When genome integrity and cell cycle decisions collide: roles of polo kinases in cellular adaptation to DNA damage

Diego Serrano · Damien D'Amours

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Abstract The drive to proliferate and the need to maintain genome integrity are two of the most powerful forces acting on biological systems. When these forces enter in conflict, such as in the case of cells experiencing DNA damage, feedback mechanisms are activated to ensure that cellular proliferation is stopped and no further damage is introduced while cells repair their chromosomal lesions. In this circumstance, the DNA damage response dominates over the biological drive to proliferate, and may even result in programmed cell death if the damage cannot be repaired efficiently. Interestingly, the drive to proliferate can under specific conditions overcome the DNA damage response and lead to a reactivation of the proliferative program in checkpoint-arrested cells. This phenomenon is known as adaptation to DNA damage and is observed in all eukaryotic species where the process has been studied, including normal and cancer cells in humans. Polo-like kinases (PLKs) are critical regulators of the adaptation response to DNA damage and they play key roles at the interface of cell cycle and checkpoint-related decisions in cells. Here, we review recent progress in defining the specific roles of PLKs in the adaptation process and how this conserved family of eukaryotic kinases can integrate the fundamental need to preserve genomic integrity with effective cellular proliferation.

Keywords DNA damage · Adaptation to checkpoint arrest · Polo kinases · Cdc5 · Genome integrity

Introduction

The precise regulation of cell cycle progression is an essential and highly conserved function in eukaryotes. During normal proliferation, cells must make regulatory decisions to either enter a proliferative state leading to cell division, or prevent entry into the cell cycle when they experience DNA damage. In the latter case, cells will block cell cycle transitions and stimulate the DNA repair machinery to reverse the damage (Hartwell and Weinert 1989). When DNA repair is completed, cells will eventually recover from their cell cycle arrest and continue cycling. In contrast, if the damage to genome integrity is too extensive or otherwise unrepairable, cells will enter a senescent state or induce a programmed cell death pathway (reviewed in Bartek and Lukas 2007; Jackson and Bartek 2009).

Maintaining genome stability in the face of extensive DNA damage is possibly one of the most challenging tasks for cells, since failure to repair DNA lesions can lead to tumorigenesis and/or cell death (Ames et al. 1993; Wallace et al. 2012). In fact, defective DNA repair mechanisms are a major cause for the accumulation of DNA mutations, and several human diseases affecting DNA repair pathways are associated with high rates of carcinogenesis (Harper and Elledge 2007; Jackson and Bartek 2009). Genome instability is not only associated with cancer initiation, but also with tumor development, which explains why this specific feature is considered a general hallmark of cancers (Hanahan and Weinberg 2011). For this reason, healthy cells spend significant resources to continuously monitor the successful completion of DNA replication and repair, as well as effective chromosome segregation. Detection of DNA damage or chromosome missegregation usually leads to the activation of checkpoints whose function is to slow

D. Serrano · D. D'Amours (✉)
Département de Pathologie et Biologie Cellulaire, Institute for Research in Immunology and Cancer, Université de Montréal,
P.O. Box 6128, Succursale Centre-Ville, Montréal, QC H3C 3J7,
Canada
e-mail: damien.damours@umontreal.ca

down cell cycle progression to allow time to reverse the damage (Bartek and Lukas 2007; Jackson and Bartek 2009).

The ability of the checkpoint machinery to halt cell cycle progression requires a very tight coupling between the checkpoint signaling cascades and the cell cycle machinery (Langerak and Russell 2011). In particular, master regulators of cell cycle progression, such as the cyclin-dependent kinases (CDKs) and the polo-like kinases (PLKs), must be connected and responsive to the checkpoint activation status within cells in order to establish a cell cycle arrest. Likewise, checkpoint mechanisms must also be responsive to the proliferative status of cells to elicit the proper, cell cycle stage-specific response to repair genome damage and to enable recovery from checkpoint arrest. These feedback mechanisms ensure that checkpoint and cell cycle processes balance each other in such a way as to allow optimal cell proliferation in the face of genomic lesions.

Interestingly, there is a specific type of response to DNA damage where the coupling between checkpoint activation and cell cycle progression is lost, thereby allowing proliferation in the presence of DNA damage. This cellular response is known as adaptation to DNA damage (reviewed in Bahassi et al. 2011; Bartek and Lukas 2007; Clemenson and Marsolier-Kergoat 2009; Swift and Golsteyn 2014; Syljuasen 2007). In this process, checkpoint-arrested cells re-enter a proliferative state even though they experience significant DNA damage. At the cellular level, the adaptation response is likely to provide a proliferative advantage to cells experiencing low levels of unrepairable DNA damage because asymmetric partition of this damage during cell division, or a change in cell cycle stage, will likely improve the chances of survival of at least one of the two daughter cells following mitosis (Galgoczy and Toczyski 2001). In contrast, none of the daughter cells would survive a permanent cell cycle arrest in the presence of DNA damage if the checkpoint response is maintained. Here, we review the mechanisms that lead to cellular adaptation to DNA damage, with a particular focus on the critical roles of PLKs in the regulation of this process.

Overview of the DNA damage response

Eukaryotic organisms have developed several mechanisms to mitigate the deleterious effects of DNA damage. In particular, complex surveillance systems continuously monitor the genome for the appearance of a wide variety of DNA lesions. These systems are collectively known as DNA damage checkpoints. Upon DNA damage formation, these checkpoints will inactivate key regulators of cell

proliferation, thereby arresting the cell cycle. Before discussing how the adaptation response can overcome checkpoint signaling in the presence of DNA damage, we will very briefly review the mechanisms leading to initial checkpoint activation and the pathways involved in the repair of DNA damage. Since the process of adaptation has been predominantly studied in the context of DNA double-strand breaks (DSBs) (see below), we will limit our overview to the cellular responses induced by this type of DNA lesion. However, it is important to keep in mind that adaptation can occur in response to a wide variety of genotoxic lesions (Kubara et al. 2012; Syljuasen et al. 2006; Yoo et al. 2004). For a detailed discussion on checkpoint responses and DNA repair pathways, we direct readers to several recent reviews (Chapman et al. 2012; Huertas 2010; Jackson and Bartek 2009; Moynahan and Jasin 2010; Panier and Durocher 2013).

Checkpoint activation and cell cycle arrest after DNA damage

The first step in the activation of the checkpoint response depends on the detection of DNA lesions by surveillance proteins that monitor for the presence of DSBs in the genome. In all eukaryotes, DSBs are specifically recognized by sensor proteins such as the Ku70/Ku80 dimer and the MRN/X (Mre11–Rad50–Nbs1/Xrs2) complex (D'Amours and Jackson 2002; Huertas 2010). These sensors bind to the DNA break, and recruit large protein kinases of the phosphoinositide 3-kinase (PI3K)-like family (i.e., ATM/ATR/DNA-PKcs). The activation of these kinases leads to the phosphorylation of histone γ -H2AX, contributing to the accumulation and stabilization of several checkpoint proteins at DSBs (Huertas et al. 2009). Moreover, this signaling pathway results in the activation of Chk1 and Chk2 (i.e., Rad53 in yeast) effector kinases, which ultimately inhibit Cdc25 phosphatases and other cell cycle regulators. Importantly, loss of Cdc25 activity prevents effective activation of the Cdk1 kinase and, as a consequence, activation of several substrates required for cell cycle progression.

Repairing the DNA lesion

Once DSBs are detected and the cell cycle is halted, the cell can heal the lesion by two different DNA repair mechanisms; homologous recombination (HR) or non-homologous end joining (NHEJ) (reviewed in Chapman et al. 2012). Interestingly, the CDK activation status operating in cells prior to the checkpoint arrest plays an active role in determining whether DSBs will be repaired through NHEJ or HR (Chapman et al. 2012; Huertas 2010; Simoneau et al. 2014). NHEJ is the primary repair pathway

used by cells when they experience DNA damage and checkpoint arrest prior to genome replication in G1 or early S phase (Chapman et al. 2012; Langerak and Russell 2011). This repair pathway provides a rapid, but sometimes imprecise, way to maintain genome integrity. In particular, DSB repair by NHEJ can have deleterious consequences if the break is located in the coding sequence of essential proteins and the repair is imprecise. On the other hand, HR is the preferred repair pathway when cells reach the G2/M stage of the cell cycle. This pathway allows for very accurate repair of DSB since sister chromatids are used as templates during the repair process (reviewed in Moynahan and Jasin 2010).

Cellular recovery from DNA damage

Once repair is completed, feedback mechanisms ensure that checkpoint signaling is shut off and cell cycle is allowed to resume in a process known as recovery from DNA damage (Bartek and Lukas 2007; Clemenson and Marsolier-Kergoat 2009). The recovery process depends on several protein kinases and phosphatases, including PLKs, which, in human cells, promote recovery from checkpoint arrest by multiple phosphorylation-induced inactivation events. These involve the Cdk1 inhibitor Wee1 (van Vugt et al. 2004), and several checkpoint effectors, including Claspin (Yoo et al. 2004), Chk2, 53PB1 (van Vugt et al. 2010) and GTSE1 (Liu et al. 2010). In the case of the Plk1-dependent phosphorylation of Wee1 and Claspin, these events are believed to create binding sites on substrates for β -TrCP, a regulatory subunit of the SCF ubiquitin ligase, thereby causing protein ubiquitylation and degradation of these Plk1 targets (Mailand et al. 2006; Mamely et al. 2006; Peschiaroli et al. 2006). Phosphorylation of GTSE1 by Plk1, on the other hand, promotes its nuclear accumulation where it can antagonize p53 activity and checkpoint maintenance (Liu et al. 2010). A key initiating event for the recovery process appears to be the reactivation of human Plk1 by Aurora A-mediated phosphorylation of its activation loop (Macurek et al. 2008). Likewise, dephosphorylation of γ -H2AX by PP4, PP2A, and Wip1 phosphatases also contributes significantly to checkpoint recovery (Chowdhury et al. 2005; Keogh et al. 2006; Macurek et al. 2010; Nakada et al. 2008). Collectively, these events act as a feedback mechanism to shut down the DNA damage signaling machinery once DNA repair is completed. Although the process of recovery is conserved in budding yeast, the sole member of the PLK family in this organism, Cdc5, and some of the homologs of the Plk1 substrates mentioned above are not involved in the recovery process in this organism (Jin and Wang 2006; Vaze et al. 2002). Additional mechanisms may be in place in lower eukaryotes to promote recovery after DNA repair is completed

(Guillemain et al. 2007; Leroy et al. 2003; O'Neill et al. 2007).

When recovery is not possible: how cells decide to die

After DNA checkpoint activation, PI3K-like kinases also trigger the activation of a key gatekeeper of genome integrity, p53. At low levels of DNA damage, p53 activation is moderate and contributes to the cell cycle arrest by lowering the levels of CDK kinase activity in cells (e.g., via p21 induction). However, in the presence of extensive or unrepairable damage, the activation of p53 in cells is much more significant (Fridman and Lowe 2003; Zhao et al. 2000). This sustained and extensive activation of p53 is a critical early event in the induction of cell death via the apoptotic pathways. The active decision to undergo cell suicide is believed to protect multicellular organisms from cells that would likely carry mutations due to the fact that they have experienced too much damage to allow effective repair. This process is in direct opposition to the adaptation response, which allows cells to re-enter the cell cycle despite carrying significant DNA damage.

The adaptation response

Systems to study the adaptation response

Several systems have been developed over the years to study cellular adaptation to the presence of persistent DNA damage. These systems fall into two general categories. Historically, the first experimental system involved the creation of persistent or irreparable DNA damage at specific regions of chromosomes, such as telomeres (generated by defective telomere-protection systems; Sandell and Zakian 1993; Toczyski et al. 1997) and/or meganuclease-specific cleavage sites introduced at unique positions in the genome (Lee et al. 1998; Sandell and Zakian 1993). Although this type of damage affects only part of the genome, it can nevertheless induce a strong checkpoint response by virtue of the fact that the lesions—DNA double-strand breaks—are continually being re-introduced and/or their repair is being prevented using genetic tools such as conditional expression of meganucleases or temperature-sensitive effectors of telomere biogenesis (Lee et al. 1998; Sandell and Zakian 1993; Toczyski et al. 1997). These systems have been primarily used in non-mammalian model organisms because of the general ease of creating conditional genetic modifications in these organisms.

The second type of system in use to study adaptation to DNA damage involves treatment of cells with non-specific DNA damaging agents. Typical agents used in adaptation studies include DNA replication-inhibiting drugs, such as

aphidicolin (Yoo et al. 2004), topoisomerase I inhibitors, such as camptothecin (Kubara et al. 2012), or non-specific DNA-damaging agents, such as ionizing radiation (Syljuasen et al. 2006). These agents are used at doses that would induce enough DNA damage to exceed (at least momentarily) the DNA repair capacity of cells, but not grossly so, in order to avoid the induction of immediate cell death. This approach is typically used to study adaptation in mammalian cells (Swift and Golsteyn 2014). The goal of both systems described above is to create a situation whereby cells could either maintain a checkpoint-induced cell cycle arrest, or take the decision to re-enter a proliferative state despite the presence of significant levels of DNA damage in their genome. This is usually detected by monitoring cell morphology (e.g., yeast budding or microcolony formation on solid medium; Lee et al. 1998; Sandell and Zakian 1993; Toczyski et al. 1997) or the appearance of changes in cell cycle status (e.g., passage from interphase to mitosis, completion of DNA replication, appearance of chromosome condensation; Kubara et al. 2012; Syljuasen et al. 2006; Yoo et al. 2004). At the molecular level, adaptation is also associated with silencing of the DNA damage signaling machinery (e.g., dephosphorylation of checkpoint effectors; Donnianni et al. 2010; Pelliccioli et al. 2001; Vidanes et al. 2010), while markers of DNA damage persist. Ultimately, cellular adaptation to DNA damage will allow cells to proliferate under conditions that might otherwise block cell cycle progression or cause cell death.

Effectors of the adaptation response to DNA damage

The mechanistic basis underpinning the adaptation response is not fully understood at present. Many different proteins involved in various cellular processes have been connected to the adaptation response, including cell cycle regulators (Jin and Wang 2006; Toczyski et al. 1997; Zhang et al. 2009), chromatin-remodeling enzymes (Papamichos-Chronakis et al. 2006), autophagy factors (Dotiwala et al. 2013), and centrosome/spindle pole body effectors (Hu et al. 2001; Valerio-Santiago et al. 2013). How this group of apparently disparate proteins regulates the adaptation to DNA damage and whether they work together or in separate pathways to overcome checkpoint-induced cell cycle arrest is still unclear.

One particular difficulty in ascertaining the role of any protein in the adaptation response is discriminating between a putative role in adaptation versus a role in checkpoint recovery after DNA repair has been completed. This is due to the fact that elimination or overexpression of effectors of either one of these processes is predicted to yield identical phenotypes (i.e., permanent arrest after DNA damage, or cell cycle re-entry, respectively; Donnianni et al. 2010; Leroy

et al. 2003; Vidanes et al. 2010; Vaze et al. 2002). Indeed, it would be surprising that mutants unable to recover from *past* DNA damage would not also be defective in adapting to the presence of *permanent* damage (as an indirect consequence of their recovery defect). Another important consideration with regards to assigning roles for proteins in adaptation is that the process appears to be highly sensitive to the extent of DNA damage, and only occurs at low-to-moderate doses of DNA damage (Lee et al. 1998). This means that proteins affecting the efficiency of DSB repair or checkpoint signaling will likely increase the steady-state levels of key repair/signaling intermediates and, as an indirect consequence, prevent effective adaptation to DNA damage. These observations suggest that careful validation is necessary to directly implicate proteins as regulators of the adaptation process, and that positive results in adaptation assays can sometime be simply explained by defects in recovery and/or DNA repair processes. Keeping these caveats in mind, we discuss below how some of the known adaptation factors might be involved in the reactivation of cell cycle progression in the presence of DNA damage.

Polo-like kinases

Polo-like kinases comprise a group of serine/threonine protein kinases carrying a unique structural domain at their C-termini, the polo-box domain (PBD) (reviewed in Archambault and Glover 2009; Lu and Yu 2009). The PBD is a phosphopeptide-binding module that is believed to target PLKs to pre-phosphorylated substrates in cells. PLKs are the most extensively characterized effectors of the adaptation response in eukaryotes (Bahassi et al. 2011). Their role in this process has been confirmed in yeast (Toczyski et al. 1997), frog cycling extracts (Yoo et al. 2004), and mammalian cell systems (Syljuasen et al. 2006). Mutations in the yeast PLK, Cdc5 (Toczyski et al. 1997), or downregulation of metazoan Plk1 (Syljuasen et al. 2006) abrogate cellular adaptation to DNA damage in all systems where this phenomenon has been studied so far.

As previously mentioned, Plk1 is also implicated in the recovery from checkpoint arrest when DNA repair is completed in higher eukaryotes (Liu et al. 2010; van Vugt et al. 2004, 2010). However, this role in recovery appears to be mechanistically distinct from the role of PLKs in adaptation, and is not conserved in budding yeast (Jin and Wang 2006; Vaze et al. 2002).

Initiating adaptation: downregulating checkpoints or re-entering the cell cycle?

The specific mechanisms used by PLKs to promote adaptation to DNA damage in metazoans are currently unknown. However, we know much more about possible

Fig. 1 Mechanisms of action of yeast PLK/Cdc5 during adaptation to DNA damage. The possible relationships between Cdc5 and the other components of the cell cycle/DNA damage machineries are illustrated using schematic diagrams. *Lines* terminating with an *arrow* indicate stimulation, whereas *lines* terminating with a *perpendicular bar* indicate inhibition. *Dotted lines* signify multistep processes. See text for a detailed explanation of individual mechanisms

targets and mechanisms used by Cdc5 to stimulate adaptation in budding yeast. In fact, multiple mechanisms have been proposed and several may act in parallel to stimulate cell cycle re-entry in the presence of DNA damage. A key question is whether Cdc5 activity itself is the target of the checkpoint response and whether a putative downregulation of Cdc5 activity might be sufficient to prevent cell cycle progression in the presence of DNA damage. This is a contentious issue since it has been initially proposed that Cdc5 kinase activity was high in checkpoint-arrested cells (Hu et al. 2001) and that Cdc5 could stimulate adaptation by phosphorylating specific effectors of cell cycle re-entry, such as the Bfa1 protein (Hu et al. 2001; Liang and Wang 2007) (Fig. 1a). In contrast, another study recently suggested that there was a Rad53-dependent reduction of Cdc5 activity by ~50–66 % in the presence of DNA damage (Zhang et al. 2009) and that this lowered Cdc5 activity might prevent normal mitotic progression by maintaining the APC activator Cdh1 (Zhang et al. 2009) and the mitotic exit inhibitor Bfa1 in hypophosphorylated states (Valerio-Santiago et al. 2013) (Fig. 1b). Since Cdh1 and Bfa1 are both inhibited by Cdc5 phosphorylation, it was proposed that lowering Cdc5 kinase activity would prevent cell cycle progression by limiting mitotic spindle elongation (a Cdh1 function; Zhang et al. 2009) and preventing exit from mitosis (a Bfa1 function; Valerio-Santiago et al. 2013).

Although a putative role for Rad53 in inhibiting Cdc5 activity provides a compelling explanation for the checkpoint-induced cell cycle arrest in response to DNA damage, it does not explain how an eventual Cdc5 “reactivation” and cell cycle re-entry would be instated to allow cellular adaptation. Indeed, if Rad53 alone controls Cdc5 activity, one would have to imagine that the inhibitory activity of Rad53 towards Cdc5 would need to be eventually curtailed in order to enable effective adaptation. Since, by definition, adaptation is initiated when cells are experiencing high Rad53 activity

due to DNA damage, it is currently unknown how Rad53-mediated checkpoint signaling would stop targeting Cdc5 to establish a transition between a checkpoint-arrested state to a proliferative state in adapting cells. It is difficult to determine whether the phosphatases that normally inactivate Rad53 during checkpoint recovery, PP2C and PP4 (Guillemin et al. 2007; Leroy et al. 2003; O’Neill et al. 2007), are also involved in adaptation since mutants in these proteins are defective in both processes. Interestingly, it has been suggested that inactivation of Rad53 during adaptation could be mediated by Cdc5 phosphorylation-induced inhibition of Rad53 (Schleker et al. 2010; Vidanes et al. 2010) (Fig. 1c). Although this might provide an attractive feedback mechanism to shut down checkpoint signaling, it is also unclear why Cdc5 would initially refrain from targeting Rad53 in the early stages of the checkpoint response, and later inhibit Rad53 efficiently to allow cellular adaptation.

The challenge in identifying the initiating event in the adaptation response depends on determining whether the central player in this process, the PLK/Cdc5 kinase, is inhibited by Rad53/Chk2 or other components of the DNA damage machinery. Resolving the conflicting results on this issue will likely require careful experiments to determine the levels of Cdc5 kinase activity in DNA-damaged cells, and whether partial inactivation of Cdc5 is sufficient to impede cell cycle progression. In light of this, it is interesting to note that the *CDC5* gene is not haploinsufficient for normal cell proliferation in yeast, which suggests that a 50 % reduction in the levels of this kinase is insufficient to delay cell cycle progression in the absence of DNA damage. In contrast, it has been shown that heterozygous mutations in *CDC5* can affect the adaptation response, which suggests that the process responds quantitatively to Cdc5 activity in cells (Vidanes et al. 2010). It is however conceivable that a change in Cdc5 substrate specificity might also be important for the initiation of the adaptation response. This would be consistent with the

observation that the adaptation-defective mutant *cdc5-ad* is semi-dominant in diploid cells, whereas a *cdc5-dg* null allele has no effect (Dotiwala et al. 2007).

Early experiments suggested that Rad53 inhibits Bfa1 activity in a Cdc5-independent manner through a downstream checkpoint kinase, Dun1 (Hu et al. 2001; Liang and Wang 2007) (Fig. 1a). If Bfa1 is not the key or sole target of Cdc5 in the adaptation response, then what is its effector in this process? A candidate-based screen has eliminated a number of key phosphatases normally involved in cell cycle progression and checkpoint recovery (Vidanes et al. 2010). In contrast, a number of DNA repair and checkpoint factors, including Sae2/CtIP, Mus81–Mms4, and Rad51 are known or proposed substrates for Cdc5 in the DNA damage response (Donnianni et al. 2010; Gallo-Fernandez et al. 2012; Matos et al. 2011, 2013; Schwartz et al. 2012; Yata et al. 2012). Since phosphorylation of the Mus81–Mms4 nuclease (Gallo-Fernandez et al. 2012; Matos et al. 2011, 2013; Schwartz et al. 2012) and Rad51 (Yata et al. 2012) recombinase stimulate their activity in DNA repair, it seems unlikely that these are critical effectors of Cdc5/Plk1 during the adaptation response, although they could be possible targets of polo kinases in recovery after DNA damage. Interestingly, it has recently been proposed that phosphorylation of Sae2 by Cdc5 would stimulate its binding to DSBs and inhibit resection at those breaks, thereby limiting the activation of the checkpoint response (Donnianni et al. 2010) (Fig. 1d). Although a definite role for Cdc5 in Sae2 phosphorylation has not been formally established in cells expressing normal levels of Cdc5, this raises the possibility that an important step in shutting off the DNA damage checkpoint during the adaptation response involves limiting the extent of end resection at DSBs. In light of this, it will be important to determine whether a general loss of Cdc5 activity would cause a stimulation of DSB end resection. Interestingly, the permanent cell cycle arrest observed in *cdc5-ad* cells experiencing persistent DNA damage was not associated with increased levels of DSB end resection nor was the adaptation defect of this mutant suppressed by limiting detection of ssDNA using the *rfa1-t11* mutation (in a *cdc5-ad* background; Lee et al. 1998), which suggests that the permanent arrest of *cdc5-ad* cells is not caused by hyperactive DSB end resection.

A possible other substrate of Cdc5 in the adaptation response is the Ino80 chromatin remodeling complex (Papamichos-Chronakis et al. 2006). Loss of Ino80 activity in yeast cells cause aberrant incorporation of histone H2AZ variant in chromatin proximal to DSBs and is associated with an inability to adapt to DNA damage. It is believed that Ino80 acts by maintaining high levels of phosphorylated histone H2AX in damaged cells. In the absence of Ino80 complex, another chromatin remodeling factor replaces phospho-H2AX with H2AZ, thereby limiting

checkpoint activation (Papamichos-Chronakis et al. 2006). It will be interesting to determine whether Ino80 complex components are substrates for Cdc5 during the cell cycle or in response to DNA damage.

Adaptation as a possible mechanism to promote repair of DNA damage

It has been argued that during adaptation a dividing cell could segregate persistent DNA lesions to one of the daughter cells, thereby allowing the survival of the other daughter cell (i.e., sick and healthy cells in Fig. 2). However, there is also another possible role for the process of adaptation. What if adaptation is acting as a mechanism to stimulate repair of DNA lesions that cannot be processed at a specific stage of the cell cycle? Indeed, progressing from a G2/M-arrested state back into interphase might create conditions that are more conducive to effective repair for some types of DNA lesions. For example, it is well known that the efficiency of specific repair pathways, such as NHEJ and HR, are regulated in a cell cycle-specific manner (reviewed in Chapman et al. 2012; Langerak and Russell 2011). It is conceivable that some types of DNA damage could be more efficiently processed by NHEJ in G1 than by HR in mitosis. In fact, it would be advantageous to attempt DNA repair at a later stage of the cell cycle, and if not possible, then activate cell death pathways (Jackson and Bartek 2009). According to this hypothesis, cells would initially impose a checkpoint-induced arrest and attempt to repair their damage using the DNA repair mechanism that is prevalent at the stage of the arrest. However, if there is failure to repair DNA damage, cells could then adapt to this damage and progress to the next stage of the cell cycle where they could use a second DNA repair pathway to attempt the repair of the persistent DNA lesions (Fig. 2b). We envision that this particular mechanism would be beneficial under circumstances of low to moderate levels of persistent DNA damage. However, in cases of massive DNA damage that overwhelms the DNA repair machinery, it would appear much less likely that progressing to the next stage of the cell cycle would improve the chances of the adapting cell to completely reverse its damage. This particular limitation also applies to the “biased segregation” model for asymmetric partition of DNA damage during adaptation.

Adaptation and the immune response

Beyond the possible impact of adaptation on DNA repair efficiency or DNA damage segregation, it is also possible that the accumulation of mutations in essential proteins in adapting cells would act as a stimulatory signal for immune surveillance system (Garrido et al. 1997). Indeed, since DNA damage often affects multiple cells simultaneously in

Fig. 2 A novel model for the DNA damage response. **a** According to the classic DNA damage response pathway, checkpoint activation and cell cycle arrest provide time to repair the DNA damage. Cells can recover if the damage is fully repaired, or can either remain permanently arrested or induce apoptosis if DNA lesions are unrepairable. **b** In the adaptation model, the cell attempts to repair the DNA lesion when the checkpoint is activated. However, if failure

to repair occurs, the cell may attempt to segregate the DNA damage asymmetrically to one of the two daughter cells. This segregation will generate one “sick” cell harboring DNA lesions, and one “healthy” daughter cell. Alternatively, by virtue of entering a new cell cycle stage, the adapting cell may stimulate DNA repair by other, more efficient mechanisms than those available when maintaining a tight checkpoint arrest. See text for more detailed information

multicellular organisms, it is possible that the “sick but surviving cells” resulting from the adaptation process could condition the immune system to recognize and eliminate cells that have evaded DNA damage-induced apoptosis. This process would be analogous to tumor antigens triggering the immune response (Coulie et al. 2014; Raulet 2006). Moreover, it is possible that the adaptation process would reduce inflammation, a well-known stimulatory factor for cancer development (Hanahan and Weinberg 2011), by limiting the extent of cell death in a tissue after extensive DNA damage. According to the model proposed above, cells might stimulate the likelihood of repairing their DNA damage (or possibly segregate it exclusively to one of the two daughter cells during mitosis) by undergoing the adaptation process. However, if they fail doing so, they still have the possibility of triggering apoptosis or survive as a “sick” cell that stimulates the immune response. The ideas and mechanisms proposed above are admittedly speculative and await experimental validation. However, these possible mechanisms are presented here in the hope that they will stimulate further work in the field.

Conclusion and outlook

Taken together, the observations and results discussed herein indicate that much work still remains to be done to

identify the mechanistic basis and specific regulators involved in the adaptation response to DNA damage. In particular, finding the specific effector substrate(s) of Cdc5/Plk1 in this process will be critical. Also, it will be important to determine whether the known regulators of adaptation work together or in multiple different pathways to regulate this important process. Validating the notion that adaptation is a unique mechanism that can—under specific circumstances—positively affect genome integrity will require much additional efforts.

Finally, it is important to mention that the process of adaptation to DNA damage could have major consequences on tumorigenesis in humans. Indeed, by maintaining cells harboring persistent DNA damage alive, adaptation may increase rates of mutagenesis and, as a consequence, stimulate malignant transformation of the damaged cells. Interestingly, human Plk1 is overexpressed in various types of cancer (Lu and Yu 2009), suggesting that this kinase could enable resistance to DNA damage-based therapeutics via a stimulation of the adaptation process. In this case, it appears that the adaptation process could be co-opted by cancer cells to evade normal checkpoint mechanisms and elude cell death pathways. For these reasons, it will be very important to understand the role of adaptation to DNA damage not only in normal or healthy circumstances, but also in the context of cancer development and treatment with genotoxic agents.

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