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Cyclin H is a new binding partner for protein kinase CK2

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Abstract

The protein kinase CK2 holoenzyme is composed of two regulatory β- and two catalytic α- or α′-subunits. There is ample evidence for the binding of individual subunits of CK2 to various cellular proteins and, moreover, for functions of the individual subunits, which are different from their roles in the holoenzyme. Here, we report that the regulatory cyclin H subunit of the cyclin H/cdk7/Mat1 complex was associated with a protein kinase activity, which shows some similarity with protein kinase CK2. Coimmunoprecipitation experiments supported the existence of complexes of cyclin H and CK2 in mammalian cells. Far Western blot experiments revealed that cyclin H bound to the α-subunit but not the α′- and β-subunits of CK2. Immunofluorescence analysis showed that cyclin H and CK2α were colocated in the nucleus. Although cyclin H functions as the regulatory subunit for the cyclin H/cdk7/Mat1 complex, it could not substitute the regulatory β-subunit of CK2 in its regulatory function of the CK2 activity.

Keywords: Phosphorylation; Kinase; Regulation; Cyclin dependent kinase; Casein kinase II

Protein kinase CK2 is a ubiquitously expressed serine/threonine protein kinase, which is composed of two regulatory β- and two catalytic α- or α′-subunits (reviewed in [1]). In addition, there is increasing evidence for the presence of the individual subunits of CK2 either in a free form or bound to other cellular proteins [2–4]. The role and function of the individual subunits of CK2, which are not in the holoenzyme, are not yet clear. Most of the more than 180 substrates of CK2 are phosphorylated only efficiently by the holoenzyme whereas only a few substrates are efficiently phosphorylated by the α-subunit alone (for review see [1]). Although CK2 seems to be absolutely required for cell viability and cell cycle progression, only little is known about the regulation of the protein kinase activity. It was believed for a long time that CK2 is not regulated by growth factors and their down-stream signalling. However, some recent reports suggest that CK2 is responsive to cellular stress in the form of heat shock [5] or agents, which activate the p38-stress-activated protein kinase pathway [6]. Moreover, we found that the growth suppressor protein p53 binds to the regulatory β-subunit of CK2 and this binding led to an inhibition of the protein kinase activity. In contrast, a mutant p53, which has lost the growth suppressor activity, was clearly unable to inhibit the CK2 kinase activity [7]. Furthermore, upon activation the growth suppressor p53 transactivates the WAF1 gene, which results in an elevated level of the $p21^{WAF1}$ protein. This $p21^{WAF1}$ protein is not only an efficient inhibitor of cyclin dependent kinases but also of protein kinase CK2 [8]. Thus, these results demonstrate a direct and an indirect regulation of protein kinase CK2 for cell cycle progression by p53 inhibiting the enzymatic activity of CK2. In addition, CK2 is at least in vitro stimulated by polyamines such as spermine [9,10] and inhibited by heparin [11]. The α-subunit was found in complex with PP2A [12], with nucleolin [13], and with tubulin [14]. The β-subunit was found in association with NOPP140 [15], c-mos [16], A-raf [17,18], p53 [19], and $p21^{WAF1}$ [20]. The interaction of NOPP140 with the β-subunit is thought to be implicated in nuclear transport of the β-subunit. The interaction of the α-subunit with nucleolin might also be implicated in directing CK2 into specific subcellular compartments. The search for

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new binding partners of the individual subunits of CK2 might help to elucidate the regulation of this enzyme and, moreover, might help to find new ways of action for the individual subunits of CK2, which differ from those in the holoenzyme.

Protein kinase CK2 phosphorylates a large number of different substrates, which are implicated in transcription, growth control, manipulation of DNA, cytoskeletal network formation, and spindle pole formation (reviewed in [1]). We recently identified cyclin H as a new substrate for CK2 [7]. Here, we report that cyclin H is tightly associated with a CK2 kinase activity. In addition, immunofluorescence analysis reveals a colocalization of CK2 and cyclin H. By using far Western blot analysis as well as by coimmunoprecipitation experiments, we show that the α-subunit but not the β-subunit of CK2 bound to cyclin H. Analysing whether cyclin H might be able to substitute the regulatory β-subunit of CK2, we found that binding of cyclin H to CK2α does not influence the CK2 kinase activity.

Materials and methods

Immunofluorescence. For immunofluorescence, cos1 cells were grown on coverslips in 100 mm petri dishes up to 50–70% confluence. We used a mouse monoclonal CK2α antibody (1A5) and a rabbit polyclonal cyclin H antibody (Fl 323, Santa Cruz Biotechnology). Cells were rinsed with phosphate-buffered saline (PBS) and fixed in 3.7% (v/v) formaldehyde in PBS for 10 min at room temperature. Cells were washed 3 × 10 min with PBS and permeabilized with 0.5% (v/v) Triton-X 100 for 10 min, then washed again 3 × 10 min with PBS, and incubated in PBS + 10% (w/v) bovine serum albumin (BSA) at room temperature for 10 min to block non-specific protein binding. Cells were incubated with the primary antibodies (mouse anti-CK2α and rabbit anti-cyclin H in a dilution of 1:10) for 1 h at room temperature or for 30 min at 37 °C. After three washes with PBS containing 0.1% (v/v) Tween 20 at room temperature, cells were incubated with an appropriate FITC- or TRITC-conjugated anti-mouse or anti-rabbit antibody (#488 or #564, Molecular Probes, The Netherlands) at room temperature for at least 30 min. Cells were washed again under the same conditions and rinsed briefly and the coverslips were mounted on a drop of mounting media and analysed under a fluorescence microscope.

Purification of proteins. Recombinant CK2 holoenzyme [21], the α-, α′-, and β-subunits of CK2 [22] were expressed in *Escherichia coli* (BL21(DE3)). The bacterially expressed proteins were purified as described [23].

Immunoprecipitation. The cos1 cells were maintained in DMEM supplemented with 10% foetal calf serum. Cells were harvested, washed with PBS, and extracted with 100 mM Tris–HCl, pH 9.0, 120 mM NaCl, and 0.5% NP40 containing complete protease inhibitor cocktail (Complete, Roche Diagnostics, Mannheim, Germany). Extracts were subjected to immunoprecipitation. For immunoprecipitation, we used monoclonal antibody (1A5) raised against a C-terminal peptide from the protein kinase CK2 α-subunit (TPSPLGPLAGSP). A protein A/G–Sepharose mixture was preincubated for 1 h with 10 μl antibody and then washed three times with PBS, pH 7.4. One milligram cell extract was preincubated with a mixture of protein A– and protein G–Sepharose (Pharmacia) to remove unspecific binding proteins. The supernatant was applied to the preincubated Sepharose–antibody-matrix and incubated for 1 h. The supernatant was removed and the antibody-matrix was washed three times with PBS, pH 7.4. The immune complex was subjected to SDS–polyacrylamide gel electrophoresis, followed by Western blot analysis with anti-cyclin H antibody Fl 323 (Santa Cruz) and with antibodies #26 directed against CK2α, #30 against CK2α′, and #32 directed against CK2β [14].

In vitro phosphorylation of cyclin H by the associated protein kinase. Recombinant cyclin H [24] was expressed in baculovirus infected insect cells (Sf9). The protein was purified by Ni^{2+}-chelate chromatography as described [25]. Cyclin H (3.5 μg) was incubated for 30 min with 2 μCi [γ-^{32}P]ATP in kinase buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, and 1 mM DTT) in the absence or in the presence of 0.5 μg polylysine, 5 mM spermidine or 0.5 μg heparin. For competition experiments, we used the CK2 substrate peptide RRRDDDSDDD in concentrations of 0.1, 0.2, and 0.4 mM. Phosphorylated proteins were separated in a 12.5% SDS–polyacrylamide gel and detected by autoradiography.

In vitro phosphorylation in the presence or absence of cyclin H. Equal amounts of CK2α or CK2 holoenzyme were incubated in kinase buffer with either 0.75 μg tubulin and 2 μCi [γ-^{32}P]ATP for 30 min at 37 °C in the absence or presence of purified recombinant cyclin H. Proteins were separated in a 12.5% SDS–polyacrylamide gel and phosphorylated proteins were detected by autoradiography.

In vivo association of protein kinase CK2 with the cyclin H/cdk7/Mat1 complex. Sf9 insect cells were triple infected with recombinant baculoviruses coding for cyclin H, cdk7, and Mat1 and the trimeric cyclin H/cdk7/Mat1 complex was isolated from the cell extract as described previously [25]. The highly purified cyclin H/cdk7/Mat1 complex was incubated with nucleophosmin/B23 and [γ-^{32}P]ATP in the presence or absence of 0.5 mM synthetic CK2 substrate peptide. Phosphorylated protein bands were then analysed by SDS–polyacrylamide gel electrophoresis. Phosphorylated protein bands were visualized by autoradiography.

SDS–polyacrylamide gel electrophoresis and far Western blot analysis. Proteins were analysed by SDS–gel electrophoresis according to the procedure of Laemmli [26]. Proteins dissolved in SDS buffer (130 mM Tris–HCl, pH 6.8, 0.02% bromophenol blue (w/v), 10% 2-mercaptoethanol, 20% glycerol (v/v), and 4% SDS) were separated in an SDS–polyacrylamide gel, renatured in 10× PBS for 2 h at 4 °C and then the refolded proteins were transferred onto a PVDF Western blotting membrane (Roche Diagnostics, Mannheim, Germany) in a buffer containing 20 mM Tris–HCl, 150 mM glycine, pH 8.9. The membrane was blocked for 1 h in binding buffer [0.05% (v/v) Tween 20 in PBS, pH 7.4] with 5% dried milk. The samples were diluted in binding buffer with 1% dried milk and incubated for 1 h at 4 °C. The membrane was washed in PBS and incubated for 1 h with an antibody against the probe, which was dissolved in binding buffer with 1% dried milk in a dilution of 1:1000. After washing with binding buffer, the blot membrane was decorated with the secondary antibody and assayed with the Lumi Light system (Roche Diagnostics, Mannheim, Germany) according to manufacturer's instructions.

Results

We have recently shown that cyclin H is a substrate for protein kinase CK2 [7] and the phosphorylation site was mapped to threonine 315 within a canonical consensus sequence for CK2 [25]. For an enzyme/substrate interaction, both partner molecules should come into close contact within the cells. Thus, we analysed the subcellular distribution of cyclin H and the catalytic α-subunit of CK2. We performed an immunofluorescence study for the localization of CK2α and cyclin H in cos1 cells. Cells were fixed and incubated with

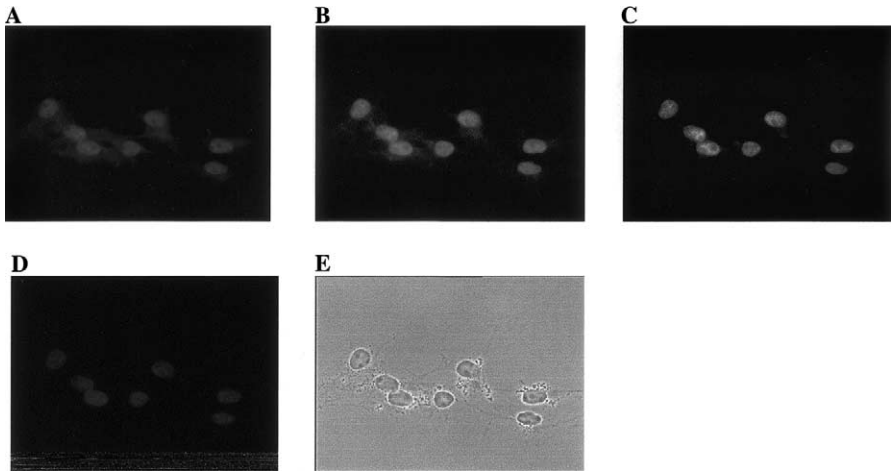

Fig. 1. Colocalization of CK2 and cyclin H. Subconfluent cos1 cells were incubated with rabbit anti-cyclin H antibody Fl 323 (A) and with mouse anti-CK2α antibody 1A5 (B), followed by TRITC-labelled goat anti-rabbit or FITC-labelled goat anti-mouse antibodies. Both immunofluorescence signals were matched in an overlay experiment (C). For control, nuclei were labelled with DAPI (D) and also a phase contrast analysis (E) is shown. Magnification is 400×.

monoclonal antibody 1A5 directed against the α-subunit of CK2, followed by FITC-labelled goat anti-mouse antibody. As shown in Fig. 1, CK2α is mainly localized in the nucleus as shown earlier [14,27,28]. The same cells were incubated with a rabbit antibody directed against cyclin H (Fl 323), followed by TRITC-labelled goat anti-rabbit antibodies. The localization of cyclin H is very similar to the CK2 staining, i.e., it is mainly nuclear. An overlay of the green and red staining revealed a colocalization of both proteins in the nucleus.

In the course of these studies, we asked further whether cyclin H might not only be a substrate for CK2 but also a specific binding partner, which might exceed an enzyme/substrate interaction. To study this interaction, we started by expressing cyclin H in insect cells infected with recombinant baculoviruses. Cyclin H from insect cells purified by Ni^{2+} agarose chromatography was incubated with [γ-^{32}P]ATP and after intensive washing proteins were analysed on an SDS–polyacrylamide gel. As shown in Fig. 2, we obtained a labelled protein band for cyclin H (lane 1), indicating that a protein kinase activity was associated with purified cyclin H. Since it is known that heparin inhibits the CK2 enzyme activity [11] and polylysine and spermidine [9] stimulate the kinase activity, the same experiment was repeated either in the presence of heparin, polylysine or spermidine. Phosphorylated proteins were again analysed on an SDS–polyacrylamide gel. As shown in Fig. 2 (lane 2), heparin efficiently inhibited the kinase activity associated with purified cyclin H whereas polylysine (lane 3) and spermidine (lane 4) stimulated the kinase activity. In all cases, equal amounts of cyclin H and [γ-^{32}P]ATP were used. Thus, purified cyclin H contains an associated protein kinase activity, which shows some properties similar to those of protein kinase CK2.

To address the kinase activity associated with cyclin H more directly to protein kinase CK2, we isolated cyclin H from insect cells infected with a recombinant baculovirus as described above. Purified cyclin H was incubated with [γ-^{32}P]ATP in the absence or in the presence of a synthetic peptide, which is usually used as a specific substrate for protein kinase CK2 [29] and which might compete with cyclin H as a substrate for the cyclin H associated protein kinase. Subsequently, cyclin H was analysed on an SDS–polyacrylamide gel followed by autoradiography. As shown in Fig. 3, cyclin H is efficiently phosphorylated by the associated protein kinase (lane 1). In the presence of increasing concentrations of the synthetic peptide with the sequence RRRDDDSDDD, there is a dose dependent reduction in the phosphorylation of cyclin H (lanes 2–4). At a concentration of 0.4 mM peptide, cyclin H phosphorylation is hardly detectable, supporting the idea that the associated protein kinase is protein kinase CK2.

In the next step, we wanted to know whether cyclin H might directly bind to CK2 and further, we were interested to know which subunit of protein kinase CK2 might bind to cyclin H. Therefore, we separated purified cyclin H from insect cells in an SDS–polyacrylamide gel. After transfer to a PVDF-filter, the membrane was blotted with purified bacterially expressed CK2α, α′ or β, followed by antibodies directed against CK2α, α′ or β. As shown in Fig. 4A, cyclin H bound only to the α-subunit of CK2 but neither to the α′- nor to the β-subunit of CK2. In a second experiment, CK2α was separated on an SDS–polyacrylamide gel transferred to a PVDF-filter and then blotted with cyclin H, followed by an antibody directed against cyclin H. As shown in Fig. 4B, there is clearly a binding of cyclin H to CK2α.

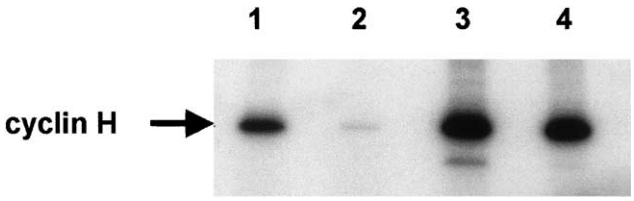

Fig. 2. A cyclin H associated protein kinase activity is inhibited by heparin and stimulated by spermidine. Cyclin H expressed in insect cells infected with a recombinant baculovirus was purified by Ni^{2+} agarose chromatography. Equal amounts of purified cyclin H were incubated with [γ-^{32}P]ATP in the absence of heparin (1), in the presence of heparin (2), in the presence of polylysine (3), and in the presence of spermidine (4) and then analysed on a 10% SDS–polyacrylamide gel. Labelled proteins were detected by autoradiography.

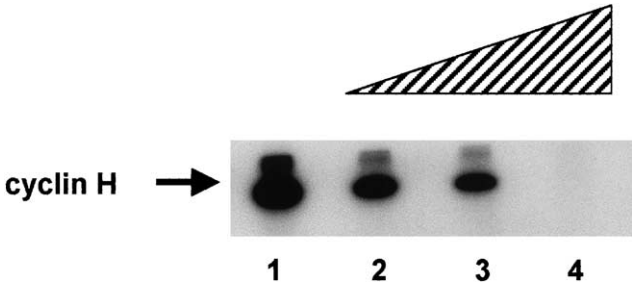

Fig. 3. Phosphorylation of cyclin H by its associated protein kinase activity is inhibited by the CK2 specific peptide substrate. Purified cyclin H from infected insect cells was incubated with [γ-^{32}P]ATP in the absence or in the presence of increasing amounts of the synthetic peptide substrate with the sequence RRRDDDSDDD. Proteins were analysed on a 12.5% SDS–polyacrylamide gel and labelled proteins were detected by autoradiography.

The same experiments were also performed with CK2α' and β without detecting a specific binding (data not shown).

Finally, we attempted to analyse the complex formation of cyclin H and CK2 in cell extracts from mammalian cells. The cos1 cells were extracted and CK2 was immunoprecipitated with monoclonal antibody 1A5 directed against the α-subunit of CK2. Immunoprecipitated proteins were analysed on a 12.5% SDS–polyacrylamide gel, followed by a transfer to a PVDF-membrane. The membrane was blotted with a rabbit anti-cyclin H antibody. As shown in Fig. 5A, cyclin H is coimmunoprecipitated with CK2, supporting the results shown above. As a control, the same filter was incubated with a mixture of rabbit antibodies against CK2α (serum #26) and CK2β (serum #32). Fig. 5B shows protein bands for CK2α- and β-subunits. Thus, from these results, one has to conclude that cyclin H binds to the CK2 holoenzyme and this binding is most probably mediated by binding to the α-subunit of CK2.

Similar to the β-subunit of CK2, cyclin H is the regulatory subunit of the cyclin H/cdk7/Mat1 complex. Thus, we wanted to analyse whether cyclin H might be able to substitute the β-subunit in the regulation of the CK2 kinase activity. For these types of analyses, we used tubulin as a substrate, which we phosphorylated with the catalytic α-subunit of CK2 alone or with the holoenzyme both, in the absence and presence of equimolar concentrations of cyclin H. As shown in Fig. 6, tubulin was weakly phosphorylated by the α-subunit of CK2 alone (lane 2) and strongly by the holoenzyme (lane 4). We found no considerable increase in the tubulin phosphorylation by CK2α in the presence of cyclin H (lanes 1 and 3). Thus, cyclin H is not able to substitute the β-subunit of CK2 in stimulating the kinase activity of the α-subunit of CK2. Moreover, there is also no gross influence of cyclin H on the phosphorylation of tubulin by the holoenzyme. In both cases, cyclin H is a substrate for the α-subunit as well as for the holoenzyme confirming previous results [25].

Cyclin H is the regulatory subunit of the cyclin H/cdk7/Mat1 complex, which phosphorylates the C-terminal domain of RNA polymerase II as well as down-stream cyclin dependent kinases. Therefore, we analysed whether protein kinase CK2 might also be associated with the cyclin H/cdk7/Mat1 complex in the cell. Insect cells were triple infected with recombinant cyclin H, cdk7, and Mat1. The trimeric complex was isolated from the cell extract as described earlier [25] and then incubated with a well known CK2 substrate namely nucleophosmin/B23 [30]. To demonstrate the specificity of the kinase reaction, the same reaction was repeated in

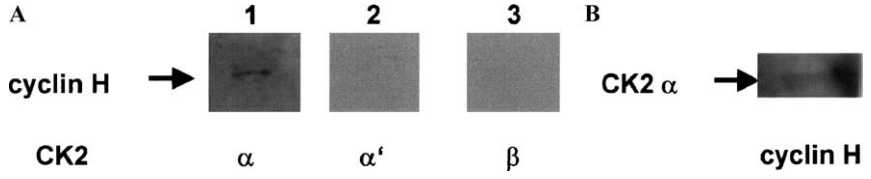

Fig. 4. Binding of cyclin H to CK2α by far Western blot analysis. (A) Cyclin H was separated on a 12.5% SDS–polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was incubated either with purified CK2 α, α' or β followed by antibodies directed against CK2α, α' or β. (B) CK2α was separated on a 12.5% SDS–polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was incubated with cyclin H, followed by antibody Fl 323 directed against cyclin H. Detection of proteins was performed using the Lumi Light system (Roche Diagnostics, Mannheim, Germany).

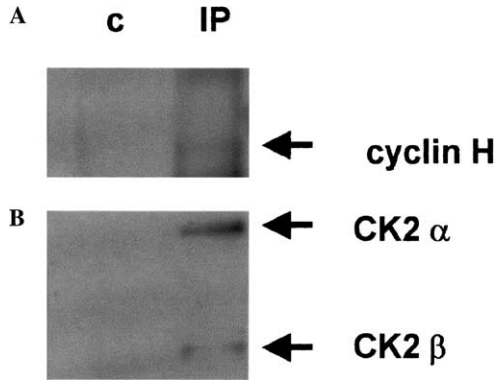

Fig. 5. Coimmunoprecipitation of cyclin H and CK2 from cos1 cells using monoclonal antibody 1A5 directed against CK2α. The immunoprecipitated proteins were separated through a 12.5% SDS–polyacrylamide gel. Proteins were transferred to a PVDF-membrane and the membrane was incubated with Fl 323 directed against cyclin H (A). As a control the same filter was incubated with a mixture of antibodies directed against CK2α and β (B). Proteins were detected by the Lumi Light system.

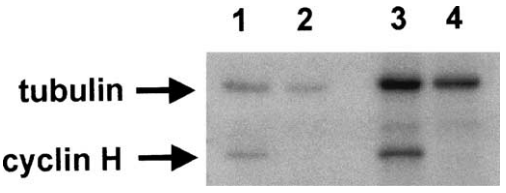

Fig. 6. Influence of cyclin H on the phosphorylation of tubulin by CK2α or by the CK2 holoenzyme. α-Tubulin (0.7 μg) was incubated with [γ-^{32}P]ATP and either 0.4 μg CK2α (1,2) or 0.45 μg CK2 holoenzyme (3,4) in the absence (2, 4) or presence of 0.2 μg cyclin H (1,3). Proteins were analysed on a 10% SDS–polyacrylamide gel. Labelled proteins were detected by autoradiography.

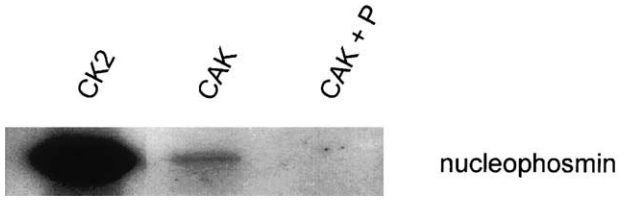

Fig. 7. Association of protein kinase CK2 with the cyclin H/cdk7/Mat1 complex. The cyclin H/cdk7/Mat1 complex was isolated from triple infected insect cells by GSH-Sepharose chromatography. After intensive washing, the complex was incubated with nucleophosmin and [γ-^{32}P]ATP in the absence (CAK) and in the presence of the synthetic CK2 specific substrate peptide (CAK + P). As a control nucleophosmin was incubated with CK2 and [γ-^{32}P]ATP (CK2). Proteins were analysed on a 10% polyacrylamide gel electrophoresis followed by autoradiography.

the presence of the CK2 specific peptide substrate. As shown in Fig. 7, nucleophosmin/B23 was phosphorylated by CK2 associated with the cyclin H/cdk7/Mat1 complex and this phosphorylation was completely inhibited by the CK2 specific substrate. Thus, these results demonstrate that CK2 is also associated with the cyclin H/cdk7/Mat1 complex in the cell and that CK2 in this form is still active.

Discussion

Protein kinase CK2 of all eukaryotes investigated so far has a tetrameric structure composed of two catalytic α- or α′-subunits and two regulatory β-subunits. Of the many proteins phosphorylated by CK2, the majority are involved in cell cycle related signalling and in the regulation of gene expression. One of the most prominent cell cycle regulators is the regulatory cyclin H subunit in the cyclin H/cdk7/Mat1 complex, which occurs as a component of the TFIIH transcription factor complex as well as in a free form [31]. The TFIIH associated form of cyclin H/cdk7/Mat1 complex is implicated in the phosphorylation of the large subunit of RNA polymerase II, thus, inducing the switch from the initiation of transcription to elongation [32]. The free cyclin H/cdk7/Mat1 form mainly functions as a cyclin activating kinase CAK that is absolutely necessary for cell cycle progression. Phosphorylation of cyclin H by CK2 seems to be required for a full activation of the cyclin H/cdk7/Mat1 complex [25]. To function as a substrate, cyclin H and CK2 should come into close contact. As shown in the present study, cyclin H and CK2 are co-located in the nuclear compartment.

In the past few years, there have been more and more observations that CK2 mediated effects other than by phosphorylation might happen. It has been reported that CK2 binds to viral proteins such as SV40 large T antigen [33], the growth suppressor protein p53 [34,35], and the cell cycle inhibitor p21^{WAF1} [8,36]. Binding of p21^{WAF1} to protein kinase CK2 inhibits the CK2 kinase activity and it also inhibits stress-activated kinases [37]. Moreover, p21^{WAF1} also binds to PCNA to block DNA replication, putting a multiple brake on cell cycle progression [38]. Binding of wild-type p53 to CK2 also resulted in an inhibition of the enzymatic activity of CK2 [7]. Since CK2 seems to be required for cell proliferation [39], there is ample evidence to believe that p53 might lead to growth arrest at least in part by inhibiting the CK2 activity. Here, we identified cyclin H as a new binding partner for protein kinase CK2. In vitro binding studies as well as pulldown experiments revealed that cyclin H bound to the catalytic α but not to the β- and also not to the α′-subunit. There are already a number of proteins known to bind to individual subunits of CK2. L5 [40], p53 [41], p21^{WAF1} [8], FGF [42], dynein [43], FAF-1 [44], NOPP140 [15], and raf [18] bind to the β-subunit of CK2 whereas Grp94 [45], nucleolin [13], Bcr-Abl [46], CKIP-1 [47], tubulin [14], and protein phosphatase 2A (PP2A) [12] bind to the α-subunit of CK2. In quiescent cells, the association between CK2α and PP2A stimulates the phosphatase activity towards

MAP/ERK1 kinase whereas mitogen stimulation of the cells disrupts the CK2α/PP2A complex allowing MEK1 activation [12]. Thus, these results support the hypothesis that the interaction of CK2 with some cellular proteins is actively implicated in the regulation of cell proliferation. Cyclin H may be another cellular binding partner of CK2, which is already known to be involved in the regulation of cell proliferation. It is known that CK2α is colocalized with RNA polymerase II [48]. Since cyclin H is a component of the TFIIH complex, which specifically interacts with RNA polymerase II, one might speculate that the interaction of cyclin H with CK2α might target protein kinase CK2 to the RNA polymerase II transcription regulation machinery. This hypothesis is supported further by the fact that the C-terminal domain (CTD) of the large subunit of RNA polymerase II contains a potential site for phosphorylation by CK2 [49]. Phosphorylation of the CTD is absolutely necessary for the switch from initiation to elongation of transcription and thus for cell proliferation. Furthermore, in the present study, we present clear evidence that CK2 is tightly associated with the cyclin H/cdk7/Mat1 complex in cells and that the CK2 kinase is still active in this form.

We generated an antibody against a peptide containing the CK2 phosphorylation site (EEEWT (P)DDDL). This antibody detected cyclin H from insect cells whereas bacterially expressed cyclin H was detected only after phosphorylation by CK2 [25]. Thus, we have to conclude that CK2 seems to bind to cyclin H in Thr-315 phosphorylated form and that CK2 phosphorylation of cyclin H does not dissociate the cyclin H/cdk7/Mat1/CK2 complex. Recently, another CK2α binding protein namely CKIP-1 was identified. It is not a substrate for CK2 and similar to cyclin H it also does not regulate the CK2 activity. It was speculated that CKIP-1 might be involved in targeting CK2 to a specific subcellular location [47]. Nucleolin is another protein that binds to the catalytic subunits of CK2 and it also seems to be implicated in targeting these subunits to particular places in the cell (for review see: [27]).

Binding of nucleolin to CK2α or α′ was inhibited by heparin, polyarginine, and histone H1 but not by the CK2 substrate casein [13], indicating that the binding of nucleolin can be separated from the enzymatic activity of CK2. Furthermore, the polylysine stretch ^{74}KKKKIKREIK^{83} which is the heparin-binding site and which is also essential for the substrate recognition is implicated in the binding of CK2α to Grp94 [45]. Since only CK2α and not α′ binds to cyclin H, we have to assume that the binding site for cyclin H might reside in the unique carboxy-terminal region of CK2α spanning from amino acids 333 to 391. From the crystal structure of human protein kinase CK2, it is evident that the C-terminus of the α-subunit is freely available for interactions with other proteins [50].

The α-subunit of protein kinase CK2 belongs to the "CMGC" group of protein kinases, which also includes the large family of cyclin dependent protein kinases, the mitogen-activated protein kinase (MAPKs and ERKs), and glycogen synthase kinase-3 [51]. Cyclin dependent kinases as well as CK2 share the property of having regulatory subunits, cyclins for cdks, and the β-subunit for CK2α. Both regulatory subunits increase the catalytic activity with most substrates. Both regulatory subunits contain a "destruction box" that may signal their degradation by the ubiquitin pathway. Since both bind to the inhibitor $p21^{WAF1}$ and to the growth suppressor p53, it was tempting to speculate that cyclin H might be able to substitute the regulatory β-subunit in the CK2 holoenzyme. However, using tubulin as a substrate for CK2α alone and for CK2α/cyclin H complexes revealed no differences in the enzymatic activity, indicating that cyclin H is unable to increase the enzymatic activity of the catalytic subunit.

Since cyclin H is also a substrate for CK2 binding of cyclin H to CK2α, it might also be a prerequisite for the correct positioning of cyclin H as a good substrate. This might be important for the holoenzyme as well as for the α-subunit alone. Since CK2 phosphorylation of cyclin H seems to be necessary for a full activity of the cyclin H/cdk7/Mat1 complex, this positioning also might be implicated in the regulation of cell proliferation.

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