Abstract: Replication fork stalling generates a variety of responses, most of which cause an increase in single-stranded DNA. ssDNA is a primary signal of replication distress that activates cellular checkpoints. It is also a potential source of genome instability and a substrate for mutation and recombination. Therefore, managing ssDNA levels is crucial to chromosome integrity. Limited ssDNA accumulation occurs in wild-type cells under stress. In contrast, cells lacking the replication checkpoint cannot arrest forks properly and accumulate large amounts of ssDNA. This likely occurs when the replication fork polymerase and helicase units are uncoupled. Some cells with mutations in the replication helicase (mcm-ts) mimic checkpoint-deficient cells, and accumulate extensive areas of ssDNA to trigger the G2-checkpoint. Another category of helicase mutant (mcm4-degron) causes fork stalling in early S-phase due to immediate loss of helicase function. Intriguingly, cells realize that ssDNA is present, but fail to detect that they accumulate ssDNA, and continue to divide. Thus, the cellular response to replication stalling depends on checkpoint activity and the time that replication stress occurs in S-phase. In this review we describe the signs, signals, and symptoms of replication arrest from an ssDNA perspective. We explore the possible mechanisms for these effects. We also advise the need for caution when detecting and interpreting data related to the accumulation of ssDNA.
Keywords: DNA replication; single-stranded DNA; replication stress; genome stability; RPA; *Schizosaccharomyces pombe*; checkpoint; MCM; helicase

1. Introduction

DNA replication stress is a significant contributor to genome instability in cancer and other diseases [1–3]. A general definition of replication stress is a condition that impairs the processivity of the normal replisome (Figure 1). Many forms of replication stress are documented, most of which trigger the Intra-S phase checkpoint to stabilize and repair stalled replication forks. Without stabilizing and repairing the causes and effects of replication stress, the genome becomes vulnerable to damage, mutation, or rearrangement. These three factors, singly or in combination with each other, describe the concept of genome instability. Genome instability carries a potential for chromosome mis-segregation and further damage to the genome and the organism.

![Figure 1. Cartoon of the replication fork showing the replicative polymerases (Pole, Polα, Polδ), the MCM helicase, single-stranded DNA binding protein RPA, and the fork protection complex (FPC) that helps couple components together. The FPC includes molecules TIMELESS (ScTof1, SpSwi1), TIPIN (ScCsm3, SpSwi3), CLASPIN (yeast Mrcl), and AND1 (ScMcf4, SpMcl1). The assignment of leading (Pole) and lagging (Polα/δ) strand polymerases remains controversial, since (Polδ) has roles copying both leading and lagging strands [4]. Numerous additional factors at the replication fork are omitted for simplicity; see [5].](image)

Many intrinsic conditions contribute to replication stress, such as late replicating regions of the genome, repetitive sequences, and collisions between the DNA replication and gene transcription mechanisms (reviewed in [6]). Stress-associated domains within the genome may vary with cell type and cell program, and can be epigenetically regulated. Frequently, replication stress regions define chromosome fragile sites (CFS) that are particularly prone to breakage and associated with accumulation of ssDNA [7]. Indeed, these may contribute to formation of ultrafine anaphase bridges (UFBs) during mitosis. UFBs are proposed to be threads of ssDNA linked to under-replicated DNA at fragile sites.
(e.g., [8,9]). CFS regions are also sensitive to external factors, such as drugs that inhibit DNA replication, disruptions in ribonucleotide metabolism, and oncogene activation [1,6,10]. Not surprisingly, mutations affecting proteins that replicate and repair DNA are also linked to cancer, neurological disorders, aging, and developmental defects (reviewed in [6]).

Whether intrinsic or extrinsic, these insults generate replication fork stress, and can lead to genetic instability. How do cells survive replication fork stress? The classic cell cycle model tells us that replication-associated damage activates a checkpoint-signaling pathway to arrest the cell cycle and promote repair. The checkpoint response should facilitate fork restart, recovery, and re-entry into the cell cycle, or promote apoptosis if the damage is irreparable (reviewed in [11–13]). The formation of single-stranded DNA (ssDNA) is a crucial effector in the response to replication stress.

2. ssDNA Is a Hallmark of Stress

Studies in multiple model systems show that ssDNA accumulation begins during replication fork arrest, when the replicative helicase and DNA polymerases become uncoupled. This leads to increased unwinding of the DNA [14–23]. However, cells without a functional replication checkpoint response cannot limit this signal. Whereas checkpoint-competent cells limit ssDNA accumulation to a few hundred base pairs, checkpoint mutants form additional ssDNA that may span 1 kb of genome between the helicase and polymerases [23–27].

The ssDNA accumulation can occur on both strands, for example, if there is unregulated unwinding ahead of a replication fork exposing both leading and lagging strands, or uncoupling of the polymerase(s) from the helicase. ssDNA may also occur if there is resection during homologous recombination. The accumulation of ssDNA is associated with increased rates of clustered point mutations in yeast and cancer cell lines [28]. Such clustered mutations suggest that exposed ssDNA is at risk for transient hypermutability that may be clinically relevant.

Disease initiation and progression may also be attributed to additional sources of ssDNA. For example, resection from broken DNA ends, replication fork regression, D-loops from recombination strand invasions, and collision between replication and transcription complexes that generates R-loops (reviewed in [6,11,29]). A failure to manage ssDNA can have serious consequences. Replication stress-associated double strand breaks are preceded by increased ssDNA [30]. Increased expression of DNA cytosine deaminases such as APOBEC, which specifically target ssDNA, is observed in some cancers [31]. Even low levels can be dangerous. Cytidine deaminase converts cytidine to uracil, resulting in C > T and C > G mutations. (e.g., [32]). Clusters of APOBEC mutational signatures described in cancer cell lines led to the development of a new descriptor: “kataegis” (Greek for “thunder”) [33,34].

Furthermore, there is evidence that cells with low levels of ssDNA can evade checkpoints, leading to abnormal mitosis, lagging chromosomes, and anaphase bridges [8,29]. These mitotic abnormalities can cause aneuploidy and overall decreased cell survival. Even so, the cells that manage to survive DNA replication stress are at risk for hyper-mutation and genome rearrangements (e.g., [35]). Therefore, managing ssDNA dynamics during genome stress is crucial to cell survival.

An under-investigated question is whether ssDNA is packaged as chromatin. Typically, regions of ssDNA, such as ultrafine anaphase bridges, can be recognized by protein binding, including the ssDNA binding protein RPA (see below), but not by histone markers (e.g., [35]). However, over
30 years ago, Alberts and colleagues suggested a model of histone octamer-ssDNA interactions that maximize charged contacts between ssDNA and histones and hold the ssDNA in place [36]. In their model, a single strand of ssDNA wrapped in a dsDNA-like conformation around a histone does not maximize charged contact zone contacts. Therefore, the interaction between ssDNA and the histone octamer is loose. Instead, they proposed two potential preferred conformations for ssDNA in a nucleosome. In the first, the ssDNA wraps almost halfway around the octamer before looping out and winding back onto the histone in a reverse direction. The second model suggests that two separate ssDNA molecules could enter mid-way along a nucleosome and wind to exit in opposite orientations, thus sharing a single nucleosome (Palter et al., 1979 [37]). The first case suggests that a longer ssDNA molecule is wrapped in a nucleosome. The second prediction might describe a situation at a DNA double strand break, particularly if a nearby octamer were co-opted to maintain ssDNA stability by balancing its charged surfaces.

These predictions have not been explored but are potentially important to our understanding of how nucleosomes repopulate replicated DNA. While little is known about ssDNA structures in chromatin, crystal structures argue that dsDNA-histone interactions are dependent on both the DNA-strand that contacts the histone (e.g., Watson or Crick strand), and the histone-protein sequence at the contact point [37]. Therefore, ssDNA versus dsDNA may have different opportunities to associate with histones. Further, charge changes to histone proteins may alter the octameric structure and affect DNA binding [37]. Clearly, any histones associated with ssDNA cannot interfere with normal ssDNA metabolism, either because they are restricted to certain regions or structures, or because the nature of the interaction leaves the ssDNA accessible.

3. RPA Is the ssDNA Sensor

The central ssDNA binding protein of eukaryotes is the trimeric replication protein A complex (RPA). RPA was first identified for its essential role in DNA replication [38]. However, RPA is multifunctional and also required for modulating DNA repair and recombination [39–46] and maintaining telomeres [47–52]. The yeast Rad52 homologous recombination protein antagonizes RPA during homologous recombination. In turn, Rad52 helps to promote Rad51 binding and RPA replacement [53–56].

RPA participates in checkpoint activation [18,46,57–59] and regulates cell cycle progression [60–62]. RPA modification patterns are complex and include phosphorylation [57,60,63–75], acetylation [76], and sumoylation [77,78].

RPA interactions with ssDNA are regulated to minimize an abundance of irreparable and under-replicated substrate, or, to avoid an accumulation of toxic recombination intermediates [44,79–81] Due to its important role sequestering and stabilizing ssDNA, RPA binding is a critical DNA damage indicator and sensor [18,82,83]. Not surprisingly, RPA is itself a target of the checkpoint [63,66,67,70]. RPA modification after checkpoint activation may limit a cell’s response to damage [82,84,85].

One of the most common agents used to induce replication stress is hydroxyurea (HU). Hydroxyurea starves the cell for nucleotides and robustly arrests DNA synthesis in wild type cells [86,87]. Hydroxyurea is generally not a lethal challenge, unless the checkpoint response system is disrupted [88]. Other DNA damaging agents such as camptothecin and methylmethane sulfonate generate other forms of stress.
Camptothecin (CPT) inhibits topoisomerase activity and generates S-phase specific DNA breaks [89]. Methylmethane sulfonate (MMS) alkylates bases, causing a variety of modifications and adducts that cause DNA replication slowing [90,91].

Mutations in replication proteins may also generate replication stress and cause cancer in human populations and vertebrate models (e.g., [82,92,93]). These genetic mutations disrupt normal replisome function. The single cell fission yeast, \textit{Schizosaccharomyces pombe}, is a convenient model organism to study replication stress. \textit{S. pombe} also has heterochromatic and chromosome features that make it an excellent model for metazoan chromosome instability, e.g., complex centromeres, Thermo-sensitive alleles of essential proteins, such as MCM helicase subunits, cause distinctive forms of stress [35,94]. The majority of temperature-sensitive MCM-helicase mutants (\textit{mcm-ts}) replicate most of their DNA before entering a lethal cell cycle arrest due to accumulated DNA damage, presumably by broken replication forks. In contrast, a \textit{mcm4-degron} mutant has an early replication-failure effect, replicates a small amount of its genome but fails to arrest. These under-replicated \textit{mcm4-degron} cells continue to divide despite accumulating RPA. Stalled and restarted forks are vulnerable to rearrangements (e.g., [95–97]) indicating that the effects of stress are intrinsically destabilizing.

In fission yeast, replication stress can be monitored in live cells by imaging foci formed by fluorescently-tagged proteins, most commonly RPA and Rad52 [35,87,98–103]. Rad52 is a well-established marker for DNA damage and repair via homologous recombination [54,103–105]. While Rad52 foci frequently denote recombination, a subset of Rad52 foci localize to stalled replication forks. These stalled forks lack Rad51 and are presumably not associated with recombination [98,106]. Rad52 signals typically overlap with RPA signal [35,87,98,99,106]. Visual RPA signals are correlated with molecular evidence for ssDNA accumulation and histone H2A.x phosphorylation [19,107,108].

A wild-type population of asynchronously growing fission yeast shows 10%–20% of cells with RPA and/or Rad52 foci [87,103,108]. These are usually single, faint foci that form and resolve during S phase. Few RPA or Rad52 foci accumulate in hydroxyurea-treated wild-type cells during drug treatment. However, wild type cells released from hydroxyurea show a transient increase of RPA and Rad52 signals thirty minutes after release, as the cells complete S phase [87,103]. These symptoms of hydroxyurea recovery are consistent with HR-mediated fork restart or short-track end resection (e.g., [95,103,108–114]). Longer end resection only occurs on collapsed forks in checkpoint mutants or after prolonged incubation [110]. A second spike of RPA and Rad52 foci are observed 3 h after release, and are likely correlated with replication during the next cell cycle.

In contrast, replication checkpoint mutants including \textit{cds1Δ} and \textit{mrc1Δ} steadily increase the numbers of RPA foci during hydroxyurea exposure. The RPA signal observed is higher than the level of Rad52. While Rad52 signal decreases in these mutants after hydroxyurea block and release, RPA levels become more intense over time, generating an bright, pan-nuclear signal [87]. This is consistent with the S-phase checkpoint limiting fork reversal through the activity of Cds1 (CHK1 in humans, Rad53 in \textit{S. cerevisiae}) and Mrc1 (CLASPIN homologue) (e.g., [115–117]). Replication mutants in the MCM helicase (e.g., \textit{mcm4-ts}, \textit{mcm4-dg}) show steadily-increasing accumulation of RPA and Rad52 during replication stress. These foci do not resolve after replication stress ends [35,87].

Significantly, the patterns of RPA accumulation are distinct for different forms of replication stress, ranging from multiple small foci in \textit{mcm4-ts}, to a single large focus in \textit{mcm4-dg}, to massive pan-nuclear staining in \textit{cds1Δ + HU} [35,87] (Figure 2). This indicates that our concepts of “replication stress” and
“fork collapse” likely encompass a range of different molecular structures, depending upon the challenge. There are different patterns of division after challenge as well. The mcm4-M68 mutant replicates much of its DNA during temperature shift and following release, and then enters a damage-checkpoint dependent cell cycle arrest. In contrast, the mcm4-ts-degron mutant synthesizes little DNA, and evade the checkpoint. The mcm4-degron cells continue to divide, causing DNA mis-segregation, aneuploidy and formation of apparent micronuclei. A subset of cds1Δ + HU cells also continues division following release, although the majority of cells remain arrested. Environmental conditions play a role and alter RPA accumulation and/or stability. In our work we have seen that replication instability induced at the same time as incubation at high temperature (37 °C) may alter RPA focus distribution and stability. This effect of temperature on RPA distribution may indicate potential changes to the DNA damage checkpoint, such as those reported by [118].

Figure 2. RPA intensity and localization patterns depend on stimulus and effect, and are visualized in live cells with RPA-CFP. For example, cds1Δ cells exposed to hydroxyurea (HU) for 3 h at 25 °C begin to accumulate pan-nuclear RPA signal, but do not form RPA foci when shifted to 36 °C for 4 h. In contrast, mcm4-ts cells only accumulate RPA foci after 4 h at 36 °C, but not in HU. Both of these mutants exhibit a late replication arrest, and develop widespread nuclear RPA signal, although the mcm4-ts mutant retains a punctate pattern while RPA coalesces into a pan-nuclear bolus in cds1Δ + HU. In contrast, the mcm4-degron is an early-replication arrest phenotype after 4 h at 36 °C, forming discrete and bright RPA foci that we believe are a signal of clustered early origins arrested in S-phase. The wild-type control cells fail to accumulate RPA signal in either HU or temperature conditions. We use a heat map to depict RPA, and foci above threshold are orange to yellow signal (heat map scale, right). Cells in these pictures were grown in minimal medium, and incubated in 12 mM HU at 25 °C, or in a 36 °C water bath.
Finally, we examined \( rad51 \Delta \) to compare replication-checkpoint deficient cells with a repair-deficient strain. We observed substantially higher endogenous levels of RPA and Rad52 in asynchronously growing \( rad51 \Delta \) cells, indicating a baseline of replication stress. The number of RPA and Rad52 foci increased after HU treatment. In contrast to the large RPA masses that accumulated in the checkpoint mutants, RPA remained in discrete puncta in the \( rad51 \Delta \) strain. RecA, the bacterial Rad51 homologue, interacts with the bacterial RPA homologue, single strand binding protein (SSB) [119] to regulate the transfer of ssDNA to RecA. This suggests that even as the \( rad51 \Delta \) strain forms DNA damage in HU, RPA accumulation is blocked, as cells cannot transfer ssDNA to recombination repair.

Clearly, the replicative MCM-helicase is important in generating ssDNA. Yet, conditions that eliminate MCM function can also generate RPA foci [35,87]. This may reflect activity of other helicases or nucleases, end resection, or strand invasion during repair and fork restart.

4. Association between ssDNA and DNA Damage

Although a threshold level of ssDNA exists that determines DNA damage checkpoint activation, the amount of ssDNA required to reach the threshold is not clear. Evidence suggests that RPA is limiting for the checkpoint response, while excessive ssDNA may exhaust RPA protective capacity [85]. Intriguing data from \( S. cerevisiae \) indicate that the enigmatic Rif1 protein may antagonize RPA early in the response to stress [81].

Excess ssDNA is associated with double strand breaks following replication stress, which may be linked to the cells entering mitosis in the presence of ssDNA [30,120–123]. There are dramatic consequences to undergoing nuclear division with persistent DNA damage or ongoing replication: prolonged M phase, lagging chromosomes, chromosome bridges, and cohesion fatigue [124–126]. It is likely that the contributions of ssDNA are under-recognized in many situations, since ssDNA can reduce the signal associated with dsDNA. Most dsDNA dyes are intercalating agents (e.g., [87]). Recent studies identified ultrafine anaphase bridges (UFB), which cannot be seen with typical DNA dyes or histone labels, but are visualized by binding by proteins including RPA and the BLM helicase [3,8,29,35]. This suggests that UFBs are threads of ssDNA. Some evidence suggests that UFBs result from under-replicated DNA at fragile sites (e.g., [8,9]). Clearly these RPA-coated structures evade typical checkpoint mechanisms, allowing mitosis to occur. This may reflect ssDNA formed as a result of mitosis (and, thus, RPA binding after mitotic entry, past the point of checkpoint restraint), or that not all forms or structures of RPA are capable of nucleating checkpoint activation.

There are practical and technical considerations in determining how ssDNA formation, RPA binding, and the DNA damage response are linked. For example, how does one choose the signal to detect when hunting for samples in a sparse field? An example of this is in spread nuclei, prepared and stained to detect dsDNA, H2Ax, RPA, and synthesis using a nucleoside analogue. Nuclei that have not spread sufficiently tend to retain the signal for the first three markers that is likely non-specific. Thus, it is vital to detect samples of similar quality and resolution. However, what channel should be used to find spread nuclei? Using dsDNA signal will exclude low-staining samples that may contain high amounts of ssDNA and/or H2Ax; in this case, the highly damaged DNA decreases the dsDNA signal using DAPI stain (Figure 3A). Alternatively, looking only for replicated samples may exclude those
that fail to synthesize large amounts of DNA (Figure 3), and so on. The important issue is to compare like sample to like, and to examine all channels under study in order to detect samples.

**Figure 3.** Detection of samples is dependent on the channel used to find the spread nuclei. (A) checkpoint-deficient mrc1Δ cells were exposed to HU and EdU to determine synthesis during HU block. After immunofluorescent detection, samples were located using the DNA synthesis marker (EdU, yellow). In this field, three spread nuclei are present, but only one is EdU-labeled (i). The others (ii, iii) are highly decorated with DNA damage signal (H2Ax, green), and ssDNA (RPA, red), but have little DNA signal (DAPI, blue). In this case, only the highly-replicated sample would have been found if the others were not nearby. Scale, 15 μm; (B) as a comparison, wild-type cells were treated with HU and EdU as in A, and processed identically. In this case there is sporadic EdU label, and very little RPA indicating that forks are restrained and/or arrested. There is high DAPI signal indicating that dsDNA is stable, and the sprinkling of DNA damage (H2Ax) suggests that the stalled and arrested forks have activated the replication checkpoint and are being monitored. Scale 10 μm.

DNA replication fork collapse is a more dynamic and longer process than previously anticipated [87]. Ongoing synthesis in checkpoint mutants released from drug treatment suggests that replication forks continue progression before finally losing their ability to synthesize DNA, at a time we call the “Fork Collapse Point”. This is an execution point that describes a point of no return, when forks are no longer able to function and/or synthesize DNA. The burden of ssDNA that accumulates during HU treatment of checkpoint mutants, and the fact that cells require RPA and Rad52 to survive replication stress, illustrates that bulk genome replication is not the only source of replicative stress during HU treatment, and this may account for the different patterns of RPA we observe. The full consequences of ssDNA accumulation may not develop until after replication stress, as cells attempt to recover and re-enter the cell cycle. It may be that division in the presence of unresolved ssDNA is the primary cause of genome instability (e.g., [120,123]).
5. Conclusions

We are poised to explore how RPA integrates its roles as a sensor, a signal, and a repair mediator after replication stress using a fission yeast model organism. By signaling damage and then mediating a transition to recombinational repair, ssDNA, and RPA likely play a pivotal role in preventing large-scale genomic mutations that contribute to cancer establishment and progression. Substantial progress in the last few years has led to exciting new questions. How is ssDNA protected to minimize the risk of DNA damage and rearrangement during stress? How does the presence of ssDNA impact the fidelity of chromosome segregation during mitosis? How does this impact genome stability in later cell cycles? Given the substantial conservation of mechanisms that respond to replication stress, studies of ssDNA and RPA are likely to provide fundamental insights into many human diseases associated with genome instability.

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Author Contributions

The authors contributed equally to the writing. Susan L. Forsburg created Figure 1; Sarah A. Sabatinos created Figures 2 and 3.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Macheret, M.; Halazonetis, T.D. DNA replication stress as a hallmark of cancer. Annu. Rev. Pathol. 2015, 10, 425–448.
2. Zhang, F.; Khajavi, M.; Connolly, A.M.; Towne, C.F.; Batiash, S.D.; Lupski, J.R. The DNA replication fostes/mmbir mechanism can generate genomic, genic and exonic complex rearrangements in humans. Nat. Genet. 2009, 41, 849–853.
3. Burrell, R.A.; McClelland, S.E.; Endesfelder, D.; Groth, P.; Weller, M.C.; Shaikh, N.; Domingo, E.; Kanu, N.; Dewhurst, S.M.; Gronroos, E.; et al. Replication stress links structural and numerical cancer chromosomal instability. Nature 2013, 494, 492–496.
4. Johnson, R.E.; Klassen, R.; Prakash, L.; Prakash, S. A major role of DNA polymerase delta in replication of both the leading and lagging DNA strands. Mol. Cell 2015, 59, 163–175.
5. Sabatinos, S.A.; Forsburg, S.L. Preserving the replication fork in response to nucleotide starvation: Evading the replication fork collapse point. In The Mechanisms of DNA Replication; Stuart, D., Ed.; Intech: Rijeka, Croatia, 2013.
6. Zeman, M.K.; Cimprich, K.A. Causes and consequences of replication stress. Nat. Cell Biol. 2014, 16, 2–9.
7. Debatisse, M.; le Tallec, B.; Letessier, A.; Dutrillaux, B.; Brison, O. Common fragile sites: Mechanisms of instability revisited. *Trends Genet.* 2012, 28, 22–32.
8. Chan, K.L.; Palmai-Pallag, T.; Ying, S.; Hickson, I.D. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. *Nat. Cell Biol.* 2009, 11, 753–760.
9. Beeharry, N.; Rattner, J.B.; Caviston, J.P.; Yen, T. Centromere fragmentation is a common mitotic defect of s and g 2 checkpoint override. *Cell Cycle* 2013, 12, 1588–1597.
10. Carr, A.M.; Lambert, S. Replication stress-induced genome instability: The dark side of replication maintenance by homologous recombination. *J. Mol. Cell Biol.* 2013, 12, 208–219.
11. Weinert, T.; Kaochar, S.; Jones, H.; Paek, A.; Clark, A.J. The replication fork’s five degrees of freedom, their failure and genome rearrangements. *Curr. Opin. Cell Biol.* 2009, 21, 778–784.
12. Sperka, T.; Wang, J.; Rudolph, K.L. DNA damage checkpoints in stem cells, ageing and cancer. *Nat. Rev. Mol. Cell Biol.* 2012, 13, 579–590.
13. Dubrana, K.; van Attikum, H.; Hediger, F.; Gasser, S.M. The processing of double-strand breaks and binding of single-strand-binding proteins RPA and Rad51 modulate the formation of atr-kinase foci in yeast. *J. Cell Sci.* 2007, 120, 4209–4220.
14. Zou, L.; Elledge, S.J. Sensing DNA damage through atrip recognition of RPA-ssDNA complexes. *Science* 2003, 300, 1542–1548.
15. Sogo, J.M.; Lopes, M.; Foiani, M. Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. *Science* 2002, 297, 599–602.
16. Walter, J.; Newport, J. Initiation of eukaryotic DNA replication: Origin unwinding and sequential chromatin association of cdc45, RPA, and DNA polymerase alpha. *Nat. Rev. Mol. Cell Biol.* 2006, 8, 617–627.
17. Byun, T.S.; Pacek, M.; Yee, M.C.; Walter, J.C.; Cimprich, K.A. Functional uncoupling of mcm helicase and DNA polymerase activities activates the atr-dependent checkpoint. *Genes Dev.* 2005, 19, 1040–1052.
18. Pacek, M.; Walter, J.C. A requirement for mcm7 and cdc45 in chromosome unwinding during eukaryotic DNA replication. *EMBO J.* 2004, 23, 3667–3676.
19. Lopes, M.; Foiani, M.; Sogo, J.M. Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable uv lesions. *Genes Dev.* 2006, 21, 15–27.
20. Feng, W.; Collingwood, D.; Boeck, M.E.; Fox, L.A.; Alvino, G.M.; Fangman, W.L.; Raghuraman, M.K.; Brewer, B.J. Genomic mapping of single-stranded DNA in hydroxyurea-challenged yeasts identifies origins of replication. *Nat. Cell Biol.* 2006, 8, 148–155.
25. Lucca, C.; Vanoli, F.; Cotta-Ramusino, C.; Pellicioli, A.; Liberi, G.; Haber, J.; Foiani, M. Checkpoint-mediated control of replisome-fork association and signalling in response to replication pausing. *Oncogene* 2004, 23, 1206–1213.

26. Namiki, Y.; Zou, L. Atrip associates with replication protein a-coated ssDNA through multiple interactions. *Proc. Natl. Acad. Sci. USA* 2006, 103, 580–585.

27. Katou, Y.; Kanoh, Y.; Bando, M.; Noguchi, H.; Tanaka, H.; Ashikari, T.; Sugimoto, K.; Shirahige, K. S-phase checkpoint proteins tof1 and mrc1 form a stable replication-pausing complex. *Nature* 2003, 424, 1078–1083.

28. Roberts, S.A.; Sterling, J.; Thompson, C.; Harris, S.; Mav, D.; Shah, R.; Klimczak, L.J.; Kryukov, G.V.; Malec, E.; Mieczkowski, P.A.; *et al*. Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Mol. Cell* 2012, 46, 424–435.

29. Sofueva, S.; Osman, F.; Lorenz, A.; Steinacher, R.; Castagnetti, S.; Ledesma, J.; Whitby, M.C. Ultrafine anaphase bridges, broken DNA and illegitimate recombination induced by a replication fork barrier. *Nucleic Acids Res.* 2011, 39, 6568–6584.

30. Feng, W.; di Rienzi, S.C.; Raghuraman, M.K.; Brewer, B.J. Replication stress-induced chromosome breakage is correlated with replication fork progression and is preceded by single-stranded DNA formation. *G3* 2011, 1, 327–335.

31. Burns, M.B.; Lackey, L.; Carpenter, M.A.; Rathore, A.; Land, A.M.; Leonard, B.; Refsland, E.W.; Kotandeniya, D.; Tretyakov, N.; Nikas, J.B.; *et al*. Apobec3b is an enzymatic source of mutation in breast cancer. *Nature* 2013, 494, 366–370.

32. Stephens, P.J.; Tarpey, P.S.; Davies, H.; van Loo, P.; Greenman, C.; Wedge, D.C.; Nik-Zainal, S.; Martin, S.; Varela, I.; Bignell, G.R.; *et al*. The landscape of cancer genes and mutational processes in breast cancer. *Nature* 2012, 486, 400–404.

33. Taylor, B.J.; Nik-Zainal, S.; Wu, Y.L.; Stebbings, L.A.; Raine, K.; Campbell, P.J.; Rada, C.; Stratton, M.R.; Neuberger, M.S. DNA deaminases induce break-associated mutation showers with implication of apobec3b and 3a in breast cancer kataegis. *eLife* 2013, 2, e00534.

34. Roberts, S.A.; Lawrence, M.S.; Klimczak, L.J.; Grimm, S.A.; Fargo, D.; Stojanov, P.; Kiezun, A.; Kryukov, G.V.; Carter, S.L.; Saksena, G.; *et al*. An apobec cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nat. Genet.* 2013, 45, 970–976.

35. Sabatinos, S.A.; Ranatunga, N.; Yuan, J.-P.; Green, M.D.; Forsburg, S.L. Replication stress in early s phase generates apparent micronuclei and chromosome rearrangement in fission yeast. *Mol. Biol. Cell* 2015, doi:10.1091/mbc.E15-05-0318.

36. Palter, K.B.; Foe, V.E.; Alberts, B.M. Evidence for the formation of nucleosome-like histone complexes on single-stranded DNA. *Cell* 1979, 18, 451–467.

37. Tsunaka, Y.; Kajimura, N.; Tate, S.; Morikawa, K. Alteration of the nucleosomal DNA path in the crystal structure of a human nucleosome core particle. *Nucleic Acids Res.* 2005, 33, 3424–3434.

38. Wold, M.S.; Kelly, T. Purification and characterization of replication protein a, a cellular protein required for *in vitro* replication of simian virus 40 DNA. *Proc. Natl. Acad. Sci. USA* 1988, 85, 2523–2527.

39. Umezu, K.; Sugawara, N.; Chen, C.; Haber, J.E.; Kolodner, R.D. Genetic analysis of yeast RPA1 and its multiple functions in DNA metabolism. *Genetics* 1998, 148, 989–1005.
40. Longhese, M.P.; Plevani, P.; Lucchini, G. Replication factor a is required in vivo for DNA replication, repair, and recombination. *Mol. Cell. Biol.* **1994**, *14*, 7884–7890.

41. Deng, S.K.; Chen, H.; Symington, L.S. Replication protein a prevents promiscuous annealing between short sequence homologies: Implications for genome integrity. *Bioessays* **2015**, *37*, 305–313.

42. Deng, S.K.; Gibb, B.; de Almeida, M.J.; Greene, E.C.; Symington, L.S. RPA antagonizes microhomology-mediated repair of DNA double-strand breaks. *Nat. Struct. Mol. Biol.* **2014**, *21*, 405–412.

43. Firmenich, A.A.; Elias-Arnanz, M.; Berg, P. A novel allele of saccharomyces cerevisiae rfa1 that is deficient in recombination and repair and suppressible by Rad52. *Mol. Cell. Biol.* **1995**, *15*, 1620–1631.

44. Wang, X.; Haber, J.E. Role of saccharomyces single-stranded DNA-binding protein RPA in the strand invasion step of double-strand break repair. *PLoS Biol.* **2004**, *2*, e21.

45. Wolner, B.; van Komen, S.; Sung, P.; Peterson, C.L. Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. *Mol. Cell* **2003**, *12*, 221–232.

46. Parker, A.E.; Clyne, R.K.; Carr, A.M.; Kelly, T.J. The *schizosaccharomyces pombe* Rad11+ gene encodes the large subunit of replication protein A. *Mol. Cell. Biol.* **1997**, *17*, 2381–2390.

47. Audry, J.; Maestrioni, L.; Delagoutte, E.; Gauthier, T.; Nakamura, T.M.; Gachet, Y.; Saintome, C.; Geli, V.; Coulon, S. RPA prevents g-rich structure formation at lagging-strand telomeres to allow maintenance of chromosome ends. *EMBO J.* **2015**, *34*, 1942–1958.

48. Kibe, T.; Ono, Y.; Sato, K.; Ueno, M. Fission yeast taz1 and RPA are synergistically required to prevent rapid telomere loss. *Mol. Biol. Cell* **2007**, *18*, 2378–2387.

49. Luciano, P.; Coulon, S.; Faure, V.; Corda, Y.; Bos, J.; Brill, S.J.; Gilson, E.; Simon, M.N.; Geli, V. RPA facilitates telomerase activity at chromosome ends in budding and fission yeasts. *EMBO J.* **2012**, *31*, 2034–2046.

50. McDonald, K.R.; Sabouri, N.; Webb, C.J.; Zakian, V.A. The pf1 family helicase pfh1 facilitates telomere replication and has an RPA-dependent role during telomere lengthening. *DNA Repair (Amst)* **2014**, *24*, 80–86.

51. Wang, X.; Baumann, P. Chromosome fusions following telomere loss are mediated by single-strand annealing. *Mol. Cell* **2008**, *31*, 463–473.

52. Ono, Y.; Tomita, K.; Matsuura, A.; Nakagawa, T.; Masukata, H.; Uritani, M.; Ushimaru, T.; Ueno, M. A novel allele of fission yeast Rad11 that causes defects in DNA repair and telomere length regulation. *Nucleic Acids Res.* **2003**, *31*, 7141–7149.

53. New, J.H.; Sugiyama, T.; Zaitseva, E.; Kowalczykowski, S.C. RAD52 protein stimulates DNA strand exchange by Rad51 and replication protein a. *Nature* **1998**, *391*, 407–410.

54. Lisby, M.; Barlow, J.H.; Burgess, R.C.; Rothstein, R. Choreography of the DNA damage response: Spatiotemporal relationships among checkpoint and repair proteins. *Cell* **2004**, *118*, 699–713.

55. Feng, Z.; Scott, S.P.; Bussen, W.; Sharma, G.G.; Guo, G.; Pandita, T.K.; Powell, S.N. Rad52 inactivation is synthetically lethal with brca2 deficiency. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 686–691.
56. Sung, P. Function of yeast Rad52 protein as a mediator between replication protein a and the Rad51 recombinase. *J. Biol. Chem.* 1997, 272, 28194–28197.

57. Wu, X.; Yang, Z.; Liu, Y.; Zou, Y. Preferential localization of hyperphosphorylated replication protein a to double-strand break repair and checkpoint complexes upon DNA damage. *Biochem. J.* 2005, 391, 473–480.

58. Xu, X.; Vaithiyalingam, S.; Glick, G.G.; Mordes, D.A.; Chazin, W.J.; Cortez, D. The basic cleft of RPA70n binds multiple checkpoint proteins, including Rad9, to regulate atr signaling. *Mol. Cell. Biol.* 2008, 28, 7345–7353.

59. Choi, J.H.; Lindsey-Boltz, L.A.; Kemp, M.; Mason, A.C.; Wold, M.S.; Sancar, A. Reconstitution of RPA-covered single-stranded DNA-activated atr-chk1 signaling. *Proc. Natl. Acad. Sci. USA* 2010, 107, 13660–13665.

60. Anantha, R.W.; Sokolova, E.; Borowiec, J.A. RPA phosphorylation facilitates mitotic exit in response to mitotic DNA damage. *Proc. Natl. Acad. Sci. USA* 2008, 105, 12903–12908.

61. Lee, S.E.; Moore, J.K.; Holmes, A.; Umezu, K.; Kolodner, R.D.; Haber, J.E. Saccharomyces Ku70, Mre11/Rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 1998, 94, 399–409.

62. Longhese, M.P.; Neecke, H.; Paciotti, V.; Lucchini, G.; Plevani, P. The 70 Kda subunit of replication protein A is required for the G1/S and intra-S DNA damage checkpoints in budding yeast. *Nucleic Acids Res.* 1996, 24, 3533–3537.

63. Brush, G.S.; Morrow, D.M.; Hieter, P.; Kelly, T.J. The ATM homologue Mec1 is required for phosphorylation of replication protein a in yeast. *Proc. Natl. Acad. Sci. USA* 1996, 93, 15075–15080.

64. Dutta, A.; Stillman, B. Cdc2 family kinases phosphorylate a human cell DNA replication factor, RPA, and activate DNA replication. *EMBO J.* 1992, 11, 2189–2199.

65. Fotedar, R.; Roberts, J.M. Cell cycle regulated phosphorylation of RPA-32 occurs within the replication initiation complex. *EMBO J.* 1992, 11, 2177–2187.

66. Liu, S.; Opiyo, S.O.; Manthey, K.; Glanzer, J.G.; Ashley, A.K.; Amerin, C.; Troksa, K.; Shrivastav, M.; Nickoloff, J.A.; Oakley, G.G. Distinct roles for DNA-pk, ATM and ATR in RPA phosphorylation and checkpoint activation in response to replication stress. *Nucleic Acids Res.* 2012, 40, 10780–10794.

67. Olson, E.; Nievera, C.J.; Klimovich, V.; Fanning, E.; Wu, X. RPA2 is a direct downstream target for atr to regulate the s-phase checkpoint. *J. Biol. Chem.* 2006, 281, 39517–39533.

68. Shi, W.; Feng, Z.; Zhang, J.; Gonzalez-Suarez, I.; Vanderwaal, R.P.; Wu, X.; Powell, S.N.; Roti Roti, J.L.; Gonzalo, S.; Zhang, J. The role of RPA2 phosphorylation in homologous recombination in response to replication arrest. *Carcinogenesis* 2010, 31, 994–1002.

69. Treuner, K.; Findeisen, M.; Strausfeld, U.; Knippers, R. Phosphorylation of replication protein a middle subunit (RPA32) leads to a disassembly of the RPA heterotrimer. *J. Biol. Chem.* 1999, 274, 15556–15561.

70. Brush, G.S.; Kelly, T.J. Phosphorylation of the replication protein a large subunit in the saccharomyces cerevisiae checkpoint response. *Nucleic Acids Res.* 2000, 28, 3725–3732.

71. Din, S.; Brill, S.J.; Fairman, M.P.; Stillman, B. Cell-cycle-regulated phosphorylation of DNA replication factor a from human and yeast cells. *Genes Dev.* 1990, 4, 968–977.
72. Kim, H.S.; Brill, S.J. Mec1-dependent phosphorylation of yeast RPA1 in vitro. *DNA Repair* 2003, 2, 1321–1335.
73. Liu, J.S.; Kuo, S.R.; Melendy, T. Phosphorylation of replication protein a by S-phase checkpoint kinases. *DNA Repair* 2006, 5, 369–380.
74. Liu, Y.; Kvaratskhelia, M.; Hess, S.; Qu, Y.; Zou, Y. Modulation of replication protein a function by its hyperphosphorylation-induced conformational change involving DNA binding domain b. *J. Biol. Chem.* 2005, 280, 32775–32783.
75. Wang, H.; Guan, J.; Wang, H.; Perrault, A.R.; Wang, Y.; Iliakis, G. Replication protein A2 phosphorylation after DNA damage by the coordinated action of ataxia telangiectasia-mutated and DNA-dependent protein kinase. *Cancer Res.* 2001, 61, 8554–8563.
76. Choudhary, C.; Kumar, C.; Gnad, F.; Nielsen, M.L.; Rehman, M.; Walther, T.C.; Olsen, J.V.; Mann, M. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 2009, 325, 834–840.
77. Burgess, R.C.; Rahman, S.; Lisby, M.; Rothstein, R.; Zhao, X. The Slx5-Slx8 complex affects sumoylation of DNA repair proteins and negatively regulates recombination. *Mol. Cell. Biol.* 2007, 27, 6153–6162.
78. Dou, H.; Huang, C.; Singh, M.; Carpenter, P.B.; Yeh, E.T. Regulation of DNA repair through desumoylation and sumoylation of replication protein a complex. *Mol. Cell* 2010, 39, 333–345.
79. Lee, S.E.; Pellicoli, A.; Vaze, M.B.; Sugawara, N.; Malkova, A.; Foiani, M.; Haber, J.E. Yeast Rad52 and Rad51 recombination proteins define a second pathway of DNA damage assessment in response to a single double-strand break. *Mol. Cell. Biol.* 2003, 23, 8913–8923.
80. Wang, X.; Ira, G.; Tercero, J.A.; Holmes, A.M.; Diffley, J.F.; Haber, J.E. Role of DNA replication proteins in double-strand break-induced recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 2004, 24, 6891–6899.
81. Xue, Y.; Rushton, M.D.; Maringele, L. A novel checkpoint and RPA inhibitory pathway regulated by rif1. *PLoS Genet.* 2011, 7, e1002417.
82. Hass, C.S.; Gakhar, L.; Wold, M.S. Functional characterization of a cancer causing mutation in human replication protein a. *Mol. Cancer Res.* 2010, 8, 1017–1026.
83. Furuya, K.; Miyabe, I.; Tsutsui, Y.; Paderi, F.; Kakusho, N.; Masai, H.; Niki, H.; Carr, A.M. Ddk phosphorylates checkpoint clamp component Rad9 and promotes its release from damaged chromatin. *Mol. Cell* 2010, 40, 606–618.
84. Fanning, E.; Klimovich, V.; Nager, A.R. A dynamic model for replication protein a (RPA) function in DNA processing pathways. *Nucleic Acids Res.* 2006, 34, 4126–4137.
85. Toledo, L.I.; Altmeyer, M.; Rask, M.B.; Lukas, C.; Larsen, D.H.; Povlsen, L.K.; Bekker-Jensen, S.; Mailand, N.; Bartek, J.; Lukas, J. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. *Cell* 2013, 155, 1088–1103.
86. Kim, S.M.; Huberman, J.A. Regulation of replication timing in fission yeast. *EMBO J.* 2001, 20, 6115–6126.
87. Sabatinos, S.A.; Green, M.D.; Forsburg, S.L. Continued DNA synthesis in replication checkpoint mutants leads to fork collapse. *Mol. Cell. Biol.* 2012, 32, 4986–4997.
88. Enoch, T.; Carr, A.M.; Nurse, P. Fission yeast genes involved in coupling mitosis to completion of DNA replication. *Genes Dev.* 1992, 6, 2035–2046.
89. Wan, S.; Capasso, H.; Walworth, N.C. The topoisomerase I poison camptothecin generates a Chk1-dependent DNA damage checkpoint signal in fission yeast. *Yeast* **1999**, *15*, 821–828.
90. Kumar, S.; Huberman, J.A. Checkpoint-dependent regulation of origin firing and replication fork movement in response to DNA damage in fission yeast. *Mol. Cell. Biol.* **2009**, *29*, 602–611.
91. Willis, N.; Rhind, N. Regulation of DNA replication by the S-phase DNA damage checkpoint. *Cell Div.* **2009**, doi:10.1186/1747-1028-4-13.
92. Bagley, B.N.; Keane, T.M.; Maklakova, V.I.; Marshall, J.G.; Lester, R.A.; Cancel, M.M.; Paulsen, A.R.; Bendzick, L.E.; Been, R.A.; Kogan, S.C.; *et al.* A dominantly acting murine allele of MCM4 causes chromosomal abnormalities and promotes tumorigenesis. *PLoS Genet.* **2012**, *8*, e1003034.
93. Shima, N.; Alcaraz, A.; Liachko, I.; Buske, T.R.; Andrews, C.A.; Munroe, R.J.; Hartford, S.A.; Tye, B.K.; Schimenti, J.C. A viable allele of Mcm4 causes chromosome instability and mammary adenocarcinomas in mice. *Nat. Genet.* **2007**, *39*, 93–98.
94. Liang, D.T.; Hodson, J.A.; Forsburg, S.L. Reduced dosage of a single fission yeast MCM protein causes genetic instability and S phase delay. *J. Cell Sci.* **1999**, *112*, 559–567.
95. Lambert, S.; Mizuno, K.; Blaisonneau, J.; Martineau, S.; Chanet, R.; Freon, K.; Murray, J.M.; Carr, A.M.; Baldacci, G. Homologous recombination restarts blocked replication forks at the expense of genome rearrangements by template exchange. *Mol. Cell* **2010**, *39*, 346–359.
96. Kaochar, S.; Shanks, L.; Weinert, T. Checkpoint genes and EXO1 regulate nearby inverted repeat fusions that form dicentric chromosomes in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21605–21610.
97. Mizuno, K.; Lambert, S.; Baldacci, G.; Murray, J.M.; Carr, A.M. Nearby inverted repeats fuse to generate acentric and dicentric palindromic chromosomes by a replication template exchange mechanism. *Genes Dev.* **2009**, *23*, 2876–2886.
98. Bass, K.L.; Murray, J.M.; O’Connell, M.J. Brc1-dependent recovery from replication stress. *J. Cell Sci.* **2012**, *125*, 2753–2764.
99. Sabatinos, S.A.; Mastro, T.L.; Green, M.D.; Forsburg, S.L. A mammalian-like DNA damage response of fission yeast to nucleoside analogs. *Genetics* **2013**, *193*, 143–157.
100. Sanchez, A.; Sharma, S.; Rozenzhak, S.; Roguev, A.; Krogan, N.J.; Chabes, A.; Russell, P. Replication fork collapse and genome instability in a deoxycytidylate deaminase mutant. *Mol. Cell. Biol.* **2012**, *32*, 4445–4454.
101. Ukimori, S.; Kawabata, N.; Shimada, H.; Imano, R.; Takahashi, K.; Yukawa, M.; Tsuchiya, E.; Ueno, M. A double mutant between fission yeast telomerase and RECQ helicase is sensitive to thiabendazole, an anti-microtubule drug. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 264–269.
102. Cavero, S.; Limbo, O.; Russell, P. Critical functions of RPA3/SSB3 in S-phase DNA damage responses in fission yeast. *PLoS Genet.* **2010**, *6*, e1001138.
103. Meister, P.; Taddei, A.; Vernis, L.; Poidevin, M.; Gasser, S.M.; Baldacci, G. Temporal separation of replication and recombination requires the intra-S checkpoint. *J. Cell Biol.* **2005**, *168*, 537–544.
104. Meister, P.; Taddei, A.; Ponti, A.; Baldacci, G.; Gasser, S.M. Replication foci dynamics: Replication patterns are modulated by S-phase checkpoint kinases in fission yeast. *EMBO J.* **2007**, *26*, 1315–1326.
105. Lisby, M.; Rothstein, R.; Mortensen, U.H. Rad52 forms DNA repair and recombination centers during S phase. *Proc. Natl. Acad. Sci. USA* 2001, 98, 8276–8282.

106. Irmisch, A.; Ampatzidou, E.; Mizuno, K.; O’Connell, M.J.; Murray, J.M. Smc5/6 maintains stalled replication forks in a recombination-competent conformation. *EMBO J.* 2009, 28, 144–155.

107. Nakamura, T.M.; Du, L.L.; Redon, C.; Russell, P. Histone H2A phosphorylation controls Crb2 recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast. *Mol. Cell. Biol.* 2004, 24, 6215–6230.

108. Bailis, J.M.; Luche, D.D.; Hunter, T.; Forsburg, S.L. Minichromosome maintenance proteins interact with checkpoint and recombination proteins to promote S-phase genome stability. *Mol. Cell. Biol.* 2008, 28, 1724–1738.

109. Steinacher, R.; Osman, F.; Dalgaard, J.Z.; Lorenz, A.; Whitby, M.C. The DNA helicase PFH1 promotes fork merging at replication termination sites to ensure genome stability. *Genes Dev.* 2012, 26, 594–602.

110. Petermann, E.; Orta, M.L.; Issaeva, N.; Schultz, N.; Hellday, T. Hydroxyurea-stalled replication forks become progressively inactivated and require two different Rad51-mediated pathways for restart and repair. *Mol. Cell* 2010, 37, 492–502.

111. Bartek, J.; Bartkova, J.; Lukas, J. DNA damage signalling guards against activated oncogenes and tumour progression. *Oncogene* 2007, 26, 7773–7779.

112. Pedersen, B.S.; De, S. Loss of heterozygosity preferentially occurs in early replicating regions in cancer genomes. *Nucleic Acids Res.* 2013, 41, 7615–7624.

113. Guerra, C.E.; Kaback, D.B. The role of centromere alignment in meiosis I segregation of homologous chromosomes in *Saccharomyces cerevisiae*. *Genetics* 1999, 153, 1547–1560.

114. Hashimoto, Y.; Puddu, F.; Costanzo, V. Rad51- and Mre11-dependent reassembly of uncoupled Cmg helicase complex at collapsed replication forks. *Nat. Struct. Mol. Biol.* 2012, 19, 17–24.

115. Hu, J.; Sun, L.; Shen, F.; Chen, Y.; Hua, Y.; Liu, Y.; Zhang, M.; Hu, Y.; Wang, Q.; Xu, W.; *et al.* The intra-S phase checkpoint targets DNA2 to prevent stalled replication forks from reversing. *Cell* 2012, 149, 1221–1232.

116. Froget, B.; Blaisonneau, J.; Lambert, S.; Baldacci, G. Cleavage of stalled forks by fission yeast MUS81/EME1 in absence of DNA replication checkpoint. *Mol. Biol. Cell* 2008, 19, 445–456.

117. Cotta-Ramusino, C.; Fachinetti, D.; Lucca, C.; Doksani, Y.; Lopes, M.; Sogo, J.; Foiani, M. EXO1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells. *Mol. Cell* 2005, 17, 153–159.

118. Janes, S.; Schmidt, U.; Ashour Garrido, K.; Ney, N.; Concilio, S.; Zekri, M.; Caspari, T. Heat induction of a novel Rad9 variant from a cryptic translation initiation site reduces mitotic commitment. *J. Cell Sci.* 2012, 125, 4487–4497.

119. Cohen, S.P.; Resnick, J.; Sussman, R. Interaction of single-strand binding protein and RECA protein at the single-stranded DNA site. *J. Mol. Biol.* 1983, 167, 901–909.

120. Lukas, C.; Savic, V.; Bekker-Jensen, S.; Doil, C.; Neumann, B.; Pedersen, R.S.; Grofte, M.; Chan, K.L.; Hickson, I.D.; Bartek, J.; *et al.* 53bp1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. *Nat. Cell Biol.* 2011, 13, 243–253.
121. Chambers, A.L.; Ormerod, G.; Durley, S.C.; Sing, T.L.; Brown, G.W.; Kent, N.A.; Downs, J.A. The Ino80 chromatin remodeling complex prevents polyploidy and maintains normal chromatin structure at centromeres. *Genes Dev.* 2012, 26, 2590–2603.

122. Chambers, A.L.; Downs, J.A. The RSC and Ino80 chromatin-remodeling complexes in DNA double-strand break repair. *Prog. Mol. Biol. Transl. Sci.* 2012, 110, 229–261.

123. Feng, W.; Bachant, J.; Collingwood, D.; Raghuraman, M.K.; Brewer, B.J. Centromere replication timing determines different forms of genomic instability in *Saccharomyces cerevisiae* checkpoint mutants during replication stress. *Genetics* 2009, 183, 1249–1260.

124. Hayashi, M.T.; Karlseder, J. DNA damage associated with mitosis and cytokinesis failure. *Oncogene* 2013, 32, 4593–4601.

125. Crasta, K.; Ganem, N.J.; Dagher, R.; Lantermann, A.B.; Ivanova, E.V.; Pan, Y.; Nezi, L.; Protopopov, A.; Chowdhury, D.; Pellman, D. DNA breaks and chromosome pulverization from errors in mitosis. *Nature* 2012, 482, 53–58.

126. Mankouri, H.W.; Huttner, D.; Hickson, I.D. How unfinished business from S-phase affects mitosis and beyond. *EMBO J.* 2013, 32, 2661–2671.

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