | 0.3−10 nM | nd | interacts with HMGB1 | 273,274 |
| p53 | tumor suppressor protein | ~150 nM | 7-fold | interacts with XPC, RPA, YB-1, HMGB1, and mtTFA | 289,291−293 |
| PARP-1 | poly(ADP-ribose) polymerase | nd | nd | interacts with DNA-PK | 197 |
| YB-1 | Y-box binding transcription factor | nd | nd | interacts with MSH2, Ku80, and p53 | 305 |

ously,[11,13,190] there has been great progress made in this area over the past few years. Various tools have been developed to identify novel platinum−DNA binding proteins. In the present section, we briefly review the identification methods and then discuss proteins that interact with platinum-modified DNA, with a focus on recent work. A list of the major human proteins that recognize platinated DNA is provided as Table 2 together with corresponding references.

## 6.1. Identification of Platinum–DNA Binding Proteins

A number of strategies have been employed to identify mammalian proteins that bind specifically to DNA adducts formed by platinum drugs. Gel mobility-shift analyses and modified western blot assays employing cisplatin-damaged DNA probes have confirmed the existence of these proteins in cell extracts.[191−193] Human cDNAs encoding cisplatin-DNA adduct recognition proteins were identified by screening a cDNA expression library with cisplatin-modified DNA.[194] Several such proteins were also discovered by affinity precipitation using cisplatin-damaged DNA cellulose.[195] Later, a more systematic isolation was carried out by fractionating human cell extracts through a cisplatin-damaged DNA-sepharose column.[196] Most recently, a photoaffinity labeling method was developed with the use of platinum complexes containing a tethered, photoreactive moiety.[197] This method allows the capture of proteins that interact weakly or even transiently with platinum-damaged DNA. A pictorial representation of these various methods is depicted in Figure 7.

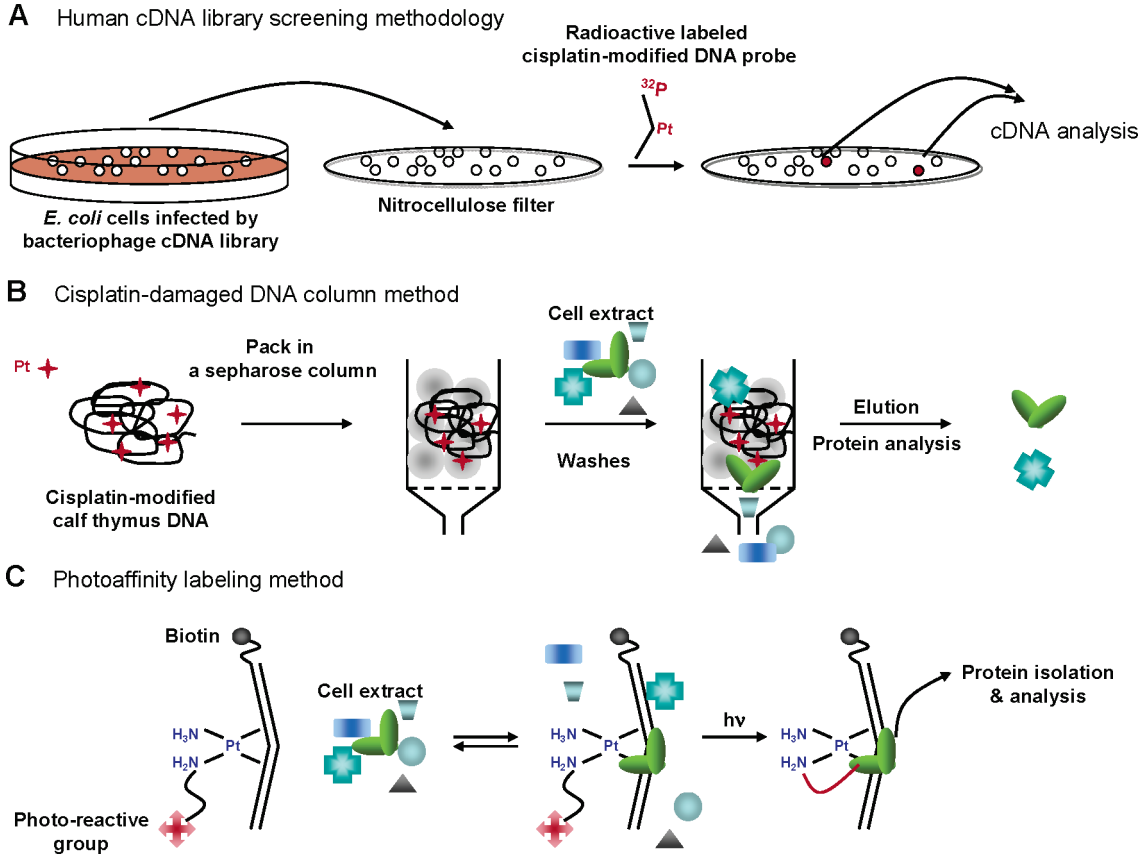


**Figure 7.** Pictorial representation of various methods used to identify proteins that bind to cisplatin-modified DNA.

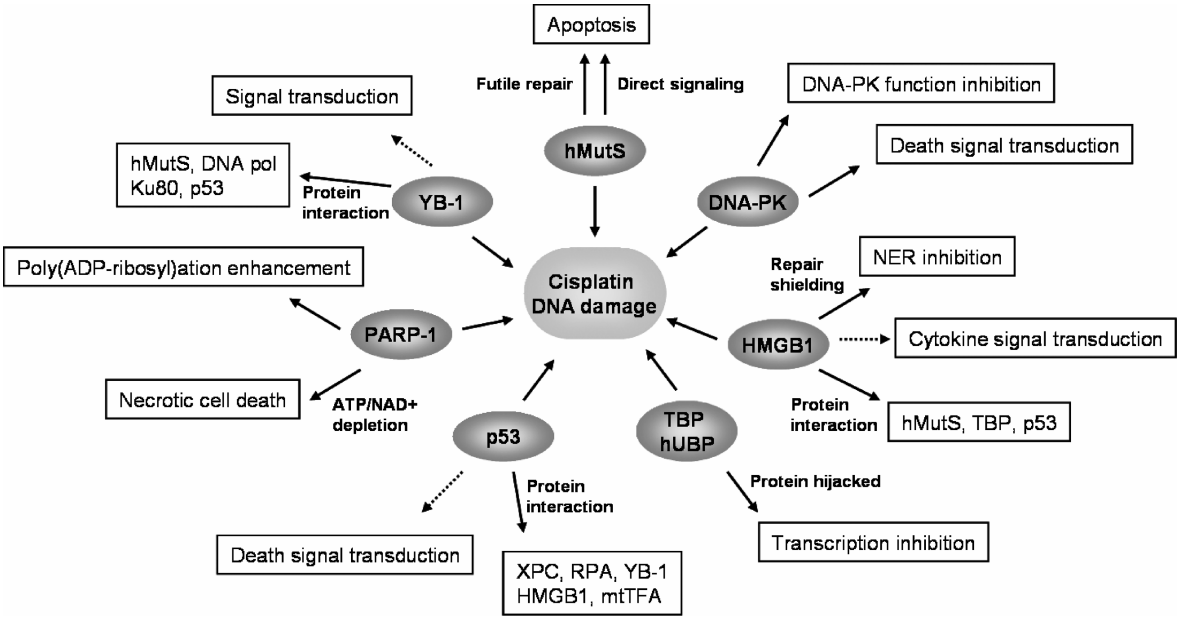


**Figure 8.** Roles of proteins that bind to DNA following cisplatin damage.

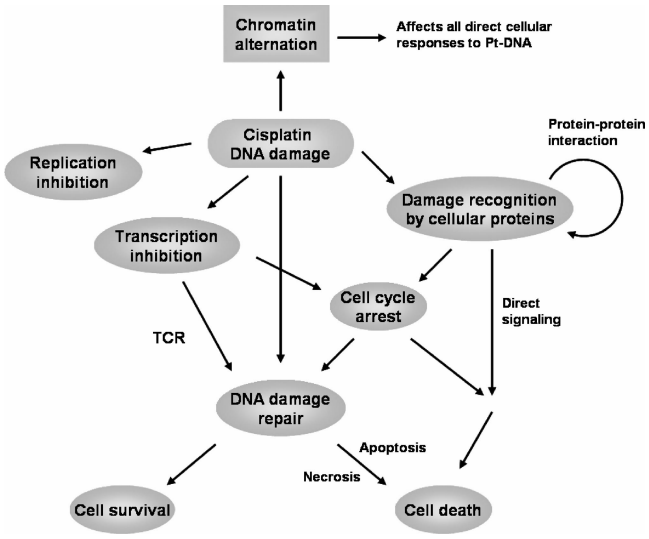


**Figure 9.** Direct cellular responses to platinum adducts: summary of our current understanding.

## 6.2. Proteins Involved in Damage Repair

Damage recognition proteins that participate in various cellular DNA repair processes are reported to bind to cisplatin-modified DNA. Their roles in modulating the biological activity of cisplatin are evidenced, as discussed above, by a diminished repair when they are mutated or absent, which generally conveys enhanced sensitivity of cells to the drug. Some of these factors, however, have distinctive properties that initiate specific cell signaling pathways.

### *6.2.1. NER Proteins*

Proteins that initiate the NER process are clear candidates for interaction with cisplatin-DNA adducts. A variety of studies indicate that XPC-hHR23B, XPA, RPA, and TFIIH recognize platinum adducts cooperatively during the early stage of NER.[151,198] Among these, XPC-hHR23B, XPA, and RPA all are reported to bind specifically to duplex DNA containing a cisplatin intrastrand cross-link.[13] Moreover, these proteins interact with each other, which additionally affects their binding to cisplatin adducts. Formation of such multiprotein complexes assures formation of a stable DNA−protein unit with specificity for the damaged site, since in general DNA-binding proteins have only weak (2- to 3-fold) binding specificity for a signal sequence on the genome compared to nonspecific binding.

XPC-hHR23B, the human homolog of yeast Rad4 and Rad23 proteins, displays a binding affinity (not to be confused with specificity) of $K_d = \sim 3$ nM for cisplatin 1,3-intrastrand adducts[199] and a faster association rate for binding the cisplatin adduct than for undamaged DNA.[200] XPC physically interacts with XPA, but the interaction does not contribute to the stability of its complex with the platinated DNA. The XPC−XPA interaction appears to be inhibited by the presence of platinated DNA.[152] The XPA protein consists of 273 amino acids (~31 kDa) and contains a zinc finger motif. Although XPA is clearly involved in the NER damage recognition process, it has the lowest binding affinity ($K_d \approx 2\ \mu$M under physiological conditions) for cisplatin-damaged DNA.[201,202] XPA, however, interacts with RPA, and the XPA−RPA complex exhibits a greater binding affinity for cisplatin-damaged duplex DNA than either XPA or RPA alone.[203] XPA modulates the RPA−DNA interaction by enhancing the stability of the ternary complex and inhibiting strand separation within the target DNA.

RPA is a heterotrimeric protein consisting of 70, 34, and 14 kDa subunits; it is an essential component of DNA repair, replication, and homologous recombination. The protein was identified as one of the cisplatin-damaged DNA recognition factors from a fractionation experiment of human cell extracts that employed cisplatin−DNA affinity chromatography.[204] RPA specifically recognizes cisplatin-damaged duplex DNA ($K_d = 25-79$ nM) with about 4−15-fold preference over undamaged DNA, but its binding to single-stranded DNA is also very strong, with $K_d$ values in the subnanomolar range.[203,205] It is proposed that, upon binding to cisplatin-modified DNA, RPA denatures the duplex DNA in the vicinity of the lesion and binds to single-stranded DNA of the unplatinated strand.[188] RPA binds to DNA containing a cisplatin 1,3-intrastrand cross-link with 1.5−2-fold higher affinity than to DNA containing a 1,2-intrastrand cross-link,

possibly due to the lower thermal stability of the 1,3-compared to the 1,2-adduct. As mentioned above, XPA enhances RPA binding to platinated DNA ($K_d = \sim 0.5$ nM) but does not affect RPA binding to single-stranded DNA.[203] The p34 subunit of RPA becomes phosphorylated in response to DNA damage in vivo as well as in vitro.[206] RPA hyperphosphorylation inhibits its duplex DNA binding, but this form of the protein retains its binding specificity for platinated DNA.[205]

XPE-deficient cells display the mildest phenotype among XP variants, retaining 40−60% of the repair capacity of normal cells.[13] An early study demonstrated that protein extracts of XPE-deficient cells lack a nuclear factor that binds specifically to cisplatin-damaged DNA.[191] This nuclear factor, called XPE binding factor or UV-damage recognition protein (UV-DRB), is a complex having two subunits with molecular masses of 127 and 48 kDa. It recognizes a broad range of DNA damage motifs,[207] but its role in damage repair is unknown. The protein is induced by cisplatin treatment,[208] and cisplatin-resistant cells express increased levels of XPE binding factor.[209]

#### 6.2.2. Mismatch Repair Proteins

Damage recognition proteins in MMR, hMutSα (MSH2−MSH6), and bacterial MutS are reported to bind to cisplatin-modified DNA. The hMutSα heterodimer consists of the MutS homologue hMSH2 and hMSH6 (GTBP/p160). hMutSα and purified hMSH2 proteins specifically recognize cisplatin-modified DNA with high binding affinities (25−67 nM; Table 2).[177,178,210] Their binding specificities to cisplatin−DNA lesions are also comparable to those of NER proteins (Table 2). As discussed above, cisplatin compound lesions, such as DNA with a CT sequence opposite a cisplatin 1,2-d(GpG) cross-link site (Pt-GG/CT), are the best binding substrates for hMutSα.[180] In addition, bacterial homolog MutS also strongly binds to cisplatin compound lesions, exhibiting almost 86-fold better binding affinity to Pt-GG/CT site than to Pt-GG/CC site.[176,179] The data suggest that MutSα may interact with cellular platinum lesions, especially compound lesions, and influence the DNA repair and signaling pathway, although a detailed mechanism of these processes is not known.

#### 6.2.3. DNA-PK

The DNA-dependent protein kinase (DNA-PK) participates in cellular DNA repair processes such as double-strand break (DSB) restoration. Recently it has become clear that the protein also plays a central role in various stress signaling pathways.[211] DNA-PK is a heterotrimeric complex comprising a large catalytic subunit (DNA-PKcs) and a Ku70/Ku80 regulatory component with DNA binding properties. Multiple studies report the involvement of DNA-PK in cisplatin action. DNA-PK mutant cell lines exhibit 3−4-fold increased sensitivity to cisplatin compared to their parental cell lines, partially because of reduced NER in the mutants.[212] Cisplatin-resistant cells overexpress the Ku80 subunit, and their extracts display increased Ku-binding activity to DNA ends.[213] Suppression of Ku70, however, was unable to affect the sensitivity of cells to cisplatin.[214] In addition, cells lacking Ku80 or DNA-PKcs are more resistant to cisplatin than wild-type cells but only when the cells are at high density prior to drug treatment.[215] The authors of this study suggested that the death signal, initiated in the damaged cell by the kinase activity of the DNA-PK complex, is passed to nearby cells by intercell communication via gap junctions. This result should alert researchers in the field of the potential importance of cell density on the measured cytotoxicity of platinum compounds.

DNA-PK binds to globally cisplatin-modified DNA, with the Ku80 subunit being responsible for the interaction.[216] Unlike undamaged DNA, which activates the kinase activity of DNA-PK through binding of Ku proteins to DNA ends,[211] cisplatin-damaged DNA fails to activate DNA-PK. Ku80 also strongly interacts with DNA containing a cisplatin 1,2-d(GpG) adduct with a $K_d$ value of 0.11 nM, which is only <2-fold weaker binding than Ku80 interaction with DNA ends.[217] Cisplatin-DNA adducts appear to inhibit translocation of Ku proteins along DNA, resulting in decreased association of DNA-PKcs to the Ku−DNA complex and therefore diminished kinase activity.[218] The position of the cisplatin adduct and sequence of the duplex DNA affect the inhibition of DNA-PK activity. It was recently reported that DSB nonhomologous endjoining, which requires DNA-PK, is also inhibited by cisplatin-damaged DNA in cell extracts.[219]

#### 6.2.4. Other Proteins

Several other proteins involved in various DNA repair processes are reported to bind to cisplatin-damaged DNA. Yeast photolyase binds to globally cisplatin-modified DNA,[220] and *E. coli* photolyase recognizes duplex DNA containing a cisplatin 1,2-d(GpG) lesion ($K_d = 50$ nM).[221] Although photolyase appears to make cells more resistant to cisplatin,[220,221] the mechanism by which it does so is unclear. T4 endonuclease VII cleaves various branched DNAs such as four-way junctions. The enzyme also recognizes and precisely cleaves duplex DNA containing cisplatin 1,2-d(GpG) and 1,2-d(ApG) adducts[222] as well as interstrand cross-links formed by both cisplatin and *trans*-DDP.[223] Finally, a recent study reported that human 3-methyladenine DNA glycosylase (AAG), a damage recognition protein involved in base excision repair, selectively binds to various cisplatin adducts.[224] The repair enzyme AAG recognizes 1,2-d(GpG), 1,2-d(ApG), and 1,3-d(GpTpG) adducts with $K_d$ values of 115, 71, and 144 nM, respectively. Cisplatin adducts inhibit the AAG repair on 1,N6-ethenoadenine, a well-known substrate of AAG, possibly by diverting the enzyme away from repair complexes.

### 6.3. HMG-Domain Proteins

High-mobility group (HMG) domain proteins, particularly HMGB1, have long been known to interact with cisplatin-modified DNA.[225,226] Our knowledge of the nature of the HMG box interaction with platinated DNA has been greatly improved in recent years. Despite the wealth of information, however, it cannot be stated with certainty that this DNA-binding protein domain plays an essential role in conveying the anticancer activity of cisplatin.

#### 6.3.1. HMGB1

High-mobility group protein 1 (HMGB1) is one of the early proteins discovered to bind cisplatin-modified DNA.[195,242] HMGB1 is an abundant ($\sim 10^6$ copies per cell) and highly conserved non-histone chromosomal protein.[227] As a non-sequence-specific DNA binding protein, it regulates numerous nuclear functions including transcription, replication, recombination, and general chromatin remodeling, serving as an architectural facilitator by assisting the assembly of

nucleoprotein complexes.[228] HMGB1 preferentially binds to DNA with bent or distorted structures, and it physically interacts with many cellular proteins such as p53, RAG1/2, TBP, MutSα, and steroid hormone receptors. For example, HMBG1 binds to MutSα and directs mismatch repair steps prior to the excision of mispaired nucleotides.[229] For the past several years, HMGB1 has also been investigated as an extracellular mediator, performing significant roles in inflammation, differentiation, migration, tumor metastasis, and the immune response.[230] Recent photobleaching[231] and cross-linking[232] experiments revealed that HMGB1 is an extremely mobile protein in the nucleus with a residence time on DNA of less than a second. Because of the high abundance of HMGB1 and affinity for bent DNA, the protein has a high probability of encountering platinum adducts and could be involved in drug action.

The varied properties of HMGB1 that suggest a likely involvement of the protein in the cisplatin mechanism of action also make it difficult to define exactly which of the HMGB1 functions might predominate in mediating drug action. It is therefore, perhaps, not surprising that attempts to correlate cellular HMGB1 levels with cisplatin sensitivity have been controversial. Studies in vitro clearly demonstrate that HMGB1 can inhibit NER of cisplatin adducts, 1,2-cross-links in particular, presumably by binding to and shielding the damage site from recognition by the repair apparatus.[157,158] Consistent with these results is the finding that an increased protein level of HMGB1 following hormone treatment sensitizes breast cancer cells to cisplatin by a factor of 2.[233] Moreover, additional expression of HMGB2, a protein over 85% identical to HMGB1, in human lung cancer cells enhanced cisplatin sensitivity more than 3-fold.[234] Conversely, HMGB1 is overexpressed in various cisplatin-resistant cell lines[235] and has been identified as a proapoptotic signaling protein.[236] In addition, mouse embryonic native and HMGB1 knockout cell lines show no significant differences in their sensitivity to cisplatin.[237] Recently, RNA interference (RNAi) was employed to silence HMGB1 in different cell lines, in which the effect of HMGB1 knock-down on cell sensitivity to cisplatin varied for the different cell lines; both increased and diminished sensitization were observed.[238] From these experiments is clear that the ability of HMGB1 to impact the cytotoxicity of cisplatin can depend upon the cell type, the experimental method used to change the protein level, and possibly even the growth conditions and number of passages of the cells.[239] An attempt to introduce foreign HMGB1 as a modulator of cytotoxicity for platinum drugs[240] failed to provide convincing evidence that HMGB1, as a DNA binding protein as well as a cytokine, can influence platinum action. Thus, unlike the order of magnitude or greater increase in cytotoxicity conveyed by compounds using the OCTs as transporters,[42] for example, the multifold (at best) sensitization of cells to cisplatin by HMGB1 upregulation make it less obvious a focus for improving chemotherapeutic action of the platinum drugs than many of the other proteins known to associate with platinated DNA.[241]

This conclusion notwithstanding, the detailed studies from several laboratories on the interactions of HMG-domain proteins, including HMGB1, with platinated DNA serve as a paradigm for investigations of this kind. We therefore discuss these results in some detail. HMGB1, a 30-kDa protein of 215 amino acids, comprises two HMG box domains A and B and an acidic C-terminal tail. Each HMG domain as well as the full-length HMGB1 protein bind selectively to cisplatin-modified DNA.[242−244] Although the two HMG box domains of HMGB1 are structurally similar and positioned in tandem, domain A interacts more strongly with cisplatin 1,2-intrastrand DNA cross-links than domain B.[243,245] The sequence context of platinum-damaged DNA modulates the binding affinity of the individual domains for cisplatin adducts. The $K_d$ value for domain A binding to a 15-bp duplex DNA containing a cisplatin 1,2-d(GpG) adduct varies from 1.6 to 517 nM depending on the flanking nucleotides.[243] Stopped-flow analysis of domain interaction with cisplatin-modified DNA reveals very rapid association ($k = 2-4 \times 10^8$ $M^{-1}$ $s^{-1}$) and dissociation ($k = 70-200$ $s^{-1}$) of the protein−DNA complex.[246] A crystal structure of domain A bound to a 16-bp DNA duplex containing a cisplatin 1,2-d(GpG) cross-link was determined.[247] The structural properties revealed in this study, and those of HMG box binding to DNA in general, are reviewed elsewhere.[11,70,241] The HMGB1 full-length protein recognizes cisplatin 1,2-intrastrand ($K_d = 0.3-370$ nM)[242,248] as well as interstrand cross-links,[249] and the interaction is unaffected by sequence context.[248] HMGB1 and its didomain component lacking the acidic tail also bind to cisplatin-modified DNA, primarily through domain A, leaving the rest of the protein available for other interactions. The acidic tail of HMGB1 is responsible for HMGB1 interaction with the TATA box binding protein (TBP).[250] In addition, the acidic tail also appears to interact with the N-terminus of histone H3, mediating the stimulation of transcription.[251] Enhanced binding of HMGB1 to cisplatin-modified DNA by protein interaction with p53 has also been reported.[252] HMGB1 binding to cisplatin-modified DNA can be modulated by post-translational modification of the protein. Lysine 2 of HMGB1 is acetylated by histone acetyltransferase CBP,[253] and the modified form of the protein shows significantly enhanced binding to cisplatin adducts.[254] HMGB1 binding to DNA modified by various cisplatin analogs reveals the significant influence of the spectator ligands on the protein−DNA interactions.[255]

### 6.3.2. SSRP1

The structure-specific recognition protein SSRP1 was discovered during early searches for factors that specifically bind to cisplatin-modified DNA by screening of a human cDNA expression library.[226] SSRP1, an 81-kDa protein, forms a heterodimer with Spt16/Cdc68, and the resulting complex FACT (facilitates chromatin transcription) is a chromatin modulator, mediating transcription, replication, and repair through reconfiguration of the nucleosome.[256] SSRP1 contains a HMG box domain, which accounts for the ability of the protein to bind to cisplatin-modified DNA. The isolated HMG domain of SSRP1 and the FACT complex selectively recognize cisplatin adducts, but SSRP1 alone fails to bind this damaged DNA.[257] Although direct evidence for SSRP1 involvement in cisplatin action is not reported, there have been several indications that FACT is involved in cellular DNA repair processes.[258,259]

Many other proteins containing one or more HMG domains bind to cisplatin-modified DNA. Included are yeast HMG-domain proteins Ixr1 ($K_d = 250$ nM),[260] cmb1,[261] and NHP6A ($K_d = 0.1$ nM),[262] mtTFA (mitochondrial transcription factor A; $K_d = \sim 100$ nM), LEF-1 (lymphoid enhancer binding protein; $K_d = \sim 100$ nM),[263] SRY (sex-determining factor; $K_d = 120$ nM),[264] tsHMG (testis-specific HMG

protein; $K_d = 24$ nM),[265] HMG-D (drosophila homologue of HMGB1; $K_d = 200$ nM),[266] and hUBF (ribosomal RNA transcription factor; $K_d = 60$ pM).[267] As in human cells, HMG-domain proteins can convey cisplatin cytotoxicity in yeast. Inactivation of the Ixr1 gene desensitizes two yeast strains to cisplatin with a decreased level of cisplatin−DNA adducts.[268] On the other hand, nhp6a/b[262] and cmb1[261] mutant cells are more sensitive to cisplatin than their parental cells, and cisplatin treatment induces cmb1 gene expression.[269] Again we see the variability in results for different systems. In HeLa cells, expression of testis-specific HMG protein tsHMG, which has a higher binding affinity to cisplatin adducts than HMGB1, enhances cisplatin cytotoxicity.[270] Finally, in an in vitro transcription assay with RNA polymerase I, cisplatin-damaged DNA inhibited rRNA synthesis by sequestering an essential transcription factor hUBF, which contains six HMG domains and displays the strongest binding affinity for cisplatin adducts.[271]

## 6.4. Other Cellular Proteins

In addition to repair and HMG-domain proteins, many other cellular proteins have been reported to preferentially recognize cisplatin-modified DNA. Since these proteins are essential for various cellular functions, their interactions with cisplatin adducts may contribute to cisplatin action. More importantly, in many cases the proteins are linked to other cisplatin damage-recognition proteins, either physically or functionally.

### *6.4.1. TBP*

The TATA-binding protein (TBP) is required for transcription initiation of all three eukaryotic RNA polymerases. The protein recognizes a TATA box of the promoter and recruits transcription initiation factors to that site. The TBP binds in the minor groove and bends the DNA duplex toward the major groove,[272] resulting in a structure resembling that of cisplatin-modified DNA. In vitro transcription is inhibited by the presence of cisplatin-damaged DNA, which directly interacts with the TBP and sequesters the protein from the TATA box.[273] Microinjection of additional TBP restores RNA synthesis in human fibroblasts. As with HMGB1, TBP preferentially binds to platinum 1,2-d(GpG) over 1,3-d(GpNpG) intrastrand cross-links.[274] Interestingly, HMGB1 binding increases the affinity of TBP for the TATA box by 20-fold,[250] indicating the strong possibility of a HMGB1/TBP complex interaction with cisplatin-modified DNA. TBP binding to cisplatin adducts is comparable to that for TATA boxes, with similar binding affinity and kinetics, characterized by relatively slow on and off rates.[275]

### *6.4.2. p53*

The tumor suppressor protein p53 is one of the most commonly mutated proteins in human cancer. The p53 protein regulates DNA repair, cell-cycle arrest, and apoptosis by modification of other genes and their products, including those involved in transcription, DNA repair, and many signaling processes.[276] Pathways related to p53 participate in transduction of DNA-damage signals upon cisplatin treatment.[18] As recently determined in 60 cell lines, expression of p53 is positively correlated to cell sensitivity to the four platinum compounds cisplatin, carboplatin, oxaliplatin, and tetraplatin (Figure 1).[277] Numerous reports support this correlation such that p53 expression enhances the sensitivity of p53-deficient cancer cells to cisplatin,[278,279] and accumulation of p53 also sensitizes cells to the drug.[280] In addition, p53 mutants have been detected in cisplatin-resistant ovarian carcinoma cells.[281] Several studies, however, reveal that p53 can have various effects on cisplatin cytotoxicity. p53-Mediated sensitization to cisplatin is reversed by altering cell growth conditions,[282] and p53 expression enhances cisplatin cytotoxicity in HeLa but not in cisplatin-resistant HeLa cells.[283] In other examples, p53-deficient and -proficient teratocarcinoma cells display the same cisplatin sensitivity,[284] and only one of two curable ovarian cancer cell lines exhibits a p53-dependent response upon cisplatin treatment.[285] Increased cisplatin cytotoxicity by the loss or abrogation of p53 function has also been reported.[286]

p53 is a protein of 393 amino acids containing two DNA-binding domains. The first domain, located in the core of the protein, binds to a specific gene sequence, whereas the C-terminal DNA-binding domain is believed to recognize damaged DNA. Under normal conditions there is a low level of a latent form of p53, which is induced, activated, and stabilized under stress conditions including DNA damage.[276] Activation of p53 occurs through post-translational modification, mainly phosphorylation. An early study demonstrated that cisplatin treatment of human ovarian cancer cells can induce the latent form of p53, which lacks a sequence-specific DNA binding ability but displays a strong affinity for cisplatin-modified DNA.[287] Cisplatin induces phosphorylation at serine 20 or 15 of the protein.[288] Both DNA binding sites are required for p53 binding to platinated DNA,[289] although the C-terminal domain is more critical for the preferential p53 binding to cisplatin-modified DNA over undamaged DNA.[290] The purified active form of p53 recognizes duplex DNA containing a cisplatin 1,2-d(GpG) intrastrand cross-link ($K_d = \sim 150$ nM) but not DNA with a 1,3-d(GpTpG) intrastrand cross-link, interstrand cross-links, or monofunctional adducts.[291] Interestingly, the binding affinity of cisplatin-modified DNA to the latent form of p53 is considerably higher than to the active form.[292] Both latent and active p53, however, do not bind to DNA modified by a trinuclear platinum compound, BBR3464,[293] which has been evaluated as a potential anticancer agent. As discussed briefly, p53 interacts with the cisplatin damage recognition proteins XPC, RPA, YB-1, HMGB1, and mtTFA, and it significantly enhances the binding affinities of HMGB1[252] and mtTFA[294] to cisplatin-modified DNA through a direct physical interaction.

### *6.4.3. PARP-1*

Poly(ADP-ribose) polymerase 1 (PARP-1) is a large (1014 amino acids) nuclear enzyme that utilizes $NAD^+$ as a substrate for the synthesis and attachment of poly(ADP-ribose) polymers to a range of target proteins as well as to itself in response to DNA damage.[295] Severe DNA damage appears to cause overactivation of PARP-1, which leads to the depletion of $NAD^+$ and ATP in cells, ultimately leading to their death by necrosis. Evidence connecting PARP-1 and cisplatin action is limited at this stage but nonetheless compelling. Cisplatin treatment increases overall poly(ADP-ribosyl)ation in O-342 rat ovarian tumor cells and CV-1 monkey cells,[296] with PARP-1 being a major contributor to the modification.[295] Moreover, PARP-1 inhibitors sensitize various human cancer cell lines to cisplatin.[297,298] Most recently, PARP-1 has been identified by photoaffinity labeling as one of several nuclear proteins that selectively

bind to cisplatin 1,2-d(GpG) adducts in cell extracts.[197,299] Possible roles of PARP-1 in cisplatin anticancer activity are discussed in a recent review,[300] but proof that such a highly abundant protein is involved in the mechanism of the drug must await further studies.

#### *6.4.4. YB-1*

YB-1 is a transcription factor that binds to the Y-box, an inverted CCAAT box sequence, and is important for signaling DNA damage and cell proliferation. This protein is overexpressed in the nucleus of cisplatin-resistant cell lines,[301,302] and suppression of the protein increases cisplatin sensitivity of human cancer cell lines or mouse embryonic stem cells.[301,303] The mRNA level of YB-1 is increased about 6-fold in response to cisplatin treatment.[304] YB-1 selectively recognizes the DNA duplex containing cisplatin 1,2-d(GpG), 1,2-d(ApG), as well as 1,3-d(GpTpG) adducts and physically interacts with PCNA, suggesting possible involvement of the protein in DNA repair.[305] YB-1 also interacts with many other cellular proteins such as MSH2, DNA polymerase $\delta$, Ku80,[306] and p53,[307] which are key proteins discussed here for their functions in cisplatin action.

## *7. Concluding Remarks*

Platinum-based anticancer drugs such as cisplatin, carboplatin, and oxaliplatin are among the most widely used chemotherapeutic agents. Challenges for researchers in this field have been to minimize side effects of the drugs while maintaining their potency against cancer cells and extend successful treatment to a wider range of human cancers. The search for novel platinum drugs and better therapeutic strategies demands a deeper understanding of how cells process platinum drugs. Recent progress provides new clues for explaining the chemistry and cellular action, including a role in specific transporters in bringing platinum complexes to the cancer tissue, the possible involvement of platinum−carbonate complexes in determining the rate of DNA modification, the nature of platinum−DNA adduct formation after drug uptake, DNA damage recognition by damage-response proteins, and cellular signaling pathways, which ultimately determine the results of drug treatment.

Proteins mediating direct cellular responses to platinum-damaged DNA include those involved in replication, transcription, repair, and chromatin structure as well as those that specifically bind to platinum−DNA adducts. Many of these proteins physically and functionally communicate with each other, which further affects their roles in mediating cisplatin anticancer activity. The consequences of altering the levels of these proteins on cisplatin cytotoxicity can vary in different cell types and the means by which the protein levels are controlled, making it difficult to arrive at a consensus mechanism for cell death. Moreover, every cell and patient has a different environment for drug uptake and cell signaling events. Better information about the tumor cell and individual patient genetics/proteomics prior to drug treatment would be valuable for tuning therapy and anticipating the anticancer activity of the drugs. Developing the optimal strategy for treating different tumors with platinum drugs based on mechanistic understanding seems an achievable objective. This goal would benefit from additional molecular studies that better define the precise contributions of damage-recognition proteins to the cellular responses against platinum complexes already in the clinic and, ultimately, elucidate the complete molecular mechanism of these compounds. In particular, studies about which of these proteins actually bind to platinum DNA damage in cancer patients and affect the DNA-mediated and transporter-specific cellular processes that lead to tumor regression are very limited at present. Post-translational modifications of proteins, such as histones, in response to drug treatment are in need of substantial further investigation. The information will allow us rationally to design new platinum compounds and combine platinum treatment with other chemical and biological agents, with the ultimate goal of improving patient care.

## *8. Abbreviations*

| | |
|---|---|
| 1,2-d(GpG) | *cis*-[Pt(NH$_3$)$_2$\{d(GpG)-N$^7$(1)-N$^7$(2)\}] |
| 1,2-d(ApG) | *cis*-[Pt(NH$_3$)$_2$\{d(ApG)-N$^7$(1)-N$^7$(2)\}] |
| AAG | 3-methyladenine DNA glycosylase |
| bp | base pair |
| carboplatin | *cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II) |
| cisplatin | *cis*-diamminedichloroplatinum(II) |
| CPD | cyclobutane pyrimidine dimmer |
| CS | Cockayne syndrome |
| CTR | copper transporter |
| DACH | 1,2-diaminocyclohexane |
| DNA-PK | DNA-dependent protein kinase |
| en | ethylenediamine |
| ERCC1 | excision repair cross-complementation group 1 |
| FACT | facilitates chromatin transcription |
| GGR | global genome repair |
| HMG | high-mobility group |
| MMR | mismatch repair |
| NER | nucleotide excision repair |
| oxaliplatin | (1*R*,2*R*-diaminocyclohexane)oxalatoplatinum(II) |
| PARP | poly(ADP-ribose) polymerase |
| PCNA | proliferating cell nuclear antigen |
| Pol II | RNA polymerase II |
| RPA | replication protein A |
| RR | recombinational repair |
| SSRP1 | structure-specific recognition protein 1 |
| TBP | TATA-binding protein |
| TCR | transcription-coupled repair |
| TLS | translesion synthesis |
| tsHMG | testis-specific HMG |
| UV-DRB | UV-damage recognition protein |
| XP | xeroderma pigmentosum |
| YB-1 | Y-box binding protein |

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