Specific Interaction between Casein Kinase 2 and the Nucleolar Protein Nopp140

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Casein kinase 2 (CK2) is a multifunctional second messenger-independent protein serine/threonine kinase that phosphorylates many different proteins. To understand the function and regulation of this enzyme, biochemical methods were used to search for CK2-interacting proteins. Using immobilized glutathione S-transferase fusion proteins of CK2, the nucleolar protein Nopp140 was identified as a CK2-associated protein. It was found that Nopp140 binds primarily to the CK2 regulatory subunit, β. The possible in vivo association of Nopp140 with CK2 was also suggested from a coimmunoprecipitation experiment in which Nopp140 was detected in immunoprecipitates of CK2 prepared from cell extracts. Further studies using an overlay technique with radiolabeled CK2 as a probe revealed a direct CK2-Nopp140 interaction. Using deletion mutants of CK2β subunits, the binding region of the CK2β subunit to Nopp140 has been mapped. It was found that the NH2-terminal 20 amino acids of CK2β are involved. Since Nopp140 has been identified as a nuclear localization sequence-binding protein and has been shown to shuttle between the cytoplasm and the nucleus, the finding of a CK2-Nopp140 interaction could shed light on our understanding of the function and regulation of CK2 and Nopp140.

Protein phosphorylation is known to be a very important means of cellular regulation, and in recent years much information about protein kinases and phosphatases, especially those involved in the mitogen-activated protein kinase pathway, has been obtained (for review, see Refs. 1–3). Little is known, however, about the function and regulation of one particular protein kinase, casein kinase 2 (CK2), although in recent years much information about its role. For example, the nucleolar protein nucleolar protein shuttling sequence-binding protein has been identified as a CK2-associating protein in previous work (16–18). In this report, we provide evidence that CK2 and the nucleolar protein Nopp140 associate as a nuclear complex in vitro and probably in vivo. These studies were performed using immobilized GST fusion proteins of CK2 subunits and a 32P-radiolabeled CK2 overlay technique and by coimmunoprecipitation of the two proteins from cell extracts. The region of the β subunit of CK2 which binds to Nopp140 was also mapped. The possible roles of CK2 in the regulation of Nopp140 as well as in rRNA synthesis and ribosomal protein transport are supported by the results of this study.

EXPERIMENTAL PROCEDURES

Materials—Nucleotide oligomers used as polymerase chain reaction primers were synthesized by Drs. Y. F. Lee and P. S. H. Chou (Biopolymer Facility, Department of Immunology, University of Washington) and by Integrated DNA Technologies, Inc. BL-21 (DE3) pLysS compe-

1 The abbreviations used are: CK2, casein kinase 2; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; DSD, CK2 peptide substrate RRKDDDSDDD.
tent cells were purchased from Novogen. [γ-32P]ATP was obtained from Amersham. The PD-10 gel filtration column and glutathione-Sepharose 4B beads were purchased from Pharmacia Biotech Inc. All other chemicals and reagents were purchased from Sigma.

**Recombinant CK2 and Nopp140**—Recombinant CK2 subunits α and α′, β, and β′ were expressed and purified from baculovirus-infected SF-9 cells as described elsewhere. Recombinant Nopp140 was expressed in E. coli BL-21 (DE3) cells transformed with pET8c/Nopp140 (Nopp140 bacterial expression vector). Since the overexpressed Nopp140 protein was predominantly insoluble and segregated into inclusion bodies, it was denatured and renatured by incubation in 6 M urea followed by extensive dilution and then purified using a hydroxyapatite column (19).

**Antibodies**—Polyclonal antibodies of Nopp140 were raised in rabbits against a synthetic peptide of Nopp140 (20). Polyclonal antibodies against CK2 subunits α, α′, and β were prepared in this laboratory (21).

**Cell Culture and Preparation of Cell Lysates**—3T3 L1 cells were grown in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum until confluence. The cells were washed by phosphate-buffered saline, harvested in lysis buffer (50 mM Tris-Cl, pH 7.5, 50 mM NaF, 0.25 mM NaCl, 0.1% Triton X-100, 2 mM EDTA, 10 μg/ml leupeptin, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride), and sonicated twice for 10 s. After centrifugation for 30 min at 13,000 rpm, the supernatants were used as cell lysates.

**CK2 Deletion Mutants**—CK2 deletion mutants of GST-CK2 were constructed by truncating the NH2-terminal (Δ1–20, Δ1–40, Δ1–80, Δ1–120, Δ1–141) and COOH-terminal (Δ161–215) amino acids of CK2β to give mutants GST-β31–215, GST-β31–215, GST-β31–215, GST-β31–215, GST-β31–215, and GST-β1–200. The cDNA for each deletion mutant was obtained by polymerase chain reaction amplification of human CK2β in BlueScript KS plasmid (22). For NH2-terminal deletion mutants, the sense primer was created from 5′-GG-GTACCCTTCCTTCTGCTAAGTATGATG-3′ (GST-β1–215), 5′-GGGATCCGGAGCTCTCCGATCTA-3′ (GST-β31–215); 5′-GGCGATCCCGCGCCGCGCG-3′ (GST-β31–215); 5′-GGCGATCCCGTGGATCGGCGG-3′ (GST-β31–215); and 5′-GGGATCCGGAGCTCTCCGATCTA-3′ (GST-β31–215). The T7 24-mer primer was used as the antisense primer for all of the NH2-terminal deletion mutants of CK2β (18). For the COOH-terminal deletion mutant, GST-β1–200, the sense primer was 5′-GGGATCCGGAGCTCTCCGATCTA-3′ and the antisense primer was 5′-GGGATCCGGAGCTCTCCGATCTA-3′. After polymerase chain reaction, the DNA fragments were purified, digested with BamHI, and ligated into pGEX-2T vector (Pharmacia).

**GST-CK2 Fusion Proteins and Their Deletion Mutants**—GST-CK2α, GST-CK2α′, and GST-CK2β and its deletion mutants were expressed in and purified from E. coli and immobilized on glutathione-Sepharose resin (18). The immobilized protein were eluted using an elution buffer containing 10 mM reduced glutathione in 50 mM Tris-Cl, pH 8.0. To remove the GST tag from GST-CK2, a thrombin cleavage procedure developed by Pharmacia was employed. GST fusion proteins of CK2 holoenzyme αβ, αβ′, and β′β were generated by mixing an equal amount of the immobilized GST-CK2αβ, GST-CK2 α′β, or GST-CK2β′β with 2 μg of anti-Nopp140 peptide IgGs in the absence and presence of 5 μM free competing peptide for 1 h at room temperature. The antibody-antigen complexes were adsorbed to 5 μl of packed protein A-Sepharose beads (Pharmacia) for an additional 1-h incubation at room temperature. The beads were washed four times with 1 ml of wash buffer (50 mM Tris, pH 7.4, 0.1% Triton X-100, 0.02% SDS, 150 mM NaCl), and the antibody-antigen complexes were eluted with Laemmli sample buffer and analyzed by SDS-PAGE (19).

**Phosphorylation of CK2**—Each of the recombinant CK2s: CK2α, CK2α′, αβ, and α′β (30–120 ng), was incubated at 30°C with 0.5 μg of recombinant Nopp140 in 25 μl of phosphorylation buffer (20 mM Tris-Cl, pH 7.5, 5 mM MgCl2, and 0.1 mM [γ-32P]ATP (2,000 cpm/pmol ATP)). After 30 min, the reaction was stopped by adding 8 μl of 4 × sample buffer, and the proteins were resolved by SDS-PAGE. The protein phosphorylation was detected by autoradiography.

**CK2 Activity Assay**—To test whether CK2-Nopp140 association would affect the catalytic activity of CK2, CK2 activity was assayed by a routine as well as a modified procedure. In the routine procedure, the assay was carried out in the same way as described previously (23, 24) in the presence and absence of Nopp140 in the reaction solution. In the modified procedure, CK2 holoenzyme was preincubated with [γ-32P]ATP in a reaction buffer (50 mM Tris-Cl, pH 7.5, 10 mM MgCl2, 0.1 mM [γ-32P]ATP (2,000 cpm/pmol ATP), and with and without Nopp140 protein (0.075 mg/ml), for 20 min at 30°C. Then, CK2 substrate peptide RRDRDDDDDD (DSS; final concentration 0.1 μmol/l) was added to the reaction mixture and incubated for another 10 min. Aliquots of reaction mixture were spotted onto P81 paper, washed, and assayed as described previously (23, 24).

To examine the activation of CK2α by deletion mutants of CK2β, CK2 assays were conducted using baculovirus-expressed CK2α (approximately 5 ng for each reaction) and bacterially expressed and purified GST-CK2β mutants. CK2α was premixed with GST-CK2β mutants for 10 min at room temperature prior to the assay to allow proper folding of the holoenzyme. An excess amount of CK2β compared with CK2α was used in this experiment to give maximal stimulation (23, 24).

**RESULTS**

**Identification of a CK2-binding Protein as Nopp140**—GST-CK2 holoenzymes were reconstituted in vitro by incubating bacterially expressed, immobilized GST-CK2α or GST-CK2α′ with CK2β (obtained by thrombin cleavage of GST tag from GST-CK2β) and immobilized on glutathione-Sepharose resin. These forms were then incubated with 3T3 L1 cell lysates for 4 h at 4°C. After extensive washing, phosphorylation reactions were initiated by adding a buffer containing [γ-32P]ATP and MgCl2 to the beads (18) and stopped using Laemmli sample buffer. The phosphorylated CK2-interacting proteins were analyzed by SDS-PAGE and autoradiography (Fig. 1). Several CK2-associated phosphoproteins were detected from the GST-CK2β resin, which had been incubated with cell lysates (Fig. 1, *lanes 1 and 2*) compared with the control experiment (*lane 3*), which shows only the autophosphorylation of the CK2 holoenzyme. Among them, one protein of 140 kDa was the most highly phosphorylated and bound to both forms of the CK2 holoenzyme.

One possible candidate for the highly phosphorylated protein p140 was a nuclear protein, Nopp140, known to be a very good CK2 substrate and known to migrate on SDS gels with a similar molecular weight (20). A polyclonal antibody against protein Nopp140 was used to test this possibility. A binding experiment using GST-CK2 fusion proteins as described above was conducted, except that the phosphorylation step was omit-
A highly phosphorylated protein p140 is associated with GST-CK2 fusion proteins. 3T3 L1 cell lysates were incubated with immobilized GST-CK2 fusion proteins: GST-CK2α + CK2β (lane 1) and GST-CK2α + CK2β (lane 2). After extensive washing, bound proteins were subjected to phosphorylating conditions using [γ-32P]ATP as the phosphate donor. The phosphorylated CK2-bound proteins were detected by autoradiography. As a control, immobilized fusion protein GST-CK2 a was used because the purified Nopp140 was ex- autophosphorylation and loaded on the same gel.

Fig. 1. A highly phosphorylated protein p140 is associated with GST-CK2 fusion proteins. 3T3 L1 cell lysates were incubated with immobilized GST-CK2 fusion proteins: GST-CK2α + CK2β (lane 1) and GST-CK2α + CK2β (lane 2). After extensive washing, bound proteins were subjected to phosphorylating conditions using [γ-32P]ATP as the phosphate donor. The phosphorylated CK2-bound proteins were detected by autoradiography. As a control, immobilized fusion protein GST-CK2 a was used because the purified Nopp140 was ex-

The CK2-Nopp140 interaction was detected when less than 2 μg of GST-CK2β was used (Fig. 2B, left lane), whereas no interaction was observed between Nopp140 and 2 μg of GST-CK2α, or Nopp140 and 2 μg of GST-CK2α (center and right lanes), suggesting that the CK2 holoenzyme-Nopp140 association most probably occurred through the β subunit of CK2. However, with a higher amount (between 5 and 10 μg) of GST-CK2α, detectable affinity toward Nopp140 was seen; GST-CK2α was associated very weakly with Nopp140 (data not shown).

CK2 Binds to Nopp140 Directly—To clarify further whether the CK2-Nopp140 interaction is a direct association, a radioactive CK2 overlay experiment was carried out using 32P-labeled purified CK2 protein as a probe (18). Bacterially expressed pure Nopp140 protein was immobilized on the membrane as described (see "Experimental Procedures"). As demonstrated in Fig. 3, lane 1, Nopp140 did bind to the radiolabeled CK2 probe, whereas a control protein, bovine serum albumin (lane 2, 1 μg of bovine serum albumin), did not bind. This indicates that the CK2-Nopp140 interaction is specific and direct. It is noteworthy that in this experiment, a dephosphorylated form of Nopp140 was used because the purified Nopp140 was expressed in bacteria, giving a band of 100 kDa on the gel, whereas in intact cells, most of the Nopp140 is in a highly phosphorylated form (20). Taken together, the experiments of

To investigate which subunits of CK2 bind to Nopp140, immobilized GST fusion proteins of each CK2 subunit, GST-CK2α, GST-CK2α′, and GST-CK2β, were used for the type of binding experiment described above. A strong CK2β-Nopp140 interaction was detected when less than 2 μg of GST-CK2β was used (Fig. 2B, left lane), whereas no interaction was observed between Nopp140 and 2 μg of GST-CK2α, or Nopp140 and 2 μg of GST-CK2α′ (center and right lanes), suggesting that the CK2 holoenzyme-Nopp140 association most probably occurred through the β subunit of CK2. However, with a higher amount (between 5 and 10 μg) of GST-CK2α, detectable affinity toward Nopp140 was seen; GST-CK2α was associated very weakly with Nopp140 (data not shown).

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mixture of anti-Ck2α, anti-Ck2α′, and anti-Ck2β antisera. The presence of protein Nopp140 was examined by immunoblotting of the CK2 immunoprecipitate with a polyclonal anti-Nopp140 antiserum (20). As shown in Fig. 4B, Nopp140 was detected in the immunoprecipitate of CK2 (lane 2) but was not detected in the preimmune sera immunoprecipitate (lane 1), indicating a specific interaction of these two proteins. Together, coimmunoprecipitation of CK2 and Nopp140 suggested an in vivo association of these two proteins.

Expression of CK2β Deletion Mutants as GST Fusion Proteins—GST-CK2β deletion mutants were prepared for mapping the region of CK2β subunit which associates with Nopp140. The constructs used are shown in Fig. 5A. Expression of these deletion constructs produced NH2-terminal and COOH-terminal truncated GST-CK2β proteins. To characterize whether these deletion mutants could stimulate the activity of CK2α as the wild type β does, the enzymatic activity of the recombinant α subunit (from SF-9 cells) was measured in the presence of an excess amount (five times more) of either wild type β subunit or the deletion mutants of β. As illustrated in Fig. 5B, at 30 °C under our assay condition (with 0.1 M NaCl in final reaction mixture), CK2α showed very low activity because of the inhibition of NaCl (Fig. 5B, far left lane). Addition of the wild type GST-β protein greatly stimulated the catalytic activity of the α subunit (second lane). However, among all of the deletion mutants of β, only the mutant with deletion of the 20 NH2-terminal amino acids, GST-β21–215, could stimulate the activity of CK2α to the same extent as the wild type β (third lane). Deletion of amino acids 1–40 (GST-β1–40) greatly decreased the ability of β to activate CK2α (fourth lane), and further deletion up to first 80 amino acids (GST-β41–215) almost totally abolished the stimulation to α (fifth lane). This suggests that deletion of amino acids 21–80 causes a major structural change in the subunit. According to Kusk et al. (26), from their study in

![Fig. 4. Coimmunoprecipitation of CK2 and Nopp140. Panel A, coimmunoprecipitation of CK2 with anti-Nopp140 peptide antibodies. Left, Amido Black stain of proteins immunoprecipitated under nondeaturing conditions from whole cell lysates after separation by SDS-PAGE and transfer to nitrocellulose. BRL cell lysates were incubated with anti-Nopp140 peptide antibodies in the absence (− lane) and presence (+ lane) of synthetic Nopp140 peptide against which the antibodies were raised. Right, immunodetection of precipitated proteins after incubation of the nitrocellulose with anti-Nopp140 (top), anti-NAP57 (middle), and anti-Ck2α antibodies (bottom). The migrating position of the IgG heavy (H) and light (L) chains are indicated in the right margin. Panel B, coimmunoprecipitation of Nopp140 with anti-CK2 antibodies. CK2 antisera were used to immunoprecipitate CK2 and its binding proteins from total lysates of 3T3 L1 cells (lane 2). A mixture of preimmune sera was used as control (lane 1). After 10% SDS-PAGE, the proteins were transferred to the membrane. Nopp140 and CK2 were detected by Nopp140 antiserum (top) and by anti-Ck2α antiserum (bottom).](image)

![Fig. 5. Mapping of the region of CK2β bound to Nopp140. Panel A, GST-CK2β deletion mutants. 1, GST-β; 2, GST-β21–215; 3, GST-β41–215; 4, GST-β1–215; 5, GST-β21–215; 6, GST-β41–215; 7, GST-β1–160. Panel B, activation of CK2α by GST-CK2β mutants. The activity of CK2α was determined in the presence of CK2β mutants as described (see “Experimental Procedures”). Panel C, mapping of the region of CK2β bound to Nopp140. 3T3 L1 cell lysates were incubated with immobilized deletion mutants of GST-CK2β (from left to right): GST-β (wild type), GST-β21–215, GST-β41–215, GST-β1–215, GST-β1–160, and GST. After extensive washing, the bound proteins were subjected to SDS-PAGE and analyzed by Nopp140 immunoblotting.](image)
the yeast two-hybrid system, amino acids 20–60 are needed for strong β-β interaction; so it is very possible that a tetrameric structure is needed to obtain full CK2 activity. Consistent with the data reported previously (27, 28), the COOH terminus of the β subunit was important for activating the α subunit because it is responsible for the α-β interaction; deletion of the COOH-terminal amino acids 160–215 caused a big decrease in the activation of the α subunit by β. Further investigation is under way to make a more thorough analysis of structure-function relationships in CK2β.

Mapping of the Binding Region of CK2β to Nopp140—The deletion mutants of CK2β were used in the in vitro CK2-Nopp140 binding studies. After incubating the immobilized GST-CK2β deletion mutants with 3T3 L1 cell lysates, the bound proteins were eluted and analyzed by SDS-PAGE and Nopp140 immunoblotting. Nopp140 was not detected in any of the eluates of the NH2-terminal deletion mutants (Fig. 5C, lanes 2–6) but was detected in the eluate from the COOH-terminal deletion mutant GST-CK2β1–160 (Fig. 5C, lane 7). Since GST-CK2β21–215 is the smallest NH2-terminal deletion, the lack of its binding ability to Nopp140 indicated that the first 20 NH2-terminal amino acids are most probably involved in the CK2-Nopp140 interaction.

Phosphorylation of Nopp140 by CK2—To understand how the CK2β subunit might affect the specificity of CK2 toward Nopp140 as a substrate, bacterially expressed Nopp140 was subjected to phosphorylation by baculovirus-expressed CK2 catalytic subunits α and α′ and by holoenzymes αβ and α′β2. The phosphorylation reactions were carried out in a buffer without NaCl in order to have maximal α or α′ catalytic activity (α and α′ are inhibited by NaCl). When a high concentration of CK2 (120 ng) was used, Nopp140 was found to be phosphorylated efficiently by all of the forms of CK2 after incubation in a phosphorylation buffer for 10 min at 30 °C; phosphorylation caused a gel mobility shift from 100 to 140 kDa (data not shown). However, when a lower amount of CK2 was used (approximately 30 ng), each form of holoenzyme, αβ2 and α′β2, showed much higher specific activity toward Nopp140 than the monomeric active subunits, α and α′, although their relative activities toward a CK2 substrate peptide, DSD, had already been deliberately adjusted to the same level by dilution. Phosphorylation of Nopp140 by α′β2 and αβ2 for 20 min at 30 °C resulted in a massive incorporation of [32P]P and a significant gel mobility shift (Fig. 6A, lanes 1 and 2), whereas phosphorylation by α and α′ under the same condition was much weaker and did not shift the band significantly (Fig. 6A, lanes 3 and 4). From the densitometric reading, phosphorylation of Nopp140 by the holoenzymes CK2 was 4-fold higher than by the monomeric enzymes (Fig. 6B), indicating that Nopp140 is a much better substrate for the holoenzyme CK2 than for the monomeric α and α′ subunit.

Effect of CK2-Nopp140 Association on CK2 Activity Using a Different Substrate—To examine the impact of the CK2-Nopp140 association on the enzymatic activity of CK2, CK2 was assayed in the presence or absence of Nopp140 using purified Sf-9 cell-expressed CK2 and bacterially expressed Nopp140. CK2 peptide substrate DSD was used for the assay. Besides the routine assay method (23, 24), a modified method in which Nopp140 was first phosphorylated for 20 min by CK2 followed by adding the DSD peptide to start the reaction, was also used to decrease the competition that might be caused by Nopp140 as an alternative substrate. In each case, no significant change of CK2 activity was detected when Nopp140 was present in the reaction mixture. This occurred even though it was possible that in the experiment using the alternative method Nopp140 phosphorylation may not have been abso-

![Figure 6: Phosphorylation of Nopp140 by CK2. Panel A, recombinant Nopp140 (0.5 μg Nopp140 for each reaction) was subjected to phosphorylation by CK2, αβ2 (lane 1), αβ2 (lane 2), α′α (lane 3), and α (lane 4). After incubation for 20 min at 30 °C, the reaction was stopped by adding sample buffer, and the proteins were resolved by SDS-PAGE. Nopp140 phosphorylation was analyzed by autoradiography. Panel B, image density from panel A.](image)

**DISCUSSION**

In this study we have identified a nucleolar protein, Nopp140, as a CK2-associated protein. The interaction of the two proteins was shown to be direct and not dependent on the phosphorylation state of Nopp140. Furthermore, a possible in vivo interaction of CK2 and Nopp140 was suggested by the coimmunoprecipitation of the two proteins from cell lysates.

Nopp140 was first isolated as a nuclear localization sequence-binding protein (29). Immunostaining and immunoelectron microscopy revealed that Nopp140 is a nucleolar protein that shuttles between the cytoplasm and the nucleolus. A possible role of Nopp140 as a chaperone for import into or export from the nucleolus was suggested (20). Having 49 phosphorylation consensus sites for CK2, and upon their phosphorylation an additional 33, Nopp140 can be highly phosphorylated by CK2 in intact cells (20), giving an apparent molecular mass of 140 kDa on SDS-PAGE. Only phosphorylated Nopp140 binds to the nuclear localization sequence-containing peptide (20). However, a precise understanding of the function of Nopp140 and its phosphorylation by CK2 is not available.

Our data showed that CK2 most probably interacts with Nopp140 through its β subunit, although some binding of the protein with the α subunit of CK2 was seen. One conceivable role of the CK2-Nopp140 interaction could be increasing the substrate specificity for Nopp140 phosphorylation. To address this point, monomeric forms of the enzyme, α and α′, and the holoenzymes, αβ and α′β2, were used for the phosphorylation of Nopp140. It was found that Nopp140 was a much better substrate for the holoenzyme form of CK2. Until now, with nearly all substrates including the routinely used CK2 substrate peptide DSD, the holoenzyme form of CK2 always exhibits a higher activity than the monomeric α or α′ subunit. An approximately 3–5-fold stimulation of CK2α activity by CK2β has normally been observed when DSD peptide is used in the assay (18, 30). In our experiment in which we compare the
ability of the α and α′ subunits to phosphorylate Nopp140 with that of the holoenzyme forms, we deliberately used a lower amount of the holoenzyme CK2 so as to give it the same catalytic activity as CK2α and CK2α′ toward the DSD peptide substrate. Under this condition, the holoenzymes still phosphorylated Nopp140 at four times the rate of either free α or α′. If normalized to the molarity level, an approximately 20-fold difference in phosphorylation could be estimated for the free catalytic subunit (α or α′) compared with holoenzyme CK2. This large difference could be partly due to the association between CK2β and Nopp140, thus favoring substrate recognition. However, because CK2 can also associate with the phosphorylated form of Nopp140, this specific CK2-Nopp140 interaction may also be correlated with other cellular functions of CK2 and Nopp140.

CK2 has been shown to be a major nuclear protein (31). Its growth-related accumulation in the nucleolus has also been observed (32, 33). The finding of the association of CK2 with Nopp140 suggests that CK2 may play an important role in the nucleolus. It has been shown that many nuclear localization sequence-interacting nucleolar proteins, including nucleolin, B23, Nopp140, and its associated protein NAP57 (25), are good substrates of CK2 and can migrate back and forth between the nucleus and cytoplasm (for review, see Ref. 34). These nucleolar proteins all have NH2-terminal domains containing stretches of acidic and serine residues with numerous CK2 phosphorylation sites, and the nuclear localization sequence binding ability of the protein seems to be dependent on their phosphorylation (for review, see Ref. 35). Recently, a major nucleolar protein, nucleolin, was also shown to be able to associate with CK2 in vitro and probably in vivo (16–18). It is possible that in addition to the phosphorylation, the association between CK2 and nucleolar proteins may represent another way of regulating the latter. The association of Nopp140 with CK2, together with phosphorylation, could affect its function in ribosomal protein transport.

Interestingly, it was found recently that Nopp140 appears to be a growth-inhibiting protein, e.g. when rat Nopp140 was overexpressed in yeast, growth impairment was observed (19). Also, SRP40, a yeast homolog of Nopp140, was identified by a genetic screen for genes that cause growth arrest when overexpressed (36). Indeed, when deletion and overexpression of SRP40 were conducted in yeast, deletion caused only minor growth impairment, but its overexpression resulted in a severe growth defect (19). It would be of interest to know if the growth inhibitory function of Nopp140 is mediated both by phosphorylation and its association with CK2.

Using deletion mutants of CK2β, the region at which CK2β binds to Nopp140 was mapped to the first 20 NH2-terminal amino acids, a domain containing the autophosphorylation sites of CK2 Ser-2 or Ser-3 (21). Since bacterially expressed GST-CK2β was used in the binding assay of these studies, the subunit should have been in its dephosphorylated form. On the other hand, in the overlay experiments, CK2 was radiolabeled by the autophosphorylation reaction in which Ser-2 and/or Ser-3 of the CK2β subunit would be phosphorylated. The CK2 bound to the membrane which was detected would have been phosphorylated. This indicates that both the dephosphorylated and the phosphorylated forms of CK2β can interact with Nopp140. From CK2 activity data (Fig. 5B), deletion of the 20 NH2-terminal amino acids gives a mutant that will still activate CK2α as well as the wild type β does. It is reasonable to assume that CK2 is still in its active form even when it is complexed with Nopp140, and this was indeed what we observed when CK2 activity toward the peptide substrate was measured in the presence of Nopp140.

One interesting observation is that when higher amounts of GST fusion proteins of CK2α and CK2α′ were used in the binding assay, CK2α did exhibit affinity for Nopp140. By contrast, CK2α′ bound very weakly to Nopp140. This CK2α-Nopp140 interaction may have occurred indirectly through a separate protein, since the association was not detected with a lower amount of GSTα. If CK2α and CK2α′ have a different affinity for the hypothetical protein, this could cause the observed difference in their binding to Nopp140. One question that could be raised is whether α and α′ subunits exhibit redundancy in vertebrates as they do in yeast (5). It is known that CK2α and CK2α′ are encoded by different genes (22) and that they are structurally very homologous (85% homology) with major differences only in the COOH terminus (4). It has been shown that the α subunit can act as a transcription factor to control β gene expression, whereas α′ cannot (37). Also, the α subunit phosphorylates the β subunit more efficiently than α′ (19). It would be interesting to know whether the two catalytic subunits are associated with different proteins in the cell and have different functions.

So far, a number of proteins have been found which interact with CK2, some with the catalytic α or α′ subunit, and some with the β subunit. For example, proteins that are reported to be able to interact with CK2α are the transcription factor ATF1 (38), nucleolin (16–18), and the heat shock protein HSP90 (39). Proteins that are shown to interact with CK2β are p53 (9), DNA topoisomerase II (11), e-Mos2, and Nopp140. Currently, it is not clear whether the binding properties of the different subunits are related to the regulation of CK2.

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