p53 Serine 392 Phosphorylation Increases after UV through Induction of the Assembly of the CK2-hSPT16-SSRP1 Complex*

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Previously, we purified a UV-responsive p53 serine 392 kinase from F9 and HeLa cells and found that its activity is attributed to a high molecular weight protein complex containing the protein kinase CK2, along with the chromatin-associated factors hSPT16 and SSRP1. Here we determine that these proteins interact in vitro and in cells via non-overlapping domains and provide evidence consistent with the idea that hSPT16 and SSRP1 change the conformation of CK2 upon binding such that it specifically targets p53 over other substrates. Also, UV irradiation apparently induces the association of the complex, thereby increasing the specificity of CK2 for p53 at the expense of other cellular CK2 substrates and leading to an overall increase in p53 serine 392 phosphorylation.

The tumor suppressor protein p53 is a highly connected cellular sensor of DNA damage and aberrant cell growth and serves a protective role by inhibiting the cell cycle or inducing apoptosis once damage occurs (1). Cellular insults that activate p53 include DNA-damaging agents such as radiation and chemical mutagens (2–5), hypoxia (6), ribonucleotide depletions (7), oxygen free radicals (8), and cellular and viral oncoproteins (9, 10). After such stresses, p53 is converted from a latent form into an active transcription factor and primarily induces the expression of numerous target genes involved in cell damage control (1, 11). Concurrently, p53 protein levels dramatically increase through relief of targeted degradation to the proteasome (12, 13) and through increased translation of the p53 mRNA (14–16). Thus many stress-activated signaling pathways connect to p53, which in turn activate expression of downstream effector pathways (1).

One of the mechanisms by which stress signals are communicated to p53 is through post-translational modifications (17). These modifications such as phosphorylation and acetylation are believed to control the protein stability of p53 and transcriptional activity by affecting protein-protein interactions and intramolecular conformational changes (11). For example, phosphorylation at several sites on the N-terminal domain in response to stress (5, 18–21) prevents binding of the MDM2 oncoprotein, an E3 ubiquitin ligase that targets p53 for degradation by the proteasomal pathway (22–24). On the C terminus, phosphorylation of Ser-392 (corresponding to murine Ser-389, for simplicity, Ser-392 will be used) enhances DNA sequence-specific binding and transcription activity in vitro (25, 26), possibly by stabilizing p53 tetramerization (27). In cells, the importance of Ser-392 phosphorylation for p53 function appears to be situation-specific. For example, overexpression of a p53 Ser-392 > Ala mutant suppressed cell growth equal to wild-type p53 in human osteosarcoma SAOS2 cells, but instead impaired the ability of p53 to suppress ras-mediated transformation in rat embryonic fibroblasts (28). Also, transient transfection of p53 with a Ser-392 > Glu substitution, but not with six other phosphorylation mutants, constitutively activated p53 as a transcription factor in NIH 3T3 mouse fibroblasts after cell growth arrest by contact inhibition (29). Taken together, these results suggest that p53 Ser-392 is important for full p53 function.

In multiple cell types, p53 Ser-392 is phosphorylated specifically after UV but not y irradiation or etoposide (3, 4). Casein kinase 2 (CK2) was originally identified as the kinase that targets this site in vitro (30). However, it was unclear what the true kinase that targets this site is in cells. Previously, we used a biochemical fractionation to purify UV-responsive p53 Ser-392 kinase activity from murine testicular carcinoma F9 cells (26). Our results showed that indeed the kinase was CK2, but it eluted from gel filtration chromatography in a high molecular weight fraction corresponding to ~700 kDa. We identified two other proteins that eluted with CK2 as hSPT16 and SSRP1. Together, these molecules are known in mammals as the chromatin associated factor, FACT (31). Interestingly, when hSPT16 and SSRP1 are complexed with CK2, they change the substrate specificity of CK2 to phosphorylate p53 over all other tested substrates. However, it remains unclear how CK2 preferentially targets p53 as a substrate after association with hSPT16 and SSRP1 and how this kinase complex is activated by DNA damaging signals.

To determine the mechanism by which the p53 Ser-392 kinase complex is activated by DNA damage, we have further characterized this complex in vitro and in cells. First, we have mapped the interaction domains between CK2, hSPT16, and SSRP1 in vitro and in cells, demonstrating that these proteins interact with each other via non-overlapping regions, consistent with the idea that they form a complex. Second, steady-state kinetic analysis of the kinase activity of CK2 shows that chromdomain-helicase-DNA binding; CK2, casein kinase 2; DTT, dithiothreitol; FACT, facilitates chromatin transcription; GST, glutathione S-transferase; SSRP1, structure-specific recognition protein; hSPT16, human ortholog of yeast suppressor of Ty insertion mutations; IP, immunoprecipitation; MDM2, a protein encoded by a gene amplified in mouse double-minute chromosome; P11, phosphocellulose; WB, Western blot; WT, wild-type; aa, amino acid(s).

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1 The abbreviations used are: E3, ubiquitin-protein ligase; CHD1, hSPT16, human ortholog of yeast suppressor of Ty insertion mutations; P11, phosphocellulose; WB, Western blot; WT, wild-type; aa, amino acid(s).
precipitation assay buffer is 50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1% 50 mM Tris/HCl (pH 8.0), 0.5% Nonidet P-40, 1 mM EDTA, 150 mM protease inhibitors including 0.2 mM phenylmethylsulfonyl fluoride, 4 °C serum. All cells were grown at 37 °C in a 5% CO 2 atmosphere.

Western Blotting (WB), Co-immunoprecipitation (co-IP), IP Kinase Assays, and in Vitro Kinase Assays—WB, co-IP, and IP kinase assays were carried out as previously described (26). The WB in Fig. 6B was analyzed by a Bio-Rad Model G7000 imaging densitometer. FLAG-N- and FLAG-C-SSRP1 RKO cell lysates were used to perform co-IPs with the anti-FLAG antibody. hSPT16, SSRP1, and CK2α' were immunoprecipitated with anti-SSRP1 from the P11 0.5 M KCl fractions of the F9 cell nuclear extract preparations as previously described. Radioactive in vitro kinase assays were performed with [γ-32P]ATP, in which the total ATP concentration (cold and hot) was 40 μM. Substrates were either 100 ng of His-p53 or 1 μg of casein. In Fig. 4C, WT-SSRP1, N-SSRP1, and mid-hSPT16 proteins are histidine-tagged. N-SSRP1, mid-SSRP1, and C-SSRP1 are thrombin-cleaved from GST while bound to the glutathione-agarose. WT-hSPT16 is FLAG-tagged. Alternatively, kinase assays were done using unlabeled ATP (1 mM) followed by SDS-PAGE and then phosphorylated His-p53 was detected by WB using the anti-Ser-392 antibody.

Kinetic Analysis—CK2 (0.5 unit) was incubated with or without 4 pmol of FACT (2 pmol of FLAG-hSPT16 and 2 pmol of His-SSRP1) on ice for 1 h. In vitro radioactive kinase assays were then carried out in the presence of 250 μM ATP (including 375 μCi of [γ-32P]ATP) for 0.5 h while titrating substrates casein and His-p53. Casein concentrations ranging from 0.25 to 64 μM and His-p53 ranging from 0.037 to 9.4 μM AMINE were added into the CK2-FACT-ATP mixture. Reactions were analyzed by SDS-PAGE, the bands were cut out, and radioactivity was quantified using a Beckman model LS 6500 scintillation counter. Reaction velocities were obtained by measuring the picomoles of ATP incorporated into substrate per second and plotted against substrate concentration. Data points were then fit to the Michaelis-Menten equation using Kaleidagraph (Synergy Software) to obtain values of V max, K m, and V/K.

RESULTS

The Recombinant FACT-C2K Complex Has the Same Apparent Molecular Weight as the Native Complex—In our previous study, the purified p53 Ser-392 kinase complex from murine testicular carcinoma F9 cells eluted from gel filtration chromatography at ~700 kDa (26). Seven polypeptides co-eluted with the kinase activity, five of which were identified as SSRP1, hSPT16, or the subunits of the CK2 heterotetramer. To determine whether hSPT16, SSRP1, and CK2 together can form a complex in vitro, we incubated these recombinant proteins together and loaded them onto a Superdex 200 size exclusion column (Fig. 1). When CK2 was run on the column alone, it eluted at close to the predicted molecular mass of the heterotetramer, 140 kDa (top panel). Interestingly, when recombinant hSPT16 and SSRP1 were mixed with CK2 and then run on the column, the p53 kinase activity of CK2 shifted to a high molecular mass fraction, at ~670 kDa, and at a similar molecular weight to the native p53 Ser-392 kinase complex (middle panel, and see Ref. 26). The bottom panel is a Western blot for hSPT16 and SSRP1 when combined with CK2. The combined molecular masses of the proteins in the FACT-C2K complex is predicted to be 360 kDa; thus we speculate that the recombinant complex contains multiple copies of some or all the components. Also, hSPT16, SSRP1, and CK2 appear to be the primary components of our previously purified native kinase complex, although it is still likely that other proteins may associate with this complex in cells.
To test whether these protein-protein interaction domains are also true in cells, we made stable cell lines with FLAG-tagged N- and C-SSRP1 in human colorectal carcinoma RKO cells and performed co-immunoprecipitations (co-IPs) with the FLAG antibody. Using these cell lines, we reproduced the results seen in the in vitro GST pull-down assay exactly. That is, FLAG-N-SSRP1 bound exclusively to endogenous hSPT16 (Fig. 3C, top panel, lane 2) and FLAG-C-SSRP1 bound exclusively to the endogenous CK2α subunit and p53 Ser-392 kinase activity (Fig. 3C, bottom two panels, lane 3). Therefore, these data confirm the SSRP1 interactions with hSPT16 and CK2 that were observed in the in vitro GST pull-down assay. Interestingly, although recombinant GST-C-SSRP1 migrates on SDS-PAGE faster than GST-N-SSRP1 (Fig. 2A, compare lanes c and e), FLAG-C-SSRP1 stably expressed in RKO cells migrates slower than FLAG-N-SSRP1 (Fig. 3C, compare lanes 2 and 3). One possibility for the slower migration of FLAG-C-SSRP1 is that the C terminus of SSRP1 is highly modified in cells by post-translational modifications; this is supported by the fact that this region has a high serine content and that this region is phosphorylated in vitro by CK2 (see Fig. 4).

Based upon our protein-protein interaction experiments, we can present a model for the binding of hSPT16 and SSRP1 to the CK2 heterotetramer (Fig. 3D). The CK2 crystal structure is solved (36) and resembles a butterfly, with the two regulatory subunits making contacts along a 2-fold axis of symmetry, and the catalytic α and α’ subunits situated like the butterfly wings making contacts only with one β subunit. Because the molecular weights of the recombinant and native complexes are similar, we speculate that there are two FACT heterodimers bound per CK2 heterotetramer, which would be a predicted size of 580 kDa, close to the 670 kDa estimated size from gel filtration chromatography.

Effect of hSPT16 and SSRP1 Deletion Mutants on CK2 Activity—Previously, we discovered that SSRP1 and hSPT16 could modulate the kinase activity of CK2 such that it phosphorylated p53 but inhibited its activity toward other substrates such as casein, histone H1, and MDM2 (26). Here we identify the p53 family member p63 as an in vitro substrate for CK2 (Fig. 4A, lane 4), although p63γ and p53 do not share sequence conservation in the C-terminal domain and there is no p63γ equivalent of Ser-392. We also tested p73α, another p53 family member, but found that CK2 does not phosphorylate this protein (Fig. 4A, lanes 7-9). Surprisingly, hSPT16 and SSRP1 inhibit the CK2-induced phosphorylation of p63γ (compare lane 4 with lanes 5 and 6), providing more evidence for the specificity of the p53 Ser-392 kinase complex.

As described above, we identified the regions of SSRP1 and hSPT16 that directly bind to CK2 and could now test whether these truncation mutants were sufficient to modulate the kinase activity of CK2 also. Kinase assays were performed using either p53 or casein as substrates, with the addition of the various WT and mutant SSRP1 and hSPT16 proteins. As shown in the bottom panel of Fig. 4C, and as seen previously (26), casein phosphorylation was strongly inhibited by His-WT-SSRP1 (compare lanes 1 and 2), although p53 phosphorylation was not affected (Fig. 4, top panel, compare lanes 1 and 2). N-SSRP1, which does not bind CK2, also did not affect CK2 activity toward casein or p53 (compare lanes 3 and 4). In contrast, both mid-SSRP1 and C-SSRP1 inhibited casein phosphorylation (compare lanes 5–8), although mid-SSRP1 inhibited to a greater degree. However, this result was surprising, because C-terminal SSRP1 bound more strongly to CK2 (Fig. 2C). As seen previously, casein phosphorylation by CK2 was inhibited by FLAG-WT-SPT16 (Fig. 4C, bottom panel, lane 9),
Although the truncation mutants appeared to inhibit only slightly (lanes 10–13). These data provide evidence that these defined protein-protein interactions of SSRP1 and hSPT16 with CK2 confer substrate specificity upon the kinase.

We also have previously observed that SSRP1 is strongly phosphorylated by CK2 (26). Our current data show that there are at least two phosphorylation sites for CK2 on SSRP1, one covered by the central domain truncation mutant and the other covered by the C-terminal truncation (Fig. 4C, top panel, lanes 4–8). Although these data will be a subject of future research, it is intriguing to speculate that phosphorylation of SSRP1 may regulate its function as a chromatin-associated transcription factor (31, 37, 38) or in its ability to bind to damaged DNA (39).

Kinetic Analysis of the Phosphorylation Reaction by the SSRP1-hSPT16-CK2 Kinase Complex—To gain insight into how SSRP1 and hSPT16 influence CK2 substrate specificity, steady-state kinetic analysis of CK2 was performed. In vitro kinase assays were performed using CK2 with or without SSRP1 and hSPT16 (together known as FACT) and using casein or p53 as substrates. Incorporation of [γ-32P]ATP into the substrates was measured per second and plotted versus substrate concentration and the data was fitted to the Michaelis-Menten equation (Fig. 5) (see “Experimental Procedures” for details). The kinetic parameters show that, when casein is used as the substrate, FACT induces a 2-fold decrease in the maximal reaction velocity ($V_{\text{max}}$) and a 7- to 8-fold increase in the Michaelis-Menten constant ($K_m$), a measure of the efficiency of substrate utilization (Fig. 5A). The efficiency of the enzymatic reaction ($V/K$) is also severely affected (~15-fold decrease) (Fig. 5A). In contrast, p53 phosphorylation by CK2 is not affected by addition of FACT (Fig. 5B), clearly demonstrating that FACT selectively modulates the kinase activity of CK2 by inhibiting its activity against other substrates. These data therefore suggest that the conformation of CK2 is modulated by FACT such that it preferentially targets p53.

The Level of the hSPT16-SSRP1-CK2 Complex Increases after UV—p53 Ser-392 phosphorylation occurs after UV but not γ irradiation in many cell types (3, 4). In our original purification of the p53 Ser-392 kinase, we observed UV-responsive kinase activity in the phosphocellulose (P11) 0.5 M KCl fraction and used this fraction to purify the SSRP1-hSPT16-CK2 complex (Fig. 6A). To understand how the P11 0.5 M KCl fraction had higher p53 Ser-392 kinase activity after UV, we repeated the F9 nuclear extract fractionation and performed WB analysis on the column input and fractions using antibodies against hSPT16, SSRP1, and CK2α'. Surprisingly, when both nontreated and UV-treated F9 nuclear extracts were run on the P11 column and fractionated, hSPT16, SSRP1, and CK2α' protein levels all were dramatically increased in the UV-treated 0.5 M KCl fraction (Fig. 6B, top two panels, lanes 3 and 4). This increase was not due to unequal loading, as demonstrated by equal levels of γ-tubulin (third panel from top), nor was it due to an overall induction of these proteins after UV,
because their levels were equal in the nuclear extract (lanes 1 and 2). Next, p53 Ser-392 kinase activity was measured using a WB kinase assay with an αSer-392 antibody (Fig. 6B, bottom panel). First, the p53 Ser-392 kinase activity is increased in the nuclear extract after UV irradiation –3-fold as measured by densitometry. Second, the activity in the 0.5 M KCl fraction is also stimulated, as would be expected due to the increase in CK2 in this fraction after UV irradiation. A co-immunoprecipitation using an αSSRP1 antibody shows that SSRP1, hSPT16, and CK2 are associated in the 0.5 M KCl fraction after UV treatment (Fig. 6C). Because the P11 0.5 M fraction is the only fraction in which hSPT16, SSRP1, and CK2 co-exist (data not shown), this suggests that these proteins form a complex in response to UV irradiation.

**DISCUSSION**

We previously identified a UV-responsive p53 Ser-392 kinase activity from F9 and HeLa cells and found that it contained protein kinase CK2 in complex with the chromatin-associated factors, hSPT16 and SSRP1 (26). There is little known concerning the biochemical properties of the hSPT16-SSRP1 heterodimer, and few reports have identified other interacting
Characterization of the CK2-hSPT16-SSRP1 Complex

SSRP1 and hSPT16 influence the substrate specificity of CK2. A, the p53 family member p63α, but not p73α, is phosphorylated by CK2, although its phosphorylation is inhibited by SSRP1 and hSPT16. In vitro kinase reactions were done for 30 min using γ-[32P]ATP and using either 50 ng of His-p53, 150 ng of His-p63α, or 500 ng of His-p73α as substrates. rFACT indicates recombinant SSRP1 and hSPT16 incubated together, titrated at 15 ng, and 30 ng of total protein. B, Coomassie-stained SDS-PAGE of the substrates used in panel A (1 μg of each protein was loaded). The asterisks indicate the proteins, and His-p73α exists as two polypeptides. C, a radioactive kinase assay was done as above with either His-p53 (100 ng, top panel) or casein (1 μg, bottom panel). CK2 was incubated along with the various SSRP1 and hSPT16 proteins as described under “Experimental Procedures.” The dots indicate that casein and the mid-SSRP1 construct are both phosphorylated by CK2 and have equal migration on SDS-PAGE. Thus, although casein phosphorylation in lane 6 is inhibited, the signal is actually due to mid-SSRP1 (see lane 6, compare the top and bottom panels).

In this study, we describe an initial biochemical analysis of hSPT16-SSRP1 and its association with CK2. We report that these proteins interact with each other via non-overlapping domains in vitro and in cells and that upon binding to CK2, hSPT16 and SSRP1 may induce a conformational change in the kinase such that it preferentially recognizes p53 as a substrate. We also present evidence that the association of the CK2-hSPT16-SSRP1 complex is increased following UV irradiation.

Biochemical Mechanism of the hSPT16-SSRP1-CK2 Kinase Complex—hSPT16 and SSRP1 form a stable heterodimer in cells that is conserved from yeast to mammalian systems (31, 37, 43) and is important for transcription and replication through chromatin. The biochemical role for this heterodimer (called FACT in mammalian cells) is not clear, although it may involve binding to histones H2A and H2B thereby disrupting the nucleosome octamer (31) and/or physically manipulating DNA by inducing negative supercoils (37). However, basic questions remain, such as the nature of their mutual protein-protein interaction domains. Here we have used truncation mutants of hSPT16 and SSRP1 to map their respective binding domains both in vitro and in cells (Figs. 2 and 3C). Using GST fusion protein binding assays we find that hSPT16 binds to the N terminus of SSRP1 and that SSRP1 binds to the central region of hSPT16 (Fig. 2, B and D). In cells, we have confirmed the N-SSRP1 and hSPT16 interaction by co-IP (Fig. 3C, top panel). Interestingly, Brewster et al. (43) demonstrated in Saccharomyces cerevisiae that POB3 (human SSRP1) failed to interact with the N terminus of SPT16, consistent with our finding that it interacts with the central domain of hSPT16 and suggesting that the protein-protein interaction domains are conserved between yeast and mammals.

Although hSPT16 and SSRP1 are a conserved heterodimer, they have also been shown to interact with other proteins, thereby providing them with additional functions. For example, in S. cerevisiae they bind to the catalytic subunit of DNA polymerase α (38), the histone acetyltransferase complex NuA3 (41), and have independently been found to elute from a size exclusion column at ~400 kDa (43), larger than the predicted size of 180 kDa for the yeast proteins. In mammals, an interaction has been described between these proteins and the transcription initiation factor TFIIE (42). Here we show that the molecular mass of the recombinant CK2-FACT complex is ~700 kDa, the same size as the purified native complex (Fig. 1) (26). This suggests that there are multiple copies of some or all of the proteins in the complex. For example, in Fig. 3D we present a model in which there are two FACT heterodimers for every CK2 heterotetramer, which would make the predicted size of the complex equal to 580 kDa and similar to the observed molecular mass as judged by gel filtration analysis. However, we cannot rule out that other proteins may be part of the native complex in cells. In fact, this appears to be the case in S. cerevisiae, demonstrated by a recent study that identified several transcription elongation complexes, including one containing CK2, SPT16, POB3 (yeast SSRP1), and CHD1, a chromodomain-helicase-DNA binding family member (44).

In this report we have performed preliminary mapping of the CK2-hSPT16-SSRP1 interactions again using GST fusion protein association and co-IP assays. We find that CK2 binds directly to the central region and C terminus of SSRP1 and to the N terminus of hSPT16 (Fig. 2, C and D). In a similar...
The CK2 subunit binds to both hSPT16 and SSRP1 and the CK2β subunit binds strongly only to SSRP1 (Fig. 3A). Likewise, in cells, CK2 binds to the C terminus of SSRP1 (Fig. 3C). Therefore, the proteins interact via non-overlapping regions, consistent with the hypothesis that they form a complex.

Using steady-state kinetic analysis of the CK2 kinase reaction, we show that hSPT16 and SSRP1 have different effects on CK2 depending upon the substrate being phosphorylated. For example, casein phosphorylation by CK2 is severely inhibited by inclusion of hSPT16 and SSRP1 into the reaction, exhibiting a decrease in the maximal reaction rate \( V_{\text{max}} \) and increase in \( K_{\text{m}} \), with a corresponding decrease in enzyme efficiency \( (V/K) \) (Fig. 5A). In contrast, p53 phosphorylation at Ser-392 is not affected (Fig. 5B). The drop in \( V_{\text{max}} \) indicates that hSPT16 and SSRP1 bind to a site on CK2 that does not overlap the substrate binding pocket, and the increase in \( K_{\text{m}} \) indicates that casein no longer binds to the substrate binding pocket efficiently, perhaps reflecting a change in the conformation of the kinase. Furthermore, the sharp decrease in enzyme efficiency seen when CK2 phosphorylates casein while complexed with hSPT16 and SSRP1 suggests that in cells p53 is preferentially being phosphorylated at the expense of other CK2 substrates. This substrate specificity even extends to the p53 family member p63γ, whose phosphorylation by CK2 is inhibited by SSRP1 and hSPT16 (Fig. 4A). Also, based on our mutational analysis, the C-terminal two-thirds of SSRP1 are necessary for influencing the substrate specificity of CK2 (Fig. 4C, bottom panel, lanes 5–8), and although full-length hSPT16 can also affect the substrate specificity (Fig. 4C, bottom panel, lane 9), the truncations do not have this ability alone (lanes 10–13).

DNA Damage-induced Activation of the CK2-hSPT16-SSRP1 Complex—Although Ser-392 was one of the first identified phosphorylation sites on p53 in cells (45), its role in p53 biology is still uncertain. It has been proposed to enhance the transcription potential of p53 based on in vitro results in which p53 phosphorylated at this site leads to increased sequence-specific DNA binding (25). Indeed, several experimental approaches in cells and in mice have supported this notion (26, 28, 29, 46), contributing to the hypothesis that phosphorylation of p53 fine-tunes the protein to respond to specific stresses. Ser-392 of p53 fits this model as well, because it is phosphorylated specifically after UV but not γ irradiation in multiple cell types (3, 4). Thus it is of particular interest in the identification of the kinase that targets this site in vivo after UV irradiation.

Here we report that phosphorylation of p53 Ser-392 increases ~3-fold in F9 cells following UV irradiation as measured by an in vitro WB-kinase assay (Fig. 6B, bottom panel). This UV-responsive kinase activity fractionated from the phosphocellulose column at 0.5 M KCl (Fig. 6A and bottom panel of 6B) and by WB analysis we detected dramatically increased protein levels of CK2, hSPT16, and SSRP1 (Fig. 6, B and C). This increase was not due to misloaded samples, because a WB for γ-tubulin shows that the total protein levels are equal in this fraction (Fig. 6B) and protein measurement by Bradford assay detected equal levels of protein (data not shown). Thus the kinase complex components change chromatographic properties identically, perhaps reflecting an increase in the CK2-hSPT16-SSRP1 complex following DNA damage. The stoichiometry of these components appears to be critical, because
elevating the SSRP1 level alone did not affect p53 Ser-392 phosphorylation nor did it affect p53 activity (47).

With the evidence in our studies, we can begin to build a model for how the CK2:hSPT16:SSRP1 complex regulates phosphorylation of p53 Ser-392 following DNA damage (Fig. 6D). First, binding of FACT to CK2 in vitro is not sufficient to increase the specific activity of CK2 toward p53 (Fig. 5B), so then why is this association needed for p53 Ser-392 phosphorylation? The reason is that the association modulates CK2 probably through a conformational change (Fig. 5A) such that it preferentially targets p53 Ser-392 at the expense of the many other CK2 substrates in cells, thus giving specificity to a kinase that is normally one of the most unspecific protein kinases known (35). In this regard, an increase in the CK2:hSPT16:SSRP1 complex following DNA damage signals would lead to a corresponding decrease in free CK2 pools in the cell. This would then result in a decrease in non-p53 cellular substrates for CK2, such that the CK2:hSPT16:SSRP1 complex now phosphorylates more p53 molecules per cell, thereby leading to the increase in p53 Ser-392 phosphorylation that we see following UV irradiation (Fig. 6D).

The mechanism for how the CK2:FACT complex assembles after DNA damage is not yet clear. We speculate that sites of DNA damage may be a trigger for bringing these proteins together. This hypothesis is based on the fact that SSRP1 is a high mobility group-box-containing protein that preferentially recognizes cisplatin-modified DNA (39). Furthermore, Yarnell et al. (48) used in vitro gel-mobility shift assays to show that the ability of SSRP1 to bind damaged DNA was increased by the addition of hSPT16. Therefore it is possible that, in addition to their role in transcription and replication, hSPT16 and SSRP1 may play a role in the cellular DNA damage response and may lead to preferential binding to CK2. This will be an area of future study.

Finally, although this study demonstrates that the CK2:hSPT16:SSRP1 complex preferentially targets p53, it is likely that the complex targets other unknown but important proteins as well. In accordance with this notion is the CK2:CHD1:hSPT16:SSRP1 interaction has recently been found in S. cerevisiae, using a low stringency affinity purification to identify regulators of transcription elongation (44). This finding implies that this complex may regulate non-p53 substrates, because there is no yeast p53 gene, and that hSPT16 and SSRP1 may themselves be regulated by CK2.

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