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HCV-induced PKR activation is stimulated by the mitogen- and stress-activated protein kinase MSK2

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ABSTRACT

The replication of viral nucleic acids triggers cellular antiviral responses. The double-stranded RNA (dsRNA)-activated protein kinase (PKR) plays a key role in this antiviral response. We have recently reported that JFH-1 HCV replication in Huh-7 cells triggers PKR activation. Here we show that the HCV-induced PKR activation is further stimulated by the mitogen- and stress-activated protein kinase 2 (MSK2), a member of the 90 kDa ribosomal S6 kinase (RSK) family that has emerged as an important downstream effector of ERK and p38 MAPK signaling pathways. We show that MSK2 binds PKR and stimulates PKR phosphorylation, whereas the closely related MSK1 and RSK2 have no effect. Our data further indicate that MSK2 functions as an adaptor in mediating PKR activation, apparently independent of its catalytic activity. These results suggest that, in addition to viral dsRNA, stress signaling contributes to the regulation of cellular antiviral response.

1. Introduction

The dsRNA-activated protein kinase (PKR) is a key mediator of the cellular antiviral action of type I interferon (IFN). PKR is expressed constitutively in many tissues at a low level, before it is transcriptionally induced upon viral infection or following IFN treatment. Binding of dsRNA at the N-terminal domain of PKR further triggers the activation of its catalytic function, which resides in the C-terminal kinase domain. Besides auto-phosphorylation of PKR itself, the alpha-subunit of the heterotrimeric eukaryotic translation initiation factor 2 (eIF2α) is the best characterized substrate of PKR. Phosphorylation of eIF2α leads to the inhibition of viral and cellular protein synthesis and the subsequent restriction of viral spread. PKR is also implicated in the response to stress signals, cell proliferation and apoptosis [1].

Hepatitis C virus (HCV) is an enveloped virus with a single-stranded, positive-sense, RNA genome. Worldwide, more than 170 million people are chronically infected with the virus, and many of them are at the risk of developing chronic liver diseases and liver cancer [2]. At present, there are limited therapies for HCV infection and many patients do not adequately respond to IFN treatment. The antiviral role of PKR against HCV has been demonstrated with the recently developed HCV replicon systems [3,4]. We have shown that HCV replication triggers phosphorylation of PKR and eIF2α, and thereby inhibits viral protein synthesis and replication [5]. Alternatively, others have shown that HCV-mediated PKR activation can lead to the attenuated translation of cellular proteins as well, including the IFN-induced antiviral proteins [6] and IFN itself [7]. In the latter cases, PKR function was considered to restrict or delay cellular antiviral responses, which potentially contributes to the establishment of persistent infection.

Although dsRNA is the typical activator of PKR, several proteins can also activate PKR. Protein activator of the IFN-inducible protein kinase (PACT) interacts with PKR and activates it in response to cellular stresses that are induced by cytokines, ceramides and other chemicals [8]. The adaptor proteins of the TNF-receptor associated factor (TRAF) family were also shown to activate PKR in LPS-mediated TLR signaling pathways [9]. The mitogen- and stress-activated protein kinase (MSK) is a serine–threonine kinase related to the 90-kDa ribosomal S6 kinase (RSK or $p90^{rsk}$) family that mediates signal transduction downstream of the MAP kinase pathways [10]. Two MSKs (MSK1 and MSK2) and four RSKs (RSK1–RSK4) have been identified in mammals. While RSKs are activated by ERK, MSKs are activated by both ERK and p38 in response to mitogens and cellular stress. It was shown that RSK2 is involved in PKR activation following UVA-induced stress signaling [11], and in the antiviral response against influenza virus [12].

In this study, we show that MSK2 specifically binds PKR and stimulates PKR phosphorylation in HCV-replicating cells. These findings provide a pathway that connects cellular stress signaling to the regulation of PKR.

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2. Materials and methods

2.1. Cells and virus

Human hepatoma Huh-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 25 mM HEPES, 50 μg/ml of gentamicin and 5% fetal bovine serum (Invitrogen) at 37 °C in 5% CO_2. JFH-1 HCV (genotype 2a) RNA was synthesized in vitro and electroporated with Gene Pulser II RF module (Bio-Rad) as described [5]. Transient suppression of RSKs was achieved by electroporation of Huh-7 cells ($\sim 4 \times 10^6$) with 1 nmol of siRNA duplexes (Sigma) of the following target sequences followed by two [dT] residues at 3′-ends of both strands: MSK1 (5′-CUGCU AAUUU GACAG GACA), MSK2 (5′-CCUUC UGUGG CACCA UCGA) and RSK2 (5′-GCAAC AUUCU UUAUG UGGA). RNA of unrelated sequence (5′-GAUCA UACGU GCGAU CAGA) was used as a negative control. Huh-7 cells stably expressing a siRNA against PKR (PKR-si cell) have been described [5]. Stable MSK2-si cells were selected with 1 μg/ml of puromycin (Sigma) after transfection of pSiMSK2. Ro 31-8220, GF109203X and H-89 were from Sigma–Aldrich. PD-98059 and SB-203580 were from Assay Designs.

2.2. Plasmid constructs

The human PKR and MxA gene constructs were described [5]. MSK1 (Accession No. NM_004755) and N- and C-terminal kinase-inactivated constructs (NT-KI, $MSK1^{D195A}$; CT-KI, $MSK1^{D565A}$; NCT-KI, $MSK1^{D195A/D565A}$) were kindly provided by Dr. J.H. Yoon [13] and subcloned into p3XFLAG-CMV-10 (Sigma), with three copies of the Flag tag at the N-terminus. MSK2 (Accession No. NM_003942) sequence was amplified from HeLa cell cDNA library and cloned into the same vector. The N- and C-terminal kinase-inactivated MSK2 (NT-KI, $MSK2^{D179A}$; CT-KI, $MSK2^{D551A}$; NT-KI, $MSK2^{D179A/D551A}$) were constructed by PCR-based mutagenesis. For stable knockdown of MSK2, pSiMSK2 was constructed to contain 5′-gcg gcc gag atc atg tgc aat tca aga gat tgc aca tga tct cgg ccg ttt ttt gga a (underlined sequences correspond to ntd 1862–1880 of MSK2 protein coding sequence) in pSilencer 2.1-U6 puro (Ambion).

2.3. Immunoblotting

Cells were lysed in RIPA buffer (10 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.1% SDS and 0.25% Sodium deoxycholate) supplemented with protease inhibitors (1 mM PMSF, 1 μg/ml Leupeptin and 4 μg/ml Aprotinin) and phosphatase inhibitor cocktails (P2850, Sigma). Proteins were separated by gel electrophoresis and transferred onto Hybond ECL membrane (GE healthcare). Blots were incubated with antibody in TBST buffer (20 mM Tris–HCl [pH 7.6], 137 mM NaCl and 0.1% Tween-20) containing 5% skim milk and visualized with SuperSignal® West Pico Chemiluminescent reagents (Thermo Scientific). Anti-PKR (sc-707), anti-MSK1 (sc-25417), anti-RSK2 (sc-9986) and anti-GAPDH (sc-47724) antibodies were purchased from Santa Cruz Biotechnology. Anti-Phospho Thr446-PKR (ab32036) and anti-MSK2 (ab42101) antibodies were from Abcam Inc. Anti-HA (H6908), anti-Flag (F3165), anti-actin (A2668) and anti-tubulin (T9026) antibodies were from Sigma.

2.4. Immunoprecipitation

Huh-7 cells transfected with plasmids by using Lipofectamine and Plus reagent (Invitrogen) were washed with ice-cold PBS and lysed for 30 min in buffer (20 mM Tris–HCl [pH 7.4], 135 mM NaCl

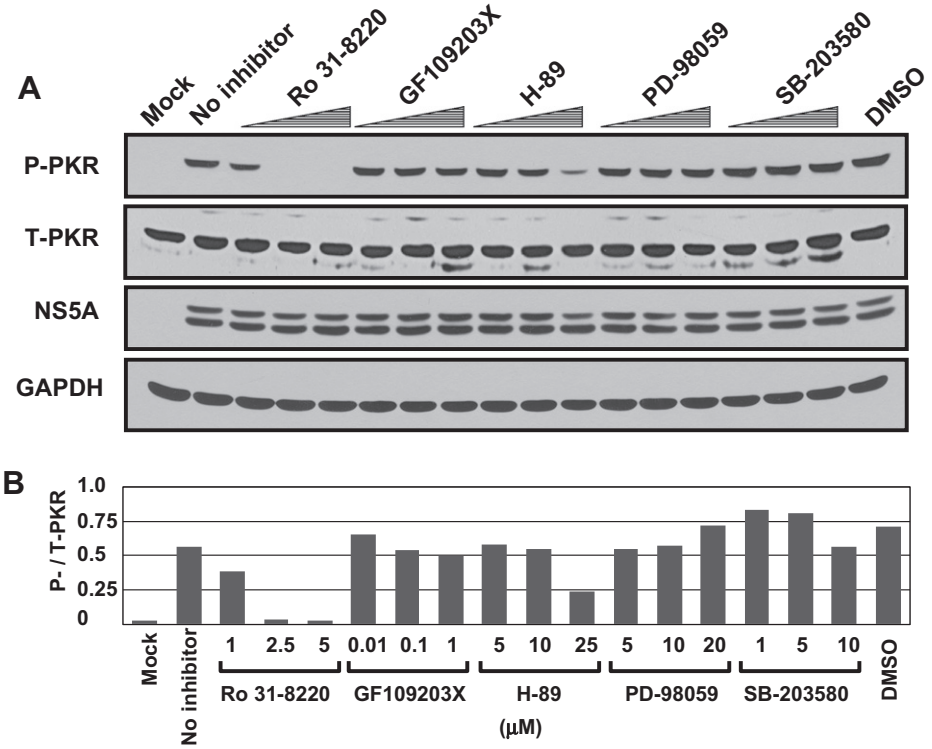

Fig. 1. PKR phosphorylation is inhibited by RSK inhibitors. Huh-7 cells carrying JFH-1 HCV RNA were treated with kinase inhibitors for 24 h: 1, 2.5, or 5 μM of Ro 31-8220; 0.5, 1, or 2 μM of GF109203X; 5, 10, or 25 μM of H89; 10, 20, or 40 μM of PD-98059; and 5, 10, or 25 μM of SB-203580. (A) Immunoblots were performed for the phosphorylated (T446) PKR, total PKR or HCV NS5A proteins. Cell without HCV RNA (first lane), and JFH-1 replicon cells, either untreated (second lane) or treated with 0.1% DMSO (last lane), respectively, were used as controls. GAPDH was used as a loading control. The blot shown here is one of more than three repeated experiments that gave similar results. (B) Intensity of phosphorylated and total PKR bands was quantitated with the ImageJ software (NIH, USA) and the ratio was shown in bar.

and 1% Triton X-100) supplemented with EDTA-free protease inhibitor cocktail (Roche Applied Science). Cell lysates were briefly sonicated and centrifuged at 14,000*g* for 10 min at 4 °C. The supernatants containing 1 mg of total protein were incubated overnight at 4 °C with 5 μl of anti-HA antibody and 50 μl Protein A Sepharose™ CL-4B (GE Healthcare). Resin-bound proteins were separated by gel electrophoresis and immunoblotted with appropriate antibodies.

2.5. In vitro kinase assays

Immunoprecipitated PKR was assayed in vitro with recombinant kinase-deficient PKR (K296R) as substrate. Cell lysates containing 100 μg of protein in IP/K lysis buffer (20 mM HEPES [pH 8.0], 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 0.5% Triton X-100, 1 mM sodium orthovanadate, 1 mM β-glycerophosphate, 1 μg/ml Pepstatin, 10 μg/ml Aprotinin and 1 μg/ml Leupeptin) were incubated with antibodies overnight at 4 °C. The immune complexes were precipitated with Protein A Sepharose, washed twice with IP/K lysis buffer and once with 1× kinase buffer, and the resin-bound PKR was assayed with 1 μg of recombinant PKR (K296R) as substrate at 30 °C for 1 h in 40 μl of 1× kinase buffer supplemented with 200 μM ATP and 5 μCi of [γ-^{32}P]-ATP as described [14]. The reactions were terminated by adding equal volumes of 2× SDS sample buffer and boiling. The ^{32}P-labeled proteins were separated by denaturing gel electrophoresis and detected by autoradiography after the gels were soaked for 1 h in 46% methanol and 8% acetic acid and dried. Recombinant PKR was expressed in the Rosetta (DE3) strain of *Escherichia coli* transformed with pET-28a (Novagen) containing PKR sequence with 6xHis tags at both the N- and C-termini. Bacterial culture was treated for 12 h at 15 °C with 0.5 mM IPTG and recombinant proteins were purified with TALON® resin (Clontech), concentrated with a spin concentrator (30 kDa MWCO; Sartorius Vivascience), and stored at −70 °C in 50 mM Tris–HCl [pH 7.5], 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF and 25% glycerol.

In another in vitro assay, recombinant PKR was incubated with recombinant MSK2 at 30 °C for 1 h in 40 μl of 1× kinase buffer (20 μM HEPES [pH 8.0], 10 mM $MgCl_2$, 0.1 mM sodium orthovanadate and 10 mM β-glycerophosphate) supplemented with 200 μM of ATP and 5 μCi of [γ-^{32}P]-ATP. The ^{32}P-labeled proteins were separated by gel electrophoresis and detected by autoradiography. Recombinant MSK2 was expressed with 6xHis tag at the N-terminus from the same vector used for PKR. Recombinant wild-type PKR protein was dephosphorylated by treatment with λ protein phosphatase (New England Biolabs) at 4 °C overnight before use.

3. Results

3.1. Stress-related kinases are involved in PKR activation

We sought cellular signals that might contribute to PKR activation in HCV-replicating cells. Since PKR activation was shown to be mediated by RSK2 following UVA-induced stress signaling [11] and in the antiviral response against influenza virus [12], we examined PKR phosphorylation in JFH-1 HCV replicon Huh-7 cells after treat-

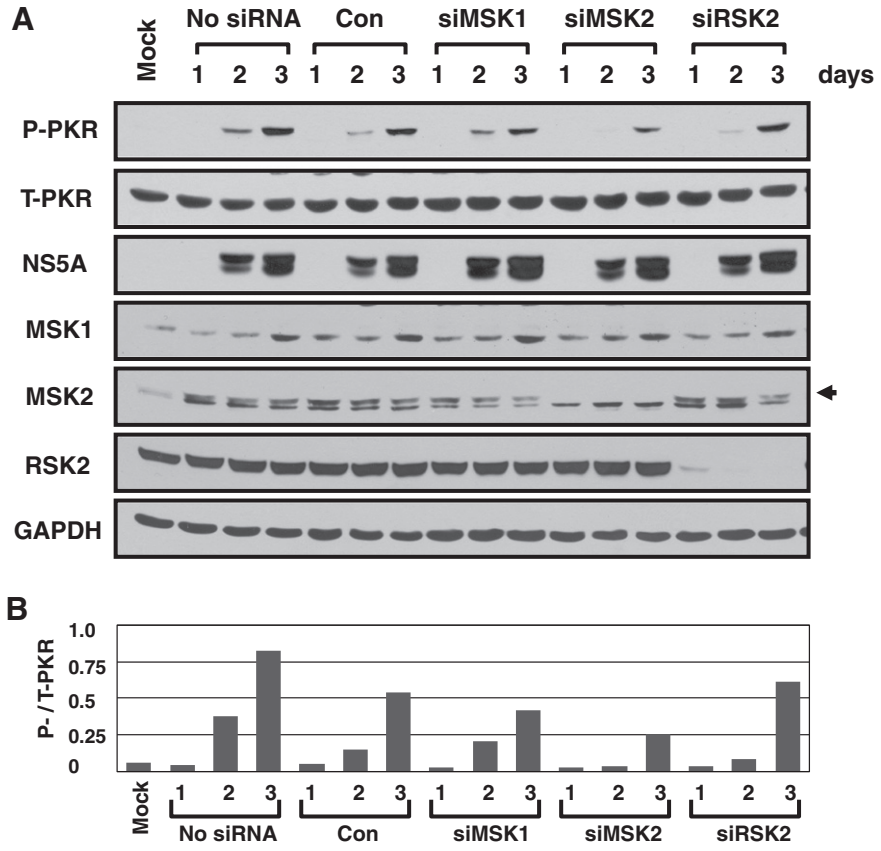

Fig. 2. MSK2 is involved in PKR phosphorylation. Huh-7 cells were transfected with siRNAs targeting RSKs and three days later with JFH-1 RNA and each individual siRNA. (A) Immunoblotting was performed after the cells were incubated for the indicated periods. The arrowhead on the right points to MSK2 above a non-specific band. Cells without HCV RNA (mock), replicon cells without siRNA treatment (No SiRNA), and replicon cells transfected with a siRNA of scrambled sequence (control) were included in the immunoblot as controls. GAPDH was used as a loading control. The blot shown here is one of more than three repeated experiments that gave similar results. (B) Phosphorylated and total PKR was quantitated and the ratio was shown in bar.

ment with kinase inhibitors including those for RSKs. Immunoblotting showed that PKR phosphorylation at threonine 446 (T446) was completely blocked with Ro 31-8220, an inhibitor for PKC, GSK3, MSK1, RSK2, and S6K1 p70 ribosomal S6 kinase [15] (Fig. 1). Phosphorylation at T446 residue, which is located in the activation loop of PKR, is essential for retaining the full activity of the protein [16]. H89, an inhibitor of PKA, ROCK-II Rho-dependent kinase, MSK1, and S6K1, also reduced PKR phosphorylation by ~60%. In contrast, inhibitors of PKC (GF 109203X), p38 kinase (SB-203580) or upstream activators of ERK1/2 (PD-98059) had no effect. These results suggested that PKR phosphorylation might be affected by extracellular stimuli- or stress-related kinases, such as RSKs. The expression of total PKR, viral NS5A and cellular GAPDH was not altered in these conditions.

RSKs constitute a family of protein kinases (RSK1–RSK4 and the related MSK1 and MSK2 proteins) that mediate signal transduction downstream of the MAP kinase pathways [10]. We have transiently suppressed MSK1, MSK2 and RSK2 expression in the JFH-1 replicon cells by RNA interference. Immunoblots indicated that the knock down of MSK2 (upper band marked with an arrowhead) blocked PKR activation completely on day 2 and by ~50% on day 3, while knock down of the closely related RSK2, despite almost complete suppression of its expression had no effect (Fig. 2). We were unable to suppress MSK1 expression. Expression of viral NS5A or PKR proteins was not altered. These results suggested that MSK2 might contribute to the HCV-mediated PKR activation.

3.2. MSK2 binds PKR and stimulates PKR activation

The interaction between transiently expressed PKR and endogenous RSKs was examined by immunoprecipitation. Immunoblotting showed that MSK2, but not MSK1, co-precipitated with transiently expressed PKR (Fig. 3A). We noted that the closely related RSK2 also co-precipitated with PKR at a lower efficiency. None of these proteins were precipitated with MxA, an IFN-inducible protein but unrelated to PKR, which was transfected as a control. To substantiate this interaction, the MSK2-bound PKR was immunoprecipitated from HCV-replicating cells and the catalytic activity of PKR was measured in vitro. A recombinant kinase-defective PKR (K296R) purified from *E. coli* was used as a substrate in this assay and phosphorylated protein was separated by gel electrophoresis. Autoradiography indicated that MSK2 co-precipitates with PKR, which is activated in JFH-1 replicating cells (compare lanes labeled J to lanes labeled G with the replication-defective GND genome), whereas such interaction was not seen with MSK1 or RSK2 (Fig. 3B). This interaction was not detected in a stable PKR knock-down cell (Fig. 3C) or significantly reduced in a stable MSK2 (upper band in the immunoblot) knock-down cell (Fig. 3D). These results together indicated that MSK2 binds PKR and contributes to the PKR activation in the HCV-dependent manner, while RSK2 binds PKR, but is not involved in PKR activation.

3.3. MSK2 functions as an adaptor in PKR activation

MSKs are serine/threonine kinases that are activated by ERK and p38 in response to mitogens and cellular stress. Cellular substrates of MSKs include CREB, NF-κB, ATF1, histone H3, and HMG-14 [17,18]. The specific interaction of MSK2 with PKR that we found raised the possibility of a catalytic role of MSK2 in PKR phosphorylation. MSKs and RSKs are unique among kinases because they contain two distinct kinase domains connected by a regulatory linker region: the N-terminal kinase domain is similar to those of AGC family kinases, and the C-terminal kinase domain is related to those of calmodulin kinase (CaMK) family kinases [19]. We generated MSK2 and MSK1 constructs with missense mutations in one or both of the two kinase domains, and measured

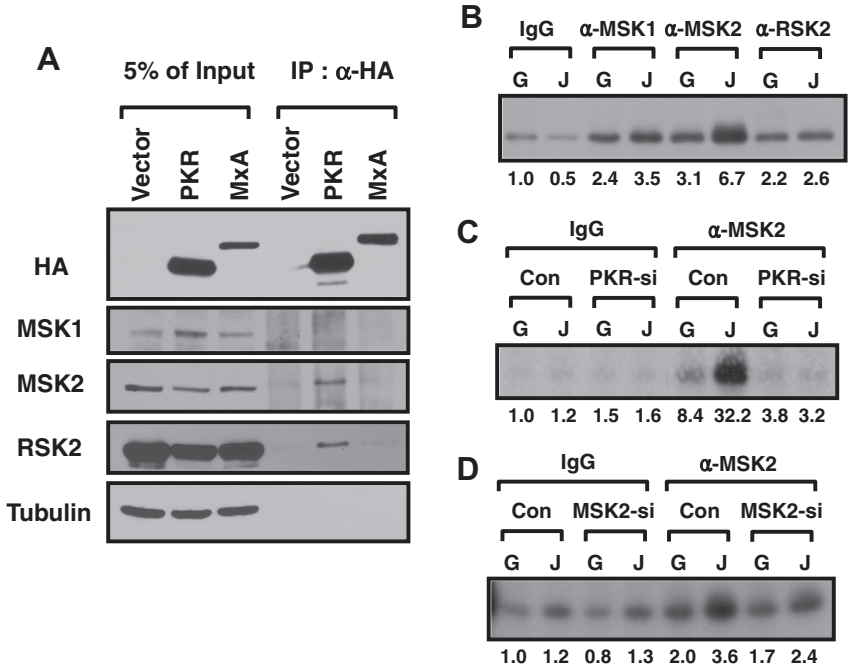

Fig. 3. MSK2 binds PKR and stimulates PKR activation. (A) Huh-7 cells were transfected with HA-tagged PKR or MxA gene constructs. After three days, proteins were precipitated with an anti-HA antibody and the co-precipitated proteins were immunoblotted with antibodies specific for the indicated RSKs. Five percent of the cell lysate was probed in parallel. Tubulin was used as a loading control. (B) RSKs were immunoprecipitated from Huh-7 cells carrying JFH-1 (lanes labeled J) or the replication-defective GND RNA (lanes labeled G) with antibodies against specific RSKs or an unrelated IgG. Activity of resin-bound PKR was assayed in vitro with a purified recombinant kinase-defective PKR (K296R) as a substrate. Autoradiographs were obtained from ^{32}P-labeled proteins separated by gel electrophoresis with the relative intensity quantitated with ImageJ software. (C and D) Immunoprecipitation was performed with an anti-MSK2 antibody and the resin-bound PKR was assayed in vitro with K296R as a substrate. Stable PKR knock-down cells (PKR-si) or MSK2-knock-down cells (MSK2-si) were used as controls.

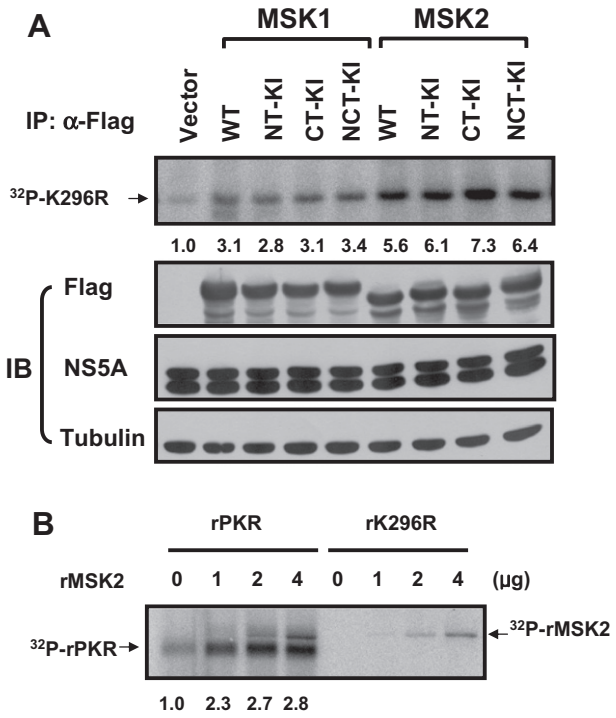

Fig. 4. MSK2 functions as an adaptor in PKR activation. (A) JFH-1 replicon cells were transfected with Flag-tagged MSK1 or MSK2 constructs expressing the wild-type or mutant proteins with an inactivated N-terminal kinase (NT-KI), an inactivated C-terminal kinase (CT-KI) or an inactivated form of both kinase motifs (NCT-KI). After two days, cell lysates were immunoprecipitated with an anti-Flag antibody, and the resin-bound PKR was assayed in vitro. The ^{32}P-labeled proteins were separated by gel electrophoresis. Immunoblots of MSKs and viral NS5A in cell lysate are shown below. Tubulin was used as a loading control. (B) Recombinant PKR (0.2 μg of wild-type PKR or kinase-deficient K296R) was incubated with [γ-^{32}P]-ATP and indicated amount of purified recombinant MSK2, and the ^{32}P-labeled proteins were separated by gel electrophoresis. Arrowheads indicate autophosphorylated PKR and MSK2, respectively.

in vitro the PKR activity co-precipitated with these constructs from HCV replicon cells. Autoradiography showed no discernible effect of those mutations on the co-precipitated PKR activity (Fig. 4A). This result, showing the stimulatory role of MSK2 being apparently independent of its kinase function, suggested that MSK2 might function as an adaptor in PKR activation. The adaptor activity of MSK2 was further tested with purified recombinant proteins. As shown in Fig. 4B, phosphorylation of the wild-type PKR was further increased by incubation with MSK2 in a dose-dependent manner, whereas no phosphorylation was observed for K296R. This result strongly suggested that MSK2 does not phosphorylate PKR directly but stimulates auto-phosphorylation of PKR. We noticed auto-phosphorylation of MSK2 in this reaction.

4. Discussion

While dsRNA is the principal activator of PKR, studies indicated that RNA binding is not absolutely required for PKR activation. Activation of PKR by PACT occurs independently of dsRNA [20]. The PKR catalytic domain, fused to a heterologous dimerization domain, undergoes auto-phosphorylation in the absence of dsRNA [21]. In addition, PKR expressed in a bacterial system can dimerize and become activated in the absence of dsRNA [22]. These results point out the importance of dimerization for proper PKR activation. An earlier study showing that dsRNA at low concentrations activates PKR but at higher concentrations the enzyme is rather inhibited [23] is also consistent with this notion. Probably, at high concentrations of dsRNA, dimerization is prevented because PKR monomers are diluted by binding different dsRNA molecules. Conversely, at high PKR concentrations, a situation often encountered in bacterial expression, PKR activation can occur through dimerization without dsRNA.

How does MSK2 enhance HCV-mediated PKR activation? It might be that while viral dsRNA is the main activator of PKR, MSK2 functions in synergy as an adaptor, holding PKR in a conformation and/or a concentration that is favorable for dimerization, which is similar to the mechanism proposed for PACT. It is considered that PACT binding to PKR within the kinase domain removes the inhibitory intramolecular interactions between the same domain and the regulatory domain of PKR, and thus facilitates dimer formation [24]. A related question is whether MSK2 is activated during PKR activation, and if so, how it is activated. Activation of MSKs and RSKs occurs in two steps. The C-terminal kinase domain is firstly activated by MAP kinase-mediated phosphorylation. The N-terminal kinase domain, which is activated through intramolecular phosphorylation by C-terminal kinase domain, is responsible for the phosphorylation of downstream substrates [25]. Although our transfection experiment with the kinase-inactivated constructs suggested that the adaptor function of MSK2 is independent of its kinase activity, activation of MSK2 by HCV cannot be ruled out completely. HCV proteins, such as core and E2, have been implicated in ERK activation [26,27]. RSKs have been shown to be activated by viral proteins, such as HIV tat and the ORF45 of the Kaposi's sarcoma-associated herpesvirus [28,29].

How does the stress-mediated PKR activation affect HCV replication? We have shown that HCV replication activates PKR without further induction of its expression, suggesting an immediate antiviral role of the latent PKR that had been present prior to HCV infection [5]. Activation of this antiviral protein can be a self-limiting mechanism for a persistent virus like HCV, a strategy by which the virus may restrict its own growth. Activation of PKR was also proposed as a mechanism beneficial for the virus by which the cellular antiviral protein synthesis can be suppressed or delayed [6,7]. In these regards, PKR activation is involved in both antiviral and virus-supportive functions. Cellular stress signals induced in HCV infection might provide additional mechanisms controlling the antiviral responses through regulation of PKR activity. Temporal regulation of PKR also seems to coordinate these seemingly conflicting activities. PKR is activated early in HCV replication, but its activity can be inhibited by viral proteins, such as NS5A, in later stages of replication. A similar mechanism was identified in influenza virus infection whereby PKR is activated by the influenza B virus RNP complex, and inhibited by the viral NS1 protein [30].

In summary, our findings indicate that HCV-mediated PKR activation is facilitated by MSK2, which links virus-induced stress to translational control. It is interesting that, as a persistently replicating virus, HCV utilizes both RNA and protein activators for regulating PKR.

Acknowledgments

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References

[1] 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.

[2] G.M. Lauer, B.D. Walker, Hepatitis C virus infection, N. Engl. J. Med. 345 (2001) 41–52.
[3] K.S. Chang, Z. Cai, C. Zhang, G.C. Sen, B.R. Williams, G. Luo, Replication of hepatitis C virus (HCV) RNA in mouse embryonic fibroblasts: protein kinase R (PKR)-dependent and PKR-independent mechanisms for controlling HCV RNA replication and mediating interferon activities, J. Virol. 80 (2006) 7364–7374.
[4] D. Jiang, H. Guo, C. Xu, J. Chang, B. Gu, L. Wang, T.M. Block, J.T. Guo, Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus, J. Virol. 82 (2008) 1665–1678.
[5] J.I. Kang, S.N. Kwon, S.H. Park, Y.K. Kim, S.Y. Choi, J.P. Kim, B.Y. Ahn, PKR protein kinase is activated by hepatitis C virus and inhibits viral replication through translational control, Virus Res. 142 (2009) 51–56.
[6] U. Garaigorta, F.V. Chisari, Hepatitis C virus blocks interferon effector function by inducing protein kinase R phosphorylation, Cell Host Microbe 6 (2009) 513–522.
[7] N. Arnaud, S. Dabo, P. Maillard, A. Budkowska, K.I. Kalliampakou, P. Mavromara, D. Garcin, J. Hugon, A. Gatignol, D. Akazawa, T. Wakita, E.F. Meurs, Hepatitis C virus controls interferon production through PKR activation, PLoS One 5 (2010) e10575.
[8] R.C. Patel, G.C. Sen, PACT, a protein activator of the interferon-induced protein kinase, PKR, EMBO J. 17 (1998) 4379–4390.
[9] J. Gil, M.A. Garcia, P. Gomez-Puertas, S. Guerra, J. Rullas, H. Nakano, J. Alcami, M. Esteban, TRAF family proteins link PKR with NF-kappa B activation, Mol. Cell. Biol. 24 (2004) 4502–4512.
[10] C. Hauge, M. Frodin, RSK and MSK in MAP kinase signalling, J. Cell Sci. 119 (2006) 3021–3023.
[11] T.A. Zykova, F. Zhu, Y. Zhang, A.M. Bode, A.M. Dong, Involvement of ERKs, RSK2 and PKR in UVA-induced signal transduction toward phosphorylation of eIF2alpha (Ser(51)), Carcinogenesis 28 (2007) 1543–1551.
[12] S. Kakugawa, M. Shimojima, H. Goto, T. Horimoto, N. Oshimori, G. Neumann, T. Yamamoto, Y. Kawaoka, Mitogen-activated protein kinase-activated kinase RSK2 plays a role in innate immune responses to influenza virus infection, J. Virol. 83 (2009) 2510–2517.
[13] K.S. Song, W.J. Lee, K.C. Chung, J.S. Koo, E.J. Yang, J.Y. Choi, J.H. Yoon, Interleukin-1 beta and tumor necrosis factor-alpha induce MUC5AC overexpression through a mechanism involving ERK/p38 mitogen-activated protein kinases-MSK1-CREB activation in human airway epithelial cells, J. Biol. Chem. 278 (2003) 23243–23250.
[14] D.C. Thomis, C.E. Samuel, Mechanism of interferon action: evidence for intermolecular autophosphorylation and autoactivation of the interferon-induced, RNA-dependent protein kinase PKR, J. Virol. 67 (1993) 7695–7700.
[15] S.P. Davies, H. Reddy, M. Caivano, P. Cohen, Specificity and mechanism of action of some commonly used protein kinase inhibitors, Biochem. J. 351 (2000) 95–105.
[16] P.R. Romano, M.T. Garcia-Barrio, X. Zhang, Q. Wang, D.R. Taylor, F. Zhang, C. Herring, M.B. Mathews, J. Qin, A.G. Hinnebusch, Autophosphorylation in the activation loop is required for full kinase activity in vivo of human and yeast eukaryotic initiation factor 2alpha kinases PKR and GCN2, Mol. Cell. Biol. 18 (1998) 2282–2297.
[17] A. Soloaga, S. Thomson, G.R. Wiggin, N. Rampersaud, M.H. Dyson, C.A. Hazzalin, L.C. Mahadevan, J.S. Arthur, MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14, EMBO J. 22 (2003) 2788–2797.
[18] G.R. Wiggin, A. Soloaga, J.M. Foster, V. Murray-Tait, P. Cohen, J.S. Arthur, MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts, Mol. Cell. Biol. 22 (2002) 2871–2881.
[19] R. Anjum, J. Blenis, The RSK family of kinases: emerging roles in cellular signalling, Nat. Rev. Mol. Cell. Biol. 9 (2008) 747–758.
[20] G.A. Peters, S. Li, G.C. Sen, Phosphorylation of specific serine residues in the PKR activation domain of PACT is essential for its ability to mediate apoptosis, J. Biol. Chem. 281 (2006) 35129–35136.
[21] T.L. Ung, C. Cao, J. Lu, K. Ozato, T.E. Dever, Heterologous dimerization domains functionally substitute for the double-stranded RNA binding domains of the kinase PKR, EMBO J. 20 (2001) 3728–3737.
[22] P.A. Lemaire, J. Lary, J.L. Cole, Mechanism of PKR activation: dimerization and kinase activation in the absence of double-stranded RNA, J. Mol. Biol. 345 (2005) 81–90.
[23] T. Hunter, T. Hunt, R.J. Jackson, H.D. Robertson, The characteristics of inhibition of protein synthesis by double-stranded ribonucleic acid in reticulocyte lysates, J. Biol. Chem. 250 (1975) 409–417.
[24] S. Li, G.A. Peters, K. Ding, X. Zhang, J. Qin, G.C. Sen, Molecular basis for PKR activation by PACT or dsRNA, Proc. Natl. Acad. Sci. USA 103 (2006) 10005–10010.
[25] C.E. McCoy, D.G. Campbell, M. Deak, G.B. Bloomberg, J.S. Arthur, MSK1 activity is controlled by multiple phosphorylation sites, Biochem. J. 387 (2005) 507–517.
[26] J. Hayashi, H. Aoki, K. Kajino, M. Moriyama, Y. Arakawa, O. Hino, Hepatitis C virus core protein activates the MAPK/ERK cascade synergistically with tumor promoter TPA, but not with epidermal growth factor or transforming growth factor alpha, Hepatology 32 (2000) 958–961.
[27] L.J. Zhao, P. Zhao, Q.L. Chen, H. Ren, W. Pan, Z.T. Qi, Mitogen-activated protein kinase signalling pathways triggered by the hepatitis C virus envelope protein E2: implications for the prevention of infection, Cell Prolif. 40 (2007) 508–521.
[28] C. Hetzer, D. Bisgrove, M.S. Cohen, A. Pedal, K. Kaehlcke, A. Speyerer, K. Bartscherer, J. Taunton, M. Ott, Recruitment and activation of RSK2 by HIV-1 Tat, PLoS One 2 (2007) e151.
[29] E. Kuang, Q. Tang, G.G. Maul, F. Zhu, Activation of p90 ribosomal S6 kinase by ORF45 of Kaposi's sarcoma-associated herpesvirus and its role in viral lytic replication, J. Virol. 82 (2008) 1838–1850.
[30] B. Dauber, L. Martinez-Sobrido, J. Schneider, R. Hai, Z. Waibler, U. Kalinke, A. Garcia-Sastre, T. Wolff, Influenza B virus ribonucleoprotein is a potent activator of the antiviral kinase PKR, PLoS Pathog. 5 (2009) e1000473.