MOLECULAR & CELLULAR ONCOLOGY
2019, VOL. 6, NO. 5, e1648027 (3 pages)
https://doi.org/10.1080/23723556.2019.1648027

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AUTHOR'S VIEWS

Yeast cells can partially revert chromosome segregation to repair late DNA double-strand breaks through homologous recombination

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ABSTRACT

DNA repair in late mitosis sets paradoxical scenarios. Cyclin-dependent kinase (CDK) activity is high, which favors homologous recombination (HR), despite a sister chromatid is not physically close to recombine with. We have found that DNA double-strand breaks partially revert chromosome segregation to find an intact template and repair through HR.

ARTICLE HISTORY
Received 17 July 2019
Revised 19 July 2019
Accepted 23 July 2019

KEYWORDS
DNA double-strand breaks; homologous recombination; chromosome segregation; kinesin; telophase

Main body

DNA double-strand breaks (DSBs) are one of the most toxic forms of DNA damage. DSBs are carcinogenic, but also the key triggering event that kills cancer cells upon radiotherapy and most chemotherapy treatments. Cells have developed several pathways to repair DSBs. They can generally be classified in (i) non-homologous end joining (NHEJ) and, (ii) homologous recombination (HR). The first one works by fusing two DNA ends lightly processed. It is an error-prone mechanism since short deletions, insertions or even chromosome translocations can be generated. On the other hand, HR is considered as error-free. To heal the DSB and restore the original sequence, HR needs the presence of a well aligned sister chromatid to be invaded with a highly processed broken end.[1]

Cells have coupled the choice between different DNA repair pathways to the cyclin-dependent kinase (CDK) activity. Thus, NHEJ is used during G_1 phase, when CDK/cyclins levels are low and no sister DNA is present. On the contrary, HR is preferred in S and G_2 phases, when DNA replication has started (or has been completed) and sister chromatids are available as templates.[2–4]

Late anaphase and telophase constitute short stages within the cell cycle where it is not clear which pathway should be chosen. On the one hand, the biochemical environment of the cell would facilitate HR, as CDK/cyclins levels are still high. Conversely, these stages are similar to G_1 as there not exist a close sister chromatid to recombine with because they have just been segregated. This paradoxical scenario, which can be found in a proportion of tumoral cells treated with chemo- and radiotherapy, has been barely studied. All efforts in understanding what happens when a DSB is generated during mitosis have mainly been focused on cells transiting prophase/metaphase. This issue is probably due to the technical caveats of specifically generating DSBs in late anaphase/telophase. Mammal and human cell cultures can be easily arrested in early mitosis (prophase/metaphase), but not in the latest stages.[5,6] This fact has hindered the study and comprehension of this unique situation.

In a recent publication,[7] we studied DSB repair in *Saccharomyces cerevisiae* cells that can be easily and stably arrested in late anaphase/telophase. Treatment of telophase-blocked cultures with the radiomimetic drug phleomycin led us to see that cells detect DSBs through the hyperphosphorylation of Rad53 kinase (CHK2 in humans). This posttranslational modification is closely linked to the activation of a specific DNA damage checkpoint (DDC). Accordingly, we observed a delay at the telophase-to-G_1 transition by analyzing the kinetics of plasma membrane abscission and the accumulation of the specific G_1 protein Sic1. Furthermore, we demonstrated that the observed delay depends on the DDC. Indeed, cells impaired for this checkpoint (*rad9Δ*; yeast Rad9 is the functional analog to human 53BP1 and BRCA1) were unable to block the G_1 entry.

Fluorescence microscopy monitorization of different chromosome loci revealed a new striking phenomenon. After DSBs, the distance between previously segregated centromere clusters was greatly reduced. Surprisingly, filming *in vivo* short movies unrevealed events of coalescence between sister chromatid loci, such as telomeres and the ribosomal DNA array (rDNA). We also observed *de novo* formation of two types of anaphase bridges (ABs): (i) thick ABs formed by bulgy nuclear masses and (ii) thin ABs with confined trafficking of less condensed DNA across the bud neck (i.e., cytokinetic plane in yeast). Coalescence was also observed when

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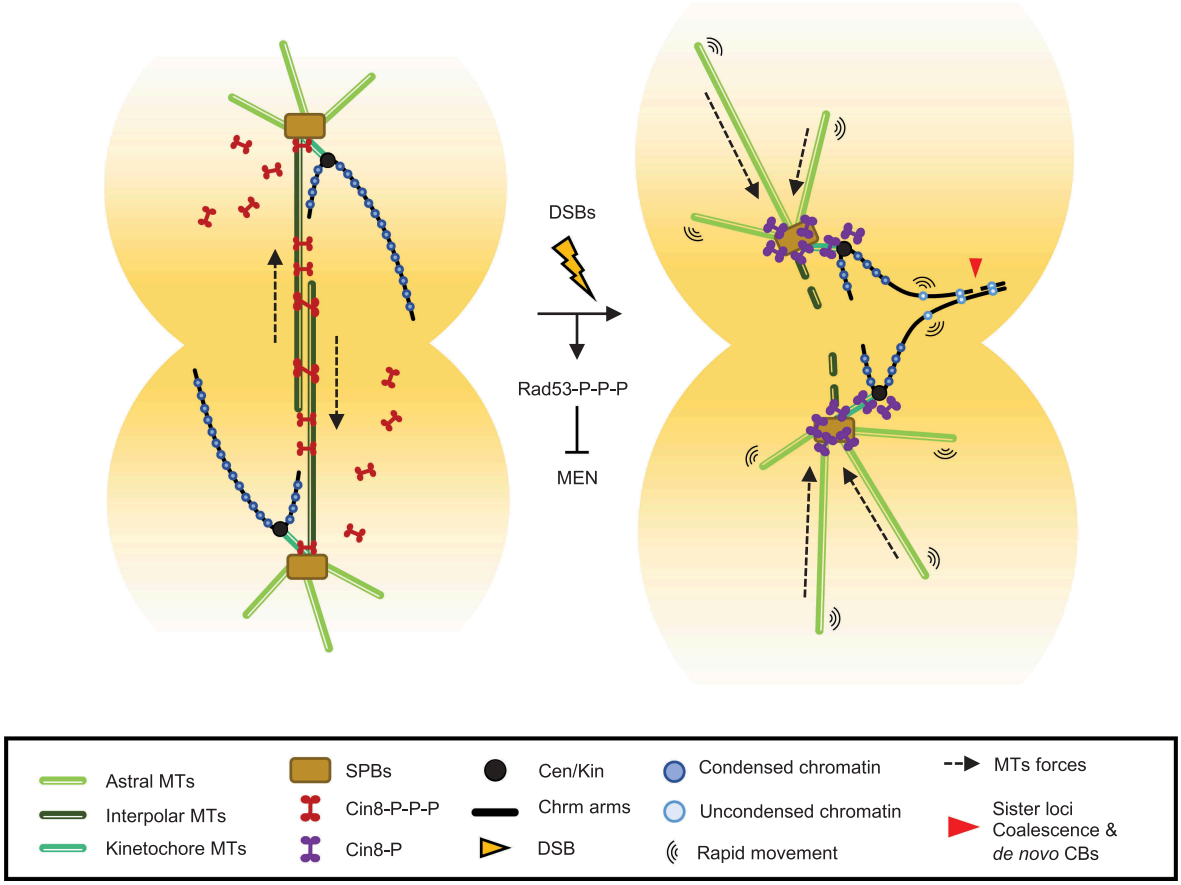

Figure 1. Events underlying sister chromatid approximation and coalescence after DNA double-strand breaks (DSBs) in late anaphase/telophase. DSBs elicit Rad53 hyperphosphorylation (Rad53-P-P-P) in telophase, inhibiting the mitotic exit network (MEN). Alongside, a partial dephosphorylation of Cin8 (from Cin8-P-P-P to Cin8-P), and its relocalization from the interpolar microtubules (MTs) to the spindle pole bodies (SPBs), drive a massive change in MT dynamics. This brings closer the segregated chromosomes (chrm), including centromeres/kinetochores (Cen/Kin) and telomeres, leading to the formation of *de novo* chromatin bridges (CBs) and coalescence between sister loci.

a single DSB was generated through the inducible expression of the sequence-specific endonuclease I-SceI.

It is known that cells mobilize damaged chromatin to the nuclear periphery, where HR is frequently used to repair.[8–10] In telophase, we also visualized a remarkable increase mobility of damaged loci. The DDC is directly responsible of this behavior, since the *rad9Δ* strain did not accelerate interloci movement. In contrast, cells deficient for HR (*rad52Δ*; analog to human BRCA2) were unable to diminish coalescence events and the interloci movement, despite we found a clear contribution in the survival to DSBs in telophase. Comparative survival between G_1, G_2/M and telophase revealed that cells use HR instead of NHEJ.

Finally, we also characterized a massive change in the morphology and dynamics of microtubules (MTs) after DSBs. The elongated mitotic spindle typical from telophase turns into a dynamic and star-shaped structure, in which interpolar MTs are no longer present. Short videomicroscopy movies showed that distances between segregated spindle pole bodies (SPBs, equivalent to human centrosomes) are also reduced, yet constrained, by the enforced astral MTs, which work as the new pushing and pulling forces inside the cell. In addition, we identified that Cin8 (kinesin-5 motor protein) is partially dephosphorylated and relocated from the mitotic spindle to SPBs/kinetochores to drive the approximation of segregated nuclear masses. A conditional Cin8-aid degron did not bring closer the DNA material, and a phosphomimic version of Cin8 for its motor domain (partially impaired to relocate after DSBs) gave rise to reduced survival rates.

Altogether, our exciting results demonstrate that DSBs in telophase lead to a partial reversion of chromosome segregation (Figure 1; adapted from[7]). Cells recognise the damage and trigger the DDC. This promotes a partial dephosphorylation and relocation of Cin8 to drive a massive change in MT dynamics. In addition, chromatin is partly decondensed and increases its mobility, while nuclear masses begin to approximate each other. Finally, coalescence events between segregated sister chromatid loci are produced, so HR can be used to repair the damage and survive.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by the Spanish Ministry of Science, Innovation and Universities (research grant BFU2017-83954-R to Félix Machín), co-financed with the European Commission's ERDF structural funds.

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