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Heat-shock inactivation of the TFIIH-associated kinase and change in the phosphorylation sites on the C-terminal domain of RNA polymerase II

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ABSTRACT

The C-terminal domain (CTD) of the RNA polymerase II largest subunit (RPB1) plays a central role in transcription. The CTD is unphosphorylated when the polymerase assembles into a preinitiation complex of transcription and becomes heavily phosphorylated during promoter clearance and entry into elongation of transcription. A kinase associated to the general transcription factor TFIIH, in the preinitiation complex, phosphorylates the CTD. The TFIIH-associated CTD kinase activity was found to decrease in extracts from heat-shocked HeLa cells compared to unstressed cells. This loss of activity correlated with a decreased solubility of the TFIIH factor. The TFIIH-kinase impairment during heat-shock was accompanied by the disappearance of a particular phosphoepitope (CC-3) on the RPB1 subunit. The CC-3 epitope was localized on the C-terminal end of the CTD and generated *in vitro* when the RPB1 subunit was phosphorylated by the TFIIH-associated kinase but not by another CTD kinase such as MAP kinase. In apparent discrepancy, the overall RPB1 subunit phosphorylation increased during heat-shock. The decreased activity *in vivo* of the TFIIH kinase might be compensated by a stress-activated CTD kinase such as MAP kinase. These results also suggest that heat-shock gene transcription may have a weak requirement for TFIIH kinase activity.

INTRODUCTION

RNA polymerase II (RNAPII) is a multisubunit enzyme (1,2). The C-terminal domain (CTD) of the largest subunit (RPB1) consists of multiple repeats of a consensus sequence and undergoes a cycle of phosphorylation/dephosphorylation during each transcription round (3). The RNAPII core enzyme, with an unphosphorylated RPB1 subunit, associates with general transcription factors and mediator proteins to form a holoenzyme (4,5). RNAPII and the general transcription factors assemble *in vitro* to form a preinitiation complex of transcription on the class II promoters. One of the general transcription factors, the TFIIH factor, phosphorylates the RPB1 subunit within the preinitiation complex (6,7). The unphosphorylated CTD interacts with the TATA box binding protein (TBP) (8,9) and may link together components of the holoenzyme (4,10). The CTD is phosphorylated during entry into elongation of transcription (3). Phosphorylation of the CTD suppresses its binding to TBP (8,9) and may contribute to disrupt the preinitiation complex thereby allowing promoter clearance.

In unstressed cells, the RPB1 subunit is found in equivalent amounts as an unphosphorylated form, IIa, and as a form phosphorylated on the CTD, IIo (11). Infection by viruses (12,13), serum stimulation of quiescent cells (14) and heat-shock (15,16) markedly alter the IIa/IIo distribution. The steady state distribution between the IIa and IIo forms of RPB1 results from the antagonist activity of CTD kinases and CTD phosphatases. A CTD phosphatase has recently been purified and characterized, but little data concern the CTD dephosphorylation step (17). In contrast, many protein kinases have been shown to phosphorylate the CTD *in vitro* (6,14,18–24), but few have gained evidence of significant CTD kinase activity *in vivo*. An attractive candidate is the cyclin dependent kinase, cdk7 (or MO15). Cdk7 associated to cyclin H is part of the vertebrate TFIIH factor and phosphorylates the RPB1 subunit *in vitro* (25–27). The yeast kin28p, a cdk7 homologue and ccl1p, a yeast cyclin H homologue are components of the yeast TFIIH (28) and functional kin28p and ccl1p proteins are essential to phosphorylate the yeast RPB1 subunit *in vivo* under exponential growth conditions (29,30). In mammalian cells, the CTD is dephosphorylated within minutes upon addition of TFIIH kinase inhibitors such as the nucleoside analogue 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) and isoquinoline sulfonamides (11,31,32). DRB-resistant and isoquinoline sulfonamide-resistant kinases have been shown to contribute to RPB1 phosphorylation in serum stimulated quiescent cells and in heat-shocked cells (14–16). In both cases, the DRB-resistant CTD kinases have been tentatively identified

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as mitogen activated protein (MAP) kinases. Studies on heat-shocked cells also suggested that the major CTD kinase operative in unstressed cells was inactivated by stress (16). Inactivation of the TFIIH kinase was hypothesized. Therefore, we investigated the TFIIH-associated CTD kinase activity both *in vitro*, in extracts from heat-shocked cells and *in vivo*, using an antibody which discriminates between the RPB1 subunit phosphorylated either by the TFIIH factor or by another CTD kinase such as a MAP kinase.

MATERIALS AND METHODS

Cells

Monolayers of human HeLa cells were cultured on tissue culture dishes or tubes in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal calf serum. Heat-shocks were performed by immersing the sealed dishes or tubes in a water bath adjusted to 45 ± 0.1°C.

Antibodies

The monoclonal antibody POL 3/3 recognizes the RNAPII largest subunit at an evolutionary conserved epitope located outside the CTD (33). The 8WG16 monoclonal antibody was directed against the CTD (34). The CC-3 monoclonal antibody was raised against a nuclear matrix phosphoprotein, p255 (35). The monoclonal antibodies raised against the various TFIIH subunits (cyclin H, cdk7, p62 and XPD) were previously described (25,36).

Whole cell lysates

After heat-shock, cells grown for 24 h were washed in chilled phosphate-buffered saline, lysed in Laemmli buffer (60 mM Tris–HCl, pH 6.8, 10% glycerol, 2% sodium dodecyl sulfate, 1% 2-mercaptoethanol and 0.002% bromophenol blue) and the lysates were heated for 8 min at 95°C.

Non denaturing cell lysis and sequential extraction of TFIIH components

HeLa cells (10^7 cells) kept at 37°C or submitted to heat-shock, were washed twice with phosphate-buffered saline and lysed in 0.2 ml of the low salt buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1% Nonidet P-40, pH 7.9) for 10 min on ice. After scraping off, the suspension was centrifuged for 5 min at 15 000 × *g* at 4°C. The resulting supernatants (S1) were frozen and the pellets (P1) were subsequently extracted in 0.2 ml of the high salt buffer B (20 mM HEPES, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, pH 7.9) by a vigorous vortexing on ice. The suspensions were centrifuged as before, and the new supernatants (S2) were frozen. For a further extraction, the high salt pellets (P2) were resuspended in 0.2 ml buffer B and reextracted at 4°C to give a supernatant S3 and a pellet P3. For Western blot, aliquots of S1, S2 or S3 were supplemented with an equal volume of 2× Laemmli buffer and 1× Laemmli buffer was added directly to the pellets.

Immunoprecipitation and CTD kinase assay

Monoclonal antibodies against various TFIIH subunits were cross-linked to protein A-Sepharose as previously described (36). Ten microliters of antibody-coated beads were added to 0.2 ml of the appropriate extracts (S1, S2 or S3). After 90 min of shaking at 4°C, the beads were washed three times in buffer C [100 mM NaCl, 20 mM Tris–HCl (pH 8), 1 mM DTT, 0.1 mM EDTA] and once in buffer D (20 mM glycerophosphate pH 7.3, 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1 mM Na_3VO_4, 10% glycerol). The beads were resuspended in 15 µl of buffer D supplemented with non-radioactive ATP at a final concentration of 0.1 mM, 0.5 mCi of [γ-^{32}P]ATP (Amersham Corp.) and 1 µg of the ctd4 oligopeptide which mimics the RPB1 C-terminal domain; this peptide consists of four of the consensus repeats (SPTSPSY) found in the CTD (37) and was synthesized by Dr O. Siffert at the Institut Pasteur, Paris. The beads were incubated for 30 min at 30°C and the reaction was arrested by the addition of 15 µl 2× Laemmli buffer. After electrophoresis on a 15% SDS-polyacrylamide gel, the gel was dried, autoradiographed and quantified with a PhosphorImager (Molecular Dynamics).

In vitro phosphorylation of the CTD from RPB1

Purified RNAPII (0.25 µl) was incubated at 25°C with purified transcription factors TFIIB, TFIIE-α, TFIIE-β, TFIIF, TFIIH, the four nucleotide triphosphates in buffer C, with or without TBP (6,25). Purified RNAPII (0.25 µl) was incubated at 30°C in the presence of purified active sea star p44mpk (Upstate Biotechnology Inc.) (1.25 µl i.e. 6.75 ng) in buffer D containing 5 mM ATP. Purified starfish cyclin B-cdc2 kinase (kindly provided by Dr M.Dorée) (38) was incubated in the presence of ATP (5 mM) at 30°C with the GST-CTD fusion proteins (0.1 µg) produced in *Escherichia coli* and purified on glutathione-Sepharose (Pharmacia). Reactions were arrested with addition of 2× Laemmli buffer and analyzed by Western blot using POL3/3, 8WG16 or CC-3 monoclonal antibodies.

GST-CTD fusion proteins

The cDNA sequence encoding the human RNA polymerase II subunit hRPB1 (Accession number X63564) (39) was mutagenized so as to introduce a unique *Nhe*I restriction site in front of the CTD (position 5157), and a *Xba*I restriction site in place of the stop codon (position 6297). A *Nhe*I–*Spe*I restriction fragment, that encodes the 26 N-terminal heptarepeats of the CTD, and a *Spe*I–*Xba*I restriction fragment, that encodes the 26 C-terminal heptarepeats as well as the extra C-terminal acidic peptide, were inserted into the unique *Nhe*I site of a PGEX-3X derivative (Pharmacia). This derivative was modified by insertion of a sequence containing six His encoding codons followed with a stop codon, added in frame 3′ of the *Nhe*I cloning site. The resulting plasmids allowed the overproduction of two chimeric proteins containing a GST peptide at their N-terminal ends and six His at their C-terminal ends.

The GST-CTD-(N-terminal) fusion protein contained a 184 aa CTD derived peptide (427 aa: calculated MW 47 470). The GST-CTD-(C-terminal) fusion contained a 198 aa CTD derived peptide (443 aa: calculated MW 49 560). The overproduced fusion proteins were retained on glutathione-Sepharose beads and next eluted from the beads with reduced glutathione yielding highly purified GST fusion proteins.

Western blots

Cell lysates, extracts generated by cell fractionation or *in vitro* reactions were analyzed by Western blot visualized using either anti-mouse IgG horseradish peroxidase conjugates (Promega) and chemiluminescence or anti-mouse IgG, ^{125}I-labelled antibodies (Amersham Corp.); the radioactivity bound to the antigens was quantified with a PhosphorImager (Molecular Dynamics).

RESULTS

Decreased TFIIH-associated CTD kinase activity in extracts from heat-shocked HeLa cells

The general transcription factor TFIIH is composed of nine subunits (40–43). Three of these subunits, cdk7, cyclin H and MAT1 form a complex, the cyclin-dependent kinase-activating kinase (CAK). The CAK complex is found in cell lysates as a 'free form' or bound to the core TFIIH composed of the other subunits (40).

To investigate changes in the TFIIH-associated kinase activity, it was necessary to ensure a complete solubilization of the TFIIH factor. This was achieved after three sequential extractions in buffers containing increasing salt concentrations as described in the Materials and Methods. Most of the CAK components were extracted in the low salt buffer (S1 extract); however, solubilisation of the TFIIH core components p62 and XPD required sequential high salt extractions (S2 and S3 extracts) of the insoluble material left over after the low salt extraction (see following paragraph).

Thus, the CAK complex was immunoprecipitated in extracts prepared from HeLa cells heat-shocked or not. Using anti-cdk7 coated beads, the highest ctd-4 kinase activities were immunoprecipitated from the low salt extracts (S1) (Fig. 1, top). In extracts from cells heat-shocked for 30 or 60 min at 45°C, the cdk7-immunoprecipitated ctd-4 kinase activity was significantly reduced (to 60 and 50% of control in S1 extracts, respectively). The anti-cdk7 antibodies retain both the free kinase complex (CAK) and the TFIIH-associated kinase (36). Therefore, to investigate the latter kinase, we used anti-p62 and anti-XPD antibodies which immunoprecipitated exclusively the TFIIH-associated cdk7 kinase (36). In this case, the highest ctd-4 kinase activities were immunoprecipitated from the high salt (S2 and S3) extracts of control cells (Fig. 1, middle and bottom). Both these immunoprecipitated kinase activities were greatly decreased in extracts from heat-shocked cells. These results show that the CTD kinase activity associated with the TFIIH factor is markedly decreased in extracts from heat-shocked cells compared to unstressed cells.

Insolubilisation of the TFIIH factor during heat-shock

Heat-shock is known to impair numerous enzymes either through a post-translational modification decreasing their specific activity or through aggregation and loss of solubility (44). To discriminate between these two possibilities, we investigated the presence of the TFIIH subunits in the extracts from control (C) or heat-shocked (HS) cells. The immunoblots were probed with antibodies directed against two subunits belonging to the core TFIIH (XPD and p62) and two subunits belonging to the CAK complex (cdk7 and cyclin H) (36).

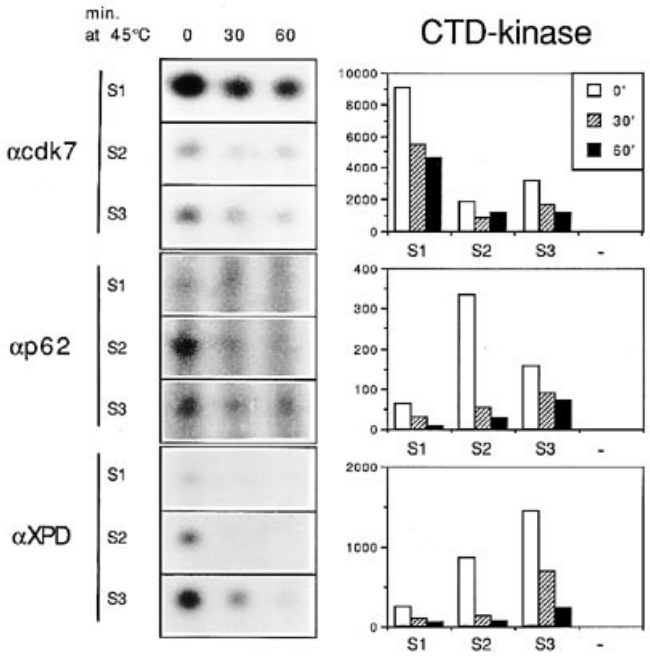

Figure 1. CTD kinase activity of cdk7 complex from control or heat-shocked HeLa cells. HeLa cells were kept at 37°C (0) or heat-shocked at 45°C for 30 min (30) or 60 min (60). Immunoprecipitations were performed using protein A-Sepharose beads coated with the anti-cdk7, anti-p62 or anti-XPD antibodies with extracts S1, S2 and S3, corresponding to 5×10^6 cells. Coated beads were then assayed for ctd4-kinase activity using the ctd4-peptide containing four repeats of the heptapeptide. Incubation mixtures were subjected to SDS-polyacrylamide gel electrophoresis, then analysed by autoradiography. The radioactivity incorporated into the ctd4-peptide was quantified with a PhosphorImager, and expressed in arbitrary units (right panels). The immunoprecipitations were repeated four times with different preparations and were very reproducible.

The cdk7 and cyclin H proteins remained mostly in the low salt extract (S1), however a significant amount (20%) appeared in the final pellet (P3) from heat-shocked cells (Fig. 2). In contrast, the solubility of XPD and p62 was greatly affected by heat-shock. Indeed, a high proportion (80%) of p62 and XPD proteins could no longer be extracted from heat-shocked cells after two high salt extractions. Including RNase A and DNase I in the high salt buffer did not improve the solubilisation of the TFIIH core subunits (data not shown).

Taken together, our data show that heat-shock leads to an important decrease in the solubility of the TFIIH factor and consistently, to a decreased activity of the corresponding CTD kinase in the cell extracts.

Decreased amounts of a phosphorylated RPB1 subunit subspecies during heat-shock

The TFIIH-associated CTD kinase phosphorylates the RPB1 subunit *in vitro* and *in vivo* (reviewed in ref. 45). Therefore, the phosphorylation state of RPB1 was investigated by Western blot in heat-shocked cells. Probing the immunoblots with a monoclonal antibody, POL3/3, directed against the core domain of RPB1, we found that phosphorylated RPB1 subunits (IIo forms) accumulated gradually in HeLa cells submitted to heat-shock while the amount of the unphosphorylated RPB1 subunit (IIa form) decreased (Fig. 3, left), as described previously (15). After 60 min of heat-shock, most of the RPB1 subunit was in a

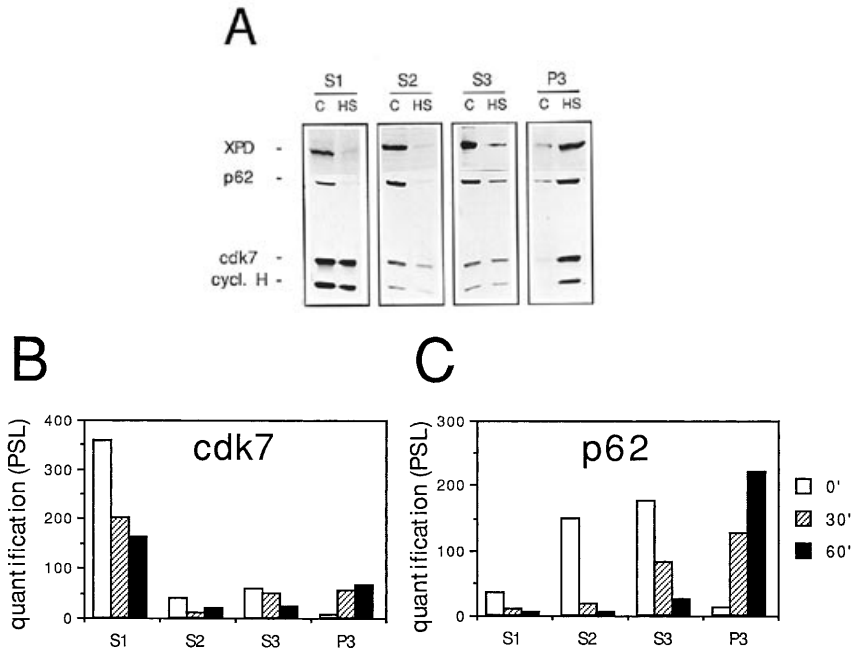

Figure 2. Presence of TFIIH subunits in extracts from control or heat-shocked HeLa cells. Proteins from HeLa cells, kept at 37°C (C) or submitted to a 45°C heat-shock during 1 h (HS), were sequentially extracted with the low salt (S1), high salt (S2) and a second high salt (S3) buffer giving a residual pellet (P3). The extracts were analyzed by Western blot after electrophoresis on a 10% SDS-polyacrylamide gel and detected with monoclonal antibodies directed against four of the TFIIH subunits: XPD, p62, cdk7 and cyclin H. Equal amounts of material corresponding to 5×10^5 cells were applied to each lane. The antigens were detected with a horseradish peroxidase-labelled secondary antibody and chemiluminescence (**A**). For quantification, the antigens were detected with a radioiodinated secondary antibody and the amount of radioactivity present in each band was quantified with a PhosphorImager and expressed in PSL. Histograms obtained for the cdk7 antigen (**B**) and for the p62 antigen (**C**) are shown. This experiment was repeated three times with different preparations and was very reproducible.

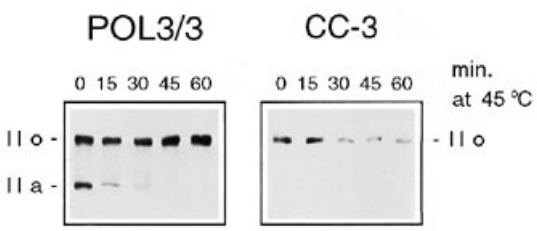

Figure 3. Modifications in the phosphorylation of the RPB1 subunit during heat-shock in HeLa cells. HeLa cells were submitted or not to a 45°C heat-shock for 15, 30, 45 or 60 min and lysed in Laemmli buffer; the lysates were analyzed by Western blot and detected with POL3/3 (left) or CC-3 (right) antibodies. Equal amounts of material corresponding to 5×10^4 cells were applied to each lane. IIa corresponds to the unphosphorylated form of RPB1, IIo to phosphorylated forms.

phosphorylated form. This result seemed in discrepancy with a decreased TFIIH kinase activity. However, we had reported previously that heat-shock also activates CTD kinase(s) distinct from TFIIH (14–16). Different CTD kinases might phosphorylate preferentially distinct sites on the CTD.

Therefore, we screened monoclonal antibodies directed against the phosphorylated CTD, which could discriminate between RPB1 subunits phosphorylated in control cells from RPB1 subunits phosphorylated in stressed cells. One of these antibodies, the CC-3 monoclonal antibody which was raised against a nuclear matrix phosphoprotein, p255 (35), recognized a phosphorylated form of RPB1 (46). The RPB1 form, recognized by the CC-3 antibody, diminished markedly in HeLa cells submitted to heat-shocks at 45°C (Fig. 4, right). This result suggested that the CC-3 antibody recognized a subset of phosphorylated RPB1 subunits which decreased during heat-shock.

The CC3 antibody binds to the CTD phosphorylated by TFIIH kinase

In an attempt to characterize the CTD kinases which might generate the phosphorylated form of RPB1 recognized by the CC-3 antibody, purified RNAPII core enzyme was phosphorylated *in vitro* with purified CTD kinases.

Purified RNAPII core enzyme contains essentially the unphosphorylated RPB1 subunit, IIa, which is detected by the POL3/3 antibody (Fig. 4A, top) as previously described (47). In contrast, the CC-3 antibody did not bind to this unphosphorylated subunit. Phosphorylation of RPB1 by TFIIH occurs within the preinitiation complex of transcription (6,25). Purified RNAPII core enzyme was therefore incubated with TFIIH in the presence of a promoter, general transcription factors and nucleotide triphosphates. When TBP, which is essential for the assembly of the preinitiation complex of transcription, was omitted from the reaction mixture, the RPB1 subunit remained unphosphorylated. In the presence of TBP, a fraction of the RPB1 subunit IIa was converted in a phosphorylated IIo form. This TFIIH-phosphorylated IIo form reacted very strongly with the CC-3 antibody.

The RPB1 phosphorylation was performed next using a purified MAP kinase. Purified RNAPII was incubated with sea star $p44^{mpk}$ in the presence of ATP. The migration of the RPB1 subunit was found to gradually slow down with increasing incubation times as detected with the POL3/3 antibody (Fig. 4A,

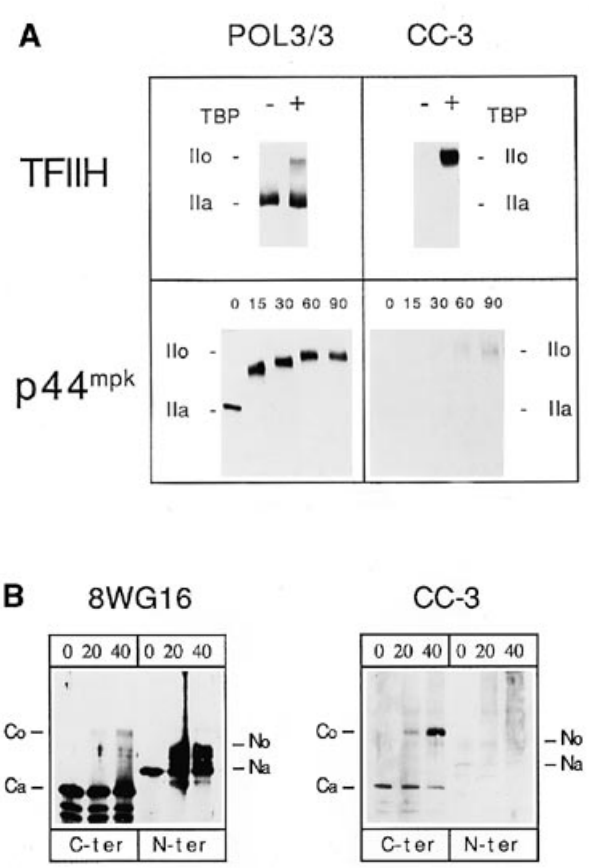

Figure 4. *In vitro* phosphorylation of the CTD with TFIIH generates the CC-3 epitope. (**A**) Purified human RNAPII core enzyme was incubated for 60 min at 25°C with TFIIH in the presence of the adenovirus major late promoter, the purified general transcription factors TFIIB, TFIIE-α, TFIIE-β, TFIIF and the four nucleotide triphosphates in the absence (–) or in the presence (+) of TBP. Alternatively, RNAPII was incubated with sea star $p44^{mpk}$ for 15, 30, 60 or 90 min at 30°C in the presence of ATP, no general transcription factors were added this time. Phosphorylation of the RPB1 human subunit was followed by Western blot using either the POL3/3 or the CC-3 antibody for detection. The positions of IIa and IIo forms are indicated. The same amount of polymerase was loaded in each lane. For a given antibody, a single exposure time is presented irrespective of the kinase assayed. (**B**) The GST-CTD-C-terminal (C-ter) and CTD-N-terminal (N-ter) fusion proteins were incubated for 0, 20 and 40 min with $p34^{cdc2}$ and ATP. After reaction, the mixtures were analyzed by Western blot with either the 8WG16 or the CC-3 antibody. The positions of the unphosphorylated (Ca and Na) and phosphorylated (Co and No) proteins are indicated.

bottom). However, the phosphorylated IIo forms obtained by incubation with $p44^{mpk}$ remained barely detectable with the CC-3 antibody even after 90 min of reaction.

To demonstrate that the CC-3 epitope was localized on the CTD, the GST was fused to fragments with 26 repeats of the CTD. The unphosphorylated fusion proteins Ca (GST fused to the CTD C-terminal fragment) and Na (GST fused to the CTD N-terminal fragment) migrated respectively like 42 and 46 kDa proteins (Coomassie blue staining data not shown). The fusion proteins reacted strongly with the 8WG16 anti-CTD monoclonal antibody (34) (Fig. 4B). Incubation of the fusion proteins with starfish cdc2 kinase and ATP generated new forms, Co and No, with lower electrophoretic mobilities. The CC-3 antibody reacted more strongly with the unphosphorylated C-terminal fusion protein than with the N-terminal fusion protein. The strongest reaction was obtained by far with the phosphorylated Co form which gave a strong signal despite its low abundance.

Taken together, these results demonstrate that *in vitro* phosphorylation of CTD by the TFIIH kinase generates its recognition by the CC-3 antibody. This antibody does not react however, with the CTD phosphorylated by MAP kinases, which have been shown to be CTD kinases activated by heat-shock (16). The increased RPB1 phosphorylation observed in heat-shocked HeLa cells is therefore consistent with the decrease in the CC-3 immunoreactivity and an impairment of the TFIIH kinase *in vivo*.

DISCUSSION

The results presented in this study strongly suggest that the TFIIH-associated kinase is impaired by heat-shock. Inactivation of the TFIIH-associated kinase in yeast kin28 or ccl1 ts-mutants shifted at non permissive temperature (29,30) or in mammalian cells treated with kinase inhibitors, results in a rapid general dephosphorylation of RPB1 (11,32). These experiments suggested that the TFIIH-associated kinase was the major kinase responsible for the phosphorylation of RPB1 *in vivo*. Heat shock is well known to reduce the extractibility of nuclear proteins (48,49) including RNA polymerase II (50). Indeed, after heat-shock, the TFIIH-associated kinase could not be released from insoluble cellular pellets. In most cases studied so far, the heat-shock induced aggregation of proteins corresponds to a loss of enzymatic activity (51–53). A decreased TFIIH-associated kinase activity has also been found in UV-irradiated cells (36). However unlike heat-shock, the UV-inactivation of the TFIIH kinase does not correspond to an insolubilisation process.

In apparent contradiction, an overall increased phosphorylation of the RPB1 subunit is observed in HeLa cells after heat-shock (15). Several kinases have been proposed to phosphorylate the CTD *in vitro* (for a review, see ref. 54). CTD kinases distinct from the TFIIH-associated kinase are activated by stress and have been tentatively identified as MAP kinases (16,55). The CC-3 antibody discriminates between the RPB1 subunit phosphorylated by the TFIIH-associated kinase and the RPB1 subunit phosphorylated by a MAP kinase. Using GST-CTD fusion proteins, the CC-3 epitope was found to be localized in the C-terminal, less conserved, part of the CTD. The disappearance of the CC-3 epitope during heat-shock is consistent with the overall decrease in TFIIH-associated kinase activity and with the phosphorylation of RPB1 by MAP kinases.

Heat-shock has been shown to promote an overall dephosphorylation of the RPB1 subunits in cells from different species such as *Drosophila*, *Chironomus*, mouse and rat (16,56). The observation of an overall phosphorylation or dephosphorylation of the RPB1 subunit would rely on the balance between impairment of the TFIIH-associated kinase and the activation of a stress-CTD kinase. Our data demonstrate that distinct phosphorylated RPB1 subunits coexist in the cells and correspond to subunits phosphorylated by different kinases. Distinct phosphorylation sites may correspond to different functions of the CTD.

The inactivation of the TFIIH-associated kinase caused by heat-shock might affect the various cellular functions in which it is involved such as transcription and the nuclear excision repair of damaged DNA. Indeed, hyperthermia seriously impairs the capacity of cells to excise DNA damage of the

5′,6′-dihydroxydihydrothymine type (57,58). Phosphorylation of the RPB1 subunit has also been associated with splicing complexes (59,60). The antigen recognized by the CC-3 antibody associates with splicing complexes (61) and colocalizes with spliceosomes (62). Splicing is interrupted during heat-shock stress (63) and it should be noted that the major eukaryotic heat-shock genes do not require splicing. Hence, it is tempting to speculate that a decreased availability of the TFIIH-associated kinase relates to the interruption of splicing.

According to most studies however, the phosphorylation of the RPB1 subunit is required for transcription: (i) microinjection of anti-cdk7 antibodies inhibit transcription in mammalian cells (25); (ii) in yeast kin28 and ccl1 ts-mutants, the steady-state levels of several mRNAs decline rapidly at non-permissive temperature as a result of reduced transcription rates (29,30,64); (iii) the TFIIH kinase activity is required for transcription in reconstituted yeast (65) and human (66) systems. Thus, the overall impairment of the TFIIH-associated kinase appears as a paradox because during stress, the heat-shock genes are transcribed with an extremely high efficiency (67,68). Furthermore, phosphorylation of the RPB1 subunit occurs on heat-shock gene promoters upon entry into elongation of transcription (69,70) which is rate-limiting (71,72). However, one cannot rule out that locally, for instance on the heat-shock genes, the TFIIH factor remains functional. Alternatively, heat-shock genes transcription might have a lower requirement for TFIIH-kinase activity than other genes. Two observations support this hypothesis: (i) transcription in reconstituted systems *in vitro* shows a requirement for CTD phosphorylation which depends on the promoter investigated (73); (ii) immunostaining of polytene chromosomes from *Drosophila* salivary glands indicates that the CTD of polymerases transcribing heat-shock genes is less phosphorylated than the CTD of polymerases transcribing genes in unstressed cells (69). The transcription of non heat-shock genes genes is decreased during heat-shock (74,75). The decreased TFIIH-associated kinase activity may therefore contribute to shut-off the non-heat shock genes transcription.

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