Heat-induced Relocalization of Protein Kinase CK2

IMPLICATION OF CK2 IN THE CONTEXT OF CELLULAR STRESS

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Delphine A. Gerber‡, Sylvie Souquere-Besse§, Fabienne Puvion§, Marie-Françoise Dubois¶, Oliver Bensaude®, and Claude Cochet††

From the ‡Laboratoire INSERM U244, CENG, 17 Avenue des Martyrs, 38000 Grenoble, France, the §Laboratoire Organisation Fonctionnelle du Noyau, Institut Fédératif CNRS, UPR 1983, 94801 Villejuif, France, and the ¶Laboratoire Régulation de l’Expression Génétique, CNRS, UMR 8541, Ecole Normale Supérieure, 75230 Paris, France

Among various other roles described so far, protein kinase CK2 has been involved in cell cycle, proliferation, and development. Here, we show that in response to specific stresses (heat shock or UV irradiation), a pool of the cellular CK2 content relocates in a particular nuclear fraction, increasing the activity of the kinase there. Electron microscopic analysis shows that upon heat shock, CK2α and CK2β subunits are both detected in similar speckle structures occurring in the interchromatin space but are differentially targeted inside the nucleolus. This CK2 relocalization process takes place in a time- and dose-dependent manner and is reversible upon recovery at 37 °C. Altogether, this work suggests CK2 be involved in the response to physiological stress in higher eukaryotic cells.

Protein kinase CK2 is a serine/threonine protein kinase, ubiquitous and highly conserved among eukaryotic organisms (reviewed in Refs. 1–3). It is composed of two catalytic subunits (α or α′) and of two regulatory subunits (β), which tetramerize to adopt an α2β structure (1–3). Protein kinase CK2 localizes both in the nucleus and in the cytoplasmic compartment where it phosphorylates a variety of substrates involved in different cellular functions. Although its precise role remains elusive, CK2 has been involved in the major cellular processes including control of cell division and proliferation (4), development, and differentiation. In yeast, in addition to being necessary for cell viability (5), CK2 has also been shown to play a role in the maintenance of ion homeostasis, as highlighted by the Na+ and Li+ stress sensitivity of the CK2α mutants in Saccharomyces cerevisiae (6), and in the control of adaptation at the yeast DNA damage checkpoint (7). In addition, the human homologue of CK2β was shown to increase resistance to UV irradiation when transfected into Xeroderma pigmentosum cells (8), raising the question of whether CK2 may play a role in the response to stress.

In challenging conditions such as exposure to heat shock, UV irradiation, heavy metals, and oxidative stress, cells react by inducing what is called a stress response (reviewed in Refs. 9 and 10). This involves activation of signaling pathways, activation of transcription factors (notably the HSFs), and post-transcriptional modifications (12) (e.g. phosphorylation and acetylation). Among all the CK2 substrates described so far, it is striking to note that many are proteins involved in the response to heat shock, including chaperon proteins (HSP56 (13) and HSP90 (14)), stress related transcription factors (HSF-1 and Egr-1 (15)), the DNA repair machinery (topoisomerase II (16), DNA ligase (17), and APE/Ref-1 (18)), or in the control of transcription (4).

In this study, we address the question of whether CK2 might be implicated in the cellular stress response and more specifically in response to heat shock. We show that brief exposure to elevated temperature dramatically affects the localization of a cellular pool of protein kinase CK2 inside the nucleus and increases the activity of the kinase up to 3-fold in a specific nuclear fraction. Electron microscopic analysis reveals a differential redistribution of CK2 subunits inside the nucleolus, upon exposure to elevated temperature. After heat shock, the α CK2 subunit colonizes the nucleolus and concentrates in dense structures at the vicinity of the perinuclear layer of condensed chromatin, whereas the β CK2 subunit is only recovered at the periphery of the condensed chromatin. In addition, we show that the stress-induced response of the kinase is dose-dependent and reversible. Finally, this redistribution of the kinase is specific to heat shock and UV irradiation, because similar effects are not observed in oxidative stress conditions. The possible implications of this CK2 relocalization in the context of cellular stress are discussed.

EXPERIMENTAL PROCEDURES

Cell Culture and Heat Shock—NIH3T3 or HeLa cells grown at 37 °C with a humidified atmosphere 0.5% CO₂ in minimal essential medium (Life Technologies Inc.) supplemented with 10% inactivated fetal bovine serum. For heat shock treatment, cells were seeded at 6 × 10⁴ cells/ml into Petri dish with medium containing 25 mM Hepes, pH 7.5, 48 h after plating, cells were submerged in water bath and heat shocked at temperatures and times as indicated in figure legends.

CK2 Extraction Protocol—Immediately after heat shock, cells were rinsed twice with ice-cold phosphate-buffered saline (PBS) and either lysed in RIPA Buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) or sequentially lysed in GPV buffer (20 mM sodium glycerophosphate, pH 7.5, 1 mM EDTA, 10 mM MgCl₂, 1 mM β-mercaptoethanol, 0.5% Triton X-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mM Na₂VO₄). The cell lysate was centrifuged at 3000 × g for 10 min at 4 °C. The clarified
supernatant (S200) was recovered, whereas the pellet (P0) was resuspended in GPV containing 200 mM NaCl, and centrifuged at 3000 × g for 10 min at 4 °C. The clarified supernatant (S200) was recovered, the pellet P200 was extracted in GPV buffer containing 400 mM NaCl and centrifuged at 14,000 × g for 10 min leading to supernatant S400. The remaining pellet P400 was finally extracted in Laemmli loading buffer. Each fraction (S0, S200, and S400) was analyzed for CK2 activity and is representative of several similar experiments. B, aliquots of each fractions (containing equal amounts of protein) were loaded on a 12% bis-acrylamide gel, transferred on to nitrocellulose membrane, and blotted with antibodies against CK2α (R403) and CK2β (b). D, extracts from cells lysed in RIPA buffer and containing the same amount of proteins were analyzed by Western blotting with R403 and β antibodies.

**RESULTS**

**CK2 Activity Increases in a Specific Subcellular Fraction after Heat Shock**—Cells were heat shocked (43 °C) for 1 h before being either extracted in GPV buffer containing increasing NaCl concentrations (see “Experimental Procedures”) or in RIPA buffer. As shown in Fig. 1, exposure of cells at 43 °C for 1 h induces a dramatic change in the pattern of CK2 activity recovered in the S0, S200, and S400 fractions, respectively (Fig. 1, A and B). In other words, after heat treatment, CK2 activity decreased in the S0 and S200 fractions, whereas it

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FIG. 1. CK2 activity in control or heat shocked cells. NIH3T3 cells were subjected to heat shock (43 °C, 1 h) and then either sequentially extracted in GPV buffer (A and B) or extracted in RIPA buffer (C and D) as described under “Experimental Procedures.” The CK2 activity presented (A and C) was measured as described under “Experimental Procedures” and is representative of several similar experiments. B, aliquots of each fractions (containing equal amounts of protein) were loaded on a 12% bis-acrylamide gel, transferred on to nitrocellulose membrane, and blotted with antibodies against CK2α (R403) and CK2β (b). D, extracts from cells lysed in RIPA buffer and containing the same amount of proteins were analyzed by Western blotting with R403 and β antibodies.
increased up to 2-fold in the S400 fraction (Fig. 1A). It was calculated that about 20% of the total CK2 activity shifted from the S0 and S200 fractions to the S400 fraction. A Western blot analysis revealed that after heat shock treatment, the level of both CK2α and CK2β subunits was sharply increased in the S400 fraction, whereas it decreased significantly in the S0 fraction (Fig. 1B). We observed that the increase in CK2 activity in the S400 fraction did not exactly reflect the high increment in the CK2 amount detected by Western blot analysis of this fraction. Yet we do not have satisfactory explanation for this light discrepancy. In all fractions, the ratio of CK2α versus CK2β subunits remained similar in control or in heat-treated cells. No significant amount of CK2 protein was detected in the P400 pellet extracted in Laemmli loading buffer (Fig. 1B).

On the other hand, the total CK2 activity measured in the extracts recovered after cell lysis in RIPA buffer was unchanged after heat treatment (Fig. 1C). Similarly, a Western blot analysis of the RIPA extracts indicated that the amount of both the catalytic and the regulatory subunits of the kinase remained constant after heat shock (Fig. 1D). Therefore, these results suggested that the change in CK2 activity observed after heat shock was correlated with an intracellular redistribution of both kinase subunits.

Stress and Dose Dependence of the CK2 Redistribution—To test whether the response of CK2 is dependent on the dose of the heat shock (i.e. on the temperature at which cells are exposed to), cells were incubated for 1 h at various temperatures ranging from 37 to 46 °C. A Western blot analysis (Fig. 2A) shows that the increase of the CK2 protein, visualized in fraction S400, was proportional to the severity of the heat shock, reaching a maximal augmentation at temperatures above 43 °C. This increase was correlated with a comparable decrease in the amount of the enzyme in fraction S0 and to a lesser extent in fraction S200. Moreover, this redistribution of the kinase was correlated with a corresponding shift of the CK2 activity from the S0 and S200 fractions to the S400 fraction (Fig. 2B). The activity measured in S400 fraction was greater at the highest temperature (45 °C). This experiment emphasizes the observation that, in response to heat shock, the localization of both CK2 subunits balances between the S0 and S200 fractions on one hand and the S400 fraction on the other hand. It was noticed that above 45 °C (severe heat shock conditions), cells started to die. Therefore, to obtain maximum effect on CK2 with limited cell death, the following heat shock experiments were performed at 43 °C (mild heat shock conditions).

To assess the specificity of CK2 response in stress conditions, NIH3T3 cells were subjected to either UV irradiation (20 and 40 J/m²) or oxidative stress (1–5 mM H₂O₂ for 2 h) (Fig. 3). It was observed that UV irradiation (40 J/m²) induced a CK2 relocalization in the S400 fraction that is analogous to the heat shock-induced CK2 relocalization (Fig. 3A). Similarly to heat shock, CK2 activity increases up to 2-fold upon UV irradiation when compared with the activity in control cells. Moreover, this augmentation of CK2 activity correlated with an increase in the amount of both CK2 subunits (Fig. 3B) and was also found to be dose-dependent (data not shown). In contrast, H₂O₂ had no effect either on the relocalization of the kinase nor on the CK2 activity measured in the S400 fraction (data not shown). Thus, we concluded that CK2 activation was specific to the type of stress and also dependent on the intensity of the stress applied.

Characteristics of Heat-induced CK2 Relocalization—To further characterize the heat-induced redistribution of CK2, time course experiments were performed. Fig. 4A shows a representative experiment of the CK2 activity, recovered in the S400 fraction from control and heat shocked cells. It was observed that CK2 activity increased in that fraction in a time-dependent manner. This increase could be detected as early as 10 min and reached a maximum after 1 h of incubation at 43 °C. The amount of both CK2α and CK2β subunits increased similarly in this fraction throughout the heat shock (Fig. 4B). This biochemical observation was correlated with an indirect immunofluorescence analysis, using a polyclonal antibody (Ra403) raised against the full-length CK2α protein (Fig. 4C). In normal growth conditions, a prominent and uniform nuclear staining was observed, indicating that the CK2α subunit localized...
mostly inside the nucleus (control). This observation was in accordance with previous immunofluorescence studies that showed a preferential labeling of CK2 in the nucleus of growing cells (22). However, soon after heat shock (15 min), some bright fluorescent speckles started to appear, becoming clearly visible after 30 min of incubation (highlighted by arrows in Fig. 4C).

Although Ra403 is a polyclonal antibody, the observed signal was specific because preabsorption of the antibody with purified CK2 abolished the staining (data not shown).

It has been shown that cell metabolism is profoundly affected by heat shock, leading to cell arrest. Nevertheless, when a moderate heat shock is applied, cells are able to fully recover over a period of 4–6 h (23). Therefore, to test the reversibility of the heat-induced CK2 response, NIH3T3 cells were subjected to mild heat shock (1 h, 43 °C) and then were incubated back at 37 °C to allow them to recover. Fig. 5 shows the time course of CK2 activity measured in the S400 fraction during recovery. CK2 activity remained high in this fraction for about 1 h after the cells were returned to 37 °C but started to decrease regularly until reaching control level, 2 h later (Fig. 5A). In parallel, the amount of both CK2 subunits, which was elevated after heat shock, decreased gradually to normal level in 3 h (Fig. 5B).

Heat Shock-induced Relocalization of CK2 Is Not Dependent on Protein Neosynthesis—Maximal heat-induced CK2 activity occurred at a temperature that virtually halts protein synthesis (23). Moreover, no major change in the amount of CK2 present in whole cell extracts between control and heat shocked cells was observed (Fig. 6). Nevertheless, the possibility still existed that CK2 neosynthesis occurred and contributed to the accumulation of the protein kinase in the S400 fraction. Thus, we tested whether inhibiting protein synthesis would interfere with the heat shock-induced response of CK2 in the S400 fraction.
fraction. Cells were incubated for 6 h with cycloheximide before being heat shocked and sequentially extracted. Under these experimental conditions, more than 90% of the protein synthesis was inhibited as determined by [35S]methionine incorporation (data not shown). In contrast, cycloheximide had no effect on the heat-induced increase of CK2 activity (Fig. 6A), nor did it affect the increase in the amount of CK2 protein recovered in the S400 fraction after heat shock (Fig. 6B). Therefore, neosynthesis of CK2 subunits could not account for the heat shock-induced CK2 activity observed in the S400 fraction. These observations were in good agreement with the fact that, apart from the heat shock proteins that are induced and specifically transcribed after heat stress, protein synthesis is rapidly stopped in eukaryotic cells during exposure to elevated temperatures (23).

Distribution of CK2 at Ultrastructural Level—To clarify the pattern of heat-induced relocalization of CK2, electron microscopy was performed on control and heat shocked cells (1 h treatment at 43 °C). Lowicryl embedded material was used for the precise localization of individual α and β subunits of CK2. Following the use of anti-CK2α antibody, labeling was more intense over the nucleus than over the cytoplasm. In untreated 3T3 cells, gold particles were scattered over the nucleoplasm but were absent over the enclosed clusters of interchromatin granules and coiled bodies. Similarly, the condensed chromatin and the nucleolus were devoid of gold particles (Fig. 7A1). Following a heat shock treatment, gold particles remained dispersed over the nucleoplasm (Fig. 7A2). Moreover, the clusters of interchromatin granules and coiled bodies were unlabeled. In contrast, under these challenging conditions, gold particles accumulated over roughly round, moderately electron opaque structures of variable sizes, from 100 nm (Fig. 7A3) to 350 nm (Fig. 7A4). These labeled structures showed a rather fibrillar configuration and were located often in the vicinity of the perinuclear layer of condensed chromatin although a few were near the nucleolus (Fig. 7A3). Strikingly, in contradistinction with nontreated cells, about 50% nucleoli were clearly labeled, preferentially at the level of the granular component.
**Fig. 7.** Electron microscope analysis. A, distribution of CK2α subunit in untreated (A1) or heat-shocked (A2—A4) NIH 3T3 cells. A1, following the use of the Ra403 antibody, gold particles are scattered in the interchromatin space but are entirely absent over the cluster of interchromatin granules (IG). The nucleolus (NU) and the condensed chromatin (CH) are unlabeled. Cytoplasm (C) is shown. Bar, 0.5 μm. A2, upon heat shock, gold particles are numerous over the nucleolus (NU) and disperse over the interchromatin space. The cluster of interchromatin granules (IG) and the condensed host chromatin (CH) are unlabeled. Cytoplasm (C) is shown. Bar, 0.5 μm. A3, gold particles accumulate over an intranuclear, small, electron-opaque dot, 200 nm in diameter, which is located either near the nuclear border or near in the nucleolus (NU). Bar, 0.5 μm. A4, the interchromatin space contains large electron-opaque dot, 350 nm in diameter, which is intensively and homogeneously labeled. The higher magnification clearly reveals its fibrillar configuration. Bar, 0.5 μm. B, distribution of CK2β subunit in untreated (B1 and B2) or heat shocked (B3—B5) NIH3T3 cells. B1, following the use of β antibody, the intranuclear distribution of labeling is similar to that obtained in A1 with Ra403 antibody; once again, gold particles are scattered over the interchromatin space but are absent over the clusters of interchromatin granules (IG). Some gold particles underline the perinuclear layer of condensed chromatin (CH). Cytoplasm (C) is shown. Bar, 0.5 μm. B2, the nucleolus is unlabeled. A cluster of interchromatin granules (IG) is shown. Bar, 0.5 μm. B3, in the nucleus, gold particles are associated with a small, fibrillar electron-opaque dot and are absent over the nucleolus (NU). Bar, 0.5 μm. B4, higher magnification. The fibrillar configuration of this large electron-opaque dot 250 nm in diameter is clearly visible. Gold particles underlined the filaments. Bar, 0.5 μm. B5, gold particles are over the filaments of the electron-opaque dot. This labeled structure is close to the perinuclear layer of condensed chromatin (CH). Bar, 0.5 μm. Cytoplasm (C) is shown.
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In untreated 3T3 cells, the distribution of the gold particles following the use of anti-CK2β antibody was similar to that obtained with the anti-CK2α antibody. Indeed, labeling occurred over the nucleoplasm and was excluded from the nucleolus (Fig. 7, B2). Occasionally, gold particles were additionally observed at the nuclear border (Fig. 7, B1). Again, heat shock treatment resulted in the additional labeling of roundish structures of variable sizes, resembling those decorated with anti-CK2α antibody (Fig. 7, B3). Those structures, once again, consisted of clustered filaments (Fig. 7, B4) and were often located close to the perinuclear condensed chromatin (Fig. 7, B5). In similarity with the anti-CK2α-labeled structures, anti-CK2β-labeled structures were heterogeneous in size; however, they were no larger than 250 nm. In contrast, no significant labeling was observed over the nucleolus (Fig. 7, B3).

**DISCUSSION**

In this study, we have shown that after heat shock, CK2 activity increases more than 2-fold in a high salt extractable fraction with characteristics of a nuclear fraction (S400). This increase of activity could reflect either an activation of the protein kinase or a redistribution of the kinase visualized by a heat shock-induced enrichment in the amount of α and β subunits in this fraction or both.

According to Fig. 1A, about 20% of the total CK2 activity relocalize to this nuclear fraction in response to heat shock. Because CK2 appears as a multifunctional protein kinase, it is expected that even a small change in the amount of CK2 in a specific subcellular fraction would be sufficient for the cell to counteract challenging conditions such as heat shock. As shown by our indirect immunofluorescence analysis, CK2 relocalizes to highly labeled speckle-like structures in the nucleus of cells subjected to elevated temperatures, whereas the enzyme exhibits a homogeneous nuclear distribution in control cells. Higher magnification, provided by the electron microscopy analysis, revealed that upon heat shock both subunits are targeted to similar dense and roundish structures 150–300 nm in size at the vicinity of the condensed chromatin. Although, the exact function of these heat-induced structures has not yet been clearly defined, they provide an opportunity for the cell to control the activity of CK2 and to target the kinase to specific substrates where their phosphorylation is required. Interestingly, many proteins involved in the heat shock response have been described to enter the nucleus and to concentrate into speckle-like structure. Indeed, in response to elevated temperatures, the heat shock transcription factor HSF relocalizes inside the nucleus to structures that also appear as granules (24, 25). Although showing some differences, the pattern of HSF relocalization is similar to the rearrangement pattern of CK2. It is tempting to relate these similar CK2 and HSF structures, because it has been recently observed that CK2 phosphorylates HSF (3). Strikingly in squamous carcinoma SC-CHN cells, CK2 also exhibits a punctuate nuclei pattern, which has been correlated with high CK2 activity and transformed phenotype (27). Remarkably, several other proteins (including GATA-1 (28), GATA-3 (29–30), p53 (31), nucleolin (32), and splicing factors (33)) have also been described to be regulated by specific accumulation into nuclear domains. Indeed, CK2 phosphorylates and regulates several nuclear proteins involved in the control of gene expression or in DNA repair in stress conditions, as exemplified by topoisomerase II (16), DNA ligase (17), or APF/REF-1 (18).

In response to heat shock, CK2α colonizes the nucleolus as shown by the electron microscopic analysis. In fact, previous immunofluorescence analysis also demonstrated a high accumulation of CK2 in the nucleoli of mouse tumor cell compared with other cellular compartment (34). Nucleolin, a major nuclear phosphoprotein, is tightly bound to and highly phosphorylated by CK2 in growing cells. This phosphorylation is required for the transcription of rRNA genes to occur. Moreover, it has been demonstrated that the catalytic α subunit of CK2 directly interacts with nucleolin and that a high percentage of CK2 in the nucleolus may be complexed with it (35). Moreover, a regulation of rDNA transcription in mammalian cells by CK2 have already been described by Belenguer et al. (36) in adult bovine aortic endothelial cells. Altogether, these observations suggest that CK2 may play a role in RNA biosynthesis during stress conditions.

It is known that the catalytic α subunit is spontaneously active and that the presence of the regulatory β subunit inside the CK2 holoenzyme positively or negatively influences the kinase activity. Consequently, any change in the stoichiometry of the tetramer may strikingly affect the pattern of phosphorylation of CK2 substrates. Strengthening this idea, experimental data are now available supporting the notion that there are free populations of both CK2 subunits, either totally on their own or in association with other cellular protein partners (37, 38). In this scenario, it is thought that pools of CK2α, CK2β, and CK2 holoenzyme would carry out distinct functions (11, 20). Our biochemical data showed that, in response to heat shock, no clear variation of the CK2 stoichiometry could be observed (Fig. 1B and data not shown). However, we cannot exclude that upon heat shock both CK2 subunits are differentially targeted but become artifactually associated after biochemical extraction (see below). Indeed, electron microscopy analysis revealed that upon heat shock, CK2α subunit localized in the granular compartment of nucleoli, whereas the regulatory subunit was not detected in this organel. This observation raise the interesting possibility that in response to heat shock the CK2 subunits are differentially targeted inside the nucleus to meet differential partners and functions.

It is known that the regulatory subunit of CK2 is implicated in the G2/M DNA damage checkpoint arrest (7) and that this CK2 subunit confers partial UV resistance on Xeroderma pigmentosum cells (8). Therefore, these findings could suggest the possibility that cellular response to DNA damage is modulated by CK2-dependent phosphorylation. Indeed our data finally show that relocalization of CK2 is a common phenomenon for cells challenged by UV irradiation or heat shock. Both stresses have been shown to induce DNA damage. Thus, one can speculate that during DNA-damaging stresses, CK2 is recruited to specifically phosphorylate and activate those proteins of the repairation machinery. Nevertheless, oxidative stress, which is also a DNA-damaging agent (26), does not induce any CK2 response under our experimental conditions. Although not necessarily conflicting, these results show that DNA damage may not be the signal that induces relocalization of CK2 and raises the question of which upstream events are involved in this cellular process.

In summary, our work demonstrated that in response to heat shock, a fraction of CK2 relocalizes to specific regions inside the nucleus, raising the CK2 activity there. Moreover, the redistribution of the kinase was specific of the stress applied and was dose-dependent. We do not know, however, the exact mechanism underlying the regulation of this heat-induced response of CK2. However, we propose that differential and specific targeting of both subunits of the kinase inside the nucleus compartment may be of importance in the cellular response to physiological stress. Whatever its significance, one can nevertheless predict that in stress conditions, CK2 may meet a totally different subset of substrates and partners and thus notably.
affect the cellular metabolism. To our knowledge this is the first demonstration of a potential role of CK2 in the stress response in higher eukaryotic cells.

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