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Heat Stress Promotes the Degradation of p53 and p300/CBP-associated Factor in Murine Embryonic Fibroblasts

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ABSTRACT

Heat stress, like genotoxic- and oxidative-stresses, has been reported to induce nuclear accumulation of p53 and its activation. However, exposure of mouse embryonic fibroblasts to elevated temperature caused insolubilization of nuclear p53 together with its co-activator, p300/CBP-associated factor (PCAF). The insolubilized proteins were translocated to the perinuclear region by CRM1-dependent export system, which was inhibited by leptomycin B. On the other hand, other co-activators, CBP and p300 remained soluble in nucleus. The insolubilization of the two proteins also occurred in other cell lines, when the cells were heat-exposed. After the heat stress, the insolubilized p53 and PCAF in the cells were degraded by proteasome, which was inhibited by pretreatment of the cells with MG132. During the degradation, Hsp70-expression was initiated and subsequently newly synthesized p53 and PCAF reappeared as soluble forms in nucleus. These results indicate that nuclear p53 and PCAF transiendly disappear prior to the induction of Hsp70-expression in response to heat stress. Conversely, genotoxic- and oxidative-stress induced neither the loss of these two proteins nor Hsp70-expression. Since p53 functions as a proapoptotic factor and a repressor of Hsp70-expression, the transient disappearance of nuclear p53 and PCAF may partly contribute to the cell survival through the enhancement of Hsp70-expression. Ryuuku Med. J., 26, 3, 113~123, 2007

Key words: heat stress; heat shock; PCAF; p53; p300; Hsp70; CBP

INTRODUCTION

A transcription factor, p53 is activated upon stress signals such as γ-irradiation, UV, hypoxia, radical oxygen, virus infection, and DNA damage by genotoxic reagents. The activated p53 is involved in cell cycle arrest, DNA repair, or apoptosis through the transcription of various genes including Bax and p21 [1,3]. Apart from its primary function as a transcription factor, it also can promote apoptosis independent of transcription [4]. p53 required posttranslational modification such as phosphorylation and acetylation for the activation [5]. The acetylation of p53 is performed by histone acetyltransferases (HATs) such as CBP (CREB-binding protein), p300, and PCAF (p300/CBP-associated factor) [6,7], which form a transcriptional complex with p53 [8]. In fact, its acetylation by PCAF and/or p300/CBP contributes to the nuclear accumulation of p53 and its activation through an enhancement of p53 sequence-specific binding to DNA [9,11]. Recent reports have shown that the acetylation of p53 either at Lys306 by PCAF or at Lys373 by p300 enhances p53-transactivating activity, resulting in promotion of transcription from a growth arrest-related gene, p21 [12,13].

Another type of stress, heat stress, induces the activation of heat shock factor-1 (HSF-1), which upregulates Hsp70 (heat shock protein 70) -expression [14,15]. Hsp70 and other Hsps assist the
recovery of cells from heat damage by processing heat-denatured proteins, that is, re-naturing the denatured proteins or assisting the degradation of irreparably denatured proteins by the ubiquitin-proteasome system. Heat stress has been also shown to induce the nuclear accumulation of p53 in various cells. The increased p53 is activated and then transactivates p21 gene expression in human glioblastoma and skin fibroblast. These lines of evidence suggest that heat stress, like the genotoxic stress, can induce the nuclear accumulation of p53 and its activation. Intriguingly, it has been reported that p53 represses the transcription from Hsp70 promoter through its association with CAAT-box binding factor (CBF) in Chinese hamster ovary cells. In addition, Quenneville et al. have reported that p53 negatively regulates Hsp70 expression in mouse keratinocytes. These facts imply that the nuclear accumulation of p53 is rather inconvenient for the expression of Hsp70 in response to heat stress. How do cells regulate the Hsp70-expression and nuclear p53-accumulation in response to heat stress? Unfortunately, the mechanism by which cells reconcile the Hsp70-expression with the nuclear p53-accumulation has not been clarified yet.

In this study, we examined in detail whether or not heat stress induces Hsp70-expression in parallel with the nuclear accumulation of p53. Consequently, we found that nuclear p53 together with PCAF become insoluble and are exported in the perinuclear region during heat stress, followed by their degradation by the proteasome after the stress. Moreover, we showed that their degradation is accompanied by the expression of Hsp70 and the subsequent reappearance of newly synthesized p53 and PCAF in the nucleus. We will discuss the biological significance of our findings and the relationship with other investigators.

**MATERIALS AND METHODS**

**Materials** Mouse monoclonal anti-Hsp70 (Stressgen, C92F3A-5), anti-Hsp90 (Transduction Laboratories, H38220), anti-p53 (SantaCruz, DO-1), anti-p53 (Stressgen, KAM-CC237), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Ambion, C6C5), anti-β-actin (Sigma, AC-74), and anti-PCAF (SantaCruz, E-8) antibodies were obtained commercially. Rabbit polyclonal anti-p300 (N-15) and anti-CBP (C-20) antibodies were purchased from SantaCruz. Goat polyclonal anti-poly (ADP-ribose) polymerase-1 (PARP-1) antibody was obtained from R & D systems. Horseradish peroxidase-conjugated rabbit anti-mouse IgG, goat anti-rabbit IgG, and rabbit anti-goat IgG antibodies were from DakoCytomation. Alexa Fluor 546 goat anti-mouse IgG and Alexa Fluor 488 goat anti-rabbit IgG antibodies were from Molecular Probes.

Leptomycin B was from LKT Laboratories, Inc. (MN, USA). Camptothecin, TLCK (p-toluenesulfonyl-L-lysine chloromethylketone, ALLM (N-acetyl-L-leucyl-L-leucyl-L-methioninal), and a cocktail of protease inhibitors were obtained from Nacalai Tesque Inc. (Japan). MG-132, leupeptin, zVADfmk, pepstatin A, and E-64 were purchased from Peptide Institute, Inc. (Japan).

**Cell Lines** Three wild-type p53-expressing cell lines, mouse embryonic fibroblast (MEF), human neuroblastoma SH-SY5Y cells, and human cervical carcinoma HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS) and 50 μg/ml of kanamycin at 37°C in a humidified CO2-incubator.

**Heat exposure** MEF and HeLa cells (1 x 10^6 cells/5 ml) and SH-SY5Y cells (2 x 10^6 cells/5 ml) were seeded in DMEM + 10% FBS on 60 mm-dishes and cultured for 24 h, respectively. At 24 h after the culture, the culture dish was immersed in a waterbath that was put in a humidified CO2-incubator set at the indicated temperature. After termination of the heat-exposure, the cells were harvested or subsequently cultured at 37°C for the indicated period.

**Cell Lysis** The harvested cells were washed once in phosphate-buffered saline (PBS) and lysed in 150 μl of buffer D (10 mM Hepes-NaOH, pH 7.6, 0.42 M NaCl, 0.5 mM EDTA, 0.5 mM EGTA, 0.5 mM DTT, 0.1% NP-40, and 1/100 vol. of the protease inhibitors cocktail), and the lysates were fractionated into buffer D-soluble and -insoluble fractions by centrifugation at 1,600 x g for 20 min. The insoluble fractions were dissolved in 100 μl of a SDS sample buffer containing 2% SDS (sodium dodecylsulfate), 62.5 mM Tris-HCl, pH 6.8, 20% glycerol, and 1/100 vol. of the protease inhibitor cocktail and sonicated using a sonicator.

The protein concentration of buffer D-soluble and -insoluble fractions was determined using a DC Protein Assay Kit (Bio-Rad Lab.) according to the manufacturer's instructions.
Isolation of nuclei from MEF cells and exposure of nuclei to heat stress

The harvested MEF cells (5.5 × 10⁶ cells) were washed once in PBS and fractionated into nuclear and cytoplasmic fractions according to a previously described method with slight modification. Briefly, the cells were lysed in 150 μl of buffer A (10 mM Hepes-NaOH, pH 7.6, 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT, 0.5 mM PMSF, and 1/100 vol. of the protease inhibitors cocktail) containing 0.1% NP-40. After standing on ice for 5 min, the cell suspensions were mixed with an iso-volume of buffer A containing 0.6 M sucrose and were fractionated into precipitates (nuclei) and the supernatant by centrifugation at 600 x g for 7 min. The nuclei were resuspended in 150 μl of buffer A containing 0.3 M sucrose. Aliquots (25 μl) of the nuclear suspensions were incubated at 0°C or 44°C for 10 min and then centrifuged at 600 x g for 7 min. The nuclear precipitates were resuspended in 50 μl of buffer D and fractionated into nuclear-soluble and-insoluble fractions by recentrifugation at 16,000 x g for 20 min. The nuclear insoluble fraction was dissolved in the SDS sample buffer as described in "Cell lysis". The protein concentrations of the two fractions were determined according to a method described above.

Western blotting

After the determination of protein concentrations, specimens prepared from cells were mixed with β-mercaptoethanol (final concentration of 5%) and boiled for 5 min. Samples (about 25 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously.

After the electrophoresis, proteins in the gel were transferred onto a polyvinylidene difluoride (PVDF) membrane, and the membrane was subjected to Western blotting as detailed previously.

RESULTS

Heat stress causes insolubilization of PCAF and p53

MEF cells were exposed to heat stress at 44°C for the indicated time (Fig.1-A). After the exposure, the cells were lysed in buffer D, and then the cell lysates were fractionated into buffer D-soluble and -insoluble fractions. In unstimulated cells, almost all of p53 and PCAF were fractionated into the soluble fraction, whereas in the cells exposed at 44°C for 60 min the proteins were detected only in the insoluble fraction (Fig.1-A). Increasing the concentration of NaCl up to 2 M in the lysis buffer failed to improve the solubility of PCAF and p53 of the heat-stressed cells (Fig.1-B). Similarly, increasing the concentration of NP-40 up to 1% was ineffective (data not shown), suggesting that the PCAF and p53 in the heat-exposed cells are probably denatured and thereby insoluble.

The evidence that almost all of the p53 and PCAF in the buffer D-soluble fraction disappeared within 15 min under heat stress indicates that the insolubilization occurred rapidly (Fig.1-A). The insolubilization of both proteins was also dependent on the temperature, since a lower temperature (40 or 42°C) was partly effective. In contrast, other
**Fig. 1** Insolubilization of PCAF and p53 by heat stress

(A) MEF cells (1 x 10^6) were cultured on 60 mm-dishes for 24 h at 37°C, and then exposed to heat stress under the indicated conditions and lysed in buffer D. After fractionation of the lysates, buffer D-soluble and -insoluble fractions were subjected to immuno-blot analyses to detect the indicated proteins as described in MATERIALS AND METHODS.

(B) MEF cells were treated with or without heat stress at 44°C for 1 h and lysed in buffer D or a modified buffer D containing an elevated concentration of NaCl. After fractionation of the lysates, the respective buffer-soluble and -insoluble fractions were subjected to immuno-blotting for PCAF and p53.

(C) SH-SY5Y and HeLa cells were seeded at the density indicated in MATERIALS AND METHODS on 60 mm-dishes and cultured for 24 h at 37°C. The cells were exposed to heat stress at 44°C for the period indicated and then lysed and fractionated into buffer D-soluble and -insoluble fractions as described in (A). Immuno-blot analyses were carried out as described in (A).

(D) Isolated nuclei of MEF cells were incubated at 0°C (-H.S.) or 44°C (+H.S.) for 10 min and then fractionated into buffer D-soluble and -insoluble fractions as described under MATERIALS AND METHODS. After SDS-PAGE, immuno-blot analyses were carried out for PCAF and p53.

HATs such as CBP/p300 and a nuclear enzyme, PARP-1 remained buffer D-soluble. In addition, the partition ratio of β-actin between the soluble and insoluble fractions remained almost constant before and after the heat stress (Fig. 1-A). These results suggest that p53 and PCAF are more heat-labile than other proteins.

The heat-induced denaturation of PCAF and p53 was not a cell type-specific event, since in two other cell lines (SH-SY5Y and HeLa) exposed to heat-stress, the two proteins were also converted to insoluble forms (Fig. 1-C).

When the nuclei isolated from MEF cells (Fig. 1-D) and SH-SY5Y cells (data not shown) were incubated at 44°C for 10 min, almost all of the PCAF and p53 became buffer D-insoluble, suggesting that the insolubilization occurs independent of the extranuclear factors.

**Insolubilized p53 and PCAF are translocated to the perinuclear region by CRM1** To clarify whether the insolubilized p53 and PCAF are redistributed in the cells or not, immunofluorescent studies were carried out. In unstressed MEF cells, p53 and PCAF, which brighten as red-fluorescent color, were mainly distributed in the nucleus, since the deeply red-stained area was consistent with the location of the DAPI-stained nucleus (upper panels in Figs. 2-A and B). On the other hand, in the cells exposed to heat stress for 1 h, almost all of nuclear PCAF and p53 disappeared, instead being detected in a region closely adjacent to the nuclear membrane, forming a doughnut-ring around the nucleus (lower panels in Figs. 2-A and B). Although PCAF still remained in nucleus at 20 min of the heat...
stress, p53 was already translocated to an extranuclear region (middle panels in Figs. 2-A and B). In contrast to the effect on PCAF and p53, heat stress did not affect the distribution of CBP or p300 (Figs. 2-C and D). These results indicate that heat stress affects not only the solubility of PCAF and p53 but also their distribution in cells.

In order to examine the mechanism by which the insolubilized PCAF and p53 are translocated to the cytoplasm, leptomycin B (LMB), which is a potent inhibitor of a nuclear export factor, CRM1 (chromosomal region maintenance 1)\(^{20}\), was used. As shown in Fig. 3-A, the distribution of PCAF was almost completely consistent with that of DAPI in unstressed MEF cells. On the other hand, PCAF in the heat-stressed cells was mainly distributed outside of the nuclear membrane, that is, the perinuclear region. The pretreatment of the cells with LMB, however, completely blocked the translocation of PCAF induced by heat-exposure (Fig. 3-A). When combined with a result showing the insolubilization of PCAF by heat stress (Fig. 1), this result suggests that the insolubilized PCAF is translocated to the perinuclear region by a CRM1-dependent nuclear export system. LMB also suppressed the translocation of the insolubilized p53 (data not shown). Although LMB blocked the nuclear export of insolubilized PCAF (Fig. 3-A), it did not affect its insolubilization (Fig. 3-B), suggesting that the insolubilization occurs in the nucleus in response to heat stress.

Neither genotoxic- nor oxidative-stress affects solubility of p53 and PCAF- Genotoxic stress and oxidative stress are well known to cause accumulation of p53 and its activation\(^{1,2}\). In order to compare with the effect of heat stress, we examined the

![Fig. 2 Immunofluorescence microscopic analysis of MEF cells exposed to heat stress](image)

![Fig. 3 Inhibition of heat-induced nuclear export of PCAF and p53 by leptomycin B](image)
Heat stress-dependent disappearance of p53 and PCAF

(A) antibody

anti-PCAF  anti-p53

none  CPT  H₂O₂

(B) Stress : none  CPT  H₂O₂  H.S.

Time (h) : 1  2  1  2  1

fraction : S. I. S. I. S. I. S. I. S. I. S. I.

PCAF  p53

Fig. 4 Effect of camptothecin and hydrogen peroxide on the location of PCAF and p53

(A) MEF cells cultured on coverslips were treated with or without 1.5 μM camptothecin (CPT) or 0.3 mM hydrogen peroxide for 4 h at 37°C. After fixation, the cells were immuno-stained with anti-PCAF antibody or anti-p53 antibody (KAM-CC237) as described in MATERIALS AND METHODS.

(B) MEF cells cultured on 60mm-dishes were treated with or without 1 μM CPT or 1 mM hydrogen peroxide for the period indicated. As a positive control, MEF cells were exposed to heat stress at 44°C for 60 min. After the treatments, the cells were fractionated into buffer D-soluble and -insoluble fractions. These fractions were subjected to immuno-blot analyses as described in Fig.1.

The fate of the insolubilized p53 and PCAF, heat-exposed MEF cells were cultured at 37°C for a given period to recover from heat damage (Fig. 5-A). Immediately after heat stress (recovery at 0 h), almost all of the PCAF and p53 proteins in MEF cells were distributed in buffer D-insoluble fraction. At 2 h after the heat stress, however, both of the proteins in the fraction were significantly reduced and completely disappeared at 6 h. In contrast, the contents of p53 and PCAF in the buffer D-soluble fraction were restored at that time (Fig. 5-A).

It is well known that the expression of Hsp70 is upregulated in response to heat stress and that the protein functions as a molecular chaperone, which refolds and/or unfolds heat-denatured proteins. Under our experimental condition, no Hsp70 was detected in unstressed MEF cells (Fig. 5-A). However, a trace amount of Hsp70 was detected...
at 2 h after the heat stress, and the expression level of Hsp70 attained a plateau at 6 h (Fig. 5-A), which was retained for further 12 h (data not shown). In contrast, treatment of MEF cells with CPT or H₂O₂, which did not cause the insolubilization of p53 (Fig. 4), failed to induce the expression of Hsp70 (Fig. 5-B).

In parallel with the restoration of PCAF and p53 in the buffer D-soluble fraction at 6 h after the heat stress (Fig. 5-A), PCAF and p53 returned to the nucleus in MEF cells (Fig. 6).

Taken together, these results suggest that heat stress causes a transient dysfunction of p53 and PCAF.

PCAF and p53 in the insoluble fraction are degraded by proteasomes—In the above section, we showed that the insolubilized PCAF and p53 disappeared at 6 h after heat stress (Fig. 5-A). To elucidate the mechanism by which the insolubilized proteins disappear, we examined whether protease-inhibitors can block the disappearance of the proteins. As shown in Fig. 7-A, the disappearance was blocked by pretreatment of MEF cells with a potent proteasome-inhibitor, MG-132[30], and to a lesser extent by its weak inhibitor, ALLM. TLCK, a trypsin-like serine protease-inhibitor, was effective on the protection of PCAF, but weakly effective on that of p53 (Fig. 7-A). Other inhibitors such as zVADfmk for caspases, leupeptin for serine/cysteine proteases, pepstatin for aspartic proteases, and E64 for cysteine proteases were ineffective. Taken together, these results suggest that PCAF and p53 in the insoluble fraction are ultimately degraded by the proteasome during the recovery of the cells from heat damage (Fig. 7-A). Since p53 and PCAF are degraded by MDM2-mediated ubiquitin-proteasome system under physiological condition[19,30], the insolubilized p53 and PCAF in MEF cells may be ubiquitinated by MDM2 and then degraded by proteasome.

PCAF and p53 restored in the soluble fraction are newly synthesized proteins—In Figs. 5 and 6, we showed that PCAF and p53 reappear as soluble forms in the nucleus during the recovery of MEF cells after heat stress. Since the insolubilized PCAF and p53 were ultimately degraded by proteasome (Fig. 7-A), it is naturally predicted that the
proteins in the soluble fraction are newly synthesized proteins. In order to test this prediction, heat-exposed MEF cells were treated with cycloheximide (CHX) to inhibit protein synthesis (Fig. 7-B). Expectedly, in the heat-exposed cells, CHX efficiently inhibited the restoration of PCAF and p53 in the soluble fraction without affecting the disappearance of the proteins in the insoluble fraction. Thus, the PCAF and p53 in the soluble fraction are newly synthesized products rather than the products renatured from the insolubilized proteins (Fig. 7-B). The amount of p53 in the cells treated with CHX alone was less than that of untreated cells (compare lane 9 with lane 1 in Fig. 7-B). The lesser amount of p53 may be explained by the fact that p53 is a short-lived protein.

**DISCUSSION**

In this study, we have demonstrated that the nuclear p53 and PCAF proteins in MEF cells become insoluble in response to heat stress and then are exported to the perinuclear region. During the recovery of the cells from the heat stress, the insolubilized proteins are degraded by proteasome, and newly synthesized p53 and PCAF re-appeared in nucleus. Conclusively, we have revealed that heat stress induces a transient dysfunction of p53 and PCAF through their denaturation and the subsequent degradation.

The heat stress-induced insolubilization of p53 and PCAF in MEF cells was not a cell-type specific event, since it also observed in other two cell lines (Fig. 1-C). The insolubilization of p53 and PCAF is probably due to denaturation of the proteins, since higher concentration of neither NaCl (Fig. 1-B) nor NP-40 in the lysis buffer (data not shown) did improve the solubility of them. When the isolated nuclei of MEF cells (Fig. 1-D) and SH-SY5Y cells (data not shown) were exposed to heat stress, p53 and PCAF became insoluble. Thus, it is likely that p53 and PCAF proteins are heat-denatured and consequently become insoluble in the nucleus. When the heat stressed-MEF cells were subjected to immunofluorescent microscopic analyses, the insolubilized p53 and PCAF were detected mainly in the perinuclear region (Figs. 2-A, -B, and 3-A). However, when the cells were pretreated with LMB that is an inhibitor of CRM1, the insoluble forms of the proteins remained in nucleus (Fig. 3). This result supports the above result that the insolubilization of the proteins occurs in the nucleus. Graeber et al. have reported that p53 is accumulated in not the nuclear but cytoplasmic fraction during exposure of colorectal carcinoma cells to heat stress and that the p53 lost transcriptional activity. Moreover, although the cause of the disappearance was not shown, Guan et al. have reported that in osteosarcoma cells, p53 protein disappeared from the nuclear fraction during heat stress. These findings may support our results that the insolubilized p53 is excluded from the nucleus during heat stress.

The insolubilized p53 and PCAF almost completely disappeared within 6 h after heat stress (Figs. 5-A). This disappearance is due to proteasomal degradation, since it was blocked by MG-132 (Fig. 7-A). The degradation of the insolubilized p53 and PCAF was followed by the restoration of soluble forms of the proteins (Figs. 5-A). This restoration was inhibited by CHX, indicating that it results from the de novo synthesis rather than the renaturation of the insolubilized p53 and PCAF (Fig. 7-B). A recent report has shown that Hsp70 assist the renaturation of heat-denatured proteins, competing with degradation of the proteins by ubiquitin-proteasome pathway. Under our experimental conditions, however, it is unlikely that Hsp70 renatures the insolubilized p53 and PCAF to the soluble forms, since Hsp70-expression was barely detectable 2 h after the heat stress, at that time the degradation of the insolubilized PCAF and p53 had fairly progressed (Fig. 5-A). Thus, we concluded that the reappearance of p53 and PCAF in the soluble fraction depends on new protein synthesis. Two previous studies showed that heat stress, like genotoxic stresses, induces the nuclear accumulation of p53 and its activation during recovery of human glioblastoma and skin fibroblast cells from heat damage. Those studies, however, did not examine the occurrence of the insolubilized p53 in the cells immediately after heat stress. Since whole cell lysates, which were prepared by using SDS-containing buffer, were used for the immunodetection of p53 in the heat-stressed cells, the investigators might overlook the emergence of the insolubilized p53. Presumably, the p53 proteins, which were accumulated in the nucleus of the glioblastoma and skin fibroblast cells during the recovery time, are newly synthesized proteins, as observed in the recovered MEF cells (Figs. 5-A and
In our results, unlike heat stress, neither treatment with CPT nor H₂O₂ affects the solubility of p53 and PCAF and their intracellular distributions (Fig. 4), suggesting that the process which p53 is accumulated in nucleus in response to heat stress is different from that in response to genotoxic and oxidative-stresses.

The biological significance of the heat stress-induced transient disappearance of p53 and PCAF remains to be elucidated. An attractive explanation for the significance is that the disappearance may be related to the expression of Hsp70, which plays as a molecular chaperone and as a suppressor of caspase-activation for cell survival. Wild-type p53 not only promotes cell death but also represses the transcription from the Hsp70 promoter by directly interacting with CBF (CAAT-box binding factor). In MEF cells, the expression of Hsp70 began at 2 h after the heat stress at which the soluble form of p53 had already become insoluble (Fig. 5-A). This result implies that the loss of p53 enhances the Hsp70-expression. In fact, two groups suggest independently that the deletion of wild-type p53 enhances Hsp70-expression. Wild-type p53, like mutant p53, associates with Hsp90 and be stabilized by the association. Geldanamycin is known to inhibit the association of Hsp90 with p53, resulting in the reduction of p53 by proteolysis. When MEF cells were treated with its analog, 17-AAG (17-allylamino-geldanamycin), Hsp70-expression and disappearance of p53 were observed (data not shown). Thus, it is plausible that the loss of p53 is involved in the initiation of Hsp70-expression. On the other hand, genotoxic stress and oxidative stress, which did not affect the solubility of p53 and its distribution (Fig. 4), failed to induce the Hsp70-expression (Fig. 5-B).

Like p53, PCAF became insoluble by heat stress (Fig. 1-A) and transiently disappeared from the nucleus (Fig. 6). Since PCAF is not only a co-activator of p53 but also a target for p53-regulated transcription, PCAF and p53 seem to be governed by a mutual fate, implying the existence of a positive autoregulatory feedback loop for p53-function. Thus, the heat stress-induced loss of PCAF may also contribute to an efficient downregulation of p53-function.

REFERENCES


36 Graeber, T.G., Peterson, J.F., Tsai, M., Monica,


