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Abstract

Cell proliferation is tightly regulated to avoid propagating DNA damage and mutations, which can lead to pathologies such as cancer. To ensure genome integrity, cells activate the DNA damage checkpoint in response to genotoxic lesions to block cell cycle progression. This surveillance mechanism provides time to repair the damage before resuming cell cycle with an intact genome. When the damage is not repaired, cells can, in some conditions, override the cell cycle arrest and proceed with proliferation, a phenomenon known as adaptation to DNA damage. A subpopulation of adapted cells might eventually survive, but only at the cost of extensive genome instability. How and in which context adaptation operates the trade-off between survival and genome stability is a fascinating question. After a brief review of the current knowledge on adaptation to DNA damage in budding yeast, we will discuss a new role of adaptation in the context of telomerase-negative cells and replicative senescence. We highlight the idea that, in all settings studied so far, survival through adaptation is a double-edged sword as it comes with increased genomic instability.

Keywords: adaptation to DNA damage, telomere, repair, genome instability, Polo kinase.**Acknowledgments**

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DNA damage checkpoint and adaptation

DNA damage is a major challenge for eukaryotic cells, which must ensure genome integrity while proliferating. The assessment of the damage and the subsequent response are critical for survival and genome stability. Cells have developed a sophisticated safeguard mechanism, called the DNA damage checkpoint, which blocks cell-cycle progression to provide time for repair (Weinert and Hartwell 1988; Hartwell and Weinert 1989). Among the variety of DNA lesions, double-strand breaks (DSBs) are particularly cytotoxic if left unrepaired and have served as a model to establish, in budding yeast, the general framework of the DNA damage response, which is conserved throughout eukaryotes. The first step of the DNA damage response is the detection of the break by the independent recruitment of the Ku complex (Yku70/Yu80) and the MRX complex (Mre11/Rad50/Xrs2). They in turn recruit protein kinases of the phosphoinositide 3-kinase-related kinase (PIKK) family, Tel1 and Mec1, which trigger the DNA damage checkpoint through a cascade of phosphorylation. The culminating step of the cascade is the activation of effector kinases, Rad53 and Chk1, which target multiple cellular processes to ultimately arrest the cell cycle (reviewed in (Finn et al. 2012)).

Two main pathways can repair the damage: non-homologous end joining (NHEJ) and homologous recombination (HR). The decision between these two mechanisms depends on the phase of the cell cycle, through Cdk1 cyclin-dependent kinase activity. NHEJ is favoured in G1 phase and ligates the two broken ends, while HR, which reseals the break using a homologous sequence, is initiated by 5'-3' resection and exposure of single-stranded DNA (ssDNA) promoted in S/G2 phases.

When repair is successful, the entire DNA damage checkpoint signalling pathway is shut down since the lesion is no longer detected, allowing the cell cycle to resume in a process called recovery. In contrast, when the damage is unreparable, yeast cells experience a much longer checkpoint arrest (~5-15 hrs) but can eventually re-enter the cell cycle despite the detectable presence of damage (Sandell and Zakian 1993; Toczyski et al. 1997; Lee et al. 1998). This process is known as adaptation to DNA damage. During adaptation, the checkpoint is partially relieved, thus allowing cell cycle progression. More precisely, Rad53 is inactivated, which alleviates the downstream inhibition of cell cycle progression (Pelliccioli et al. 2001). The upstream part of the checkpoint remains largely unaffected, including the sensors Ddc1 (subunit of the 9-1-1 clamp complex) and Ddc2 (in complex with Mec1), and the adaptor Rad9, although a very slight drop in Ddc2 foci intensity in a subset of cells and in Rad9 hyperphosphorylation has been reported (Melo et al. 2001; Donnianni et al. 2010; Vidanes et al. 2010).

While many mutants defective for adaptation have been described and have contributed to its characterization, the molecular mechanisms underlying the process are not yet completely elucidated (Harrison and Haber 2006). The conserved family of Polo-like kinases has been implicated in adaptation and recovery to DNA damage checkpoint (Serrano and D'Amours 2014). In budding yeast, Cdc5 is the only Polo kinase and is essential for several cell-cycle-related processes (Botchkarev and Haber 2018). The involvement of Cdc5 in adaptation was first demonstrated with the isolation of the L251W point mutant allele, called *cdc5-ad*, which is defective for adaptation (Toczyski et al. 1997). Cells carrying the *cdc5-ad* allele and challenged by an unreparable DNA damage stay arrested in G2/M and maintain Rad53 in an activated state (Pelliccioli et al. 2001). Other recently identified adaptation mutants of *CDC5* (*cdc5-16* and *cdc5-T238A*) have also contributed to the dissection of Cdc5's role in adaptation (Ratsima et al. 2011; Ratsima et al. 2016; Rawal et al. 2016). However, despite its central role in adaptation, the exact mechanisms by which Cdc5 exerts its function and which Cdc5 targets are affected in response to DNA damage are still unclear (Hu et al. 2001; Zhang et al. 2009; Donnianni et al. 2010; Dotiwala et al. 2010; Schleker et al. 2010; Vidanes et al. 2010; Valerio-Santiago et al. 2013; Ratsima et al. 2016; Rawal et al. 2016; Botchkarev et al. 2017).

Not all unreparable DNA damages seem to be amenable to adaptation. For instance, while a single unrepaired DSB allows adaptation, two simultaneously induced DSBs do not (Lee et al. 1998). One important factor appears to be the amount of single-stranded DNA that accumulates during the arrest, as supported by the fact that the adaptation defect of *yku70Δ* mutant, which exhibits increased resection, is suppressed by limiting resection through deletion of *MRE11* (Lee et al. 1998). Consistently, *cdc13-1* mutant cells placed at restrictive temperature, in which telomeres are uncapped, resected and thus detected as DNA damage, is a widely used setting to study adaptation but the fraction of cells that are able to eventually adapt decreases with increasing temperatures (Toczyski 2006). However, the global amount of ssDNA induced at restrictive temperature in *cdc13-1* cells might exceed that found in cells with two DSBs (Garvik et al. 1995; Westmoreland et al. 2018), suggesting that the nature of the damage, and not only the amount of exposed ssDNA, might be important for adaptation. Besides, the *cdc13-1*-induced telomere dysfunction at higher temperature also involves more complex molecular mechanisms, such as defective nuclear homeostasis of Cdc13-1 proteins and confounding

temperature-dependent defect of the telomeres themselves (Paschini et al. 2012; Mersaoui and Wellinger 2018), reinforcing the idea that the molecular nature of the damage might impact on adaptation efficiency. Other types of damage, induced by specific treatments, have also been used in vertebrate systems and suggest that adaptation operates only within a range DNA damage load (Yoo et al. 2004; Syljuasen et al. 2006; Rezacova et al. 2011; Kubara et al. 2012).

We recently investigated whether telomerase-negative cells might also undergo adaptation when they activate the DNA damage checkpoint in response to critically short telomeres in a process known as replicative senescence. This new experimental setting we tested is physiologically relevant for human somatic tissues where telomerase is repressed and where cells eventually enter senescence due to telomere exhaustion.

Adaptation in telomere-induced replicative senescence

Telomeres are repeated sequences found at chromosome extremities. By nature, they resemble one side of a DSB but specific proteins are bound to telomeres and collectively form a capping structure that prevents DNA damage checkpoint activation and unwanted repair by NHEJ or HR (Jain and Cooper 2010; Wellinger and Zakian 2012). The length of a telomere is dynamic and varies with each cell cycle. On the one hand, telomeres shorten due to the DNA-end-replication problem. Conversely, as they become shorter, telomeres can be extended by telomerase, a dedicated reverse transcriptase able to add *de novo* telomeric repeats. Telomeres are thus kept at a steady-state length distribution, the homeostasis of which is regulated by hundreds of genes (Kupiec 2014; Harari and Kupiec 2018). In the absence of telomerase, telomeres get continuously shorter as cells divide until the shortest one reaches a critical threshold, activating the DNA damage checkpoint and arresting the cells in replicative senescence (Teixeira 2013).

Replicative senescence is a heterogeneous process at several scales. Independent telomerase-negative cultures display variable senescence onset timings. Even within a telomerase-negative cell culture, subcloned single cells give rise to colonies of heterogeneous sizes, reflecting complex cell proliferation dynamics (Lundblad and Szostak 1989; Enomoto et al. 2002). Thus, a telomerase-negative culture is always a mixture of cells at different stages of senescence. The lack of synchrony in reaching senescence precludes the use of standard assays to study the role of adaptation (Toczyski 2006).

To circumvent this issue, we took advantage of a single-cell microfluidics-based method coupled to live microscopy, which previously allowed us to characterize the dynamics of cell divisions in individual cell lineages in which telomerase activity was experimentally repressed (Xu et al. 2015). In particular, we evidenced in individual lineages the presence of frequent cell-cycle arrests that were followed by more cell divisions and were consequently not terminal senescence arrests. We termed them non-terminal arrests and showed that they were, at least partially, Mec1- and Pol32-dependent. Combined with evidence that recombination factors such as Rad51 and Rad52 are important for cell growth not only as an alternative telomere maintenance mechanism but even early after telomerase inactivation (Lundblad and Blackburn 1993; Le et al. 1999; Khadaroo et al. 2009; Churikov et al. 2014; Fallet et al. 2014; Xu et al. 2015; Claussin and Chang 2016; Lue and Yu 2017), this result suggests that telomerase-negative cells frequently experience telomere-related damage, activate the DNA damage checkpoint and undergo repair events such as break-induced replication (BIR).

In a recent report, we demonstrated that, in addition to repair, adaptation is also a relatively frequent response to checkpoint arrests in telomerase-negative cells, occurring in 2-7% of all cell cycles (Coutelier et al. 2018). Two independent approaches were used: a genetic approach using the adaptation-deficient mutants *cdc5-ad* and *tid1Δ* and a functional approach using a fluorescent reporter to assess cell division with maintenance of the upstream part of the checkpoint pathway, typical of adaptation. Importantly, we did not use strains impaired for repair pathways, as in some adaptation assays (Toczyski 2006). That telomerase-negative cells undergo adaptation even in the presence of functional repair mechanisms begs the question of the cell fate decision between the two pathways. It is possible that the choice simply depends on the type of detected telomeric damage and on how repairable it is. Regarding the nature of the damage, while we do not exclude progressively shortening telomeres reaching a critical threshold as a potential signal, the early timing of the non-terminal compared to terminal arrests makes them hard to reconcile with a model of progressive telomere shortening. We rather speculate that telomere breaks due to replication fork stalling and collapse might trigger these arrests. Whether the molecular nature of the telomere breaks somehow inhibits repair factors and thus channels the cell toward adaptation remains to be investigated further.

Cell survival and genome instability: adaptation as a double-edged sword

The body of evidence supporting a functional role of adaptation in yeast has grown over the past years. It was initially shown that adaptation is important for survival of irradiated diploid cells when HR is compromised, while at the same time promoting genomic instability in the surviving progeny (Galgoczy and Toczyski 2001). This is supported by a recent study showing that adaptation to genotoxic challenge of HR-deficient diploid cells leads to aneuploid progeny that develops some form of resistance to a second genotoxic challenge (Bender et al. 2018). To follow on a similar idea, in another setting, adaptation is required to survive telomere uncapping by loss of Cdc13 in established telomerase-independent survivors (Mersaoui et al. 2015). The Cdc13-independent cells grow in the presence of permanent damage that is tolerated because of mutations in checkpoint genes. In contrast to surviving telomere uncapping, adaptation is dispensable for the emergence of post senescence telomerase-independent survivors (Coutelier et al. 2018).

While adaptation can be thought of as a last-ditch effort to survive, it is also a strong commitment as the cost of bearing extensive genome instability is great. For instance, the longer cells stay with uncapped telomeres (*cdc13-1* at restrictive temperature), the less viable they are once they return to permissive temperature with functional telomeres, and this loss of viability is partially rescued in an adaptation mutant (Toczyski et al. 1997; Klermund et al. 2014). Maintenance of the checkpoint arrest is thus beneficial for cell survival only if the initial damage is eventually removed or repaired. It is interesting to suggest that adaptation, with its typically long timescale, may be part of a bet edging mechanism to maximize survival in the face of DNA damage with unpredictable persistence. This possibility may be tested experimentally and mathematically modelled.

Our recent study extends the functional role of adaptation to telomerase-negative cells and replicative senescence (Coutelier et al. 2018). After adapting to a prolonged cell-cycle arrest, telomerase-negative cells still had substantial proliferation potential, which added to the relatively high frequency of adaptation events makes post-adapted cells a non-negligible fraction of a heterogeneous population of senescing cells. Adaptation is thus a true survival mechanism for telomerase-negative cells experiencing telomere damage. Consistent with the notion that cell proliferation with unrepaired DNA damage is detrimental for genome stability, adaptation contributes to roughly half of the mutation rate observed in senescent cells (Coutelier et al. 2018). Cells undergoing non-terminal arrests followed by adaptation, which occur with increasing frequency as cells divide, would mutate and progressively accumulate in the senescing population. This model partially explains the finding that the mutation rate of telomerase-negative cells increases over time as they lose proliferation potential (Hackett et al. 2001; Hackett and Greider 2003; Coutelier et al. 2018). Because precancerous cells can likewise accumulate high level of genome instability due to dysfunctional telomeres (Maciejowski and de Lange 2017), we suggest that similar adaptation events could occur in aging human cells, contributing to the early stages of tumorigenesis when checkpoints are still functional and leading to genome instability. Consistent with this idea, Plk1 overexpression has been found in numerous human tumours and seems to be involved in neoplastic transformation as well as in resistance to several chemotherapy drugs (Strebhardt and Ullrich 2006; Gutteridge et al. 2016). Plk1-induced genome instability might also be detrimental though if too important, even to cancer cells, as recently reported (de Carcer et al. 2018).

As for other types of damage, adaptation in telomerase-negative cells leads to a wide range of mutations and genome rearrangements (Coutelier et al. 2018). By promoting cell division, adaptation might cause global genome rearrangement starting at the telomeres and initiating a cascade of instabilities through a myriad of possible mechanisms (Galgoczy and Toczyski 2001; Hackett and Greider 2003; Kaye et al. 2004; Vasan et al. 2014; Lopez et al. 2015; Beyer and Weinert 2016; Millet and Makovets 2016). Adapted cells could also explore repair pathways that were not available or utilized during the initial G2/M arrest, such as NHEJ in G1 and BIR or microhomology-mediated end joining (MMEJ) in S/G2 (Galgoczy and Toczyski 2001; Wang et al. 2018). A direct link between the adaptation mechanism and repair pathway choice could be Cdc5/Plk1, since many repair factors are possible substrates for Cdc5 kinase activity, including Sae2 and Mms4 (Donnianni et al. 2010; Matos et al. 2011; Gallo-Fernandez et al. 2012; Schwartz et al. 2012; Szakal and Branzei 2013). Similarly, in human cells, CtIP (ortholog of Sae2), Mre11, Rad51 and BRCA1 are Plk1 targets (Yata et al. 2012; Chabalier-Taste et al. 2016; Li et al. 2017; Wang et al. 2018). Cdc5/Plk1 might therefore preferentially activate some repair pathways while concurrently enabling adaptation.

Overall, adaptation is a double-edged sword for cells experiencing unrepairable DNA damage. The trade-off is that adapted cells may eventually generate a viable progeny but only at the cost of widespread genome instability. The balance between survival and genome instability might depend on the type of damage. Notably, telomere damage in telomerase-negative cells seems amenable to adaptation with substantial survival rate in the progeny, despite extensive genome instability. For unicellular organisms, increased genomic and phenotypic diversity can drive evolution and “adaptation” in a broader sense. However, adapting to DNA damage that could potentially be repaired can be detrimental. In multicellular organisms and in particular mammals, the benefits of

cell survival through adaptation appear to be largely outweighed by the potential for cancer development, although a positive role of adaptation in the elimination of damaged cells through defective cell cycle progression might also be plausible. The cell fate decision between repair and adaptation is thus critical and although it is achieved, as a first approximation, through timescale separation, more investigation is needed to understand the intricate relationship between adaptation, repair and genome instability.

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