J. Biochem. 2011;149(5):563–568 doi:10.1093/jb/mvr005

JB THE JOURNAL OF BIOCHEMISTRY

Cyclin-dependent kinase 5 phosphorylates mammalian HMGB1 protein only if acetylated

Received December 6, 2010; accepted January 11, 2011; published online January 20, 2011

Iva Ugrinova, Iliya G. Pashev and Evdokia A. Pasheva*

Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113, Sofia, Bulgaria

*Evdokia A. Pasheva, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113, Sofia, Bulgaria, Tel: +359 2 979 2668, Fax: +359 2 872 35 07, email: eva@obzor.bio21.bas.bg

High mobility group box 1 (HMGB1) protein is the most abundant chromatin-associated non-histone protein expressed in all nucleated eukaryotic cells. We examined the phosphorylation of mammalian HMGB1 by testing the ability of the cyclin-dependent kinase 5 (Cdk5) to use as substrates native protein, either unmodified or *in vivo* acetylated and recombinant HMGB1. It turned out that Cdk5 was active on the *in vivo* acetylated HMGB1 only. We studied the effect of the phosphorylation on the 'architectural' properties of the acetylated HMGB1. The treatment with Cdk5 of the acetylated HMGB1 inhibited its capacity to induce DNA end-joining but had no effect on its ability to recognize distorted DNA structures.

Keywords: acetylation/cyclin dependent kinase 5/ end-joining/HMGB1 protein/phosphorylation.

Abbreviations: CBP, CREB binding protein; CREB, cAMP response element binding protein; HDAC, histone deacetylase; PCAF, p300/CBP associated factor.

The high mobility group box 1(HMGB1) protein is an abundant chromatin-associated non-histone protein. Discovered in the early seventies, the protein's first name was HMG1 because the mobility in the gel was its characteristic feature at that time (*1*). Almost forgotten for more than a decade, it became an attractive object of investigation with the discovery in 1990 of a novel DNA binding motif, displaying sequence similarity to two homologous sequence repeats in HMG1 (*2*). Due to its later established ability to bend DNA and to preferentially bind prebent DNA, it was soon recognized as an 'architectural factor', facilitating the assembly of certain nucleoprotein complexes involved in fundamental nuclear events (*3*, *4*). Presently, HMGB1 is gaining an increasing interest with its extra cellular functions as a key cytokine that mediates the response to infection and inflammation (*5*–*7*). Intensively studied, its precise biological role is still obscure. One approach to enlarge our knowledge is to investigate its post-translational modifications. HMGB1 can undergo phosphorylation, acetylation, methylation, ADP-ribosylation and glycosylation (*8*), but attention is paid so far to phosphorylation and acetylation. Studies on acetylation have been focused exclusively on vertebrate HMGB1 proteins. *In vivo* experiments identified Lys2 as a target site for this modification (*9*, *10*) and further demonstrated the consequences of acetylation for the DNA binding properties of HMGB1 (*10*–*13*) and for its effect on replication and repair of DNA (*12*, *14*). Recently, the acetylation of HMGB1 was found to affect strongly the manner of protein binding to DNA: unlike the parental protein, shown to bind mainly within internal sequences, acetylated protein bound preferentially to DNA ends (*13*). As a result, HMGB1 lost its DNA bending ability and acquired DNA end-joining activity. As far as the latter property is involved in both double-strand break repair and V(D)J recombination, these findings suggest a mechanism for switching the protein either to assistance in formation of various nucleoprotein complexes (parental form), or to double strand brake repair (acetylated form). Unlike acetylation, phosphorylation has been studied mainly with HMGB proteins from plants and from dipteran insects *Chironomus* and *Drosophila* (*15*) with a few papers only communicating data on HMGB1 from mammals (*16*, *17*). The HMGB proteins in higher plants are differentially phosphorylated by various protein kinases (*18*, *19*) that affect the protein thermal stability, DNA binding properties (*18*) and the interaction with transcription factors (*20*). The HMGB1 proteins in the insects are phosphorylated at serine residue(s) located in the region of their acidic C-terminal tails. The modification alters substantially the conformation and DNA binding properties of these proteins and therefore appears to be essential for their function (*15*). In our previous works we demonstrated that the C-terminal domain of mammalian HMGB1 modulated the acetylation of the protein by the histone acetyltransferase CREB binding protein (CBP) and was responsible for the inhibitory effect of HMGB1 protein on the repair of cis-platinated DNA (*11*, *12*). Therefore we searched for a serine kinase with a putative target site in a close proximity to the regulatory C-tail.

We examined the phosphorylation of mammalian HMGB1 by testing the ability of cyclin-dependent kinase 5 (Cdk5) to use as substrates several HMGB1 preparations: native protein, either unmodified or *in vivo* acetylated and recombinant HMGB1. We showed that Cdk5 was active on the acetylated HMGB1 only. The phosphorylation had no effect on the binding affinity of the acetylated HMGB1 to *cis*-platinated DNA while the DNA end-joining

activity of the protein was inhibited upon phosphorylation with Cdk5.

Materials and Methods

Preparation of DNA probes

Plasmid pUC19 DNA (2.69 kb) is digested with *Pvu*II and *Bam*H1 to produce 211 and 111 bp fragments, respectively. The 111 bp fragment was purified from agarose gel and labelled at its protruding 5′-ends with [α-^{32}P] dCTP using the Klenow fragment (Promega). The affinity of HMGB1 to cis-platin damaged DNA was assayed with site-specifically platinated oligonucleotide prepared by using chemically synthesized 40 bp oligonucleotide with a single GpG for adduct formation. Probe preparation and the binding affinity determinations are described elsewhere (*10*).

Preparation of proteins

HMGB1 proteins, either unmodified or *in vivo* acetylated, were isolated by non-denaturing salt extraction procedure from Guerin ascites tumour cells grown in the absence or presence of butyrate, respectively (*10*). To obtain recombinant HMGB1, cDNA encoding full-length rat HMGB1 (lib. N 961, RZPD) was amplified by PCR using the following primers: forward 5′-TGCACTGGAATTCATG GGCAAAGAGATCC-3′ and reverse 5′-CAGTGCACTCGAG-TT ATTCATCATCATCATCTTC-3′ according to a published protocol (*14*), subcloned in pET28a expression vector, expressed in modified *Escherichia coli* BL21 Poly Lys S. and purified on a HIS Select HF Nickel Affinity gel (Sigma). The purity of the proteins was assessed by SDS-18% polyacrylamide gel electrophoresis (PAGE). *In vitro* phosphorylation of various HMGB1 proteins was assayed with Cdk5 and carried out as described elsewhere (*21*). Briefly, 20 pmol Cdk5 (human recombinant, Calbiochem) were preincubated with 120 pmol Cdk5 activator p35 (bovine recombinant, Calbiochem) in 20 µl phosphate-buffered saline, pH 7.2, containing 1 mM dithiothreitol (DTT) and 1 mM EDTA for 2 h at 22°C. Then, 5 µl from the preincubation mixture was diluted to 20 µl with phosphorylation reaction mixture containing 5 mM $MgCl_2$, 30 mM MOPS, pH 7.2 and 100 pmol of protein. The reaction was started by adding 10 nmol [γ-^{32}P]ATP (3000 mCi/mmol) and incubated at 30°C for different periods of time. The phosphorylation reactions were terminated by adding SDS sample buffer and fractionated by PAGE. The gels were stained with Coomassie blue R250, dried and autoradiographed.

Ligase-mediated circularization assay

[α-^{32}P] dCTP labelled probes were used generally following a published protocol (*13*). The products of ligation were separated on 5% PAGE in 0.5× TBE at 10 V/cm, and the vacuum-dried gel was autoradiographed and scanned with a Gel Pro analyzer. Resistance to digestion with exonuclease III (Boehringer, 5 U/reaction, 37°C, 30 min) was used to discriminate between linear and circular DNA.

Exo III mapping

The 147 bp DNA fragment was obtained by PCR amplification from plasmid pGEM3Z-601. Both primers: forward 5′-CAGGATGTAT ATATCTGACACGTGCCT-3′ and reversed 5′-CGAGAGAATCC CGGTGCCGAGGCC-3′ were 5′-end labelled by polynucleotide kinase (Invitrogen) following a standard protocol. The labelled DNA fragment was gel purified and incubated with different HMGB1 proteins for 10 min in a buffer containing 10 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and 50 mM NaCl. HMGB1-DNA complexes were digested with exonuclease III for 30 min at 37°C and treated with proteinase K (0.2 mg/ml) in the presence of SDS. Following deproteinization and precipitation with ethanol, DNA was separated on 8% denaturing polyacrylamide gel. After electrophoresis, the gels were dried and autoradiographed.

Electrophoretic mobility shift assay

Binding of various HMGB1 preparations to cis-platin damaged DNA was monitored by electrophoretic mobility shift assay (EMSA) using 5% PAGE in 0.5× TBE (0.045 M Tris–borate, 1 mM EDTA) as described (*22*) except that the incubation mixture was supplemented with 10 mM sodium n-butyrate. Upon completion of electrophoresis, the gel was dried and exposed to Kodak XAR-5 film at −70°C. The autoradiographies were scanned with Gel-Pro Analyzer.

Results

In vitro *phosphorylation of HMGB1 protein samples by Cdk5*

We applied the bioinformatical approach using the program http://mgc.nci.nih.gov/ that predicted the modification status of the protein. According to this program the kinase Cdk5 had only one favorable target site, the serine at position 180 situated just before the HMGB1 C-terminus peptide comprising of 30 acidic amino acids and this fact defined Cdk5 as the suitable kinase. The ability of the enzyme to phosphorylate mammalian HMGB1 was assayed using the following protein substrates: HMGB1 isolated from rat ascites tumour cells, *in vivo* monoacetylated HMGB1 isolated from the same cells grown in the presence of sodium butyrate, an inhibitor of histone deacetylase (HDAC), and recombinant HMGB1. The monoacetylated position in HMGB1 protein was previously detected as Lys2 (*10*). The purified proteins (in triplicate) were subjected to SDS–PAGE (Fig. 1A) to demonstrate their purity and to justify that equal quantities used to perform the kinetics of phosphorylation with [γ-^{32}P] ATP in the presence Cdk5 (Fig. 1B). It was interesting to note that the acetylated protein was the unique substrate for Cdk5 in a time dependent manner. Neither the recombinant HMGB1 nor the native non-acetylated protein incorporated radioactive phosphate.

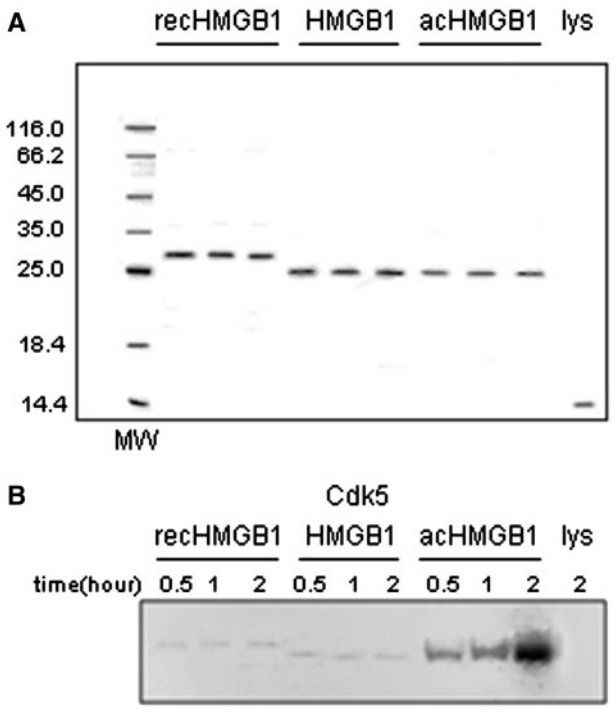

Fig. 1 ***In vitro*** **phosphorylation of recombinant HMGB1 (recHMGB1), native HMGB1and** ***in vivo*** **acetylated HMGB1 (acHMGB1).** The proteins were treated with Cdk5, aliquots were taken at times indicated, run on SDS–18% PAGE and visualized by staining with Coomassie blue (A) and by autoradiography (B) MM, molecular mass markers; Lys, Lysozyme.

The effect of phosphorylation on DNA end-joining activity of acetylated HMGB1 protein

Regarding the *in vivo* acetylated HMGB1, previous experiments demonstrated that due to its preferential association with DNA ends it failed to bend DNA but, instead, it acquired the capacity to stimulate DNA end-joining. The increase in protein concentration resulted in overloading of DNA ends with protein molecules that prevented the free termini to be in spatial proximity for an efficient ligase reaction (*13*). The ability of native HMGB1 to bend linear DNA fragments or to stimulate their end-joining when the protein is acetylated was tested by the ligase-mediated DNA circularization assay. The same approach was used for the Cdk5-phosphorylated *in vivo* acetylated HMGB1. The experiment was carried out with a radiolabelled 111 bp DNA fragment in the presence of *in vivo* acetylated HMGB1 and Cdk5-phosphorylated/acetylated HMGB1. The end-joining activity of the acetylated protein was inhibited upon phosphorylation with Cdk5 (Fig. 2, compare lanes 3 and 5 as well as lanes 4 and 6). A control experiment with heat-inactivated Cdk5 (lane 7) demonstrated that the observed inhibitory effect was not due to the presence of Cdk5 itself. Another control experiment was carried out with exoIII to discriminate between circular and linear DNA (Fig. 2, lane 8). The findings presented in Fig. 2 suggest two possible effects of phosphorylation on the acetylated HMGB1. One is an abrogation of its ability to bind DNA ends and, as a result, the two DNA fragments cannot be brought together. Alternatively, the affinity to DNA termini is further enhanced so that at higher protein concentration, the phosphorylated/acetylated HMGB1 molecules associate with both ends of DNA fragment thus blocking the dimer formation. To distinguish between the two possibilities, the interactions of 5′-labelled DNA fragments with native non-acetylated HMGB1, *in vivo* acetylated HMGB1 and Cdk5 phosphorylated/acetylated protein were analysed by cleavage with exo III. Fig. 3A presents a denaturing gel of DNA fragments obtained after exoIII digestion of free DNA (lane 2) and DNA protein complexes (lanes 3–8). ExoIII digestion of native protein–DNA complex produced DNA fragments between 50 and 100 nt (lanes 3 and 4). Digestion of acetylated HMGB1-DNA complex, however, resulted in the appearance of a full-length 147 nt fragment in addition

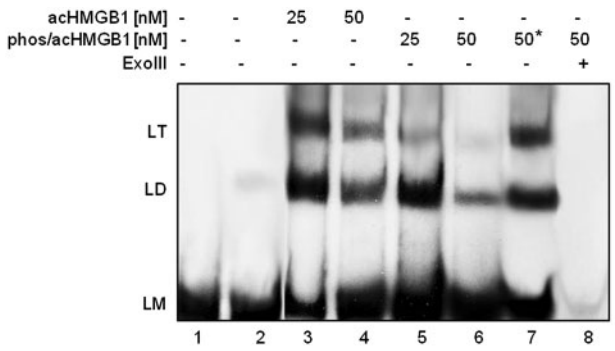

Fig. 2 Effect of Cdk5 phosphorylation on the DNA end-joining activity of *in vivo* acetylated HMGB1 (acHMGB1). ^{32}P-labelled 111 bp DNA fragment was incubated with either acHMGB1 or Cdk5-treated acHMGB1(phos/acHMGB1) in the presence of T4 DNA ligase and the products obtained analysed on a 5% native PAGE. Lane 1, 111 bp DNA; lane 2, T4 ligase treated 111 bp DNA fragment; Lane 7, a control experiment carried out with heat-inactivated Cdk5; lane 8, digestion with exonuclease III (exo III). LM, linear monomer, LD, linear dimmer, LT, linear trimmer.

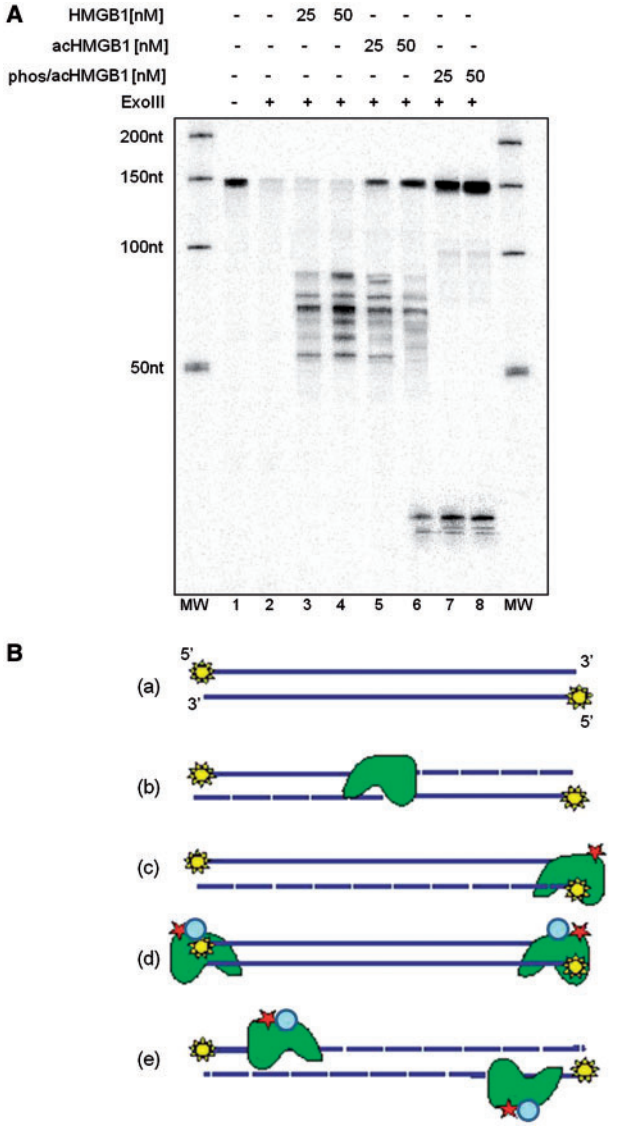

Fig. 3 Exonuclease III digestion of the complexes of ^{32}P-labelled 147 bp DNA fragment with native HMGB1, acetylated HMGB1 (acHMGB1) and Cdk5-phosphorylated acHMGB1 (phos/acHMGB1). The labelled DNA fragments were incubated with different HMGB1 samples at the indicated concentrations and the complexes formed digested with exonuclease III. Following treatment with proteinase K (0.2 mg/ml) in the presence of SDS, DNA was deproteinized, ethanol precipitated and fractionated by 8% denaturing PAGE. After electrophoresis, the gel was dried and autoradiographed. (A lanes 1 and 2): 147 bp DNA fragment before and after exo III digestion, respectively; MW, molecular weight markers. (B) Schematic presentation of various HMGB1-DNA complexes, corresponding to the exo III digestion pattern shown in A (a) 5′-labelled 147 bp DNA; (b) HMGB1-DNA complex; (c) acHMGB1-DNA complex; (d) and (e), complexes of DNA with Cdk5-phosphorylated acHMGB1. The dotted lines represent DNA accessible for exo III digestion in the protein-DNA complexes. The asterisk and the circles mark the acetylated and Cdk5-phosphorylated HMGB1, respectively.

to the set of 50–100 nt fragments (lanes 5 and 6). At higher protein concentrations, the amount of full length DNA increased and a set of small DNA fragments also appeared (lane 6). Exo III digestion of Cdk5-phosphorylated/acetylated HMGB1-DNA complex produced only the full-length 147 nt fragments and the small ones, entirely lacking the set of 50–100 nt fragments (Fig. 3A, lanes 7 and 8). The interpretation of these data is illustrated by the scheme in Fig. 3B. The DNA fragments between 50 and 100 nt are probably obtained when the protein is bound in the middle of the fragment (Fig. 3B-b). The full-length 147 nt DNA appears if the protein is bound to one or both DNA ends (Fig. 3B-c and d). The small DNA fragments we attribute to the situation shown in Fig. 3B-e, when the protein does not cover the very ends of the fragment. One may speculate on the basis of the above described results that the native HMGB1 is bound within the middle part of the fragment thus restricting the exoIII digestion to the production of 50–100 nt long DNA (Fig. 3A, lanes 3 and 4 correspond to Fig. 3B, b). If acetylated, however, the protein is directed to the fragment's end, protecting it from exoIII digestion and resulting in the appearance of 147 nt signal which increases at higher protein concentration because the protein covers both DNA ends (Fig. 3A, lanes 5 and 6 correspond to Fig. 3B-c and d). Yet, some molecules remain middle-positioned since faint 50–100 nt fragments are still observed. Similar situation was demonstrated in our previous studies on the binding of acetylated HMGB1 to DNA by atomic force microscopy where the statistics of acHMGB1/DNA complexes revealed terminal binding of ~75% of the protein molecules but still in the 25% of the complexes the acetylated HMGB1 protein was internally bound (*13*). This pattern is entirely missing when DNA is incubated with phosphorylated/acetylated HMGB1: the presence of full-length DNA undoubtedly suggests that the protein is bound to DNA ends only (Fig. 3A, lanes 7 and 8 correspond to Fig. 3B-d and e). The observed small DNA fragments (Fig. 3A, lanes 7 and 8) we attribute to the situation presented in Fig. 3B-e. In other words, phosphorylation and acetylation of HMGB1 exert synergistical effect by forcing HMGB1 to associate with DNA ends.

The effect of phosphorylation on the binding affinity to cis-platin damaged DNA of acetylated HMGB1

The interaction of Cdk5-phosphorylated acetylated HMGB1 (phos/acHMGB1) with the site-specifically platinated 40 bp oligonucleotide was assayed by EMSA (Fig. 4A). The EMSA-based binding curve phos/acHMGB1 (Fig. 4B) was also presented. The calculated constants for the acetylated HMGB1 and its Cdk5-phosphorylated form, the respective values were rather close (0.1 and 0.08 μM, respectively). We may draw the conclusion that the modification of acetylated HMGB1 caused by Cdk5 does not influence the affinity of the protein to DNA damaged by the anti-tumour drug *cis*-platinum.

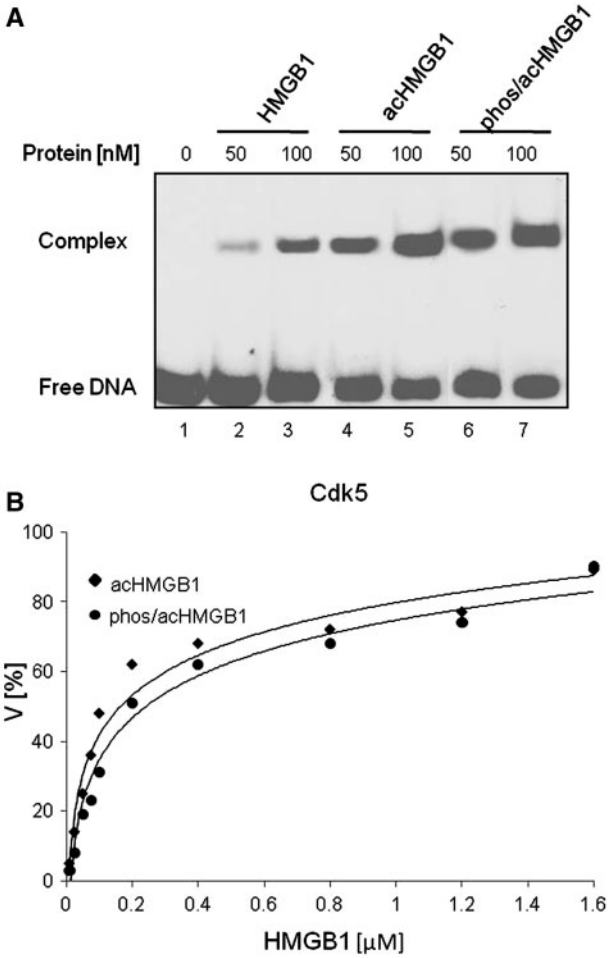

Fig. 4 Binding affinities to *cis*-platin damaged DNA of *in vitro* Cdk5-phosphorylated acetylated HMGB1 (phos/acHMGB1). (A) EMSA of the interactions of cis-platinated DNA with various HMGB1 preparations (each one in two concentrations): native HMGB1 (lanes 2 and 3), acetylated HMGB1 (acHMGB1, lanes 4 and 5), Cdk5-phosphorylated acHMGB1 (phos/acHMGB1, lanes 6 and 7), (B) binding curves of acHMGB1 and phos/acHMGB1 to cis-platinated DNA. V stands for the fraction of bound protein molecules to a single molecule of cis-platinated DNA.

Discussion

First identified as a chromatin factor assisting in formation of nucleoprotein complexes, HMGB1 was later recognized as a cytokine that mediated inflammatory responses, activated endothelial cells, enhanced haematopoietic stem cell migration and transduced cellular signals through various receptors (*7*). As a protein with rather unusual biology, all cellular events that may affect the properties of HMGB1 and among them the post-translational modifications, would be of particular interest.

This work reports our data on phosphorylation of mammalian HMGB1 protein *in vitro* by the kinase Cdk5. The enzyme was chosen using program http://mgc.nci.nih.gov. Cdk5 was predicted to modify only one serine at position 180, situated immediately before the acidic tail, known to act as a modulator of HMGB1 properties. This is a serine threonine kinase,

phosphorylating cytoskeletal elements, signalling molecules or regulatory proteins. Although it appears to be indispensable for normal neural development and function (*23*), accumulating evidence indicates that Cdk5 affects also non-neuronal cells (*24*, *25*). Three HMGB1 proteins were tested as substrates: native HMGB1 purified from Guerin ascites tumour cells, *in vivo* acetylated HMGB1 protein isolated from the same cells grown in the presence of butyrate and recombinant HMGB1. We show that unlike the recombinant and native HMGB1 which cannot be phosphorylated by Cdk5, *in vivo* monoacetylated protein at Lys2 is a good substrate for this enzyme. The reason for this effect is not clear. One possibility suggests that it might result from acetylation-phosphorylation interplay, leading to enhanced binding of Cdk5 and/or its activator p35 to acetylated HMGB1. Similar situation was suggested to account for the stabilization and activation of p53 in a process leading to neuronal cell death (*26*) or as a transcription factor in response to certain stresses (*27*). In these two cases, phosphorylation enhanced the interactions of the histone acetyltransferases p300 and p300/CBP associated factor with p53, respectively, inducing its acetylation which in turn enhanced both DNA binding ability and repair activity of p53 (*27*, *28*). Another example was the demonstration that the phosphorylation status of p65 subunit of nuclear NF-kB determined its association with CBP/p300 or HDAC-1 thus modulating its activity (*29*) as well as the case with the human positive coactivator 4 (PC4). This protein closely resembles HMGB1 by its property to bind DNA in a sequence-independent manner. Phosphorylation of PC4 by casein kinase II was shown to inhibit the p300-mediated acetylation of PC4, while acetylation did not influence phosphorylation of PC4 (*30*). Next, we analysed the effect of phosphorylation on the architectural properties of monoacetylated HMGB1 such as affinity to damaged DNA and DNA bending ability. Phosphorylation by Cdk5, similar to acetylation, stimulated the binding of HMGB1 to DNA ends, but unlike acetylation, it inhibited DNA end-joining. One explanation of this finding suggests a saturation of DNA ends with the protein and, as a result, the two DNA fragments cannot be brought together thus blocking the end-joining. It is already shown that the acetylation at Lys2 increases the affinity of HMGB1 protein to *cis*-platinated DNA up to 7–8 times (*10*). The additional phosphorylation by Cdk5 had no further effect.

The function of Cdk5 has been implicated in Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, etc. (*31*). HMGB1 is considered one of the potentially amyloidogenic proteins that contains an amyloidogenic peptide fragment highly homologous to the Alzheimer's amyloid β-peptide (*32*). Our findings that *in vivo* acetylated HMGB1 is a substrate of Cdk5 might place both proteins in a common signalling mechanism.

Conflict of Interest
None declared.

References

1. Goodwin, H., Sanders, C., and Johns, E.W. (1973) A new group of chromatin-associated proteins with high content of acidic and basic amino acids. *Eur. J. Biochem.* **38**, 14–19
2. Jantzen, H.M., Admon, H., Bell, S.P., and Tjian, R. (1990) Nucleolar transcription factor hUBF contains a DNA binding motif with homology to HMG proteins. *Nature* **344**, 830–836
3. Thomas, J.O. and Travers, A.A. (2001) HMG1 and 2, and related structural DNA-binding proteins. *Trends Biochem. Sci.* **26**, 167–174
4. Mitsouras, K., Wong, B., Arayata, C., Johnson, R.C., and Carey, M. (2002) The DNA architectural protein HMGB1 displays two modes of action that promote enhanseosome assembly. *Mol. Cell. Biol.* **22**, 4390–4401
5. Wang, H., Bloom, O., Zhang, M., Vishnubbakat, J.M., Ombrellino, M., Che, J., Frazier, A., Yang, H., Ivanova, S., Borovikova, L., Manogue, K.R., Faist, E., Abraham, E., Andersson, J., Andersson, U., Molina, P.E., Abumrad, N.N., Sama, A., and Tracey, K.J. (1999) HMG-1 as a late mediator of endotoxin lethality in mice. *Science* **285**, 248–251
6. Scaffidi, P., Misteli, T., and Bianchi, M.E. (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* **418**, 191–195
7. Lotze, M.T. and Tracey, K.J. (2005) High mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nature Rev. Immunol.* **5**, 331–342
8. Einck, L. and Bustin, M. (1985) The intracellular distribution and function of the high mobility group chromosomal proteins. *Exp. Cell Res.* **156**, 295–310
9. Sterner, R., Vidali, G., Heinrikson, R.L., and Allfrey, V.G. (1978) Postsynthetic modification of high mobility group proteins. Evidence that high mobility group proteins are acetylated. *J. Biol. Chem.* **253**, 7601–7609
10. Ugrinova, I., Pasheva, E., Armengaud, J., and Pashev, I.G. (2001) In vivo acetylation of HMGB 1 protein enhances its binding affinity to distorted DNA structures. *Biochemistry* **41**, 14655–14660
11. Pasheva, E.A., Sarov, M., Bidjekov, K., Ugrinova, I., Sarg, B., Lindner, H., and Pashev, I.G. (2004) In vitro acetylation of HMGB-1 and -2 proteins by CBP: the role of the acidic tail. *Biochemistry* **43**, 2935–2940
12. Mitkova, E., Ugrinova, I., Pashev, I.G., and Pasheva, E.A. (2005) Effect of HMGB-1 protein on the repair of cisplatin-damaged DNA is accomplished through the acidic domain. *Biochemistry* **44**, 5893–5898
13. Ugrinova, I., Mitkova, E., Moskalenko, C., Pashev, I.G., and Pasheva, E.A. (2007) DNA bending versus DNA end joining activity of HMGB1 protein is modulated in vitro by acetylation. *Biochemistry* **46**, 2111–2117
14. Topalova, D., Ugrinova, I., Pashev, I.G., and Pasheva, E.A. (2008) HMGB1 protein inhibits DNA replication in vitro: a role of the acetylation and the acidic tail. *Int. J. Biochem. Cell. Biol.* **40**, 1536–1542
15. Wiśniewski, J.R., Szewczuk, Z., Petry, I., Schwanbeck, R., and Renner, U. (1999) Constitutive phosphorylation of the acidic tails of the high mobility group 1 proteins by casein kinase II alters their conformation, stability, and DNA binding specificity. *J. Biol. Chem.* **274**, 20116–20122
16. Poser, I., Golob, M., Buettner, R., and Bosserhoff, A. (2003) Upregulation of HMG1 leads to melanoma inhibitory activity expression in malignant melanoma cells and contributes to their malignancy phenotype. *Mol. Cell. Biol.* **23**, 2991–2998

17. Oh, Y.J., Youn, J.H., Ji, Y., Lee, S.E., Lim, K.J., Choi, J.E., and Shin, J.S. (2009) HMGB1 is phosphorylated by classical protein kinase C and is secreted by a calcium-dependent mechanism. *J. Immunol.* **182**, 5800–5809
18. Stemmer, C., Schwanders, A., Bauw, G., Fojan, P., and Grasser, K.D. (2002) Protein kinase CK2 differentially phosphorylates maize chromosomal high mobility group B (HMGB) proteins modulating their stability and DNA interactions. *J. Biol. Chem.* **277**, 1092–1098
19. Stemmer, C., Leeming, D.J., Franssen, L., Grimm, R., and Grasser, K.D. (2003) Phosphorylation of maize and arabidopsis HMGB proteins by protein kinase CK2α. *Biochemistry* **42**, 3503–3508
20. Krohn, N.M., Yanagisawa, S., and Grasser, K.D. (2002) Specificity of the stimulatory interaction between chromosomal HMGB proteins and the transcription factor Dof2 and its negative regulation by protein kinase CK2-mediated phosphorylation. *J. Biol. Chem.* **277**, 32438–32444
21. Lew, J., Huang, Q., Qi, Z., Winkfein, R.J., Aebersold, R., Hunt, T., and Wang, J.H. (1994) A brain- specific activator of cyclin-dependent kinase 5. *Nature* **371**, 423–426
22. Pasheva, E.A., Pashev, I.G., and Favre, A. (1998) Preferential binding of high mobility group 1 protein to UV damaged DNA. *J. Biol. Chem.* **273**, 34730–34736
23. Dhariwala, F.A. and Rajadhyaksha, M.S. (2008) An unusual member of the Cdk family: Cdk5. *Cell. Mol. Neurobiol.* **28**, 351–369
24. Sandal, T., Stapnes, C., Kleivdal, H., Hedin, L., and Doskoland, S.O. (2002) A novel, extraneuronal role for cyclin-dependent protein kinase 5 (CDK5): modulation of cAMP-induced apoptosis in rat leukemia cells. *J. Biol. Chem.* **277**, 20783–20793
25. Lin, H., Juang, J.L., and Wang, P.C. (2004) Involvement of Cdk5/p25 in digoxin-triggered prostate cancer cell apoptosis. *J. Biol. Chem.* **279**, 29302–29307
26. Lee, J.H., Kim, H., Lee, S.J., and Kim, K.T. (2007) Stabilization and activation of p53 induced by Cdk5 contributes to neuronal cell death. *J. Cell Sci.* **120**, 2259–2271
27. Sakaguchi, K., Herrera, J., Saito, S., Miki, T., Bustin, M., Vassilev, A., Anderson, C.W., and Appella, E. (1998) DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* **12**, 2831–2841
28. Gu, W. and Roeder, R.G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* **90**, 595–605
29. Zhong, H., May, M.J., Jimi, E., and Ghosh, S. (2002) The phosphorylation status of Nuclear NF-kB determines its association with CBP/p300 or HDAC-1. *Mol. Cell* **9**, 625–636
30. Kumar, B., Swaminathan, V., Banerjee, S., and Kundu, T.K. (2001) p300-mediated acetylation of human transcriptional coactivator PC4 is inhibited by phosphorylation. *J. Biol. Chem.* **276**, 16804–16809
31. Dhariwala, F.A. and Rajadhyaksha, M.S. (2008) An unusual member of the Cdk family: Cdk5. *Cell. Mol. Neurobiol.* **28**, 351–369
32. Kallijärvi, J., Haltia, M., and Baumann, M.H. (2001) Amphoterin includes a sequence motif which is homologous to the Alzheimer's ß-amyloid peptide (Aß), forms amyloid fibrils in vitro, and binds avidly to Aß. *Biochemistry* **40**, 10032–10037