Interaction of FACT, SSRP1, and the High Mobility Group (HMG)
Domain of SSRP1 with DNA Damaged by the Anticancer Drug
Cisplatin*

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The structure-specific recognition protein SSRP1, initially
isolated from expression screening of a human B-cell cDNA library for proteins that bind to cisplatin
(cis-diaminedichloroplatinum(II))-modified DNA, contains a single DNA-binding high mobility group (HMG)
domain. Human SSRP1 purifies as a heterodimer of SSRP1 and Spt16 (FACT) that alleviates the nucleo-
osomal block to transcription elongation by RNAPII in vitro. The affinity and specificity of FACT, SSRP1, and
the isolated HMG domain of SSRP1 for cisplatin-damaged DNA were investigated by gel mobility shift assays.
FACT exhibits both affinity and specificity for DNA damaged globally with cisplatin compared with unmodified
DNA or DNA damaged globally with the clinically ineffective trans-DDP isomer. FACT binds the major 1,2-
d(GpG) intrastrand cisplatin adduct, but its isolated SSRP1 subunit fails to form discrete, high affinity com-
plexes with cisplatin-modified DNA under similar conditions. These results suggest that Spt16 primes SSRP1
for cisplatin-damaged DNA recognition by unveiling its HMG domain. As expected, the isolated HMG domain
of SSRP1 is sufficient for specific binding to cisplatin-damaged DNA and binds the major cisplatin 1,2-d(GpG)
intrastrand cross-link. The affinity and specificity of FACT for cisplatin-modified DNA, as well as its impor-
tance for transcription of chromatin, suggests that the interaction of FACT and cisplatin-damaged DNA may
be crucial to the anticancer mechanism of cisplatin.

Eukaryotic cells package DNA into chromatin, the structure
of which impedes essential cellular processes that require DNA
for function. Such processes include replication, recombination,
and transcription. The regulation of gene expression is there-
fore intimately tied to chromatin structure.

DNA transcription in vitro can be reconstituted by a minimal
set of general transcription factors and RNA polymerase II (1).
These minimal components are insufficient for transcription
from reconstituted nucleosomal templates, however, implying
the existence of cellular mechanisms for chromatin remodeling
to facilitate access of the transcription machinery to DNA (2).
Two classes of nuclear factors, ATP-dependent chromatin remod-
eling enzymes and histone acetyltransferases, allow the
transcription machinery to assemble and initiate transcription
from chromatin templates (3, 4). Recently, a novel factor,
FACT (Facilitates Chromatin Transcription), which enables
transcription elongation past nucleosomes, was isolated from
HeLa nuclear extracts (5, 6). This factor is a heterodimer of
human Spt16/Cdc68 and human SSRP1 proteins (7). FACT is
inactivated by chemical cross-linking of the histone octamer,
suggesting that it may function to unravel H2A/H2B histone
dimers from nucleosome cores (7). The remaining H3/H4
tetramer is itself insufficient to repress the elongating RNA
polymerase (8, 9).

FACT is a very abundant nuclear protein complex with an
estimated 100,000 copies/HeLa cell (7). The complex is con-
served across a diverse range of organisms, analogous com-
plexes of SPT16/SSRP1 homologs having been isolated from
Xenopus and Saccharomyces cerevisiae (10–12). Although dis-
tinct roles for the two protein components of FACT have yet to
be elucidated, the DNA-binding high-mobility group (HMG)
domain of SSRP1 (13) may target the complex to nucleosomes
(7). Circumstantial evidence suggests that HMG domain pro-
teins bind to DNA as it enters and exits the nucleosome (14).
Consistent with the hypothesis that the HMG domain of
SSRP1 is responsible for FACT binding to DNA, FACT activity
is abolished by addition of a 5-fold excess of superhelical plas-
mid DNA competitor (5). The mechanistic details of derepres-
sion of transcription elongation by FACT remain to be
elucidated.

Human Cdc68/Spt16 is a 119.9-kDa nuclear protein with
36% identity to its yeast homolog. Previous genetic studies with
yeast Cdc68 suggested a role in modulating chromatin struc-
ture to effect both gene repression and activation (15–17). The
domain features of the 81-kDa human SSRP1 protein are
shown in Fig. 1 (18). Initially isolated from expression screening
of a human B-cell cDNA library for proteins that bind DNA
modified by the antitumor agent cisplatin (cis-diaminedichlo-
roplatinum(II)), SSRP1 is expected to bind distorted DNA
structures through its DNA-binding HMG domain (18). The
ability of SSRP1 to recognize DNA modified by cisplatin and
the deleterious effect of cisplatin on transcription suggest a

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1 The abbreviations used are: FACT, Facilitates Chromatin Trans-
cription; HMG, high mobility group; cisplatin and cis-DDP,
cis-diaminedichloroplatinum(II); bp, base pair(s); trans-DDP,
trans-diaminedichloroplatinum(II); nt, nucleotide(s); PCR, poly-
merase chain reaction; PAGE, polyacrylamide gel electrophoresis;
cis127-bp, 127-bp probe damaged globally with cisplatin; trans-127-
bp, 127-bp probe damaged globally with trans-DDP.
possible role for SSRP1 and its physiologically relevant complex FACT in the cisplatin anticancer mechanism (19–21). Although the success of cisplatin therapy on a variety of cancers, including testicular, ovarian, and head and neck, has been remarkable since its introduction in 1979, there are several drawbacks to cisplatin treatment. These include toxic side effects, inherent or acquired resistance, and efficacy in only a handful of tumor types (22). A detailed understanding of its mechanism of action may allow for the rational design of new antitumor therapies.

Cisplatin reacts with a number of cellular components, but it is generally accepted that the biologically relevant target is DNA. After loss of the chloride ligands due to the relatively low concentration of Cl⁻ ions in the cell, cisplatin coordinates preferentially to the N-7 atoms of two adjacent purine nucleotides by forming mainly intrastrand d(GpG) and d(ApG) DNA cross-links (23, 24). Such adducts bend the duplex toward the major groove and unwind the DNA helix. The minor groove is consequently flattened and widened. Delineation of the cellular processing of cisplatin-DNA adducts is of great importance to unraveling its mechanism of cytotoxicity. Therefore, much work has focused on identifying and characterizing proteins that bind 1,2-intrastrand cross-links with high affinity and specificity (25). Structural distortions imposed on DNA by cisplatin 1,2-intrastrand cross-links are recognized by members of the HMG domain protein family including SSRP1 with notable specificity over unmodified double-stranded DNA.

Like other members of the HMG-domain protein family, SSRP1 is expected to recognize cisplatin-modified DNA through this DNA-binding component. Here we demonstrate the affinity and specificity of FACT for cisplatin-modified DNA and the major 1,2-intrastrand cisplatin-DNA lesion in particular. Electrophoretic mobility shift assays reveal that both the SSRP1 and Spt16 subunits of FACT are necessary for high affinity binding to cisplatin-modified DNA. The isolated HMG domain of SSRP1, however, is sufficient for binding the major 1,2-d(GpG) intrastrand cisplatin-DNA cross-link. The affinity and specificity of FACT for cisplatin-modified DNA and its role in modulating chromatin structure during transcription suggest that the interaction of FACT and cisplatin-modified DNA may be important in the cisplatin anticancer mechanism.

EXPERIMENTAL PROCEDURES

Oligonucleotide Probes—A 127-base pair probe was constructed by digesting a commercially available 123-base pair ladder (Life Technologies, Inc.) with Avai restriction enzyme (New England Biolabs). The digested 123-base pair fragment containing 4-bp 5'-overhangs was purified on a 15% native polyacrylamide gel, ethanol-precipitated, and quantitated by UV-vis spectroscopy (Hewlett Packard 8453). The purified probe was modified with cisplatin or trans-DTP at various platinum:nucleotide ratios (r_p, bound platinum:nucleotide ratio) following published procedures (26). The extent of platination (r_p, bound platinum:nucleotide ratio) was determined by flameless atomic absorption spectroscopy (PerkinElmer Life Sciences HGA-800 Aanalyst 300) and UV-vis spectroscopy. Platinated and unplatinated 123-base pair probes having recessed 3'-ends were further labeled with Klenow fragment of DNA polymerase (New England Biolabs) and [α-32P]dCTP (PerkinElmer Life Sciences), cold dATP, dTTP, and dGTP to give 127-base pair oligonucleotide duplexes. Radiolabeled probes were purified with G25 Quick Spin columns (Roche Molecular Biochemicals), ethanol-precipitated, and quantitated by scintillation counting (Beckman LS7500). The probe mixtures were denatured polyacrylamide gels. Subsequent autoradiography allowed single-stranded 156-mers to be excised and isolated from the gel by electroelution and ethanol precipitation. DNA duplexes were obtained by renaturing single-stranded 156-nt oligonucleotides, followed by purification on 10% native polyacrylamide gels, electroelution, and ethanol precipitation. The resulting 156-bp probes were quantitated by scintillation counting.

Plasmids—cDNA encoding the HMG (residues 539–614) domain of SSRP1 was amplified by PCR using human SSRP1 cDNA and the primers 5'-GCTCTAGAAGGAGGTTGAGACAGTAAGGCGAAAAGA C-3' and 5'-CTCGCTCTGCGCATGTTAAATTCTTCTGACCTT-3'. The PCR primers contained restriction sites for XhoI and NdeI (italicized) as well as initiation and termination codons (underlined); the last, 5'-AAGGAG, was positioned nine base pairs upstream of the initiation codon. PCR was performed with Taq DNA polymerase (Life Technologies, Inc.) under the following thermal cycler conditions: 5 min at 94 °C (denaturation cycle); 1 min at 94 °C, 2 min at 50 °C, and 3 min at 72 °C (25 cycles); 10 min at 72 °C (extension cycle). The resulting fragments were digested with XhoI and NdeI (New England Biolabs), purified by using a PCR purification kit (Qiagen), and ligated into the XhoI and NdeI sites of pET3a (Novagen) to give pSSRP.d1 for expression of the HMG domain. Correct insertion of the PCR fragment was confirmed by restriction enzyme mapping and DNA sequencing.

Expression and Purification of Recombinant SSRP1 HMG-Domain Peptides—BL21 (DE3) Codon Plus RIL (Stratagene) cells harboring pSSRP.d1 were grown at 37 °C in LB containing 100 μg/ml ampicillin and 20 μM methionine. Protein production was induced at an A₆₀₀ of 0.7 by addition of isopropyl β-D-thiogalactopyranoside to a final concentration of 1 mM. After an induction period of 5 h, cells were collected by centrifugation, resuspended in cold lysis buffer (50 mM Tris, pH 7.0, 20 mM NaCl, 10 mM EDTA, 10 mM EGTA, 10 mM MgCl₂, 0.05% β-mercaptoethanol, 1 mM Pefabloc, 1 mM Dnase I, 2 mM dithiothreitol, 20 μM methionine, 2 mM Na₃O₂SO₄, and lysed by sonication. Debris was removed by centrifugation, and cellular proteins were precipitated with 55% saturated ammonium sulfate. Recombinant protein was precipitated with 98% saturated ammonium sulfate, collected by centrifugation, resuspended in buffer A (20 mM Tricine, pH 8.3, 5 mM dithiothreitol, 1 mM EDTA, 20 mM methionine, 2 mM Na₃O₂SO₄, and dialyzed against the same buffer. The desalted protein solution was loaded onto a cation-exchange SP-Sepharose Fast Flow (Amersham Pharmacia Biotech) column washed with buffer A. Proteins were eluted with a linear gradient of 0–1% NaCl in buffer A. Eluted proteins were detected by SDS-PAGE follow by Coomassie staining. Fractions containing SSRP1 HMG domain were pooled, dialyzed, concentrated, and applied to a Superdex 75 column (Amersham Pharmacia Biotech) washed with buffer B (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 1.4 mM KH₂PO₄, 20 mM methionine, 2 mM Na₃O₂SO₄, pH 7.3). Proteins were eluted with column buffer, and fractions containing pure SSRP1 HMG domain were pooled, concentrated, frozen in liquid nitrogen, and stored at −80 °C. SSRP1 HMG domain concentrations were determined from A₂₈₀ values by using an extinction coefficient (ε₂₈₀ = 18,600 M⁻¹ cm⁻¹) calculated by quantitative amino acid analysis.

Isolation of FACT from HeLa Cells—FACT was isolated from HeLa cells as described (5). The purity of native FACT (hFACT) was assessed by denaturing and native PAGE.

Production of Recombinant SSRP1, Spt16, and FACT—Methods for recombinant SSRP1 (rSSRP1) and Spt16 (rSpt16) production and purification will be reported elsewhere. Recombinant FACT (rFACT) was obtained by mixing equimolar amounts of pure rSSRP1 and pure rSpt16, followed by gel filtration chromatography.

**Gel Mobility Shift Assays**—Gel mobility shift assays with SSRP1

8 S. S. Marla and S. J. Lippard, personal communication.

9 S. Oh and D. Reinberg, personal communication.
HMG domain and protein were performed as follows. To assay for protein recognition of cisplatin-damaged DNA, cisplatin-modified or unmodified 127-base pair duplexes (0.2 nm, 40,000 cpm) were titrated with protein in buffer PP250 (10 mM Tris, pH 7.5, 250 mM NaCl, 10 mM MgCl₂, 0.5 mM EDTA, 5% glycerol, 1 mM dithiothreitol) containing 0.2 mg/ml of chicken erythrocyte genomic DNA. Samples were incubated on ice for 1 h and made 10% in sucrose immediately prior to loading onto pre-equilibrated native 4% polyacrylamide gels (29:1 acrylamide:bisacrylamide, 3.3% cross-linking, 45 mM Tris borate, 1 mM EDTA, pH 8.3). Electrophoresis was continued for 2 h at 215 V and 4 °C. To assay for binding of the major 1,2-d(GpG) intrastrand cisplatin-DNA crosslink, AG*G*A and AGGA 15-bp oligonucleotide duplexes (0.4 nm, 20,000 cpm) were titrated with protein in binding buffer SD250 (10 mM Hepes, pH 7.5, 10 mM MgCl₂, 50 mM LiCl, 100 mM NaCl, 1 mM spermidine, 0.2 mg/ml bovine serum albumin, 0.05% Nonidet P-40). Samples were incubated on ice for 30 min and made 10% in sucrose immediately prior to loading onto pre-equilibrated native 12% polyacrylamide gels (29:1 acrylamide:bisacrylamide, 3.3% cross-linking, 45 mM Tris borate, 1 mM EDTA, pH 8.3). Electrophoresis was continued for 1.5–2 h at 300 V and 4 °C. Following electrophoresis, gels were dried and exposed to a phosphorimaging plate for 12–24 h. The amount of bound and free oligonucleotide was assessed with a Bio-Rad GS-525 phosphorimaging device.

To examine the affinity and specificity of FACT for cisplatin-damaged DNA, 127-bp duplexes (0.02–2 nm, 20,000 cpm) undamaged or globally damaged with cisplatin or trans-DDP, or 156-bp site-specific modified or unmodified duplexes (0.1 nm, 20,000 cpm) were combined with hFACT, rFACT, rSpt16, or rSSRP1 in binding buffer PP50 (10 mM Tris, pH 7.5, 10 mM MgCl₂, 50 mM NaCl, 1 mM dithiothreitol) containing 0.2 mg/ml of chicken erythrocyte genomic DNA competitor. The HMG domain retains affinity for cisplatin-modified DNA, as evidenced by gel-mobility shifts of the domain with a 127-bp probe damaged globally with cisplatin (Fig. 3A). The interaction between the domain and cisplatin-modified DNA is not a result of general DNA affinity for the probe because the domain fails to bind the unmodified 127-mer under identical conditions (Fig. 3A). The domain does exhibit very low affinity for probes damaged with the clinically ineffective trans-dddP isomer (data not shown); however, protein affinity for the cisplatin-damaged probe is significantly higher than that for the corresponding trans-dddP-damaged DNA.

The mobility of protein-DNA complexes formed upon incubation of hSSRP1 HMG domain with the cisplatin-modified 127-bp probe decreases with increasing protein concentration (Fig. 3A). A similar pattern of mobility shifts occurs when probes with increasing damage levels (0.003–0.044 platinum atoms/nucleotide, 1–12 platinum atoms/duplex DNA) are used (data not shown). Both of these results are consistent with multiple proteins binding to these long probes at high platinisation or protein levels.

Recognition of the Major 1,2-d(GpG) Intrastrand Cisplatin-DNA Adduct by hSSRP1 HMG Domain—To confirm that the minimal HMG domain of hSSRP1 can bind the major 1,2-d(GpG) intrastrand cisplatin-DNA cross-link, the affinity of the domain for the oligonucleotide duplex AG*G*A, 5’-CCTCTAGAG*GATCCTC-3’, where asterisks denote the sites of platinum coordination to the N-7 positions of adjacent guanines, was investigated. A representative gel mobility shift with AG*G*A and hSSRP1 HMG domain is shown in Fig. 3B. The domain fails to shift the corresponding unplatinated duplex under identical conditions (data not shown).

Isolation of hFACT from HeLa Cells—Human FACT, the heterodimer of hSpt16 and hSSRP1, was isolated from HeLa cells as described (5). The complex contains hSpt16 and hSSRP1, as well as a minor contaminant at ~40 kDa as judged by SDS-PAGE followed by silver staining. This contaminant is not necessary for FACT activity (7). Native PAGE demonstrates that hFACT isolated in this manner contains no free hSSRP1 or hSpt16.

Gel Mobility Shift Assays of hFACT with DNA Damaged with Cisplatin—To assess the ability of hFACT to bind specifically to cisplatin-modified DNA, gel mobility shift assays were performed with a series of platinated or unplatinated 127-bp probes (Fig. 4A). Cisplatin- and trans-DDP-damaged probes used in these experiments were modified at similar levels. FACT retains some nonspecific affinity for all probes in the
absence of competitor; however, the band of lowest mobility observed in lanes 2, 5, and 8 corresponds to the position of free protein (data not shown) and disappears in the presence of excess competitor (Fig. 4A). A specific protein-DNA complex is formed upon incubation of FACT with the 127-bp probe modified globally with cisplatin. This complex persists even in the presence of a 3.6 \times 10^5-fold excess of nonspecific competitor DNA (Fig. 4A, lanes 5 and 6) and remains robust in NaCl concentrations of at least 250 mM (data not shown). The mobility of the putative FACT-cis127-bp complex is unchanged by increasing platinum damage levels within the range of 1–8 platinum atoms/127-bp duplex (data not shown).

Fig. 3. Affinity and specificity of SSRP1 HMG domain for cisplatin-damaged DNA. A, gel mobility shift assays of SSRP1 HMG domain with a 127-bp DNA probe undamaged or damaged globally with cisplatin. ^32P-Labeled 127-bp or cis127-bp (0.012 Pt/nt, 3 Pt/duplex) probe (0.2 nM) was incubated with 0, 260 nM, or 2,600 nM SSRP1 HMG domain as indicated and subjected to electrophoresis in the presence of 0.2 mg/ml chicken erythrocyte genomic DNA. B, gel mobility shift assay of SSRP1 HMG domain and AG^*G*A ^32P-labeled probe demonstrates ability of domain to bind the major 1,2-d(GpG) intrastrand cisplatin-DNA adduct. Radiolabeled AG^*G*A probe (0.4 nM) was incubated with 0 or 100 nM SSRP1 HMG domain as indicated and electrophoresed.

Fig. 4. Affinity and specificity of hFACT for cisplatin-damaged DNA. A, native hFACT demonstrates both high affinity and specificity for cisplatin-damaged DNA with respect to undamaged DNA or DNA damaged with the clinically ineffective trans-DDP isomer. Native FACT (130 nM) was incubated with ^32P-labeled 127-bp, cis127-bp (0.012 Pt/nt, 3 Pt/duplex) or trans127-bp (0.015 Pt/nt, 3–4 Pt/duplex) probe (0.2 nM) in the presence or absence of 2 mg/ml nonspecific chicken erythrocyte genomic DNA. B, native hFACT recognizes the major 1,2-d(GpG) intrastrand cisplatin-DNA lesion. Native hFACT (130 nM) was subjected to gel mobility shift assay with ^32P-labeled 156-bp probe containing a single, centered 1,2-d(GpG) intrastrand cisplatin adduct (0.1 nM) or the corresponding undamaged 156-bp duplex (0.1 nM).

The recombinant complex yields a bandshift pattern with the cis127-bp probe identical to that of native FACT (compare Figs. 4A and 4B). As with native FACT, rFACT fails to bind the trans-DDP-modified probe under these conditions (data not shown). In contrast, gel mobility assays with each of the recombinant FACT subunits indicate that the Spt16 subunit fails to bind the cis127-bp probe (Fig. 6B) and that the SSRP1 subunit gives rise to only a very weak shift that disappears in the presence of excess competitor (Fig. 6C). Although native and recombinant FACT form specific protein-cis127-bp complexes at protein concentrations as low as 3 nM (data not shown), rSSRP1 fails to yield a bandshift pattern similar to that of rFACT or native FACT even at protein concentrations of 900 nM (Fig. 6C).

**DISCUSSION**

**Interaction of the Isolated HMG Domain of SSRP1 with Cisplatin-damaged DNA**—Fragments of SSRP1 interact with...
cisplatin-modified DNA binding, a fragment corresponding to residues 539–614 was expressed and purified from *Escherichia coli*. This recombinant HMG domain was used in gel mobility shift experiments with globally cisplatin-modified and unmodified probes. The isolated domain is sufficient for specific binding to DNA damaged globally with cisplatin. The domain is selective for cisplatin-modified DNA with respect to both unmodified DNA and DNA containing trans-DDP adducts. In addition, like other isolated HMG domains (27, 40), the HMG domain of SSRP1 binds oligonucleotide probes containing the major 1,2-(d(GpG)) intrastrand cisplatin-DNA cross-link. The affinity of this interaction depends on the DNA sequence flanking the drug-DNA lesion as seen for HMG domains A and B of HMG1 (27).

Interaction of FACT and SSRP1 with Cisplatin-damaged DNA—Both SSRP1 and its physiologically relevant complex with Spt16, FACT, are expected to bind distorted DNA structures including cisplatin-DNA cross-links by means of the HMG domain of SSRP1. Here we show that FACT has both affinity and specificity for DNA damaged globally with cisplatin with respect to undamaged DNA. A tightly bound complex results from incubation of FACT with cisplatin-modified DNA as evidenced by its resilience in the presence of 3.6 × 10⁵-fold excess of nonspecific DNA or >50-fold excess of unplatinated competitor DNA. Like other HMG-domain proteins, FACT binds the major 1,2-intrastrand cross-links of cisplatin-modified DNA. The 1,2-intrastrand cisplatin-DNA cross-links are the most abundant DNA adducts formed following cisplatin treatment (41). These adducts are thought to be crucial to cisplatin toxicity because geometric constraints prohibit the clinically inactive trans-DDP from forming 1,2-intrastrand adducts, although trans-DDP can form DNA cross-links similar to the less abundant cisplatin-DNA lesions (21). Notably, FACT fails to bind DNA damaged with the clinically ineffective trans-DDP isomer.

We then sought to confirm that the SSRP1 subunit confers the ability to recognize cisplatin-modified DNA on the FACT complex. As anticipated, Spt16, which contains no putative DNA-binding domains, has no affinity for either cisplatin-damaged or undamaged probes. Unexpectedly, SSRP1 fails to form a high-affinity complex with the same cisplatin-modified probe in gel mobility shift assays under conditions sufficient for hFACT binding. The complex of rSSRP1 and rSpt16, however, is capable of binding cisplatin-damaged DNA with affinity and specificity comparable with hFACT in identical bandsift assays. A >300-fold excess of rSSRP1 fails to afford a protein-DNA complex of affinity comparable with that of hFACT or rFACT, suggesting that protein concentration discrepancies are unlikely to explain the lack of a bandsift with rSSRP1. The observed differences in affinity between the protein-DNA complex formed by hFACT or rFACT and that formed by SSRP1 suggest that Spt16 primes SSRP1 for cisplatin-modified DNA recognition. We suggest that, in the absence of Spt16, SSRP1 adopts a fold that renders its HMG domain inaccessible to cisplatin-modified DNA. When Spt16 is present, a conformational change occurs unveiling the HMG domain of SSRP1 and leading to the observed binding interaction.

Functional Implications—Because modulation of chromatin structure is essential to many cellular processes that use DNA as a substrate, including replication and transcription, FACT may effect such processes. The specific interaction of SSRP1 and FACT with DNA damaged with the anticancer drug cisplatin as judged by electrophoretic mobility shift assays implicates both SSRP1 and FACT in the mechanism of cisplatin cytotoxicity. The binding of SSRP1 or FACT to cisplatin-damaged DNA could mediate cellular sensitivity to the drug by
shielding its DNA cross-links from repair, allowing the drug-DNA lesions to persist and eventually leading to cell death (28, 29, 35, 39, 42–44). Moreover, presence of cisplatin-DNA adducts could titrate SSRP1 or FACT from its normal binding sites, thereby disrupting SSRP1/FACT function(s) (45, 46). Finally, the formation of stable FACT/SSRP1 complexes at cisplatin-DNA cross-links could lead to stalling of the RNAPII transcription machinery, ubiquitination of RNAPII, and proteolysis resulting ultimately in cell death (30, 31). Any or all of these activities may contribute to the mechanism of cisplatin-mediated cytotoxicity.

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