Cell. Mol. Life Sci. (2013) 70:4009–4021
DOI 10.1007/s00018-013-1307-3

Cellular and Molecular Life Sciences

REVIEW

Checkpoint kinase 1 in DNA damage response and cell cycle regulation

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Received: 6 November 2012 / Revised: 28 January 2013 / Accepted: 18 February 2013 / Published online: 19 March 2013

Abstract Originally identified as a mediator of DNA damage response (DDR), checkpoint kinase 1 (Chk1) has a broader role in checkpoint activation in DDR and normal cell cycle regulation. Chk1 activation involves phosphorylation at conserved sites. However, recent work has identified a splice variant of Chk1, which may regulate Chk1 in both DDR and normal cell cycle via molecular interaction. Upon activation, Chk1 phosphorylates a variety of substrate proteins, resulting in the activation of DNA damage checkpoints, cell cycle arrest, DNA repair, and/or cell death. Chk1 and its related signaling may be an effective therapeutic target in diseases such as cancer.

Keywords Chk1 · Cell cycle · DNA damage · Checkpoint

Abbreviations

Chk1	Checkpoint kinase 1
Chk2	Checkpoint kinase 2
Chk1	S-checkpoint kinase 1-short
DDR	DNA damage response
ATM	Ataxia telangiectasia mutated
ATR	ATM and Rad3 related
Cdc25	Cell division cycle 25
CDK	Cyclin-dependent kinases

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Introduction

Checkpoint kinase 1 (Chk1) was initially identified in fission yeast as a serine/threonine protein kinase that is essential for DNA damage-induced cell cycle arrest [1]. Chk1 homologs were subsequently identified in other species such as *Drosophila*, *Xenopus*, mouse, and human [2–4]. Functional studies in these model systems showed that Chk1 phosphorylates the key regulators of cyclin-dependent kinase 1 (CDK1) during DNA damage, resulting in CDK1 inactivation and blockade of G2/M transition. More recent work has established important roles of Chk1 not only in DNA damage response (DDR) but also in unperturbed cell cycle. In the normal cell cycle, Chk1 mediates the checkpoints in S and M phases as well as G2/M transition. In this review, we discuss Chk1 regulation, its role in DDR and unperturbed cell cycle, and the possibility of targeting Chk1 for cancer therapy.

Cell cycle and DNA damage response

The cell cycle consists of a series of highly ordered, sequential phases that lead to cell division [5]. A notable feature of cell cycle progression is that cells do not enter the next phase until the previous phase is completed. This feature, known as cell cycle checkpoints, provides an important surveillance mechanism for faithful replication and division of the cells [5] (Fig. 1). Progression from one phase of cell cycle to the next is governed by Cdks, their cyclin partners,

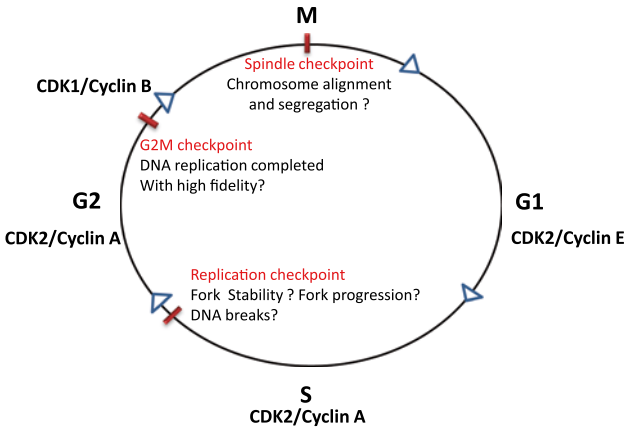

Fig. 1 Cell cycle checkpoints. The cell cycle consists of G1, S, G2, and M phases, which are driven by various cyclin/CDK complexes. Progression from one phase to the next phase in the cell cycle is monitored by different checkpoints. S phase is regulated by replication checkpoint that monitors the initiation of replication, replication fork stability, fork progression, and DNA lesions. G2/M checkpoint monitors the completion of DNA replication with high fidelity. Spindle checkpoint makes sure that chromosomes are aligned and segregated for even distribution into two daughter cells

protein kinases, and phosphatases [6]. Specifically, the cell cycle is driven by temporal activation of Cdks, which depends on the association of cyclin partners, phosphorylation by specific protein kinases, and dephosphorylation by Cdc25 family phosphatases. For example, CDK1, the critical CDK for G2/M phase transition, is activated by cyclin B binding, phosphorylation at serine-161, and dephosphorylation of the inhibitory tyrosine-14 and -15 sites by Cdc25.

DNA damage response is a network of signal transduction pathways activated in a cell that leads to checkpoint activation, regulate cell cycle transitions, DNA repair and apoptosis in response to DNA damage [7, 8]. DNA damage is sensed by several molecular complexes or pathways, the most notable of which include ATM and ATR that activate DNA damage checkpoint (Fig. 2). In general, double-strand DNA breaks are sensed by MRE11-Rad 50-Nbs1 (MRN) complex, which localizes to the DNA damage site and recruits ATM [9, 10]. ATM usually exists as homo-dimers that are inactive; however, upon localization to DNA damage sites, ATM is autophosphorylated to become monomers leading to its activation [11]. Activated

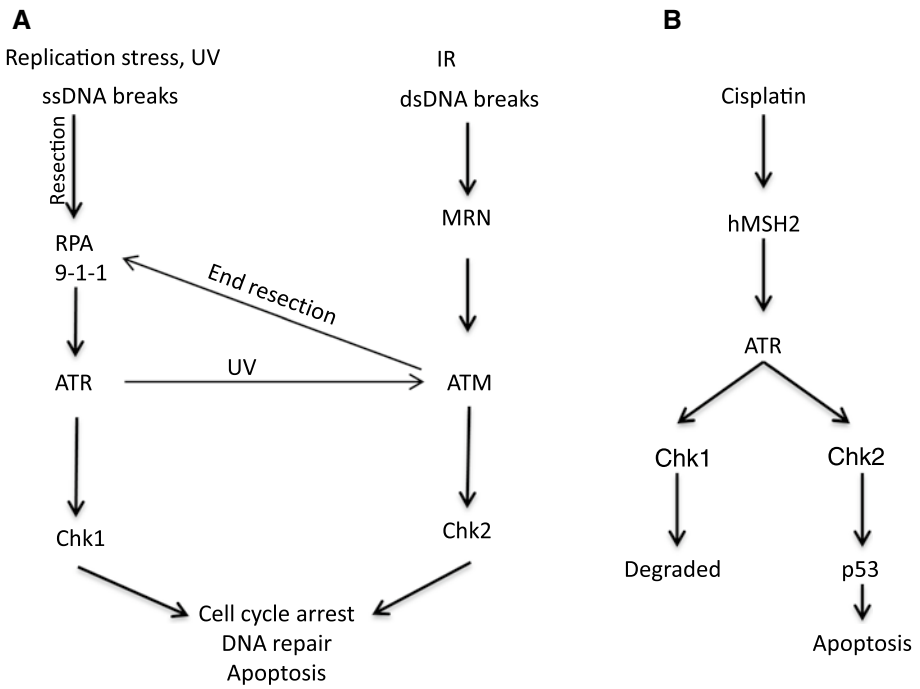

Fig. 2 Chk1 and Chk2 activation in response to DNA damage. **a** Double-strand DNA (dsDNA) breaks are sensed by the MRN complex, which recruits and activates ATM. MRN-mediated nuclease activity generates SS DNA, which can activate ATR. ATM is also activated in UV-induced DNA damage in ATR-dependent manner. Activated ATM phosphorylates several substrates including Chk2. RPA senses and binds single-strand DNA (ssDNA) lesions, which recruit ATR through ATRIP. 9-1-1 (Rad9-Rad1-Hus1) complex binds to ssDNA and dsDNA junction, which recruits TOPBP1 to activate ATR, which phosphorylates Chk1. **b** ATR is also activated by DNA damage induced by cisplatin. Cisplatin-induced DNA lesion recruits MSH2 (mismatch repair protein 2), which in turn helps in the recruitment and activation of ATR. Activated ATR phosphorylates both Chk1 and Chk2, however, Chk1 is degraded and Chk2 remains active, which phosphorylates and activates p53 leading to apoptosis

ATM phosphorylates downstream targets including Chk2, H2AX, and MDC1 etc. [12]. Different from ATM, ATR is mainly activated by single-stand DNA generated as a result of replication stress, UV induced DNA damage, nuclease activity consequent to double-strand breaks, enzymatic and helicase remodelling following ICL [13]. The single-strand DNA is recognized and bound by replication protein A (RPA), resulting in the recruitment of ATR through its interacting partner protein called ATRIP [7, 14, 15]. Independently, RAD9-RAD1-HUS1 (9-1-1) complex binds to the ssDNA and dsDNA junction through Rad17 [16]. The 9-1-1 complex in turn helps to recruit topoisomerase binding protein 1 (TOPBP1), an activator of ATR, resulting in ATR activation [17, 18]. In addition to this canonical pathway, recent work has revealed a novel pathway of ATR activation involving mismatch repair proteins [19] (Fig. 2). Upon activation, ATR can phosphorylate downstream targets including Chk1. ATR-mediated Chk1 phosphorylation also requires another intermediate protein called claspin, which binds and stabilizes activated Chk1. As a result, Chk1 phosphorylation was abolished in the absence of claspin in *Xenopus* egg extracts [20]. Although ATM and ATR are generally known to respond to double- and single-strand DNA damage respectively, their signaling pathways are not mutually exclusive. For example, double-strand breaks activate ATM, which along with MRE11 nuclease activity, generates single-strand DNA leading to consequent ATR activation [7, 21]. ATR activated in response to UV-induced DNA damage activate ATM by phosphorylating ATM at its autophosphorylation site S1981 [22]. In addition, the downstream signaling molecules are not exclusively responsive to ATM or ATR. For example, in cisplatin-induced DNA cross-linking and replication stress, both Chk1 and Chk2 are activated in an ATR-dependent manner. While Chk1 is degraded after initial activation, Chk2 phosphorylates p53 leading to its stabilization and activation to induce apoptosis [23].

Intertwined relationship between the cell cycle and DDR

DNA damage response and the cell cycle are two intertwined cellular processes. On the one hand, as discussed above, DDR leads to cell cycle arrest by activating checkpoint kinases. On the other hand, an emerging idea is that even the unperturbed cell cycle has a constitutive surveillance mechanism that is related to DDR [24, 25]. This is particularly relevant to DNA replication in S-phase, when single-strand DNA (ssDNA) and DNA breaks may be induced in several ways. During replication, template DNA is unwound at the replication fork by DNA helicases resulting in ssDNA. ssDNA is highly susceptible to damage such as those induced by free radicals. Breaks in ssDNA at the replication fork can be converted to a double-strand break following replication. As a result, both single-strand and double-strand DNA breaks-associated DDR may be activated to slow down the progression of the cell cycle to repair the damage to ensure a faithful DNA replication and genome integrity. In this process, ATR and Chk1 play a critical role in sensing the initial ssDNA breaks to activate DDR. Consistently, deficiency in either ATR or Chk1 leads to embryonic lethality in mice and embryonic stem cells of these models show cell cycle abnormalities, accumulate double-stand breaks and fragile site breaks [26–29]. The intertwined relationship between DDR and cell cycle provides an explanation as to why DDR proteins, such as ATR and Chk1, play such critical roles in the unperturbed cell cycle.

Chk1 in cell cycle regulation and tissue physiology

Checkpoint kinase 1 was originally identified as a gene that can rescue a CDK1/cdc2 mutant in fission yeast [1]. Constitutive overexpression of Chk1 in fission yeast leads to mitotic delay, whereas loss of Chk1 has no effect on cell cycle progression. However, in the absence of Chk1, the yeast cells become more sensitive to UV-induced DNA damage and fail to arrest in G2-phase following DNA damage [1]. In *Drosophila*, Grapes (Chk1 homolog in *Drosophila*) regulates syncytial cell division fidelity, mitotic entry, and cyclin A degradation and as a result, it is indispensable for embryogenesis [3, 30]. In addition, Grapes may delay the accumulation of cyclin B1 in the nucleus to prevent nuclear CDK1 activation and premature entry into mitosis in *Drosophila* embryos [31, 32], supporting a role of Chk1 in cell cycle in early stages of embryogenesis in *Drosophila*. In *Xenopus* eggs, addition of exogenous, active Chk1 delays mitosis whereas immunodepletion of Chk1 results in an early entry into mitosis [2]. Unlike yeast, mammalian cells do not show noticeable phenotypes following Chk1 overexpression; however Chk1 knockdown and inhibition leads to reduced proliferation and cell death [33]. In mice, Chk1 knockout leads to embryonic death in utero at E6.5 stage [28, 29]. Chk1 knockout blastocysts as well as embryonic stem cells also fail to proliferate and fail to activate the G2/M checkpoint in response to DNA damage and die shortly in culture [28, 29]. Embryonic death of Chk1-null mice is independent of p53 since p53-deficiency fail to rescue the phenotype [28]. Although viable, Chk1-deficient chicken lymphoma DT40 cells are deficient in G2 checkpoint in response to DNA damage and are defective in both DNA replication and cell cycle [34]. Collectively, these studies indicate that Chk1 is master regulator of cell cycle, cell survival and embryogenesis.

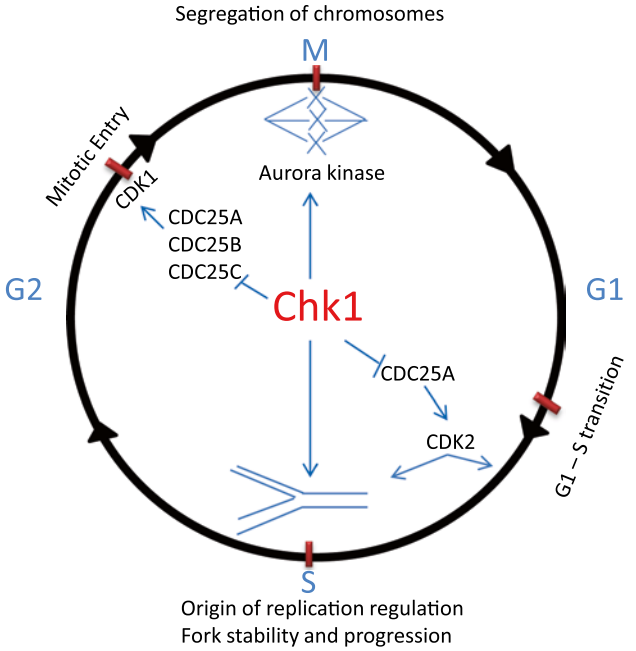

Fig. 3 Cell cycle checkpoints regulated by Chk1. Chk1 helps maintain genomic integrity by regulating DNA replication, G2/M, and spindle checkpoints. Chk1 monitors DNA replication, slows down the replication to favor fork stability, prevent stalling of forks and DNA breaks. Chk1 prevents premature entry into mitosis before DNA replication is completed with high fidelity by inhibiting Cdc25 family phosphatases. Chk1 also regulates segregation of chromosomes through activation of aurora kinase to prevent genomic instability

In unperturbed cell cycle, Chk1 regulates DNA replication in S phase, G2/M transition or mitotic entry, and spindle checkpoint in M phase (Fig. 3). In S phase, Chk1 arrests cell cycle for DNA replication mainly by inducing Cdc25A degradation, resulting in the inhibition of CDK2. In U2OS cells, Chk1 phosphorylates Cdc25A at multiple sites and inhibition of Chk1 results in a marked accumulation of Cdc25A, suggesting that phosphorylation by Chk1 targets Cdc25A for degradation [35–37]. Consistently, conditional deletion of Chk1 from mammary cells in mice results in Cdc25A accumulation, which is accompanied by increases in S phase cells, accumulation of DNA damage, premature mitotic entry, and cell death [38]. Knockdown and inhibition of Chk1 lead to increased initiation of DNA synthesis, increased single-strand DNA, aberrant fork structures, accumulation of double-strand breaks, DDR, and increased phosphorylation of ATR targets [33, 39]. These studies suggest that Chk1 may suppress late initiation of replication to prevent DNA damage for normal progression of S phase. However, the ATR/Chk1 pathway can also sense the strand stability and progression and initiate late replication to repair DNA breaks [40]. Collectively, all these observations suggest that Chk1 regulates replication checkpoint and is required for normal S phase progression and cell survival.

Mitotic entry is promoted by the activation of the cyclin B-CDK1 complex, which also involves the regulation by Chk1 [41]. Briefly, in late G2-phase, Chk1 accumulates at centrosome to phosphorylate Cdc25B (the phosphatase and activator of CDK1), resulting in the inhibition of Cdc25B and consequent suppression of CDK1. This regulation is critical to the prevention of premature mitotic entry in unperturbed cell cycle [41].

Finally, Chk1 has a critical role in spindle checkpoint in M-phase of the cell cycle. In support of this conclusion, Chk1-deficient chicken lymphoma DT 40 cells are viable, but they have increased levels of chromosome mis-segregation and genomic instability and fail to arrest in mitosis when treated with Taxol, a microtubule-stabilizing agent [42]. Similarly, Chk1 haploinsufficient primary mammary epithelial cells also exhibit misaligned chromosomes, chromosome missegregation, and enhanced binucleation [43]. Checkpoint kinase 1 knockdown U2OS cells undergo aberrant mitosis and fail to activate spindle checkpoint in response to Nocodazole treatment [37]. These studies suggest defective spindle checkpoint in the absence of Chk1. Mechanistically, Chk1 regulates spindle checkpoint through the activation and localization of Aurora B kinase, a regulator of spindle checkpoint that recruits BubR1 and MAD1 to the kinetochores [42, 43] to prevent APC/C activation and delay anaphase onset [44]. Chk1 deficiency leads to mislocalization of Aurora B, BubR1 and MAD1 proteins, resulting in spindle checkpoint failure [42, 43] (Fig. 3).

Chk1 in DNA damage response

Originally identified in yeast, Chk1 is now recognized as an important mediator and signal transducer in DDR in other eukaryotes including mammals. In response to DNA damage, Chk1 is rapidly phosphorylated at serine-317 and serine-345 by ATR and becomes highly activated, resulting in the activation of DNA damage checkpoints [28, 45, 46]. Activated Chk1 phosphorylates several downstream targets to bring about cell cycle arrest, activate DNA repair pathways, and induce apoptosis when DNA damage is severe [8, 47–49].

Chk1-induced cell cycle arrest in DDR

Checkpoint kinase 1 induces cell cycle arrest in DDR mainly by phosphorylating Cdc25 family phosphatases, WEE1 kinase and controlling PLK1 (Fig. 4). The Cdc25 family is dual-specificity phosphatases that can dephosphorylate Cdks in their ATP-binding loop, resulting in the activation of Cdks and cell cycle progression. In mammalian cells, there are three (A, B and C) isoforms of Cdc25, all

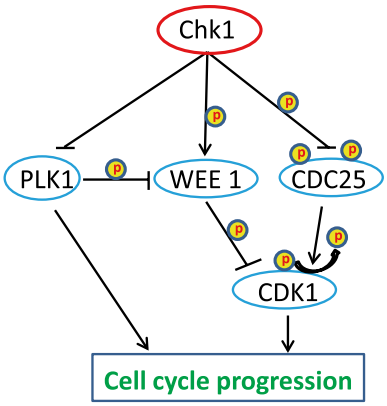

Fig. 4 Chk1 regulation of cell cycle progression. Chk1 regulates progression of cell cycle by inhibiting Cdc25 family phosphatases and polo-like kinase 1(PLK1), and activating WEE 1 kinase. Cdc25 is a phosphatase and activator of CDK1, whereas WEE1 inhibits CDK1 by phosphorylation. PLK1 can activate CDK1 by inhibiting WEE1. PLK1 can also directly promote cell cycle progression

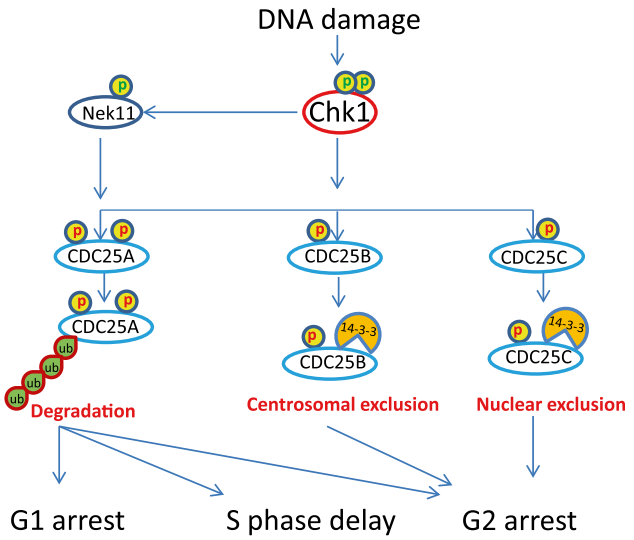

Fig. 5 Chk1-mediated cell cycle arrest in response to DNA damage. Chk1 phosphorylates Cdc25 family resulting in their inhibition. Chk1 can also activate Nek11, which further phosphorylates Cdc25A on multiple sites to target it for degradation. Cdc25A is crucial for G1-S transition, S phase progression, and mitotic entry; as a result, Cdc25A inhibition leads to G1 arrest, slows down replication, and G2 arrest. Chk1 phosphorylates Cdc25B to promote its binding to 14-3-3 protein and sequestration from centrosome. Chk1 also phosphorylates Cdc25C to induce its binding to 14-3-3 protein and nuclear exclusion. Cdc25B and Cdc25C inhibition mainly causes G2 arrest

of which can be phosphorylated by Chk1 in DDR. Interestingly, upon phosphorylation Cdc25A, B and C may employ distinct mechanisms to arrest the cell cycle [50, 51] (Fig. 5). For Cdc25A, phosphorylation by Chk1 targets it for proteasomal degradation, which leads to the inhibition of Cdk1 and Cdk2 resulting in cell cycle arrest at G1/S transition, S phase, and G2/M transition [52, 53]. Interestingly, besides direct phosphorylation, Chk1 can also activate Nek11, which in turn phosphorylates Cdc25A to induce cell cycle arrest during DDR [54]. For Cdc25C, a main regulation seems to be its cellular localization. Specifically, phosphorylation of Cdc25C by Chk1 promotes its binding to 14-3-3 proteins leading to the sequestration of Cdc25C from Cdk1, Cdk1 inactivation and G2 arrest [4]. Cdc25B regulation by Chk1 seems to occur at centrosomes, where Chk1 phosphorylates Cdc25B leading to its sequestration from centrosome and inhibition of centrosomal Cdk1 [41].

In addition to Cdc25 phosphotases, Chk1 phosphorylates WEE1, the protein kinase that is responsible for the inhibitory phosphorylation of Cdk1 (Fig. 4). As a result, in response to DNA damage, the phosphorylation and activation of WEE1 by Chk1 leads to the inhibition of Cdk1 and cell cycle arrest at G2 phase [55, 56]. Chk1 is also a negative regulator of PLK1 (polo like kinase 1), a mitotic kinase involved in centrosome maturation, spindle formation, and cytokinesis [57]. PLK1 phosphorylates WEE1 and targets it for degradation, leading to Cdk1 activation and mitotic entry (Fig. 4). In addition, recent work suggests that, in response to DNA damage, Chk1 dissociates from the chromatin leading to decreased phosphorylation and acetylation of histone H3, which may contribute to DNA damage-associated transcriptional repression [58]. Interestingly, the repressed genes include cyclin B and Cdk1, although the role of the transcriptional repression in cell cycle arrest remains unclear.

Chk1-mediated DNA repair during DDR

As discussed above, Chk1 induces checkpoint activation resulting in cell cycle arrest. In addition, in S-phase, Chk1 may slow down DNA replication by blocking the initiation factor CDC45 in a Cdk2-independent manner [59]. Together, these mechanisms provide time for DNA repair to maintain genomic integrity in response to DNA damage. Notably, emerging evidence from the past few years has suggested a more direct role of Chk1 in DNA repair (Fig. 6).

In this regard, Chk1 induces post-translational modification and activation of several important DNA repair factors. (1) Proliferating cell nuclear antigen (PCNA). One of the mechanisms of Chk1-mediated DNA repair is by inducing monoubiquitination of PCNA [60]. Upon ubiquitination, PCNA can activate translesion synthesis, a process that allows the replication machinery to pass or tolerate DNA lesions for replication. Specifically, at DNA damage sites, monoubiquitinated PCNA recruits specific translesion synthesis DNA polymerases to replace classical polymerases [61, 62], which can bypass or repair the damaged sites (lesion) for DNA replication [63]. (2) FANCE. In the repair of DNA cross-links, Chk1 phosphorylates the FANCE

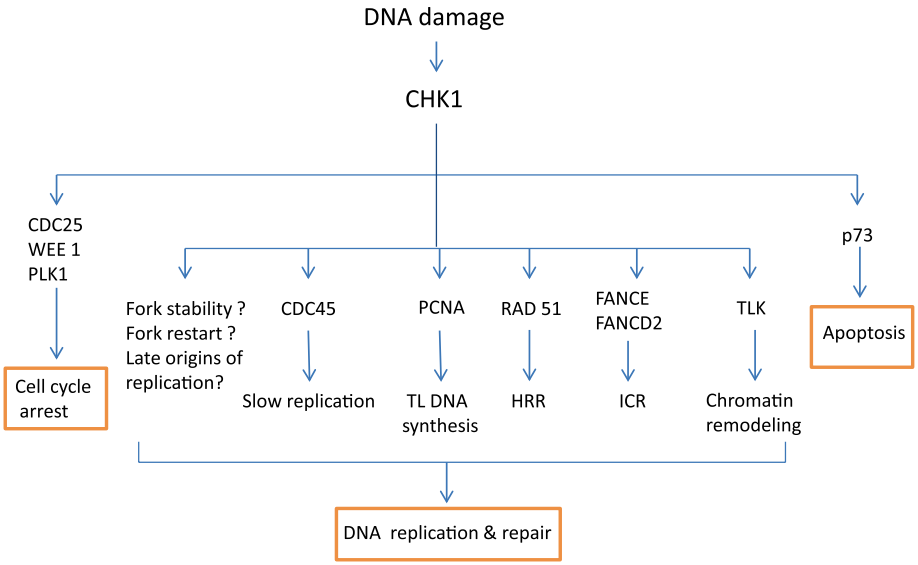

Fig. 6 Chk1-mediated DNA repair. Activated Chk1 phosphorylates several substrates to mediate DNA repair and apoptosis. Chk1 slows down DNA replication directly by affecting CDC45 loading on replication complex to allow time for DNA repair. Chk1 promotes monoubiquitination of PCNA, which recruits translesion polymerases to help translesion DNA synthesis. Chk1 phosphorylates TLK-tousled like kinase to promote chromatin assembly. Chk1 phosphorylates Rad51 to target it to double-strand breaks to promote HRR-homologous recombination and repair. Chk1 phosphorylates FANCE and monoubiquitinates FANCD2, which help resolve ICR-interstrand crosslink DNA lesion. Chk1 also regulates replication fork stability, fork restart, and late origins of replication, the mechanistic details of which are currently unclear. In addition to DNA repair, Chk1 may also induce cell cycle arrest and apoptosis by regulating different substrates

subunit of the Fanconi anemia (FA) core complex on two conserved sites serine-346 and serine-S374, resulting in its co-localization with FANCD2 at nuclear foci or DNA damage sites [44, 64]. Together with BRCA1 and 2, FANCE and ubiquitinated FANCD2 help resolve interstrand DNA crosslinks [44]. (3) Rad51. Checkpoint kinase 1 participates in homologous recombination repair by regulating Rad51. Knockdown or inhibition of Chk1 leads to accumulation of double-strand breaks and increased cell death in response to double-strand breaks induced by hydroxyurea and camptothecin. Mechanistically, Chk1 phosphorylates Rad51 at serine-309 and recruits it to DNA repair foci to promote homologous recombination repair and cell survival [65]. (4) TLK. In response to double-strand DNA breaks, Chk1 can also phosphorylate the serine-695 site of TLK (tousled-like kinase), an evolutionarily conserved serine/threonine kinase that is involved in chromatin assembly, DNA replication, and repair. Interestingly, the phosphorylation of TLK by Chk1 depends on ATM [66]. Finally, it is noteworthy that Chk1 has also been suggested to stabilize replication forks and/or restart stalled replication forks; however, the underlying mechanisms remain unclear.

Chk1-induced cell death in DDR

There are few reports implicating Chk1 in apoptosis or cell death. Chk1 phosphorylates p73 at serine-47, resulting in a drastic increase in p73 expression and transcription activity in response to DNA damage. Although p73 is a p53-related protein, it induces apoptosis independently of p53 [67, 68]. In contrast, there are instances where Chk1 inhibits apoptosis through ATM/ATR-caspase2 and RPA-caspase3 pathway [69, 70]. Therefore, it remains unclear whether (and to what extent) Chk1 contributes to apoptosis and its regulation.

Regulation of Chk1

At the sequence level, Chk1 is highly conserved from yeast to humans [52] (Fig. 7). Structurally, Chk1 has a conserved N-terminal kinase domain, a linker region, a regulatory SQ/TQ domain, and a C-terminal domain with unclear functions [52, 71]. It is noteworthy that Chk1 and Chk2 are not homologous in sequence, although they both have checkpoint function and share some phosphorylation substrate proteins.

It is well recognized that Chk1 is activated upon phosphorylation at two conserved sites, serine-317 and serine-345 [28, 46, 72]. Although phosphorylation at these two sites is indispensable for the function of Chk1, it remains elusive as to how exactly the phosphorylation activates Chk1 [45]. The study of crystallographic structure revealed that in the absence of phosphorylation, the Chk1 kinase domain assumes an open, active conformation [73]. Indeed, the

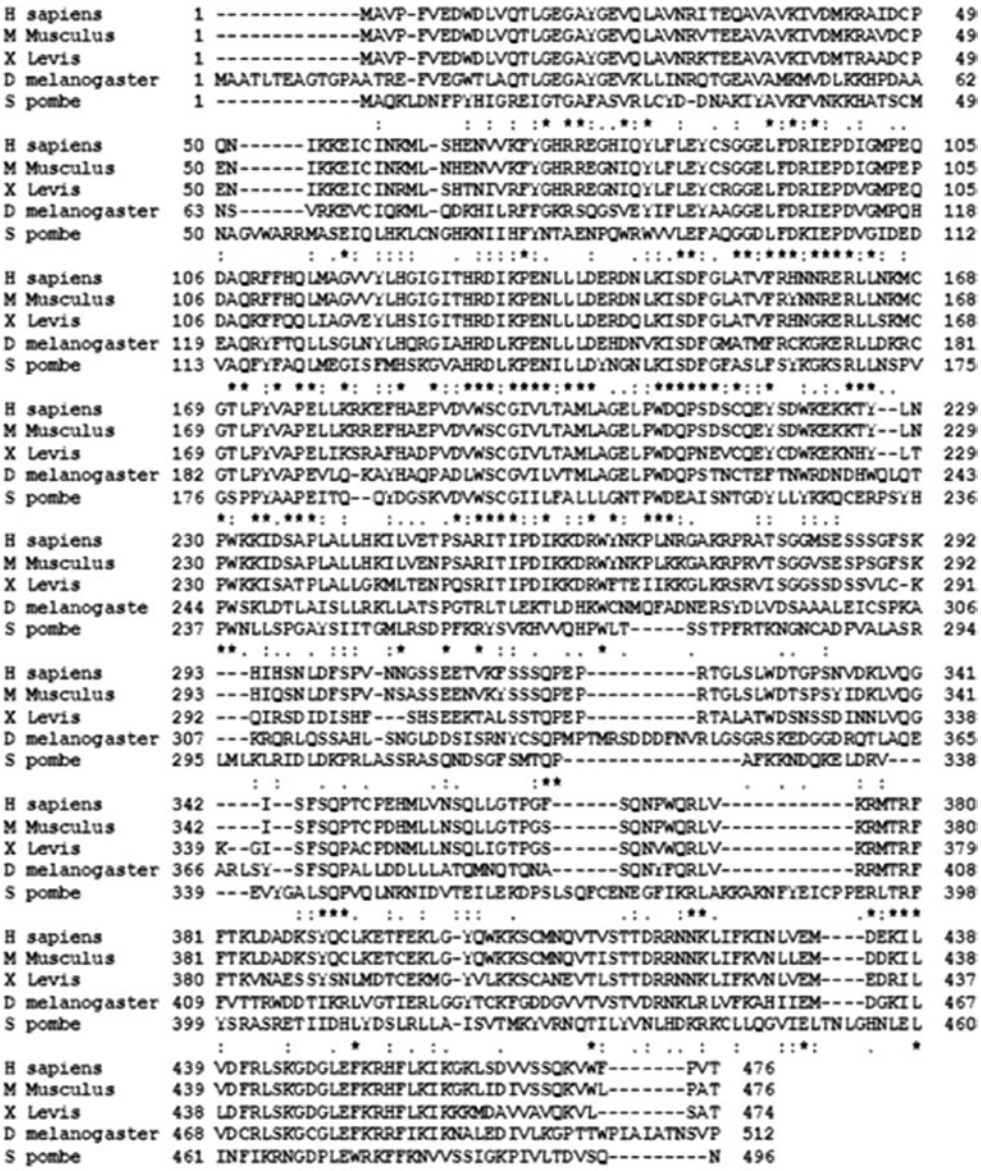

Fig. 7 CHk1 sequence alignment between human, mice, fruit fly, *Xenopus* and yeast. Chk1 sequences of human, mice, *Drosophila*, *Xenopus*, and yeast were analyzed using T-coffee, a Web server for multiple sequence alignment tool. The N-terminal kinase domain and regulatory SQ/TQ domain of Chk1 are highly conserved, while the C-terminal domain is less conserved. *Asterisk* indicates highly conserved sequence, *Colon* indicates conserved substitution, *Dot* indicate semi conserved substitutions

kinase domain has significantly higher kinase activity when compared to full-length Chk1. These observations indicate that Chk1 kinase domain has a constitutively active conformation that does not rely on phosphorylation for activation. One model of Chk1 activation proposes that normally the C-terminal domain interacts with the kinase domain to mask the active site and the phosphorylation at serine-317 and serine-345 dissociates these two domains leading to Chk1 activation. This model is supported by several lines of evidence. First, C-terminal truncation mutants show higher catalytic activity than intact Chk1 [73]. Second, in binding assays, ectopically expressed C-terminal domain of rat Chk1 binds to the N-terminal kinase domain [74]. In addition, in *Xenopus* egg extracts, ectopically expressed C-terminal domain can interact with N-terminal Chk1 domain and inhibit its activity but not full-length Chk1 [71]. The inhibitory effect of C-terminal Chk1 domain on N-terminal kinase domain can be overcome by treatment with aphidicolin and phosphomimetic mutations of ATR [71], suggesting that the C-terminal domain indeed inhibits kinase activity. Together, these observations suggest that the C-terminal domain is inhibitory to the kinase domain in Chk1 and, upon phosphorylation, this inhibition is disabled. While this "intramolecular inhibition" model is supported by several lines of evidence, it has been challenged recently. For example, several C-terminal truncation mutants lead to loss of function in Chk1 [45, 75], suggesting that removal of the C-terminal domain is not sufficient for Chk1 activation and Chk1 regulation may be more complex than "intramolecular inhibition". In addition to serine-317 and serine-345, Chk1

has also been reported to have autophosphorylation at serine-296, which plays a role in G2 checkpoint [76]. Interestingly, the autophosphorylation is consequent to phosphorylation at serine-317 and serine-345 through intramolecular mechanisms. It is noteworthy that serine-296 phosphorylation does not affect the kinase activity of Chk1 [77].

Interestingly, a recent study by Walker and colleagues indicates that Chk1 may be subjected to a new mechanism of regulation, called "de-repression". In this model, Chk1 is antagonized by an inhibitory or repressing factor(s), the dissociation of which leads to Chk1 activation. In support of this possibility, Chk1 immunoprecipitated from untreated cells demonstrates significantly higher kinase activity after being washed with a more stringent buffer. Notably, Chk1 immunoprecipitated from DNA damaged (by aphidicolin) cells also shows significantly higher kinase activity when washed with the stringent buffer, although the increase is somewhat smaller than that of untreated cells. In addition, stringent buffer washes also increase the kinase activity of Chk1 (S345A) mutant from transfected cells [78], suggesting that phosphorylation at serine-345 is not obligatory for Chk1 kinase activity per se, although it plays a role in Chk1 activation in cells. Furthermore, Chk1 can be activated in a claspin-dependent manner in response to genotoxic stress independently of ATM/ATR and associated phosphorylation [79]. Together, these studies support the possibility of additional regulatory mechanisms for Chk1, in addition to phosphorylation. The "de-repression" model is intriguing and tantalizing, but the identity of the "repressing" factor(s) of Chk1 is not known.

Our latest work has identified a splice variant of Chk1, which may act as an endogenous repressor of Chk1 [80]. This variant is spliced in a way that the exon 3 of Chk1 is deleted, leading to the production of an N-terminally truncated protein, which we call Chk1-short or Chk1-S. Due to the truncation, Chk1-S lacks part of the kinase domain including the ATP-binding (Fig. 8a) and, as expected, does not possess protein kinase activity. Importantly, a series of in vitro and in vivo tests showed that Chk1-S directly interacts with Chk1 at its N-terminal domain and inhibits Chk1 kinase activity. In addition, we confirmed the finding by Walker and colleagues that stringent buffer washes can increase the activity of Chk1 immunoprecipitated from cells. Moreover, we showed that the addition of exogenous

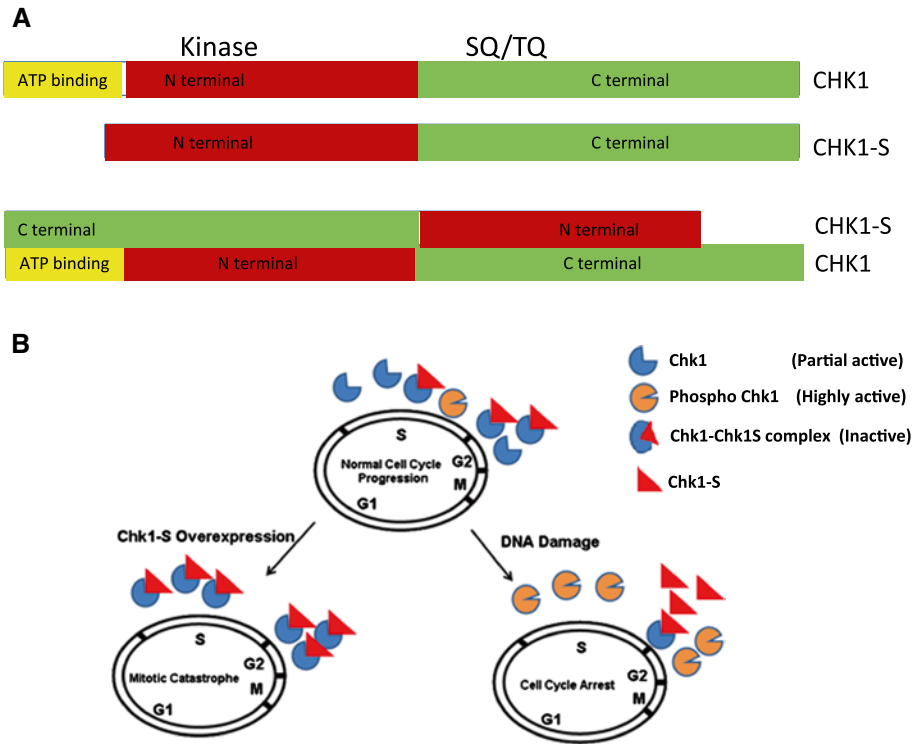

Fig. 8 Physical and functional interactions between Chk1 and Chk1-S. **a** Schematic representation of Chk1 and Chk1S domains and their interaction. Checkpoint kinase 1 has ATP binding domain, N-terminal kinase domain, SQ/TQ domain, and C-terminal domain. Chk1S lacks ATP binding domain. C-terminal domain of Chk1 interacts with N-terminal domain of CHK1-S. **b** Regulation of Chk1 by Chk1-S. Chk1-S interacts with Chk1 to inhibit its kinase activity. In unperturbed cell cycles, Chk1-S is expressed in late S to G2 phase to inhibit Chk1 and promote mitotic entry. In DNA damage, Chk1 is phosphorylated to prevent Chk1-S binding and inhibition, resulting in Chk1 activation. When Chk1-S is overexpressed, it antagonizes Chk1, leading to premature mitotic entry and consequent cell death via mitotic catastrophe. Figure 8b is adopted from Pabla et al. PNAS 109, 197-202, 2012

Chk1-S can reverse the effect of stringent buffer washing. Together, these results suggest that Chk1-S may be one of the key endogenous repressing factors of Chk1. In an unperturbed cell cycle, Chk1-S expression increases significantly in late G2 and early M phases coinciding with the decrease in Chk1 activity for mitotic entry. Overexpression of Chk1-S leads to premature mitotic entry followed by cell death in the form of mitotic catastrophe. These observations support an important role of Chk1-S in the regulation of normal cell cycles by binding and antagonizing Chk1. In response to DNA damage, the interaction between Chk1 and Chk1-S is lost in an ATR-dependent manner (Fig. 8b). Remarkably, mutation of Chk1 at serine-317 and serine-345 prevents the dissociation of Chk1 from Chk1-S during DNA damage, indicating that the dissociation of Chk1 from Chk1-S in DDR depends on Chk1 phosphorylation. Based on these observations, we propose that Chk1-S is a key regulator of Chk1 in both normal cell cycles and DDR. Mechanistically, Chk1-S binds to Chk1 to act as a repressor to block Chk1 activity. In normal cell cycles, Chk1-S is drastically increased at late G2 phase to repress Chk1 to promote mitotic entry. In DDR, ATR-mediated Chk1 phosphorylation at serine-317 and serine-345 leads to the dissociation of Chk1-S from Chk1, resulting in Chk1 activation and cell cycle arrest (Fig. 8b).

In addition to the above-described regulatory mechanisms, Chk1 is also known to be subjected by proteasomal degradation. Interestingly, proteasomal degradation of Chk1 may depend on its phosphorylation status. In camptothecin-induced DNA damage, Chk1 phosphorylation at serine-345 targets the protein for proteosomal degradation [81]. It was later shown that the phosphorylation exposes a dragon-like region at the C-terminus, which is recognized by the F-box protein called Fbx6, leading to Chk1 ubiquitination and proteasomal degradation and termination of the checkpoint [82]. Consistently, there is an inverse correlation between Chk1 and Fbx6 expression in cultured cancer cells and breast cancer tissues. In addition, overexpression of Fbx 6 leads to degradation of chk1 and increased cellular sensitivity to camptothecin [82].

Chk1 substrates

Despite the multiple roles of Chk1 in normal cell cycles and DDR, only a few phosphorylation substrates of Chk1 have been identified thus far. As a result, identification of functional substrates of Chk1 is currently a subject of intense investigation. The consensus sequence of Chk1-specific substrates has been suggested by analyzing the peptide derived from *Xenopus*. Cdc25C containing serine-287, a typical Chk1-phosphorylation site. Indeed, the consensus sequence has been useful for predicting Chk1 substrates including PDS1 (an anaphase inhibitor) and Wee1 [83]. Nonetheless, the consensus sequence is insufficient to explain other Chk1 substrates. Some of the Chk1 substrates may be context-dependent and do not rely on the consensus sequence for phosphorylation. In this regard, a substrate may be phosphorylated by Chk1 through interaction with adaptor proteins. A recent study has conducted a global phospho-proteomic screen using analogue-sensitive Chk1 and suggested 171 proteins as its potential phosphorylation substrates. Moreover, KAP1, a protein phosphorylated in response to DNA damage, has been validated to be a Chk1 substrate in both in vitro and in vivo studies and KAP1 phosphorylation can be used as a read out for Chk1 activation [47]. However, most of the substrates implied in this study remain to be validated. It is expected that new and important functional substrates of Chk1 will be discovered in the next few years to provide new insights into the multiple functions of Chk1 in the cell cycle and DDR.

Chk1 in cancer

Checkpoint kinase 1 mutations or loss are very rare in cancer. A logical explanation is that Chk1 plays an essential role in cell cycle regulation, cell proliferation and survival [28, 29, 80], and as a result, cells with defective Chk1 are eliminated during tumorigenesis. Nonetheless, partial ablation of Chk1 (heterozygous) favors tumor formation in WNT1 oncogenic mice [28]. In addition, loss-of-function Chk1 mutations have been reported in stomach, endometrial, and colorectal cancers [84–87]. Mutations have been mapped to the microsatellite instability region in the coding region of Chk1 with a stretch of nine adenine nucleotides [86]. Frame-shift mutations lead to the formation of a truncated protein in colorectal and endometrial cancers lacking part of the kinase domain and the C-terminal domain [86]. Intriguingly, in these cancers is that the second allele is normal, resulting in the expression of functional Chk1 albeit at a lower level than that of normal tissues [88]. Monoallelic mutations leading to partial loss of expression are also observed in ATR in endometrial and stomach cancers [85]. These studies support the haploinsufficient tumor model in which monoallelic mutations in several genes of the same pathway favor tumorigenesis [88]. In mice, conditional deletion of Chk1 in mammary epithelial cells fails to produce tumors; instead it leads to cell death [38]. However, the mice haploinsufficient for both Chk1 and p53 develop tumors in mammary glands [89]. Similarly, complete loss of Chk1 in skin suppresses chemically induced carcinogenesis, whereas partial (haploinsufficient) deletion of Chk1 promotes benign malignant tumor progression [90]. Triple-negative (Estrogen-/progesterone-/HER2-) breast cancers express a very high

level of Chk1 and have poor clinical outcomes, suggesting that Chk1 favors cell proliferation [91]. Together, these studies indicate that Chk1 is essential for cell survival and its haploinsufficiency may promote cancer, especially in the presence of mutations of other relevant genes. Of note, Chk1-S, the splice variant and endogenous inhibitor of Chk1, is expressed at significantly higher levels in cancer tissues than normal tissues and notably, Chk1-S expression correlates with the degree of malignancy in ovarian and testicular tumors [80]. Interestingly, Chk1 expression is also higher in cancer. It is suggested that relatively high levels of Chk1 and Chk1-S may favor cell proliferation in cancer.

Targeting Chk1 for cancer therapy

DNA damaging agents are the most commonly used drugs for cancer therapy. By damaging DNA, these chemotherapy drugs induce cell cycle arrest to prevent cell proliferation and trigger cell death in cancers. Many of these agents induce G1 arrest in a p53-dependent manner and G2 arrest in a Chk1-dependent manner. G1 arrest is defective in many cancers since more than 50 % of cancers are defective in p53; under these situations, G2 arrest becomes a main pathway for cancer cell survival. Hence, Chk1-dependent G2 checkpoint is a major target for chemotherapy [92–95]. In this regard, Chk1 inhibition or knockdown increases the sensitivity of p53-deficient cancer cells to DNA damage agents [33, 96]. However, it has also been reported in certain cell lines that Chk1 is essential for G2 checkpoint irrespective of the p53 status and hence Chk1 inhibition may not preferantially kill p53-deficient cells [97]. It has also been shown that Chk1 inhibition increases the sensitivity of cancer cells to anti-mitotic agents [98, 99]. In addition, Chk1 is a client of HSP90 and inhibition of HSP90 leads to the loss of Chk1 resulting in the sensitization of cancer cells to Gemcitabine, an S phase chemotherapeutic agent [100]. Chk1 and Wee1 kinase inhibitors in combination have more than additive effect on different cancer cell line proliferation and human xenograft models [101]. Therefore, Chk1 inhibitors may target multiple cell cycle phases to override the checkpoint responses during cancer therapy, leading to increased therapeutic efficacy. Currently, Chk1 inhibitors are being developed and tested alone or in combination with DNA damaging agents in clinical trials for cancer therapy [8, 36]. However, so far, none of these have passed phase III clinical trials. Obviously, the specificity and potency of Chk1 inhibitors are critical to their clinical use. In addition, their side effects have to be carefully evaluated because Chk1 has an important role in cell cycle regulation and its inhibition may adversely affect cell viability and function in normal tissues.

Concluding remarks

Checkpoint kinase 1 plays an essential role in cell cycle regulation and DNA damage response. In unperturbed cell cycle, Chk1 regulates G1/S transition, S phase, mitotic entry, and mitosis. In DDR, Chk1 is an important signal transducer and the trigger of G2 checkpoint activation. The role of Chk1 in unperturbed cell cycle and tissue physiology is only beginning to be understood. Chk1 regulates S phase progression by controlling DNA replication, fork stability and late origins of replication the mechanistic details of which are yet to be identified. The role of Chk1 in the development and pathogenesis in different tissues are yet to be investigated. In addition, although Chk1 regulates several checkpoints, only a few Chk1 substrate proteins have been identified. The discovery of Chk1-S as an important regulator of Chk1 in both unperturbed cell cycle and DDR has opened new areas of investigation. Finally, Chk1 may be an effective therapeutic target in diseases. In this regard, Chk1 inhibitors, when used together with other therapeutic agents, may significantly enhance the chemotherapy efficacy in cancer treatment.

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