Biochemical and Biophysical Research Communications 450 (2014) 885–890

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

# v-Src inhibits the interaction between Rad17 and Rad9 and induces replication fork collapse

Yasunori Fukumoto *, Takahito Miura, Mariko Morii, Sho Kubota, Takuya Honda, Shoichi Kubota, Takao Morinaga, Noritaka Yamaguchi, Yuji Nakayama [1], Naoto Yamaguchi *

*Department of Molecular Cell Biology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan*

ARTICLE INFO

*Article history:*
Received 30 May 2014
Available online 24 June 2014

*Keywords:*
v-Src
DNA damage checkpoint
ATR
Rad17
Rad9

ABSTRACT

ATR-dependent DNA damage checkpoint is crucial to maintain genomic stability. Recently, we showed that Src family kinases suppress ATR-dependent checkpoint signaling in termination of DNA damage checkpoint. However, the precise molecular mechanism is unclear. Therefore, we examined the role of oncogenic v-Src on ATR-Chk1 signaling. We show that v-Src suppresses thymidine-induced Chk1 phosphorylation and induces replication fork collapse. v-Src inhibits interaction between Rad17 and Rad9 in chromatin fraction. By contrast, v-Src does not inhibit RPA32 phosphorylation, ATR autophosphorylation, or TopBP1–Rad9 interaction. These data suggest that v-Src attenuates ATR-Chk1 signaling through the inhibition of Rad17–Rad9 interaction.



## 1. Introduction

DNA replication stress activates ataxia telangiectasia mutated (ATM) – and Rad3-related protein (ATR) –dependent DNA damage checkpoint. The activated ATR pathway prevents firing of late replication origins, and also stabilizes stressed replication forks to prevent collapse of replication forks [1–3]. ATR pathway is mediated by assembly of protein complex on damaged chromatin. ATR–ATRIP complex is recruited to replication protein A (RPA)-coated single-stranded DNA (ssDNA) through interaction between ATRIP and RPA [1,3–6]. Rad17 composes replication factor C (RFC)-like protein complex (Rad17-RFC) in which RFC1 is replaced by Rad17. Rad9, Hus1, and Rad1 compose proliferating cell nuclear antigen (PCNA)-like heterotrimeric complex (9–1–1) [1,3]. In responses to DNA damage and replication stress, Rad17-RFC loads 9–1–1 onto damaged chromatin [7–11]. 9–1–1 recruits TopBP1 through interaction between Rad9 and TopBP1 [12–14]. On damaged chromatin, TopBP1 interacts with ATR and stimulates kinase activity of ATR [15–17]. In addition to the role in chromatin loading of 9–1–1, Rad17 also has a role in mediating signal transduction from ATR to Chk1. Rad17 is a phosphorylation substrate of ATR, and the phosphorylation is required for ATR-dependent Chk1 phosphorylation and activation [18–20]. Furthermore, Rad17-RFC, 9–1–1, and TopBP1 form a tripartite protein complex on damaged chromatin to mediate ATR-dependent Chk1 activation [17,21].

Once DNA repair is completed, activated checkpoint proteins are inactivated by protein phosphatases and ubiquitin ligases [22,23]. Rad17 is degraded by APC/Cdh1 and the degradation promotes recovery from ATR-dependent DNA damage checkpoint [24]. However, little is known about regulation of the interaction among Rad17-RFC, 9–1–1 and TopBP1 in checkpoint termination.

Src family kinases (SFKs) are the largest family of non-receptor tyrosine kinases. SFKs are mainly located on the cytoplasmic side of the plasma membrane, however a fraction of the SFKs are expressed in the nucleus [25–29]. Furthermore, Lyn, one of the SFK members, is activated and translocated into the nucleus upon DNA damage induction [30,31]. Involvement of a viral form of Src, v-Src in DNA damage responses has also been reported [32–34]. However, little is known about the involvement of SFKs in p53-independent and ATR-dependent DNA damage checkpoint pathway.

Recently, we reported that SFKs suppress ATR-Chk1 signaling during recovery from G2 checkpoint arrest or during exposure to replication stress [35]. We also showed that v-Src suppresses thymidine-induced Rad17 phosphorylation, however the precise molecular mechanism is unclear. Here, we show that in the presence of replication stress, v-Src suppresses Chk1 phosphorylation and induces Kap1 phosphorylation, suggesting that v-Src induces

* Corresponding authors. Address: Department of Molecular Cell Biology, Graduate School of Pharmaceutical Sciences, Chiba University, Inohana 1-8-1, Chuo-ku, Chiba 260-8675, Japan. Fax: +81 43 226 2869 (Y. Fukumoto). Fax: +81 43 226 2868 (N. Yamaguchi).

*E-mail addresses:* fukumoto@faculty.chiba-u.jp (Y. Fukumoto), nyama@faculty.chiba-u.jp (N. Yamaguchi).

[1] Present address: Department of Biochemistry & Molecular Biology, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan.

http://dx.doi.org/10.1016/j.bbrc.2014.06.078
0006-291X/

replication fork collapse through inhibition of replication checkpoint. Moreover, we also show that the underlying mechanism involves inhibition of the interaction between Rad17 and Rad9. By contrast, v-Src does not inhibit RPA32-S33 phosphorylation, ATR autophosphorylation, or TopBP1–Rad9 interaction. These results suggest that v-Src attenuates ATR-Chk1 signaling through the inhibition of Rad17–Rad9 interaction, and shed light on a possible mechanism by which endogenous SFKs suppress ATR-Chk1 signaling.

## 2. Materials and methods

### 2.1. Cell lines, cell culture, and plasmids

HeLa S3 cells carrying an inducible v-Src allele (HeLa S3-TR/v-Src) was described previously [35]. Parental HeLa S3 and HeLa S3-TR/v-Src cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 5% bovine serum. The constitutively active mutant of Lyn, LynΔC-HA was described previously [29,35]. The cDNA encoding human Rad17 was isolated from a cDNA library derived from HEK293T cells, and cloned into pcDNA3 vector with N-terminal FLAG tag. Gene transfection was performed using acidified polyethylenimine [36].

### 2.2. Analysis of checkpoint proteins

For analysis of checkpoint proteins, cells were exposed to 4 mM thymidine for 24 h. Expression of v-Src was induced with 1 μg/ml doxycycline for 12 or 24 h, and 5 μM SU6656 was added simultaneously with doxycycline. Flow cytometric analysis was described previously [35,37].

### 2.3. Cell lysates and solubilized chromatin fractions

For immunoprecipitation of ATR, cells were lysed in a high salt buffer [50 mM HEPES-NaOH (pH 7.8), 400 mM NaCl, 1% Triton X-100, 10% glycerol] for 20 min at 4 °C. The lysates were cleared by centrifugation and used for immunoprecipitation. Solubilized chromatin fraction was prepared as described previously [35]. After cell lysis, the soluble fraction was collected as nucleoplasmic/cytoplasmic fraction.

### 2.4. Antibodies

The following antibodies were used in this study: ATR (N-19, Santa Cruz Biotechnology, sc-1887), ATR-pT1989 (KeraFAST, EVU001), ATRIP (Cell Signaling Technology, 2737), Rad1 (N-18, Santa Cruz Biotechnology, sc-14314), Rad9 (Bethyl Laboratory, A300-890), RPA32-pS33 (Bethyl Laboratory, A300-246A), RPA32 (cl. RPA34-20, Merck Millipore, NA19L), Src (N-16, Santa Cruz Biotechnology, sc-19; cl. GD11, Merck Millipore, 05-184), TopBP1 (abcam, ab2402). Actin, Chk1, Chk1-pS345, Cyclin A, FLAG (M2), Hsc70, Kap1, Kap1-pS824, Lamin A/C, Rad17, Rad17-pS645, and phosphotyrosine (4G10) antibodies were described previously [35,38].

## 3. Results

### 3.1. v-Src suppresses ATR-dependent Rad17 phosphorylation in a kinase-dependent manner

Recently we reported that SFKs suppress ATR-Chk1 signaling induced by replication stress and Adriamycin exposure [35]. We showed that regulation of Rad17-S645 phosphorylation is the key event and that shRNA-mediated knockdown of Lyn increases Rad17 phosphorylation. However, the precise molecular mechanism is unclear. Proteasomal degradation of Rad17 was reported [24], but we showed that proteasomal activity is not required for SFKs to suppress Rad17 phosphorylation [35]. Therefore, to gain more insight into the molecular mechanism, we examined the role of oncogenic v-Src in suppression of ATR-dependent Rad17 phosphorylation, since v-Src has constitutively active kinase activity.

We showed that ectopic expression of v-Src suppressed Rad17 phosphorylation [35]. However, we had not examined whether the effect is kinase dependent. We used HeLa S3 cell line because it can easily be adapted to cell cycle synchronization method. We used thymidine to induce replication stress, since it is widely accepted as a method of cell synchronization. Replication stress induces ATR-dependent Rad17 phosphorylation at S645 [1,3,6]. As reported, thymidine treatment induced Rad17-S645 phosphorylation in HeLa S3 cells (Fig. 1A). In HeLa S3-TR/v-Src cells, induction of v-Src for 24 h suppressed the Rad17 phosphorylation (Fig. 1A and B), as we showed previously [35]. The suppression of Rad17 phosphorylation during the time course were presented previously [35]. Moreover, induction of v-Src from 12 h after thymidine addition also suppressed Rad17 phosphorylation (Fig. 1A and B). Doxycycline alone did not affect Rad17 phosphorylation in parental HeLa S3 cells (Fig. 1C). A specific SFK inhibitor SU6656 prevented v-Src-induced Rad17 dephosphorylation. For an unknown reason, SU6656 also decreased protein amount of v-Src, but a comparison of lane 4 with lane 7 in Fig. 1A indicates that the inhibition of Rad17 phosphorylation is dependent on the kinase activity. SU6656 induced faster migration of v-Src protein on SDS–PAGE (Fig. 1A), which should reflect inhibition of the kinase activity as we showed previously [39]. These data suggest that SFKs regulate Rad17 phosphorylation in a kinase-dependent manner.

To interpret the data more precisely, the effect of v-Src on thymidine-induced cell cycle arrest was examined. Induction of v-Src for 24 h reduced cyclin A accumulation to around 80% (Fig. 1A) and slightly increased G2 phase cells on FACS analysis (Fig. 1D), suggesting that the cells are partly released from thymidine block. Induction of v-Src for 12 h prevented neither cyclin A accumulation (Fig. 1A) nor cell cycle synchronization (Fig. 1D), indicating that the effect of v-Src on Rad17 phosphorylation is not due to possible inhibition of thymidine-induced cell cycle arrest.

### 3.2. v-Src promotes replication fork collapse under replication stress

Phosphorylation of Chk1 at S345 by ATR is a reliable indicator of activation of Chk1 in vivo [1,40]. To examine the biological significance of the v-Src-induced Rad17 dephosphorylation, the effect of v-Src on Chk1 phosphorylation was examined. v-Src suppressed Chk1-S345 phosphorylation to around 70% (Fig. 2A and B). Inhibition of replication checkpoint induces replication fork collapse, and the collapsed forks are often converted to DNA double strand breaks which induce ATM-dependent phosphorylation of downstream substrates [1,2]. As reported, caffeine and a specific ATR inhibitor VE-821 [41] inhibited thymidine-induced Chk1 phosphorylation (Fig. 2C), and hence induced Kap1 phosphorylation (Fig. 2D) which is an indicative of DNA double strand breaks and ATM activation [42]. In the presence of thymidine, v-Src also induced Kap1 phosphorylation (Fig. 2E). These data suggest that v-Src induces replication fork collapse through the inhibition of replication checkpoint. This is also supported by the increased RPA32-S33 phosphorylation as shown below. One of the possible explanations for the mild effect of v-Src on Chk1 phosphorylation is that the replication fork collapse stimulates Chk1 phosphorylation, because we previously observed that high-level ectopic expression of Lyn re-stimulated Chk1 phosphorylation while low-level expression suppressed it [35].

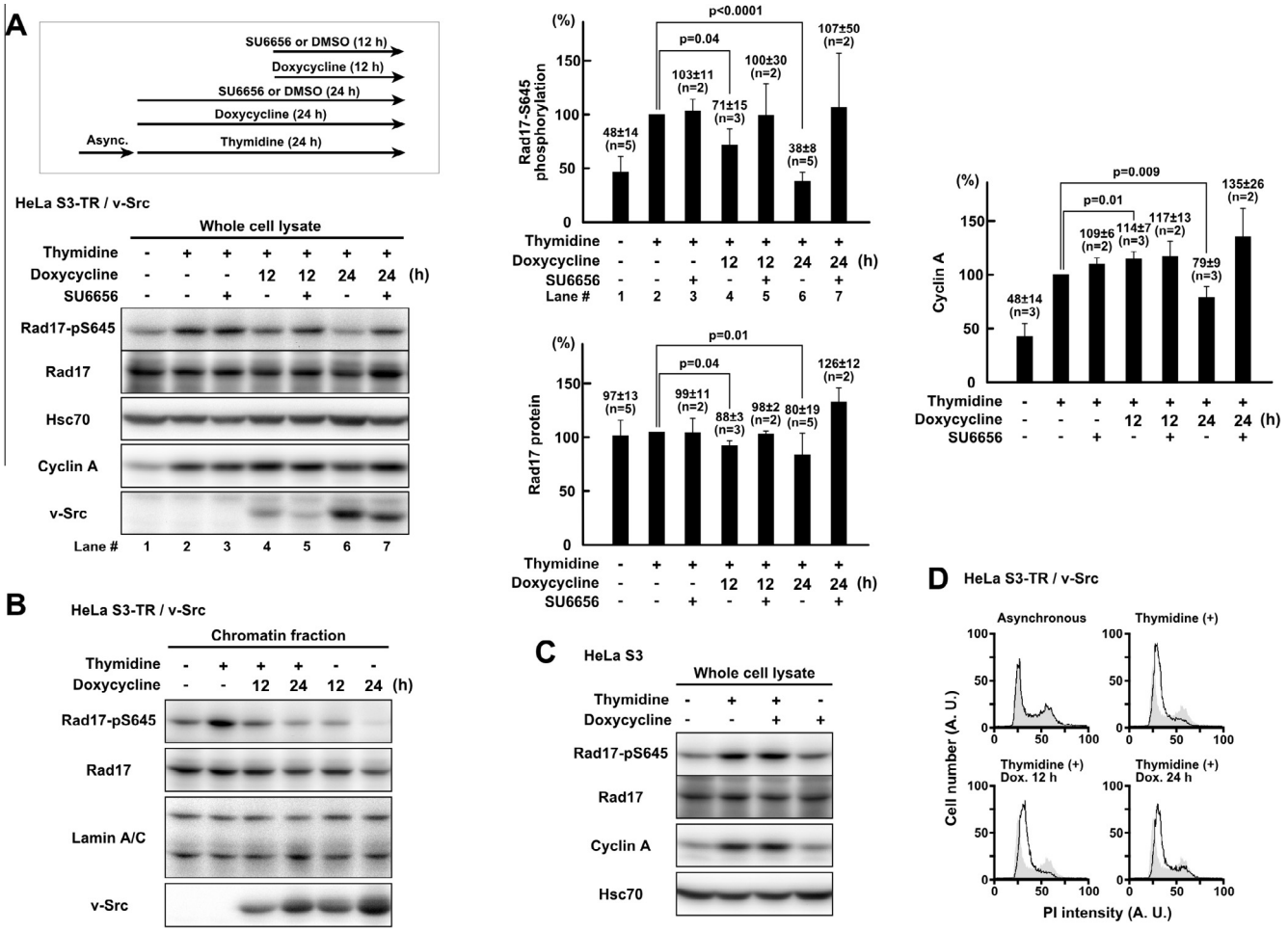


**Fig. 1.** v-Src suppresses Rad17 phosphorylation in a kinase-dependent manner. (A) Asynchronously growing HeLa S3-TR/v-Src cells were exposed to 4 mM thymidine for 24 h. Expression of v-Src was induced with 1 μg/ml doxycycline for 12 or 24 h before harvesting. Simultaneously with doxycycline, 5 μM SU6656 was added. Whole cell SDS lysates were prepared and probed with the indicated antibodies. Async., asynchronous. The graph represents results from more than two independent experiments. *P* values were calculated using *t*-test. (B) The same experiment as in A, except that chromatin fractions were prepared as described in Section 2. (C) HeLa S3 cells were exposed to 4 mM thymidine and/or 1 μg/ml doxycycline for 24 h. (D) HeLa S3-TR/v-Src cells were treated as in A, and fixed for flow cytometric analysis. A.U., arbitrary unit. Dox., doxycycline.

### 3.3. v-Src does not inhibit the kinase activity of ATR

To further examine the effect of v-Src on ATR signaling, we examined the effect of v-Src on RPA phosphorylation by ATR, since RPA32-S33 phosphorylation is induced by replication stress or fork collapse in a manner dependent on ATR and TopBP1 but independent of Rad17 [1,43,44]. In the presence of thymidine, v-Src did not inhibit but promoted thymidine-induced RPA32-S33 phosphorylation and electrophoretic mobility shift of RPA32 protein (Fig. 3A and B). Probably, the v-Src-induced replication fork collapse should promote RPA phosphorylation by ATR and DNA-PK, since previous works showed RPA32-S33 phosphorylation by ATR and DNA-PK [43,44]. These data raise a possibility that v-Src does not inhibit the kinase activity of ATR even though v-Src prevents the ATR-dependent phosphorylation of Rad17 and Chk1.

Next we examined the effect of v-Src on the kinase activity of ATR. Autophosphorylation of ATR at T1989 is a reliable indicator of the kinase activity [45]. Thymidine treatment induced ATR-T1989 phosphorylation as reported previously (Fig. 3C). However, v-Src did not affect the ATR-T1989 phosphorylation (Fig. 3D), suggesting that v-Src does not inhibit the kinase activity of ATR. We confirmed that most of all the ATR protein was extracted with a high salt buffer (data not shown). Furthermore, these data also indicate that replication stress is induced even after v-Src expression.

### 3.4. v-Src inhibits interaction between Rad17 and Rad9

The above results suggest that v-Src attenuates ATR-Chk1 signaling downstream of ATR. ATR-Chk1 signaling is mediated by assembly of protein complex on damaged chromatin [1,3], and 9–1–1, Rad17-RFC, and TopBP1 form a tripartite protein complex on damaged chromatin to mediate the checkpoint signaling [17,21]. Therefore, chromatin fraction was prepared from the thymidine-treated cells, and the effect of v-Src on Rad17 and 9–1–1, and TopBP1 was examined. In our preparation, the chromatin-enriched fraction contains chromosomal proteins, cytoskeletons, but not cytoplasmic and nucleoplasmic proteins [38]. v-Src did not affect chromatin loading of Rad17, Rad9, Rad1, and TopBP1 at least as much as Rad17-S645 phosphorylation (Figs. 1B and 4A–C). Unlike previous reports [7–11,21], thymidine exposure did not promote chromatin loading of Rad17, Rad9, Rad1, and TopBP1 (Figs. 1B and 4A–C), which might be due to the difference in extraction condition. Next, protein–protein interactions among 9–1–1, Rad17-RFC, and TopBP1 were examined. For this purpose, Rad9 was precipitated from solubilized chromatin fraction, since previous reports suggested that Rad9 interacts directly with Rad17 as well as TopBP1 [10,12–14]. v-Src expression did not affect Rad9–TopBP1 interaction, but it markedly inhibited Rad9–Rad17 interaction (Fig. 4D). On the other hand, the interaction between

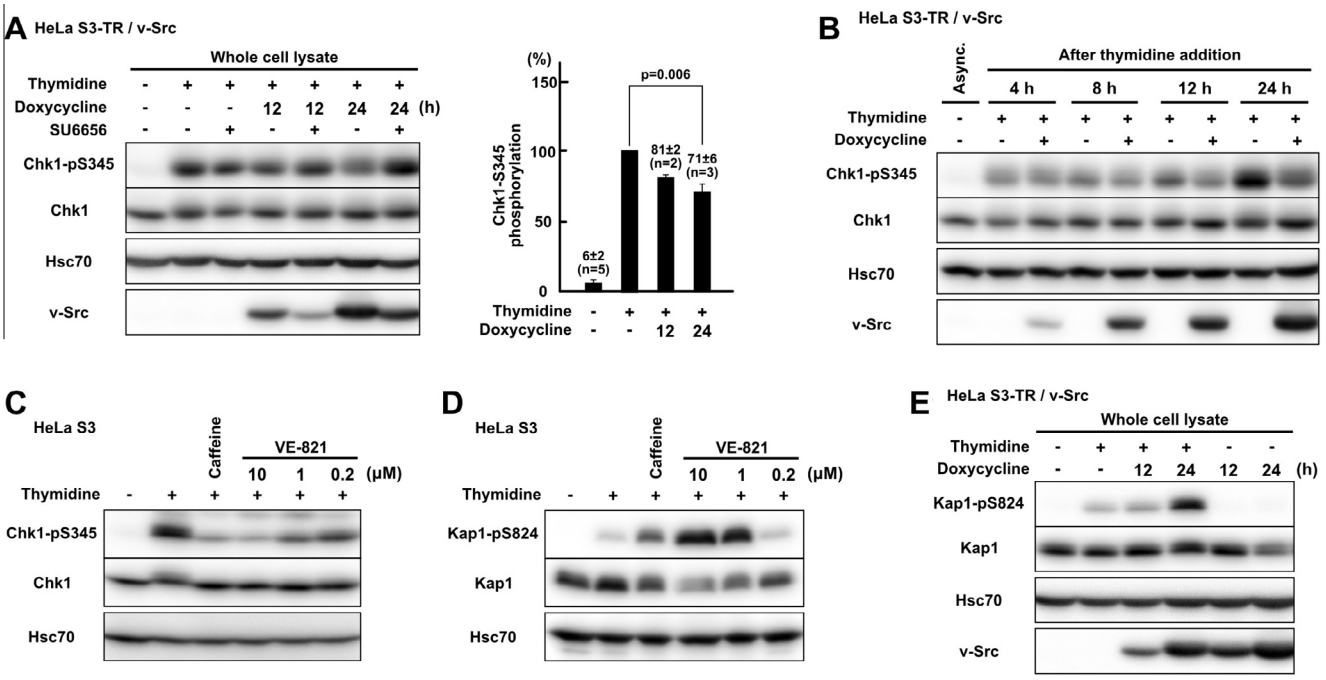


**Fig. 2.** v-Src promotes replication fork collapse under replication stress. (A and E) The same experiment as in Fig. 1A. HeLa S3-TR/v-Src cells were exposed to 4 mM thymidine for 24 h. At 12 or 24 h before harvesting, 1 μg/ml doxycycline and/or 5 μM SU6656 were added. Whole cell lysates were prepared and probed with the indicated antibodies. The graph represents results from more than two independent experiments. *P* values were calculated using *t*-test. (B) The same experiment as in A. Thymidine and doxycycline were added simultaneously, and whole cell lysates were prepared at the indicated times. Async., asynchronous. (C and D) HeLa S3 cells were exposed to 4 mM thymidine for 24 h, and whole cell lysates were prepared. At 2 h before harvesting, 5 mM caffeine or 0.5, 1, or 10 μM VE-821 were added.

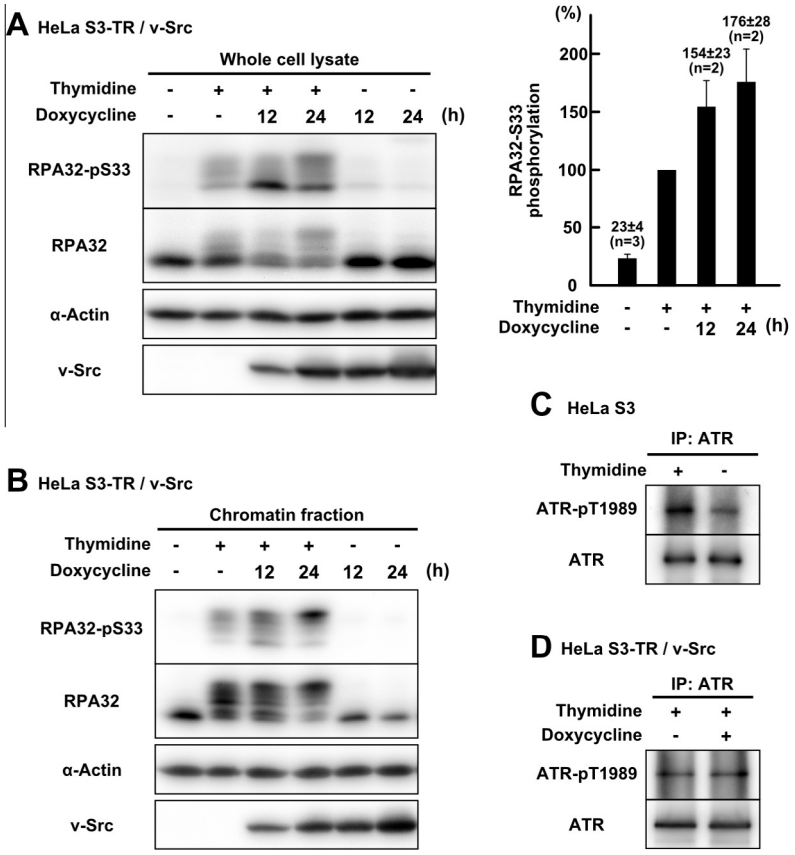


**Fig. 3.** v-Src does not inhibit the kinase activity of ATR. (A) The same experiment as in Fig. 1A. HeLa S3-TR/v-Src cells were exposed to 4 mM thymidine for 24 h. At 12 or 24 h before harvesting, 1 μg/ml doxycycline was added. Whole cell lysates were prepared and probed with the indicated antibodies. The graph represents results from two independent experiments. (B) The same experiment as in A, except that chromatin fractions were prepared. (C and D) HeLa S3 or HeLa S3-TR/v-Src cells were exposed to 4 mM thymidine and/or 1 μg/ml doxycycline for 24 h, and high salt lysates were prepared. The lysates were precipitated with anti-ATR antibody, and autophosphorylation of ATR was detected. Representative results from two independent experiments are shown.

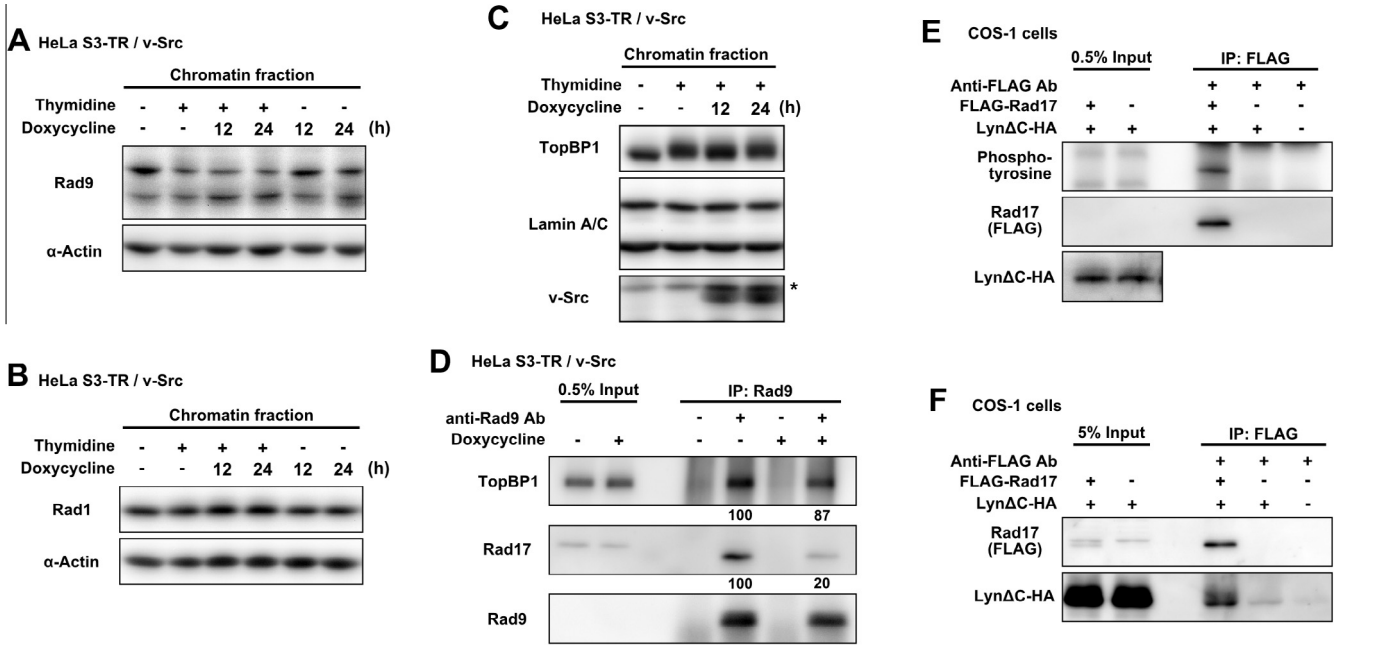


**Fig. 4.** v-Src inhibits interaction between Rad17 and Rad9. (A–C) HeLa S3-TR/v-Src cells were exposed to 4 mM thymidine and/or 1 μg/ml doxycycline for 24 h. Solubilized chromatin fractions were prepared as described in Section 2, and probed with the indicated antibodies. (D) Rad9 was precipitated from the solubilized chromatin fraction, and co-precipitation of the indicated proteins were detected. v-Src expression was induced for 24 h. Representative results from two independent experiments are shown. (E and F) In COS-1 cells, FLAG-tagged Rad17 was coexpressed with LynΔC-HA, and high salt lysates were prepared. Rad17 was precipitated, and tyrosine phosphorylation of Rad17 (E) and co-precipitation of LynΔC-HA (F) were detected.

Rad17 and Rad9 was not detected in low salt buffer-soluble nucleoplasmic fraction (data not shown), suggesting that the Rad9–Rad17 interaction in the chromatin fraction reflects protein complex assembly on damaged chromatin. Rad17 protein migrated faster after immunoprecipitation for an unknown reason (Fig. 4D), but we confirmed the specificity of anti-Rad17 antibody (cl. 31E9) in HeLa S3 cells by showing that the intensity of the Rad17 band was decreased by UV irradiation and increased by MG132 ([35] Fig. 5E), as reported previously [24]. Furthermore, Rad17 protein can be detected with anti-Rad17-pS645 antibody after immunoprecipitation with the anti-Rad17 antibody, although Rad17 protein also migrated faster in this condition (data not shown). Since Rad17 has a long unstructured region in its N-terminus, one of the possible explanations for the difference would be proteolytic digestion of the N-terminal region. Rad9 gives two bands in solubilized chromatin (Fig. 4A) as shown previously [7–11], however, Rad9 showed only lower band after immunoprecipitation (Fig. 4D), which might be due to dephosphorylation during immunoprecipitation as suggested by others [11,46]. These data suggest that v-Src attenuates ATR-Chk1 signaling through the inhibition of interaction between Rad9 and Rad17.

Finally, we examined which proteins are phosphorylated by SFKs in replication checkpoint. Rad17, but not Rad9, Rad1 or Hus1 was phosphorylated by Lyn (Fig. 4E and data not shown). Moreover, Lyn was coprecipitated with Rad17 (Fig. 4F). These data raise a possibility that v-Src regulates replication checkpoint through tyrosine phosphorylation of Rad17 protein.

## 4. Discussion

Rad17-RFC plays a role in mediating Chk1 phosphorylation by ATR, in addition to the role in chromatin loading of 9–1–1 [17–21]. The present data suggest that v-Src inhibits ATR signaling through disruption of the interaction between Rad17-RFC and 9–1–1 on damaged chromatin (Fig. 4D). Since, v-Src does not inhibit signaling from RPA-ssDNA filaments to Rad9–TopBP1 interaction (Figs. 3A, B, D and 4D), the reduced Rad17 phosphorylation should be the consequence of the inhibition of Rad17–Rad9 interaction. Currently, the disappearance of DNA lesions is considered to bring about the end of checkpoint signaling [22,23]. However, the present results proved the presence of an active process that is triggered by v-Src and terminates the checkpoint signaling. Moreover, previous works showed that ATP binding of Rad17 is required for its interaction with Rad9 [10,21,46,47], but the negative regulation of the interaction had not been reported so far. Therefore, our results would add a new layer of regulation to ATR-Chk1 signaling.

Given that the interaction between Rad17-RFC and 9–1–1 is required for 9–1–1 loading and TopBP1 recruitment [10,21], it is curious that v-Src does not perturb Rad9–TopBP1 interaction (Fig. 4D). One of the possible explanations is that the residual interaction between Rad17-RFC and 9–1–1 is sufficient for 9–1–1 loading and TopBP1 interaction. Another possibility is that TopBP1 is recruited to ATR–ATRIP complex independently of Rad17, since Rad17-independent and TopBP1-dependent RPA32-S33 phosphorylation was reported [43].

It is unclear whether the effect of v-Src on Rad17–Rad9 interaction is direct or indirect. We previously showed that ectopic expression of Lyn in the nucleus suppresses Chk1 phosphorylation more efficiently than that in the cytoplasm [35]. Therefore, one of the possibility is that v-Src regulates replication checkpoint through the tyrosine phosphorylation of Rad17. It is also unclear whether endogenous SFKs regulate the Rad17-S645 phosphorylation by the same mechanism. Further work will clarify these issues.

## Acknowledgments

This work was supported in part by grant-in-aid for Scientific Research, Global COE Program (Global Center for Education and Research in Immune Regulation and Treatment), and Program for

Leading Graduate Schools (Nurture of Creative Research Leaders in Immune System Regulation and Innovative Therapeutics) from the Japanese Ministry of Education, Culture, Sports, Science and Technology. S.K. was Global-COE research assistants. M.M. and T.H. are research assistants of LGS.

## References

[1] K.A. Cimprich, D. Cortez, ATR: an essential regulator of genome integrity, Nat. Rev. Mol. Cell Biol. 9 (2008) 616–627.
[2] D. Branzei, M. Foiani, Maintaining genome stability at the replication fork, Nat. Rev. Mol. Cell Biol. 11 (2010) 208–219.
[3] R.L. Flynn, L. Zou, ATR: a master conductor of cellular responses to DNA replication stress, Trends Biochem. Sci. 36 (2011) 133–140.
[4] D. Cortez, S. Guntuku, J. Qin, S.J. Elledge, ATR and ATRIP: partners in checkpoint signaling, Science 294 (2001) 1713–1716.
[5] L. Zou, S.J. Elledge, Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes, Science 300 (2003) 1542–1548.
[6] T.S. Byun, M. Pacek, M. Yee, J.C. Walter, K.A. Cimprich, Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint, Genes Dev. 19 (2005) 1040–1052.
[7] M.A. Burtelow, S.H. Kaufmann, L.M. Karnitz, Retention of the human Rad9 checkpoint complex in extraction-resistant nuclear complexes after DNA damage, J. Biol. Chem. 275 (2000) 26343–26348.
[8] P. Roos-Mattjus, B.T. Vroman, M.A. Burtelow, M. Rauen, A.K. Eapen, L.M. Karnitz, Genotoxin-induced Rad9–Hus1–Rad1 (9–1–1) chromatin association is an early checkpoint signaling event, J. Biol. Chem. 277 (2002) 43809–43812.
[9] L. Zou, D. Cortez, S.J. Elledge, Regulation of ATR substrate selection by Rad17-dependent loading of Rad9 complexes onto chromatin, Genes Dev. 16 (2002) 198–208.
[10] V.P. Bermudez, L.A. Lindsey-Boltz, A.J. Cesare, Y. Maniwa, J.D. Griffith, J. Hurwitz, A. Sancar, Loading of the human 9–1–1 checkpoint complex onto DNA by the checkpoint clamp loader hRad17-replication factor C complex in vitro, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 1633–1638.
[11] R.E. Jones, J.R. Chapman, C. Puligilla, J.M. Murray, A.M. Car, C.C. Ford, H.D. Lindsay, XRad17 is required for the activation of XChk1 but not XCds1 during checkpoint signaling in Xenopus, Mol. Biol. Cell 14 (2003) 3898–3910.
[12] M. Mäkiniemi, T. Hillukkala, J. Tuusa, K. Reini, M. Vaara, D. Huang, H. Pospiech, I. Majuri, T. Westerling, T.P. Mäkelä, J.E. Syväoja, BRCT domain-containing protein TopBP1 functions in DNA replication and damage response, J. Biol. Chem. 276 (2001) 30399–30406.
[13] R.P. St Onge, B.D.A. Besley, J.L. Pelley, S. Davey, A role for the phosphorylation of hRad9 in checkpoint signaling, J. Biol. Chem. 278 (2003) 26620–26628.
[14] K. Furuya, M. Poitelea, L. Guo, T. Caspari, A.M. Carr, Chk1 activation requires Rad9 S/TQ-site phosphorylation to promote association with C-terminal BRCT domains of Rad4TOPBP1, Genes Dev. 18 (2004) 1154–1164.
[15] A. Kumagai, J. Lee, H.Y. Yoo, W.G. Dunphy, TopBP1 activates the ATR–ATRIP complex, Cell 124 (2006) 943–955.
[16] S. Delacroix, J.M. Wagner, M. Kobayashi, K. Yamamoto, L.M. Karnitz, The Rad9–Hus1–Rad1 (9–1–1) clamp activates checkpoint signaling via TopBP1, Genes Dev. 21 (2007) 1472–1477.
[17] J. Lee, A. Kumagai, W.G. Dunphy, The Rad9–Hus1–Rad1 checkpoint clamp regulates interaction of TopBP1 with ATR, J. Biol. Chem. 282 (2007) 28036–28044.
[18] S. Bao, R.S. Tibbetts, K.M. Brumbaugh, Y. Fang, D.A. Richardson, A. Ali, S.M. Chen, R.T. Abraham, X.F. Wang, ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress responses, Nature 411 (2001) 969–974.
[19] S. Post, Y.C. Weng, K. Cimprich, L.B. Chen, Y. Xu, E.Y.H.P. Lee, Phosphorylation of serines 635 and 645 of human Rad17 is cell cycle regulated and is required for G(1)/S checkpoint activation in response to DNA damage, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 13102–13107.
[20] X. Wang, L. Zou, T. Lu, S. Bao, K.E. Hurov, W.N. Hittelman, S.J. Elledge, L. Li, Rad17 phosphorylation is required for claspin recruitment and Chk1 activation in response to replication stress, Mol. Cell 23 (2006) 331–341.
[21] J. Lee, W.G. Dunphy, Rad17 plays a central role in establishment of the interaction between TopBP1 and the Rad9–Hus1–Rad1 complex at stalled replication forks, Mol. Biol. Cell 21 (2010) 926–935.
[22] J. Bartek, J. Lukas, DNA damage checkpoint: from initiation to recovery or adaptation, Curr. Opin. Cell Biol. 19 (2007) 238–245.
[23] C. Clémenson, M.C. Marsolier-Kergoat, DNA damage checkpoint inactivation: adaptation and recovery, DNA Repair 8 (2009) 1101–1109.
[24] L. Zhang, C.H. Park, J. Wu, H. Kim, W. Liu, T. Fujita, M. Balasubramani, E.M. Schreiber, X.F. Wang, Y. Wan, Proteolysis of Rad17 by Cdh1/APC regulates checkpoint termination and recovery from genotoxic stress, EMBO J. 29 (2010) 1726–1737.
[25] V. Radha, S. Nambirajan, G. Swarup, Association of Lyn tyrosine kinase with the nuclear matrix and cell-cycle-dependent changes in matrix-associated tyrosine kinase activity, Eur. J. Biochem. 236 (1996) 352–359.
[26] A.K. Jain, A.K. Jaiswal, GSK-3β acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2, J. Biol. Chem. 282 (2007) 16502–16510.
[27] P. Paliwal, V. Radha, G. Swarup, Regulation of p73 by Hck through kinase-dependent and independent mechanisms, BMC Mol. Biol. 8 (2007) 45.
[28] K. Ikeda, Y. Nakayama, Y. Obata, T. Kuga, K. Kasahara, Y. Fukumoto, N. Yamaguchi, Nuclear localization of Lyn tyrosine kinase mediated by inhibition of its kinase activity, Exp. Cell Res. 314 (2008) 3392–3404.
[29] A. Takahashi, Y. Obata, Y. Fukumoto, Y. Nakayama, K. Kasahara, T. Kuga, Y. Higashiyama, T. Saito, K.K. Yokoyama, N. Yamaguchi, Nuclear localization of Src-family tyrosine kinases is required for growth factor-induced euchromatinization, Exp. Cell Res. 315 (2009) 1117–1141.
[30] S. Kharbanda, Z.M. Yuan, E. Rubin, R. Weichselbaum, D. Kufe, Activation of Src-like p56/p53 Lyn tyrosine kinase by ionizing radiation, J. Biol. Chem. 269 (1994) 20739–20743.
[31] S. Kharbanda, A. Saleem, Z.M. Yuan, S. Kraeft, R. Weichselbaum, L.B. Chen, D. Kufe, Nuclear signaling induced by ionizing radiation involves colocalization of the activated p56/p53 Lyn tyrosine kinase with p34 cdc2, Cancer Res. 56 (1996) 3617–3621.
[32] N. Masumoto, S. Nakano, H. Fujishima, K. Kohno, Y. Niho, V-Src induces cisplatin resistance by increasing the repair of cisplatin-DNA interstrand cross-links in human gallbladder adenocarcinoma cells, Int. J. Cancer 80 (1999) 731–737.
[33] B.L. Webb, E. Jimenez, G.S. Martin, V-Src generates a p53-independent apoptotic signal, Mol. Cell. Biol 20 (2000) 9271–9280.
[34] A. Vigneron, I.B. Roninson, E. Gamelin, O. Coqueret, Src inhibits adriamycin-induced senescence and G2 checkpoint arrest by blocking the induction of p21waf1, Cancer Res. 65 (2005) 8927–8935.
[35] Y. Fukumoto, M. Morii, T. Miura, S. Kubota, K. Ishibashi, T. Honda, A. Okamoto, N. Yamaguchi, A. Iwama, Y. Nakayama, N. Yamaguchi, Src family kinases promote silencing of ATR-Chk1 signaling in termination of DNA damage checkpoint, J. Biol. Chem. 289 (2014) 12313–12329.
[36] Y. Fukumoto, Y. Obata, K. Ishibashi, N. Tamura, I. Kikuchi, K. Aoyama, Y. Hattori, K. Tsuda, Y. Nakayama, N. Yamaguchi, Cost-effective gene transfection by DNA compaction at pH 4.0 using acidified, long shelf-life polyethylenimine, Cytotechnology 62 (2010) 73–82.
[37] Y. Nakayama, A. Igarashi, I. Kikuchi, Y. Obata, Y. Fukumoto, N. Yamaguchi, Bleomycin-induced over-replication involves sustained inhibition of mitotic entry through the ATM/ATR pathway, Exp. Cell Res. 315 (2009) 2515–2528.
[38] S. Kubota, Y. Fukumoto, K. Aoyama, K. Ishibashi, R. Yuki, T. Morinaga, T. Honda, N. Yamaguchi, T. Kuga, T. Tomonaga, N. Yamaguchi, Phosphorylation of KRAB-associated protein 1 (KAP1) at Tyr-449, Tyr-458, and Tyr-517 by nuclear tyrosine kinases inhibits the association of KAP1 and heterochromatin protein 1α (HP1α) with heterochromatin, J. Biol. Chem. 288 (2013) 17871–17883.
[39] T. Kuga, Y. Nakayama, M. Hoshino, Y. Higashiyama, Y. Obata, D. Matsuda, K. Kasahara, Y. Fukumoto, N. Yamaguchi, Differential mitotic activation of endogenous c-Src, c-Yes, and Lyn in HeLa cells, Arch. Biochem. Biophys. 466 (2007) 116–124.
[40] Y. Katsuragi, N. Sagata, Regulation of Chk1 kinase by autoinhibition and ATR-mediated phosphorylation, Mol. Biol. Cell 15 (2004) 1680–1689.
[41] P.M. Reaper, M.R. Griffiths, J.M. Long, J.D. Charrier, S. MacCormick, P.A. Charlton, J.M.C. Golec, J.R. Pollard, Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR, Nat. Chem. Biol. 7 (2011) 428–430.
[42] Y. Ziv, D. Bielopolski, Y. Galanty, C. Lukas, Y. Taya, D.C. Schultz, J. Lukas, S. Bekker-Jensen, J. Bartek, Y. Shiloh, Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway, Nat. Cell Biol. 8 (2006) 870–876.
[43] B. Shiotani, H.D. Nguyen, P. Håkansson, A. Maréchal, A. Tse, H. Tahara, L. Zou, Two distinct modes of ATR activation orchestrated by Rad17 and Nbs1, Cell Rep. 30 (2013) 1651–1662.
[44] S. Liu, S.O. Opiyo, K. Manthey, J.G. Glanzer, A.K. Ashley, C. Amerin, K. Troksa, M. Shrivastav, J.A. Nickoloff, G.G. Oakley, Distinct roles for DNA-PK, ATM and ATR in RPA phosphorylation and checkpoint activation in response to replication stress, Nucleic Acids Res. 40 (2012) 10780–10794.
[45] S. Liu, B. Shiotani, M. Lahiri, A. Maréchal, A. Tse, C.C. Leung, J.N.M. Glover, X.H. Yang, L. Zou, ATR autophosphorylation as a molecular switch for checkpoint activation, Mol. Cell 43 (2011) 192–202.
[46] L.A. Lindsey-Boltz, V.P. Bermudez, J. Hurwitz, A. Sancer, Purification and characterization of human DNA damage checkpoint Rad complexes, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 11236–11241.
[47] M. Rauen, M.A. Burtelow, V.M. Dufault, L.M. Karnitz, The human checkpoint protein hRad17 interacts with the PCNA-like proteins hRad1, hHus1, and hRad9, J. Biol. Chem. 275 (2000) 29767–29771.