Protein Kinase CK2 Phosphorylates the High Mobility Group Domain Protein SSRP1, Inducing the Recognition of UV-damaged DNA*

Received for publication, January 9, 2003, and in revised form, February 4, 2003
Published, JBC Papers in Press, February 4, 2003, DOI 10.1074/jbc.M300250200

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The structure-specific recognition protein SSRP1 plays a role in transcription and replication in the chromatin context. Mediated by its C-terminal high mobility group (HMG) box domain, SSRP1 binds DNA non-sequence specifically but recognizes certain DNA structures. Using acetic acid urea polyacrylamide gel electrophoresis and mass spectrometry, we have examined the phosphorylation of maize SSRP1 by protein kinase CK2α. The kinase phosphorylated several amino acid residues in the C-terminal part of the SSRP1 protein. Two phosphorylation sites were mapped in the very C-terminal region next to the HMG box domain, and about seven sites are localized within the acidic domain. Circular dichroism showed that the phosphorylation of the two C-terminal sites by CK2α resulted in a structural change in the region of HMG box domain, because the negative peak of the CD spectrum at 222 nm was decreased by −10%. In parallel, the phosphorylation induced the recognition of UV-damaged DNA, whereas the non-phosphorylated protein does not discriminate between UV-damaged DNA and control DNA. The affinity of CK2α-phosphorylated SSRP1 for the DNA correlates with the degree of UV-induced DNA damage. Moreover, maize SSRP1 can restore the increased UV-sensitivity of a yeast strain lacking the NHP6a/B HMG domain proteins to levels of the control strain. Collectively, these findings indicate a role for SSRP1 in the UV response of eukaryotic cells.

In eukaryotes, the packaging of genomic DNA with histones and other proteins into chromatin affects DNA-dependent processes, including transcription, recombination, replication, and repair. In many cases, chromatin represses these processes by restricting the access of DNA binding regulatory factors to their DNA target sites. Numerous nuclear activities have been identified that can overcome the repressive effects of chromatin. Post-translational modification of histones and ATP-dependent, chromatin-remodeling complexes are involved in altering the chromatin structure so that the DNA becomes more accessible to the DNA-binding proteins required for transcription initiation (1, 2). DNA repair and other DNA-dependent processes occurring in the chromatin context are also coupled to the structural properties of chromatin.

DNA is frequently damaged by a variety of agents that are of endogenous or environmental origin. For many organisms, the most important environmental mutagen is the ultraviolet component of sunlight. The DNA lesions induced by UV light (mainly dipyrimidine photoproducts) are both mutagenic and cytotoxic. Therefore, constant removal and replacement of damaged nucleotide residues by DNA repair mechanisms is required to maintain the DNA as a carrier of stable genetic information (3, 4). DNA damage and repair processes are influenced to a great extent by the packaging of the DNA into chromatin. The repair machinery is therefore dependent on the assistance of ATP-dependent, chromatin-remodeling complexes (5–7).

In human cells, a protein complex termed FACT (facilitates chromatin transcription) has been identified that is a chromatin-specific elongation factor required for transcription of chromatin templates in vitro (8). The FACT complex interacts with the H2A/H2B dimers of the nucleosome, indicating that it may promote transcription by nucleosome remodeling. Biochemical analyses have revealed that the FACT of HeLa cells consists of the general transcriptional regulator CDC68/Spt16 and the SSRP1 protein containing an HMG box DNA-binding domain (9). In Xenopus, a homologous complex termed DUF (DNA unwinding factor) has been identified that is involved in DNA replication in cell-free extracts (10). Extensive genetic and biochemical work in yeast has demonstrated the existence of the CP complex consisting of CDC68 and Pob3 (which is homologous to SSRP1) and shown that both proteins are encoded by essential genes. The CP complex plays a role in transcription and replication (11, 12). Yeast Pob3 lacks the HMG box DNA binding domain of the homologous SSRP1 occurring in higher eukaryotes, and the small HMG-type protein NHP6 appears to provide the HMG domain function, mediating the interaction with nucleosomes (13, 14).

In plants, SSRP1 proteins (~72 kDa) with similarity to the vertebrate counterparts have been identified from both monocotyledinous and dicotyledonous species (15). Mediated by its HMG box domain, maize SSRP1 binds non-sequence specifically to linear DNA and recognizes nucleosomes and certain DNA structures such as DNA minicircles and supercoiled DNA. Moreover, maize SSRP1 can bend DNA and facilitate the formation of specific nucleoprotein structures (16, 17). Here we demonstrate that maize SSRP1 is a substrate for protein kinase CK2α and that the phosphorylated protein (but not the unphosphorylated one) increases the rate of DNA bending up to 10%. In parallel, the phosphorylation increased the recognition of UV-damaged DNA, whereas the non-phosphorylated protein does not discriminate between UV-damaged DNA and control DNA. The affinity of CK2α-phosphorylated SSRP1 for UV-damaged DNA correlates with the degree of UV-induced DNA damage. Moreover, maize SSRP1 can restore the increased UV-sensitivity of a yeast strain lacking the NHP6a/B HMG domain proteins to levels of the control strain. Collectively, these findings indicate a role for SSRP1 in the UV response of eukaryotic cells.

* This work was supported by a grant from the Danish Research Council (to K. D. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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** The abbreviations used are: SSRP1, structure-specific recognition protein 1; HMG, high mobility group; AU, acetic acid urea; CD, circular dichroism; YPD, yeast extract-peptone-dextrose; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight.
non-phosphorylated protein) recognizes UV-damaged DNA. Furthermore, we show that a yeast nhp6Δb double mutant is sensitive toward UV irradiation compared with the wild type strain and that the expression of maize SSRP1 can complement the increased UV sensitivity of the yeast Δnhp6Δb strain.

EXPERIMENTAL PROCEDURES

Expression, Purification and Phosphorylation of SSRP1 Proteins—The region encoding SSRP1-(Asp455–Asp639) was amplified from the maize SSRP1 cDNA using Deep Vent DNA polymerase (New England Biolabs) and the primers 5′-AAGAGGCCAGGTTCAGGAGACACTGAT-3′ and 5′-CACTGACCTGACATTGACCTGGTGCAAA-3′. The amplified product was ligated into the BamHI and EcoRI sites of pBluescript KmI/KpnI, creating blunt ends using the Klenow DNA polymerase and subsequent digestion with BamHI and EcoRI, because the UV-induced dimerization of the thymine bases occurring within the GAATTC EcoRI recognition sequence abolished the endonuclease digestion of the fragment (21). For the control experiments with non-phosphorylated proteins, the substrate proteins were incubated in mock phosphorylation reactions lacking either ATP or the protein kinase. The phosphorylation state of the SSRP1 proteins was examined by mass spectrometry and acetic acid urea (AU)-PAGE (20).

Mass Spectrometric Analyses of SSRP1 Proteins and Tryptic Peptides—Mass spectrometric analyses of SSRP1 proteins and tryptic peptides were performed on a Voyager-DE PRO system equipped with a nitrogen laser (Applied Bio-systems) using the linear and the reflector mode. The system was calibrated with standards provided by the supplier. As matrix sinapinic acid (20 mg/ml in 50% acetonitrile, 0.1% trifluoroacetic acid) was used. Samples were mixed 1:1 with the matrix on the target plate and air-dried.

Circular Dichroism—CD spectra of non-phosphorylated and CK2α-phosphorylated SSRP1(K532-D639) were recorded and analyzed as described previously (19).

Analysis of DNA Binding by Electrophoretic Mobility Shift Assays (EMSAs)—The purified 78-bp XbaI/KpnI fragment of pBluescript (Stratagene) was UV irradiated with 5 or 10 J/m² at 254 nm. The UV-induced DNA damage was analyzed by determining the cleavability of the DNA fragment with EcoRI, because the UV-induced dimerization of the thymine bases occurring within the GAATTC EcoRI recognition sequence abolished the endonuclease digestion of the fragment (21). For instance, at an UV dose of 5 J/m², ~50% of the fragment was cleavable with EcoRI (data not shown). Binding reactions contained binding buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 5% (w/v) glycerol, 0.05% (w/v) bromophenol blue, 0.05% (w/v) xylene cyanol), various amounts of protein, and 15 ng of the 78-bp fragment. Binding reactions were incubated for 5 min at room temperature before loading of the samples onto 5% polyacrylamide gels in 1× TBE buffer (90 mM Tris borate, pH 7.5, 1 mM EDTA). The DNA was stained with SYBR Gold (Molecular Probes) and detected with the Typhoon 8600 Photomager (Amersham Biosciences). Data were analyzed using the Image Quant software.

Analysis of the UV Sensitivity of Yeast Strains—The coding region for maize SSRP1 was obtained by digesting the plasmid pQE9cm-SSRP1 with PstI, creating blunt ends using the Klenow DNA polymerase and subsequent digestion with BamHI. The pMTL1 yeast expression vector (22) was prepared by digestion with HindIII, creating blunt ends using the Klenow DNA polymerase and subsequent digestion with BamHI. The SSRP1 coding region was ligated with pMTL1, resulting in the expression plasmid pMTL1-SSRP1, which was checked by DNA sequencing. The plasmids pMTL1 and pMTL1-SSRP1 were transformed into the yeast strain Y18 (MATa ura3–52 trpl–289 his3–112 leu2–3 112 gal2 gal10 and U82 (MATa trpl–289 his3–112 leu2–3 112 gal2 gal10 nhp6A–Δ:URA3 nhp6B–1::HIS3) by the lithium acetate method. The increased generation time and the temperature sensitivity of the Δnhp6ΔaΔb strain (Y82) was examined and proved to match the results published previously (23). The UV-sensitivity of the different strains was determined by calculating the survival rates for Y92 cells on solid YPD medium (24). Single colonies were grown to an A600 of 1 in selective liquid medium. The cells were harvested by centrifugation and washed with TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). After serial dilution in TE buffer, the cells were spread onto YPD plates (strains transformed with pMTL1 plasmids on galactose-containing plates), which were irradiated using a UV Stratalinker 2400 (Stratagene) with different UV doses of UV 254 nm. The plates were incubated at 30 °C for 5 days before scoring of the surviving colonies.

RESULTS

Protein Kinase CK2 Phosphorylates Several Residues in SSRP1—Human SSRP1 can occur in an UV-induced complex with Spt16, p53, and CK2, resulting in specific CK2-mediated Ser359 phosphorylation of p53 (26, 27). Analysis of the amino acid sequence of maize SSRP1 using the program PhosphoBase 2.0 (28) revealed that maize SSRP1 contains a number of potential phosphorylation sites for protein kinase CK2. Therefore, we examined whether maize SSRP1 (schematically depicted in Fig. 1A) is also a substrate of CK2. In contrast to most other protein kinases, CK2 can utilize both ATP and GTP as phosphate donors (29–31). The phosphorylation of SSRP1 by recombinant maize CK2α was analyzed for different reaction times in the presence of [γ-32P]GTP or [γ-32P]ATP (Fig. 1B). Both phosphate donors were utilized by CK2α to phosphorylate SSRP1 with similar efficiency. To determine which domain(s) of SSRP1 are phosphorylated, full-length SSRP1 (residues
Met<sup>1</sup>–Asp<sup>629</sup> and the truncated proteins SSRP1-(Asp<sup>455</sup>–Asp<sup>629</sup>) lacking the large N-terminal domain, and SSRP1(Lys<sup>552</sup>–Asp<sup>639</sup>), consisting essentially of the HMG box DNA-binding domain (Fig. 1A), were tested for phosphorylation by CK2α in the presence of [γ<sup>32</sup>P]ATP (Fig. 1C). Both full-length SSRP1 and SSRP1(Asp<sup>455</sup>–Asp<sup>639</sup>) were readily phosphorylated by CK2α to similar extents, suggesting that the C-terminal part of the protein is phosphorylated. The very C-terminal end of the protein, SSRP1(Lys<sup>552</sup>–Asp<sup>639</sup>), is phosphorylated by CK2α to a lesser extent (Fig. 1B), demonstrating that SSRP1 is mainly phosphorylated within the acidic domain (residues Asp<sup>455</sup>–Gln<sup>531</sup>). To estimate the number of amino acid residues phosphorylated in SSRP1, truncated versions of SSRP1 were phosphorylated for different times by CK2α in the presence of unlabelled ATP. The phosphorylated proteins were analyzed by AU-PAGE, which can resolve proteins depending on their phosphorylation state. Phosphorylation of the C-terminal protein constructs SSRP1(Lys<sup>552</sup>–Asp<sup>639</sup>) and SSRP1(Lys<sup>552</sup>–Asp<sup>619</sup>) resulted in the appearance of two protein bands with reduced electrophoretic mobility (relative to the non-phosphorylated protein) upon CK2α phosphorylation, indicating the presence of two phosphorylation sites in these proteins (Fig. 2). The same experiment was performed with the C-terminal portion of SSRP1 including the acidic domain (Fig. 2). The number of phosphorylated residues in SSRP1 (Lys<sup>552</sup>–Asp<sup>639</sup>) and SSRP1(Asp<sup>455</sup>–Asp<sup>639</sup>) was determined using MALDI/TOF mass spectrometry. Comparison of the masses determined for non-phosphorylated and CK2α-phosphorylated SSRP1-(Lys<sup>552</sup>–Asp<sup>639</sup>) and SSRP1-(Asp<sup>455</sup>–Asp<sup>639</sup>) revealed mass differences of 161 and 729 Da, respectively (data not shown). This indicates the presence of two phosphorylated residues in SSRP1-Lys<sup>552</sup>–Asp<sup>639</sup> (and depending on the number of sodium adducts, Ref. 19) up to nine phosphorylated residues in SSRP1-(Asp<sup>455</sup>–Asp<sup>639</sup>), which is in line with the analysis of the phosphorylated proteins by AU-PAGE. To determine more precisely which amino acid residues of the C-terminal part of SSRP1 are phosphorylated by CK2α, non-phosphorylated and CK2α-phosphorylated SSRP1-(Lys<sup>552</sup>–Asp<sup>639</sup>) were digested with trypsin, and the resulting peptides were examined by MALDI/TOF mass spectrometry (Table I). This analysis revealed that the very C-terminal peptide Gly<sup>626</sup>–Asp<sup>639</sup> (comprising the peptide C-terminal of the HMG box domain) occurs in the double-phosphorylated form. This peptide contains one threonine and two serine residues, which theoretically could be phosphorylated. However, the threonine Thr<sup>629</sup> is unlikely to be phosphorylated, first because serine residues are by far preferred over threonine residues as phosphor acceptor sites of CK2 and, second, because the sequence context of the two serine residues more closely resembles typical CK2 phosphorylation sites (29–31). Moreover, the two serine residues are well conserved among SSRP1 proteins of different plant species, whereas the threonine residue Thr<sup>629</sup> occurs exclusively in maize SSRP1 (17). Therefore, Ser<sup>634</sup> and Ser<sup>638</sup> are most likely phosphorylated by CK2α. Because of the unusual acidity of the involved peptides (which poses a problem for mass spectrometry), we were, to date, unable to determine precisely which of the 14 possible Ser/Thr residues (of which –8 are situated in a CK2 consensus sequence) in the acidic region (Asp<sup>455</sup>–Gln<sup>531</sup>) are actually phosphorylated by CK2α.

| Peptide               | Calculated mass<sup>a</sup> | Untreated<sup>b</sup> | CK2α<sup>c</sup> |
|----------------------|-----------------------------|---------------------|-----------------|
| Lys<sup>552</sup>–Lys<sup>554</sup> | 2167.0                      | 2165.7              | 2165.0          |
| Ala<sup>560</sup>–Arg<sup>572</sup> | 1581.7                      | ND                  | 1581.9          |
| Ser<sup>577</sup>–Lys<sup>589</sup> | 1372.7                      | 1372.6              | 1373.0          |
| Lys<sup>592</sup>–Lys<sup>607</sup> | 1019.6                      | 1019.5              | 1019.7          |
| Met<sup>598</sup>–Lys<sup>603</sup> | 680.3                       | 680.1               | ND              |
| Gln<sup>604</sup>–Lys<sup>614</sup> | 1317.7                      | 1317.6              | 1317.8          |
| Lys<sup>615</sup>–Lys<sup>619</sup> | 723.4                       | 724.3               | 724.5           |
| Gly<sup>621</sup>–Arg<sup>625</sup> | 724.4                       | 724.3               | 724.5           |
| Gly<sup>626</sup>–Asp<sup>639</sup> | 1394.6                      | 1394.6              | 1555.7          |

<sup>a</sup> The mass values (in Da) were calculated based on the known protein sequence.

<sup>b</sup> The masses of the tryptic peptides (in Da) were determined by MALDI-TOF mass spectrometry.

<sup>c</sup> Tryptic peptides of SSRP1-(Lys<sup>552</sup>–Asp<sup>639</sup>) phosphorylated by CK2α.

<sup>d</sup> The N-terminal peptide includes also the 6×His-tag.

<sup>e</sup> Not determined.
creases (~10%) of the negative peak at 222 nm in the CD spectrum of the phosphorylated protein (relative to the non-phosphorylated protein) indicates loss of α-helical structure. The structure of the HMG box domain is ~75% α-helical, suggesting that there may be phosphorylation-induced structural changes in the DNA-binding domain.

Because the C-terminal phosphorylation of maize chromosomal HMGB proteins by CK2 reduces the affinity of the HMGB1 and HMGB2 proteins for linear DNA (19), we examined whether the phosphorylation of the two amino acid residues at the C-terminal end of SSRP1 influences the DNA binding of SSRP1-(Lys532–Asp639). The interaction of non-phosphorylated and CK2a-phosphorylated SSRP1-(Lys532–Asp639) with a 78-bp DNA fragment was analyzed in electrophoretic mobility shift assays. Various concentrations of the protein were incubated with the DNA fragment, and the formation of protein/DNA-complexes was examined using native PAGE (Fig. 4). The non-phosphorylated and the CK2a-phosphorylated SSRP1-(Lys532–Asp639) bound the DNA-fragment with similar affinity, as can be seen by the disappearance of the unbound fragment and the appearance of a rather diffuse complex of lower electrophoretic mobility (Fig. 4, top panels; lanes 5 and 6). To examine whether UV-damaged DNA is a preferred target for SSRP1, the DNA fragment was treated with different doses of UV prior to the protein binding studies. The UV-damage of the DNA is evident from the more diffuse and slightly reduced electrophoretic mobility of the DNA fragment (32) relative to the untreated DNA (compare Fig. 4, lanes 1) and from its reduced cleavability by EcoRI (data not shown). The phosphorylated protein bound the UV-treated DNA fragment at significantly lower protein concentrations when compared with the non-phosphorylated protein (Fig. 4), demonstrating that the phosphorylated protein displayed a higher affinity for the UV-damaged DNA. This effect was dependent on the UV dose used to irradiate the DNA. When the DNA was irradiated with 5 J/m², the phosphorylated protein displayed a ~5-fold higher affinity for the DNA than the non-phosphorylated protein, whereas with the DNA that was irradiated with 10 J/m² there was a ~10-fold difference in affinity. Unlike the CK2-

phosphorylated protein that discriminated UV-damaged DNA and control DNA, non-phosphorylated SSRP1-(Lys532–Asp639) bound the UV-damaged DNA and the control DNA with the same affinity.

SSRP1 Can Complement the UV Sensitivity of the Yeast NHP6p6p6p Mutant—The yeast homologue of animal and plant SSRP1, termed Pob3, lacks an HMG box DNA-binding domain. Recently, it was demonstrated that the HMGB-type protein NHP6p/interacts with the CDC68/Pob3 complex (CP complex) to provide an HMG box domain so that Pob3 and NHP6p function as a bipartite analogue of SSRP1 (13, 14). An artificial SSRP1-like protein, created by fusing Pob3 and NHP6p, is able to perform several in vivo functions (13). Yeast mutant strains lacking either NHP6p or NHP6p (88% amino acid sequence identity) grow normally, whereas the strain lacking both proteins displays, among other defects, a temperature-sensitive growth (23). After confirming some of these published growth characteristics (data not shown), we have monitored the sensitivity of the double mutant strain Δnhp6a/b (Y82) toward UV irradiation in comparison to the control strain (Y18). The yeast strains were treated with different doses of UV light, and the colony formation of surviving cells was scored on YPD plates. The survival rate of the Δnhp6a/b strain was ~2.5-fold lower than that of the control strain at an UV irradiation of 25 and 50 J/m² (Fig. 5A). To examine whether maize

![Fig. 4. CK2a-mediated phosphorylation of C-terminal amino acid residues results in the recognition of UV-damaged DNA by SSRP1. Increasing concentrations (0, 50, 100, 250, 500 nM, and 1 μM, lanes 1–6, respectively) of CK2a-phosphorylated (left panels) or non-phosphorylated SSRP1-(Lys532–Asp639) (right panels) were incubated with a 78-bp DNA fragment. The DNA was either untreated (top panels) or irradiated with 5 J/m² (middle panels) or 10 J/m² (bottom panels). The binding reactions were separated by native PAGE, stained with SYBR Gold, and scanned using a PhosphorImager.](http://www.jbc.org/)

![Fig. 5. The increased UV-sensitivity of the yeast Δnhp6a/b strain can be complemented by maize SSRP1. A, colonies of the Δnhp6a/b strain (Y82) and the control strain (Y18) were grown in liquid medium, spotted onto YPD plates in serial dilutions, and irradiated with various UV doses. Following UV irradiation, colonies were scored after 5 days of growth at 30 °C. The graphs depict the mean values of the survival rates of at least 10 individual clones of the two strains (relative to the non-irradiated controls). The formation of colonies was counted from each 10 starting colonies. The graph depicts the mean values of the survival rates of cells of the two strains relative to the non-irradiated cells. B, the survival rates of the Δnhp6a/b strain (Y82), the control strain (Y18), the strain Y82 bearing the insert-less pMTL1 plasmid, and the strain Y82 bearing the pMTL-SSRP1 plasmid were scored after UV irradiation of 25 J/m². The mean value of the survival rates was determined as described above, and, in the case of Y82-pMTL1 and Y82-pMTL1-SSRP1, four clones of three independent transformants were analyzed.](http://www.jbc.org/)

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**SSRP1 Binding to UV-damaged DNA**

1. **Fig. 4.** CK2α-mediated phosphorylation of C-terminal amino acid residues results in the recognition of UV-damaged DNA by SSRP1. Increasing concentrations (0, 50, 100, 250, 500 nM, and 1 μM, lanes 1–6, respectively) of CK2α-phosphorylated (left panels) or non-phosphorylated SSRP1-(Lys532–Asp639) (right panels) were incubated with a 78-bp DNA fragment. The DNA was either untreated (top panels) or irradiated with 5 J/m² (middle panels) or 10 J/m² (bottom panels). The binding reactions were separated by native PAGE, stained with SYBR Gold, and scanned using a PhosphorImager.

2. **Fig. 5.** The increased UV-sensitivity of the yeast Δnhp6a/b strain can be complemented by maize SSRP1. A, colonies of the Δnhp6a/b strain (Y82) and the control strain (Y18) were grown in liquid medium, spotted onto YPD plates in serial dilutions, and irradiated with various UV doses. Following UV irradiation, colonies were scored after 5 days of growth at 30 °C. The graphs depict the mean values of the survival rates of at least 10 individual clones of the two strains (relative to the non-irradiated controls). The formation of colonies was counted from each 10 starting colonies. The graph depicts the mean values of the survival rates of cells of the two strains relative to the non-irradiated cells. B, the survival rates of the Δnhp6a/b strain (Y82), the control strain (Y18), the strain Y82 bearing the insert-less pMTL1 plasmid, and the strain Y82 bearing the pMTL-SSRP1 plasmid were scored after UV irradiation of 25 J/m². The mean value of the survival rates was determined as described above, and, in the case of Y82-pMTL1 and Y82-pMTL1-SSRP1, four clones of three independent transformants were analyzed.
SSRP1 had an effect on the increased UV sensitivity of the Δnhp6Δb (Y82) strain, the survival upon UV irradiation with 25 J/m² of the non-transformed Y82 strain and Y82 bearing pMTL1-SSRP1 or pMTL1 was analyzed in comparison to the control strain Y18. Y82 bearing pMTL1-SSRP1 displayed UV sensitivity comparable with that of the Y18 control strain (Fig. 5B), indicating that the expression of SSRP1 could complement the lack of NHP6A/B with respect to UV sensitivity, whereas the presence of the insert-less plasmid pMTL1 in Y82 had no significant effect.

**DISCUSSION**

Protein kinase CK2, typically found in a heterotrimeric (α₂β₂) complex, is a Ser/Thr kinase conserved in a wide variety of eukaryotes, including plants, mammals, and yeast (29–31). CK2 phosphorylates predominantly serine residues that occur in an acidic environment within the substrate proteins and is involved in cell growth and proliferation but also in stress response and cell survival (33, 34). Plant CK2 from various species occurs as heterotrimeric enzyme but also as catalytically active monomeric CK2α (35–37). Here we have shown that CK2α phosphorylates several residues of the maize SSRP1 protein. Two phosphorylation sites were mapped within the very C-terminal tryptic peptide of SSRP1, which are in agreement with typical CK2 phosphorylation sites (29–31). The two serine residues are well conserved among different plant SSRP1 proteins but not in mammalian or *Drosophila* SSRP1 (17). However, the mammalian and *Drosophila* SSRP1 proteins (in comparison to the plant counterparts) a more extended domain C-terminal of the HMG box domain, which contains serine residues that perfectly match CK2 consensus recognition sites. Accordingly, CK2 can phosphorylate the C-terminal part of human SSRP1 (amino acid residues 471–709), but the exact phosphorylation site(s) have not yet been mapped (27). Therefore, CK2-mediated phosphorylation of residues C-terminal of the HMG box DNA-binding domain could be a general mechanism of SSRP1 regulation.

Like the non-sequence-specific HMG domain proteins of the HMG family, SSRP1 appears to be involved in a variety of DNA-dependent processes such as replication and transcription (38–41). In complex with CDC68/Spt16, SSRP1 (Pob3 in yeast) can act as facilitator of transcription and recombination in the chromatin context (9, 11). Mediated by its HMG box domain, SSRP1 binds DNA non-sequence specifically but has the ability to recognize certain DNA structures (minicircles, cisplatin-modified DNA, four-way junctions, and supercoiled DNA) and to bend DNA (17, 42–44). As shown here, the phosphorylation of two amino acid residues at the C-terminal end of maize SSRP1 resulted in an increased binding to UV-damaged DNA, whereas the interaction with untreated DNA was unchanged. SSRP1 may recognize the bend or kink introduced by the UV-induced DNA lesion. Depending on the type of lesion, a cytocobaltum pyrimidine dimer or a pyrimidine (4–6) pyrimidone photoproduc, bends of 7°–9° or 44°, respectively, are inserted (3, 7). In contrast to the situation with SSRP1, HMBG proteins that are phosphorylated by CK2 C-terminal of the HMG domain display (depending on the DNA substrate) a rather reduced affinity for DNA (19, 45). The yeast Pob3 protein can be considered an HMG domain-less SSRP1 protein. Pob3 interacts with the small HMG-type protein NHP6A (containing an HMG domain) and may form a higher eukaryote type “bipartisan SSRP1 protein” (13, 14). A yeast strain lacking both the NHP6A and NHP6B proteins (Δnhp6Δa/b strain, Y82) displays an increased UV-sensitivity, as evident from the reduced survival rates of the mutant cells upon UV irradiation, relative to control cells. Expression of maize SSRP1 in the Δnhp6Δa/b strain restored the UV sensitivity comparable with the levels of the control strain. An artificial Pob3-NHP6A fusion protein proved to be functional in yeast cells (13), and such a fusion protein has, over the entire sequence, a high degree of amino acid sequence similarity to maize SSRP1. Moreover, the C-terminal region of the NHP6 proteins (46) contains (similar to maize SSRP1) a serine residue that is compatible with a CK2 phosphorylation site. CK2-mediated phosphorylation is correlated with a UV-response in two ways. CK2 activity can be induced by UV irradiation (47), and CK2 can associate in an UV-dependent way with SSRP1/ Spt16/53 (26, 27). The CK2-mediated phosphorylation of SSRP1 induces the recognition of UV-damaged DNA by SSRP1 and may link SSRP1 to an UV damage response. Therefore, in addition to its established functions in transcription and replication (9–11, 41, 48), SSRP1 may also play a role in DNA repair.

**Acknowledgments—**We thank Diana J. Leeming for preparing CD samples, Hanne Krone Nielsen for excellent technical assistance and Dr. W. Horz for the yeast strains used in this study.

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J. Biol. Chem. 2003, 278:12710-12715.
doi: 10.1074/jbc.M300250200 originally published online February 4, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300250200

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