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Behind the wheel *and* under the hood: functions of cyclin-dependent kinases in response to DNA damage

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Abstract

Cell division and the response to genotoxic stress are intimately connected in eukaryotes, for example, by checkpoint pathways that signal the presence of DNA damage or its ongoing repair to the cell cycle machinery, leading to reversible arrest or apoptosis. Recent studies reveal another connection: the cyclin-dependent kinases (CDKs) that govern both DNA synthesis (S) phase and mitosis directly coordinate DNA repair processes with progression through the cell cycle. In both mammalian cells and yeast, the two major modes of double strand break (DSB) repair—homologous recombination (HR) and non-homologous end joining (NHEJ)—are reciprocally regulated during the cell cycle. In yeast, the cell cycle kinase Cdk1 directly promotes DSB repair by HR during the G2 phase. In mammalian cells, loss of Cdk2, the CDK active throughout S and G2 phases, results in defective DNA damage repair and checkpoint signaling. Here we provide an overview of data that implicate CDKs in the regulation of DNA damage responses in yeast and metazoans. In yeast, CDK activity is required at multiple points in the HR pathway; the precise roles of CDKs in mammalian HR have yet to be determined. Finally, we consider how the two different, and in some cases opposing, roles of CDKs—as targets of negative regulation by checkpoint signaling and as positive effectors of repair pathway selection and function—could be balanced to produce a coordinated and effective response to DNA damage.

Introduction

CDKs are protein Ser/Thr kinases that play a central role in cell division in eukaryotes. The tight regulation of CDK activity during the cell cycle helps to ensure proper alternation of S phase and mitosis, and is enforced by several mechanisms. CDKs are allosterically activated by binding to cyclins, which are strictly controlled by timed expression, degradation and localization. In addition, CDKs must be phosphorylated within the activation loop of the kinase domain by a CDK-activating kinase (CAK) to attain full activity. Conversely, CDK activity is inhibited by phosphorylation near the N-terminus of the protein or by binding of CDK inhibitor (CKI) proteins (reviewed in [1]).

In both the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, a single CDK catalytic subunit triggers both G1/S and G2/M transitions. In metazoans, different CDKs are active at distinct times in the cell cycle. Normal progression through G1 phase of the mammalian cell cycle is dependent on the activities of Cdk4/cyclin D, Cdk6/cyclin

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D and Cdk2/cyclin E complexes. Cdk2 also binds to cyclin A and is the major kinase active throughout S phase, thought to regulate both the onset of DNA replication and its completion. Cdk1 binds both cyclins A and B, and its activation late in G2 phase triggers mitosis (reviewed in [1]).

The expansion of the CDK family in metazoans has fostered the specialization of its members to perform different tasks. Nonetheless, Cdk1 has retained the ability to perform most or all of the basic functions needed for mouse cell proliferation in culture when all other CDKs normally active in interphase are removed by gene disruptions [2]. Compensation is incomplete *in vivo*, however, because mice lacking Cdk2, -4 and -6 are inviable [2], and those lacking even a single interphase CDK, Cdk2, are infertile due to a defect in meiosis [3,4].

Cells respond to DNA damage in many ways, which include activating specific repair pathways, inducing transcription and, in metazoan cells, undergoing apoptosis (reviewed in [5]). A common response in all eukaryotes is to arrest the cell cycle by activating a checkpoint. In most organisms, checkpoint signaling targets CDK activity, thereby holding the cell in interphase to allow for repair of the damaged DNA (reviewed in [6-8]). Not so in budding yeast, despite conservation of the DNA damage-sensing and checkpoint signaling pathways themselves. *S. cerevisiae* cells respond to DSBs not by inhibiting CDK activity and arresting in interphase, but instead by arresting at metaphase through checkpoint kinase-dependent stabilization of the anaphase inhibitor Pds1 (securin) and inhibition of the kinase Cdc5 (Polo), which promotes mitotic exit (reviewed in [6]). Recent studies in multiple organisms have revealed that, besides their established but essentially passive role as targets of checkpoint signaling, CDKs act positively, to regulate the choice of repair pathway activated in response to a DSB. Here we review these findings, and attempt to integrate the emerging roles of CDKs as regulators of DNA repair into existing models of their function in cell cycle control.

The two major modes of DSB repair are reciprocally regulated during the cell cycle

DSBs are the most dangerous DNA lesions if left unrepaired or repaired improperly, causing potentially lethal or oncogenic chromosomal aberrations such as translocations, amplifications or deletions [9,10]. DSBs can be formed by reactive oxygen species, by ionizing radiation (IR) or as unresolved intermediates of physiologic topoisomerase and nuclease reactions. Perhaps the most common cause of DSBs is stalling and collapse of DNA replication forks (reviewed in [11]).

The two major pathways of DSB repair are homologous recombination (HR) and nonhomologous end joining (NHEJ) (reviewed in [8,11]). HR is initiated by the 5'-to-3' resection of DNA from the DSB to yield single-stranded (ss) DNA, which becomes coated by the ssDNA binding protein RPA (Fig. 1). Rad52 displaces RPA and loads Rad51 recombinase to form a nucleoprotein filament capable of strand invasion at a homologous sequence, leading to repair by gene conversion (reviewed in [12]). HR is generally error-free. NHEJ, on the other hand, repairs DSBs by capturing the broken DNA ends and directly ligating them together. This pathway can do without homology and frequently results in deletions or insertions at the break site (reviewed in [13,14]).

In both yeast and vertebrate cells, the two modes of DSB repair are regulated reciprocally, with NHEJ predominating in the G0/G1, and HR in the S/G2, intervals of the cell cycle (Fig. 2) (reviewed in [15]). In *S. pombe*, NHEJ frequency is 10-fold higher in early G1 than in other cell cycle stages; HR frequency, conversely, is low in G1 and up-regulated in G2 cells [16]. In budding yeast cells, repair of a single DSB by NHEJ [17-19], and NHEJ-dependent telomeric DSB fusions [20], are mostly limited to G1. The formation of IR-induced Rad51 nuclear foci

—indicators of DSB repair by HR—is largely restricted to S and G2 phases in yeast and mammalian cells [21-24]. Moreover, DSBs are preferentially repaired by HR in human cells synchronized in S phase [25]. Despite the preference for HR, NHEJ remains active during S and G2 phases of the mammalian cell cycle [26-30] and is the favored, if not sole pathway for repair of damage caused by IR in G1 (reviewed in [15,31]). Consequently, NHEJ deficiency has a greater impact on survival when cells are exposed to IR in G1, as opposed to G2 [30, 32-34]. Multiple mechanisms enforce this bias. For example, the NHEJ factor Ku80 binds to DSBs preferentially in G1 [35]. Also, during human lymphoid cell development, immunoglobulin V(D)J recombination by NHEJ is restricted to G0/G1 phases by the cell-cycle regulation of RAG2 recombinase [36].

One reason for the shift towards HR during S/G2 in diploid cells is that sister chromatids, which are only available from S phase onwards, are preferred over homologous chromosomes as templates for HR repair [37,38]. Recent work indicates, however, that this is not the sole explanation, and that CDKs might directly influence the channeling of DSBs into different repair pathways.

CDKs regulate DSB repair

The evidence implicating CDKs in DNA repair comes mainly from studies in yeast. In *S. cerevisiae*, loss of the cyclins Clb5 and Clb6, which promote S-phase progression in complex with Cdk1, renders cells sensitive to DNA damaging agents such as methylmethanesulfonate (MMS), ultraviolet (UV) light and IR [39]. Deletion of *CLB5* and *CLB6* delays, but does not prevent S phase in budding yeast [40,41]. This suggests two possible, not mutually exclusive, explanations for increased DNA damage sensitivity of *clb5 clb6* mutant strains: 1) perturbation of normal replication timing might make cells more vulnerable to genotoxic stress; or 2) Cdk1 complexes with Clbs 5 and 6 play specific roles, not redundant with those of other CDK/cyclin pairs, in DNA damage responses. Genetic evidence that CDK plays a direct role in DNA repair emerged in *S. pombe*, where a hypomorphic mutation in *cdc13*—encoding the essential mitotic cyclin, cyclin B—hindered repair of radiation-induced DSBs at two distinct points in the HR pathway (Fig. 1). First, it decreased the formation of Rad51 foci in response to IR, by a function redundant with that of Rad50. At a later stage it impaired resolution of HR intermediates by topoisomerase III and Rqh1 [42], a helicase in the RecQ family (reviewed in [43]). Therefore, cyclin B, which activates Cdk1 to drive cells into mitosis, also influences DSB repair. Because *S. pombe* cells, like metazoan somatic cells, respond to DNA lesions by arrest in interphase [7], there is a paradox: Cdk1/cyclin B regulates HR while being restrained from triggering mitosis, which would be lethal in the presence of an unrepaired DSB. To perform this balancing act, *S. pombe* does not rely on specialized CDK/cyclin pairs, but might instead depend on specialized CDK activation *pathways*. Perhaps in support of this idea, one of two CAKs in fission yeast, the non-essential Csk1, is required for normal HR frequency and resistance to DNA damage [44].

In *S. cerevisiae*, Cdk1/Clb activity is required for recombinational repair of DSBs [17,18]. At the restrictive temperature in a temperature-sensitive *cdc28* (encoding Cdk1) mutant or in the presence of CDK inhibitors (small molecules or proteins), cells were unable to repair an HO endonuclease-induced DSB by HR. Inhibition of Cdk1 led to failures in checkpoint activation and DSB end-resection. Consistent with a defect in resection, Cdk1 inactivation blocked recruitment of RPA and Rad51 to the DSB. Inability to recruit RPA might in turn explain the faulty checkpoint, because RPA-bound ssDNA is a necessary signal for binding and activation of the damage-sensing kinase Mec1, which is homologous to mammalian ATR and ATM (reviewed in [6]). The role of Cdk1 in HR is not limited to end-resection—turning off activity at later times after DSB induction led to blocks further downstream in the pathway [18]—but

may be selectively required for resection at DSBs induced by endonuclease, as opposed to IR [45].

Why Cdk2 matters

In metazoans, roles of CDK in the DNA damage response are not as clearly defined, and it is unclear which CDK/cyclin pairs, if any, perform functions analogous to those of budding yeast Cdk1/Clb in protecting genomic integrity. Cyclin A is the major cyclin in active CDK complexes from late G1 through early G2 in human cells [46], and the major CDK bound to cyclin A throughout S phase is Cdk2 [47], which is therefore likely to regulate DNA repair and responses to replication stress. Cdk2 phosphorylates core components of cell division and DNA replication machineries (reviewed in [48]), but also targets proteins that may function primarily in DNA damage repair [49-52]. Homozygous *Cdk2* knockout mice are viable, but have defects in male and female gametogenesis [3,4]. This implicates Cdk2 specifically in the regulation of meiotic cell cycles, which require induction and subsequent repair of DSBs to ensure their fidelity. Similar phenotypes arise in mice lacking components of the DNA repair machinery [53,54].

Cdk2^{-/-} mice and MEFs derived from them exhibited increased sensitivity to radiation-induced DSBs, and delayed DNA repair [55]. In human U2OS cells exposed to IR, RPA-coated ssDNA formation and ATR-dependent phosphorylation of the checkpoint kinase Chk1 were sensitive to the CDK inhibitor roscovitine and restricted to S and G2 phase when cyclin A expression is high [56]. In addition, reduction of Cdk2 levels by RNAi in human cells led to a decrease in IR-induced Chk1 phosphorylation, which correlated with a slower rate of DNA repair [57]. Together, these findings implicate mammalian interphase CDKs, specifically Cdk2/cyclin A, in the control of DSB repair. Moreover, they suggest a role in the early steps of HR repair, possibly similar to that of yeast Cdk1.

CDK targets that function in DSB resection

CDK-dependent regulation of DSB repair pathway choice is both direct and indirect. In both mammalian and yeast cells, the expression of repair proteins fluctuates depending on the cell cycle stage, which is in turn a function of CDK activity. For example, the protein levels of HR factors Rad51 and Rad52 peak during S phase in mammalian cells [58]. A number of other HR proteins, including fission yeast Ctp1 and its mammalian homolog CtIP [59,60], as well as the tumor suppressor protein BRCA1 [61,62], are expressed periodically with a maximum during S/G2. On the other hand, a number of key players in DSB repair pathways are direct targets of CDK-dependent phosphorylation, which might directly influence pathway choice and/or efficiency.

CDKs phosphorylate several proteins involved in HR (Fig. 1). For example, Cdk1 is needed for the induction of DSBs in budding yeast meiosis; one of its required functions is to phosphorylate Mer2 and thereby modulate its interactions with proteins essential in break formation [63]. As mentioned above, Cdk1 activity is also necessary in *S. cerevisiae* for the DNA resection step that initiates repair by HR or single-strand annealing (SSA) [17,18]. A recently identified CDK target involved in this process is Sae2, a homolog of Ctp1 and CtIP. CDK-dependent phosphorylation of Sae2 at Ser267 is needed for proper DNA-end resection and HR [64].

Another possible target of CDKs in budding yeast is Mre11/Rad50/Xrs2 (MRX), which is one of the first complexes recruited to the DSB site, thought to act together with Sae2 in DSB end-resection [65]. The MRX complex has multiple functions in meiotic and mitotic cells, including DSB repair, telomere maintenance, and DNA damage checkpoint activation (reviewed in [66,67]). Mre11 and Xrs2 both have consensus sites for phosphorylation by CDK, but mutating

those sites did not cause hypersensitivity to DNA-damaging agents or defects in checkpoint activation after DSBs occurred in G2 [18]. This suggests that any functions of CDK-dependent MRX phosphorylation are redundant with those of other proteins or pathways. Finally, the *S. cerevisiae* checkpoint protein Rad9 is phosphorylated by Cdk1 in vitro [68,69] and regulates DSB resection at telomeres [70].

In mammalian cells, there is so far only indirect evidence that CDKs play a role in DSB resection, in that proteins involved in resection have also been identified as CDK targets. The HR factor CtIP interacts directly with Nbs1—a member of the Mre11-Rad50-Nbs1 (MRN) complex, analogous to budding yeast MRX—and promotes RPA focus formation [71]. CtIP is phosphorylated at Ser327 during S/G2 of an unperturbed cell cycle and, dependent on ATM or ATR, at Ser664 and Ser745 in response to DNA damage [71,72]. CtIP interacts with another DNA repair protein, BRCA1 [73-75], dependent on phosphorylation of CtIP-Ser327 by CDK [76]. BRCA1 also associates with the MRN complex—an interaction enhanced by IR and diminished by roscovitine treatment. Depletion of CtIP by RNAi decreased BRCA1/MRN complex formation [77]. Therefore, CtIP and CDK activity are both needed for BRCA1 to interact with MRN. Furthermore, human U2OS cells expressing a mutant version of CtIP, in which the putative CDK phosphorylation site (Thr847, corresponding to Ser267 of Sae2) was changed to Ala, displayed enhanced sensitivity to camptothecin [64], suggesting an important role for that phosphorylation in DSB repair, conserved between budding yeast and mammals.

Fission yeast Ctp1 is also required for the function of Mre11 (a nuclease) in the 5'-3' resection of DSBs [59], and potentially has two Rad3/Tel1 (fission yeast homologs of ATM/ATR) and two CDK recognition sites [78]. However, *ctp1* mutant strains in which all four sites were mutated had no detectable defect in DNA repair, so it remains uncertain what role, if any, Ctp1 phosphorylation plays in DNA repair in *S. pombe* [78].

CDK functions further downstream in HR

In yeast, Cdk1 activity seems to regulate early steps in HR such as resection, intermediate events such as Rad51 recruitment and DNA synthesis, and later stages such as resolution [17,18,42]. Predictably, proteins implicated in later events have also been identified as potential CDK substrates in both yeast and metazoans. For example, the human breast cancer susceptibility gene products BRCA1 and BRCA2 are phosphorylated by CDK [49,51]. BRCA1 is phosphorylated at Ser1497, which is part of a CDK consensus site [51], but the function of this modification remains unknown. BRCA2 is phosphorylated on Ser3291 by CDK in a cell cycle-dependent manner. The phosphorylation site lies within a region that confers the ability to interact with multimeric Rad51, thereby stabilizing the Rad51 nucleoprotein filament, possibly to stimulate HR repair. CDK-dependent phosphorylation of BRCA2 at Ser3291 prevented interaction with Rad51, providing a potential switch to down-regulate recombinase activity [49,79]. Consistent with this interpretation, phosphorylation on Ser3291 increased during G2 phase and peaked in mitosis, but decreased when cells were irradiated. The decrease in phosphorylation correlated with increased BRCA2-Rad51 association. These data suggest an HR-inhibitory role for CDK activity during late G2 phase and mitosis in mammalian cells, which runs counter to the generally pro-recombinogenic influences imputed to CDKs in other settings.

In fission yeast, the checkpoint mediator Crb2 (also known as Rhp9), ortholog of *S. cerevisiae* Rad9, is a confirmed target of Cdk1 [80]. Crb2 and Rad9 both contain BRCA1 C-terminal homology (BRCT) motifs: phosphopeptide-binding domains present in a number of nuclear proteins involved in DNA repair [81]. Phosphorylation of Crb2-Thr215 by Cdk1 is required for reentering the cell cycle after DNA damage-induced checkpoint arrest [80], and promotes the recruitment of Crb2 to DSB sites induced by IR [82]. Crb2 was also implicated

in the CDK-dependent regulation of a late step in HR, after Rad51 loading; the *crb2-T215A* mutation was epistatic to a *cdc13* (cyclin B) mutation proposed to impair resolution of HR intermediates by the topoisomerase Top3 and the ReqQ helicase Rqh1 [42].

In budding yeast, Cdk1 might regulate HR by phosphorylating the DNA helicase Srs2, which has similarity to the bacterial UvrD/Rep helicases (reviewed in [83]). Srs2 is a negative regulator of HR, which prevents potentially deleterious recombination events. One mechanism by which Srs2 is thought to downregulate HR is dissociation of Rad51 from ssDNA (reviewed in [84,85]). Srs2 was phosphorylated in response to DNA damage in a Mec1- and Cdk1-dependent manner [86], and was identified in a proteomic screen for direct targets of budding yeast Cdk1 [68]. An *srs2* mutant strain in which all seven putative Cdk1 phosphorylation sites were mutated to non-phosphorylatable Ala or Val residues exhibited hypersensitivity to intra S-phase DNA damage. In fission yeast, the hyper-recombination phenotype caused by *srs2* deletion was suppressed by loss of the CAK Csk1 [44], suggesting that Srs2 normally antagonizes a function of a CDK activated by Csk1, possibly Cdk1, in promoting HR.

A role for CDKs in telomere maintenance

Cdk1 also functions in end resection of DNA at telomeres in *S. cerevisiae* [87,88]. Likewise, in mammalian cells, CDK activity has a role in telomere repair. In mouse embryonic fibroblasts (MEFs), inactivation of a temperature-sensitive (ts) variant of the telomere binding protein TRF2, which protects telomeres from nucleolytic degradation, results in rapid telomere failure. Telomere repair by NHEJ, which leads to telomere fusions, occurs primarily in G1. This is due, at least in part, to an NHEJ-inhibitory effect of high CDK activity later in the cell cycle, because treatment with roscovitine induced telomere fusions when cells with ts TRF2 were shifted to non-permissive temperature in G2/M [89].

CDK targets with functions in NHEJ

CDK substrates have also been identified in the NHEJ pathway. For example, RAG2 has been proposed to be negatively regulated by Cdk2/cyclin A, through phosphorylation on Thr490 and consequent destabilization upon S phase entry [36,90,91]. In addition, Ku70 was reported to be a substrate of Cdk2 in complex with cyclin A1, a cyclin A isoform expressed preferentially in the germ line [52]. Another putative CDK substrate, implicated in NHEJ as well as base excision repair (BER), is DNA Polymerase λ (Pol λ), which belongs to the X family of DNA polymerases (reviewed in [92]). Pol λ could be co-immunoprecipitated with Cdk2 from HeLa cell extracts, and was phosphorylated by Cdk1 and Cdk2 in vitro. In vivo, phosphorylation of Pol λ is cell cycle regulated [93], and stabilizes the protein, possibly to promote its specific functions in DNA damage repair in late S and G2 phases [94].

Roles for CDKs in regulating checkpoint function

In addition to serving as targets of checkpoint signaling, CDKs have been implicated in the regulation of checkpoint function in vertebrate cells. Tipin, a protein with suspected functions in checkpoint responses in *Xenopus*, was recently identified as a substrate of Cdk2/cyclin E [95]. A central player in checkpoint signaling, the kinase ATR, is recruited to RPA-coated ssDNA by its interacting protein Atrip, which binds to RPA. Atrip is a Cdk2/cyclin A substrate in vitro and is phosphorylated on Ser224 in a cell cycle-dependent manner in vivo [50]. Activation of ATR/Atrip results in phosphorylation of the checkpoint kinase Chk1 and other effectors. One outcome of this signaling cascade is cell cycle arrest (reviewed in [96]). Phosphorylation of Atrip-Ser224 is necessary for maintenance of the G2/M checkpoint in response to IR and UV light [50]. Therefore, CDKs play an active role at an upstream point in a signaling pathway that ultimately inhibits CDKs.

Conclusions and Perspectives

HR and NHEJ are the two major pathways for repairing DSBs. In yeast, HR is the prevalent mechanism, whereas mammalian cells rely more heavily on NHEJ. This difference could reflect the higher compaction of chromatin in higher eukaryotic cells that might impede access to homologous sequences. There are several factors known to regulate the NHEJ-versus-HR pathway choice. One is the nature of the DSB. In mammalian cells, blunt-ended DSBs, such as those produced by some nucleases and by IR, can be repaired by either pathway, whereas DSBs produced by replication fork collapse are repaired almost exclusively by HR (reviewed in [31]). In budding yeast, NHEJ is more restricted, being efficient at rejoining ends with cohesive overhangs, but not those with noncohesive or no overhangs (reviewed in [13]). In addition, cell type influences pathway selection. For example, in budding yeast, NHEJ and HR are both active in haploid cells, but NHEJ is suppressed in diploids by repression of the haploid-specific gene *NEJ1* [97-99]. HR is more robust in chicken B cells and mouse embryonic stem cells than in most other types of vertebrate cells [100,101].

Although differences in template availability could in theory produce the observed biases towards HR in diploid and G2 cells, mounting evidence suggests that the cell cycle machinery actively regulates DSB repair pathway choice. The initial step of processing a DSB seems to be a decision point between HR and NHEJ. In budding yeast, the resection of a DSB prevents NHEJ, which cannot simply ligate 3'-overhangs. Therefore, once a DSB is resected to generate 3'-ssDNA, it must be repaired by HR or by SSA, which likewise relies on sequence homology between the broken DNA ends [102]. Lack of end-processing, on the other hand, preserves an appropriate substrate for NHEJ [17,18]. Results in budding and fission yeast indicate that Cdk1 activity is essential for the 5'-3' resection of DSB ends and important for subsequent recruitment of RPA and Rad51 [17,18,42]. It is therefore likely to be a determinant of pathway selection, promoting HR and inhibiting NHEJ during S and G2 phases of the cell cycle.

Whether CDKs play a similar role in mammalian cells has yet to be established. Because both HR and NHEJ are active during S and G2 in mammalian cells, the two pathways might coordinate their functions in the repair of DSBs. Also, in contrast to the situation in yeast, NHEJ in mammals can process both cohesive and noncohesive ends and might therefore compete more effectively with HR for certain substrates. The Ku70/Ku80 heterodimer is recruited to DSBs more rapidly than are the HR factors, and it has been proposed that the binding of Ku specifically interferes with the initiation of HR at IR- or endonuclease-induced DSBs (reviewed in [15]). Despite these differences, there is growing circumstantial evidence that CDKs play active roles in coordinating DNA repair processes in metazoans, as they do in yeast.

As mentioned above, whereas budding yeast Cdk1 is not directly inhibited in response to DNA damage, fission yeast and mammalian CDKs are the primary targets of inhibitory signaling by DNA structure checkpoints. In mammalian cells, both G1/S and intra-S-phase checkpoints are thought to target Cdk2 (reviewed in [8]), which normally governs origin licensing and firing and E2F-dependent transcription to promote S-phase entry and DNA replication. The principal target of the G2/M checkpoint is the mitotic inducer Cdk1/cyclin B; checkpoint activation causes increased inhibitory phosphorylation of a conserved Tyr residue in the protein substrate-binding region of Cdk1, by altering the balance of activity between the kinases and phosphatases that work on this site (reviewed in [8]).

An important question for future study is how seemingly opposing roles of CDKs—as both targets for inhibition by checkpoint signaling and positive regulators of DNA repair—are coordinated (Fig. 3). In one simple mechanism, a lag between checkpoint activation and CDK shutoff might allow sufficient time for CDK to execute its function in regulating DNA repair

(Fig. 3A). Another model invokes temporal separation between the phosphorylation event and repair pathway activation (Fig. 3B). In other words, the phosphorylation by CDK of a substrate involved in HR might be a constitutive event that occurs during normal S-phase progression. DSBs incurred during S phase would therefore be effectively channeled into the appropriate pathway (i.e. HR), before they happen. An attractive feature of this model is that it could reconcile cases in which phosphorylations by CDK appear to promote HR with those in which they seem to act negatively. The difference would reside in the substrate being phosphorylated, not the CDK doing the modification, such that, for certain substrates, phosphorylation by CDK prior to damage would promote HR, while for other substrates (e.g. BRCA2 [49,79]), the HR-promoting event might be *dephosphorylation* by phosphatases after CDK activity is shut off by the checkpoint.

Neither of these scenarios can account for the CDK requirement in budding yeast cells that have sustained an endonuclease-induced DSB. In that case, acute inhibition of CDK up to two hours after the induction of the DSB still slowed completion of HR repair, by inhibiting DNA synthesis after synapsis [18]. This implies an ongoing need for the active kinase during repair. If a similar requirement exists in fission yeast or metazoan cells, it would suggest that these organisms have evolved mechanisms to channel *CDK activity* into functions required in the DNA damage response, while restraining mitosis.

We propose that the CDK activity needed for repair of DNA damage in checkpoint-arrested cells could be generated by distinct subpathways of CDK activation (Fig. 3C). In vertebrate cells, this might be accomplished by continued activation of Cdk2, which cannot trigger mitosis [103], while Cdk1 is inhibited. Recent work in fact suggests that Cdk2 and Cdk1 follow kinetically distinct paths to activation in human cells [47], and that the CDKs active during S phase might be relatively resistant to inhibitory phosphorylations in both budding yeast and metazoans [104,105]. How *S. pombe* could achieve a similar insulation of DNA repair-promoting and mitosis-inducing functions is less clear, because the same CDK/cyclin complex that triggers mitosis also regulates HR [42]. Despite having only one CDK to drive the cell cycle, however, fission yeast is unique in having two CAKs [106,107], and could thereby activate that CDK by different mechanisms. Perhaps consistent with this idea, one CAK—the essential Mcs6 complex—has been implicated genetically in the control of mitotic entry [108], whereas the other—the nonessential Csk1—has been shown to function in the DNA damage response, as described above [44]. Precise temporal control over CDK activity was instrumental in uncovering its roles in meiotic DSB formation [63] and DSB end-resection [17,18] in budding yeast. The development of similar tools in vertebrate cells will be needed to reveal whether CDKs govern the choice of DSB repair pathways in all eukaryotes, and how that function is coordinated with the primary one of driving the cell cycle forward.

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Figure 1. Interactions of CDKs with homologous recombination (HR) proteins in eukaryotes
In this schematic diagram, simplified to highlight targets of the cell-cycle machinery in multiple organisms, proteins that are probable substrates of CDKs (CtIP/Sae2/Ctp1, Rad9/Crb2, BRCA1, BRCA2, Srs2), or which interact genetically with CDKs (Crb2, Rqh1, Top3), are indicated in red (mammals), green (*S. cerevisiae*) or blue (*S. pombe*). Dotted arrows indicate either that the function influenced by the phosphorylation event is not yet known (Srs2), or that the CDK-dependent influence might be indirect (Crb2, Rqh1, Top3). See text for details of individual interactions.

Figure 2. HR and NHEJ are differentially regulated with progression through the cell cycle

In metazoans different CDKs are active in distinct cell cycle phases, whereas in budding yeast a single CDK promotes cell cycle progression in complex with different cyclins. NHEJ predominates during G1 and early S phase whereas HR is upregulated during late S and G2. NHEJ stays active throughout the mammalian cell cycle.

Figure 3. Models of coordination between cell cycle progression and DNA repair

(A) A lag between checkpoint activation and CDK shutoff allows sufficient time for CDK to execute its function in repair before being inactivated by the checkpoint. (B) Phosphorylation of substrates involved in HR is constitutive, occurring during normal S-phase progression. In the cases illustrated, one substrate (S1) promotes HR in its phosphorylated form, whereas the other (S2) must be dephosphorylated after CDK activity is turned off by the checkpoint in order to exert a pro-recombinogenic effect. (C) Subpathways of CDK activation allow repair of DNA damage in arrested cells. Checkpoint activation inhibits the mitotic inducer Cdk1 by up-regulation of the kinase Wee1 and down-regulation of the phosphatase Cdc25, leading to

increased Tyr15 phosphorylation. In this scenario, Cdk2 escapes inhibition because it follows a distinct pathway, in which activation by CAK and inhibition by Wee1 are uncoupled.