Biochemical and Biophysical Research Communications 377 (2008) 231–235

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Nck-1 interacts with PKR and modulates its activation by dsRNA

Eric Cardin, Louise Larose *

Polypeptide Laboratory, Experimental Medicine Department, McGill University, Strathcona Building, 3640 University Street, W315, Montreal, Que., Canada H3A2B2

ARTICLE INFO

Article history:
Received 22 September 2008
Available online 1 October 2008

Keywords:
Nck-1
dsRNA
PKR
p38MAPK
Cell death

ABSTRACT

Activation of the double-stranded RNA (dsRNA)-activated protein kinase PKR results in inhibition of general translation through phosphorylation of the eukaryotic initiation factor 2 alpha-subunit on serine 51 (eIF2αSer51). Previously, we have reported that the adaptor protein Nck-1 modulates eIF2αSer51 phosphorylation by a subset of eIF2α kinases, including PKR. Herein, we demonstrate that Nck-1 prevents efficient activation of PKR by dsRNA, revealing that Nck-1 acts at the level of PKR. In agreement, Nck-1 impairs p38MAPK activation and attenuates cell death induced by dsRNA, in addition to diminish eIF2αϖ Ser51 phosphorylation. Our data show that the inhibitory effect of Nck-1 on PKR is reversible, as it could be overcome by increasing levels of dsRNA. Interestingly, we found that Nck-1 interacts with the inactive form of PKR, independently of its Src homology domains. Furthermore, we uncovered that Nck-1 is substrate of PKR *in vitro*. All together, our data provide the first evidence identifying Nck-1 as a novel endogenous regulator of PKR and support the notion that Nck-1–PKR interaction could be a way to limit PKR activation.

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The adaptor proteins Nck are recognized to mainly mediate signaling pathways linking membrane receptors to cytoskeleton rearrangement [1]. However, we have previously implicated Nck in signaling pathways regulating phosphorylation of the α-subunit of the eukaryotic initiation factor eIF2 on serine 51 (eIF2αSer51) by a subset of eIF2α kinases [2–4]. Among these, we established that Nck-1 modulates eIF2α phosphorylation by the dsRNA-activated protein kinase PKR. However, beside its eIF2α kinase activity, PKR also affects the activation of JNK, p38MAPK, and NFκB [5]. PKR has been implicated in several biological processes, but its main function is to mediate the interferons antiviral host response [6].

Activation of PKR involves its dimerization and autophosphorylation induced upon binding of viral or synthetic dsRNA [7], but cellular endogenous PKR activators also exist [5]. On the other hand, inhibitors of PKR were first identified from viruses which developed mechanisms to bypass PKR control in order to replicate [8], but only few endogenous inhibitors of PKR are known. In this study, we demonstrate that increased expression levels of Nck-1 impair activation of PKR by dsRNA. This results in attenuated PKR-dependent signaling events and associated cellular death, thus revealing Nck-1 as a novel endogenous modulator of PKR.

Materials and methods

Cell culture. HeLa cells were grown as described [4]. COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with antibiotics and 10% heat-inactivated fetal bovine serum (Invitrogen, USA).

Transfection. Lipofectamine-Plus reagent (Invitrogen) was used according to the manufacturer's instructions to transfect the following plasmids (1 μg): pRK5/HA-tagged human Nck-1 (provided by Dr. Li [9]), pcDNA 3.1/Flag-tagged human PKR wild type or dominant negative PKRΔ_6 (provided by Dr. Koromilas [10]) or corresponding empty vectors.

PKR activation. PKR activation was achieved by transfecting cells with 0.3 μg/ml (or as indicated) of the synthetic double-stranded RNA poly IC (GE Healthcare, USA) during 2 h using Lipofectamine-Plus according to the manufacturer's instructions. At the end of the transfection period, cells were washed once and kept in growth medium for an additional 2 h.

Antibodies. Antibodies used for Western blot of either total cell lysates normalized for protein content, GST pull down or coimmunoprecipitation assays were: anti-phospho-PKR (pT451) (BioSource™, Invitrogen), anti-phospho-p38MAPK (Thr180/Tyr182) (Cell Signaling Technology Inc., USA), anti-total PKR (Sigma, USA), anti-total p38MAPK (Santa Cruz Biotechnology, USA), phosphospecific eIF2αSer51 antibody (BioSource™, Invitrogen),

Abbreviations: dsRNA, double-stranded RNA; eIF2α, eukaryotic initiation factor 2 alpha-subunit; PKR, protein kinase RNA-activated; SH2, Src homology 2 domain; SH3, Src homology 3 domain; Poly IC or pIC, polyriboinosinic:polyribocytidylic acid; WB, Western blot; TCL, total cell lysate; MTT, methylthiazolyldiphenyl-tetrazolium bromide; DSP, dithiobis(succinimidyl)propionate; PP1c, protein phosphatase type 1 catalytic subunit; PAK1, p21-activated protein kinase 1.

* Corresponding author. Fax: +1 514 398 3923.
E-mail address: louise.larose@mcgill.ca (L. Larose).

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doi:10.1016/j.bbrc.2008.09.112

anti-total eIF2α antibody (Santa Cruz), anti-HA (Santa Cruz) and anti-Flag antibody (Sigma). Nck was immunoprecipitated using our anti-Nck antibody as previously reported [11]. Goat anti-rabbit or goat-anti mouse conjugated horseradish peroxidase were used as secondary antibodies (Bio-Rad, USA) and ECL detection performed as previously described [4].

Cell survival. Cells were plated in triplicate at 1.2×10^4 cells per well in 96 well plate 24 h following Nck-1 transfection and then subjected to transfection with poly IC as described above. Cell survival was measured using Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay [12].

Pull-down assay. Cells first transfected with Flag-PKR or Flag-PKRΔ_6, 36 h later were transfected again for 2 h with or without poly IC. PKRΔ_6, which lacks six amino acids (361–366) between catalytic domains IV and V, behaves as a dominant negative PKR [10]. Two hours post PKR activation, cells were harvested in lysis buffer (10 mM Tris–HCl, pH 7.5, 50 mM KCl, 1% Triton, 10 mM NaPPi, 100 mM NaF, 1 mM DTT, 1 mM activated orthovanadate, 17.5 mM β-glycerophosphate, 4 μg/ml aprotinin, 2 μg/ml leupeptin, 100 μg/ml pefablock, 1 mM benzamidine). For pull-down assays, 600 μg proteins (1.2 ml, HeLa) or 2 mg (1 ml, COS-7) of total cell lysate were mixed with bacterial recombinant GST or GST–Nck-1 immobilized on glutathione-agarose beads (20 μg) for 2 h at 4 °C. Pulled down proteins were analyzed for the presence of PKR by Western blot as described above.

Immunoprecipitation. COS-7 cells transfected with plasmids containing wild type or mutants HA-Nck-1 cDNA were treated with 2 mM of the reversible crosslinker agent dithiobis(succinimidyl)propionate (DSP) for 30 min at room temperature. Cells were then washed with PBS and lysed in RIPA buffer (50 mM Hepes, pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 10% Glycerol, 1.5 mM $MgCl_2$, 1 mM EGTA, 1 mM activated orthovanadate, 10 mM NaPPi, 100 mM NaF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, pefablock SC 0.1 μg/ml). Protein samples corresponding to 3 mg (1.3 ml) were immunoprecipitated with anti-HA antibodies, followed by the addition of protein A-agarose beads and further incubated for 1 h at 4 °C. Proteins bound on beads were processed for Western blot using indicated antibodies.

In vitro kinase assay. Bacterial recombinant GST-PKR (500 ng) was incubated with either GST–Nck-1 or GST (500 ng) in 30 μl of kinase reaction buffer (10 mM Tris–HCl, pH 7.7, 50 mM KCl, 2 mM $MgCl_2$, 5 mM β-mercaptoethanol, 2 μg/ml aprotinin, 1 μg/ml leupeptin, 0.1 mM PMSF). As control, GST-PKR (200 ng) was incubated with His-eIF2α (500 ng). Prewarmed samples were further incubated for 30 min at 30 °C following the addition of [γ-^{32}P]ATP (10 μCi). Reactions were stopped by adding Laemmli buffer and further processed by SDS–PAGE. Protein phosphorylation was visualized by autoradiography.

Results

PKR activation is impaired in cells overexpressing Nck-1

We have previously reported that overexpression of Nck-1 reduces PKR-mediated eIF2α phosphorylation [4]. To delineate the mechanism by which Nck-1 affects PKR-mediated eIF2α phosphorylation, we examined PKR activity in response to synthetic dsRNA in HeLa cells overexpressing Nck-1. Levels of phosphorylated PKR on Thr451, which correlates with PKR activation [7], were significantly increased by 4.6-fold in control mock transfected cells following poly IC treatment. In contrast, phosphorylation of PKR induced by poly IC was significantly reduced in Nck-1 overexpressing cells. Indeed, it was only induced by 1.6-fold and did not reach statistical significance (Fig. 1A). These results demonstrate that increased levels of Nck-1 in Hela cells prevent efficient PKR activation by dsRNA.

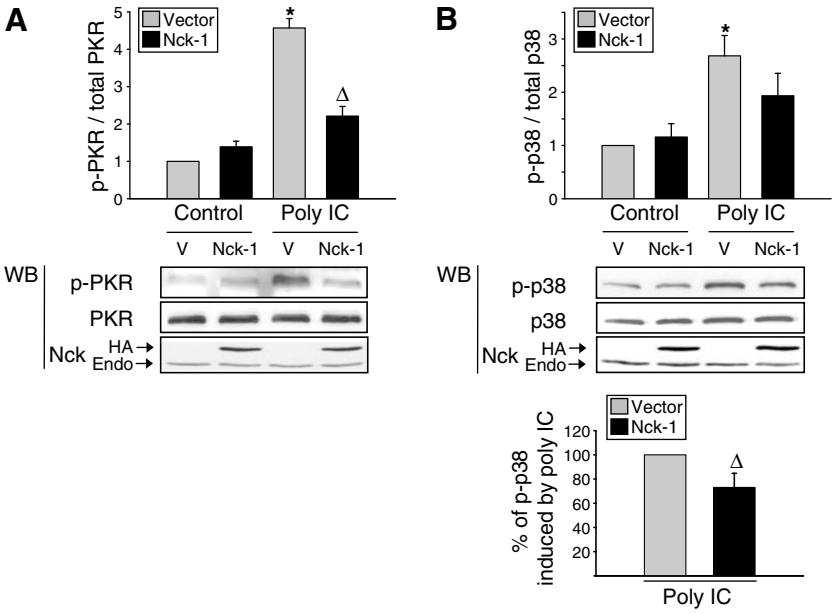

Fig. 1. Nck-1 reduces PKR and p38 MAPK activation induced by dsRNA. (A) HeLa cells were transfected with empty vector (V) or vector containing HA-Nck-1 (Nck-1) cDNA prior to be subjected 24 h later to a second transfection with synthetic dsRNA (poly IC). Indicated proteins were detected by Western blotting of cell lysates adjusted for protein content using specific antibodies (lower panel). Densitometry and statistical analyses (Student's *t*-test) were performed on data obtained from three independent experiments. Results are reported as the means ± SEM of the ratio phospho-PKR (p-PKR) over total PKR normalized to control empty vector in each experiment (upper panel). $^{*}p < 0.001$ relative to vector control, $^{\Delta}p < 0.01$ relative to vector with poly IC. (B) Following the strategy described in (A), the indicated proteins were detected by Western blotting of cell lysates (middle panel). Densitometry and statistical analyses (Student's *t*-test) were performed on data obtained from seven independent experiments. Results are presented as the means ± SEM of the ratio phospho-p38 MAPK (p-p38) over total p38 MAPK (p38) normalized to control empty vector in each experiment (upper panel). $^{*}p < 0.001$ relative to vector control. Effect of Nck-1 on phopho-p38 MAPK induced by poly IC was calculated as a percentage induction relative to vector-transfected cells (lower panel). $^{\Delta}p < 0.01$ relative to vector with poly IC.

dsRNA-induced p38MAPK activation is impaired in cells overexpressing Nck-1

To assess whether limited PKR activation in Nck-1 overexpressing cells also results in concomitant decrease in phosphorylation and activation of PKR downstream effectors other than eIF2α, we investigated the levels of p38MAPK phosphorylation in response to dsRNA. As shown in Fig. 1B, phosphorylation of p38MAPK, which correlates with p38MAPK activation, was significantly increased by 2.7-fold in control mock transfected cells treated with poly IC. However, in Nck-1 overexpressing cells, induction of p38MAPK phosphorylation by poly IC was lower than 2-fold and not statistically significant (upper panel). The inhibitory effect of Nck-1 was further revealed by showing that poly IC-induced p38MAPK activation was significantly reduced in Nck-1 overexpressing cells compared to control cells (lower panel). These data reveal that transient overexpression of Nck-1 in HeLa cells results in impaired PKR-mediated p38MAPK phosphorylation/activation in response to dsRNA.

dsRNA-induced cell death is attenuated in cells overexpressing Nck-1

Long term activation of PKR by dsRNA is recognized to induce cell death [13]. Therefore, we next assessed whether overexpressing Nck-1 could protect cells from dsRNA-induced death. As shown in Fig. 2A, poly IC-induced cell death was significantly decreased by 45% in cells overexpressing Nck-1 when poly IC was transfected at 0.2 μg/ml. However, when poly IC during transfection was increased to 2 μg/ml to further induce PKR activation, overexpression of Nck-1 still decreased cell death by 27%, although this effect was no more statistically significant. Interestingly, activation of PKR with higher concentrations of poly IC also resulted in the loss of the effect of Nck-1 on PKR-mediated eIF2αSer51 phosphorylation (Fig. 2B). These data indicate that increased levels of Nck-1 protect cells from dsRNA-induced cell death. In addition, these reveal that the inhibitory effect of Nck-1 on PKR is reversible, as it is could be overcome in stress conditions involving higher levels of PKR activators.

Nck-1 interacts with PKR

PKR amino acid sequence is devoid of classical consensus binding site for SH3 domains, but numerous atypical SH3 domain binding motifs are noticed [14]. In addition, PKR has been recently reported to become phosphorylated on tyrosine residues upon activation [15], creating potential binding sites for SH2 domain-containing proteins [16]. These observations prompted us to determine whether Nck-1 through it SH2 or SH3 domains could interact with PKR. For this, we performed *in vitro* pull-down experiments using GST fusion protein of Nck-1 and lysates prepared from cells transiently expressing Flag-tagged wild type PKR, transfected or not with poly IC. As shown in Fig. 3A, GST–Nck-1 binds Flag-PKR from control mock transfected cells while GST does not. Surprisingly, PKR binding to GST–Nck-1 was not observed in cells transfected with poly IC, suggesting that Nck-1 interacts only with inactive PKR. To further test whether dsRNA-mediated PKR activation abrogates Nck-1 interaction, we subjected lysates from cells transiently expressing Flag-tagged dominant negative PKR ($PKR\Delta_6$) transfected or not with poly IC to pull-down assays with GST–Nck-1 (Fig. 3B). In contrast to what was observed for PKR WT, Flag-$PKR\Delta_6$ although interacting slightly with GST alone, binds GST–Nck-1 independently of poly IC transfection. These results strongly support that Nck-1 interacts exclusively with inactive PKR and suggest that Nck-1 does not compete with dsRNA for PKR binding.

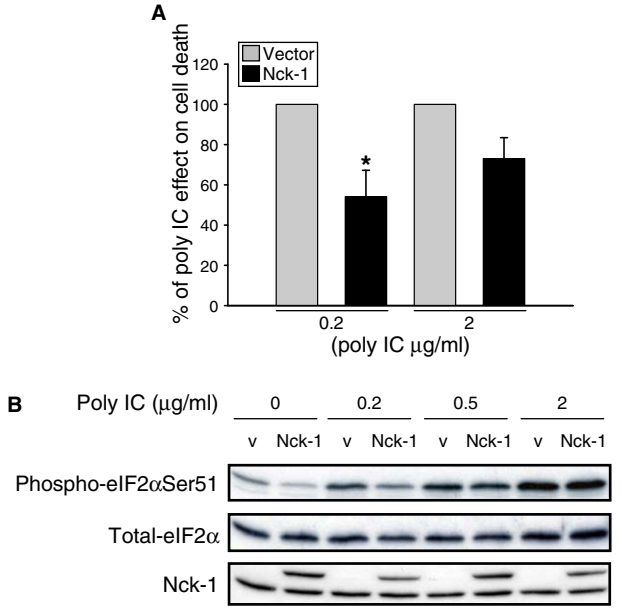

Fig. 2. Nck-1 reduces cell death induced by dsRNA. (A) HeLa cells were transfected with empty vector or vector containing HA-Nck-1 cDNA, and grown for 24 h prior to be plated in 96 well plates and transfected with poly IC at the indicated concentrations. After 24 h, cell viability was measured using MTT assay as described in Materials and methods. Results are presented as the means ± SEM of the percentage (%) of poly IC-induced cell death normalized to empty vector-transfected cells in each experiment. Statistical analyses (Student's *t*-test) were performed on data obtained from three independent experiments. $^{*}p < 0.05$ relative to vector with 0.2 μg/ml poly IC). (B) Following the strategy described in Fig. 1, cells were treated with increasing concentration of poly IC and the indicated proteins were detected by Western blotting.

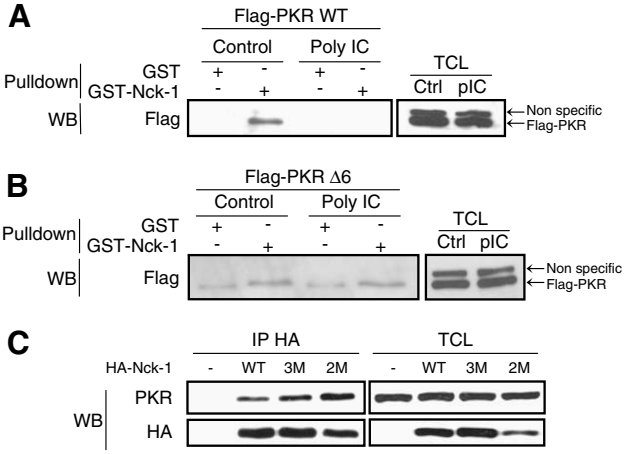

Fig. 3. Nck-1 binds to PKR. (A) Pull-down of Flag-PKR with GST-Nck-1. HeLa cells transiently expressing wild type human Flag-PKR were transfected with poly IC (pIC) or Mock transfected (Control or Ctrl). GST or GST–Nck-1 were used in pull-down and Flag-PKR was detected by Western blotting (WB) using a Flag specific antibody. TCL, total cell lysate. (B) Pull-down of dominant negative Flag-PKR with GST-Nck-1. COS-7 cells transiently expressing dominant negative Flag-PKRΔ6 (Δ6) were transfected with poly IC (pIC) or Mock transfected (Control or Ctrl). GST or GST–Nck-1 were used in pull-down and Flag-PKR was detected by Western blotting (WB) using a Flag specific antibody. (C) Coimmunoprecipitation of PKR with wild type or mutants HA-Nck-1. COS-7 cells were transfected with wild type HA-Nck-1 (WT) or HA-Nck-1 mutants with all three SH3 mutated (3M) or only the SH2 mutated (2M). Using an anti-HA antibody, HA immunoprecipitates were prepared using lysates from COS-7 cells previously exposed to the cross linker agent DSP. PKR and HA-Nck-1 proteins in the immunoprecipitated samples were detected by Western blotting using specific antibodies.

We next addressed which Nck-1 SH domain(s) mediate(s) its interaction with PKR by using HA-tagged Nck-1 wild type and mutants. We observed equivalent amount of endogenous PKR coimmunoprecipitated with HA-Nck-1 wild type compared to HA-Nck-1 mutated in the three SH3 domains (3M) or the SH2 domain (2M) (Fig. 3C). Western blotting of total cell lysate (TCL) using an anti-HA antibody indicated similar amount of HA-Nck-1 proteins immunoprecipitated except for the HA-Nck-1 2 M, which appears to be expressed at a lower level. All together these results indicate that Nck-1 binds to inactive PKR through a mechanism that is apparently independent of the SH domains.

Nck-1 is phosphorylated by PKR in vitro

Having discovered Nck-1 and PKR interaction, and shown that this interaction is lost upon PKR activation, we then proposed that Nck-1 could be phosphorylated by PKR. To determine whether Nck-1 is a substrate of PKR, we assembled *in vitro* kinase assays involving purified recombinant activated GST-PKR incubated with GST or GST–Nck-1 fusion proteins in presence of [γ-^{32}P]ATP. In these assays, we observed that GST–Nck-1 was clearly phosphorylated by GST–PKR while GST was not (Fig. 4, upper panels). Interestingly, a degradation product of GST–Nck-1 detected by Coomassie blue staining of the GST–Nck-1 preparation was also found phosphorylated by GST-PKR. A rough estimation of the size of this degradation product suggests that Nck-1 phosphorylation site(s) could be localized in its amino terminal portion that encompasses the three SH3 domains. At last, as expected in similar assay we showed that GST-PKR phosphorylates eIF2α, confirming the proper activation state of GST-PKR. These results provide evidence suggesting that Nck-1 might be a novel PKR substrate.

Discussion

PKR, a dual protein kinase activated by double-stranded RNA [15], is well known to play an important role in host antiviral response [6]. dsRNA-mediated PKR activation involves conformational change leading to PKR dimerization and autophosphorylation on Thr446 and Thr451 in its activation loop [7]. PKR inhibitors have been identified from viruses and recently, few were uncovered from mammalian cells. In this study, we identify the adaptor protein Nck-1 as a reversible inhibitor of PKR. Indeed, we have shown a clear effect of Nck-1 in limiting PKR activation and signaling induced by dsRNA. Our findings that Nck-1 and PKR exist in a common molecular complex strongly suggest that Nck-1 presumably by interacting with PKR, limits PKR activation. In addition, the exclusive interaction between Nck-1 and the inactive form of PKR further suggests that PKR activation promotes dissociation of Nck-1 from this complex. Therefore, Nck-1 binding to inactive PKR could be a way to limit PKR activation.

The exact mechanism by which Nck-1 modulates PKR activation remains to be established. Among potential mechanisms, Nck-1 could directly inhibit PKR. Alternatively, Nck-1 could recruit PKR inhibitor(s) in PKR close proximity. Supporting this, we previously demonstrated that Nck-1 participates in the assembly of a molecular complex containing the serine/threonine protein phosphatase PP1c [3]. Although it has been previously reported that PP1c inhibits PKR by directly interacting with PKR [17], we cannot exclude a role for Nck-1 in targeting PP1c to PKR as reported for CReP and GADD34, which both target PP1c to eIF2α [18] and [19]. Finally, Nck-1 could limit PKR activation by acting as a pseudosubstrate. Supporting this, we report that Nck-1 interacts exclusively with the inactive form of PKR, independently of the SH domains, in addition to be substrate for PKR. Further investigation aims to define

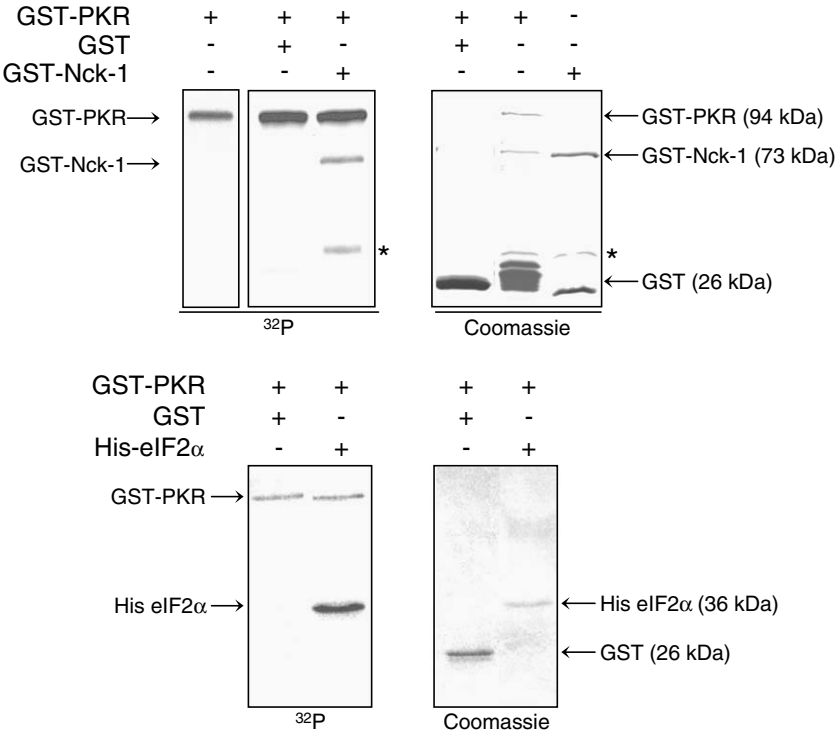

Fig. 4. Nck-1 is phosphorylated by PKR *in vitro*. Autoradiography of *in vitro* kinase assay using GST-PKR alone and with either GST or GST–Nck-1. Samples were prepared as described in Material and methods, and subjected to SDS–PAGE and autoradiography (upper left panels). Coomassie blue staining of GST, GST–PKR or GST–Nck-1 preparations are also presented (upper right panel). GST-PKR with either GST or His-eIF2α were used as positive control in an *in vitro* kinase assay processed as mentioned above, before being subjected to autoradiography (lower left panel). Coomassie blue staining of GST–PKR with either GST or His-eIF2α is also presented (lower right panel). * designate a degradation product of GST–Nck-1 that is phosphorylated by PKR.

the molecular determinants mediating recruitment of Nck-1 in a PKR complex are necessary to understand the underlying mechanism and establish the significance of this complex.

Interestingly, our findings describing a novel link between Nck-1 and PKR, parallel the established relationship between Nck-1 and the p21-activated protein kinase PAK1 [20]. In fact, Nck-1 directly binds to inactive PAK1, becomes phosphorylated by PAK1 and dissociates from activated PAK1 [21]. Nck-1/PAK1 dissociation has been proposed as a mechanism facilitating PAK1 relocalization to focal complexes [22]. In contrast to Nck-1/PAK1 interaction, which is mediated through the SH3 domains of Nck-1, we reported that Nck-1/PKR interaction does not involve the SH domains of Nck-1. However, analogous to its interaction with PAK1, Nck-1 interacts exclusively with the inactive PKR. It would be then interesting to determine whether PKR change conformation and/or autophosphorylation abolish binding of Nck-1 and whether Nck-1/PKR dissociation plays an important role in PKR maximal activation and/or subcellular relocalization. In parallel, it is of high interest to pursue the significance of Nck-1 phosphorylation by PKR based on growing evidence suggesting that phosphorylation of adaptor proteins regulate molecular complex formation and protein localization, both mechanisms by which adaptor proteins mediate cellular signaling [23]. Nck-1 contains numerous potential serine, threonine and tyrosine phosphorylation sites [24] and has been shown to be phosphorylated upon activation of numerous growth factor receptors [25–27]. Considering this, it would be significant to identify PKR phosphorylation site(s) on Nck-1 and assess their importance in Nck-1 ability to limit PKR activation and signaling.

In this study, we have shown a clear effect of Nck-1 in limiting PKR activation and signaling induced by dsRNA. Because these processes are crucial in mediating the interferons antiviral host response, it would be of importance to further investigate the contribution of Nck-1 in regulating viral replication.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada (RGPN 250215-02 to L.L.). E.C. is supported by the MUHC-RI from McGill University and L.L. is Chercheur National of the Fonds de la Recherche en Santé du Québec (FRSQ).

References

[1] W. Li, J. Fan, D.T. Woodley, Nck/Dock: an adapter between cell surface receptors and the actin cytoskeleton, Oncogene 20 (2001) 6403–6417.
[2] S. Kebache, E. Cardin, D.T. Nguyen, E. Chevet, L. Larose, Nck-1 antagonizes the endoplasmic reticulum stress-induced inhibition of translation, J. Biol. Chem. 279 (2004) 9662–9671.
[3] M. Latreille, L. Larose, Nck in a complex containing the catalytic subunit of protein phosphatase 1 regulates eukaryotic initiation factor 2alpha signaling and cell survival to endoplasmic reticulum stress, J. Biol. Chem. 281 (2006) 26633–26644.
[4] E. Cardin, M. Latreille, C. Khoury, M.T. Greenwood, L. Larose, Nck-1 selectively modulates eIF2alphaSer51 phosphorylation by a subset of eIF2alpha-kinases, FEBS J. 274 (2007) 5865–5875.
[5] A.J. Sadler, B.R. Williams, Structure and function of the protein kinase R, Curr. Top Microbiol. Immunol. 316 (2007) 253–292.
[6] M.A. Garcia, J. Gil, I. Ventoso, S. Guerra, E. Domingo, C. Rivas, M. Esteban, Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action, Microbiol. Mol. Biol. Rev. 70 (2006) 1032–1060.
[7] F. Zhang, P.R. Romano, T. Nagamura-Inoue, B. Tian, T.E. Dever, M.B. Mathews, K. Ozato, A.G. Hinnebusch, Binding of double-stranded RNA to protein kinase PKR is required for dimerization and promotes critical autophosphorylation events in the activation loop, J. Biol. Chem. 276 (2001) 24946–24958.
[8] R.J. Schneider, I. Mohr, Translation initiation and viral tricks, Trends Biochem. Sci. 28 (2003) 130–136.
[9] M. Chen, H. She, A. Kim, D.T. Woodley, W. Li, Nckbeta adapter regulates actin polymerization in NIH 3T3 fibroblasts in response to platelet-derived growth factor bb, Mol. Cell. Biol. 20 (2000) 7867–7880.
[10] A.E. Koromilas, S. Roy, G.N. Barber, M.G. Katze, N. Sonenberg, Malignant transformation by a mutant of the IFN-inducible dsRNA-dependent protein kinase, Science 257 (1992) 1685–1689.
[11] G. Lussier, L. Larose, A casein kinase I activity is constitutively associated with Nck, J. Biol. Chem. 272 (1997) 2688–2694.
[12] M.B. Hansen, S.E. Nielsen, K. Berg, Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill, J. Immunol. Methods 119 (1989) 203–210.
[13] G.N. Barber, The dsRNA-dependent protein kinase, PKR and cell death, Cell Death Differ. 12 (2005) 563–570.
[14] C.Y.H. Jia, J. Nie, C. Wu, C. Li, S.S.C. Li, Novel Src homology 3 domain-binding motifs identified from proteomic screen of a pro-rich region, Mol. Cell. Proteomics 4 (2005) 1155–1166.
[15] Q. Su, S. Wang, D. Baltzis, L.K. Qu, A.H. Wong, A.E. Koromilas, Tyrosine phosphorylation acts as a molecular switch to full-scale activation of the eIF2alpha RNA-dependent protein kinase, Proc. Natl. Acad. Sci. USA 103 (2006) 63–68.
[16] L.E. Marengere, T. Pawson, Structure and function of SH2 domains, J. Cell Sci. Suppl. 18 (1994) 97–104.
[17] S.L. Tan, S.U. Tareen, M.W. Melville, C.M. Blakely, M.G. Katze, The direct binding of the catalytic subunit of protein phosphatase 1 to the PKR protein kinase is necessary but not sufficient for inactivation and disruption of enzyme dimer formation, J. Biol. Chem. 277 (2002) 36109–36117.
[18] C. Jousse, S. Oyadomari, I. Novoa, P. Lu, Y. Zhang, H.P. Harding, D. Ron, Inhibition of a constitutive translation initiation factor 2alpha phosphatase, CReP, promotes survival of stressed cells, J. Cell Biol. 163 (2003) 767–775.
[19] I. Novoa, Y. Zhang, H. Zeng, R. Jungreis, H.P. Harding, D. Ron, Stress-induced gene expression requires programmed recovery from translational repression, EMBO J. 22 (2003) 1180–1187.
[20] W. Lu, S. Katz, R. Gupta, B.J. Mayer, Activation of Pak by membrane localization mediated by an SH3 domain from the adaptor protein Nck, Curr. Biol. 7 (1997) 85–94.
[21] G.M. Bokoch, Y. Wang, B.P. Bohl, M.A. Sells, L.A. Quilliam, U.G. Knaus, Interaction of the Nck adapter protein with p21-activated kinase (PAK1), J. Biol. Chem. 271 (1996) 25746–25749.
[22] Z.S. Zhao, E. Manser, L. Lim, Interaction between PAK and Nck: a template for Nck targets and role of PAK autophosphorylation, Mol. Cell. Biol. 20 (2000) 3906–3917.
[23] L. Cherezova, A. Gatesman, D.C. Flynn, The effects of phosphorylation on adaptor protein function, Front. Biosci. 7 (2002) d164–203.
[24] J.M. Lehmann, G. Riethmuller, J.P. Johnson, Nck, a melanoma cDNA encoding a cytoplasmic protein consisting of the src homology units SH2 and SH3, Nucleic Acids Res. 18 (1990) 1048.
[25] W. Li, P. Hu, E.Y. Skolnik, A. Ullrich, J. Schlessinger, The SH2 and SH3 domain-containing Nck protein is oncogenic and a common target for phosphorylation by different surface receptors, Mol. Cell. Biol. 12 (1992) 5824–5833.
[26] J. Meisenhelder, T. Hunter, The SH2/SH3 domain-containing protein Nck is recognized by certain anti-phospholipase C-gamma 1 monoclonal antibodies, and its phosphorylation on tyrosine is stimulated by platelet-derived growth factor and epidermal growth factor treatment, Mol. Cell. Biol. 12 (1992) 5843–5856.
[27] D. Park, S.G. Rhee, Phosphorylation of Nck in response to a variety of receptors, phorbol myristate acetate, and cyclic AMP, Mol. Cell. Biol. 12 (1992) 5816–5823.