

Polo-like kinases and DNA damage checkpoint: beyond the traditional mitotic functions

El Mustapha Bahassi

Division of Hematology and Oncology, Department of Internal Medicine, University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267-0562, USA

Email: bhassiem@uc.edu

Abstract

Polo-like kinases (Plks) are a family of serine–threonine kinases that play a pivotal role in cell cycle progression and in cellular response to DNA damage. The Plks are highly conserved from yeast to mammals. There are five Plk family members (Plk1–5) in humans, of which Plk1, is the best characterized. The Plk1 isoform is being aggressively pursued as a target for cancer therapy, following observations that this protein is overexpressed in human tumors and is actively involved in malignant transformation. The roles of Plks in mitotic entry, spindle pole functions and cytokinesis are well established and have been the subject of several recent reviews. In this review, we discuss functions of Plks other than their classical roles in mitotic progression. When cells incur DNA damage, they activate checkpoint mechanisms that result in cell cycle arrest and allow time for repair. If the damage is extensive and cannot be repaired, cells will undergo cell death by apoptosis. If the damage is repaired, cells can resume cycling, as part of the process known as checkpoint recovery. If the damage is not repaired or incompletely repaired, cells can override the checkpoint and resume cycling with damaged DNA, a process called checkpoint adaptation. The Plks play a role in all three outcomes and their involvement in these processes will be the subject of this review.

Keywords: polo-like kinases, DNA damage, checkpoint activation, apoptosis

Experimental Biology and Medicine 2011; **236**: 648–657. DOI: 10.1258/ebm.2011.011011

Introduction

Polo-like kinases (Plks) are a conserved family of serine–threonine kinases with highly related catalytic domains.^{1–3} Their conservation is evident from yeast to mammals. The first Plk to be described was *Drosophila melanogaster* Polo, a mutant of which produced mitotic and meiotic defects.^{4,5} Budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) each have a single Plk designated Cdc5 and Plo1, respectively, which functions to regulate mitotic entry, mitotic exit and cytokinesis. In *Xenopus laevis*, there are three known Plks (Plx1–3)⁶ and in mammals there are five known mammalian Plks (Plk1–5). Plk1, Plx1, Polo, Cdc5 and Plo1 are orthologs and their multiple contributions to cell cycle progression reveal their conserved and essential cellular roles. The functions of their paralogs (Plk2–5 and Plx2, 3), however, are less well understood.

The Plks1, 2, 3 and 5 each have two conserved ‘polo box’ motifs while Plk4 (Sak), a divergent member of the Plk family, possesses only one of the two bipartite polo-box motifs. In Plk1, the two polo boxes comprise the polo box domain (PBD) that coordinates protein–protein interactions

and subcellular localization.^{7–14} As in Plk1, the Plk2 and Plk3 PBDs preferentially bind phosphoserine and phosphothreonine motifs.^{15–22} The Plks appear to regulate the G1/S transition, the entry of cells into mitosis, cytokinesis and the cellular response to DNA damage. These functions have been deduced in part by their localization to mitotic structures and by the phosphorylation of their specific substrates via their PBD and catalytic domains, respectively.^{11,12,23–27}

The best characterized member of the Plk family is Plk1, which serves as a key positive regulator of mitosis, meiosis and cytokinesis.^{2,25} Various types of cancers overexpress Plk1, and its elevated expression is associated with poor prognosis.^{28–33} Thus Plk1 is considered a candidate target for anticancer therapy.^{34–38} Within the past few years, a number of Plk1 substrates has been identified revealing signaling pathways by which Plk1 can regulate mitotic entry, DNA damage checkpoint responses, spindle formation and mitotic exit (reviewed by Strebhardt).³⁹

The Plk2 (serum-inducible kinase, Snk) and Plk3 (also designated FGF-inducible kinase, Fnk or Prk) kinases are products of early-response genes that are induced when

quiescent mouse fibroblasts are stimulated by serum.^{40–43} The Plk2 gene is a transcriptional target of p53 and is also reported to be elevated in cancer cells. The Plk2 kinase interacts with Chk1 and Chk2 kinases and participates in S-phase arrest.⁴⁴ Both Plk2 and Plk4 appear to be involved in centriole duplication.^{45,46}

Plk3 is considered to be a tumor suppressor and a regulator of cellular responses to DNA damage and angiogenesis.^{40–43,47,48} Some studies report that Plk3 expression remains relatively constant during normal cell cycle progression⁴² while others argue that Plk3 is active mainly during entry of cells into S phase⁴⁹ or in mitosis.⁵⁰ While expression of Plk1 is down-regulated following DNA damage,⁵¹ Plk3 expression is induced and accompanied by an activating phosphorylation that is ataxia telangiectasia mutated (ATM) dependent.^{41–43} When Plk3 is activated, it mediates phosphorylation of Chk2 by ATM and subsequent checkpoint activation.⁵² Chk2 physically interacts with and stimulates Plk3 activity. The interaction of Chk2 and Plk3 is enhanced following DNA damage, suggesting that Chk2 may mediate direct activation of Plk3 in response to genotoxic stress.⁴³ Plk3 also phosphorylates DNA polymerase δ , suggesting that it may be involved in DNA repair.⁴¹ Plk3 also participates in the onset of mitosis via inhibitory phosphorylation of Cdc25c.⁵³

The Plk4 (Sak) kinase is required for late mitotic progression, cell survival and postgastrulation embryonic development.⁵⁴ It is a structurally divergent member of the Plk family, having only one polo-box domain that can form two adjacent and functional PBDs by homodimerization *in vivo*.⁵⁵ Functionally, Plk4 is more closely related to Plk1 than to the other Plk members. Like Plk1, it localizes to centrosomes and the cleavage furrow during cytokinesis, and its role in centriole duplication is well established.⁴⁵ Also like Plk1, cells and mice that are heterozygous for Plk4 display haploinsufficiency with increased probability of mitotic errors and cancer development. Consistent with this observation, there is loss of heterozygosity at Plk4 in about 50% of human hepatocellular carcinomas (HCC).⁵⁶ It appears that a tight balance of the Plk4 protein level is requisite for sustained genomic instability since overexpression of Plk4 leads to the appearance of multinucleated cells⁵⁷ and may be associated with colon tumors.⁵⁸

We have recently cloned the cDNA that encodes a fifth member of the Plk family, designated *Plk5*.¹⁴ Based on its nucleotide sequence, there are two distinct domains within the Plk5 protein. The amino-terminal portion has features characteristic of the catalytic domain of a serine/threonine kinase and shows strong homology to other kinases of the polo family. The carboxy-terminal part of the protein harbors the putative regulatory domain, which contains the PBD and shares extensive homology with the carboxy-terminal domains of the other Plk proteins. Based on sequence similarities at the DNA and protein levels, Plk5 shares greater similarity with Plk2 and Plk3 than with Plk1 and Plk4. Consistent with this observation, we have shown that the mouse *Plk5* gene is activated following DNA damage. Interestingly, human *Plk5* but not mouse *Plk5* contains a stop codon at position 807 of the nucleotide sequence (exon 6) that leads to a truncated protein. A second

open reading frame starts just after this stop codon and extends through the end of the human *Plk5* gene. *Plk5* mRNA levels become elevated following introduction of DNA damage or disruption of microtubules, suggesting that Plk5 is a stress-induced protein that is involved in preserving cell integrity and that responds to a broad range of insults. Mouse Plk5 localizes predominantly in the nucleolus, and deletion of a putative nucleolar localization signal within its N-terminal region disrupts this subcellular localization. Overexpression of Plk5 leads to G1 cell cycle arrest, decreased DNA synthesis and apoptosis, a characteristic it shares with Plk3.¹⁴

Plks are regulated at several levels including transcription, phosphorylation and ubiquitination-mediated degradation. At the transcriptional level, Plks are regulated in a cell-cycle-dependent manner.⁵⁹ Plk1 and its orthologs are regulated by a CDE/CHR (cell-cycle-dependent element/cell cycle gene homology region) element, which represses the transcription of Plk1 (in mammals) and *plp1* (in fission yeast) and involves unknown factors.^{60–63} Forkhead transcription factors (FKH-TFs) stimulate the transcription of Plk1, *cdc5* and *plp1*, which in turn activate FKH-TF-dependent transcription.^{64–66} In *Drosophila melanogaster* and humans, retinoblastoma protein and E2F can inhibit polo or Plk1 transcription.^{61,67,68} The tumor suppressor protein p53 and its downstream effector p21 also repress Plk1 transcription. The transcriptional activator protein MYB has also been shown to activate polo transcription in *D. melanogaster*.⁶⁹ p53 regulates the transcription of PLK2, PLK3 and PLK4 especially in response to DNA damage. Both Plk2 and Plk5 have been shown to be regulated at the transcriptional level through CpG methylation of their promoters.⁷⁰ The role of p53 in the transcription regulation of these genes is further detailed below. Plks are also activated by phosphorylation in their T-loop (or activation loop) domain.^{12,71} This phosphorylation activates the kinase domain of these proteins and relieves an intramolecular inhibitory interaction with the Polo-box domain (PBD).^{3,72} In human cells, Plk1 is activated at mitotic entry by Aurora A kinase and its adaptor BORA, which phosphorylate Plk1 in its T-loop.^{73,74} Plk1 has been proposed to be dephosphorylated in the T-loop by protein phosphatase 1.⁷⁵ Other kinases and phosphatases probably regulate the T-loop phosphorylation of other Plks. Plk activity is also regulated by ubiquitin-mediated degradation.⁷⁶ Plks are targeted for degradation by different ubiquitin ligases including anaphase promoting complex (APC^{Cdh1}) and the ubiquitin ligase SCF (SKP1, Cullin, F-box).^{9,77–81} Polyubiquitylation is recognized by the 26S proteasome, which destroys the Plk. Plk1 and Cdc5 are targets of the Cdc20 homolog 1 (Cdh1)-activated APC. Plk4 and probably Plk2–5 are targets of the ubiquitin ligase SCF (SKP1, Cullin, F-box).^{9,77–81} Plks engage in protein interactions through the binding of their PBD to targets previously primed by phosphorylation. This increases the kinase domain activity and positions Plks favorably for phosphorylation of either the same target or another proximal target.^{7,13,82,83}

The role of Plks, especially Plk1, in the cell cycle including entry into mitosis, centrosome maturation, assembly of the bipolar spindle, sister chromatid splitting, activation of the

anaphase promoting complex and exit from mitosis is well understood and has been the subject of several excellent reviews.^{1,2,39,84–89} Although data supporting a role of Plks in the DNA damage response and repair are still scarce, the Plks are emerging as important regulators of checkpoint modulation in response to DNA damage. In this review we discuss evidence linking Plks to the DNA damage response, particularly in checkpoint adaptation, checkpoint recovery and apoptosis. The recent findings about the interplay between Plks and p53 in the regulation of these processes will also be addressed.

Plks and DNA damage response

The DNA damage checkpoint represents a vital cellular process that coordinates cell cycle progression (or its transient cessation) with DNA repair in response to DNA damage. When there is DNA damage or replication stress, checkpoint responses prevent further cell divisions to allow time for DNA repair and/or DNA replication to be completed.^{90–93} The checkpoint machinery is highly conserved in eukaryotes and defects in this machinery lead to sensitivity to damage in yeast and to cancer susceptibility in humans. The PI3-kinase proteins that include ATM (Tel1 in *S. cerevisiae* and *S. pombe*) and ataxia telangiectasia and Rad3-related (ATR) (Mec1 in *S. cerevisiae* and Rad3 in *S. pombe*) play important roles as the initial sensor of DNA damage that initiate the signal transduction pathways, resulting in the transcriptional induction of DNA repair genes and the slowing of cell cycle progression.^{94–96} The DNA damage response involves a number of factors that ultimately coordinate the spatiotemporal assembly of protein complexes at the site of DNA damage to initiate and maintain the checkpoint. Depending on the type of genotoxic stress, different checkpoint pathways are activated. Ultraviolet (UV) irradiation and stalled replication forks preferentially activate the ATR-Chk1 pathway, whereas double-strand breaks (DSBs) result in the activation of both the ATM-Chk2 and ATR-Chk1 pathways. In mammals, ATM is recruited to the site of DNA damage where it phosphorylates and activates the effector kinase Chk2, while ATR phosphorylates and activates the effector kinase Chk1, a process that requires the mediator protein Claspin. Important downstream targets of Chk1 and Chk2 include p53 and Cdc25A. The p53 protein can serve as a transcription factor and Cdc25A phosphatase can activate cyclin-dependent kinase 1 (Cdk1), by dephosphorylating the Cdk tyrosine 15 and threonine 14. Chk1-mediated phosphorylation induces the stabilization of p53 (with the consequent expression of the CDK inhibitor p21) and is required for the SCF^{βTrcp}-mediated degradation of Cdc25A. Thus, activation of Chk1 and Chk2 results in the attenuation of Cdk1 activity with consequent inhibition of mitosis.

Following arrest in G2 as a result of DNA damage, cells will undergo one of three possible scenarios. The first, known as checkpoint recovery, involves the complete repair of the damaged DNA before cells can continue to divide. The second is a potentially deleterious option known as checkpoint adaptation, which involves cell cycle

progression with damaged DNA. The third is apoptosis, which allows elimination of cells with heavily damaged DNA. The Plks have been shown to be involved in all three processes.

Plks in checkpoint adaptation

When DNA damage occurs in interphase, ATR- and ATM-mediated checkpoints maintain the integrity of the genome by arresting the cell cycle. These signaling pathways provide time for DNA repair prior to the onset of mitosis and thus prevent the catastrophic chromosome breakage that can occur when damaged chromatids are segregated. 'Recovery' from damage-induced arrest results from relaxation of the checkpoint after DNA repair is complete. In *S. cerevisiae*, there is a mechanism for 'adaptation' to the G2/M DNA damage checkpoint that also permits a cell with irreparable damage to re-enter the cell cycle.⁹⁷ Adaptation may be beneficial for a unicellular organism because it provides opportunities for the cell to repair the damaged chromosome in a subsequent cell cycle, enhancing its chances for survival.^{98,99} The use of the word 'adaptation' to describe the process of overriding a checkpoint was first introduced by Leland Hartwell.¹⁰⁰ Adaptation is a common component of many disparate signal transduction systems, such as bacterial chemotaxis¹⁰¹ and vertebrate vision,¹⁰² and renders these pathways less sensitive to a given stimulus upon continued exposure to that signal.

In *S. cerevisiae*, adaptation to the G2/M arrest of the DNA damage checkpoint was demonstrated using a haploid yeast strain containing a non-essential extra copy of chromosome VII with a cleavage site for the homothallic switching endonuclease (HO). When HO was induced in a mutant (*rad52*) unable to repair double-strand DNA (dsDNA) breaks, cells arrested in G2/M for approximately 10 h, then adapted and resumed division in the presence of the broken chromosome.⁹⁷ This checkpoint adaptation was later shown to be mainly mediated by the yeast Plk Cdc5.¹⁰⁰ Hartwell's group used the same system described in Sandell and Zakarian⁹⁷ and isolated two adaptation-defective mutants that remain permanently arrested as large-budded cells when faced with an irreparable dsDNA break in chromosome VII. This adaptation-defective phenotype was entirely relieved by deletion of *Rad9*, a gene required for the G2/M DNA damage checkpoint arrest. Loss of adaptation was conferred by a mutant *Cdc5*. A second less penetrant, adaptation-defective mutant was due to mutation at the *CKB2* locus, which encodes a non-essential specificity subunit of casein kinase II.¹⁰⁰ Failure of the *cdc5-ad* mutants to adapt to DNA damage was due to mitotic exit defects.¹⁰³ The involvement of Cdc5 in checkpoint adaptation is corroborated by recent data showing that overproduction of the Cdc5 protein overrides the checkpoint signaling induced by dsDNA breaks, preventing the phosphorylation of several Mec1/ATR targets, including Ddc2/ATRIP, the checkpoint mediator Rad9, and the transducer kinase Rad53/CHK2.¹⁰⁴ High levels of Cdc5 also slow down DSB processing in a Rad9-dependent manner, but do not prevent the binding of checkpoint factors to a

single DSB. Cdc5 also appears to regulate Sae2, the functional ortholog of human CtIP, which in turn regulates DSB processing and inhibits checkpoint signaling. One proposition is that Cdc5 interferes with the checkpoint response to DSBs by acting at multiple levels in the signal transduction pathway and at an early step required to resect DSB ends.¹⁰⁴

A similar scenario is observed in *Xenopus* egg extracts. Nuclei in extracts containing the replication inhibitor aphidicolin can adapt to the signal created by replication stress and enter into mitosis in the presence of unreplicated DNA.¹⁰⁵ This effect is mediated by the *Xenopus* homolog of Plk, Plx1, which inactivates Chk1 by regulating Claspin.¹⁰⁵ Claspin is an adaptor protein necessary for Chk1 activation.¹⁰⁶ It is a replication-dependent chromatin-binding protein that becomes highly phosphorylated in an ATR-dependent manner after genotoxic stress. This phosphorylation appears to be necessary for activation of Chk1 by ATR.¹⁰⁶ Furthermore, ATR-dependent phosphorylation of Claspin at Thr906 creates a docking site for Plx1 to bind via its phosphopeptide-binding polo-box domains.¹⁰⁵ This binding event facilitates phosphorylation of Claspin by Plx1 on a nearby residue, Ser934, phosphorylation of which causes the dissociation of Claspin from chromatin, leading to inactivation of Chk1 and entry of cells into mitosis. When endogenous Claspin is depleted and replaced with Claspin that is mutant at either Thr906 or Ser934, the mutant Claspin accumulated on chromatin, Chk1 remained phosphorylated, and nuclei in the extract remained arrested in interphase. While adaptation in *X. laevis* and *S. cerevisiae* is well established, adaptation would appear to be unfavorable for mammalian systems because of the obvious mutator effect and the enhanced risk of cancer. Several observations suggest that this pathway would require additional levels of control if it indeed exists in mammals. For example, the phosphorylation site in human Claspin that corresponds to *Xenopus* Claspin Thr906 is not an ATR consensus site but rather a consensus Cdk/MAPK site. Perhaps this site is regulated by another kinase or mediates a different outcome such as recovery. Furthermore, although *Xenopus* Plx1 activity continues to increase following aphidicolin treatment, in human cells its Plk1 ortholog is down-regulated following UV irradiation or adriamycin treatment.^{49,75} This finding raises the question of how Plk1 would mediate adaptation to these types of genotoxic stress in mammalian cells. The situation in *Xenopus* is also likely to be more complex. Clearly, phosphorylation at Thr906 and Ser934 is involved in Claspin regulation, but there is a significant delay between Plx1-mediated phosphorylation of Claspin and mitotic entry, suggesting additional steps may be involved.

Despite the argument against checkpoint adaptation in humans, Syljuåsen *et al.*¹⁰⁷ have shown that human osteosarcoma cells enter mitosis with γ -H2AX foci, a marker for unrepaired DNA DSBs, following ionizing radiation-induced G₂ checkpoint arrest. Exit from the G₂ checkpoint was accelerated by inhibiting Chk1 and was delayed by overexpressing wildtype Chk1 or depleting Plk1 with an inhibitory RNA. Chk1 and Plk1 seem to control this process, at least partly, via independent

signaling pathways. These results suggest that human cells are able to exit the checkpoint arrest and divide before DNA damage has been fully repaired. Such cell division in the presence of damaged DNA may be detrimental for genetic stability and could potentially contribute to cancer development.¹⁰⁷ It is, therefore, somewhat surprising that this process occurs in higher eukaryotes. At least upon first glance, the obvious alternative to adaptation is apoptosis, which seems a safer route for a multicellular organism when replication is incomplete. An apoptotic endpoint would prevent the accumulation of cells containing potentially cancer-inducing mutations. An argument for adaptation in multicellular organisms, however, can be proposed, and indeed adaptation could be a preliminary step for initiating cell death. For example, mammalian cells initially arrested in mitosis with nocodazole eventually move into G₁ where they arrest again in a p53-dependent manner.¹⁰⁸ By analogy, it is perhaps essential that a mammalian cell move into or through mitosis in order to induce apoptosis when replication forks are stalled. Adaptation could be necessary to convert the stalled fork into a different type of signal that is better able to induce death by apoptosis (e.g. DSBs) or to move the cell into another phase of the cell cycle where apoptosis can occur. Alternatively, adaptation could lead to death by mitotic catastrophe if the replication blockage is extensive. Less extensive blockage, however, might not be sufficient to cause cell death, and damaged cells could survive, ultimately leading to cancer.

Plks in checkpoint recovery

Single-celled organisms can clearly adapt to DNA damage to override checkpoint arrest. In contrast, multicellular organisms prefer a less mutagenic strategy that involves complete repair of damaged DNA prior to cell cycle resumption; namely, checkpoint recovery. In response to DNA damage in the G₂ phase, activation of the DNA damage ATM/ATR-Chk2/1-p53 leads to cell cycle arrest and Plk1 inactivation.^{51,109} During the recovery from DNA replication and DNA damage stress, the G₂ checkpoint needs to be silenced. In mammals, this process involves the degradation of Claspin mediated by the SCF ^{β Trcp} ubiquitin ligase following the phosphorylation of Claspin by Plk1.^{110–112} Plk1 also contributes directly to abolishing the inhibition of Cdk1/cyclin B through regulation of the phosphatase Cdc25B and the kinase Wee1.^{111,113} While the destruction of Claspin removes the essential co-activator of Chk1 (thereby allowing re-accumulation of Cdc25A and activation of Cdc25B and Cdc25C), degradation of Wee1 eliminates a direct CDK inhibitor. Both processes converge on activation of the mitosis-promoting cyclin-CDK complexes.

In a recent study, a combination of bioinformatics and biochemical approaches was used to identify Plk1 and Cdk1 targets within the ATM-Chk2 pathway that are involved in G₂ DNA damage checkpoint silencing.¹¹⁴ Plk1 was found to interact and phosphorylate 53BP1 protein and expression of a 53BP1 mutant that is unable to interact

with Plk1 prevents checkpoint release and fails to restart cell cycle after ionizing radiation-mediated cell cycle arrest. Plk1 was also shown to phosphorylate Chk2 to inactivate its forkhead-associated (FHA) domain and inhibit its kinase activity in mammalian cells. Thus, a mitotic kinase-mediated negative feedback loop regulates the ATM-Chk2 branch of the DNA damage signaling network by phosphorylating conserved sites in 53BP1 and Chk2 to inactivate G2 checkpoint signaling and control checkpoint duration.¹¹⁶

Clarification of the mechanism underlying the reactivation of Plk1 at the time of checkpoint recovery has resolved a longstanding point of confusion where Plk1 is inactivated in response to DNA damage and yet is responsible for recovery from the DNA damage checkpoint. During G2, Aurora A, in complex with its co-factor hBora, phosphorylates Plk1 at Thr-210. Activated Plk1 phosphorylates Cdc25C and Wee1, which in turn induces activation of cyclin B-Cdk1 complexes and promotes mitotic entry. Although Plk1 is activated in G2 both during unperturbed growth and after recovery from a DNA damage checkpoint, it is only essential for mitotic entry during checkpoint recovery.^{75,115}

Checkpoint recovery requires silencing of key checkpoint proteins, principally the p53 tumor suppressor. Sustained G2 arrest following DNA damage requires functional p53.¹¹⁶ How p53 is inactivated during G2 checkpoint recovery, however, is unknown. A recent study by Liu *et al.*¹¹⁷ identified G2- and S-phase-expressed 1 (GTSE1) protein as both a negative regulator of p53 and a Plk1 substrate during the recovery process. The GTSE1 protein is expressed specifically during G2 and S phases of the cell cycle, and is localized mainly in the cytoplasm, apparently associated with microtubules.^{118,119} This protein is able to down-regulate p53 by translocating into the nucleus, binding to and shuttling p53 out of the nucleus and inducing its degradation.^{120,121} In the later stage of DNA-damage-induced arrest, GTSE1 starts accumulating in the nucleus,¹²¹ suggesting that it might be involved in the checkpoint recovery process through down-regulation of p53. Plk1 phosphorylates GTSE1 at Ser 435 both during normal cell cycle progression and G2 checkpoint recovery. As a negative regulator of p53, GTSE1 is essential for eliminating active p53 from the nucleus in the later stage of the recovery process. Thus, the G2 arrest by p53 is relieved, thereby allowing increased Cdk1/cyclin B activity to promote mitotic entry. The function of Plk1-mediated phosphorylation of GTSE1 is to activate the nuclear import signal of GTSE1 and promote its nuclear accumulation. Furthermore, cells expressing the phosphorylation-deficient mutant GTSE1-S435A failed to enter mitosis after caffeine treatment, suggesting that phosphorylation of GTSE1 is required for G2 checkpoint recovery. This finding provides suggestive evidence that Plk1 facilitates p53 elimination during the checkpoint recovery process. A similar p53-mediated inactivation mechanism involving Wip1, the p53-induced phosphatase, has been described by Medema and co-workers¹²² who showed that Wip1 is responsible for retaining checkpoint recovery competence by counteracting p53 function. The role of Plks in checkpoint adaptation and checkpoint recovery is summarized in Table 1.

Table 1 Mechanisms underlying checkpoint adaptation and checkpoint recovery in different species

Checkpoint adaptation <i>S. cerevisiae</i> ; <i>X. levis</i> ; <i>H. sapiens</i> ?	Checkpoint recovery <i>S. Pombe</i> ; <i>H. sapiens</i>
Degradation of Claspin	Degradation of Claspin
Inactivation of Cdc25B and Wee1	Regulation of Sae2/CtIP function
Inactivation of Chk2 and 53BP1	Prevention of the phosphorylation of Mec1/ATR targets (ATRIP, Rad9, Rad53/Chk2)
Nuclear accumulation of GTSE1	

All these processes are regulated by polo-like kinases

Plks: p53-mediated apoptotic and antiapoptotic functions

Available evidence indicates that Plk orthologs (Plk1, Plx1, Plo1, Polo, Cdc5) function differently from Plk paralogs (Plk2–5 and Plx2, 3) in regulating mammalian cell proliferation and oncogenesis (reviewed in Strebhardt).³⁹ While the oncogenic role of Plk1 is well documented, other Plk family members appear to have tumor suppressor functions. Overexpression of Plk1 in NIH3T3 cells produces oncogenic foci and is tumorigenic in nude mice.¹²³ In relation to their implication in carcinogenesis, there is less known about the oncogenic or tumor suppressive potential of Plk2, Plk3, Plk4 and Plk5. Recent findings indicate that Plk2 is down-regulated by promoter hyper-methylation in primary lymphomas and that its overexpression in B-cells lymphomas leads to apoptosis, implying that Plk2 acts as a *bona fide* tumor suppressor.¹²⁴ Expression of Plk3 is also diminished in several human tumor types, which may contribute to the generation of genetic instability due to its role in DNA damage response.¹²⁵ The antineoplastic function of Plk3 is further substantiated by the observation that Plk3-deficient mice can spontaneously develop tumors in various organs, including the liver.⁴⁸ Similarly, it appears that Plk4 has a role of tumor suppressor in hepatocarcinogenesis, since mice heterozygous for Plk4 spontaneously develop liver and lung tumors.¹²⁶ A comprehensive analysis of Plk proteins in HCC indicates that the Plks are deregulated in human HCC. The data suggest that in this disease Plk1 can be oncogenic, while Plk2, Plk3 and Plk4 behave more like tumor suppressors.¹²⁷

Consistent with its role in cell survival, Plk1 has been shown to play an antiapoptotic role, while the other Plk members promote apoptosis under conditions of stress. The apoptotic process involves the interaction of Plk1 with the tumor suppressor protein p53. Depletion of Plk1 by siRNA depletion or its drug-mediated inactivation stabilizes p53 and results in cell death. Significantly, there appears to be reduced amounts of Plk1 in cancer cells with defective p53. Two independent RNAi studies showed that the loss of Plk1 function preferentially reduced the survival of cells with mutant p53.^{128,129} There are also reports that anaplastic thyroid carcinoma, which has high frequency of p53 mutation (70–90%) and carries a chromosomal instability signature, is profoundly sensitive to Plk1 inhibition by both siRNA knockdown¹³⁰ and compound-mediated inhibition.¹³¹ Interestingly, the Aurora kinase inhibitor VX-680

also preferentially kills cancer cells with compromised p53 function, leading to the proposition that a similar mechanism of weakened pseudo-G₁ tetraploidy checkpoint in p53-defective cells may be operative.¹³² Aurora A is the upstream kinase that phosphorylates and activates Plk1.⁷³ Thus, it remains to be determined whether or not the observed increase in cell death is mediated through Plk1 inhibition. Finally,¹³³ treatment with Nutlin-3a (a small-molecule inhibitor of MDM2 that activates the p53 pathway) or UV irradiation renders cells that lack wildtype p53 more sensitive to Plk1 inhibition than cells with wildtype p53.¹³⁴

The observation that the kinase domain of Plk1 physically binds to the DNA-binding domain of p53, thereby inhibiting both the transactivation activity and the apoptosis-inducing function of p53, provided the first evidence of a direct relationship between Plk1 and p53.¹³⁴ Other evidence supports the antiapoptotic effect of Plk1. The topoisomerase-I-binding protein topors, which has both ubiquitin and SUMO E3 ligase activity, is phosphorylated on Ser718 by Plk1.^{135–137} The phosphorylation of topors by Plk1 inhibits sumoylation of p53 and simultaneously enhances the ubiquitinylation and subsequent degradation of p53. Furthermore, long-term depletion or inactivation of Plk1 correlates with reduced levels of MDM2 leading to stabilization and activation of p53.¹³⁸ Furthermore, the transactivation domain of the transcription factor p73, another member of the p53 family, is phosphorylated by Plk1,^{139–141} and depletion of Plk1 in p53-deficient cells upregulates p73 and the pro-apoptotic protein p53AIP1.¹⁴⁰ Upregulation of Plk1 reduces p73-mediated promoter activity of the pro-apoptotic protein BAX,¹⁴¹ suggesting that regulation of p73 function by Plk1 provides a mechanism to inhibit p53-independent apoptosis. Plk1 is also overabundant in cells that express E6AP or HPV E7, with upregulation of Plk1 dependent upon the degradation of p53.¹⁴²

Transcription of *Plk1* is also dependent upon p53.¹⁴³ The p53 protein is both necessary and sufficient to mediate Plk1 inhibition in response to DNA damage through direct repression of *Plk1* expression. There are two distinct sites within the *Plk1* promoter to which p53 binds. The recruitment of p53 to one of these sites is further stimulated by DNA damage in a manner that is coincident with local changes in histone deacetylation favoring a closed chromatin structure.¹⁴³

The role of Plk2 expression in apoptosis has also been documented. Ectopic expression of Plk2 in Burkitt's lymphoma cells results in apoptotic cell death, which provides a potential mechanism to explain the strong selective pressure for loss of Plk2 function in B-cell neoplasia. This apoptotic effect likely involves p53. The interaction between Plk2 and p53 has been shown in several instances. An Affymetrix analysis of gene expression patterns in wildtype and p53-null animals before and after irradiation identified a p53-dependent induction of Plk2. Furthermore, Nutlin-3a upregulates Plk2 in wildtype p53 cells compared with p53-null cells.¹⁴⁴ In other experiments, depletion of Plk2 with siRNA oligonucleotides in different cell lines when combined with treatment with spindle poisons

paclitaxel or nocodazole, hypersensitizes the cells and is associated with a significant increase in apoptosis, similar to what is observed when p53 is mutant.¹⁴⁴ This finding indicates that a Plk2-controlled mitotic checkpoint may represent an alternative mechanism by which p53 can prevent mitotic catastrophe and preserve genome integrity. The data also suggest the possibility that disruption of *Plk2* may be of therapeutic value in sensitizing paclitaxel-resistant tumors. Similar to Plk1, *Plk2* is a transcriptional target of p53 after genotoxic stress.¹⁴⁴

Unlike Plk1, whose overexpression leads to cell transformation,¹²⁵ Plk3 overexpression leads to cell death by apoptosis.^{41,145} Furthermore, while G2 arrest in response to DNA damage correlates with the inhibition of Plk1,⁵¹ the activity of Plk3 rapidly increases in an ATM-dependent manner following DNA damage.⁴¹ Plk3 physically interacts with p53 and this interaction is enhanced upon DNA damage. An *in vitro* kinase assay followed by immunoblot analysis has shown that p53 serine 20, a Chk2 target site, is also a residue that is phosphorylated by Plk3 following DNA damage,¹⁴⁶ suggesting that Plk3 functionally links DNA damage to cell cycle arrest and apoptosis via the ATM/p53 pathway.^{41,47,145} Furthermore, *Plk3* gene expression is upregulated in primary fibroblasts two hours after treatment with ionizing radiation.^{147,148} The *Plk3* gene is a target of p53 as revealed by several microarray experiments designed to identify p53 target genes that are upregulated in response to various types of stress.^{48,149–151} RNAi-based depletion of Plk3, in cells that were arrested by serum deprivation and then released by addition of serum, resulted in the failure of cells to enter the S phase, suggesting that Plk3 is required in G1.⁴⁹

Similar to Plk2 and Plk3, it is likely that Plk4 functions within or is a target of DNA damage pathways. This proposition is supported by the observation that Plk4 interacts with and phosphorylates p53.^{126,152} Expression of Plk4 is repressed in a p53-dependent manner in response to DNA damaging agents. This repression may be mediated through the recruitment of a histone deacetylase that represses transcription.¹⁵³ While total loss of Plk4 is lethal in mice, mouse embryonic fibroblasts that are heterozygous at *Plk4* display a phenotype typified by multiple centrosomes, which lead to multipolar spindles, mitotic failure and delayed proliferation.¹²⁶

The newly identified member of the Plk family, Plk5 behaves more like Plk2–4 than Plk1. Ectopic expression of murine Plk5 induces the accumulation of HEK293 and NIH3T3 cells in G1 followed by cell death by apoptosis. The Plk5 protein is localized at nucleoli and is induced by various stress-promoting agents. In contrast to Plk2 and Plk3, the stress response of Plk5 seems to be independent of p53.¹⁴ Further analysis is needed to better understand how Plk5 is activated in response to DNA damage and the downstream targets it regulates in order to induce cell cycle arrest and apoptosis.

Summary

Beyond the classical mitotic functions of Plks and the emerging roles of the Plk paralogs in DNA damage response,

Plks play a role in checkpoint activation, checkpoint maintenance and checkpoint silencing. Combination of Plk1 up-regulation and Plk2-5 down-regulation may have a central role in unrestrained cell cycle progression and, consequently, in cell proliferation. Thus therapeutic approaches aimed at suppressing Plk1 and/or reactivating Plk2-5 genes might be highly beneficial for the treatment of human cancers. The role of Plk1 in checkpoint recovery is being exploited for the design of new therapeutic approaches in the treatment of cancer as its inhibition induces profound mitotic delay and apoptosis in several tumor cell lines. Combination therapies based on conventional spindle poisons and Plk1 inhibitors may exacerbate this mitotic delay leading to even more extensive cell death in mitosis. Similarly, a combination of chemotherapeutics such as cisplatin or doxorubicin and inhibitors of Plk2-5 may attenuate checkpoint response and affect the repair of damaged DNA leading to cell death by apoptosis.

ACKNOWLEDGEMENTS

I thank Dr Peter Stambrook and Dr Susan Robbins for reading the manuscript. I apologize for all those whose excellent work was not cited because of the limited space. This work was supported in part by NIH grants R03 ES015307, RO1 ES012695 and RO1 ES016625.

REFERENCES

- Glover DM, Hagan IM, Tavares AA. Polo-like kinases: a team that plays throughout mitosis. *Genes Dev* 1998;**12**:3777-87
- Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division. *Nat Rev Mol Cell Biol* 2004;**5**:429-40
- Lowery DM, Lim D, Yaffe MB. Structure and function of Polo-like kinases. *Oncogene* 2005;**24**:248-59
- Sunkel CE, Glover DM. Polo, a mitotic mutant of *Drosophila* displaying abnormal spindle poles. *J Cell Sci* 1988;**89**(Pt 1):25-38
- Llamazares S, Moreira A, Tavares A, Girdham C, Spruce BA, Gonzalez C, Karess RE, Glover DM, Sunkel CE. Polo encodes a protein kinase homolog required for mitosis in *Drosophila*. *Genes Dev* 1991;**5**:2153-65
- Duncan PI, Pollet N, Niehrs C, Nigg EA. Cloning and characterization of Plx2 and Plx3, two additional Polo-like kinases from *Xenopus laevis*. *Exp Cell Res* 2001;**270**:78-87
- Lee KS, Grenfell TZ, Yarm FR, Erikson RL. Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk. *Proc Natl Acad Sci USA* 1998;**95**:9301-6
- Logarinho E, Sunkel CE. The *Drosophila* POLO kinase localises to multiple compartments of the mitotic apparatus and is required for the phosphorylation of MPM2 reactive epitopes. *J Cell Sci* 1998;**111**:2897-909
- Shirayama M, Zachariae W, Ciosk R, Nasmyth K. The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J* 1998;**17**:1336-49
- Qian YW, Erikson E, Li C, Maller JL. Activated polo-like kinase Plx1 is required at multiple points during mitosis in *Xenopus laevis*. *Mol Cell Biol* 1998;**18**:4262-71
- Moutinho-Santos T, Sampaio P, Amorim I, Costa M, Sunkel CE. *In vivo* localisation of the mitotic POLO kinase shows a highly dynamic association with the mitotic apparatus during early embryogenesis in *Drosophila*. *Biol Cell* 1999;**91**:585-96
- Qian YW, Erikson E, Maller JL. Mitotic effects of a constitutively active mutant of the *Xenopus* polo-like kinase Plx1. *Mol Cell Biol* 1999;**19**:8625-32
- van de Weerd BC, Littler DR, Klompmaker R, Huseinovic A, Fish A, Perrakis A, Medema RH. Polo-box domains confer target specificity to the Polo-like kinase family. *Biochim Biophys Acta* 2008;**1783**:1015-22
- Andryskis Z, Bernstein WZ, Deng L, Myer DL, Li YQ, Tischfield JA, Stambrook PJ, Bahassi el M. The novel mouse Polo-like kinase 5 responds to DNA damage and localizes in the nucleolus. *Nucleic Acids Res* 2010;**38**:2931-43
- Lin CY, Madsen ML, Yarm FR, Jang YJ, Liu X, Erikson RL. Peripheral Golgi protein GRASP65 is a target of mitotic polo-like kinase (Plk) and Cdc2. *Proc Natl Acad Sci USA* 2000;**97**:12589-94
- Sutterlin C, Lin CY, Feng Y, Ferris DK, Erikson RL, Malhotra V. Polo-like kinase is required for the fragmentation of pericentriolar Golgi stacks during mitosis. *Proc Natl Acad Sci USA* 2001;**98**:9128-32
- Yarm FR. Plk phosphorylation regulates the microtubule-stabilizing protein TCTP. *Mol Cell Biol* 2002;**22**:6209-21
- Elia AE, Cantley LC, Yaffe MB. Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates. *Science* 2003;**299**:1228-31
- Elia AE, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, Mohammad D, Cantley LC, Smerdon SJ, Yaffe MB. The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell* 2003;**115**:83-95
- Neef R, Preisinger C, Sutcliffe J, Kopajtich R, Nigg EA, Mayer TU, Barr FA. Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis. *J Cell Biol* 2003;**162**:863-75
- Zhou T, Aumais JP, Liu X, Yu-Lee LY, Erikson RL. A role for Plk1 phosphorylation of NudC in cytokinesis. *Dev Cell* 2003;**5**:127-38
- Liu X, Zhou T, Kuriyama R, Erikson RL. Molecular interactions of Polo-like-kinase 1 with the mitotic kinesin-like protein CHO1/MKLP-1. *J Cell Sci* 2004;**117**:3233-46
- Lane HA, Nigg EA. Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. *J Cell Biol* 1996;**135**:1701-13
- do Carmo Avides M, Tavares A, Glover DM. Polo kinase and Asp are needed to promote the mitotic organizing activity of centrosomes. *Nat Cell Biol* 2001;**3**:421-4
- Golsteyn RM, Schultz SJ, Bartek J, Ziemiecki A, Ried T, Nigg EA. Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases *Drosophila* polo and *Saccharomyces cerevisiae* Cdc5. *J Cell Sci* 1994;**107**(Pt 6):1509-17
- Lee KS, Yuan YL, Kuriyama R, Erikson RL. Plk is an M-phase-specific protein kinase and interacts with a kinesin-like protein, CHO1/MKLP-1. *Mol Cell Biol* 1995;**15**:7143-51
- Song S, Grenfell TZ, Garfield S, Erikson RL, Lee KS. Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol Cell Biol* 2000;**20**:286-98
- Wolf G, Elez R, Doerner A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger HM, Rübsamen-Waigmann H, Strebhardt K. Prognostic significance of polo-like kinase (Plk) expression in non-small cell lung cancer. *Oncogene* 1997;**14**:543-9
- Weichert W, Kristiansen G, Winzer KJ, Schmidt M, Gekeler V, Noske A, Müller BM, Niesporek S, Dietel M, Denkert C. Polo-like kinases isoforms in breast cancer: expression patterns and prognostic implication. *Virchows Arch* 2005;**446**:442-50
- Takahashi T, Sano B, Nagata T, Kato H, Sugiyama Y, Kunieda K, Kimura M, Okano Y, Saji S. Polo-like kinase 1 (PLK1) is overexpressed in primary colorectal cancers. *Cancer Sci* 2003;**94**:148-52
- Weichert W, Kristiansen G, Schmidt M, Gekeler V, Noske A, Niesporek S, Dietel M, Denkert C. Polo-like kinase 1 expression is a prognostic factor in human colon cancer. *World J Gastroenterol* 2005;**11**:5644-50
- Kneisel L, Strebhardt K, Bernd A, Wolter M, Binder A, Kaufmann R. Expression of polo-like kinase (PLK1) in thin melanomas: a novel marker of metastatic disease. *J Cutan Pathol* 2002;**29**:354-8
- Takai N, Hamanaka R, Yoshimatsu J, Miyakawa I. Polo-like kinases (Plks) and cancer. *Oncogene* 2005;**24**:287-91
- Fabbro D, Garcia-Echeverria C. Targeting protein kinases in cancer therapy. *Curr Opin Drug Discov Dev* 2002;**5**:701-12
- Noble ME, Endicott JA, Johnson LN. Protein kinase inhibitors: insights into drug design from structure. *Science* 2004;**303**:1800-5
- Stout TJ, Foster PG, Matthews DJ. High-throughput structural biology in drug discovery: protein kinases. *Curr Pharm Des* 2004;**10**:1069-82

- 37 Lenart P, Petronczki M, Steegmaier M, Di Fiore B, Lipp JJ, Hoffmann M, Rettig WJ, Kraut N, Peters JM. The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. *Curr Biol* 2007;**17**:304–15
- 38 Steegmaier M, Hoffmann M, Baum A, Lenart P, Petronczki M, Krssak M, Gurtler U, Garin-Chesa P, Lieb S, Quant J, Grauert M, Adolf GR, Kraut N, Peters JM, Rettig WJ. BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth *in vivo*. *Curr Biol* 2007;**17**:316–22
- 39 Strebhardt K. Multifaceted polo-like kinases: drug targets and antitargets for cancer therapy. *Nat Rev Drug Discov* 2010;**9**:643–60
- 40 Donohue PJ, Alberts GF, Guo Y, Winkles JA. Identification by targeted differential display of an immediate early gene encoding a putative serine/threonine kinase. *J Biol Chem* 1995;**270**:10351–7
- 41 Xie S, Wu H, Wang Q, Cogswell JP, Husain I, Conn C, Stambrook P, Jhanwar-Uniyal M, Dai W. Plk3 functionally links DNA damage to cell cycle arrest and apoptosis at least in part via the p53 pathway. *J Biol Chem* 2001;**276**:43305–12
- 42 Bahassi el M, Conn CW, Myer DL, Hennigan RF, McGowan CH, Sanchez Y, Stambrook PJ. Mammalian Polo-like kinase 3 (Plk3) is a multifunctional protein involved in stress response pathways. *Oncogene* 2002;**21**:6633–40
- 43 Xie S, Wu H, Wang Q, Kunicki J, Thomas RO, Hollingsworth RE, Cogswell J, Dai W. Genotoxic stress-induced activation of Plk3 is partly mediated by Chk2. *Cell Cycle* 2002;**1**:424–9
- 44 Matthew EM, Yen TJ, Dicker DT, Dorsey JF, Yang W, Navaraj A, El-Deiry WS. Replication stress, defective S-phase checkpoint and increased death in Plk2-deficient human cancer cells. *Cell Cycle* 2007;**6**:2571–8
- 45 Bettencourt-Dias M, Rodrigues-Martins A, Carpenter L, Riparbelli M, Lehmann L, Gatt MK, Carmo N, Balloux F, Callaini G, Glover DM. SAK/PLK4 is required for centriole duplication and flagella development. *Curr Biol* 2005;**15**:2199–207
- 46 Warnke S, Kemmler S, Hames RS, Tsai HL, Hoffmann-Rohrer U, Fry AM, Hoffmann I. Polo-like kinase-2 is required for centriole duplication in mammalian cells. *Curr Biol* 2004;**14**:1200–7
- 47 Bahassi el M, Myer DL, McKenney RJ, Hennigan RF, Stambrook PJ. Priming phosphorylation of Chk2 by polo-like kinase 3 (Plk3) mediates its full activation by ATM and a downstream checkpoint in response to DNA damage. *Mutat Res* 2006;**596**:166–76
- 48 Yang Y, Bai J, Shen R, Brown SA, Komissarova E, Huang Y, Jiang N, Alberts GF, Costa M, Lu L, Winkles JA, Dai W. Polo-like kinase 3 functions as a tumor suppressor and is a negative regulator of hypoxia-inducible factor-1 alpha under hypoxic conditions. *Cancer Res* 2008;**68**:4077–85
- 49 Zimmerman WC, Erikson RL. Polo-like kinase 3 is required for entry into S phase. *Proc Natl Acad Sci USA* 2007;**104**:1847–52
- 50 Chase D, Feng Y, Hanshaw B, Winkles JA, Longo DL, Ferris DK. Expression and phosphorylation of fibroblast-growth-factor-inducible kinase (Fnk) during cell-cycle progression. *Biochem J* 1998;**333**(Pt 3):655–60
- 51 Smits VA, Klompmaier R, Arnaud L, Rijkse G, Nigg EA, Medema RH. Polo-like kinase-1 is a target of the DNA damage checkpoint. *Nat Cell Biol* 2000;**2**:672–6
- 52 Bahassi el M, Penner CG, Robbins SB, Tichy E, Feliciano E, Yin M, Liang L, Deng L, Tischfield JA, Stambrook PJ. The breast cancer susceptibility allele CHEK2*1100delC promotes genomic instability in a knock-in mouse model. *Mutat Res* 2007;**616**:201–9
- 53 Bahassi el M, Hennigan RF, Myer DL, Stambrook PJ. Cdc25C phosphorylation on serine 191 by Plk3 promotes its nuclear translocation. *Oncogene* 2004;**23**:2658–63
- 54 Hudson JW, Kozarova A, Cheung P, Macmillan JC, Swallow CJ, Cross JC, Dennis JW. Late mitotic failure in mice lacking Sak, a polo-like kinase. *Curr Biol* 2001;**11**:441–6
- 55 Leung GC, Hudson JW, Kozarova A, Davidson A, Dennis JW, Sicheri F. The Sak polo-box comprises a structural domain sufficient for mitotic subcellular localization. *Nat Struct Biol* 2002;**9**:719–24
- 56 Rosario CO, Ko MA, Haffani YZ, Gladly RA, Paderova J, Pollett A, Squire JA, Dennis JW, Swallow CJ. Plk4 is required for cytokinesis and maintenance of chromosomal stability. *Proc Natl Acad Sci USA* 2010;**107**:6888–93
- 57 Fode C, Binkert C, Dennis JW. Constitutive expression of murine Sak suppresses cell growth and induces multinucleation. *Mol Cell Biol* 1996;**16**:4665–72
- 58 Macmillan JC, Hudson JW, Bull S, Dennis JW, Swallow CJ. Comparative expression of the mitotic regulators SAK and PLK in colorectal cancer. *Ann Surg Oncol* 2001;**8**:729–40
- 59 Uchiumi T, Longo DL, Ferris DK. Cell cycle regulation of the human polo-like kinase (PLK) promoter. *J Biol Chem* 1997;**272**:9166–74
- 60 Alvarez B, Martinez AC, Burgering BM, Carrera AC. Forkhead transcription factors contribute to execution of the mitotic programme in mammals. *Nature* 2001;**413**:744–47
- 61 Laoukili J, Kooistra MR, Brás A, Kauw J, Kerckhoven RM, Morrison A, Clevers H, Medema RH. FoxM1 is required for execution of the mitotic programme and chromosome stability. *Nat Cell Biol* 2005;**7**:126–36
- 62 Zhu G, Spellman PT, Volpe T, Brown PO, Botstein D, Davis TN, Futcher B. Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth. *Nature* 2000;**406**:90–94
- 63 Buck V, Ng SS, Ruiz-Garcia AB, Papadopoulos K, Bhatti S, Samuel JM, Anderson M, Millar JB, McInerney CJ. Fkh2p and Sep1p regulate mitotic gene transcription in fission yeast. *J Cell Sci* 2004;**117**:5623–32
- 64 Darieva Z, Bulmer R, Pic-Taylor A, Doris KS, Geymonat M, Sedgwick SG, Morgan BA, Sharrocks AD. Polo kinase controls cell-cycle-dependent transcription by targeting a coactivator protein. *Nature* 2006;**444**:494–98
- 65 Papadopoulos K, Ng SS, Ohkura H, Geymonat M, Sedgwick SG, McInerney CJ. Regulation of gene expression during M-G1-phase in fission yeast through Plo1p and forkhead transcription factors. *J Cell Sci* 2008;**121**:38–47
- 66 Fu Z, Malureanu L, Huang J, Wang W, Li H, van Deursen JM, Tindall DJ, Chen J. Plk1-dependent phosphorylation of FoxM1 regulates a transcriptional programme required for mitotic progression. *Nat Cell Biol* 2008;**10**:1076–82
- 67 Ren B, Cam H, Takahashi Y, Volkert T, Terragni J, Young RA, Dynlacht BD. E2F integrates cell cycle progression with DNA repair, replication, and G2/M checkpoints. *Genes Dev* 2002;**16**:245–56
- 68 Gunawardena RW, Siddiqui H, Solomon DA, Mayhew CN, Held J, Angus SP, Knudsen ES. Hierarchical requirement of SWI/SNF in retinoblastoma tumor suppressor-mediated repression of Plk1. *J Biol Chem* 2004;**279**:29278–85
- 69 Wen H, Andrejka L, Ashton J, Karess R, Lipsick JS. Epigenetic regulation of gene expression by *Drosophila* Myb and E2F2–RBF via the Myb–MuvB/dREAM complex. *Genes Dev* 2008;**22**:601–14
- 70 de Cárcer G, Escobar B, Higuero A, García L, Anson A, Pérez G, Mollejo M, Manning G, Meléndez B, Abad Rodríguez J, Malumbres M. Plk5, a Polo-box domain-only protein with specific roles in neuron differentiation and glioblastoma suppression. *Mol Cell Biol* 2011; Jan 18. [Epub ahead of print]
- 71 Jang YJ, Ma S, Terada Y, Erikson RL. Phosphorylation of threonine 210 and the role of serine 137 in the regulation of mammalian polo-like kinase. *J Biol Chem* 2002;**277**:44115–20
- 72 Jang YJ, Lin CY, Ma S, Erikson RL. Functional studies on the role of the C-terminal domain of mammalian polo-like kinase. *Proc Natl Acad Sci USA* 2002;**99**:1984–89
- 73 Macurek L, Lindqvist A, Lim D, Lampson MA, Klompmaier R, Freire R, Clouin C, Taylor SS, Yaffe MB, Medema RH. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. *Nature* 2008;**455**:119–23
- 74 Seki A, Coppinger JA, Jang CY, Yates JR, Fang G. Bora and the kinase Aurora A cooperatively activate the kinase Plk1 and control mitotic entry. *Science* 2008;**320**:1655–58
- 75 Yamashiro S, Yamakita Y, Totsukawa G, Goto H, Kaibuchi K, Ito M, Hartshorne DJ, Matsumura F. Myosin phosphatase-targeting subunit 1 regulates mitosis by antagonizing polo-like kinase 1. *Dev Cell* 2008;**14**:787–97
- 76 Ferris DK, Maloid SC, Li CC. Ubiquitination and proteasome mediated degradation of polo-like kinase. *Biochem Biophys Res Commun* 1998;**252**:340–4
- 77 Engelbert D, Schnercher D, Baumgarten A, Wasch R. The ubiquitin ligase APC^{Cdh1} is required to maintain genome integrity in primary human cells. *Oncogene* 2008;**27**:907–17

- 78 Garci-Higuera I, Manchado E, Dubus P, Cañamero M, Méndez J, Moreno S, Malumbres M. Genomic stability and tumour suppression by the APC/C cofactor Cdh1. *Nat Cell Biol* 2008;**10**:802–11
- 79 Charles JF, Jaspersen SL, Tinker-Kulberg RL, Hwang L, Szidon A, Morgan DO. The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr Biol* 1998;**8**:497–507
- 80 Visintin C, Tomson BN, Rahal R, Paulson J, Cohen M, Taunton J, Amon A, Visintin R. APC/C–Cdh1-mediated degradation of the Polo kinase Cdc5 promotes the return of Cdc14 into the nucleolus. *Genes Dev* 2008;**22**:79–90
- 81 Yamashita Y, Kajigaya S, Yoshida K, Ueno S, Ota J, Ohmine K, Ueda M, Miyazato A, Ohya K, Kitamura T, Ozawa K, Mano H. Serine–threonine kinase acts as an effector of Tec tyrosine kinase. *J Biol Chem* 2001;**276**:39012–20
- 82 Lens SM, Voest EE, Medema RH. Shared and separate functions of polo-like kinases and aurora kinases in cancer. *Nat Rev Cancer* 2010;**10**:825–41
- 83 Seong YS, Kamijo K, Lee JS, Fernandez E, Kuriyama R, Miki T, Lee KS. A spindle checkpoint arrest and a cytokinesis failure by the dominant-negative polo-box domain of Plk1 in U-2 OS cells. *J Biol Chem* 2002;**277**:32282–93
- 84 Strebhardt K, Ullrich A. Targeting polo-like kinase 1 for cancer therapy. *Nat Rev Cancer* 2006;**6**:321–30
- 85 van de Weert BC, Medema RH. Polo-like kinases: a team in control of the division. *Cell Cycle* 2006;**5**:853–64
- 86 Petronczki M, Lenart P, Peters JM. Polo on the rise – from mitotic entry to cytokinesis with Plk1. *Dev Cell* 2008;**14**:646–59
- 87 Takaki T, Trenz K, Costanzo V, Petronczki M. Polo-like kinase 1 reaches beyond mitosis – cytokinesis, DNA damage response, and development. *Curr Opin Cell Biol* 2008;**20**:650–60
- 88 Archambault V, Glover DM. Polo-like kinases: conservation and divergence in their functions and regulation. *Nat Rev Mol Cell Biol* 2009;**10**:265–75
- 89 Barr FA, Gruneberg U. Cytokinesis: placing and making the final cut. *Cell* 2007;**131**:847–60
- 90 Bartek J, Lukas J. DNA damage checkpoints: from initiation to recovery or adaptation. *Curr Opin Cell Biol* 2007;**19**:238–45
- 91 Harper JW, Elledge SJ. The DNA damage response: ten years after. *Mol Cell* 2007;**28**:739–45
- 92 Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. *Nature* 2004;**432**:316–23
- 93 Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000;**408**:433–9
- 94 Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. *Genes Dev* 2001;**15**:2177–96
- 95 Bakkenist CJ, Kastan MB. Initiating cellular stress responses. *Cell* 2004;**118**:9–17
- 96 Lambert S, Carr AM. Checkpoint responses to replication fork barriers. *Biochimie* 2005;**87**:591–602
- 97 Sandell LL, Zakian VA. Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* 1993;**75**:729–39
- 98 Galgoczy DJ, Toczyski DP. Checkpoint adaptation precedes spontaneous and damage-induced genomic instability in yeast. *Mol Cell Biol* 2001;**21**:1710–8
- 99 Lee SE, Pelliccioli A, Malkova A, Foiani M, Haber JE. The *Saccharomyces* recombination protein Tid1p is required for adaptation from G2/M arrest induced by a double-strand break. *Curr Biol* 2001;**11**:1053–7
- 100 Toczyski DP, Galgoczy DJ, Hartwell LH. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* 1997;**90**:1097–106
- 101 Parkinson JS. Signal transduction schemes of bacteria. *Cell* 1993;**73**:857–71
- 102 Yarfitz S, Hurley JB. Transduction mechanisms of vertebrate and invertebrate photoreceptors. *J Biol Chem* 1994;**269**:14329–32
- 103 Jin F, Wang Y. Budding yeast DNA damage adaptation mutants exhibit defects in mitotic exit. *Cell Cycle* 2006;**5**:2914–9
- 104 Donnianni RA, Ferrari M, Lazzaro F, Clerici M, Tamilselvan Nachimuthu B, Plevani P, Muzi-Falconi M, Pelliccioli A. Elevated levels of the polo kinase Cdc5 override the Mec1/ATR checkpoint in budding yeast by acting at different steps of the signaling pathway. *PLoS Genet* 2010;**6**:e1000763
- 105 Yoo HY, Kumagai A, Shevchenko A, Dunphy WG. Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase. *Cell* 2004;**117**:575–88
- 106 Kumagai A, Dunphy WG. Repeated phosphopeptide motifs in Claspin mediate the regulated binding of Chk1. *Nat Cell Biol* 2003;**5**:161–5
- 107 Syljuasen RG, Jensen S, Bartek J, Lukas J. Adaptation to the ionizing radiation-induced G2 checkpoint occurs in human cells and depends on checkpoint kinase 1 and Polo-like kinase 1 kinases. *Cancer Res* 2006;**66**:10253–7
- 108 Lanni JS, Jacks T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol Cell Biol* 1998;**18**:1055–64
- 109 Taylor WR, Stark GR. Regulation of the G2/M transition by p53. *Oncogene* 2001;**20**:1803–15
- 110 Mailand N, Bekker-Jensen S, Bartek J, Lukas J. Destruction of Claspin by SCFbetaTrCP restrains Chk1 activation and facilitates recovery from genotoxic stress. *Mol Cell* 2006;**23**:307–18
- 111 Mamely I, van Vugt MA, Smits VA, Semple JL, Lemmens B, Perrakis A, Medema RH, Freire R. Polo-like kinase-1 controls proteasome-dependent degradation of Claspin during checkpoint recovery. *Curr Biol* 2006;**16**:1950–5
- 112 Peschiaroli A, Dorrello NV, Guardavaccaro D, Venere M, Halazonetis T, Sherman NE, Pagano M. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. *Mol Cell* 2006;**23**:319–29
- 113 van Vugt MA, Bras A, Medema RH. Polo-like kinase-1 controls recovery from a G2 DNA damage-induced arrest in mammalian cells. *Mol Cell* 2004;**15**:799–811
- 114 van Vugt MA, Gardino AK, Linding R, Ostheimer GJ, Reinhardt HC, Ong SE, Tan CS, Miao H, Keezer SM, Li J, Pawson T, Lewis TA, Carr SA, Smerdon SJ, Brummelkamp TR, Yaffe MB. A mitotic phosphorylation feedback network connects Cdk1, Plk1, 53BP1, and Chk2 to inactivate the G(2)/M DNA damage checkpoint. *PLoS Biol* 2010;**8**:e1000287
- 115 Kelm O, Wind M, Lehmann WD, Nigg EA. Cell cycle-regulated phosphorylation of the *Xenopus* polo-like kinase Plx1. *J Biol Chem* 2002;**277**:25247–56
- 116 Bunz F, Dutriaux A, Lengauer C, Waldman T, Zhou S, Brown JP, Sedivy JM, Kinzler KW, Vogelstein B. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* 1998;**282**:1497–501
- 117 Liu XS, Li H, Song B, Liu X. Polo-like kinase 1 phosphorylation of G2 and S-phase-expressed 1 protein is essential for p53 inactivation during G2 checkpoint recovery. *EMBO Rep* 2010;**11**:626–32
- 118 Utrera R, Collavin L, Lazarevic D, Delia D, Schneider C. A novel p53-inducible gene coding for a microtubule-localized protein with G2-phase-specific expression. *EMBO J* 1998;**17**:5015–25
- 119 Collavin L, Monte M, Verardo R, Pfeleger C, Schneider C. Cell-cycle regulation of the p53-inducible gene B99. *FEBS Lett* 2000;**481**:57–62
- 120 Monte M, Benetti R, Buscemi G, Sandy P, Del Sal G, Schneider C. The cell cycle-regulated protein human GTSE-1 controls DNA damage-induced apoptosis by affecting p53 function. *J Biol Chem* 2003;**278**:30356–64
- 121 Monte M, Benetti R, Collavin L, Marchionni L, Del Sal G, Schneider C. hGTSE-1 expression stimulates cytoplasmic localization of p53. *J Biol Chem* 2004;**279**:11744–52
- 122 Lindqvist A, de Bruijn M, Macurek L, Bras A, Mensinga A, Bruinsma W, Voets O, Kranenburg O, Medema RH. Wip1 confers G2 checkpoint recovery competence by counteracting p53-dependent transcriptional repression. *EMBO J* 2009;**28**:3196–206
- 123 Smith MR, Wilson ML, Hamanaka R, Chase D, Kung H, Longo DL, Ferris DK. Malignant transformation of mammalian cells initiated by constitutive expression of the polo-like kinase. *Biochem Biophys Res Commun* 1997;**234**:397–405
- 124 Syed N, Smith P, Sullivan A, Spender LC, Dyer M, Karran L, O’Nions J, Allday M, Hoffmann I, Crawford D, Griffin B, Farrell PJ, Crook T. Transcriptional silencing of Polo-like kinase 2 (SNK/PLK2) is a frequent event in B-cell malignancies. *Blood* 2006;**107**:250–6
- 125 Winkles JA, Alberts GF. Differential regulation of polo-like kinase 1, 2, 3, and 4 gene expression in mammalian cells and tissues. *Oncogene* 2005;**24**:260–6

- 126 Ko MA, Rosario CO, Hudson JW, Kulkarni S, Pollett A, Dennis JW, Swallow CJ. Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. *Nat Genet* 2005;**37**:883–8
- 127 Pellegrino R, Calvisi DF, Ladu S, Ehemann V, Staniscia T, Evert M, Dombrowski F, Schirmacher P, Longerich T. Oncogenic and tumor suppressive roles of polo-like kinases in human hepatocellular carcinoma. *Hepatology* 2010;**51**:857–68
- 128 Guan R, Tapang P, Levenson JD, Albert D, Giranda VL, Luo Y. Small interfering RNA-mediated Polo-like kinase 1 depletion preferentially reduces the survival of p53-defective, oncogenic transformed cells and inhibits tumor growth in animals. *Cancer Res* 2005;**65**:2698–704
- 129 Liu X, Lei M, Erikson RL. Normal cells, but not cancer cells, survive severe Plk1 depletion. *Mol Cell Biol* 2006;**26**:2093–108
- 130 Salvatore G, Nappi TC, Salerno P, Jiang Y, Garbi C, Ugolini C, Miccoli P, Basolo F, Castellone MD, Cirafo AM, Melillo RM, Fusco A, Bittner ML, Santoro M. A cell proliferation and chromosomal instability signature in anaplastic thyroid carcinoma. *Cancer Res* 2007;**67**:10148–58
- 131 Nappi TC, Salerno P, Zitzelsberger H, Carlomagno F, Salvatore G, Santoro M. Identification of Polo-like kinase 1 as a potential therapeutic target in anaplastic thyroid carcinoma. *Cancer Res* 2009;**69**:1916–23
- 132 Gizatullin F, Yao Y, Kung V, Harding MW, Loda M, Shapiro GI. The Aurora kinase inhibitor VX-680 induces endoreduplication and apoptosis preferentially in cells with compromised p53-dependent postmitotic checkpoint function. *Cancer Res* 2006;**66**:7668–77
- 133 Sur S, Pagliarini R, Bunz F, Rago C, Diaz LA Jr, Kinzler KW, Vogelstein B, Papadopoulos N. A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. *Proc Natl Acad Sci USA* 2009;**106**:3964–9
- 134 Ando K, Ozaki T, Yamamoto H, Furuya K, Hosoda M, Hayashi S, Fukuzawa M, Nakagawara A. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. *J Biol Chem* 2004;**279**:25549–61
- 135 Yang X, Li H, Zhou Z, Wang WH, Deng A, Andrisani O, Liu X. Plk1-mediated phosphorylation of Topors regulates p53 stability. *J Biol Chem* 2009;**284**:18588–92
- 136 Weger S, Hammer E, Heilbronn R. Topors acts as a SUMO-1 E3 ligase for p53 *in vitro* and *in vivo*. *FEBS Lett* 2005;**579**:5007–12
- 137 Rajendra R, Malegaonkar D, Pungaliya P, Marshall H, Rasheed Z, Brownell J, Liu LF, Lutzker S, Saleem A, Rubin EH. Topors functions as an E3 ubiquitin ligase with specific E2 enzymes and ubiquitinates p53. *J Biol Chem* 2004;**279**:36440–4
- 138 Momand J, Wu HH, Dasgupta G. MDM2 – master regulator of the p53 tumor suppressor protein. *Gene* 2000;**242**:15–29
- 139 Kaghad M, Bonnet H, Yang A, Creancier L, Biscan JC, Valent A, Minty A, Chalon P, Lelias JM, Dumont X, Ferrara P, McKeon F, Caput D. Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell* 1997;**90**:809–19
- 140 Koida N, Ozaki T, Yamamoto H, Ono S, Koda T, Ando K, Okoshi R, Kamijo T, Omura K, Nakagawara A. Inhibitory role of Plk1 in the regulation of p73-dependent apoptosis through physical interaction and phosphorylation. *J Biol Chem* 2008;**283**:8555–63
- 141 Soond SM, Barry SP, Melino G, Knight RA, Latchman DS, Stephanou A. p73-mediated transcriptional activity is negatively regulated by polo-like kinase 1. *Cell Cycle* 2008;**7**:1214–23
- 142 Patel D, Incassati A, Wang N, McCance DJ. Human papillomavirus type 16 E6 and E7 cause polyploidy in human keratinocytes and up-regulation of G2-M-phase proteins. *Cancer Res* 2004;**64**:1299–306
- 143 McKenzie L, King S, Marcar L, Nicol S, Dias SS, Schumm K, Robertson P, Bourdon JC, Perkins N, Fuller-Pace F, Meek DW. p53-dependent repression of polo-like kinase-1 (PLK1). *Cell Cycle* 2010;**9**:4200–12
- 144 Burns TF, Fei P, Scata KA, Dicker DT, El-Deiry WS. Silencing of the novel p53 target gene Snk/Plk2 leads to mitotic catastrophe in paclitaxel (taxol)-exposed cells. *Mol Cell Biol* 2003;**23**:5556–71
- 145 Conn CW, Hennigan RF, Dai W, Sanchez Y, Stambrook PJ. Incomplete cytokinesis and induction of apoptosis by overexpression of the mammalian polo-like kinase, Plk3. *Cancer Res* 2000;**60**:6826–31
- 146 Chehab NH, Malikzay A, Appel M, Halazonetis TD. Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. *Genes Dev* 2000;**14**:278–88
- 147 Zhou T, Chou JW, Simpson DA, Zhou Y, Mullen TE, Medeiros M, Bushel PR, Paules RS, Yang X, Hurban P, Lobenhofer EK, Kaufmann WK. Profiles of global gene expression in ionizing-radiation-damaged human diploid fibroblasts reveal synchronization behind the G1 checkpoint in a G0-like state of quiescence. *Environ Health Perspect* 2006;**114**:553–9
- 148 Kis E, Szatmari T, Keszei M, Farkas R, Esik O, Lumniczky K, Falus A, Safrany G. Microarray analysis of radiation response genes in primary human fibroblasts. *Int J Radiat Oncol Biol Phys* 2006;**66**:1506–14
- 149 Staib F, Robles AI, Varticovski L, Wang XW, Zeeberg BR, Sirotnin M, Zhurkin VB, Hofseth LJ, Hussain SP, Weinstein JN, Galle PR, Harris CC. The p53 tumor suppressor network is a key responder to microenvironmental components of chronic inflammatory stress. *Cancer Res* 2005;**65**:10255–64
- 150 Han ES, Muller FL, Perez VI, Qi W, Liang H, Xi L, Fu C, Doyle E, Hickey M, Cornell J, Epstein CJ, Roberts LJ, Van Remmen H, Richardson A. The *in vivo* gene expression signature of oxidative stress. *Physiol Genomics* 2008;**34**:112–26
- 151 Wang L, Gao J, Dai W, Lu L. Activation of Polo-like kinase 3 by hypoxic stresses. *J Biol Chem* 2008;**283**:25928–35
- 152 Swallow CJ, Ko MA, Siddiqui NU, Hudson JW, Dennis JW. Sak/Plk4 and mitotic fidelity. *Oncogene* 2005;**24**:306–12
- 153 Li J, Tan M, Li L, Pamarthy D, Lawrence TS, Sun Y. SAK, a new polo-like kinase, is transcriptionally repressed by p53 and induces apoptosis upon RNAi silencing. *Neoplasia* 2005;**7**:312–23