Cooperation between Small Nuclear RNA-activating Protein Complex (SNAPc) and TATA-box-binding Protein Antagonizes Protein Kinase CK2 Inhibition of DNA Binding by SNAPc*

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Protein kinase CK2 regulates RNA polymerase III transcription of human U6 small nuclear RNA (snRNA) genes both negatively and positively depending upon whether the general transcription machinery or RNA polymerase III is preferentially phosphorylated. Human U1 snRNA genes share similar promoter architectures as that of U6 genes but are transcribed by RNA polymerase II. Herein, we report that CK2 inhibits U1 snRNA gene transcription by RNA polymerase II. Decreased levels of endogenous CK2 correlates with increased U1 expression, whereas CK2 associates with U1 gene promoters, indicating that it plays a direct role in U1 gene regulation. CK2 phosphorylates the general transcription factor small nuclear RNA-activating protein complex (SNAPc) that is required for both RNA polymerase II and III transcription, and SNAPc phosphorylation inhibits binding to snRNA gene promoters. However, restricted promoter access by phosphorylated SNAPc can be overcome by cooperative interactions with TATA-box-binding protein at a U6 promoter but not at a U1 promoter. Thus, CK2 may have the capacity to differentially regulate U1 and U6 transcription even though SNAPc is universally utilized for human snRNA gene transcription.

Protein kinase CK2 is an important regulator of cellular growth (1–4), and abnormal CK2 activity may contribute to tumor progression (5). CK2 is a tetrmeric enzyme composed of two catalytic subunits, α and α′, and two copies of the regulatory β subunit (6). One role for CK2 is to function as a regulatory protein that controls gene transcription. For example, general RNA synthesis in yeast is impaired when a temperature-sensitive mutant of the CK2 α′ subunit is shifted to a restrictive temperature (7). This decline in total RNA synthesis also suggests that expression of highly transcribed genes encoding ribosomal (r), transfer (t), and small nuclear RNA (snRNA)1 is sensitive to levels of functional CK2.

In yeast CK2 is important for active RNA polymerase III transcription (8) and yet, paradoxically, CK2 has been proposed to be the terminal effector in a DNA damage response pathway that represses RNA polymerase III transcription (9). In humans, CK2 exhibits differential effects on gene transcription during the cell cycle. During mitosis, CK2 inhibits RNA polymerase III transcription, whereas at other stages CK2 can stimulate transcription (10). The nature of the regulation is dictated by CK2 target selection. One key target for CK2 is the general transcription factor TFIIIB (11–13). There are at least two versions of human TFIIIB that function for transcription of distinct classes of genes (14). The Brf1-TFIIIB complex functions for 5 S rRNA and tRNA transcription and is composed of the TATA-box-binding protein (TBP) and the TBP-associated factors, Bdp1 and Brf1. The Brf2-TFIIIB complex functions for U6 snRNA transcription and is composed of TBP plus Bdp1 but Brf2 instead of Brf1. Brf1-TFIIIB phosphorylation during M phase results in the selective release of Bdp1 from tRNA promoters (15). Hernandez and co-workers (10) further demonstrated that Bdp1 is the critical CK2 target within Brf2-TFIIIB for mitotic repression of U6 transcription. Because Bdp1 is a shared component of both TFIIIB complexes, CK2 may target this factor to repress global RNA polymerase III transcription. However, CK2 inhibitors also interfere with Brf1-TFIIIB binding to the TFIIIC complex (12), which itself recognizes intragenic promoter elements of 5 S rRNA and tRNA genes, suggesting that CK2 also has a stimulatory role in RNA polymerase III transcription through enhanced preinitiation complex assembly. Consistent with this positive role, CK2 can also activate RNA polymerase III transcription in human cells (12) and in this process may additionally phosphorylate RNA polymerase III itself (13). Together, these data point to an important but complex role for CK2 control of RNA polymerase III transcription.

Human U6 snRNA genes are interesting because they are transcribed by RNA polymerase III and yet their promoters are similar to other snRNA genes, such as U1 and U2, which are transcribed by RNA polymerase II (16–18). Consequently, the mechanisms regulating human snRNA gene transcription by RNA polymerases II and III may also be shared. Nonetheless, the RNA polymerase II-transcribed genes do not use TFIIIB and, thus, rely on other factors for regulatory intervention. Regardless of polymerase specificity, all human snRNA genes contain a distal sequence element encompassing an octamer element that is recognized by Oct-1. Additional sites for the Sp1 (19) and STAF (20) transcriptional activator proteins are adjacent located to the distal sequence element at some snRNA genes (21). Oct-1 activates snRNA transcription by direct protein contacts (22–24) with the basal transcription factor called the snRNA-activating protein complex (SNAPc) (25), which is also referred to as the proximal sequence element transcription...
SNAPC plays a pivotal role in snRNA gene transcription by extracts that have been depleted of endogenous TBP (25). Thus, TATA-less promoters is unclear. Nonetheless, it is likely that RNA polymerase II (25), but how TBP is recruited to these III transcription (35, 38). The striking parallel between RNA polymerase II and III transcription, CK2 also has a complex role in including TFIIA, TFIIB, TFIIE, and TFIIF (42). As in RNA polymerase III transcription activation by Oct-1, and coordinating TBP activation (24). TBP co-purify extensively during the biochemical fractionation of U1 but not at U6 distal sequence element and stimulate the binding of Oct-1 to the U6 TATA box as an early critical step in RNA polymerase transcription (18, 25, 27–31). SNAPC contains at least five proteins called SNAP190 (PTFα), SNAP50 (PTFβ), SNAP45 (PTFγ), and SNAP19 (27–33). The largest subunit SNAP190 plays a centrally important role in human snRNA gene transcription first by serving as the scaffold for SNAPC assembly though interactions with most other members of SNAPC (34, 35). Once the complex is assembled, SNAP190 further recognizes the PSE through its Myb DNA binding domain (30) and is also the direct target for Oct-1 (23, 36). In an unexpected twist, SNAP190 can make DNA contacts within the U1 distal sequence element and stimulate the binding of Oct-1 to this enhancer, suggesting that in some contexts coordinated binding of the activator and general transcription machinery is important for transcriptional activation (24).

Human U6 snRNA genes, but not U1 genes, also contain a TATA box that is located adjacent to the PSE, and this promoter arrangement dictates that transcription occurs by RNA polymerase III (37). The TATA box is recognized by the TBP component of the Brf2-TFIIB complex (35, 38–41). SNAPC, through its SNAP190 subunit, stimulates TBP binding to the U6 TATA box as an early critical step in RNA polymerase III transcription (35, 38).

TBP is also required for human snRNA gene transcription by RNA polymerase II (25), but how TBP is recruited to these TATA-less promoters is unclear. Nonetheless, it is likely that SNAPC contributes to TBP activity at these genes. SNAPC and TBP co-purify extensively during the biochemical fractionation of SNAPC (27), and those fractions enriched for SNAPC and TBP can reconstitute U1 snRNA transcription in vitro from extracts that have been depleted of endogenous TBP (25). Thus, SNAPC plays a pivotal role in snRNA gene transcription by providing core promoter recognition, serving as a target for transcription activation by Oct-1, and coordinating TBP activity and preinitiation complex assembly for both RNA polymerases II and III. Additional RNA polymerase II general transcription factors are also required for U1 transcription including TFIIB, TFIIE, and TFIIF (42). As in RNA polymerase III transcription, CK2 also has a complex role in regulating RNA polymerase II transcription. CK2 phosphorylation of TFIIB and TFIIE stimulates preinitiation complex assembly at the adenovirus major late promoter, whereas TFIIF phosphorylation can stimulate RNA polymerase II elongation. In contrast, CK2 phosphorylation of RNA polymerase II inhibits transcription, potentially by impairing elongation (43).

The striking parallel between RNA polymerase II and III transcription of human snRNA genes prompted an investigation into the role of phosphorylation in U1 transcription. In this study we report that CK2 inhibits overall U1 snRNA gene transcription by RNA polymerase II and can phosphorylate SNAPC to inhibit its DNA binding. Interestingly, cooperative interactions of SNAPC with TBP at U6 but not at U1 promoter DNA can overcome the repressive effects of CK2. Together, these data suggest that CK2 may differentially affect preinitiation complex assembly for RNA polymerase II and III transcription of human snRNA genes depending upon the promoter architecture.

**MATERIALS AND METHODS**

**Chromatin Immunoprecipitation Assays—**Chromatin immunoprecipitation assays from HeLa cells were performed using the anti-CK2α (Ab245), anti-CK2β (Ab278) antibodies (44) as well as anti-SNAP43 (CS48) and anti-TBP antibodies described previously (45). Enrichment of genomic sequences in the immunoprecipitation reactions was measured by PCR as previously described (45).

**RNA interference (RNAi)—**CK2α and CK2α cDNA were generated with a T7 promoter at both ends by reverse transcription (RT)-PCR using total RNA from HeLa cells as a template. The primers for CK2α are CK2α forward, 5’-GCCATATATGCAGCTACTATAGGAAATG-3’ and CK2α reverse, 5’-GCCATATATGCAGCTACTATAGGAAATG-3’. The primers for CK2β are CK2β forward, 5’-GCCATATATGCAGCTACTATAGGAAATG-3’ and CK2β reverse, 5’-GCCATATATGCAGCTACTATAGGAAATG-3’. LacZ cDNA with a T7 promoter at both ends was generated by PCR using pBelican-lacZ as a template. The primers used were lacZ forward, 5’-TATAATACGACTATAGGAGAATTTAC-3’ and lacZ reverse, 5’-GCCATATATGCAGCTACTATAGGAAATG-3’. Resulting cDNAs were subjected to in vitro transcription with T7 polymerase to produce double-stranded RNA. After DNase I treatment, double-stranded RNA was incubated with recombinant Dicer, and resultant Dicer-generated small interfering RNA were purified according to the manufacturer’s instructions (Invitrogen). Approximately 250 ng of Dicer-generated small interfering RNA for lacZ, CK2α, or CK2β plus 250 ng of CK2β Dicer-generated small interfering RNA were transfected into HeLa cells using Lipofectamine 2000 (Invitrogen). Cells were harvested 30 h later, and total RNA was extracted using TRIzol (Invitrogen). RT-PCR was carried out using Titan One Tube RT-PCR System (Roche Applied Science). The primers used to amplify U1 pri-mRNA, forward, 5’-ACAGCCTCATACGCCTCACT-3’ and U1 reverse, 5’-ACAGCCTCATACGCCTCACT-3’. The primers used to amplify the total U1 snRNA population are U1 forward, 5’-ATACGTACCTGGCAGGGGAG-3’ and U1 reverse, 5’-ACAGGAGAAGGGGACAGGCG-3’. RT-PCR products were separated by 3% Tris borate EDTA-agarose electrophoresis, stained with ethidium bromide, and visualized with Kodak imaging software.

**In Vitro Transcription Assays—**In vitro transcription of human U1 and U6 snRNA genes were performed as described previously (37) with the following modifications. The HeLa cell nuclear extracts were pre-incubated with Dignam buffer D either with or without recombinant CK2 and kinase inhibitors for 60 min at 30 °C before initiating transcrip-tion by the addition of transcription buffers, nucleotide triphosphates, and DNA templates. The amounts of recombinant CK2 and kinase inhibitors used are indicated in the legend to Fig. 2. Transcripts were separated by denaturing PAGE and visualized by PhosphorImager analysis (Amer sham Biosciences).

**Expression and Purification of Recombinant Proteins—**GST-SNAP190 (1–719) was expressed in Escherichia coli BL21 (DE3) using the vector pSBet-GST-SNAP190 (1–719) and was purified for in vitro kinase assays by affinity chromatography using glutathione-Sepharose beads (Pharmacia Biosciences). Recombinant mini-SNAP containing SNAP190 (1–719), SNAP43, and SNAP50 was co-expressed in E. coli using the vector combination pSBet-GST-SNAP190 (1–719) and pET21-His-SNAP43/HA-SNAP50. Recombinant mini-SNAP was affinity-purified using glutathione-agarose beads followed by digestion with thrombin to release the complex from the GST tag and dialysis against Dignam buffer D containing 80 mM KCl.

**Immunoprecipitation and in Vitro Kinase Assays—**For the experiment presented in Fig. 3A, 180 μl of HeLa cell nuclear extract (10 mg/ml) was incubated with 20 μl of rabbit anti-SNAP43 (CS48 (27)), anti-SNAP190 (CS398, CS402 (30)), anti-CK2α (Ab245 (44)), or preimmune antibodies covalently coupled to protein-G agarose beads. Recovered proteins were analyzed by Western blot using a mouse monoclonal antibody against CK2α (Transduction Laboratories). For Fig. 3C, 40 μl of HeLa cell nuclear extract was used for each immunoprecipitation. After extensive washing with HEMGT-150 buffer (20 mM Heps, pH 7.9, 0.1 mM EDTA, 5 mM MgCl₂, 10% glycerol, 0.5% Tween 20, 150 mM KCl), the beads were suspended in 40 μl of HEMGT-150 buffer containing 2 μl of [γ-32P]ATP (6000 Ci/mmol, 150 μCi/ml), and the samples were incubated at 30 °C for 15 min. The beads were then washed extensively in HEMGT-150 buffer, and proteins were separated by 12.5% SDS-PAGE. Radiolabeled proteins were visualized by autoradiography. For Fig. 3C, 100 μl of HeLa cell nuclear extracts were used for immunoprecipitation. After kinase reactions, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Radiolabeled proteins were detected first by autoradiography. Subsequent Western blot analyses were performed using anti-SNAP190 (CS402) antibodies.

For Fig. 5, B and C, ~5 μg of GST-SNAP190 (1–719) was bound to glutathione-agarose beads (10 μl). Immobilized GST-SNAP190 (1–719) was incubated with 10 μl of HeLa cell nuclear extract for 30 min at 30 °C. The beads were washed extensively with HEMGT-150. In vitro kinase assays were then performed directly on the beads. Kinase reactions were also performed using untreated GST-SNAP190 (1–719) plus

27698

**Human snRNA Gene Regulation by CK2**
ether CK2 associates with snRNA gene promoters. Chromatin immunoprecipitation experiments were performed using HeLa cell chromatin and the indicated antibodies. Enrichment of the U6 and U1 promoter regions was detected by PCR and was compared with recovery of the U1 upstream regions and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exon 2 as negative controls. B, U1 primary transcripts accumulate after CK2 reduction. CK2 levels were reduced by transient transfection of Dicer-generated small interfering RNA (d-siRNA) (52) corresponding to CK2α (lane 7) or CK2α plus CK2β (lane 8). Cells were also treated with LacZ Dicer-generated small interfering RNA as a reference (lane 6). Levels of the U1 primary transcript and total U1 population were monitored by RT-PCR (top). Endogenous CK2 and GAPDH levels were measured by Western analysis (bottom). For reference, lanes 1–5 contain 2-fold decreasing increments of material harvested from untreated cells to serve as a standard curve for each assay.

A

B

FIG. 1. CK2 inhibits U1 snRNA gene expression. A, endogenous CK2 associates with snRNA gene promoters. Chromatin immunoprecipitation experiments were performed using HeLa cell chromatin and the indicated antibodies. Enrichment of the U6 and U1 promoter regions was detected by PCR and was compared with recovery of the U1 upstream regions and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) exon 2 as negative controls. B, U1 primary transcripts accumulate after CK2 reduction. CK2 levels were reduced by transient transfection of Dicer-generated small interfering RNA (d-siRNA) (52) corresponding to CK2α (lane 7) or CK2α plus CK2β (lane 8). Cells were also treated with LacZ Dicer-generated small interfering RNA as a reference (lane 6). Levels of the U1 primary transcript and total U1 population were monitored by RT-PCR (top). Endogenous CK2 and GAPDH levels were measured by Western analysis (bottom). For reference, lanes 1–5 contain 2-fold decreasing increments of material harvested from untreated cells to serve as a standard curve for each assay.
transcription assays were performed in the absence (lanes 1) or presence of DRB (lanes 2, 1 μM DRB; lanes 3 and 4, 7 μM DRB). The reaction shown in lane 4 also contains 10 units of recombinant CK2. In vitro U1 transcription assays were performed using a U1 G-less cassette in the absence (lanes 5 and 7) or presence of 10 units of recombinant CK2 (lanes 6 and 8) either in the absence (lanes 5 and 6) or presence of 7 μM DRB (lanes 7 and 8). IC, internal control. B, in vitro U1 transcription is sensitive to the CK2 inhibitor quercetin. Additional U1 transcription assays were performed in the absence (lane 1) or presence of 1 μM (lanes 2) and 7 μM quercetin (lanes 3–5), respectively, whereas an additional 10 and 100 units of CK2 were added to reactions shown in lanes 4 and 5, respectively.

Endogenous CK2 Targets SNAP190 for Phosphorylation at Multiple Sites—SNAPC recognizes the core promoters of human snRNA genes and plays an important early role in coordinating transcription of snRNA genes by both RNA polymerases II and III. The findings that CK2 can affect both human U1 and U6 transcription (Fig. 2 and Ref. 13) implicates SNAPC as a potential target for CK2. First, we examined whether CK2 co-purifies with SNAPC. As shown in Fig. 3A, endogenous CK2 from HeLa cell nuclear extract was recovered with SNAPC, during immunoprecipitation using anti-SNAP190 (lanes 4 and 5) or anti-SNAP43 (lane 3) antibodies but not while using IgG antibodies (lane 2). These levels of recovered CK2 are significantly less than that observed in reactions using antibodies against CK2α (lane 7), suggesting that only a minor proportion of SNAPC is associated with CK2 or that the interaction between SNAPC and CK2 is not stable. In separate experiments, recombinant CK2 alone did not cross-react with the anti-SNAP43 antibodies (data not shown), suggesting that recovery of CK2 in these assays requires SNAPC. These results indicate that endogenous CK2 associates with SNAPC.

Next, to determine whether any subunits of SNAPC can be phosphorylated by SNAPC-associated kinase(s), including CK2, the anti-SNAP43 immunopurified proteins were directly assayed for kinase activity by incubation with [γ-32P]ATP. As shown in Fig. 3B, robust phosphorylation of a 190-kDa protein was observed in the anti-SNAP43 immunopurified samples (lane 1), suggesting that SNAP190 is extensively phosphorylated in this assay. After a longer exposure (as shown in Fig. 3B), proteins of 60 kDa, 43 kDa (labeled SNAP43), and 19 kDa (labeled with an asterisk (*)) in size were additionally observed. The identity of the 60-kDa protein is unknown; however, these results suggest that SNAP43 and SNAP190 were also phosphorylated in these assays but to a much lesser extent than SNAP190.

To confirm the identity of the proteins phosphorylated by the SNAPC-associated kinase, immunopurified SNAPC was used for in vitro kinase assays followed by Western blot analysis using SNAPC-specific antibodies (Fig. 3C). A 190-kDa protein is phosphorylated in kinase assays using material recovered by either anti-SNAP43 (lane 2) or anti-SNAP190 (lane 3) immunoprecipitation but not by immunoprecipitation with premun antibodies (lane 1). As expected, no phosphorylation was observed when [γ-32P]ATP was not included in the kinase reaction (lane 4). Lanes 5 and 6 show the results of anti-SNAP190 Western blot analysis for the same reactions shown in lanes 3 and 4. SNAP190 is detected in both reactions regardless of whether [γ-32P]ATP is added or not. Importantly, SNAP190 as detected by Western blot analysis co-migrated with the 190-kDa protein that is phosphorylated by the SNAPC-associated kinase, indicating that the 190-kDa phosphoprotein is indeed SNAP190. In similar experiments, SNAP43 co-migrated with this inhibitor may have nonspecific positive effects on transcription possibly from cryptic promoters on the reporter plasmid. Although quercetin can inhibit a variety of kinases, the increase in U1-specific transcription was reversed by the addition of increasing amounts of recombinant CK2 (lanes 4 and 5), suggesting that CK2 has a direct and overall negative role in controlling U1 transcription. Previously, U2 snRNA gene transcription by RNA polymerase II in nuclear run-on assays was not sensitive to DRB (48, 49), suggesting that CK2 is not involved in the transcription of these genes. The possibility remains that U1 and U2 gene transcription is differentially sensitive to regulation by CK2. Nonetheless, because U1 and U2 genes utilize similar promoter elements and general transcription factors for efficient transcription, a role for CK2 in U2 transcription cannot yet be dismissed.

Human snRNA Gene Regulation by CK2

A

In vitro transcription

B

In vitro transcription

Fig. 2. CK2 represses U1 transcription. A, CK2 reverses inhibition of U6, but not U1, transcription by the kinase inhibitor DRB. In vitro U6 transcription assays were performed in the absence (lane 1) or presence of DRB (lane 2, 1 μM DRB; lanes 3 and 4, 7 μM DRB). The reaction shown in lane 4 also contains 10 units of recombinant CK2. In vitro U1 transcription assays were performed using a U1 G-less cassette in the absence (lanes 5 and 7) or presence of 10 units of recombinant CK2 (lanes 6 and 8) either in the absence (lanes 5 and 6) or presence of 7 μM DRB (lanes 7 and 8). IC, internal control. B, in vitro U1 transcription is sensitive to the CK2 inhibitor quercetin. Additional U1 transcription assays were performed in the absence (lane 1) or presence of 1 μM (lanes 2) and 7 μM quercetin (lanes 3–5), respectively, whereas an additional 10 and 100 units of CK2 were added to reactions shown in lanes 4 and 5, respectively.
the 43-kDa phosphoprotein (data not shown), suggested that SNAP43 is also phosphorylated by the SNAPC-associated kinase. A similar experiment performed to determine whether the 19-kDa phosphoprotein observed in the in vitro kinase assays is SNAP19 was inconclusive because our anti-SNAP19 antibodies were not sensitive enough to detect SNAP19 in this assay. Taken together, these results demonstrate that a SNAPC-associated kinase phosphorylates SNAP190 and SNAP43.

To determine whether endogenous SNAPC is phosphorylated, αSNAP43 immunoprecipitation reactions were performed from HeLa cell nuclear extracts, and recovered proteins were directly analyzed by Pro-Q diamond staining (Molecular Probes), which specifically detects phosphorylated proteins. As shown in Fig. 4, a protein of ~190 kDa was detected in the anti-SNAP43-immunoprecipitated sample (lane 5) that was not observed in a similarly immunoprecipitated sample treated with phosphatase (lane 6), indicating that this protein is phosphorylated. This 190-kDa protein was not detected in the sample recovered by immunoprecipitation with nonspecific antibodies (lane 4). Furthermore, the 190-kDa protein co-migrated with HA-tagged SNAP190 recovered by anti-HA immunoprecipitation from transiently transfected HeLa cells (lane 7), suggesting that the endogenous 190-kDa phosphoprotein is SNAP190. This same gel was analyzed by Sypro ruby staining to detect the total level of recovered proteins. The 190-kDa protein was detected in both the untreated (lane 2) and phosphatase-treated (lane 3) samples, although SNAP190 staining was reduced in the latter sample. In all immunoprecipitated samples, significant levels of IgG heavy chain were detected by Sypro ruby staining (bottom panel) but not by Pro-Q diamond staining, further demonstrating that phosphorylated proteins are specifically detected in this assay. Therefore, we conclude that endogenous SNAP190 is phosphorylated. Other subunits of SNAPC were not detected in these assays (not shown) perhaps because they are not phosphorylated in vivo or the recovered levels of phosphorylated protein in these assays were below the threshold of detection using the Pro-Q diamond stain.

We had previously observed that recombinant SNAP43 was preferentially phosphorylated when assembled into SNAPC, and furthermore, efficient SNAP43 phosphorylation required SNAP190-(1–719), suggesting that SNAP190 is responsible for recruiting a kinase activity to the complex (data not shown). In those experiments, SNAP190-(1–719) was also extensively phosphorylated. Therefore, to determine whether CK2 interacts with SNAP190, GST-SNAP190-(1–719) was used to affinity-purify kinase(s) activity from HeLa nuclear extracts, and anti-CK2 Western analysis was performed (Fig. 5A). Significant amounts of CK2 associated with GST-SNAP190-(1–719)
SNAP190 was acid-hydrolyzed and dissolved in pH 1.9 buffer containing radiolabeled endogenous major phosphopeptides are indicated. The directions of electrophoresis fragments obtained from radiolabeled GST-SNAP190-(1–719) phosphorylate the same regions within SNAP190-(1–719). Analysis of tryptic SNAP190-associated kinase from HeLa cell nuclear extracts phosphorylated by the HeLa cell-derived kinase was investigated. As shown in Fig. 5B (top panel), both DRB (lanes 2–4) and quercetin (lanes 5–7) were effective in limiting the extent of GST-SNAP190-(1–719) phosphorylation as compared with the untreated sample (lane 1). A second hallmark of CK2 is that it is capable of utilizing GTP as a phosphoryl group donor. Indeed, robust phosphorylation of GST-SNAP190-(1–719) was observed when $\gamma^{32P}GTP$ was included in the reaction (bottom panel, lane 1), and this GTP-based phosphorylation was inhibited by DRB (lanes 2–4) and quercetin (lanes 5–7), consistent with the idea that endogenous CK2 can associate with and phosphorylate SNAP190.

To further examine the extent of SNAP190 phosphorylation by endogenous CK2, GST-SNAP190-(1–719) was phosphorylated either by recombinant CK2 or by endogenous kinase(s) present in the HeLa cell nuclear extract that are capable of associating with SNAP190. Subsequently, phosphorylated GST-SNAP190-(1–719) was digested with trypsin, and the radiolabeled peptides were compared by two-dimensional TLC (Fig. 5C). This analysis revealed two major SNAP190 tryptic peptides that were phosphorylated by the kinase recruited from HeLa cell extracts (left panel) and by recombinant CK2 (middle panel). The tryptic peptides from both these kinase reactions were then mixed and analyzed as before (right panel). As had been observed for the individual analysis of the tryptic peptides, only two predominate spots were observed, suggesting that recombinant CK2 and the kinase activity from HeLa cells phosphorylate SNAP190 within the same regions, thus providing additional evidence confirming that the HeLa cell-derived kinase is CK2. Endogenous SNAP190 that was phosphorylated by HeLa cell-derived CK2 was also hydrolyzed for phosphoamino acid analysis (Fig. 5D), which revealed that phosphorylation occurs predominately on serine residues in this assay. Given the above observations, we conclude that the major SNAP190-associated kinase in HeLa nuclear extracts is CK2.

**CK2 Restricts SNAP C Promoter Recognition**—An examination of the amino acid sequence of SNAP190-(1–719) revealed a total of 13 CK2 consensus motifs containing serines, most of which are clustered around regions involved in cooperative promoter recognition by SNAP C in the presence of TBP (35, 38). Other CK2 sites are contained within the Myb DNA binding domain of SNAP190, in particular, the Rh and Ra Myb repeats (30). Because CK2 had previously been shown to inhibit DNA binding by other Myb-domain proteins (51), these observations immediately suggested the possibility that CK2 could potentially inhibit both DNA binding and TBP recruitment by SNAP C.

To test whether CK2 could affect DNA binding by SNAP C, electrophoretic mobility shift assays were performed with DNA probes resembling either a U6 (wild-type PSE and TATA) or U1 promoter (wild-type PSE with mutant TATA). The recombinant SNAP C, used in these reactions is a partial complex containing full-length SNAP43 and SNAP50 along with SNAP190-(1–719), hereafter referred to as mini-SNAP C, and this complex tyrosine ($\text{pTyr}$). The mixture was separated by one-dimensional thin layer electrophoresis (TLE), and phosphoamino acids were visualized with ninhydrin (lane 1). Subsequently, radiolabeled phosphoamino acids were detected by PhosphorImager analysis (lane 2). Identical results were obtained with full-length HA-SNAP190 and GST-SNAP190-(1–719) (not shown).
SNAPC and TBP (reactions did not affect cooperative DNA binding by mini-SNAPC, to the U6-specific probe either in the presence or absence of CK2 and ATP (lane 11) contained 150 units of CK2. The positions of the mini-SNAPC-DNA and mini-SNAPC-TBP-DNA complexes are indicated. EMSA, electrophoretic mobility shift assay.

In our assays we observed that two subunits of SNAPC, SNAP190 and SNAP43, were phosphorylated by CK2. Both SNAP43 and SNAP190 interact with TBP and are candidates for regulatory intervention by CK2 to influence TBP recruitment at snRNA gene promoters. However, we favor the idea that SNAP190 plays a dominant role in this process. First, SNAP190 is a better substrate for CK2. Second, SNAP190 contains an unusual Myb DNA binding domain consisting of four and a half Myb repeats (51). That phosphorylated SNAPC can bind DNA cooperatively with TBP in reactions wherein SNAPC is unable to bind DNA alone (Fig. 6) argues that most SNAPC in these reactions is not a target for CK2 regulation. As noted by the authors of that study, the recombinant SNAPC used was expressed in insect cells, and it could have already phosphorylated and, thus, refractive to further effects of CK2. In that system CK2 stimulated transcription by phosphorylating RNA polymerase III but also inhibited transcription by phosphorylating the Bdp1 subunit of TFIIIB (10). To determine whether CK2 affects SNAPC function we examined the effect of phosphorylation on recombinant SNAPC, expressed in E.coli. Our studies indicate that CK2 does impair SNAPC binding to the PSE within the U6 promoter but adjacent TBP binding to the TATA-box can rescue SNAPC recruitment. Thus, CK2 may play an important role in ensuring that SNAPC is not engaged in nonproductive preinitiation complex formation at inappropriate sites in the genome by restricting DNA binding and requiring multiple factors for promoter recognition. In contrast, TBP did not rescue SNAPC binding to DNA containing the U1-like arrangement of promoter elements, suggesting that SNAPC phosphorylation by CK2 could be important for the repressive effects of CK2 observed in U1 transcription. Whether CK2 can disable SNAPC already bound to DNA is not known, but if so, this would suggest that CK2 could act after preinitiation complex assembly. It will be important to determine the cellular context for CK2 action on SNAPC.

In our assays we observed that two subunits of SNAPC, SNAP190 and SNAP43, were phosphorylated by CK2. Both SNAP43 and SNAP190 interact with TBP and are candidates for regulatory intervention by CK2 to influence TBP recruitment at snRNA gene promoters. However, we favor the idea that SNAP190 plays a dominant role in this process. First, SNAP190 is a better substrate for CK2. Second, SNAP190 contains an unusual Myb DNA binding domain consisting of four and a half Myb repeats (51). That phosphorylated SNAPC can bind DNA cooperatively with TBP in reactions wherein SNAPC is unable to bind DNA alone (Fig. 6) argues that most SNAPC in these reactions is phosphorylated and argues against the idea that CK2 inhibits SNAPC, though phosphorylation of a residue that is critical for DNA interaction. Instead, we speculate that phosphorylation...
induces a conformational change in SNAP190, rendering it unable to recognize the PSE. Interestingly, a number of CK2 sites within SNAP190 are located adjacent to the TBP-recruiting region (TRR)-1 and TRR-2 that are involved in TBP recruitment to the U6 promoter (35, 38). TRR-2 coincides with the SNAP190 Rc and Rd Myb repeats, and interaction with TBP may unveil the SNAP190 Myb DNA binding domain to U1-like arrangement of promoter elements. This notion further suggests that CK2 may have the capacity to differentially regulate U1 and U6 transcription even though SNAP_C is universally used for snRNA gene transcription. As with human U6 transcription, it remains possible that CK2 phosphorylates different factors during the cell cycle to enact either positive or negative outcomes on U1 transcription.

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REFERENCES

1. Meggio, F., and Pinna, L. A. (2003) FASEB J. 17, 349–368
2. Pinna, L. A. (2002) J. Cell Sci. 115, 3873–3878
3. Guerra, B., and Issinger, O. G. (1999) Electrophoresis 20, 391–408
4. Pinna, L. A., and Meggio, F. (1997) Prog. Cell Cycle Res. 3, 77–97
5. Tawfic, S., Yu, S., Wang, H., Faust, R., Davis, A., and Ahmed, K. (2001) Histol. Histopathol. 16, 573–582
6. Niefeld, K., Guerra, B., Ermakowa, I., and Issinger, O. G. (2001) EMBO J. 20, 5320–5331
7. Hanka, D. E., Rethinaswamy, A., and Glover, C. V. (1995) J. Biol. Chem. 270, 25905–25914
8. Heckman, D. J., and Schultz, M. C. (1996) Mol. Cell. Biol. 16, 892–896
9. Ghavidel, A., and Schultz, M. C. (2001) Cell 106, 575–584
10. Hu, P., Samudre, K., Wu, S., Sun, Y., and Hernandez, N. (2004) Mol. Cell 16, 81–92
11. Ghavidel, A., and Schultz, M. C. (1997) Genes Dev. 11, 2780–2789
12. Johnston, I. M., Allison, S. J., Morton, J. P., Schramm, L., Scott, P. H., and White, R. J. (2002) Mol. Cell. Biol. 22, 3757–3768
13. Hu, P., Wu, S., and Hernandez, N. (2003) Mol. Cell 12, 699–703
14. Schramm, L., and Hernandez, N. (2002) Genes Dev. 16, 2593–2600
15. Fairley, J. A., Scott, P. H., and White, H. J. (2003) EMBO J. 22, 5841–5850
16. Lobo, S. M., and Hernandez, N. T. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. C., and Conaway, J. W., eds) pp. 127–159, Raven Press, Ltd., New York
17. Hernandez, N. (2001) J. Biol. Chem. 276, 26733–26736
18. Henry, R. W., Ford, E., Mittal, R., Mittal, V., and Hernandez, N. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 111–120
19. Ares, M., Jr., Chung, J. S., Giglio, L., and Weiner, A. M. (1987) Genes Dev. 1, 808–817
20. Schaub, M., Myslinski, E., Schuster, C., Krol, A., and Carbon, P. (1997) EMBO J. 16, 173–181
21. Hernandez, N. (1992) in Transcriptional Regulation (McKnight, S., and Yamamoto, K., eds) pp. 281–313, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
22. Mittal, V., Ma, B., and Hernandez, N. (1999) Genes Dev. 13, 1807–1821
23. Ford, E., Strubin, M., and Hernandez, N. (1998) Genes Dev. 12, 3528–3549
24. Hovde, S., Hinkley, C. S., Strong, K., Brooks, A., Gu, L., Henry, R. W., and Geiger, J. (2002) Genes Dev. 16, 2772–2777
25. Sadowski, C. L., Henry, R. W., Lobo, S. M., and Hernandez, N. (1993) Genes Dev. 7, 1535–1548
26. Murphy, S., Yoon, J. B., Gerster, T., and Roeder, R. G. (1992) Mol. Cell. Biol. 12, 3247–3261
27. Henry, R. W., Sadowski, C. L., Kobayashi, R., and Hernandez, N. (1995) Nature 374, 653–656
28. Henry, R. W., Ma, B., Sadowski, C. L., Kobayashi, R., and Hernandez, N. (1996) EMBO J. 15, 7129–7136
29. Henry, R. W., Mittal, V., Ma, B., Kobayashi, R., and Hernandez, N. (1998) Genes Dev. 12, 2664–2673
30. Li, W., Wang, H., Frago, A., Kanelas, N., Matthias, P., Strubin, M., and Hernandez, N. (1998) Mol. Cell. Biol. 18, 368–377
31. Sadowski, C. L., Henry, R. W., Kobayashi, R., and Hernandez, N. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 4289–4293
32. Bai, L., Wang, Z., Yoon, J. B., and Roeder, R. G. (1996) Mol. Cell. Biol. 16, 5419–5426
33. Yoon, J. B., and Roeder, R. G. (1996) Mol. Cell. Biol. 16, 1–9
34. Ma, B., and Hernandez, N. (2001) J. Biol. Chem. 276, 5027–5035
35. Ma, B., and Hernandez, N. (2002) Mol. Cell. Biol. 22, 8067–8078
36. Mittal, V., Cleary, M. A., Herr, W., and Hernandez, N. (1996) Mol. Cell. Biol. 16, 1955–1965
37. Lobo, S. M., and Hernandez, N. (1989) Cell 58, 55–67
38. Hinkley, C. S., Hirsch, H. A., Gu, L., LaMere, B., and Henry, R. W. (2003) J. Biol. Chem. 278, 18649–18657
39. Zhao, X., Schramm, L., Hernandez, N., and Herr, W. (2003) Mol. Cell 11, 151–161
40. Cabraro, P., and Murphy, S. (2002) J. Biol. Chem. 277, 28681–28688
41. Cabraro, P., and Murphy, S. (2001) J. Biol. Chem. 276, 43056–43064
42. Kuhlman, T. C., Cho, H., Reinberg, D., and Hernandez, N. (1999) Mol. Cell. Biol. 19, 2130–2141
43. Cabrejos, M. E., Allende, C. C., and Maldonado, E. (2004) J. Cell Biochem. 93, 2–10
44. Yu, I. J., Spector, D. L., Bae, Y. S., and Marshak, D. R. (1991) J. Biol. Chem. 266, 2130–2141
45. Hirsch, H. A., Jawdekar, G. W., Lee, K. A., Gu, L., and Henry, R. W. (2004) Mol. Cell. Biol. 24, 5989–5999
46. van der Geer, P., and Hunter, T. (1994) Electrophoresis 15, 544–554
47. Mittal, V., and Hernandez, N. (1997) Science 275, 1136–1140
48. Jacobs, E. Y., Ogtuwaru, I., and Weiner, A. M. (2004) Mol. Cell. Biol. 24, 846–855
49. Medlin, J. E., Uguen, P., Taylor, A., Bentley, D. L., and Murphy, S. (2003) EMBO J. 22, 925–934
50. Price, D. H. (2000) Mol. Cell. Biol. 20, 2629–2634
51. Luscher, B., Christenson, E., Litchfield, D. W., Krebs, E. G., and Eisenman, R. N. (1990) Nature 344, 517–522
52. Myers, J. W., Jones, J. T., Meyer, T., and Ferrell, J. E., Jr. (2003) Nat. Biotechnol. 21, 324–328