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Heterogeneous Nuclear Ribonucleoprotein A2 Interacts with Protein Kinase CK2

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The catalytic subunit of protein kinase CK2 (CK2α) was found associated with heterogeneous nuclear ribonucleoprotein particles (hnRNPs) that contain the core proteins A2 and C1-C2. High levels of CK2 activity were also detected in these complexes. Phosphopeptide patterns of hnRNP A2 phosphorylated *in vivo* and *in vitro* by protein kinase CK2 were similar, suggesting that this kinase can phosphorylate hnRNPA2 *in vivo*. Binding experiments using human recombinant hnRNP A2, free human recombinant CK2α or CK2β subunits, reconstituted CK2 holoenzyme and purified native rat liver CK2 indicated that hnRNP A2 associated with both catalytic and regulatory CK2 subunits, and that the interaction was independent of the presence of RNA. However, the capability of hnRNP A2 to bind to CK2 holoenzyme was lower than its binding to the isolated subunits. These data indicate that the association of CK2α with CK2β interferes with the subsequent binding of hnRNP A2. HnRNP A2 inhibited the autophosphorylation of CK2β. This effect was stronger with reconstituted human recombinant CK2 than with purified native rat liver CK2.

Protein kinase CK2 from nuclear extracts of rat liver cells phosphorylates the heterogeneous nuclear ribonucleoprotein proteins (hnRNPs) A2 and C (C1-C2) (1, 2). These proteins belong to a family that form complexes with RNA polymerase II transcripts known as premessenger ribonucleoprotein complexes. About 20 protein members of this family have been described in HeLa cells. The more abundant are A, B and C groups, also called the core proteins (3). HnRNPs have been involved in structural roles, in RNA protection against degradation and more recently they have been viewed as a subset of the transacting pre-mRNA processing factors. These proteins recognise defined regions in pre-mRNAs, and through specific interactions contribute to the targeting of processing factors to defined sites (4, 5). Moreover, some of the hnRNPs were shown to shuttle between nucleus and cytoplasm (6, 7), suggesting a possible role of these proteins in mature mRNA export (8, 9). In these complexes RNA is likely to play a bridging effect although direct protein-protein interactions could be also possible (10).

Several hnRNPs are susceptible of phosphorylation what inhibits their binding to pre-mRNA and affects their participation in the spliceosome (11, 12). *In vivo* phosphorylation of hnRNP C1-C2 seems to involve several protein kinases, one of which is protein kinase CK2 (11). HnRNP A1 is also a substrate for several protein kinases, including cAMP-dependent protein kinase (PKA), protein kinase C (PKC) and protein kinase CK2 (13). HnRNP A2 is phosphorylated *in vitro* by CK2 (2, 14). However, the kinase activity responsible of its *in vivo* phosphorylation is still unknown.

Protein kinase CK2 is a multipotential serine/threonine kinase, widely distributed in eukaryotes, located in both cytoplasm and nucleus, and highly conserved during evolution (15). The classic conformation of CK2 in mammalian cells is an heterotretamer formed by two catalytic subunits, α or α' of 42-44 kDa and 38 kDa, respectively and two regulatory β subunits of 28 kDa (16, 17). However, dimers of β-β subunits can also be found, as well as free catalytic subunits bound to intranuclear components (18). CK2β subunit participates in the assembly of the holoenzyme, in the regulation of the catalytic activity and confers specificity for the protein substrates. Furthermore, CK2β can be phosphorylated by the catalytic subunit at serines 2 and 3. Autophosphorylation also occurs in CK2α but in

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a lower extent. A streaking characteristic of CK2 is that it can use either ATP or GTP, as phosphate donor, what distinguishes it from other multipotential serine/threonine protein kinases (19, 20).

A broad range of CK2 substrates have been identified, most of which are proteins involved in different nuclear activities, such as nucleolar organization, DNA topology and replication, gene transcription, and RNA processing (15). These data indicate that CK2 may play an important role in the nucleus by regulating a broad spectrum of nuclear functions. In this work we obtained evidences that CK2α is present in hnRNA-hnRNPs complexes and that hnRNP A2 could be an *in vivo* substrate for protein kinase CK2. Furthermore, both proteins form complexes *in vitro* that result in a reduced autophosphorylation of CK2β.

EXPERIMENTAL PROCEDURES

Materials. Purified CK2 from rat liver was obtained as previously described (21). Monoclonal antibody 4F4 was the kind gift of Dr. Gydeon Dreyfuss (Philadelphia). Polyclonal antibody against CK2α was obtained from UBI. The antibody against hnRNP A2 was obtained as described (2). Monoclonal antibody against human cytokeratine 18 (anti-p45) was obtained according to (22). Protein A-trisacryl was purchased from Pierce (Rockford, USA). RNA from *S. pombe* was the kind gift of Dr. Rosa Aligué (Barcelona, Spain). $^{32}P_i$ and radioactive nucleotides [γ-^{32}P]ATP or [γ-^{32}P]GTP were purchased from Amersham (Buckinghamshire, UK). Plasmid pET9c-hnRNPA2 (a kind gift of Dr. Adrian R. Krainer, Cold Spring Harbor Laboratory, NY) was expresed in E. coli strain BL21 (D3). HnRNP A2 protein was purified from the supernatant of sonicated cells by phenyl-Superose and ssDNA column chromatographies. The purified protein was concentrated using Centricon 10 and dialyzed against buffer A plus 0.1 M KCl. The recombinant subunits of protein kinase CK2 were obtained as described (23). To reconstitute the holoenzyme, equimolar quantities of each subunit (0.1 nmol) were incubated during 1 h at 30°C in 200 μl of reconstitution buffer (50 mM Tris-HCl pH 7.0, 150 mM NaCl, 0.1% BSA).

Cells cultures and preparation of cell lysates. HeLa and NP18 cells were grown at 37°C and 5% CO_2 in a humidified atmosphere in Dulbecco's modified Eagle's medium (Biological Industries, Israel), supplemented with 10% of fetal calf serum, glutamine, non-essential aminoacids, piruvic acid and antibiotics. Confluent cultures ($\sim 10^7$ cells) were harvested and washed twice in cold phosphate-buffered saline (PBS). Then, the cells were scrapped and the pellets were frozen at −80°C until they were used. Total soluble extracts from HeLa or NP18 cells were obtained from frozen pellets (−80°C) that were resuspended in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 250 mM NaCl, 50 mM NaF, 0.1% Triton X-100, 2 μg/ml leupeptin, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 2 μg/ml pepstatin A, 10 μg/ml aprotinin) and kept on ice for 30 min. Then, the samples were centrifuged for 10 min at 10,000 rpm and 4°C. The pellets were discarded and the supernatants collected and kept at −80°C until they were used.

In vivo labeling of HeLa cells and obtention of nuclear extracts. HeLa cells (two dishes of 100 mm of diameter with confluent cells) were subjected to depletion of endogenous phosphates by incubation for 2 h with supplemented D-MEM without phosphate. Labeling was performed by adding 150 μCi/ml of $^{32}P_i$ (Amersham) and keeping the cells for 3 h in culture conditions. To obtain the nuclear extracts, the cells were washed twice with cold PBS, scrapped in 5 ml of cold PBS and centrifuged at 2600 rpm for 5 min. Cells were resuspended in 1 ml of RSB buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) plus 0.1 mM Na_3VO_4, 0.1 mM PMSF, 5 mM NaF, 2 μg/ml leupeptin, and 10 μg/ml aprotinin. Nonidet P40 was added until reach a final concentration of 0.5%. The tube was vortexed for 20 s and the suspension was centrifuged for 7 min at 3100 rpm at 4°C and the nuclear pellet collected and washed twice with 1 ml of RSB. Nuclei were resuspended in 250 μl of buffer A (10 mM Tris-HCl pH 7.4, 100 mM NaCl, 2.5 mM $MgCl_2$) plus Na_3VO_4, PMSF, NaF, leupeptin and aprotinin as indicated above. Nuclei were sonicated with 4 bursts (5 s each), with the sonicator set at scale 2. The sonicated nuclei were decanted over buffer A plus 30% sucrose and centrifuged for 15 min at 7000 rpm. The supernatant corresponded to the nucleoplasmic fraction.

Immunoprecipitation, gel electrophoresis, and immunoblotting. 2-3 mg of soluble extracts were immunoprecipitated by adding 2 μl of 4F4 antibody while keeping the tubes in rotation for 3 h at 4°C. Then, 30 μl of protein A-trysacryl were added and the tube was kept in rotation for 1 h. The tube was spun down for 5–10 s and washed twice in 1 ml of lysis buffer. Finally, the pellets were resuspended in sample buffer, subjected to electrophoresis and transferred onto an Immobilon P membrane for immunodetection assays.

Samples were electrophoresed in SDS-polyacrylamide gels essentially as described by Laemmli (24). Non-equilibrium pH electrophoresis (NEPHGE) were run as described by O'Farrell (25) with a SDS-10% polyacrylamide gel as the second dimension. Gels were stained with Coomassie Blue G-250 or transferred onto Immobilon-P membranes (Millipore) and used for immunodetection with either 4F4 antibody (1:1000 dilution), anti hnRNP A2 (1:500 dilution), anti CK2α or anti CK2β (1:500 dilution), following standard procedures.

Phosphorylation assays and protein determination. Phosphorylation assays were carried out as reported previously (23) except for the presence of 100 mM KCl and 12 μM of either [γ-^{32}P]GTP or [γ-^{32}P]ATP (2.8 Ci/mmol). When hnRNP A2 was used as substrate, its concentration ranged from 1.25 to 10 μg/ml. CK2 activity assays in the immunoprecipitates were performed under similar conditions but the reaction mixture contained also 1 μM okadaic acid and 100 μg/ml of β-casein. In all cases, after 20 min of incubation at 30°C, the reaction was stopped by adding SDS-PAGE sample buffer and analyzed by electrophoresis and autoradiography.

RESULTS

Protein kinase CK2 is present in hnRNP complexes. Total extracts from asynchronously growing HeLa cells were immunoprecipitated using 4F4 monoclonal antibodies which are specific for hnRNP C1-C2 proteins. These antibodies immunoprecipitate the complexes containing these and other associated hnRNPs (26). As shown in Fig. 1A, hnRNPs C1-C2 and hnRNP A2 were detected in the 4F4 immunoprecipitates. Western blot analyses using specific antibodies against CK2α revealed its presence in these immunoprecipitates (Fig. 1B). Similar results were obtained using extracts from a different cell line, the human pancreatic carcinoma cell line NP18 (Fig. 1B). These data demonstrated that the presence of CK2α in hnRNPs complexes is not restricted to HeLa cells, but may be regarded as a more general feature. In contrast, similar experiments performed to detect the presence of CK2β in these complexes gave negative results (data not shown). The possibility that the presence of CK2α subunit in the immunoprecipitates could be due to a non-specific binding of this catalytic subunit to protein A-trisacryl was discarded since immunoprecipitates obtained with control antibodies (anti-p45) did not con-

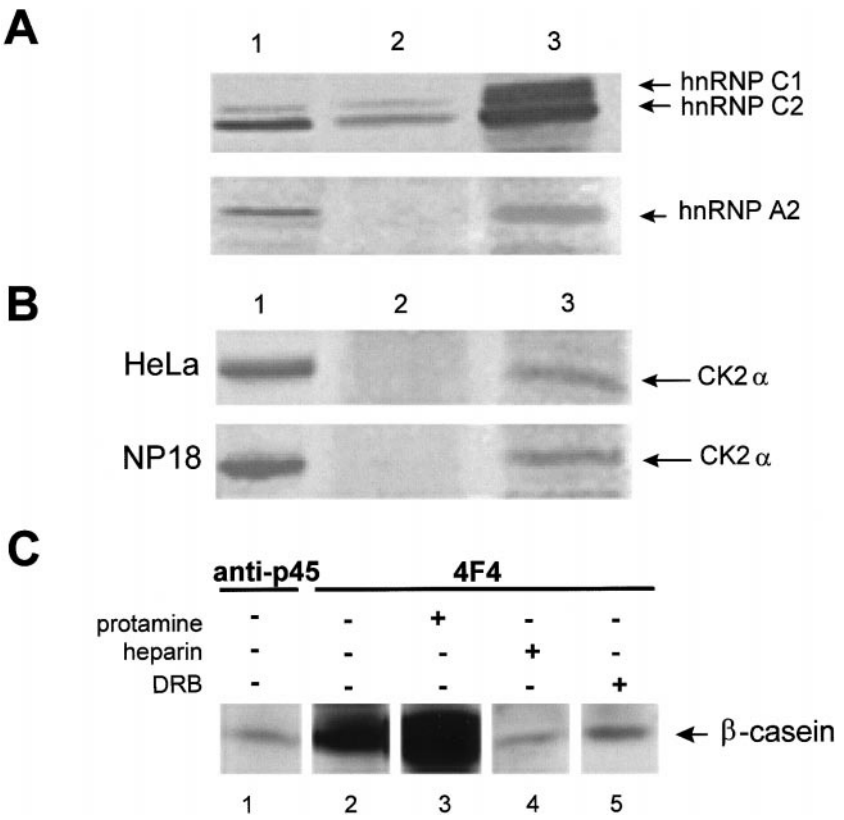
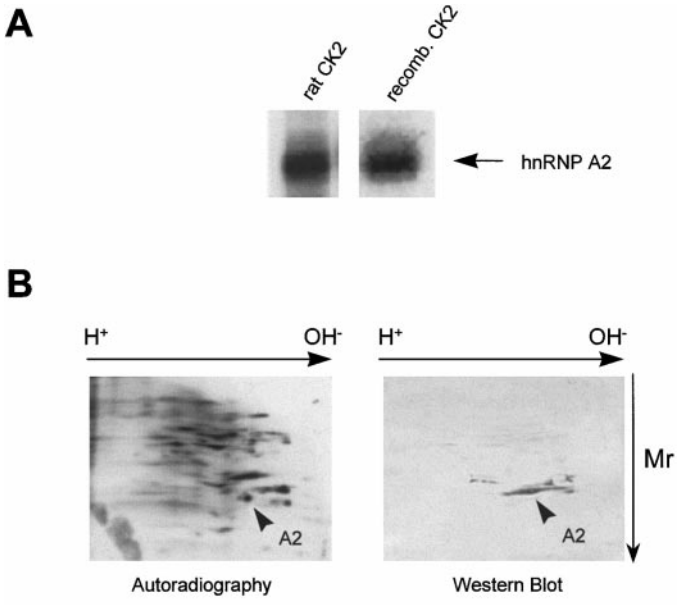


FIG. 1. Identification of protein kinase CK2 in the hnRNP complexes. (A) 30 μg of nucleoplasmic extract from HeLa cells were subjected to SDS-PAGE and transferred onto Immobilon-P membranes either directly (pre-immunoprecipitation, lane 1), or after immunoprecipitation with the control antibody anti-p45 (lane 2) or with 4F4 antibody (lane 3). The membranes were incubated with specific antibodies in order to detect the hnRNPs C1-C2 and A2 (4F4 and anti-p36, respectively). (B) Membranes correspond to replicates of that shown in A, but in this case they were incubated with antibodies anti-CK2α subunit. (C) Immunoprecipitates obtained with the control antibody anti-p45 (lane 1) or with 4F4 antibody (lanes 2 to 5) were assayed for CK2 activity on β-casein as substrate, either without additions (lanes 1 and 2), in the presence of 1.2 mg/ml of protamine (lane 3), 5.4 μg/ml of heparin (lane 4), or 40 μM DRB (lane 5).

tain CK2α (Fig. 1B). To confirm the presence of CK2α in the hnRNP complexes the activity of CK2 was measured in the immunoprecipitates obtained with 4F4 antibodies, using [γ-^{32}P]GTP and β-casein as substrates. As shown in Fig. 1C, CK2 activity was clearly detected in the immunoprecipitates obtained with 4F4 antibodies (lane 2) but not when a control antibody (anti-p45) was used (lane 1). The effects of protamine (a CK2 activator) and of heparine and DBR (CK2 inhibitors) on this kinase activity were also analysed. As shown in Fig. 1C, protamine induced a marked increase in the phosphorylation of β-casein (lane 3). In contrast, heparin and DBR produced a clear inhibitory effect (lanes 4 and 5).

HnRNP A2 protein can be phosphorylated in vivo by protein kinase CK2. The presence of hnRNP A2 and CK2 in the same hnRNP complexes and the fact that hnRNP A2 and hnRNPs C1-C2 are phosphorylated by CK2 in rat liver cells extracts (2) strongly suggested that this protein could be phosphorylated *in vivo* by CK2. To clarify this point, *in vivo* and *in vitro* phosphorylation experiments were performed. As shown in Fig. 2A both purified rat liver and reconstituted human recombinant CK2 holoenzyme were able to phosphorylate purified recombinant hnRNP A2. To analyse whether hnRNP A2 was an *in vivo* substrate for CK2, asynchronously growing HeLa cells were incubated with ^{32}Pi (150 μCi/ml). Then, a nuclear fraction, enriched in hnRNPs proteins was obtained (27) and the proteins of this fraction resolved in two-dimensional (NEPHGE) gels. These gels were done in duplicate. The first gel was dried without staining and exposed to an X-rays film. The second gel was transferred to an Immobilon-P membrane and the spot corresponding to hnRNP A2 was immunodetected by western blotting (Fig. 2B). This membrane was also exposed to an X-ray film and the spot corresponding to hnRNP A2 protein was identified in this autoradiography. It was then possible to identify the spot corresponding to hnRNP A2 in the first dried and exposed gel. The hnRNP A2 spot was cut out from the dried gel and digested with 2 μg of V8 protease in the stacking gel. The proteolytic products were resolved by SDS-PAGE using a 14% (w/v) polyacrylamide gel. The phosphopeptides were



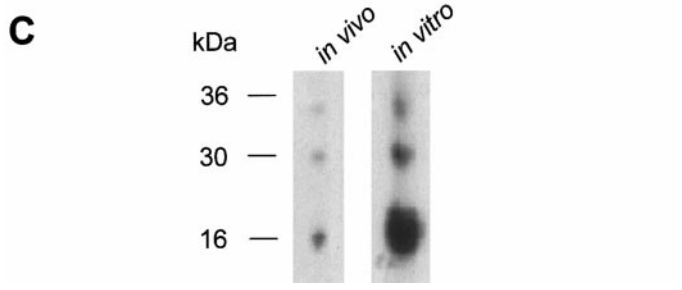


FIG. 2. Phosphorylation of hnRNP A2 by protein kinase CK2 *in vitro* and *in vivo*. (A) Purified hnRNP A2 was incubated under phosphorylation assay conditions with either native protein kinase CK2 purified from rat liver (left) or reconstituted human recombinant protein kinase CK2 (right) and subjected to SDS-PAGE and autoradiography. (B) (Left) ^{32}P-labelled proteins present in nucleoplasmic extracts from asinchronic HeLa cell cultures were subjected to a NEPHGE and autoradiography (right). Immunoblot of a duplicate of the gel immunodeted with anti-hnRNP A2 antibodies. In both cases, the spot corresponding to hnRNP A2 is shown by an arrowhead. (C) hnRNP A2 phosphorylated *in vivo* (left) or *in vitro* by CK2 (right) were subjected to SDS-PAGE. Then, the ^{32}P-labelled bands were digested with V8 protease and the phosphopeptides obtained were analysed by SDS-PAGE and autoradiography.

compared with those obtained from hnRNP A2 protein phosphorylated *in vitro* by purified rat liver CK2. As shown in Fig. 2C, the phosphopeptide patterns obtained were identical. These results strongly suggest that CK2 can phosphorylate hnRNP A2 *in vivo* in HeLa cells.

In vitro interaction of CK2 with the hnRNP A2 protein. In order to establish the kind of interaction between hnRNP A2 and protein kinase CK2, and its possible dependence on the presence of nucleic acids, *in vitro* binding assays were performed. Human recombinant CK2α (25 pmols) or CK2β (20 pmols) were incubated overnight at 4°C and gentle rotation with 25 or 20 pmols of human recombinant hnRNP A2 either in the absence or in the presence of increasing concentrations of RNA (10, 100 and 1,000 μg/ml). Then, the samples were subjected to immunoprecipitation using antibodies against either CK2α or CK2β (Fig. 3A). The immunoprecipitates were resolved in SDS-PAGE and transferred to Immobilon P membranes. The blots were developed with antibodies against hnRNP A2. Control immunoprecipitations were made in the absence of RNA. Figure 3A shows that hnRNP A2 associates with CK2α and CK2β equally well in the absence or in the presence of RNA, since bands of similar intensity of immunoprecipitated hnRNP A2 were found in the blots at the different concentrations of RNA used.

To better define the interaction between hnRNP A2 and CK2, hnRNP A2 (28 pmols) was incubated overnight in the presence of rat liver protein kinase CK2, reconstituted human recombinant protein kinase CK2

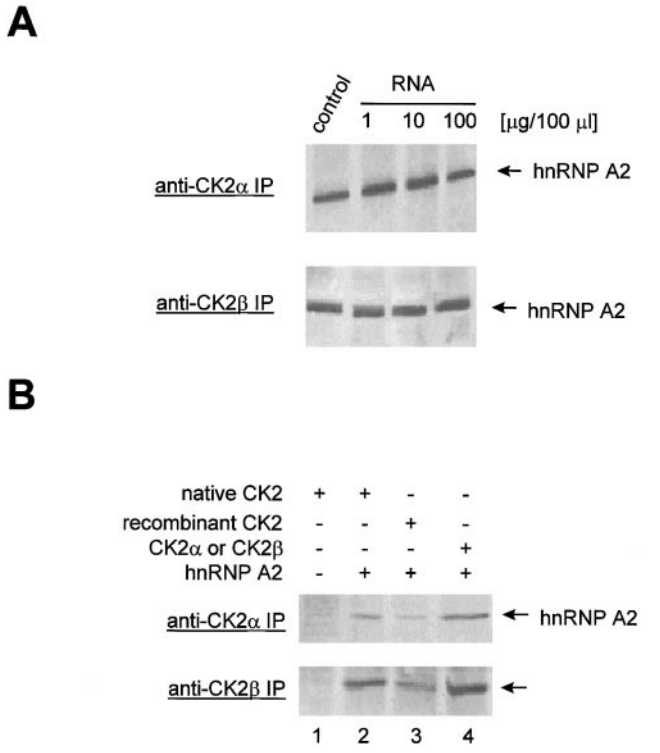


FIG. 3. *In vitro* association of hnRNP A2 and protein kinase CK2. (A) CK2α (upper blot) or CK2β subunits (lower blot) were incubated plus hnRNP A2 either in the absence (control) or in the presence of the indicated concentrations of RNA from *S. pombe*. The samples were immunoprecipitated with anti-CK2α antibodies (upper blot) or anti-CK2β (lower blot) and subjected to SDS-PAGE and Western blot, revealed with anti-hnRNP A2 antibodies. (B) The Western blots correspond to immunoprecipitations with anti-CK2α antibodies (upper blot) or anti-CK2β (lower blot) from an *in vitro* mixture containing either purified rat liver CK2 (lanes 1 and 2), recombinant human CK2 reconstituted *in vitro* (lane 3) and either CK2α (lane 4, upper blot) or CK2β (lane 4, lower blot), in the absence (lane 1) or in the presence (lanes 2 to 4) of hnRNP A2. The blots were revealed with anti-hnRNP A2 antibodies. Both incubations were made using equimolar quantities of CK2α or CK2β subunits.

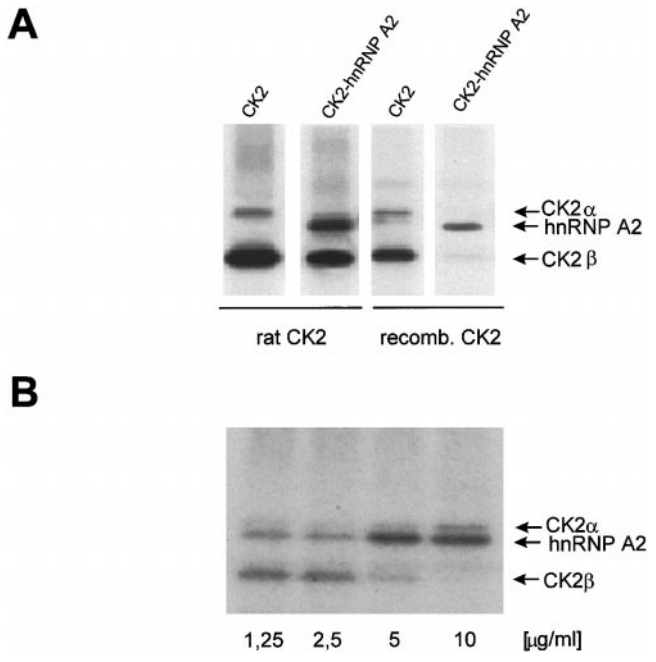


FIG. 4. Effect of hnRNP A2 on protein kinase CK2 autophosphorylation. (A) Purified rat liver CK2 (rat CK2) and human recombinant CK2 reconstituted *in vitro* (recomb. CK2) were incubated under phosphorylation conditions either alone (CK2) or in the presence of hnRNP A2 (CK2-hnRNP A2). The mixture was then analyzed by SDS-PAGE and autoradiography. Arrows indicate the mobility corresponding to hnRNP A2 and CK2α and CK2β subunits. (B) Human recombinant CK2, reconstituted *in vitro*, was incubated under phosphorylation conditions in the presence of the indicated amounts of hnRNP A2. The phosphorylated proteins were resolved as in (A).

holoenzyme or free human recombinant CK2α subunit, being in all cases constant the total amount of catalytic subunits present (12 pmols) (Fig. 3B, upper blot). Lane 1 corresponds to rat liver protein kinase CK2 alone (without hnRNP A2) used as a control. All the samples where immunoprecipitated with 2 μl of anti-CK2α polyclonal antibody. A similar experiment was performed using CK2β instead of CK2α (Fig. 3B, lower blot). In this case 20 pmols of CK2β were present in each point, and the samples were immunoprecipitated with 2 μl of anti-CK2β polyclonal antibody. In both cases, the higher levels of recombinant hnRNP A2 protein were detected when using free subunits (lane 4), indicating that hnRNP A2 can interact with both CK2α and CK2β. Lower levels of hnRNP A2 protein were detected in lanes 2 and 3, suggesting that when CK2 acquires the holoenzyme conformation the interaction of the catalytic and regulatory subunits interferes with the subsequent binding of hnRNP A2.

The presence of hnRNP A2 inhibits the autophosphorylation of CK2β subunit. The possibility that the interaction with hnRNP A2 affected the characteristics of CK2 was explored on the bases of its effect on CK2 autophosphorylation. As it can be observed in Fig. 4A the presence hnRNP A2 (5 μg/ml) inhibited CK2 autophosphorylation on CK2β, both with purified rat liver (100 mU) and recombinant protein kinase CK2 (1 pmol, that corresponds approximately to 100 mU). However, this effect was rather slight with native rat liver CK2 and much more evident with human recombinant CK2. Furthermore, the effect on recombinant CK2β phosphorylation depended on the concentration of hnRNP A2 used in the assay (Fig. 4B). When increasing concentrations of hnRNP A2 were added to the assay (1.25 μg/ml to 10 μg/ml) the phosphorylation of CK2β decreased progressively until disappearing in the presence of high concentrations of hnRNP A2. An increase in hnRNP A2 phosphorylation was also observed, what suggests that hnRNP A2 can compete with CK2β to serve as substrate for CK2α.

DISCUSSION

In previous studies, we have demonstrated that hnRNPs A2 and C1-C2 can be phosphorylated by protein kinase CK2 in nuclear extracts from rat liver cells (1, 2). The results reported here indicate that in cell extracts CK2α subunit is present in the pre-mRNA-hnRNPs complexes that contain hnRNP A2 and hnRNP C1-C2 proteins. This is also in agreement with previous observations showing that a fraction of CK2α subunit is tightly bound to intranuclear components (18), and it may explain the ability of CK2 to phosphorylate hnRNPs *in vivo*.

In vitro binding experiments show that hnRNP A2 associates with both free catalytic CK2α and free regulatory CK2β, indicating that both CK2 subunits have hnRNP A2 binding sites. The association of any of these CK2 subunits with hnRNP A2 is independent of the presence of RNA, suggesting that it results from a direct protein-protein interaction and it is not due to a bridging effect of the nucleic acid. The interaction of hnRNP A2 to each one of the free CK2 subunits is clearly stronger than the interaction with native or recombinant CK2 holoenzyme. This suggests that the specific binding sites are fully accessible for hnRNP A2 when interacting independently but they would be partially occluded when CK2α and CK2β subunits associate.

The inhibitory effect of hnRNP A2 on CK2β autophosphorylation in the reconstituted recombinant CK2 suggests that hnRNP A2 might modulate the accessibility of the N-terminal region of CK2β (which contains the autophosphorylation sites) to CK2α catalytic center. However, it is interesting to remark that this effect was very slight when native CK2 purified from rat liver cytosol was used. Differences in the behaviour of the native rat liver CK2 enzyme and reconstituted recombinant CK2 holoenzyme have also been reported previously (28). Our present data would give support to the idea that the quaternary structure of the native holoenzyme could be more stable than that of reconstituted CK2.

The possible functional implications of hnRNP A2 phosphorylation by protein kinase CK2 are unknown. Basal phosphorylation by this enzyme has previously been detected with hnRNP C1 (11). This phosphorylation may be related to protein maturation and folding to acquire the functional structure or to serve as a determinant to render the protein susceptible to phosphorylation by other protein kinases, as reported previously for other protein substrates (29). HnRNP A2 phosphorylation by CK2 might also result in regulation of its nuclear-cytoplasmic transport. It has been described that hnRNP A1 and hnRNP A2 translocate between the nucleous and cytoplasm. The hnRNP A1 shuttling seems to be controlled by phosphorylation of a single peptide, which is a target for the ζ isoform of protein kinase C, cAMP dependent protein kinase (PKA) and protein kinase CK2 (12, 30), indicating that phosphorylation on these proteins may regulate their intracellular location.

Alterations in the pl of the core hnRNPs have been observed in HeLa cells in mitosis, when hnRNPs disperse throughout the cell (6). The return to the nucleus of hnRNP A1, A2, B1 and B2, among others, is blocked by actinomycin D and also by 5,6-dichlorobenzimidazole (DRB) (6), effects which are probably linked to inhibition of RNA polymerase II transcription. DRB prevents RNA polymerase II phosphorylation on its carboxyl-terminal domain, what is necessary for the transition from the initiation phase to the elongation phase (31, 32). Protein kinases distinct from protein kinase CK2 have been invoked in the response to DRB (33, 34). Nonetheless, protein kinase CK2 is known to phosphorylate the carboxyl-terminal domain of RNA polymerase II at a position that is believed as normally phosphorylated *in vivo* (31) and DRB is also a quite specific and potent inhibitor of protein kinase CK2 (35). Thus, the possibility that this basal phosphorylation by protein kinase CK2 may influence the ability of RNA polymerase II to be activated by other protein kinases is attractive and would provide a more integrated view of the mechanisms by which DRB exerts its effects on hnRNP return to the nucleus.

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REFERENCES

1. Bosser, R., Aligué, R., Guerini, D., Agell, N., Carafoli, E., and Bachs, O. (1993) *J. Biol. Chem.* **268,** 15477–15483.
2. Bosser, R., Faura, M., Serratosa, J., Renau-Piqueras, J., Pruschy, M., and Bachs, O. (1995) *Mol. Cell. Biol.* **15,** 661–670.
3. Dreyfuss, G., Matunis, M. J., Piñol-Roma, S., and Burd, C. G. (1993) *Annu. Rev. Biochem.* **62,** 289–321.
4. Bennet, R., Piñol-Roma, S., Stakins, D., Dreyfuss, G., and Reed, R. (1992) *Mol. Cell. Biol.* **12,** 3165–3175.
5. Matunis, E. L., Matunis, M. J., and Dreyfuss, G. (1993) *J. Cell Biol.* **121,** 219–228.
6. Piñol-Roma, S., and Dreyfuss, G. (1991) *Science* **253,** 312–314.
7. Piñol-Roma, S., and Dreyfuss, G. (1992) *Nature* **355,** 730–732.
8. Michael, W. M., Choi, M., and Dreyfuss, G. (1995) *Cell* **83,** 415–422.
9. Visa, N., Alzhanova, A. T., Sun, X., Kiseleva, E., Björkroth, B., Wurtz, T., and Daneholt, B. (1996) *Cell* **84,** 253–264.
10. Huang, M., Rech, J. E., Northington, S. J., Flicker, P. F., Mayeda, A., Krainer, A. R., and LeStourgeon, W. M. (1994) *Mol. Cell. Biol.* **14,** 518–533.
11. Mayrand, S. H., Dwen, P., and Pederson, T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7764–7768.
12. Municio, M. M., Lozano, J., Sanchez, P., Moscat, J., and Diaz-Meco, M. T. (1995) *J. Biol. Chem.* **270,** 15884–15891.
13. Idriss, H., Kumar, A., Casas-Finet, J. R., Guo, H., Damuni, Z., and Wilson, S. H. (1994) *Biochem.* **33,** 11382–11390.
14. Pancetti, F., Bosser, R., Itarte, E., and Bachs, O. (1996) *Biochem. Biophys. Res. Commun.* **218,** 35–39.
15. Allende, J. E., and Allende, C. C. (1995) *Faseb J.* **9,** 313–323.
16. Litchfield, D. W., Lozeman, F. J., Piening, C., Sommercorn, J., Takio, K., Walsh, K. A., and Krebs, E. G. (1990) *J. Biol. Chem.* **265,** 7638–7644.
17. Chester, N., Yu, I. J., and Marshak, D. R. (1995) *J. Biol. Chem.* **270,** 7501–7514.
18. Stigare, J., Buddelmeijer, N., Pigon, A., and Egyhazi, E. (1993) *Mol. Cell. Biochem.* **129,** 77–85.
19. Pinna, L. A. (1990) *Biochim. Biophys. Acta* **1054,** 267–284.
20. Issinger, O. G. (1993) *Pharmac. Ther.* **59,** 1–30.
21. Molina, E., Plana, M., and Itarte, E. (1991) *Biochem. J.* **277,** 811–818.
22. Bastos, R., Engel, P., Pujades, C., Falchetto, R., Aligué, R., and Bachs, O. (1992) *Hepatology* **16,** 1434–1446.
23. Bodenbach, L., Fauss, J., Robitzki, A., Krehan, A., Lorenz, P., Lozeman, F., and Pyerin, W. (1994) *Eur. J. Biochem.* **220,** 263–273.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. O'Farrell, P. Z., Goodman, H. M., and O'Farrell, P. H. (1977) *Cell* **43,** 1133–1142.
26. Choi, Y. D., and Dreyfuss, G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7471–7475.
27. Piñol-Roma, S., Choi, Y. D., Matunis, M. J., and Dreyfuss, G. (1988) *Genes Dev.* **2,** 215–227.
28. Marin, O., Meggio, F., Sarno, S., and Pinna, L. A. (1997) *Biochemistry* **36,** 7192–7198.
29. Roach, P. J. (1991) *J. Biol. Chem.* **266,** 14139–14142.
30. Cobianchi, F., Calvio, C., Stoppini, M., Buvoli, M., and Riva, S. (1993) *Nucleic Acids Res.* **21,** 949–955.
31. Payne, J. M., Laybourn, P. J., and Dahmus, M. E. (1989) *J. Biol. Chem.* **264,** 19621–19629.
32. Dubois, M. F., Nguyen, V. T., Bellier, S., and Bensaude, O. (1994) *J. Biol. Chem.* **269,** 13331–13336.
33. Vincent, M., Lauriault, P., Dubois, M. F., Lavoie, S., Bensaude, O., and Chabot, B. (1996) *Nucleic Acids Res.* **24,** 4649–4652.
34. Marshall, N. F., Peng, J., Xie, Z., and Price, D. H. (1996) *J. Biol. Chem.* **271,** 27176–27183.
35. Zandomeni, R., and Weinmann, R. (1984) *J. Biol. Chem.* **259,** 14804–14811.