DNA damage triggers increased mobility of chromosomes in G1-phase cells

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ABSTRACT **During S phase in *Saccharomyces cerevisiae*, chromosomal loci become mobile in response to DNA double-strand breaks both at the break site (local mobility) and throughout the nucleus (global mobility). Increased nuclear exploration is regulated by the recombination machinery and the DNA damage checkpoint and is likely an important aspect of homology search. While mobility in response to DNA damage has been studied extensively in S phase, the response in interphase has not, and the question of whether homologous recombination proceeds to completion in G1 phase remains controversial. Here, we find that global mobility is triggered in G1 phase. As in S phase, global mobility in G1 phase is controlled by the DNA damage checkpoint and the Rad51 recombinase. Interestingly, despite the restriction of Rad52 mediator foci to S phase, Rad51 foci form at high levels in G1 phase. Together, these observations indicate that the recombination and checkpoint machineries promote global mobility in G1 phase, supporting the notion that recombination can occur in interphase diploids.**

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INTRODUCTION

After DNA damage, cells must pursue timely repair to preserve the integrity of their genomes. Developmental factors, signaling milieu, cell type, and the characteristics of the lesion play a role in the repair systems employed. One of the critical determinants in repair pathway choice is progression through the cell cycle, which introduces complex challenges to nuclear organization and DNA metabolism (Mathiasen and Lisby, 2014; Hustedt and Durocher, 2016). The two main repair strategies used to resolve double-strand breaks (DSBs) are ligation via nonhomologous end joining (NHEJ) and homologous recombination (HR). During NHEJ in *Saccharomyces cerevisiae*, DSB ends are first bound by the Ku70/Ku80 complex before ligation is catalyzed by Dnl4, Lif1, and Nej1 (Palmbos *et al.*, 2005). HR, however, requires a homologous template elsewhere in the genome, for example, either the sister chromatid in S phase or the homologue in a diploid. The commitment to HR is thought to occur following resection of the 5′ ends of the DSB (Mathiasen and Lisby, 2014). The MRX complex (Mre11, Rad50, and Xrs2) is critical for initiating initial resection, while Sgs1, Exo1, and Dna2 are responsible for more extensive resection (Mathiasen and Lisby, 2014). Following single-stranded DNA (ssDNA) generation, replication protein A (RPA) is recruited to the 3′ ends and catalyzes ATR/Mec1 checkpoint signaling (Zou and Elledge, 2003), the recruitment of the Rad52 recombination mediator, and the mitotic recombinase Rad51 (Sung *et al.*, 2003; Lisby *et al.*, 2004). Rad51 filaments then search the genome for homology and catalyze strand invasion and repair (Qi *et al.*, 2015).

The differences in the repair of DSBs in G1 and S and in haploid and diploid cells have been well studied. It has long been appreciated that diploid cells are more resistant to DSBs, which may be a result of the presence of a homologous template throughout the cell cycle (Friis and Roman, 1968; Heude and Fabre, 1993). This difference extends to the G1 phase of the cell cycle, where evidence indicates that G1 diploids are competent for HR and gene conversion (Luchnik *et al.*, 1977; Esposito, 1978; Fabre, 1978; Lee and Petes, 2010). The ability of both haploid and diploid cells to repair DSBs depends on the characteristics of the break itself. So-called "dirty" DSBs that require end processing are resected and prepared

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Abbreviations used: CFP, cyan fluorescent protein; DIC, differential interference contrast; DSB, double-strand break; GFP, green fluorescent protein; HR, homologous recombination; MSD, mean-square displacement; NHEJ, nonhomologous end joining; RFP, red fluorescent protein; ssDNA, single-stranded DNA; WT, wild type; YFP, yellow fluorescent protein.

for HR, while "clean" breaks (formed by endonuclease cutting) are predominantly repaired by NHEJ in haploids (Barlow *et al.*, 2008). In diploid cells, NHEJ is blocked by the a1/α2 repression of *NEJ1* expression (Kegel *et al.*, 2001), suggesting that even clean-break repair events in G1 phase must occur by HR. However, other reports indicate that HR requires S-phase CDK1 activation (Aylon *et al.*, 2004; Ira *et al.*, 2004). In addition, the recruitment of Rad52 to repair centers is cell cycle restricted to S phase in haploid cells (Lisby *et al.*, 2004; Barlow *et al.*, 2008). Thus, it is unclear how recombination is coordinated in the G1 phase.

Proper repair via HR requires the coordination of many enzymatic and cell biological steps. One aspect of this process that has remained poorly understood is the search for homologous sequence in the crowded nucleus following DSB formation (reviewed in Smith and Rothstein, 2017). This search is especially critical in G1-phase diploids, which are limited to interhomologous repair. Time-lapse imaging studies have provided the most insight into this question on a cell biological level. Yeast chromosomal loci are confined to a small volume during S phase (Mine-Hattab and Rothstein, 2012) and to a slightly larger volume during G1 phase (Dion *et al.*, 2013; Lawrimore *et al.*, 2017). The motion regime of yeast chromosomes is essentially subdiffusive (Mine-Hattab *et al.*, 2017), but can be approximated at longer timescales as undergoing Brownian diffusion (Marshall *et al.*, 1997). Following the induction of a site-specific DSB in S-phase cells, loci proximal to the break expand their explored volume 10-fold, in a process known as local mobility. Interestingly, undamaged loci throughout the nucleus also become more mobile, although to a lesser extent, in a process known as global mobility. These increases in explored volume may underlie the homology search process, allowing highly mobile sequences close to the break to move throughout the nucleus to seek homology, aided in the search by the nucleus-wide increased motion permitted by global mobility (Mine-Hattab and Rothstein, 2013).

The mechanisms of these mobility responses have not been definitively identified, although the regulatory underpinnings are becoming clearer. The DNA damage checkpoint activated by Mec1 is critical for both global and local mobility, while the recombination machinery itself, particularly Rad51, Rad52, and Rad54, likely regulates the ability of the checkpoint to trigger increased mobility (Dion *et al.*, 2012; Mine-Hattab and Rothstein, 2012; Smith *et al.*, 2018). Downstream of checkpoint activation, a diverse array of factors have been implicated in the mobility response, including microtubules (Strecker *et al.*, 2016; Lawrimore *et al.*, 2017), actin (Spichal *et al.*, 2016), and chromatin remodelers (Hauer *et al.*, 2017). Importantly, increased chromosomal mobility after DNA damage seems to be remarkably well conserved, and has been observed in human and insect cells, with regulation similar to yeast (Dimitrova *et al.*, 2008; Chiolo *et al.*, 2011; Lottersberger *et al.*, 2015).

Most studies of chromosomal mobility have been performed in S-phase cells, but the response to DNA damage in the G1 phase is less clear. Recent work has indicated that G1-phase haploid cells treated with phleomycin are able to undergo a global mobility response, but the response in diploids, where a repair template is available, has not been examined. To gain insight into G1-phase repair dynamics, we explored whether G1-phase diploid cells undergo global mobility. We find that, compared with S-phase cells, G1-phase diploid cells have an elevated baseline mobility that undergoes a further increase following irradiation, demonstrating that G1-phase diploid cells also induce global mobility. This increase in mobility is regulated similarly as in S-phase cells and is dependent on the DNA damage checkpoint and the recombinase Rad51, consistent with the idea that homology search can occur in the G1 phase of the cell cycle. Surprisingly, despite a strong defect in Rad52 recruitment, we find that Rad51 is recruited to sites of DNA damage in G1 phase, further supporting the notion of interphase recombination. Thus, our results demonstrate that global increased DNA mobility is part of the response to DSBs in interphase diploid cells and that checkpoint and recombination factors regulate this process.

RESULTS AND DISCUSSION

Increased chromosomal mobility after DNA damage occurs in G1-phase cells

To gain insight into G1-phase repair dynamics, we made use of a previously described system (Mine-Hattab and Rothstein, 2012). We imaged cells containing a multiple tandem array of the bacterial *tetO* sequence bound by red fluorescent protein (RFP)-tagged TetR. To correct for the motion of the cell or the movement of the nucleus, we also tagged a structural component of the spindle pole body, Spc110, with yellow fluorescent protein (YFP). As the SPB is embedded in the nuclear wall and largely immobile (Berger *et al.*, 2008), we corrected positional measurements of the *tetO* array, taken every 10 s for 30–70 time points, by the position of the SPB. Using these positional measurements, we calculated a metric known as mean-square displacement (MSD), which models how displacement lengths change over given time intervals (Heun *et al.*, 2001). Previous work has shown that yeast chromosomes undergo confined Brownian diffusion within a small volume at this timescale and thus display plateaued MSD curves (Marshall *et al.*, 1997). The radius of that confined volume (R_c) can be calculated based on the height of the plateau. The *URA3* locus in particular is confined to a volume with an R_c of ~450 nm in S-phase cells (Mine-Hattab and Rothstein, 2012; Smith *et al.*, 2018).

To analyze the mobility of the *URA3* locus in G1-phase cells, we restricted our analysis to unbudded cells with an undivided spindle pole body. We find that G1-phase diploids, like haploids (Heun *et al.*, 2001; Lawrimore *et al.*, 2017), exhibit a higher baseline R_c (Figure 1A, R_c = 570 ± 70 nm) than S-phase cells, possibly due to differences in cohesin loading between G1 phase and S phase (Dion *et al.*, 2013). To examine the mobility of *URA3* in an HR-specific context, we used ionizing radiation to create "dirty" (Barlow *et al.*, 2008), which are preferentially repaired by HR in haploid cells. Breaks formed in this way in G1-phase cells show markers of resection, such as ssDNA formation (through the appearance of RPA foci) and Mec1-dependent checkpoint activation (through the formation of Ddc1 foci), indicating the engagement of the HR pathway (Table 1). We therefore detected damaged G1-phase cells via these Ddc1–cyan fluorescent protein (CFP) foci (Lisby *et al.*, 2004; Barlow *et al.*, 2008), and measured the mobility of the *URA3* locus. Following DSB formation, G1-phase diploid cells undergo an additional increase in R_c (Figure 1A, R_c = 730 ± 100 nm, p value compared with undamaged = 0.02), indicating that global mobility also occurs during G1 phase. This increase in R_c corresponds to a two- to threefold increase in nuclear volume explored.

Genotype	0 Gy	Cells	40 Gy	Cells
RFA1-YFP	30%	59	82%	45
DDC1-CFP	7.0%	143	56%	108
DDC1-CFP+20 mM caffeine	9.2%	109	46%	97
DDC1-CFP rad51Δ	10%	108	54%	97

TABLE 1: Percent of G1 cells with DNA damage foci.

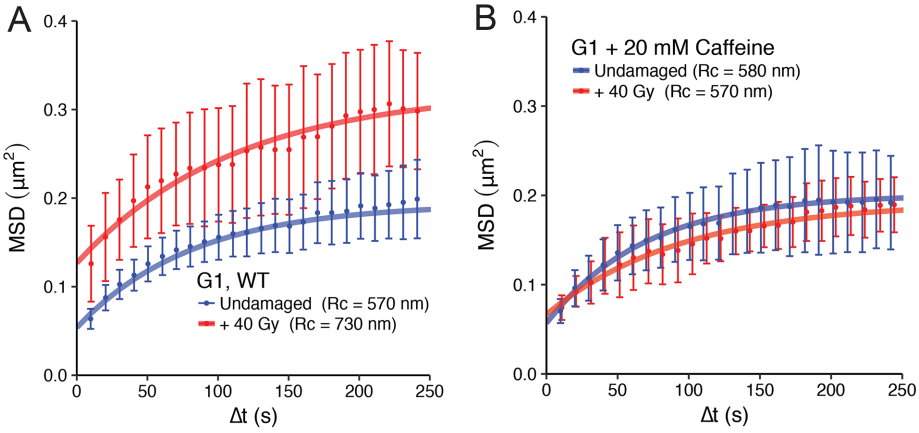

FIGURE 1: Global mobility occurs in G1-phase diploids and is regulated by the DNA damage checkpoint. (A) Undamaged (blue) G1-phase diploids show mobility that is slightly elevated compared with S-phase cells (Mine-Hattab and Rothstein, 2012; Smith *et al.*, 2018). After irradiation (red) there is a further increase in exploration (Wilcoxon rank-sum test *p* value = 0.02). (B) Caffeine treatment blocks global mobility in G1-phase cells, with irradiated cells (red) showing no difference in mobility compared with undamaged cells (blue) (Wilcoxon rank-sum test *p* value = 0.8).

Recent evidence has demonstrated that the DNA damage checkpoint is necessary and sufficient for global mobility in both diploid and haploid cells during S phase (Seeber *et al.*, 2013; Smith *et al.*, 2018). Moreover, damaged G1-phase haploid cells exhibit a Rad9-dependent checkpoint arrest (Siede *et al.*, 1993). To examine whether or not G1-phase global mobility in diploids is regulated by the checkpoint, we treated cells with the PI3K-like kinase inhibitor caffeine (Gentner and Werner, 1975; Hall-Jackson *et al.*, 1999; Heffernan *et al.*, 2002) in the presence and absence of damage to block checkpoint activation. Interestingly, caffeine treatment did not affect Ddc1 focus recruitment (Table 1). However, as in S-phase cells, global mobility was blocked in damaged cells subjected to caffeine treatment (Figure 1B, undamaged: $R_c = 580 \pm 80$ nm, damaged: 570 ± 40 nm, *p* value = 0.8), indicating that the regulatory mechanisms of mobility present in S phase are preserved in G1 phase.

G1-phase global mobility requires the recombinase RAD51

In S-phase cells, global mobility is controlled by a regulatory circuit established by the recombination machinery and the DNA damage checkpoint (Smith *et al.*, 2018). The recruitment of Rad51 to resected DNA stimulates global mobility signaling alongside the DNA damage checkpoint. To test whether these regulatory systems are also present in G1 phase, we examined *rad51*Δ cells. As shown in Table 1, *rad51*Δ did not affect recruitment of the Ddc1 checkpoint protein. When assaying cells for global mobility, we noted a slight increase in the baseline R_c of *rad51*Δ G1-phase cells compared with wild type (WT) cells (Figure 2, $R_c = 670 \pm 40$ nm, *p* value compared with undamaged WT = 0.06). This increase is consistent with earlier reports that *RAD51* deletion in S phase leads to elevated baseline mobility (Dion *et al.*, 2013; Lawrimore *et al.*, 2017). However, following irradiation, there was no further change in mobility (Figure 2, $R_c = 640 \pm 50$ nm, *p* value compared with undamaged *rad51*Δ = 0.7), indicating that Rad51, as in S-phase cells, is required for the global mobility response.

Global mobility in G1-phase diploids is not a consequence of changes in nuclear volume

A simple explanation for changes in the volume explored during global mobility is that the nucleus changes in size or shape following a DSB. An expansion in nuclear volume following damage could contribute to an expansion in the volume that loci explore. Recent work has shed light on a possible link between the DNA damage response and nuclear plasticity (Kumar *et al.*, 2014; Kidiyoor *et al.*, 2016); thus, we wanted to investigate whether global mobility is related to changes in nuclear volume. To address this question, we tagged Nic96, a component of the nuclear pore complex, with green fluorescent protein (GFP) and used it to estimate nuclear volumes in G1-phase diploid cells before and after irradiation. As depicted in Figure 3A, we calculated volumes by assuming a spherical nucleus and measuring the inner diameter of the Nic96 ring. When we applied this method to undamaged cells (Figure 3B), we found that our median volume calculations were only slightly larger than the mean values reported for haploid nuclei (Winey *et al.*, 1997; Jorgensen *et al.*, 2007). Importantly, we observed no change in median nuclear volume following irradiation (0 Gy = 2.7 μm³, 40 Gy = 2.6 μm³, unpaired *t* test *p* value = 0.87), indicating that global mobility is not mediated by gross changes in nuclear morphology.

Rad51 forms foci in G1-phase cells without concomitant formation of Rad52 foci

Previous evidence in haploid cells has suggested that Rad52 activity is restricted to S phase and that Rad52 foci do not form on G1-phase DSBs until Cdc28 activity allows cells to become competent for HR (Barlow *et al.*, 2008). Because we observed Rad51-dependent global mobility in G1-phase diploids, we were curious whether Rad52 foci form in G1-phase diploids and whether they recruit Rad51. To answer this question, we examined the appearance of Rad51 and Rad52 foci in G1- and S-phase diploid cells before and after irradiation. Singly tagged (*YFP-RAD51/RAD51* or *RAD52-CFP/RAD52*; Figure 4B, black points) and doubly tagged (*YFP-RAD51/RAD51 RAD52-CFP/RAD52*; Figure 4B, red points) strains were used. The doubly tagged cells were used to show that neither

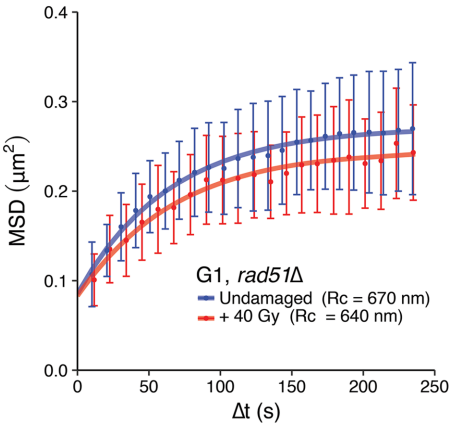

FIGURE 2: *rad51*Δ cells display no global mobility response. Both undamaged (blue) and damaged (red) cells exhibit similar radii of confinement (Wilcoxon rank-sum test *p* value = 0.7).

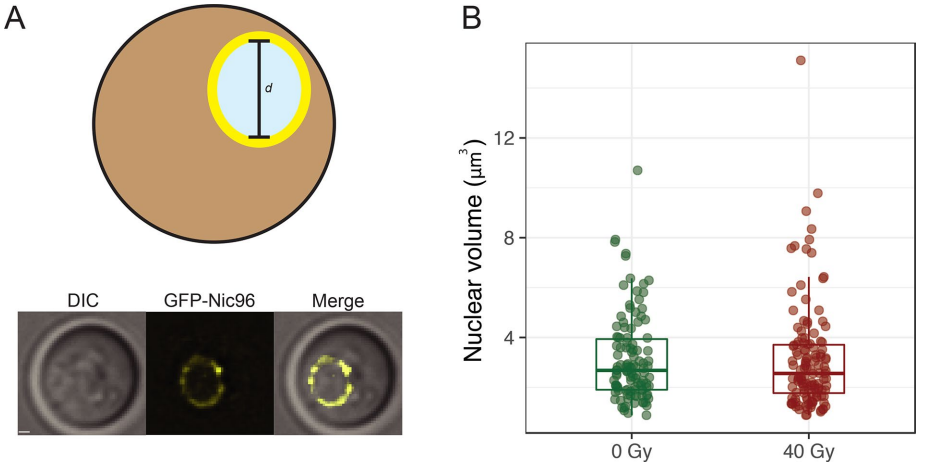

FIGURE 3: Nuclear volume does not change following irradiation of G1-phase diploid cells. (A) A schematic of the method used to estimate nuclear volumes. The inner diameter of the Nic96 ring is used to calculate a spherical volume (see *Materials and Methods*). In the case of ellipsoid nuclei, the longest available diameter is used. Scale bar: 0.6 microns. (B) Scatter plot of calculated nuclear volumes from undamaged (left, median = 2.7 μm^3, N = 114 cells) and irradiated (right, median = 2.6 μm^3, N = 129 cells) (unpaired t test p value = 0.87). Box plots represent median and interquartile range.

tagged protein affects localization of the other. As previously reported, we observe fewer Rad52 foci in G1-phase cells after 40 Gy (Figure 4, A and B). However, Rad51 foci form at high levels in damaged diploid G1-phase and S-phase cells (Figure 4, A and B). These data indicate that, despite the relative scarcity of Rad52 foci, Rad51 is able to access damaged sites in G1 phase.

Implications for the control of HR in G1 cells

While it has long been appreciated that DSBs can be repaired in G1-phase diploid cells, the regulation of DSB repair in interphase is not well understood, and the similarities and differences from S phase remain to be delineated. We show here that the mechanisms of one facet of HR, increased chromosomal mobility, are preserved in G1-phase diploids, and the regulation of chromosome mobility is similar to that observed in S phase. Additionally, we find that, despite a cell cycle restriction of Rad52 foci to S, Rad51 foci frequently form, demonstrating that recombination proteins can be loaded in G1 phase and suggesting that the mobility processes we observe are a product of HR. We have previously shown that the recruitment of recombination factors to sites of damage is critical for mobility, and these observations are consistent with those findings (Mine-Hattab and Rothstein, 2012; Smith *et al.*, 2018). Therefore, we propose that DSBs formed in G1 phase are resected to yield ssDNA overhangs that catalyze the recruitment of checkpoint and recombination complexes. The interactions between these two complexes drive increases in chromosomal mobility to promote HR.

This model raises several interesting questions. First, does the complete HR reaction occur in G1-phase cells? Supporting this view, we observe the loading of Rad51 as well as the induction of global mobility, a possible prerequisite for homology search. On the other hand, the recombination machinery may be loaded in G1 phase, yet remain inactive until S phase begins and Cdk1 activity increases, as observed in haploid cells (Barlow *et al.*, 2008). It is also possible that global mobility is induced alongside checkpoint activation but that S-phase entry is required to stabilize Rad51 presynaptic filaments to drive local mobility and repair. We favor the hypothesis that repair reactions are proceeding to completion in G1 phase based both on previous reports of interphase gene conversion and repair (Luchnik *et al.*, 1977; Esposito, 1978; Fabre, 1978; Brunborg *et al.*, 1980; Kadyk and Hartwell, 1992; Lee and Petes, 2010) and our observations of Rad51 loading and increased chromosomal mobility (Figures 1 and 4). However, as Lawrimore and colleagues also observed global mobility in haploid G1-phase cells following treatment with the radiomimetic drug phleomycin, as well as after endonuclease cutting, it is also possible that the signaling reactions and

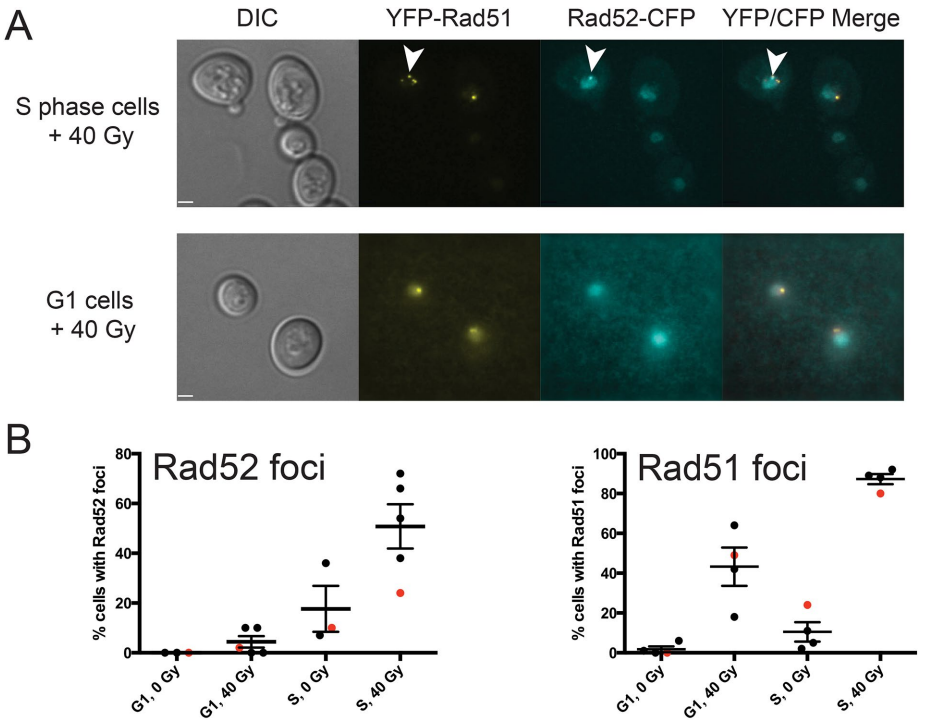

FIGURE 4: Rad51 forms repair foci in G1 phase. (A) Representative images of G1- and S-phase cells depicting YFP-Rad51 and Rad52-CFP foci each tagged in the same strain. White arrowhead indicates a Rad52 focus colocalizing with a Rad51 focus. Scale bar: 2 μm. (B) Measurements of Rad52 (left) or Rad51 (right) focus formation in G1- and S-phase cells, with and without treatment with 40 Gy of gamma radiation. Black points represent the percent foci for each tagged protein in independent experiments. Red points represent an independent experiment in which Rad52 and Rad51 are both tagged in the same cells. Error bars represent 1 SEM for each group of experiments.

downstream mobility evoked by DSB formation are not directly coupled to the completion of interhomologous recombination, as these cells lack a viable repair template (Lawrimore *et al.*, 2017). Still, it seems unlikely that diploid cells would delay repair until S phase, given that the homologue is always available as a template, and the alternative repair strategy, NHEJ, is down-regulated (Kegel *et al.*, 2001).

Second, why is Rad52 focus formation restricted to S? The finding that Rad51 foci form in G1 phase despite low levels of Rad52 foci is, at first, incongruous with the notion that Rad52 foci are required for Rad51 recruitment. However, it is possible that Rad52 is still functional in G1 phase but fails to form a focus, that is, there are not enough molecules to be visualized. In this way, Rad52 could be stimulating Rad51 filament formation and providing regulatory input into the control of global mobility despite a lower level of expression or of binding to resected ssDNA. Alternative mediators such as Rad55, Rad57, and Rad59 are unlikely to play a role in Rad51 focus formation in G1 phase, because Rad51 foci require Rad52 irrespective of the cell cycle (Smith *et al.*, 2018). Thus, we suggest that Rad52 remains functional in G1-phase diploids and promotes the formation of the HR machinery.

The effect of the cell cycle on DSB repair has been long appreciated, but many of the precise details still remain to be resolved. Do broken chromosomes in G1 phase undergo local as well as global mobility? The recruitment of Rad51 to sites of damage suggests that local mobility and homology search are occurring. What are the differences in mobility and repair between G1-phase haploids and diploids? Heterozygosity of the mating-type locus or the presence of a sister homologue may have broad effects on repair pathway choice and attendant phenomena such as increased mobility. How are recombination proteins loaded to different types of breaks in G1 phase, and how does that loading differ from S phase? The reduced recruitment of a variety of repair factors in G1 phase (Barlow *et al.*, 2008) indicates that the cell may take a separate approach to DSBs that form in interphase. In any case, the results presented here show that global increased chromosomal mobility following DSB formation is a facet of the DNA repair response that is present in both G1 and S phases and suggest that the mechanisms of homology search are preserved.

MATERIALS AND METHODS

Strains

All strains are *RAD5+* derivatives of W303 (Thomas and Rothstein, 1989; Zhao *et al.*, 1998). Strains were created as listed in Supplemental Table S1 (Jiang *et al.*, 1996; Ryan *et al.*, 2003; Lisby *et al.*, 2004; Mine-Hattab and Rothstein, 2012; Reid *et al.*, 2016).

Caffeine treatment

Caffeine treatment was performed as described in Barlow and Rothstein (2009). Cells were treated for 30 min with 20 mM caffeine, which was diluted from a freshly prepared 100 mM stock, and irradiated in the presence of caffeine.

γ-Irradiation

Overnight cultures of strains were diluted slightly in fresh medium and allowed to grow for 1 h at 23°C. Aliquots were exposed to radiation using a Nordion 220 ^{60}Co irradiator and were then prepared for imaging.

Microscopy

Microscopy was performed as described previously (Lisby *et al.*, 2004; Mine-Hattab and Rothstein, 2012; Smith *et al.*, 2018). Cells were resuspended at higher density from overnight cultures before being placed upon a 1.4% agarose slab for visualization. Images were acquired on a Leica DM5500B upright microscope using a 1.46 numerical aperture 100× magnification Plan Apochromat lens illuminated with a 100-W mercury arc lamp (Leica Microsystems). High-efficiency filter cubes were used for fluorophore imaging (Chroma 41028, Chroma 31044v2, and Chroma 41002C, for YFP, CFP, and RFP, respectively), and images were captured with a Hamamatsu Orca AG cooled digital CCD (charged-coupled device). All microscopy was performed at 23°C. Analysis of image data was performed with Volocity software (Perkin-Elmer). For mobility experiments, we captured 15 *z*-stacks spaced by 300 nm every 10 s for 70 time points. Exposure times were as follows: differential interference contrast (DIC) (30 ms), YFP (100 ms), RFP (100 ms), and CFP (2s for Ddc1-CFP). DIC and CFP images were taken once before time-lapse imaging began. For Rad51 and Rad52 focus experiments, we captured 21 *z*-stacks spaced as for mobility experiments. DIC exposure time was 30 ms, and the YFP and CFP exposure time was 800 ms.

Nuclear volume calculations

Multiple *z*-stacks were obtained as for mobility experiments. The section with the largest diameter was selected, and three inner-diameter measurements were made and averaged for the GFP-Nic96 ring. When the nucleus was ellipsoid, the longest available diameter was measured. These diameters were used to calculate spherical volumes and are likely to be overestimates. Statistical comparisons between irradiated and undamaged cells were made using an unpaired *t* test.

Data analysis and statistics

Analysis and statistics were performed as previously described (Smith *et al.*, 2018). We calculated mean MSD plots from the population of cells in each experiment as well as values for each individual cell. These individual values were used to calculate ±SEM values for each experiment and Wilcoxon rank-sum tests (Mann and Whitney, 1947) to evaluate significance. A table of all results and test values can be found in Supplemental Table S2. Analyses were performed in R (R Core Team, 2016). All code is available upon request.

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