CELL STRUCTURE AND FUNCTION 40: 43–50 (2015)

Mini-review and Review

Novel Insights into Chk1 Regulation by Phosphorylation

Hidemasa Goto[1,2], Kousuke Kasahara[1,3], and Masaki Inagaki[1,2*]

[1]*Division of Biochemistry, Aichi Cancer Center Research Institute, Nagoya, Aichi 464-8681, Japan,* [2]*Department of Cellular Oncology, Nagoya University Graduate School of Medicine, Nagoya, Aichi 466-8550, Japan,* [3]*Department of Oncology, Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Aichi, 467-8603, Japan*

ABSTRACT. **Checkpoint kinase 1 (Chk1) is a conserved protein kinase central to the cell-cycle checkpoint during DNA damage response (DDR). Until recently, ATR, a protein kinase activated in response to DNA damage or stalled replication, has been considered as the sole regulator of Chk1. Recent progress, however, has led to the identification of additional protein kinases involved in Chk1 phosphorylation, affecting the subcellular localization and binding partners of Chk1. In fact, spatio-temporal regulation of Chk1 is of critical importance not only in the DDR but also in normal cell-cycle progression. In due course, many potent inhibitors targeted to Chk1 have been developed as anticancer agents and some of these inhibitors are currently in clinical trials. In this review, we summarize the current knowledge of Chk1 regulation by phosphorylation.**

Key words: Chk1, cell-cycle checkpoint, phosphorylation

Introduction

DNA damage or stalled replication is sensed by cell-cycle checkpoints, which arrest the cell cycle to allow the time necessary to repair damaged DNA or to relieve the stalled replication fork (Ciccia and Elledge, 2010; Jackson and Bartek, 2009). These checkpoint signals are mediated by two evolutionally conserved protein kinases, ataxia-telangiectasia mutated (ATM) (Cremona and Behrens, 2014; Shiloh and Ziv, 2013)/ATM- and Rad3-related (ATR; Fig. 1) (Cimprich and Cortez, 2008; Flynn and Zou, 2011; Zeman and Cimprich, 2014). ATM and ATR phosphorylate and then activate checkpoint kinase 2 (Chk2) (Antoni *et al.*, 2007) and Chk1(Lukas *et al.*, 2003; Reinhardt and Yaffe, 2009; Zhang and Hunter, 2014), respectively (Fig. 1). The ATM-Chk2 pathway primarily reacts to DNA double strand break (DSB; Fig. 1). With regard to cell-cycle arrest, the transcription factor p53 is one of the most important substrates for ATM and Chk2 (Fig. 1) (Appella and Anderson, 2001; McGowan, 2002; Polager and Ginsberg, 2009; Riley *et al.*, 2008). These phosphorylations block p53 degradation by E3 ligase MDM2 (Fig. 1) (Appella and Anderson, 2001; Polager and Ginsberg, 2009; Riley *et al.*, 2008; Wade *et al.*, 2013). The stabilized p53 induces p21, which binds and then inhibits cyclin-dependent kinases (CDKs; Fig. 1) (Polager and Ginsberg, 2009; Riley *et al.*, 2008). Unlike ATM-Chk2, the ATR-Chk1 pathway detects a broader spectrum of DNA abnormalities (e.g. UV light, DNA replication inhibition, interstrand DNA crosslinking, or DSB end resection; Fig. 1). With regard to cell-cycle arrest, Cdc25A phosphatase is an essential target for Chk1 (Fig. 1) (Boutros *et al.*, 2007; Goto *et al.*, 2012; Neely and Piwnica-Worms, 2003). Chk1-induced phosphorylation triggers Cdc25A polyubiquitination and degradation (Fig. 1) (Boutros *et al.*, 2007; Busino *et al.*, 2004; Jin *et al.*, 2003; Neely and Piwnica-Worms, 2003). Since Cdc25A is a critical phosphatase for CDK activation (Boutros *et al.*, 2007; Goto *et al.*, 2012), the activation of ATR-Chk1 pathway results in cell-cycle arrest (Fig. 1).

In cancer cells, the ATM-Chk2-p53 pathway is often impaired due to the mutations or deletions of *ATM*, *CHEK2*, and/or *TP53* (Antoni *et al.*, 2007; Cremona and Behrens, 2014; Goto *et al.*, 2012; Riley *et al.*, 2008; Shiloh and Ziv, 2013). These mutations are also identified as can-

*To whom correspondence should be addressed: Masaki Inagaki, Division of Biochemistry, Aichi Cancer Center Research Institute, Nagoya, Aichi 464-8681, Japan.
Tel: +81–52–762–6111 (ext. 7020), Fax: +81–52–763–5233
E-mail: minagaki@aichi-cc.jp

Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ATM- and Rad3-related; β-TrCP, β-transducin repeated-containing protein; CDK, cyclin-dependent kinase; Chk, Checkpoint kinase; DDR, DNA damage response, DSB, DNA double strand break; MAPK, mitogen-activated protein kinase; MK2, MAPK-activated protein (MAPKAP) kinase-2; NES, nuclear export signal; NLS, nuclear localization signal; RSK, ribosomal S6 kinase; PKB, protein kinase B; ssDNA, single strand DNA; UV, ultraviolet.

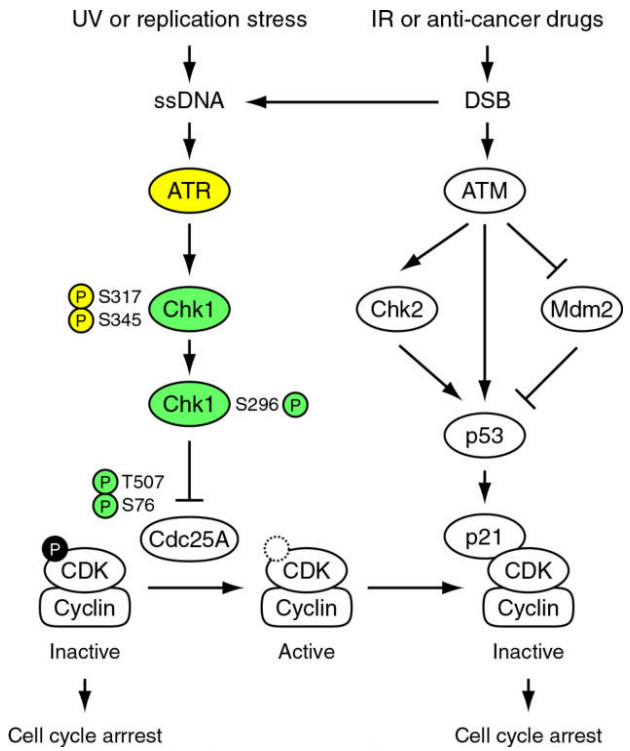

Fig. 1. A schema of DNA damage/replication checkpoint responses. The ATM-Chk2-p53 pathway is activated by DNA double strand breaks (DSBs), whereas the activation of ATR-Chk1-Cdc25A pathway is induced by single strand DNA (ssDNA) adjacent to double strand DNA. At the S or G2 phase of the cell cycle, DSBs are processed to ssDNA, which secondly activates the ATR-Chk1-Cdc25A pathway. The activation of these pathways ultimately suppresses cyclin-dependent kinases (CDKs).

cer driver mutations involved in tumor initiation and progression (Kandoth *et al.*, 2013; Lawrence *et al.*, 2014; Vandin *et al.*, 2012). Due to this defect, cancer cells rely much more heavily on the ATR-Chk1-Cdc25A pathway for survival than normal cells (Goto *et al.*, 2012; Zhang and Hunter, 2014). Chk1 inhibitors were originally developed and tested to sensitize tumors to existing cancer therapies, such as radiation therapy and chemotherapy with various DNA-damaging agents and/or anti-metabolites (Carrassa and Damia, 2011; Dai and Grant, 2010; Ma *et al.*, 2011). Since Chk1 also regulates normal (unperturbed) cell-cycle progression (Goto *et al.*, 2012; Zhang and Hunter, 2014), Chk1 inhibitors are also expected to kill cancer cells as single agents (Carrassa and Damia, 2011; Maugeri-Sacca *et al.*, 2013; Thompson and Eastman, 2013). Due to the fact that the mechanism underlying Chk1 function in normal (unperturbed) cell-cycle progression remains poorly understood (Goto *et al.*, 2012; Zhang and Hunter, 2014), little is known about the mechanisms by which Chk1 inhibitors preferentially kill cancer cells without inducing exogenous DNA damages.

Chk1 phosphorylation by ATR is essential for cell-cycle checkpoint (especially at the intra-S phase or at the G2/M transition) (Capasso *et al.*, 2002; Niida *et al.*, 2007; Wilsker *et al.*, 2008) and conserved from yeast to mammals (Rhind and Russell, 2000). Recent studies have also revealed that Chk1 is phosphorylated and regulated by various types of protein kinases other than ATR (Goto *et al.*, 2012; Zhang and Hunter, 2014). In this review, we will summarize functional change of Chk1 by phosphorylation with a particular focus on human Chk1.

Chk1 Phosphorylation by ATR

ATR activation requires the generation of structures containing single strand DNA (ssDNA) adjacent to double strand DNA (dsDNA; Fig. 1) (Cimprich and Cortez, 2008; Flynn and Zou, 2011; Zeman and Cimprich, 2014). Such ssDNA is coated with replication protein A (RPA) complex and then attracts the complex of ATR and its regulatory protein ATR-interacting protein (ATRIP) (Cimprich and Cortez, 2008; Flynn and Zou, 2011; Zeman and Cimprich, 2014). The accumulation of ATR/ATRIP complex to damage sites results in initial (weak) activation of ATR (Cimprich and Cortez, 2008; Flynn and Zou, 2011; Zeman and Cimprich, 2014). The ssDNA-RPA structure also functions as a platform to recruit a number of checkpoint regulators including Rad17, Rad9-Hus1-Rad1 (9-1-1) complex, DNA topoisomerase II binding protein 1 (TopBP1), Timeless (TIM), TIM-interacting protein (TIPIN), and Claspin to damaged sites (Ciccia and Elledge, 2010; Jackson and Bartek, 2009). ATR phosphorylates some of these recruited proteins (Cimprich and Cortez, 2008; Flynn and Zou, 2011; Zeman and Cimprich, 2014). The accumulation and phosphorylation of these checkpoint regulators further stimulates the catalytic activity of ATR (Ciccia and Elledge, 2010; Cimprich and Cortez, 2008; Flynn and Zou, 2011; Jackson and Bartek, 2009; Zeman and Cimprich, 2014). For the efficient phosphorylation of Chk1 by ATR, Chk1 needs to form a complex with Claspin (Chini and Chen, 2004; Kumagai and Dunphy, 2000; Kumagai *et al.*, 2004). Based on the localization of these proteins, ATR-induced Chk1 phosphorylation likely occurs at the sites of DNA damage on chromatin (Fig. 2) (Kasahara *et al.*, 2010; Smits *et al.*, 2006; Zhang *et al.*, 2005).

The activated ATR phosphorylates Chk1 at Ser317 and Ser345 in its C-terminal regulatory domain (Fig. 2 and Table I) (Zhao and Piwnica-Worms, 2001). Chk1 phosphorylation at these sites (especially at Ser345) is critical for DNA damage checkpoint activation, replication control, and cell viability (Capasso *et al.*, 2002; Niida *et al.*, 2007; Wilsker *et al.*, 2008). Functionally, ATR-induced phosphorylation elevates Chk1 catalytic activity (Fig. 2 and Table I) (Katsuragi and Sagata, 2004; Walker *et al.*, 2009; Zhao and Piwnica-Worms, 2001): Ser345 is a more critical phosphor-

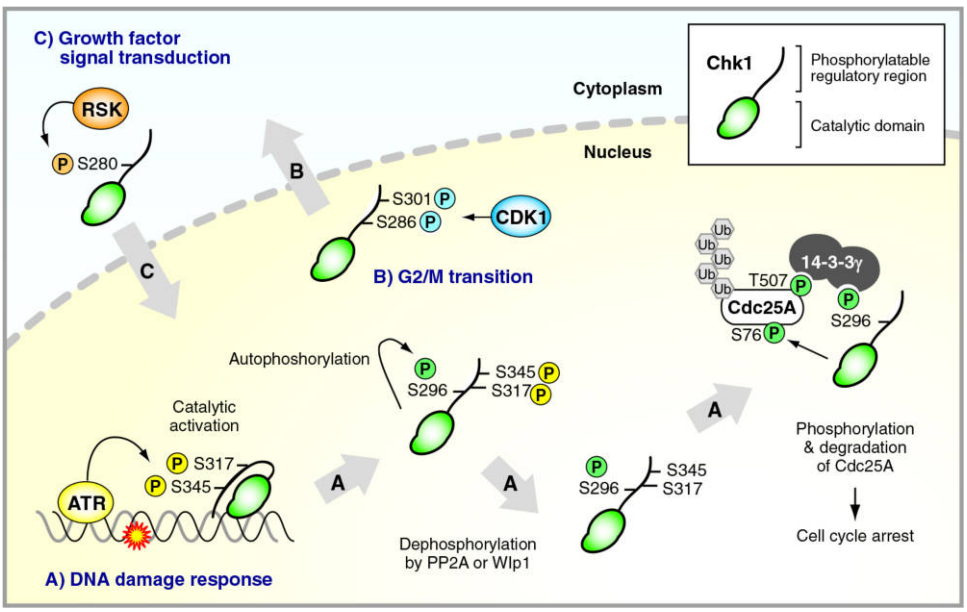

Fig. 2. Functional change of Chk1 by phosphorylation. A schematic representation of functional change by Chk1 phosphorylations during DNA damage response (DDR; A), at the G2/M transition (B), or in response to growth factor stimulation (C).

Table I. FUNCTIONAL CHANGE BY PHOSPHORYLATION AT ITS C-TERMINAL REGULATORY DOMAIN

Site	Stimulus	Kinase	Interactor	Effect on Chk1 function
Ser-280	Growth factor	p90 RSK		Transport from cytoplasm to nucleus
	UV irradiation	p90 RSK		Acceleration of activation processes
Ser-286 & Ser-301	G2/M transition	CDK1		Transport from nucleus to cytopasm
Ser-296	DNA damage response (DDR)	Chk1	14-3-3γ	1) Interaction with Cdc25A via 14-3-3γ
				2) Release from DNA damage sites to entire nucleus
Ser-317 & Ser-345	DDR	ATR		Catalytic activation
Ser-345	DDR	ATR	14-3-3β/ζ	Nuclear retention
Ser-345	DDR	ATR		Polyubiquitylation & degradation

ylation site for catalytic activation (Walker *et al.*, 2009). The N-terminal catalytic domain of Chk1 adopts an open kinase conformation (Chen *et al.*, 2000) and the deletion of C-terminal domain increases Chk1 catalytic activity (Chen *et al.*, 2000; Katsuragi and Sagata, 2004). In fission yeast, the C-terminal domain contains a pseudo-substrate motif that facilitates the intra-molecular interaction (Biggins *et al.*, 2008). These observations suggest a model in which the phosphorylation at two residues relieves an auto-inhibitory effect by the C-terminal regulatory domain (Biggins *et al.*, 2008; Chen *et al.*, 2000; Katsuragi and Sagata, 2004; Walker *et al.*, 2009; Zhang *et al.*, 2009). However, the pseudo-substrate motif does not appear to be conserved in Chk1 from other species. In addition, the deletion of the C-terminus abrogates Chk1 function in fission yeast (Caparelli and O'Connell, 2013; Kosoy and O'Connell, 2008), suggesting that the regulatory domain may play diverse roles in Chk1 activity. Therefore, much is still unknown about molecular mechanism by which Chk1 is structurally activated by phosphorylation.

Chk1-Ser345 phosphorylation regulates Chk1 function in addition to the catalytic activation. This phosphorylation creates a docking site for 14-3-3 β or ζ (Table I) (Jiang *et al.*, 2003). Since putative nuclear export signal (NES) sequence is located near Ser345 on Chk1 (Jiang *et al.*, 2003; Wang *et al.*, 2012), 14-3-3 β or ζ competes with Crm-1 (a protein required for nuclear export; also called exportin 1) to the binding to Chk1 (Jiang *et al.*, 2003), resulting in nuclear retention of Chk1 during DNA damage response (DDR; Table I) (Sanchez *et al.*, 1997). Since nuclear Chk1 activity is critical to establish a checkpoint (Matsuyama *et al.*, 2011; Reinhardt *et al.*, 2010), the checkpoint activation processes are accelerated by nuclear accumulation of Chk1, together with its catalytic activation.

At later stage of DDR, Chk1 binds E3 ligases (such as SCF^{Fbx6} in the cytoplasm and $Cul4A^{CDT2}$ in the nucleus),

which requires preceding Chk1-Ser345 phosphorylation (Table I) (Huh and Piwnica-Worms, 2013; Leung-Pineda *et al.*, 2009; Zhang *et al.*, 2009, 2005). The interaction with E3 ligases induces proteasome-dependent Chk1 degradation (Table I), resulting in checkpoint termination and cell-cycle resumption (Huh and Piwnica-Worms, 2013; Leung-Pineda *et al.*, 2009; Zhang *et al.*, 2009; Zhang *et al.*, 2005). However, there is ~4-hr difference between Chk1 phosphorylation and the onset of degradation (Zhang *et al.*, 2005). Thus, much is still unknown about a signaling pathway from Chk1 phosphorylation to degradation during DDR.

Chk1 Autophosphorylation (Especially at Ser296)

During DDR, Chk1 is autophosphorylated at several sites including Ser296 just after Chk1 phosphorylation at Ser317 and Ser345 by ATR (which implies Chk1 catalytic activation; Fig. 2) (Clarke and Clarke, 2005; Kasahara *et al.*, 2010). After Chk1 autophosphorylation, ATR sites are rapidly dephosphorylated by phosphatases such as protein phosphatase 2A (PP2A) (Kasahara *et al.*, 2010; Leung-Pineda *et al.*, 2006) or PPM1D (also called Wip1 or PP2Cδ; Fig. 2) (Lu *et al.*, 2005). Ser296-phosphorylated Chk1 is diffusely detected in the entire nucleus (nucleoplasm; soluble nuclear fraction), whereas Ser345-phosphorylated Chk1 is predominantly observed at DNA damage foci (Kasahara *et al.*, 2010). These observations lead to a model in which this phosphorylation shift promotes the release of Chk1 from DNA damage foci on the chromatin (Fig. 2 and Table I). Since ATR-mediated phosphorylation induces Chk1 autophosphorylation (the most likely direct trigger to spread Chk1 signals over nucleus) (Kasahara *et al.*, 2010), this model fits with the observation that Ser345 phosphorylation triggers Chk1 release from the chromatin (Shimada *et al.*, 2008; Smits *et al.*, 2006; Zhang *et al.*, 2005). Since important downstream effectors such as Cdc25A are distributed diffusely in the nucleus (Bekker-Jensen *et al.*, 2006), the redistribution to the entire nucleus is essential for Chk1 to deliver checkpoint signals (Smits *et al.*, 2006).

Among Chk1 autophosphorylation sites, Ser296 is the most critical phosphorylation site for the checkpoint function (Kasahara *et al.*, 2010). Ser296 phosphorylation creates a docking site for 14-3-3γ (Fig. 2 and Table I) (Kasahara *et al.*, 2010). Since Chk1 also generates 14-3-3-binding sites on Cdc25A (Chen *et al.*, 2003), dimerization of 14-3-3 proteins (Mohammad and Yaffe, 2009) facilitates the formation of ternary complex among Chk1, Cdc25A, and 14-3-3γ (Fig. 2) (Kasahara *et al.*, 2010). This complex formation is essential for Chk1 to phosphorylate Ser76 on Cdc25A (Fig. 2), whereas it is dispensable for Cdc25 phosphorylation at Thr507 (a phosphorylation site required for 14-3-3 binding) (Chen *et al.*, 2003) by Chk1 (Kasahara *et al.*, 2010). Ser76 is known as a rate-limiting phosphorylation site for Cdc25A degradation (Boutros *et al.*, 2007; Busino *et al.*, 2004; Jin *et al.*, 2003; Neely and Piwnica-Worms, 2003). Ser76 phosphorylation triggers additional Cdc25A phosphorylations by NEK11 (Melixetian *et al.*, 2009) and casein kinase 1 (CK1) (Honaker and Piwnica-Worms, 2010), which generate a β-TrCP (E3 ligase) recognition motif (phosphodegron) on Cdc25A. This proteasome-dependent Cdc25A degradation prevents premature activation of CDKs, resulting in cell-cycle arrest. Thus, Chk1-Ser296 autophosphorylation is one of critical steps for checkpoint signaling during DDR.

Chk1 Phosphorylation by CDKs

Chk1 also plays critical roles in normal (unperturbed) cell-cycle progression. Essential functions of Chk1 during development are well documented by the early embryonic lethality of Chk1-knockout mice (Liu *et al.*, 2000; Takai *et al.*, 2000). It is conceivable that Chk1 monitors replication forks in S phase (Maya-Mendoza *et al.*, 2007; Syljuasen *et al.*, 2005; Takai *et al.*, 2000). Chk1 activity is necessary to prevent late-origin firing and then irreversible replication fork collapse (Maya-Mendoza *et al.*, 2007). At the G2 phase, Chk1 inhibits premature mitotic entry (Enomoto *et al.*, 2009; Kramer *et al.*, 2004; Matsuyama *et al.*, 2011). Thus, Chk1 has an activity to negatively regulate cell-cycle progression even without exogenously introducing DNA damage. However, there is a debate as to whether or not ATR participates in Chk1 activity during normal (unperturbed) cell-cycle progression (Brown and Baltimore, 2000; de Klein *et al.*, 2000; Shimada *et al.*, 2008).

At the G2/M transition, Chk1 is phosphorylated at Ser286 and Ser301 by CDK1 (Shiromizu *et al.*, 2006), a mitotic inducer kinase (Fig. 2 and Table I). This phosphorylation stimulates Chk1 transport from the nucleus to the cytoplasm in a Crm-1-dependent manner (Fig. 2 and Table I) (Enomoto *et al.*, 2009; Matsuyama *et al.*, 2011). This transport relieves Chk1 inhibitory activity against CDK1 in the nucleus, leading to mitotic progression (Table I) (Enomoto *et al.*, 2009; Matsuyama *et al.*, 2011). This nuclear model appears to conflict with reports that a small fraction of Chk1 localizes to the centrosome to block premature mitotic entry (Kramer *et al.*, 2004; Tibelius *et al.*, 2009). However, an anti-Chk1 antibody used for immunostaining in these studies (Kramer *et al.*, 2004; Tibelius *et al.*, 2009) clearly cross-reacts with a centrosomal protein other than Chk1 (Matsuyama *et al.*, 2011). In addition, forced immobilization of Chk1 to the centrosome has little impact on the timing of mitotic entry, whereas Chk1-NLS (a mutant localized predominantly in the nucleus) clearly delays mitotic entry in the same experimental condition (Matsuyama *et al.*, 2011). Therefore, nuclear but not centrosomal Chk1 prevents CDK1 from unscheduled activa-

tion before mitosis. CDK1 relieves the inhibitory effect though nuclear exclusion of Chk1 by phosphorylation. In other words, there is a positive feedback loop between CDK1 and Chk1 at the G2/M transition.

As described above, accumulating data support the view that Chk1 functions as a negative cell-cycle regulator mainly in the nucleus. However, CDK1 is first activated at the centrosome during the G2/M transition (Jackman *et al.*, 2003), raising the question as to which factor(s) transduce checkpoint signals to the cytoplasm and the centrosome. Several groups pointed out a novel checkpoint pathway mediated by p38 and MK2 that operates parallel to Chk1 and is activated downstream of ATM and ATR (Bulavin *et al.*, 2001; Manke *et al.*, 2005; Raman *et al.*, 2007; Reinhardt *et al.*, 2007; Reinhardt and Yaffe, 2009). Chk1 controls nuclear events but p38-MK2 pathway regulates cytoplasmic events in the checkpoint responses (Reinhardt *et al.*, 2010). Since p38 phosphorylates and then inhibits Cdc25B (a centrosomal subtype of Cdc25) (Bulavin *et al.*, 2001), the p38-MK2 pathway may be one of likely candidates to transmit checkpoint signals to the cytoplasm and the centrosome.

Ser286 and Ser301 are also phosphorylated during DDR (Ikegami *et al.*, 2008). CDK2 or CDK1 is responsible for Chk1 phosphorylation at Ser286 and Ser301 during DDR (Ikegami *et al.*, 2008; Xu *et al.*, 2012, 2011). Unlike in mitosis, CDK-induced phosphorylation occurs simultaneously with the phosphorylation at Ser317 and Ser345 by ATR during DDR (Ikegami *et al.*, 2008). The co-existence of Ser345 phosphorylation likely turns off Crm-1-mediated nuclear export by CDK-induced phosphorylation, because Ser345 phosphorylation promotes Chk1 binding to 14-3-3 β or ζ, which competes with Crm-1 (Jiang *et al.*, 2003). Although CDK-mediated Chk1 phosphorylation was reported to reinforce checkpoint signaling (Xu *et al.*, 2012, 2011), less is known about the significance of this phosphorylation during DDR than at the G2/M transition.

Chk1 Phosphorylation at Ser280

In quiescent cells (G0 phase cells), Chk1 is localized diffusely both in the nucleus and the cytoplasm (Li *et al.*, 2012). At the G0/G1 transition, Chk1 is phosphorylated predominantly at Ser280 by p90 RSK downstream of the Ras-MAPK cascade (Fig. 2 and Table I) (Li *et al.*, 2012). Ser280 phosphorylation promotes Chk1 translocation from the cytoplasm to the nucleus (Fig. 2 and Table I) (Li *et al.*, 2012). Since Chk1 is activated in the nucleus during DDR (Jiang *et al.*, 2003; Sanchez *et al.*, 1997), such nuclear accumulation is likely to be of great use to maintain genomic integrity during cell-cycle progression. In support of this hypothesis, Ser280 phosphorylation accelerates Chk1 activation processes after UV irradiation (Table I) (Li *et al.*, 2012).

In response to DNA damage during the G2 phase, Akt/PKB was also reported to induce Chk1 phosphorylation at Ser280 (King *et al.*, 2004; Shtivelman *et al.*, 2002) and to reduce nuclear localization of Chk1 (Puc *et al.*, 2005). However, the *in vitro* analysis reveals that Akt/PKB phosphorylates Chk1 at several sites, among which Ser280 is only a minor phosphorylation site (Li *et al.*, 2012). In addition, Chk1-Ser280 mutants behaved like Chk1 wild type in the G2/M checkpoint (Tonic *et al.*, 2010; Xu *et al.*, 2010). Although Akt/PKB negatively regulates the G2/M checkpoint (Henry *et al.*, 2001; Nimbalkar and Quelle, 2008; Shtivelman *et al.*, 2002; Xu *et al.*, 2010), there is a debate as to whether G2/M checkpoint is negatively regulated by Chk1-Ser280 phosphorylation.

Conclusion

Remarkable progress has been made in understanding Chk1 regulation. Chk1 is phosphorylated by a variety of protein kinases. The resulting phosphorylations change catalytic activity, binding partners and/or localization of Chk1. The functional change(s) by phosphorylation(s) enable Chk1 to control not only DDR but also normal cell-cycle progression. The development of Chk1-targeted molecular therapies calls for a more advanced molecular understanding of Chk1 especially in normal cell-cycle progression in the absence of exogenous DNA damages.

Acknowledgments. This work was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and by the Takeda Science Foundation. We thank Dr. T. Magin (University Leipzig, Germany) for critical comments. We sincerely apologize to all our colleagues whose work we were unable to cite due to space limitations. H.G. and M.I. has received research funding form the Takeda Science Foundation, which was established in 1963 with an endowment from Takeda Pharmaceutical Company Ltd.

References

Antoni, L., Sodha, N., Collins, I., and Garrett, M.D. 2007. CHK2 kinase: cancer susceptibility and cancer therapy - two sides of the same coin? *Nat. Rev. Cancer*, **7**: 925–936.

Appella, E. and Anderson, C.W. 2001. Post-translational modifications and activation of p53 by genotoxic stresses. *Eur. J. Biochem.*, **268**: 2764–2772.

Bekker-Jensen, S., Lukas, C., Kitagawa, R., Melander, F., Kastan, M.B., Bartek, J., and Lukas, J. 2006. Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. *J. Cell Biol.*, **173**: 195–206.

Biggins, S., Palermo, C., Hope, J.C., Freyer, G.A., Rao, H., and Walworth, N.C. 2008. Importance of a C-Terminal Conserved Region of Chk1 for Checkpoint Function. *PLoS ONE*, **3**: e1427.

Boutros, R., Lobjois, V., and Ducommun, B. 2007. CDC25 phosphatases in cancer cells: key players? Good targets? *Nat. Rev. Cancer*, **7**: 495–507.

Brown, E.J. and Baltimore, D. 2000. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. *Genes Dev.*, **14**: 397–402.

Bulavin, D.V., Higashimoto, Y., Popoff, I.J., Gaarde, W.A., Basrur, V., Potapova, O., Appella, E., and Fornace, A.J., Jr. 2001. Initiation of a G2/M checkpoint after ultraviolet radiation requires p38 kinase. *Nature*, **411**: 102–107.

Busino, L., Chiesa, M., Draetta, G.F., and Donzelli, M. 2004. Cdc25A phosphatase: combinatorial phosphorylation, ubiquitylation and proteolysis. *Oncogene*, **23**: 2050–2056.

Caparelli, M.L. and O'Connell, M.J. 2013. Regulatory motifs in Chk1. *Cell Cycle*, **12**: 916–922.

Capasso, H., Palermo, C., Wan, S., Rao, H., John, U.P., O'Connell, M.J., and Walworth, N.C. 2002. Phosphorylation activates Chk1 and is required for checkpoint-mediated cell cycle arrest. *J. Cell Sci.*, **115**(Pt 23): 4555–4564.

Carrassa, L. and Damia, G. 2011. Unleashing Chk1 in cancer therapy. *Cell Cycle*, **10**: 2121–2128.

Chen, M.S., Ryan, C.E., and Piwnica-Worms, H. 2003. Chk1 Kinase Negatively Regulates Mitotic Function of Cdc25A Phosphatase through 14-3-3 Binding. *Mol. Cell. Biol.*, **23**: 7488–7497.

Chen, P., Luo, C., Deng, Y., Ryan, K., Register, J., Margosiak, S., Tempczyk-Russell, A., Nguyen, B., Myers, P., Lundgren, K., and others. 2000. The 1.7 A crystal structure of human cell cycle checkpoint kinase Chk1: implications for Chk1 regulation. *Cell*, **100**: 681–692.

Chini, C.C. and Chen, J. 2004. Claspin, a regulator of Chk1 in DNA replication stress pathway. *DNA Repair (Amst)*, **3**: 1033–1037.

Ciccia, A. and Elledge, S.J. 2010. The DNA damage response: making it safe to play with knives. *Mol. Cell*, **40**: 179–204.

Cimprich, K.A. and Cortez, D. 2008. ATR: an essential regulator of genome integrity. *Nat. Rev. Mol. Cell Biol.*, **9**: 616–627.

Clarke, C.A. and Clarke, P.R. 2005. DNA-dependent phosphorylation of Chk1 and Claspin in a human cell-free system. *Biochem. J.*, **388**(Pt 2): 705–712.

Cremona, C.A. and Behrens, A. 2014. ATM signalling and cancer. *Oncogene*, **33**: 3351–3360.

Dai, Y. and Grant, S. 2010. New insights into checkpoint kinase 1 in the DNA damage response signaling network. *Clin. Cancer Res.*, **16**: 376–383.

de Klein, A., Muijtjens, M., van Os, R., Verhoeven, Y., Smit, B., Carr, A.M., Lehmann, A.R., and Hoeijmakers, J.H. 2000. Targeted disruption of the cell-cycle checkpoint gene ATR leads to early embryonic lethality in mice. *Curr. Biol.*, **10**: 479–482.

Enomoto, M., Goto, H., Tomono, Y., Kasahara, K., Tsujimura, K., Kiyono, T., and Inagaki, M. 2009. Novel positive feedback loop between Cdk1 and Chk1 in the nucleus during G2/M transition. *J. Biol. Chem.*, **284**: 34223–34230.

Flynn, R.L. and Zou, L. 2011. ATR: a master conductor of cellular responses to DNA replication stress. *Trends Biochem. Sci.*, **36**: 133–140.

Goto, H., Izawa, I., Li, P., and Inagaki, M. 2012. Novel regulation of checkpoint kinase 1: Is checkpoint kinase 1 a good candidate for anticancer therapy? *Cancer Sci.*, **103**: 1195–1200.

Henry, M.K., Lynch, J.T., Eapen, A.K., and Quelle, F.W. 2001. DNA damage-induced cell-cycle arrest of hematopoietic cells is overridden by activation of the PI-3 kinase/Akt signaling pathway. *Blood*, **98**: 834–841.

Honaker, Y. and Piwnica-Worms, H. 2010. Casein kinase 1 functions as both penultimate and ultimate kinase in regulating Cdc25A destruction. *Oncogene*, **29**: 3324–3334.

Huh, J. and Piwnica-Worms, H. 2013. CRL4(CDT2) targets CHK1 for PCNA-independent destruction. *Mol. Cell. Biol.*, **33**: 213–226.

Ikegami, Y., Goto, H., Kiyono, T., Enomoto, M., Kasahara, K., Tomono, Y., Tozawa, K., Morita, A., Kohri, K., and Inagaki, M. 2008. Chk1 phosphorylation at Ser286 and Ser301 occurs with both stalled DNA replication and damage checkpoint stimulation. *Biochem. Biophys. Res. Commun.*, **377**: 1227–1231.

Jackman, M., Lindon, C., Nigg, E.A., and Pines, J. 2003. Active cyclin B1-Cdk1 first appears on centrosomes in prophase. *Nat. Cell Biol.*, **5**: 143–148.

Jackson, S.P. and Bartek, J. 2009. The DNA-damage response in human biology and disease. *Nature*, **461**: 1071–1078.

Jiang, K., Pereira, E., Maxfield, M., Russell, B., Goudelock, D.M., and Sanchez, Y. 2003. Regulation of Chk1 includes chromatin association and 14-3-3 binding following phosphorylation on Ser-345. *J. Biol. Chem.*, **278**: 25207–25217.

Jin, J., Shirogane, T., Xu, L., Nalepa, G., Qin, J., Elledge, S.J., and Harper, J.W. 2003. SCFbeta-TRCP links Chk1 signaling to degradation of the Cdc25A protein phosphatase. *Genes Dev.*, **17**: 3062–3074.

Kandoth, C., McLellan, M.D., Vandin, F., Ye, K., Niu, B., Lu, C., Xie, M., Zhang, Q., McMichael, J.F., Wyczalkowski, M.A., and others. 2013. Mutational landscape and significance across 12 major cancer types. *Nature*, **502**: 333–339.

Kasahara, K., Goto, H., Enomoto, M., Tomono, Y., Kiyono, T., and Inagaki, M. 2010. 14-3-3gamma mediates Cdc25A proteolysis to block premature mitotic entry after DNA damage. *EMBO J.*, **29**: 2802–2812.

Katsuragi, Y. and Sagata, N. 2004. Regulation of Chk1 kinase by autoinhibition and ATR-mediated phosphorylation. *Mol. Biol. Cell*, **15**: 1680–1689.

King, F.W., Skeen, J., Hay, N., and Shtivelman, E. 2004. Inhibition of Chk1 by activated PKB/Akt. *Cell Cycle*, **3**: 634–637.

Kosoy, A. and O'Connell, M.J. 2008. Regulation of Chk1 by its C-terminal domain. *Mol. Biol. Cell*, **19**: 4546–4553.

Kramer, A., Mailand, N., Lukas, C., Syljuasen, R.G., Wilkinson, C.J., Nigg, E.A., Bartek, J., and Lukas, J. 2004. Centrosome-associated Chk1 prevents premature activation of cyclin-B-Cdk1 kinase. *Nat. Cell Biol.*, **6**: 884–891.

Kumagai, A. and Dunphy, W.G. 2000. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in Xenopus egg extracts. *Mol. Cell*, **6**: 839–849.

Kumagai, A., Kim, S.M., and Dunphy, W.G. 2004. Claspin and the activated form of ATR-ATRIP collaborate in the activation of Chk1. *J. Biol. Chem.*, **279**: 49599–49608.

Lawrence, M.S., Stojanov, P., Mermel, C.H., Robinson, J.T., Garraway, L.A., Golub, T.R., Meyerson, M., Gabriel, S.B., Lander, E.S., and Getz, G. 2014. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature*, **505**: 495–501.

Leung-Pineda, V., Ryan, C.E., and Piwnica-Worms, H. 2006. Phosphorylation of Chk1 by ATR is antagonized by a Chk1-regulated protein phosphatase 2A circuit. *Mol. Cell. Biol.*, **26**: 7529–7538.

Leung-Pineda, V., Huh, J., and Piwnica-Worms, H. 2009. DDB1 targets Chk1 to the Cul4 E3 ligase complex in normal cycling cells and in cells experiencing replication stress. *Cancer Res.*, **69**: 2630–2637.

Li, P., Goto, H., Kasahara, K., Matsuyama, M., Wang, Z., Yatabe, Y., Kiyono, T., and Inagaki, M. 2012. P90 RSK arranges Chk1 in the nucleus for monitoring of genomic integrity during cell proliferation. *Mol. Biol. Cell*, **23**: 1582–1592.

Liu, Q., Guntuku, S., Cui, X.S., Matsuoka, S., Cortez, D., Tamai, K., Luo, G., Carattini-Rivera, S., DeMayo, F., Bradley, A., and others. 2000. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev.*, **14**: 1448–1459.

Lu, X., Nannenga, B., and Donehower, L.A. 2005. PPM1D dephosphorylates Chk1 and p53 and abrogates cell cycle checkpoints. *Genes Dev.*, **19**: 1162–1174.

Lukas, C., Falck, J., Bartkova, J., Bartek, J., and Lukas, J. 2003. Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat. Cell Biol.*, **5**: 255–260.

Ma, C.X., Janetka, J.W., and Piwnica-Worms, H. 2011. Death by releasing the breaks: CHK1 inhibitors as cancer therapeutics. *Trends Mol.*

Med., **17**: 88–96.

Manke, I.A., Nguyen, A., Lim, D., Stewart, M.Q., Elia, A.E., and Yaffe, M.B. 2005. MAPKAP kinase-2 is a cell cycle checkpoint kinase that regulates the G2/M transition and S phase progression in response to UV irradiation. *Mol. Cell*, **17**: 37–48.

Matsuyama, M., Goto, H., Kasahara, K., Kawakami, Y., Nakanishi, M., Kiyono, T., Goshima, N., and Inagaki, M. 2011. Nuclear Chk1 prevents premature mitotic entry. *J. Cell Sci.*, **124**(Pt 13): 2113–2119.

Maugeri-Sacca, M., Bartucci, M., and De Maria, R. 2013. Checkpoint kinase 1 inhibitors for potentiating systemic anticancer therapy. *Cancer Treat. Rev.*, **39**: 525–533.

Maya-Mendoza, A., Petermann, E., Gillespie, D.A., Caldecott, K.W., and Jackson, D.A. 2007. Chk1 regulates the density of active replication origins during the vertebrate S phase. *EMBO J.*, **26**: 2719–2731.

McGowan, C.H. 2002. Checking in on Cds1 (Chk2): A checkpoint kinase and tumor suppressor. *Bioessays*, **24**: 502–511.

Melixetian, M., Klein, D.K., Sorensen, C.S., and Helin, K. 2009. NEK11 regulates CDC25A degradation and the IR-induced G2/M checkpoint. *Nat. Cell Biol.*, **11**: 1247–1253.

Mohammad, D.H. and Yaffe, M.B. 2009. 14-3-3 proteins, FHA domains and BRCT domains in the DNA damage response. *DNA Repair (Amst)*, **8**: 1009–1017.

Neely, K.E. and Piwnica-Worms, H. 2003. Cdc25A regulation: to destroy or not to destroy--is that the only question? *Cell Cycle*, **2**: 455–457.

Niida, H., Katsuno, Y., Banerjee, B., Hande, M.P., and Nakanishi, M. 2007. Specific role of Chk1 phosphorylations in cell survival and checkpoint activation. *Mol. Cell. Biol.*, **27**: 2572–2581.

Nimbalkar, D. and Quelle, F.W. 2008. Phosphoinositide 3-kinase signaling overrides a G2 phase arrest checkpoint and promotes aberrant cell cycling and death of hematopoietic cells after DNA damage. *Cell Cycle*, **7**: 2877–2885.

Polager, S. and Ginsberg, D. 2009. p53 and E2f: partners in life and death. *Nat. Rev. Cancer*, **9**: 738–748.

Puc, J., Keniry, M., Li, H.S., Pandita, T.K., Choudhury, A.D., Memeo, L., Mansukhani, M., Murty, V.V., Gaciong, Z., Meek, S.E., and others. 2005. Lack of PTEN sequesters CHK1 and initiates genetic instability. *Cancer Cell*, **7**: 193–204.

Raman, M., Earnest, S., Zhang, K., Zhao, Y., and Cobb, M.H. 2007. TAO kinases mediate activation of p38 in response to DNA damage. *EMBO J.*, **26**: 2005–2014.

Reinhardt, H.C., Aslanian, A.S., Lees, J.A., and Yaffe, M.B. 2007. p53-Deficient Cells Rely on ATM- and ATR-Mediated Checkpoint Signaling through the p38MAPK/MK2 Pathway for Survival after DNA Damage. *Cancer Cell*, **11**: 175–189.

Reinhardt, H.C. and Yaffe, M.B. 2009. Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2, and MK2. *Curr. Opin. Cell Biol.*, **21**: 245–255.

Reinhardt, H.C., Hasskamp, P., Schmedding, I., Morandell, S., van Vugt, M.A., Wang, X., Linding, R., Ong, S.E., Weaver, D., Carr, S.A., and others. 2010. DNA damage activates a spatially distinct late cytoplasmic cell-cycle checkpoint network controlled by MK2-mediated RNA stabilization. *Mol. Cell*, **40**: 34–49.

Rhind, N. and Russell, P. 2000. Chk1 and Cds1: linchpins of the DNA damage and replication checkpoint pathways. *J. Cell Sci.*, **113**(Pt 22): 3889–3896.

Riley, T., Sontag, E., Chen, P., and Levine, A. 2008. Transcriptional control of human p53-regulated genes. *Nat Rev Mol. Cell Biol.*, **9**: 402–412.

Sanchez, Y., Wong, C., Thoma, R.S., Richman, R., Wu, Z., Piwnica-Worms, H., and Elledge, S.J. 1997. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science*, **277**: 1497–1501.

Shiloh, Y. and Ziv, Y. 2013. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. *Nat. Rev. Mol. Cell Biol.*, **14**: 197–210.

Shimada, M., Niida, H., Zineldeen, D.H., Tagami, H., Tanaka, M., Saito, H., and Nakanishi, M. 2008. Chk1 is a histone H3 threonine 11 kinase that regulates DNA damage-induced transcriptional repression. *Cell*, **132**: 221–232.

Shiromizu, T., Goto, H., Tomono, Y., Bartek, J., Totsukawa, G., Inoko, A., Nakanishi, M., Matsumura, F., and Inagaki, M. 2006. Regulation of mitotic function of Chk1 through phosphorylation at novel sites by cyclin-dependent kinase 1 (Cdk1). *Genes Cells*, **11**: 477–485.

Shtivelman, E., Sussman, J., and Stokoe, D. 2002. A role for PI 3-kinase and PKB activity in the G2/M phase of the cell cycle. *Curr. Biol.*, **12**: 919–924.

Smits, V.A., Reaper, P.M., and Jackson, S.P. 2006. Rapid PIKK-dependent release of Chk1 from chromatin promotes the DNA-damage checkpoint response. *Curr. Biol.*, **16**: 150–159.

Syljuasen, R.G., Sorensen, C.S., Hansen, L.T., Fugger, K., Lundin, C., Johansson, F., Helleday, T., Sehested, M., Lukas, J., and Bartek, J. 2005. Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol. Cell. Biol.*, **25**: 3553–3562.

Takai, H., Tominaga, K., Motoyama, N., Minamishima, Y.A., Nagahama, H., Tsukiyama, T., Ikeda, K., Nakayama, K., Nakanishi, M., and Nakayama, K. 2000. Aberrant cell cycle checkpoint function and early embryonic death in Chk1(–/–) mice. *Genes Dev.*, **14**: 1439–1447.

Thompson, R. and Eastman, A. 2013. The cancer therapeutic potential of Chk1 inhibitors: how mechanistic studies impact on clinical trial design. *Br. J. Clin. Pharmacol.*, **76**: 358–369.

Tibelius, A., Marhold, J., Zentgraf, H., Heilig, C.E., Neitzel, H., Ducommun, B., Rauch, A., Ho, A.D., Bartek, J., and Kramer, A. 2009. Microcephalin and pericentrin regulate mitotic entry via centrosome-associated Chk1. *J. Cell Biol.*, **185**: 1149–1157.

Tonic, I., Yu, W.N., Park, Y., Chen, C.C., and Hay, N. 2010. Akt activation emulates Chk1 inhibition and Bcl2 overexpression and abrogates G2 cell cycle checkpoint by inhibiting BRCA1 foci. *J. Biol. Chem.*, **285**: 23790–23798.

Vandin, F., Upfal, E., and Raphael, B.J. 2012. De novo discovery of mutated driver pathways in cancer. *Genome Res.*, **22**: 375–385.

Wade, M., Li, Y.C., and Wahl, G.M. 2013. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat. Rev. Cancer*, **13**: 83–96.

Walker, M., Black, E.J., Oehler, V., Gillespie, D.A., and Scott, M.T. 2009. Chk1 C-terminal regulatory phosphorylation mediates checkpoint activation by de-repression of Chk1 catalytic activity. *Oncogene*, **28**: 2314–2323.

Wang, J., Han, X., Feng, X., Wang, Z., and Zhang, Y. 2012. Coupling cellular localization and function of checkpoint kinase 1 (Chk1) in checkpoints and cell viability. *J. Biol. Chem.*, **287**: 25501–25509.

Wilsker, D., Petermann, E., Helleday, T., and Bunz, F. 2008. Essential function of Chk1 can be uncoupled from DNA damage checkpoint and replication control. *Proc. Natl. Acad. Sci. USA*, **105**: 20752–20757.

Xu, N., Hegarat, N., Black, E.J., Scott, M.T., Hochegger, H., and Gillespie, D.A. 2010. Akt/PKB suppresses DNA damage processing and checkpoint activation in late G2. *J. Cell Biol.*, **190**: 297–305.

Xu, N., Libertini, S., Zhang, Y., and Gillespie, D.A. 2011. Cdk phosphorylation of Chk1 regulates efficient Chk1 activation and multiple checkpoint proficiency. *Biochem. Biophys. Res. Commun.*, **413**: 465–470.

Xu, N., Libertini, S., Black, E.J., Lao, Y., Hegarat, N., Walker, M., and Gillespie, D.A. 2012. Cdk-mediated phosphorylation of Chk1 is required for efficient activation and full checkpoint proficiency in response to DNA damage. *Oncogene*, **31**: 1086–1094.

Zeman, M.K. and Cimprich, K.A. 2014. Causes and consequences of replication stress. *Nat. Cell Biol.*, **16**: 2–9.

Zhang, Y. and Hunter, T. 2014. Roles of Chk1 in cell biology and cancer therapy. *Int. J. Cancer*, **134**: 1013–1023.

Zhang, Y.W., Otterness, D.M., Chiang, G.G., Xie, W., Liu, Y.C., Mercurio, F., and Abraham, R.T. 2005. Genotoxic stress targets human Chk1 for degradation by the ubiquitin-proteasome pathway. *Mol. Cell*, **19**: 607–618.

Zhang, Y.W., Brognard, J., Coughlin, C., You, Z., Dolled-Filhart, M., Aslanian, A., Manning, G., Abraham, R.T., and Hunter, T. 2009. The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. *Mol. Cell*, **35**: 442–453.

Zhao, H. and Piwnica-Worms, H. 2001. A TR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Mol. Cell. Biol.*, **21**: 4129–4139.

(Received for publication, November 8, 2014, accepted, December 18, 2014 and published online, December 25, 2014)