Biochemical and Biophysical Research Communications 381 (2009) 59–64

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

hnRNP-U is a specific DNA-dependent protein kinase substrate phosphorylated in response to DNA double-strand breaks

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ARTICLE INFO

Article history:
Received 19 January 2009
Available online 11 February 2009

Keywords:
DNA-PK
hnRNP-U
SAF-A
DNA damage
Etoposide

ABSTRACT

Cellular responses to DNA damage are orchestrated by the large phosphoinositol-3-kinase related kinases ATM, ATR and DNA-PK. We have developed a cell-free system to dissect the biochemical mechanisms of these kinases. Using this system, we identify heterogeneous nuclear ribonucleoprotein U (hnRNP-U), also termed scaffold attachment factor A (SAF-A), as a specific substrate for DNA-PK. We show that hnRNP-U is phosphorylated at Ser59 by DNA-PK in vitro and in cells in response to DNA double-strand breaks. Phosphorylation of hnRNP-U suggests novel functions for DNA-PK in the response to DNA damage.

Introduction

DNA damage in the form of strand breaks or adducts, or stalled replication forks, activates evolutionarily conserved signalling pathways. Important components are the large protein-serine/threonine kinases Ataxia Telangiectasia Mutated (ATM) and ATM- and Rad3-Related (ATR), which possess catalytic domains related to phosphoinositide 3-kinase (PI3 K) [1]. ATM is an important regulator of cellular responses to double strand breaks (DSB), whereas ATR is activated by single stranded DNA (ssDNA) formed when DNA replication is stalled. ATM and ATR activate cell cycle checkpoints that delay DNA replication and entry into mitosis in response to DNA damage or replication arrest [2].

DNA-dependent protein kinase (DNA-PK) is another PI3K-like kinase (PIKK) in vertebrates that consists of a large catalytic subunit (DNA-PKcs) and a heterodimer of Ku70 and Ku80 subunits that detects DSB. DNA-PK is a key enzyme in the repair of DSB by non-homologous end joining (NHEJ) and has a critical role in V(D)J recombination [3]. DNA-PK may also have roles in DNA damage checkpoint signalling [4]. Although DNA-PK recognises certain Ser/Thr-Gln sites like ATM and ATR [5], it has a distinct substrate specificity and can also phosphorylate other Ser/Thr residues [6].

In order to characterise the different functions of ATM, ATR and DNA-PK it is necessary to identify their specific substrates. Recently, we have developed a human cell-free system in which DNA damage pathways are activated by oligonucleotides [7]. Here, we use this system to identify heterogeneous nuclear ribonucleoprotein U (hnRNP-U) as a novel substrate for DNA-PK that is not targeted by ATM or ATR. We show that hnRNP-U is phosphorylated at Ser59 by DNA-PK in vitro and in cells in response to DSB. The selective phosphorylation of hnRNP-U by DNA-PK suggests novel functions for this kinase in the cellular response to DNA damage and provides a marker for DNA-PK activity in cells.

Material and methods

Cell-free system for DNA damage responses. HeLa nuclear (HNE) and cytoplasmic extracts (S100) were purchased from Cilbiotech (Mons, Belgium). Ten microliters of each were mixed with the addition of ATP (1 mM), creatine kinase (10 ng ml^{-1}), creatine phosphate (5 mM) to a final volume of 25 μl (at approximately 15 mg protein ml^{-1}). To induce a DNA damage response, 50 ng pre-annealed $dA_{70}dT_{70}$ or 50 ng 5′-biotinylated dA_{70} pre-annealed with dT_{70} (5′-bio-$dA_{70}dT_{70}$) were added. 1 μM okadaic acid was also added to the extracts to inhibit protein phosphatase activity. Where indicated, 5′-biotinylated dAdT oligos were retrieved onto streptavidin beads (Dynabeads) and washed and eluted according to manufacturer's guidelines. Protein kinase inhibitors dissolved in DMSO (final concentration 0.1%) were NU7441 (10 μM) [8], KU55933 (10 μM) [9] (both gifts of G.C. Smith, Kudos, Cambridge, UK) and staurosporine (3 μM) (Calbiochem). The extracts were typically incubated for 60 min at 30 °C. 1U alkaline phosphatase (Roche) was used in reactions to reverse protein phosphorylation (37 °C, 60 min).

SDS–PAGE and Western blot. Samples were separated on standard SDS–PAGE and transferred onto nitrocellulose membranes

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doi:10.1016/j.bbrc.2009.02.019

by Western blotting. To visualise a phosphorylation event, 25–50 μM PhosTag acrylamide (PhosTag, Hiroshima, Japan) was added to the resolving gel in the presence of 50–100 μM $MnCl_2$. To chelate Mn^{2+} ions the gels were washed in western transfer buffer plus 1 mM EDTA for 10 min, and then in transfer buffer without EDTA for 10 min. Antibodies used were directed against actin (Roche, used at 1:5000), Chk1 (Santa Cruz, 1:1000), Chk1 pS296, pS317, p345 (Santa Cruz, 1:1000), DNA-PKcs (Abcam, 1:500), pS2056 DNA-PK (Abcam, 1:500), γH2A.X (Abcam, 1:1000), or hnRNP-U (Abcam, 1:1000). Horseradish peroxidase-conjugated anti-mouse or rabbit IgG was used as a secondary antibody and peroxidase activity was visualised in an ECL reaction.

Mass spectrometry. Mass spectrometry analysis was performed at the College of Life Sciences, University of Dundee. Protein identification was performed by 1D nLC (nano liquid chromatography) MS/MS and phosphopetides were analysed using a 4000 QTrap mass spectrometer (Applied Biosciences).

Generation of a polyclonal antibody against the phosphorylated Ser59 site of hnRNP-U. Peptides (MEPGNGSLDLGGD and MEPGNGpSLDLGGD, where pS is the phosphorylated residue) were produced at the Cancer Research UK London Research Institute. The peptides were used by Moravian Biotech (Brno, Czech Rep.) for the generation of a polyclonal antibody which was purified using affinity chromatography by negative selection against the dephosphorylated peptide and by positive selection against the phosphorylated peptide, each coupled to Reacti-Gel 6X beads (Pierce).

Expression and purification of His_6-tagged hnRNP-U. hnRNP-U was amplified by PCR from cDNA and cloned into the pET21-α protein expression vector. Recombinant His_6-hnRNP-U was expressed in BLR-DH3 competent bacteria and purified according to standard protocols.

Kinase assay. Three hundred nanograms purified His_6-hnRNP-U was incubated with 1 μl DNA-PK enzyme (Promega), 1 mM ATP with 5 μl (10 μg/μl) sheared herring sperm DNA (Promega) in the specified buffer. The reaction was incubated for 15 min at 30 °C.

Cell culture. Human osteosarcoma-derived U2OS cells were grown in DMEM (Gibco 1×) supplemented with 10% foetal bovine serum (Biosera), 2 mM glutamine (1%) (Gibco), 1% penicillin/streptomycin (P/S) (Gibco, 5000 U/ml). Human glioblastoma-derived MO59J and MO59K cells were grown in DMEM:Ham's F12 (1:1) mix (Invitrogen) with the same supplements. Cells were grown at 37 °C, 5% CO_2 to sub-confluency (70–90% coverage) before treatment for 60 min with etoposide (40 μM), camptothecin (3 μM), cisplatin (10 μg/ml), hydroxyurea (3 mM), bleomycin (5 μg/ml), aphidicolin (3 μM), or nocodazole (100 nM) where indicated. Protein kinase inhibitors were used as described for cell extracts.

Results

Activation of both ATM–ATR–Chk1 and DNA-PK pathways in a cell-free system

A cell-free system made from equal volumes of HeLa nuclear and S100 cytoplasmic extracts supplemented with the protein phosphatase inhibitor okadaic acid reproduces certain DNA damage responses in response to double-stranded oligonucleotides [10]. Incubation of the extracts with pre-annealed 70mers of poly(dA) and poly(dT) caused the DNA-dependent activation of ATR, as detected by the phosphorylation of Claspin and the effector kinase Chk1 (Fig. 1A). Phosphorylation of Chk1 occurred at two activating sites, Ser317 and Ser345, which are targeted by ATR [11]. Consistent with its activation, Chk1 also became phosphorylated at Ser296, an autophosphorylation site [10].

Poly(dAdT) also stimulated the activation of DNA-PK, as indicated by increased autophosphorylation on Ser2056 of DNA-PKcs

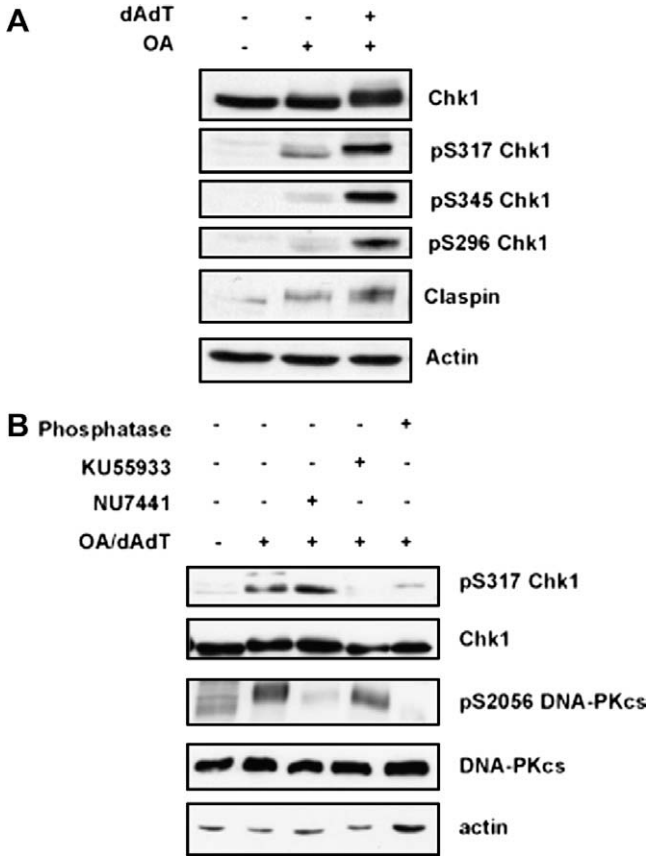


Fig. 1. Activation of ATM–ATR–Chk1 and DNA-PK pathways in human cell-free system. (A) Mixed HeLa nuclear and cytoplasmic extracts were incubated with the protein phosphatase inhibitor okadaic acid (OA) plus poly(dAdT) to mimic DNA damages responses. Samples were then analysed by SDS–PAGE and Western blotting with the indicated antibodies directed against Claspin, Chk1, specific Chk1 phosphorylation sites and actin as a loading control. Activation of ATR causes phosphorylation of Claspin and Chk1, indicated by their upshift. Phosphorylation Chk1 at Ser 317 and Ser345 leads to its activation, indicated by Ser296 phosphorylation. (B) Poly(dAdT) activates DNA-PK, detected by autophosphorylation of the catalytic subunit (DNA-PKcs) at S2056 (pS2056 DNA-PKcs). DNA-PK activity is blocked by NU7441, whereas Chk1 phosphorylation is blocked by the ATM inhibitor KU55933.

(Fig. 1B). Phosphorylation of DNA-PKcs Ser2056 was prevented by the DNA-PK inhibitor NU7441 [8], which did not inhibit phosphorylation of Chk1 Ser317. By contrast, the ATM inhibitor KU55933 blocked Chk1 Ser317 phosphorylation, demonstrating that Chk1 phosphorylation is dependent on ATM in this system, whereas DNA-PK activation was not blocked by KU55933. As expected, both Chk1 Ser317 and DNA-PKcs Ser2056 phosphorylation were inhibited by alkaline phosphatase. Thus, both the ATM–ATR–Chk1 and the DNA-PK pathways are activated in response to double-stranded DNA (dsDNA) in this cell-free system.

Identification of hnRNP-U as a prominent dsDNA-binding protein

We wished to exploit the cell-free system to identify novel components of DNA damage signalling pathways by purifying proteins that co-precipitate with poly(dAdT). To facilitate purification, a 5'-biotin moiety was added to the dA_{70} strand, which did not impede the phosphorylation of Chk1 (data not shown). After incubation in extracts, 5′-biotin-poly(dAdT) retrieved on streptavidin-beads specifically coprecipitated a prominent polypeptide of apparent M_r 120,000 (Supplementary Fig. 1A). Excision of this polypeptide, its tryptic digestion and analysis by mass spectrometry identified it as heterogeneous nuclear ribonucleoprotein U

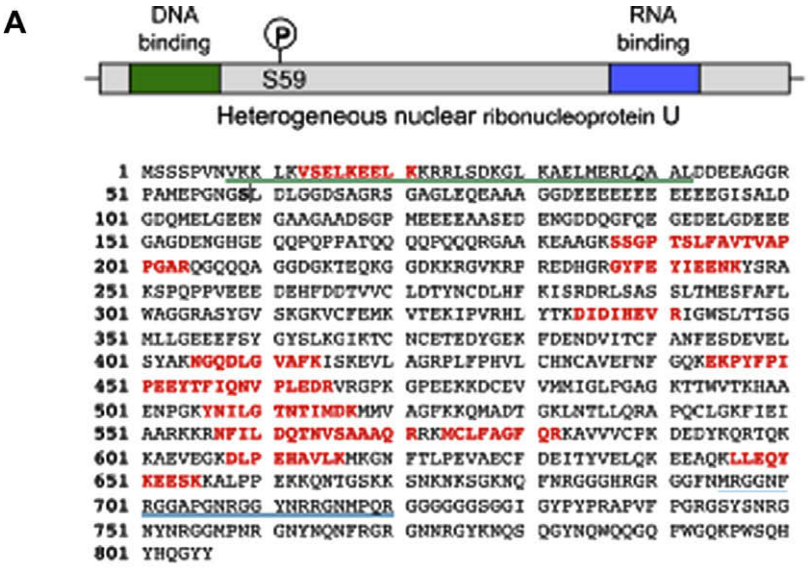
(hnRNP-U), also termed scaffold attachment factor A (SAF-A). hnRNP-U is an abundant nuclear phosphoprotein with regions required for DNA and RNA binding, respectively [12]. The protein has a predicted M_r 88,939 but migrates with apparent M_r 120,000 on SDS–PAGE [12] (Fig. 2).

hnRNP-U is phosphorylated at Ser59 by DNA-PK in response to poly(dAdT) in vitro

To determine if hnRNP-U might be a substrate for DNA-activated protein kinases involved in damage signalling, we analysed hnRNP-U phosphorylation by SDS–PAGE using PhosTag, which specifically retards the migration of phosphorylated proteins [13]. We found that hnRNP-U was indeed phosphorylated in the cell-free system in response to poly(dAdT), but not single-stranded dA_{70} (Fig. 3A). The upshift was abolished by treatment with alkaline phosphatase, confirming that it is due to phosphorylation. Interestingly, the shift was also blocked by NU7441, showing that hnRNP-U phosphorylation was dependent on DNA-PK activity. The amount of phosphorylated hnRNP-U was reduced by the ATM inhibitor KU55933, but was not inhibited by staurosporine, which inhibits Chk1 and other kinases [14].

Analysis of hnRNP-U precipitated by 5′-biotin-poly(dAdT) by QTrap mass spectrometry identified a single major phosphorylated residue at Ser59 (Supplementary Fig. 1). The primary sequence context of the phosphorylation site is perfectly conserved in different mammals (data not shown) and has a leucine immediately C-terminal to the phosphorylated residue followed by an aspartate, similar to sites in some other proteins that are phosphorylated by DNA-PK (Fig. 2B). The absence of glutamine at position 60 in hnRNP-U indicates that Ser59 is unlikely to be also phosphorylated directly by ATM or ATR.

To study the phosphorylation of Ser59 in hnRNP-U a polyclonal antibody was raised against a phosphopeptide corresponding to the site and purified it so that it only recognised phosphoryated hnRNP-U (Supplementary Figs. 2A and B). Using this antibody, we confirmed that hnRNP-U was phosphorylated in response to activation of DNA damage responses in the cell-free system (Fig. 3B). Phosphorylation of Ser59 was dependent on DNA-PK, since it was completely inhibited by NU7441. As expected, Chk1 phosphorylation at Ser317 and Ser296 was not affected by NU7441, but was strongly inhibited by KU5933, while phosphorylation of Ser296 was also blocked by staurosporine, consistent with this being an autophosphorylation event. These results demonstrate that hnRNP-U phosphorylation and Chk1 phosphorylation in response to DNA damage signalling in the cell-free system are dependent on the DNA-PK and ATM–ATR pathways, respectively. However, phosphorylation of Ser59 of hnRNP-U was also partially



B

Protein	Site	Sequence	Reference
hnRNP U	S59	PAMEPGNGSLDLGGDSAG	this paper
XRCC4	S260	SKDDSIISSLDVTDIAPS	[6]
XLF/Cernunnos	S245	DPHTSNSASLQGIDSQCV	[24]
Cds1(Xenopus)	S39	SSSSGTLSSLDTVPVQDL	[25]
DNA-PKcs	S2056	TGVQSYSYSQDPRPATGR	[26]
	T2609	VLTPMFVETQASQGTLQT	[26]
H2A.X	S139	GGKKATQASQEY-COOH	[27]
Artemis	S516	PSSTVAGGSQSPKLFSDS	[28]
	S645	LNLSTNADSQSSSDFEVP	[28]
DNA ligase IV	T260	EHLKAPNLTNVNKISNIF	[29]

Fig. 2. The structure and primary sequence of hnRNP-U. (A) Top, linear representation showing site of phosphorylation (S59) and DNA and RNA binding domains (green and blue, respectively). Bottom, amino acid sequence showing peptides identified by mass spectrometry (red), DNA binding domain (underlined in green) and RNA binding domain (underlined in blue). The phosphorylated residue is boxed. (B) Comparison of sites phosphorylated by DNA-PK in selected proteins [6,24–29]. Phosphorylated residues are underlined. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

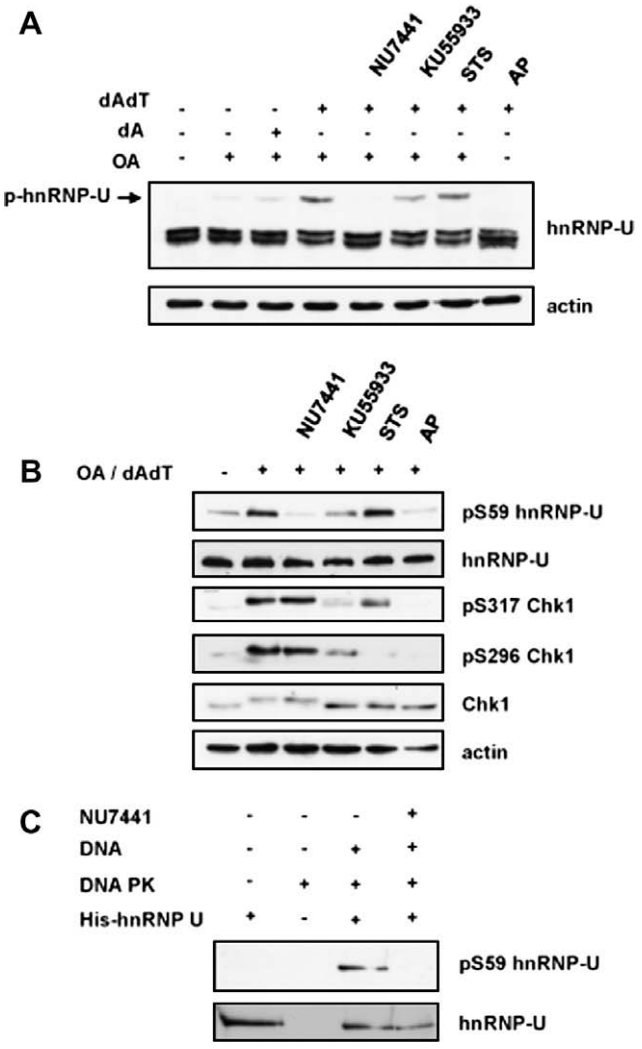


Fig. 3. hnRNP-U is phosphorylated by DNA-PK in a cell-free system. (A) Effects of protein kinase inhibitors on the phosphorylation of hnRNP-U. Phosphorylated hnRNP-U (indicated by arrow) was visualised by the upshift of the protein detected by Western blotting following separation on a PhosTag acrylamide gel. STS, staurosporine; AP, alkaline phosphatase. (B) Effects of protein kinase inhibitors on the phosphorylation of hnRNP-U at Ser59. Phosphorylation of hnRNP-U and Chk1 was detected by a site-specific antibodies. (C) Direct phosphorylation of hnRNP-U Ser59 by DNA-PK.

inhibited by KU5933 (Fig. 3B), consistent with the effect on hnRNP-U phosphorylation analysed using PhosTag (Fig. 3A), showing that the DNA-PK-activation is partially dependent on ATM activity in this system, as in cells [15].

To test if DNA-PK can directly phosphorylate Ser59 of hnRNP-U, we performed an *in vitro* kinase assay using recombinant His_6-hnRNP-U and purified DNA-PK. We confirmed that DNA-PK catalyses the phosphorylation of Ser59 using the specific antibody and that this reaction was completely inhibited by NU7441 (Fig. 3C).

Phosphorylation of hnRNP-U at Ser59 in human cells in response to DNA double-strand breaks requires DNA-PK

To study the phosphorylation of hnRNP-U in human cells, we treated U2OS cells with a variety of different drugs that elicit DNA damage or DNA replication stress responses, namely: etoposide, a topoisomerase II inhibitor causing DSB; camptothecin, a topoisomerase I inhibitor causing SSB; cisplatin, a DNA interchelator; bleomycin, another DNA interchelator; hydroxyurea, an inhibitor of dNTP synthesis that blocks DNA replication; aphidicolin, an inhibitor of DNA polymerases. The microtubule poison nocodazole was used as a control. As expected, only etoposide induced substantial DSB responses within 1 h, as indicated by the phosphorylation of H2A.X (γH2AX), whereas Chk1 was equally well phosphorylated at Ser317 in response to etoposide and aphidicolin, and was more weakly phosphorylated in response to other DNA damaging drugs. There was strong autophosphorylation of DNA-PK on Ser2056 only in response to etoposide, showing that DNA-PK was activated specifically in response to DSB generated by this drug (Fig. 4A). Similarly, we found that hnRNP-U was phosphorylated on Ser59 only in response to etoposide (Fig. 4A). hnRNP-U

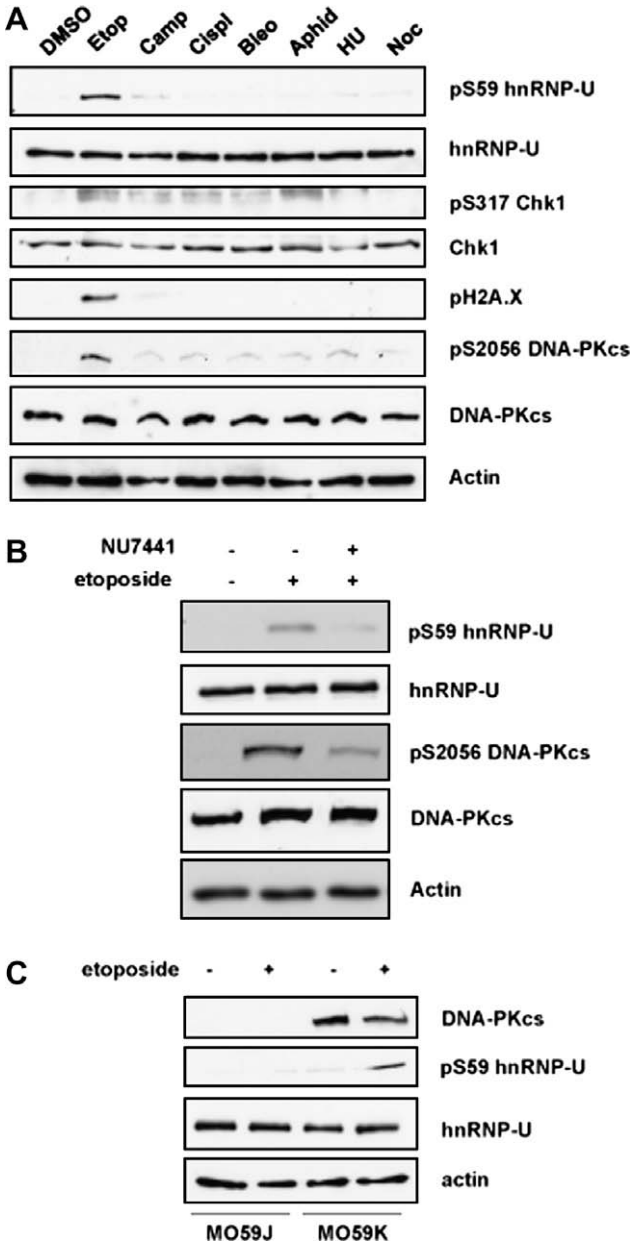


Fig. 4. Phosphorylation of hnRNP-U Ser59 in cells in response to etoposide is dependent on DNA-PK. (A) Analysis of the phosphorylation of hnRNP-U Ser59 in U2OS cells treated with DNA damaging agents (Etop, etoposide; Camp, camptothecin; Cispl, cisplatin; Bleo, bleomycin; Aphid, aphidicolin, HU, hydroxyurea) or nocodazole (Noc). The carrier DMSO (0.1%) was used as a control. (B) Phosphorylation of hnRNP-U Ser59 in U2OS cells in response to etoposide was inhibited by the DNA-PK inhibitor NU7441. (C) Phosphorylation of hnRNP-U Ser59 in response to etoposide was abolished in MO59J cells that lack DNA-PKcs, but not matched MO59K cells that express DNA-PKcs.

was also phosphorylated in response to ionizing radiation, which creates DSBs (data not shown). These results show that hnRNP-U is phosphorylated in human cells in response to DSB when DNA-PK is activated and not in response to other forms of DNA damage or DNA replication stress.

Phosphorylation of hnRNP-U at Ser59 in U2OS cells in response to etoposide was strongly inhibited by NU7441 to a similar extent as DNA-PKcs autophosphorylation (Fig. 4B). Furthermore, the phosphorylation of hnRNP-U at Ser59 after etoposide treatment was absent in MO59J cells that lack DNA-PKcs [16], but was still apparent in the matched cell line MO59K that expresses DNA-PKcs (Fig. 4C). Thus, phosphorylation of hnRNP-U at Ser59 is dependent on DNA-PK in human cells. These results indicate that the Ser59 site is phosphorylated directly and exclusively by DNA-PK in response to DSB.

Discussion

Cellular responses to DNA damage are important for surveillance against genomic instability. Components of DNA damage signalling pathways are potential targets for drugs that could sensitize cancer cells to radiation or chemotherapy, although they may be aberrant in certain cancer cells. In order to define the components of these pathways in cancer cells and how they might be targeted to improve anti-cancer therapies, it is important to identify specific substrates of the kinases ATM, ATR and DNA-PK, which have distinct biological roles. Here, we have identified hnRNP-U as a *bona fide* cellular substrate for DNA-PK that is targeted at Ser59 solely by this kinase and not ATM or ATR in response to DSB. Thus, phosphorylation of hnRNP-U Ser59 may be used as a specific marker for DNA-PK activity in cells.

We identified hnRNP-U as a DNA-PK substrate initially using a cell-free system that we show reproduces both the Chk1 pathway [7] and the DNA-PK pathway (this paper). We find that Chk1 phosphorylation in response to dsDNA molecules in this system is ATM-dependent, being strongly inhibited by KU55933. This is consistent with ATR activation being dependent on ATM [17]. Interestingly, the activation of DNA-PK by poly(dA/dT) is also partially dependent on ATM activity, suggesting a role for ATM in the recognition of DSB or the subsequent activation of DNA-PK. The extracts offer a useful biochemical system for the dissection of these divergent pathways.

The phosphorylation of hnRNP-U Ser59 by DNA-PK in cells suggests that hnRNP-U may play a role in mediating those cellular responses to DSBs that are mediated specifically by DNA-PK. hnRNP-U, also known as SAF-A, is an abundant nuclear protein which is essential in development [18] and has had several functions ascribed to it, including regulation of transcription and RNA processing [19,20]. Interestingly, hnRNP-U has been found associated with WT1 (Wilms' tumour 1) protein and is itself encoded by a potential Wilms' tumour gene [21]. hnRNP-U has also been reported to function as a pseudosubstrate inhibitor of $SCF^{\beta\text{-}TRCP}$ [22], an E3 ubiquitin ligase that has roles in DNA damage responses through degradation of cell cycle regulators such as Cdc25A and Claspin [2]. Furthermore, hnRNP-U interacts with MDM2, an E3 ubiquitin ligase that is a negative regulator of p53, which plays a central role in transcriptional responses to DNA damage [23]. Each of these interactions is potentially regulated by hnRNP-U phosphorylation in response to DNA-PK activation by DSB.

Acknowledgments

We thank Dr. Lindsey Allan (University of Dundee) for assisting with the hnRNP-U constructs. Professor Carl Smythe (University of Sheffield) and Dr. Graeme Smith (Kudos) kindly provided MO59J cells and protein kinase inhibitors, respectively. This work was funded by Association of International Cancer Research (AICR) and a Royal Society-Wolfson Research Merit Award (to P.R.C.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.02.019.

References

[1] K. Hiom, DNA repair: how to PIKK a partner, Curr. Biol. 15 (2005) R473–R475.
[2] J.W. Harper, S.J. Elledge, The DNA damage response: 10 years after, Mol. Cell. 28 (2007) 739–745.
[3] S.J. Collis, T.L. DeWeese, P.A. Jeggo, A.R. Parker, The life and death of DNA-PK, Oncogene 24 (2005) 949–961.
[4] B. Muller, J. Blackburn, C. Feijoo, X. Zhao, C. Smythe, DNA-activated protein kinase functions in a newly observed S phase checkpoint that links histone mRNA abundance with DNA replication, J. Cell Biol. 179 (2007) 1385–1398.
[5] S.T. Kim, D.S. Lim, C.E. Canman, M.B. Kastan, Substrate specificities and identification of putative substrates of ATM kinase family members, J. Biol. Chem. 274 (1999) 37538–37543.
[6] Y. Yu, B.L. Mahaney, K. Yano, R. Ye, S. Fang, P. Douglas, D.J. Chen, S.P. Lees-Miller, DNA-PK and ATM phosphorylation sites in XLF/Cernunnos are not required for repair of DNA double strand breaks, DNA Repair (Amst.) 7 (2008) 1680–1692.
[7] C.A.L. Clarke, P.R. Clarke, DNA-dependent phosphorylation of Chk1 and Claspin in a human cell-free system, Biochem. J. 388 (2005) 705–712.
[8] J.J. Leahy, B.T. Golding, R.J. Griffin, I.R. Hardcastle, C. Richardson, L. Rigoreau, G.C. Smith, Identification of a highly potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor (NU7441) by screening of chromenone libraries, Bioorg. Med. Chem. Lett. 14 (2004) 6083–6087.
[9] I. Hickson, Y. Zhao, C.J. Richardson, S.J. Green, N.M. Martin, A.I. Orr, P.M. Reaper, S.P. Jackson, N.J. Curtin, G.C. Smith, Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM, Cancer Res. 64 (2004) 9152–9159.
[10] C.A.L. Clarke, L.N. Bennett, P.R. Clarke, Cleavage of claspin by caspase-7 during apoptosis inhibits the Chk1 pathway, J. Biol. Chem. 280 (2005) 35337–35345.
[11] H. Zhao, H. Piwnica-Worms, ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1, Mol. Cell. Biol. 21 (2001) 4129–4139.
[12] M. Kiledjian, G. Dreyfuss, Primary structure and binding activity of the hnRNP U protein: binding RNA through RGG box, EMBO J. 11 (1992) 2655–2664.
[13] E. Kinoshita, E. Kinoshita-Kikuta, K. Takiyama, T. Koike, Phosphate-binding tag, a new tool to visualize phosphorylated proteins, Mol. Cell. Proteomics 5 (2006) 749–757.
[14] B. Zhao, M.J. Bower, P.J. McDevitt, H. Zhao, S.T. Davis, K.O. Johanson, S.M. Green, N.O. Concha, B.B. Zhou, Structural basis for Chk1 inhibition by UCN-01, J. Biol. Chem. 277 (2002) 46609–46615.
[15] B.P. Chen, N. Uematsu, J. Kobayashi, Y. Lerenthal, A. Krempler, H. Yajima, M. Lobrich, Y. Shiloh, D.J. Chen, Ataxia telangiectasia mutated (ATM) is essential for DNA-PKcs phosphorylations at the Thr-2609 cluster upon DNA double strand break, J. Biol. Chem. 282 (2007) 6582–6587.
[16] S.P. Lees-Miller, R. Godbout, D.W. Chan, M. Weinfeld, R.S. Day 3rd, G.M. Barron, J. Allalunis-Turner, Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line, Science 267 (1995) 1183–1185.
[17] A. Jazayeri, J. Falck, C. Lukas, J. Bartek, G.C. Smith, J. Lukas, S.P. Jackson, ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks, Nat. Cell Biol. 8 (2006) 37–45.
[18] M.J. Roshon, H.E. Ruley, Hypomorphic mutation in hnRNP U results in post-implantation lethality, Transgenic Res. 14 (2005) 179–192.
[19] A.M. Krecic, M.S. Swanson, HnRNP complexes: composition, structure, and function, Curr. Opin. Cell Biol. 11 (1999) 363–371.
[20] M. Yugami, Y. Kabe, Y. Yamaguchi, T. Wada, H. Handa, HnRNP-U enhances the expression of specific genes by stabilizing mRNA, FEBS Lett. 581 (2007) 1–7.
[21] L. Spraggon, T. Dudnakova, J. Slight, O. Lustig-Yariv, J. Cotterell, N. Hastie, C. Miles, HnRNP-U directly interacts with WT1 and modulates WT1 transcriptional activation, Oncogene 26 (2007) 1484–1491.
[22] M. Davis, A. Hatzubai, J.S. Andersen, E. Ben-Shushan, G.Z. Fisher, A. Yaron, A. Bauskin, F. Mercurio, M. Mann, Y. Ben-Neriah, Pseudosubstrate regulation of the SCF(beta-TrCP) ubiquitin ligase by hnRNP-U, Genes Dev. 16 (2002) 439–451.
[23] H. Shimizu, L.R. Burch, A.J. Smith, D. Dornan, M. Wallace, K.L. Ball, T.R. Hupp, The conformationally flexible S9–S10 linker region in the core domain of p53 contains a novel MDM2 binding site whose mutation increases ubiquitination of p53 in vivo, J. Biol. Chem. 277 (2002) 28446–28458.
[24] Y. Yu, W. Wang, Q. Ding, R. Ye, D. Chen, D. Merkle, D. Schriemer, K. Meek, S.P. Lees-Miller, DNA-PK phosphorylation sites in XRCC4 are not required for survival after radiation or for V(D)J recombination, DNA Repair (Amst.) 2 (2003) 1239–1252.
[25] T.D. McSherry, P.R. Mueller, Xenopus Cds1 is regulated by DNA-dependent protein kinase and ATR during the cell cycle checkpoint response to double-stranded DNA ends, Mol. Cell. Biol. 24 (2004) 9968–9985.

[26] B.P. Chen, D.W. Chan, J. Kobayashi, S. Burma, A. Asaithamby, K. Morotomi-Yano, E. Botvinick, J. Qin, D.J. Chen, Cell cycle dependence of DNA-dependent protein kinase phosphorylation in response to DNA double strand breaks, J. Biol. Chem. 280 (2005) 14709–14715.
[27] T. Stiff, M. O'Driscoll, N. Rief, K. Iwabuchi, M. Lobrich, P.A. Jeggo, ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation, Cancer Res. 64 (2004) 2390–2396.
[28] S. Soubeyrand, L. Pope, R. De Chasseval, D. Gosselin, F. Dong, J.P. de Villartay, R.J. Hache, Artemis phosphorylated by DNA-dependent protein kinase associates preferentially with discrete regions of chromatin, J. Mol. Biol. 358 (2006) 1200–1211.
[29] Y.G. Wang, C. Nnakwe, W.S. Lane, M. Modesti, K.M. Frank, Phosphorylation and regulation of DNA ligase IV stability by DNA-dependent protein kinase, J. Biol. Chem. 279 (2004) 37282–37290.