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REVIEW

Assembling nuclear domains: Lessons from DNA repair

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Eukaryotic nuclei are organized into nuclear domains that unite loci sharing a common function. These domains are essential for diverse processes including (1) the formation of topologically associated domains (TADs) that coordinate replication and transcription, (2) the formation of specialized transcription and splicing factories, and (3) the clustering of DNA double-strand breaks (DSBs), which concentrates damaged DNA for repair. The generation of nuclear domains requires forces that are beginning to be identified. In the case of DNA DSBs, DNA movement and clustering are driven by actin filament nucleators. Furthermore, RNAs and low-complexity protein domains such as RNA-binding proteins also accumulate around sites of transcription and repair. The link between liquid–liquid phase separation and actin nucleation in the formation of nuclear domains is still unknown. This review discusses DSB repair domain formation as a model for functional nuclear domains in other genomic contexts.

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Nuclear domains: Organizing genomic transactions

Advances in chromosome conformation capture are yielding increasingly comprehensive three-dimensional views of eukaryotic chromatin and have established nuclear domains as a principal organizational element of the genome. At present, efforts to understand the factors involved in domain assembly and how domain structure dictates genomic function are well underway.

The past 30 years have witnessed increased use of cytological and microscopy techniques that offer visual clues into the large-scale organization of the nucleus. Pioneering studies by the Cremer laboratory garnered the first evidence that chromosomes occupy fixed positions during interphase (Cremer et al., 1982). Chromosome painting subsequently found that small, gene-dense chromosomes tend to congregate in the center of the nucleus while gene-poor chromosomes localize to the nuclear periphery (Croft et al., 1999). Moreover, within a given chromosome, gene-rich euchromatin is spatially segregated from gene-poor heterochromatin. More recently, advances in live-cell imaging have allowed for the direct visualization of genome architecture. Using superresolution microscopy, multiple nonrepetitive targets from adjacent loci can be resolved with accuracy (Beliveau et al., 2015; Boettiger et al., 2016; Kundu et al., 2017). In the setting of DNA damage, chromosomal domains undergoing repair may be visualized by studying discrete structures termed DNA repair foci that arise and resolve following the application of genotoxic agents. Foci are thought to represent large numbers of double-strand break (DSB) repair proteins interacting at the site of DNA damage. Notably, these large repair domains are often organized into distinct subdomains within foci or stripes (Bekker-Jensen et al., 2006; Altmeyer et al., 2015; Tsouroula et al., 2016).

In contrast to the large-scale view of chromatin organization provided by microscopy, high-throughput chromosome capture generates global interaction contact maps that show genomic loci are clustered in large compartments (A and B) and subcompartments termed topologically associated domains (TADs; Dekker et al., 2013). TADs delineate interaction domains on the scale of hundreds of kilobases. While much effort over the past decade has focused on understanding TAD formation, the impact of foci clustering on higher-order genome organization is still unclear. For example, TADs maintain epigenetic control over gene expression by facilitating local interactions between promoter and enhancer regions; however, at the level of megabases, transcribed genes assemble within a limited number of specialized protein domains (Altmeyer et al., 2015; Chong et al., 2018; Sabari et al., 2018). These "transcription factories" are thought to concentrate machinery at active genes to coordinate transactions at hundreds of gene promoters. Thus, distinct drivers of chromatin organization are likely at play. Recent observations suggest that the CCCTC-binding factor CTCF and structural maintenance of chromosome cohesin complexes work together to establish chromatin loops within TADs (for review, see Szabo et al., 2019). In contrast, actin proteins and

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intrinsically disordered proteins (IDPs) also accumulate at transcription sites, where they may drive large-scale movements. IDPs promote phase separation or demixing into liquid droplets; for example, FUS/TLS (fused in sarcoma/translocated in sarcoma) contains positively charged low-complexity repeats (e.g., RGG) that promote aggregation in concert with poly (ADP-ribose) polymerase activity (Mastrocola et al., 2013; Altmeyer et al., 2015; Singatulina et al., 2019). Nuclear actin polymerization is also required for the movement of active genes from the nuclear periphery to the interior (Tumbar and Belmont, 2001; Chuang et al., 2006; Percipalle and Visa, 2006; Dundr et al., 2007). Thus, extrinsic forces applied to chromatin are a defining aspect of large-scale nuclear domain assembly.

Similar to the assembly of nuclear domains during transcription, DNA DSB repair occurs on hierarchical scales from the pairing of juxtaposed damaged and undamaged loci in S phase to the clustering of spatially proximate DSBs in G1 phase (Aymard et al., 2017) to the merging of DSB foci across micron distances in S/G2 phase (Lisby et al., 2003; Aten et al., 2004; Neumaier et al., 2012; Caron et al., 2015; Caridi et al., 2018; Schrank et al., 2018). Akin to transcription, multiple factors contribute to chromatin motion. The degree to which movement reflects intrinsic changes to the chromatin fiber (e.g., by chromatin remodeling complexes) or extrinsic forces applied to chromatin (e.g., IDPs and the nuclear cytoskeleton) is a subject of rigorous study and debate (Hurst et al., 2019). A functional role for the actin-nucleating Arp2/3 complex has emerged as a driver of large-scale chromosomal movements during DSB repair. In this review, we will primarily discuss the role of nuclear actin polymerization in generating domains where multiple DSBs are processed for repair and address the possible role of phase separation in this process.

Determinants of DNA DSB repair pathway choice

DSB repair is performed by multiple pathways. The decision to use one pathway over another is influenced by cell cycle stage, the chromatin state, and the complexity of the lesion itself (Symington and Gautier, 2011). A critical determinant of DSB repair pathway choice is the processing of the broken DNA ends, an event that can be used to classify repair pathways: pathways that require end-processing via resection, the process that generates a 3′ single-strand DNA (ssDNA) overhang, and pathways that proceed with minimal or no end-processing. End-joining mechanisms involve unprocessed ends (e.g., nonhomologous end-joining [NHEJ]) or minimally processed ends. In contrast, mechanisms that rely on long tracks of homologous sequences for repair (e.g., homology-directed repair [HDR] or single-strand annealing [SSA]) involve extensive DNA end-resection.

NHEJ promotes rapid ligation of DSB ends and is the prominent repair pathway employed throughout the cell cycle apart from S phase. NHEJ is initiated when the Ku70–Ku80 heterodimer (KU) loads onto DSB ends and recruits the catalytic subunit of DNA-PK (DNA-PKcs). KU competes with the Mre11–Rad50–Nbs1 (MRN) complex at DSB ends (Mimitou and Symington, 2010). NHEJ requires DNA-PK, the Artemis nuclease, DNA ligase IV, Xrcc4, and Xlf (Neal et al., 2014; Chang and Lieber, 2016). NHEJ is active throughout interphase and thus provides the critical mode of DSB repair before DNA replication and the generation of a sister chromatid, the homologous template used in vertebrates for HDR.

DNA end-resection is initiated by the MRN complex in which Mre11 acts as an endo- and exonuclease to generate a 3′ DNA overhang. The Nbs1 subunit (Xrs2 in yeast) recruits the phosphoinositide 3-kinase (PI3K)–like kinase ATM (Tel1 in yeast) to the DSB site. ATM, an upstream checkpoint kinase, phosphorylates multiple substrates involved in the DNA damage response (Matsuoka et al., 2007), including the H2AX histone variant on Ser139 (called γH2AX), which spreads for megabases around the DSB site (Rogakou et al., 1999). Endonucleolytic cleavage by Mre11 occurs upstream of DSBs bound by the KU complex and induces a nick in the 5′ strand via the endonucleolytic activity of Mre11 (Garcia et al., 2011). Mre11 processing is potentiated by CtIP (Sae2 in yeast). Oligonucleotides are subsequently exonucleolytically cleaved in a 3′ to 5′ direction toward the break site, which generates a short single-stranded 3′ DNA overhang. Similarly, DSB lesions that are chemically modified or blocked by bulky adducts cannot be ligated by the NHEJ machinery and instead are resected to remove the adduct (Aparicio et al., 2016; Hoa et al., 2016). This activity is regulated by both cyclin-dependent kinase 2 in S and G2-phase cells (Huertas et al., 2008; Peterson et al., 2011; Cannavo and Cejka, 2014; Anand et al., 2016) and by the ataxia telangiectasia and Rad3-related protein (ATR) following DNA damage (Peterson et al., 2012). By restricting cyclin-dependent kinase–mediated activation of CtIP to S and G2, resection is coordinated to occur after DNA replication when a sister chromatid is present for repair. Thus, DSB resection and consequent repair by homology-directed mechanisms compensates when KU is unable to orchestrate the joining of complex ends. Down-regulation of the MRN complex or CtIP by siRNA or shRNA inhibits early resection (Zhou et al., 2014). In addition, Mre11 endonuclease and exonuclease activities may be abrogated by the small-molecule inhibitors mirin and PFM01, respectively (Dupré et al., 2008; Shibata et al., 2014).

Following processing by the MRN/CtIP nuclease, long-range resection is performed by the exonuclease Exo1 or the combined efforts of Dna2 and the Sgs1–Top3–Rmi1 complex (Zhu et al., 2008; Mimitou and Symington, 2010). As ssDNA is exposed, it is rapidly bound by replication protein A (RPA). In addition to protecting ssDNA from degradation, RPA facilitates DNA unwinding by Sgs1 and directs the nuclease activity of Dna2 in a 5′ to 3′ orientation (Cejka et al., 2010). RPA-bound ssDNA also activates the PI3K-like kinase ATR (Mec1 in yeast; Costanzo et al., 2003; Zou and Elledge, 2003).

It is unclear why long-range resection subsequently proceeds over thousands of bases (Zhou et al., 2014) since limited resection is sufficient to expose sequence homology between meiotic chromosomes (Zakharyevich et al., 2010). Moreover, cells successfully undergo HDR between sister chromatids in the absence of Exo1 or Sgs1 (Westmoreland and Resnick, 2016). Extensive resection could facilitate efficient homology search by allowing the generation of longer Rad51 filaments, which may stiffen the DNA fiber (van der Heijden et al., 2007). Rad51 filament nucleation starts when a few Rad51 monomers bind to ssDNA and

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displace RPA. This process is mediated by BRCA2 in mammals and Rad52 in yeast (Sugiyama and Kowalczykowski, 2002; Jensen et al., 2010). Rad51 expression is cell cycle regulated and peaks in S/G2 phase when end resection is taking place and the complementary strand is readily found on the sister chromatid (Johnson and Jasin, 2000). This ensures that in mammalian cells HDR takes place between sister chromatids. Notably, the movement of induced DSBs requires Rad51 (Dion et al., 2012; Miné-Hattab and Rothstein, 2012; Cho et al., 2014).

Whereas resection in the context of adjacent sister chromatids promotes repair fidelity by destining breaks for HDR, resection within highly repetitive sequences infrequently allows SSA of complementary strands (Bhargava et al., 2016). This process leading to DNA deletions is facilitated by Rad52 (Rothenberg et al., 2008). Furthermore, the persistence of DSBs bearing short overhangs favors microhomology-mediated end-joining (MMEJ), a process that restores a linear DNA molecule by eliminating non-homologous sequences flanking the DSB (Bennardo et al., 2008). Given that MMEJ may occur throughout the cell cycle, its mutagenic potential is regulated by RPA, which inhibits the annealing of small microhomologies in *Saccharomyces cerevisiae* (Deng et al., 2014). The spatial segregation of resected DSB ends may shield DSBs from MMEJ in G1, thereby limiting the interactions of resected DSB ends until S phase (Aymard et al., 2017).

Genetic requirements for chromatin motion during repair

The study of chromatin motion during DSB repair provides insight into how cells assemble nuclear domains on a hierarchical scale. DSB mobility and subsequent clustering can be evaluated by studying fixed cells or by live-cell imaging (Fig. 1). Chromatin motion assessed using mean-square displacement (MSD) analysis measures the position of repair foci over time, whereas the anomalous diffusion coefficient α characterizes the degree to which foci explore their environment (Tarantino et al., 2014). In diploid and haploid yeast, a single DSB triggers two responses in the genome: (1) increased local mobility (the MSD of a tagged DSB is significantly higher than that of an undamaged region) and (2) increased global mobility (undamaged chromosomes also move, albeit to a lesser degree; Dion et al., 2012; Miné-Hattab and Rothstein, 2012). The movements of DSB loci in yeast produce MSD curves that plateau at later time points, indicating that damaged sites undergo confined Brownian motion (Marshall et al., 1997; Dion et al., 2012). This increase in chromosome dynamics is time dependent and highly specific for DSBs. Immediately after DSB induction by HO cleavage, DSB movements are actually more confined relative to undamaged chromosomes (Saad et al., 2014). Similarly, single-strand breaks induced by bleomycin or spontaneous lesions in S-phase cells do not exhibit increased motion (Dion et al., 2012, 2013). This suggests that the machinery that elicits chromatin movement is tightly regulated by the DNA damage response that occurs downstream of DSB generation.

In mammalian cells, the amplitude of DSB movements correlates with break complexity and the pathway activated. In contrast to yeast, which repair DSBs predominantly by HDR, mammalian cells repair restriction endonuclease breaks primarily by NHEJ (Manivasakam et al., 2001). Calculation of the MSD of I-SceI–induced DSBs in NIH3T3 cells indicates that these breaks exhibit similar dynamics as intact chromosomes (Roukos et al., 2013). Furthermore, DNA breaks induced by ultrasoft x-rays, which yield predominantly simple DSBs, are rapidly bound by KU and are repaired by NHEJ (Reynolds et al., 2012). Like I-SceI-induced breaks, ultrasoft x-ray–induced DSBs remain positionally stable (Nelms et al., 1998). These early studies suggested that DSBs in yeast exhibit enhanced motion, whereas DSBs in mammalian cells do not (Lemaître and Soutoglou, 2015).

In fact, DSBs undergoing HDR in mammalian cells exhibit enhanced mobility similar to their yeast counterpart. HDR-specific DSB movements can be monitored (1) at DSBs occurring within telomeres of alternative lengthening of telomeres (ALT) cells; (2) by following DNA damage markers specific for HDR, such as Rad51; or (3) by monitoring repair of complex breaks, such as those harboring protein–DNA adducts during S phase. In the absence of telomerase, some transformed cells maintain telomeres by a process called ALT in which telomere ends recombine by homology-directed synthesis (Fasching et al., 2007). Fusion of the nuclease Fok1 to TRF1, a component of the shelterin complex, induces a DSB response within telomeric DNA (Tang et al., 2013). Similar to DSBs undergoing HDR in yeast, ALT telomeres are highly mobile in U2OS cells that express TRF1-FokI (Cho et al., 2014). Interestingly, broken telomeres that merge for recombination exhibit distinct motion properties; "incoming" telomeres have an α coefficient of ~2, indicating directed movement, while "recipient" telomeres have an α coefficient of ~0.8, indicating confined motion. This suggests that telomeres undergoing homology search undergo rapid directional movements that culminate in synapsis. Alternatively, C-rich extrachromosomal circles, which can be formed in ALT cells (Zhang et al., 2019), could potentially undergo directed movements.

DSB movements related to HDR may be observed in mammalian cells by generating breaks with chemically modified complex ends. For example, etoposide, a DNA topoisomerase 2 poison, traps topoisomerase 2 adducts on DNA, which require resection by MRN and CtIP for removal (Aparicio et al., 2016; Hoa et al., 2016). In contrast, DSBs induced by ionizing radiation (IR) are simpler and predominantly repaired by NHEJ in mammalian cells (Mahaney et al., 2009). Relative to the movement of IR-induced DSBs, etoposide breaks exhibit substantially greater mobility in U2OS cells (Krawczyk et al., 2012). Similarly, DSBs generated by the radiomimetic drug neocarzinostatin (NCS) are more mobile in G2 when repaired by HDR, as monitored by Rad52 or RPA, than in G1 when repaired by NHEJ and marked by 53BP1 (Schrank et al., 2018). Interestingly, restriction endonuclease-generated DSBs within transcriptionally active genes load Rad51 in G2 and repair by HDR (Aymard et al., 2014). These HDR breaks migrate into clusters in G1, whereas NHEJ-prone DSBs do not (Aymard et al., 2017). These studies support the notion that DSBs slated for HDR exhibit greater motion than those undergoing NHEJ.

DSB dynamics are often visualized by single-particle tracking of chromosomes tagged by LacO and TetO arrays. This has the

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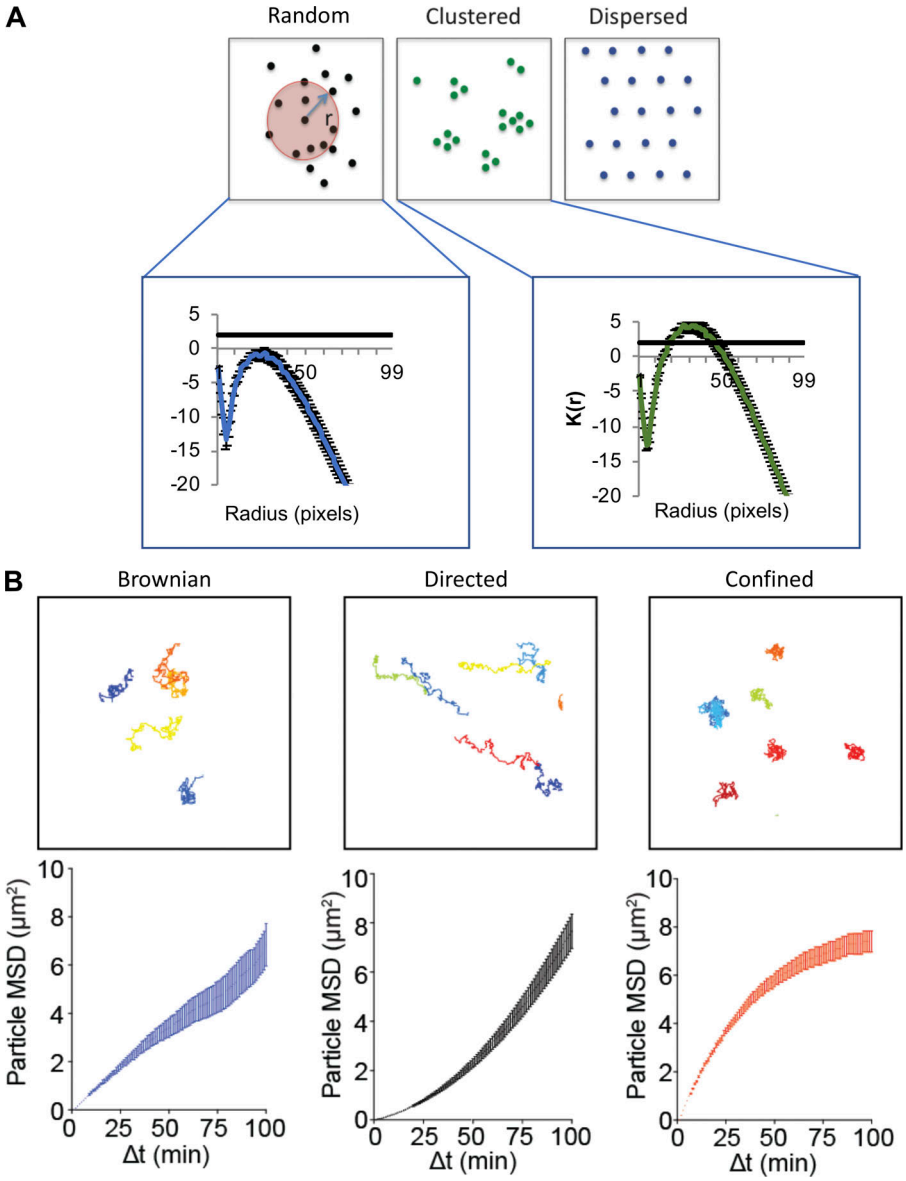

Figure 1. **Tools to characterize DSB movements in fixed and living cells. (A)** Icy bioimaging software (Quantitative Image Analysis Unit, Institut Pasteur) uses the spot detector plug-in and spatial analysis plug-in to assess the clustering of DSB foci in fixed cells (Lagache et al., 2013). The spatial analysis plug-in utilizes Ripley's K-function to assess the deviance of paired points from total randomness. When sufficient pairs of points congregate within a given radius, the software generates a K-function, which crosses a threshold of statistical significance indicating clustering events (green dots and representative green curve). This allows for analysis of DNA damage clustering using markers that are currently not compatible with live-cell imaging, such as Rad51 or γH2AX. **(B)** In living cells, DSB movements may be measured as individual foci using the MATLAB plug in @msdanalyzer (Tarantino et al., 2014). MSD values are calculated using the equation MSD = $\{[x(t + \Delta t) - x(t)]^2\}$, where x is the position of the DSB focus and t is the time. Particle tracking is shown in the top panels. MSD curves are shown in the bottom panels. Freely diffusing particles display unconfined Brownian motion (top left). The MSD plots the average squared distance that particles travel over increasing time intervals. MSD curves that increases proportionally with time are characteristic of Brownian motion (bottom). Particle movements that exceed diffusion are called directed motions (top middle). MSD curves that increase without bounds as time approaches positive infinity are characteristic of particles with directed motion (bottom). Subdiffusive particles moving within a limited territory are said to display confined Brownian motion (top right). Particles undergoing subdiffusion generate MSD curves that plateau at later time intervals.

benefit of tracking the dynamics of undamaged chromosomes; however, it does not inform on the mode of repair at the break site (Roukos et al., 2013). Taken together, these data highlight the need to consider repair pathway choice when interpreting the mobility of damaged chromosomes in mammalian cells. Furthermore, DSB motion may be tracked at different timescales that reveal unique properties of chromatin movement (Fig. 1). For example, when DSB movements are acquired at short time intervals (e.g., 10 or 100 ms) in yeast, broken chromosomes appear less mobile than undamaged loci (Miné-Hattab et al., 2017). This could reflect the stiffening of the DNA structure upon Rad51 loading (Herbert et al., 2017; Miné-Hattab et al., 2017). DNA stiffening may decrease its motion on millisecond timescales. In turn, a stiffened DNA end can navigate the chromatin meshwork more effectively (similar to a needle in a ball of yarn) on minute timescales.

Experiments in yeast established genetic connections between repair by HDR and chromosomal mobility (Fig. 2). The mobility of damaged chromosomes requires several central HDR proteins including the Rad51 recombinase, the Rad54 ATPase, Rad52, and ATR (Dion et al., 2012; Miné-Hattab and Rothstein, 2012; Smith et al., 2018). Importantly, increased global mobility upon DSB generation is observed in cells expressing Rad51 mutants that lack recombinase activity yet form nucleoprotein filaments (Smith et al., 2018). This suggests that global chromatin motion is triggered downstream of resection yet upstream of recombination and may reflect the eviction of nucleosomes by chromatin remodelers (Hauer and Gasser, 2017). Intriguingly, cells that lack both Rad51 and Rad52 exhibit elevated global mobility even in the absence of DNA damage. This effect is blocked by caffeine, an inhibitor of PI3K-like kinases, including Tel1/ATM and Mec1/ATR activity (Smith et al., 2018). These results indicate that (1) the DNA damage checkpoint is sufficient to trigger chromatin movement and (2) Rad52 might restrain chromatin movement until checkpoint activation promotes the loading of Rad51. The HDR machinery is also required for enhanced chromatin movement in other eukaryotic species. The diffusive and directed movements of ALT telomere DSBs require Rad51 in U2OS (Cho et al., 2014).

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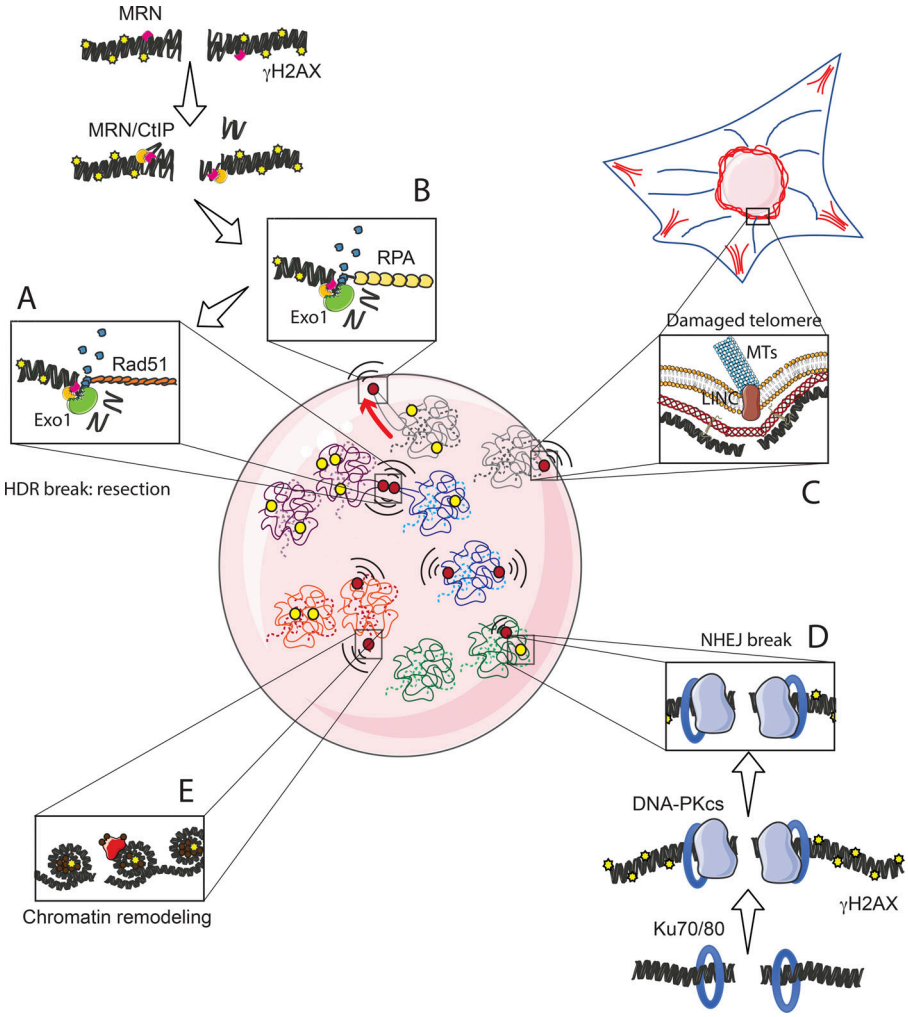

Figure 2. **Modalities and genetic requirements for DSB movement.** A mammalian cell nucleus is shown in the center with damaged and undamaged sites indicated by red and yellow circles, respectively. Dashed lines indicate sister chromatids, while homologous chromosomes share identical colors. Different modalities for DNA DSB mobility are shown: (A) clustering of HDR breaks in mammalian cells, (B) relocalization of heterochromatic DSB in *Drosophila*, (C) cytoplasmic microtubule-driven motion of damaged telomeres, (D) limited mobility of NHEJ breaks, and (E) chromatin remodeling–dependent DSB motion. The initial steps of HDR are shown. The MRN/CtIP nuclease initiates DNA end-resection at sites of DNA DSBs marked by γH2AX. Further resection is brought about by additional nucleases, including Exo1. The resulting ssDNA 3′ overhang is coated with the trimeric ssDNA-binding protein RPA followed by the assembly of the Rad51 recombinase. **(A)** Rad51 is associated with enhanced DSB mobility in yeast and mammalian cells. Rad51, Mre11, and CtIP are required for this enhanced mobility. **(B)** In *Drosophila*, the activities of MRN, CtIP, Exo1, and Blm are required for DSB relocation outside the heterochromatic domain, which takes place before the assembly of Rad51 chromatin filaments (red arrow). Movements in A and B both require nuclear actin polymerization. **(C)** Microtubule (MT) polymerization in the cytoplasm transduces forces to damaged chromatin that generates movement. Microtubule-driven forces are relayed via the LINC complex. **(D)** DSBs that undergo NHEJ have limited mobility. **(E)** DSBs recruit chromatin remodeling complexes, which reorganize nucleosomes, thereby increasing access of repair machinery to the damaged site. Chromatin decompaction facilitated by chromatin remodelers promotes chromosome mobility.

Similarly, DSB movement in *Drosophila melanogaster* requires the activities of the end-processing machinery, including MRN, CtIP, Exo1, and Blm (Chiolo et al., 2011; Caridi et al., 2018), as well as in mammals (Tsouroula et al., 2016). However, relocalization of heterochromatic breaks in *Drosophila* occurs before Rad51 focus formation, suggesting that unlike in yeast, DSB movement precedes Rad51 recruitment and is not driven by homology search (Fig. 2). Notably, the initial step of resection is also required for DSB mobility in U2OS cells. Down-regulation of MRN inhibits clustering of AsiSI-induced DSBs in U2OS cells (Aymard et al., 2017). Furthermore, treatment of U2OS cells with Mirin, a small-molecule inhibitor of Mre11 nuclease (Dupré et al., 2008), inhibits the motion and clustering of Rad52 foci following NCS treatment in U2OS cells (Schrank et al., 2018). The impact of Rad51 loss in these cells is not known.

In contrast, the NHEJ machinery may constrain movement. Upon DSB generation, DNA ends are initially tethered together by a complex containing KU and DNA-PKcs (Soutoglou et al., 2007; Graham et al., 2016). Upon loss of Ku80, the motion of I-SceI–induced DSBs increases from 50 nm/min to 80 nm/min (Soutoglou et al., 2007). Moreover, cells depleted of DNA-PKcs exhibit increased movement and damaged chromosome pairing (Yamauchi et al., 2017). Notably, knockdown of NHEJ machinery also increases the efficiency of HDR (Bennardo et al., 2008). Thus, the increase in movement observed upon depletion of Ku80 or DNA-PKcs might reflect a shift toward HDR-based motion at the break site (Fig. 2).

Roles of the nuclear cytoskeleton in chromosome movements

Work from multiple laboratories has recently defined several roles for nuclear actin in regulating the movements of damaged chromosomes. Since actin was isolated from nuclear extracts of the slime mold *Physarum polycephalum*, it has been identified in nuclei throughout Eukarya (Lestourgeon et al., 1975). Given its abundance in the cytoplasm, biochemical studies implicating actin in nuclear transactions were initially panned as artifacts of contamination (Egly et al., 1984). This skepticism gradually abated as the discovery that actin associates with ARP4/BAF53 in chromatin remodeling complexes and RNA polymerases renewed interest in the field. Notably, while the actin monomer is an essential component of chromatin remodeling complexes, chromatin remodelers do not polymerize actin.

In mammals, actin is shuttled into and out of the nucleus by importin-9 and exportin-6, which bind profilin–actin and

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cofilin–actin complexes, respectively (Stüven et al., 2003; Bohnsack et al., 2006). While specialized nuclei without exportin-6 like *Xenopus laevis* oocytes contain a meshwork of polymerized actin, the majority of somatic nuclei have low levels of F-actin, indicating that most of the pool is globular actin. However, the machinery that polymerizes actin in the cytoplasm is also found in the nucleus (Weston et al., 2012; Virtanen and Vartiainen, 2017; Caridi et al., 2018; Schrank et al., 2018). Specifically, the Arp2/3 complex, WASP, and formins are located in both compartments in somatic cells (Wu et al., 2006; Yoo et al., 2007; Taylor et al., 2010; Belin et al., 2015). The Arp2/3 complex contains seven proteins, including the actin-related proteins Arp2 (44 kD) and Arp3 (47 kD), which share 50% homology with conventional actin (42 kD). In human cells, Arp2 and Arp3, together with the Arp complex subunits ARPC1, ARPC2, ARPC3, ARPC4, and ARPC5, mediate the formation of branched actin structures. Arp2/3 binds to the side of a preexisting mother filament and polymerizes daughter filaments at 70-degree angles. In addition to its nucleation activity, Arp2/3 crosslinks newly nucleated actin filaments with older ones generating a dendritic network of Y-branched structures (Mullins et al., 1998). Polymerization at the barbed ends of this network generates propulsive forces that drive movement (Yarar et al., 1999). In addition, F-actin works with myosins for cargo transport (Pollard, 2016).

Nuclear actin structures arise in mammalian cells following DNA damage with a variety of genotoxic agents including NCS (a radiomimetic antibiotic), methyl-methane sulfonate (an alkylating agent), IR, and restriction endonucleases (Andrin et al., 2012; Belin et al., 2015; Caridi et al., 2018; Schrank et al., 2018). Wiskott–Aldrich syndrome (WAS)–related proteins and the Arp2/3 complex were recently found to localize to DNA during DSB repair (Caridi et al., 2018; Schrank et al., 2018). Nuclear actin polymerization at DSBs was proposed to occur by a two-step mechanism in which WASP localizes to DSB sites and activates Arp2/3 specifically during HDR (Schrank et al., 2018). Notably, when WASP and Arp2/3 were inactivated, DSB movements were dramatically attenuated, and DSB clustering failed to occur. Nuclear actin polymerizes in close proximity to DNA damage foci (as seen by Rad51 staining). Moreover, actin foci track with RPA foci, suggesting that the forces generated by Arp2/3-dependent actin polymerization move DNA damage foci (Fig. 2). These actin foci likely contain short-lived branched actin networks that polymerize in the vicinity of DSBs, thereby enhancing chromatin motion.

Arp2/3 activity does not appear to generate directed break movement in mammalian cells likely reflecting lamins and structural maintenance of chromosome complexes that crosslink and thereby globally constrain chromatin; however, in *Drosophila*, DSBs arising within heterochromatin migrate to the nuclear periphery in a process requiring resection and the activities of CtIP, Exo1, and MRN (Chiolo et al., 2011). This movement occurs before Rad51 loading and is thought to prevent aberrant interchromosomal recombination between repetitive sequences inside the heterochromatic compartment (HC; Fig. 2). Consistent with studies in mammalian cells, *Drosophila* cells polymerize nuclear actin filaments upon DSB generation (Caridi et al., 2018). These filaments are nucleated by the Arp2/3 complex, which colocalizes with DSB sites within the HC domain. Notably, inactivation of Arp2/3 blocks the movement of heterochromatic DSBs and reduces the clustering of euchromatic DSBs induced by IR. These data suggest that nuclear Arp2/3 polymerizes actin to move DSBs to nuclear compartments that are more permissive for HDR. Importantly, the relocalization of heterochromatic DSBs to the periphery is an example of directed movement, while the clustering of euchromatic DSBs occurs by confined Brownian motion (Fig. 1). While Arp2/3 activity is solely required to promote clustering, the directed movement of heterochromatic breaks also requires the activities of myosin I and V, which colocalize with HC sites. Myosin I is thought to serve as molecular dock, while myosin V directs processive movement along actin filaments (Mehta et al., 1999; McIntosh and Ostap, 2016). Accordingly, the directed motion of heterochromatic breaks may reflect their association with myosins that transit along Arp2/3-polymerized filaments.

Diverse actin-based mechanisms for DSB mobility might exist. Cell exposure to methyl-methane sulfonate, which generates primarily single-strand breaks, triggers the polymerization of short nuclear actin filaments (Belin et al., 2015). In this setting, nuclear actin assembly requires formin 2 and Spire 1/2 nucleation factors. Similarly, clustering of DSBs induced in G1 in actively transcribed genes also requires formin 2 (Aymard et al., 2017). Interestingly, F-actin polymerizes in early G1 cells in concert with nuclear expansion and chromatin decompaction (Baarlink et al., 2017). The link between nuclear actin polymerization in postmitotic cells and movement of DSBs in G1 cells remains to be elucidated.

Nuclear actin also forms an integral structural component within a variety of chromatin remodeling complexes, including the INO80, RSC, SWI–SNF, and SWR1-C complexes, which reorganize nucleosomes in an ATP-dependent manner (Hauer and Gasser, 2017). Recent studies have unraveled the mechanism by which INO80-actin/ARP4/BAF53 facilitates nucleosome sliding (Knoll et al., 2018; Zhang et al., 2018). To date, however, these functions have not been shown to directly require polymerized actin and are regulated by actin-related proteins distinct from Arp2/3. It is thought that chromatin decompaction increases the fluidity of chromatin both locally at the break site and globally (Neumann et al., 2012; Seeber et al., 2013).

In contrast to DSBs, the movement of yeast telomeres that localize to the nuclear periphery involves crosstalk between the cytoplasmic and nuclear cytoskeleton. Loss of telomere tethering to the nuclear envelope in yeast enhances DSB motion. Telomeres attach to the nuclear periphery via the KU complex as well as Sir4 and Esc1 (Taddei et al., 2004). DSB generation in budding yeast releases telomeres from the nuclear periphery that diffuse into the interior (Lawrimore et al., 2017). This migration is mediated in part by microtubule polymerization, which is thought to transmit forces generated in the cytoplasm to chromatin by the nucleoskeleton (Fig. 2). Notably, microtubule polymerization is required for the mobility of uncapped telomeres, which resemble DSB ends in mammalian cells (Lottersberger et al., 2015). In this setting, telomere movements also require components of the linker of the nucleoskeleton and

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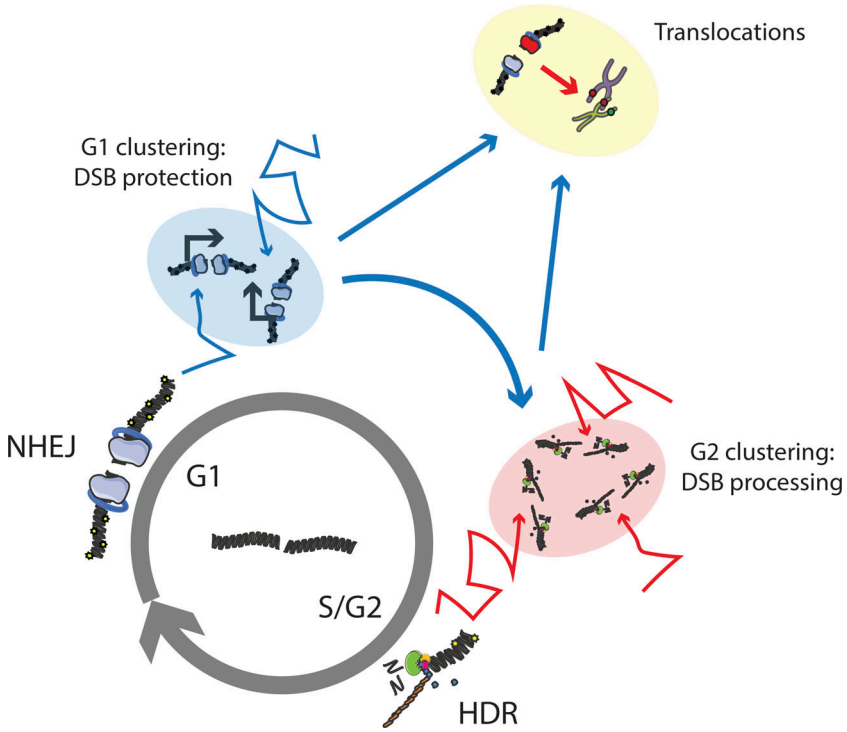

Figure 3. **Roles and risks of DSB repair factories in mammalian cells.** DSBs occurring in the G2 phase of the cell cycle and repaired by HDR cluster into repair domains or factories (shown in pink) to facilitate repair reactions, such as DNA end-processing, the initial step of HDR. DSBs occurring in G1 that cannot be routinely repaired by NHEJ, such as DSBs generated within highly transcribed genes, are thought to cluster into domains (shown in blue) for temporary shielding from repair until G2, when HDR can be initiated. Bringing DSBs in close proximity within spatially distinct domains (shown in yellow) could also favor abnormal chromosome rearrangements, such as translocations.

cytoskeleton (LINC) complex, including SUN1/2 and nesprin-4 as well as the plus-ended microtubule motor kinesin-1. These data suggest that cytoplasmic microtubules distort the nuclear envelope, thereby increasing DSB dynamics at LINC sites. In addition, small microtubules may polymerize from within the nucleoplasm following DNA damage to promote DSB relocalization. One-ended DSBs resolved by break-induced replication were found to travel along microtubules to the nuclear pore subcomplex NUP84 for repair (Chung et al., 2015; Oshidari et al., 2018). These movements require the Kar3 kinesin motor, which captures DNA ends. Taken together, these studies highlight the diverse ways in which the cytoskeleton forms nuclear domains in an effort to preserve genome integrity.

Functions of DSB mobility and clustering

DSB motion is thought to serve multiple purposes. First, it could facilitate homology search, particularly when the homologous chromosome is used as template for repair. Second, DSB motion drives clustering of multiple DNA damage sites into larger repair domains, which could facilitate repair. Finally, movement and/or clustering of DSBs could protect DSBs from inappropriate repair (Fig. 3).

In yeast, DSBs roam a nuclear volume that is 10-fold more expansive than the volume occupied by undamaged chromosomes (Lisby et al., 2003; Dion et al., 2012; Miné-Hattab and Rothstein, 2012). Here, DSB mobility facilitates the search for a homologous chromosome during HDR (Smith and Rothstein, 2017). This concept is supported by studies in ALT cells in which Rad51-coated telomeres undergo directed movements over long distances to find templates for repair (Cho et al., 2014). In contrast to recombination between homologues, chromosomal DSBs undergoing HDR primarily use the sister chromatid as a repair template (Johnson and Jasin, 2000). However, homologue pairing has been reported in thyroid cell lines undergoing DNA damage in G1 in actively transcribed genes. Interestingly, pairing has been shown to be dependent, at least in part, on actin polymerization (Evdokimova et al., 2018).

Given that sister chromatids are tightly bound by cohesin, a genome-wide search for a homologous template is not warranted (Krawczyk et al., 2012). Instead, DSBs in mammalian cells show a tendency to merge into repair clusters (Aten et al., 2004; Caron et al., 2015; Aymard et al., 2017; Schrank et al., 2018). Unlike homology search, which pairs damaged and undamaged loci, DSB clustering occurs when multiple migrating breaks coalesce (Caron et al., 2015). This movement preferentially occurs at DSBs slated for HDR in G1 and during HDR in G2 (Aymard et al., 2017; Schrank et al., 2018).

DSB resection is a critical determinant of repair pathway choice that commits DSBs to HDR. As discussed above, DNA resection and other downstream events in HDR are required for DSB movements. Notably, inhibition of nuclear actin polymerization via Arp2/3 inhibition significantly impairs resection (Schrank et al., 2018). This suggests positive feedback between resection and movement to ensure tight control of resection. DSB resection must be restrained to prevent mutagenic SSA between complementary strands (Ochs et al., 2016). In contrast, sufficient end-processing is required to reveal sequences for homologue recognition. The degree to which a DSB is resected may depend on the extent of DNA damage. Excess DNA damage may destroy target templates and necessitate more resection to reveal distinct sequences for homology search. DSB clustering is one mechanism by which end-processing might be tuned to the degree of damage incurred by the genome. While mammalian cells with a handful of breaks exhibit minimal chromosomal mobility (Roukos et al., 2013), cells that suffer hundreds of DSBs have enhanced motion and clustering (Aten et al., 2004;

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Neumaier et al., 2012; Caron et al., 2015; Aymard et al., 2017). Similarly, DSB movement in yeast positively correlates with more extensive DNA damage (Miné-Hattab and Rothstein, 2012). Increased chromosomal motion could promote clustering of damaged chromosomes, leading in turn to more efficient end-processing by concentrated repair machinery within repair domains (Fig. 3). Indeed, cells expressing the AsiSI restriction enzyme (which generates ~100 DSBs) exhibit nuclear domain formation (Caron et al., 2015; Aymard et al., 2017; Schrank et al., 2018). Thus, DNA repair domains may predominate in the setting of high levels of DNA damage and preferentially form to regulate end-processing. For example, DSBs broken in G1 may cluster to be resected in G2 (Aymard et al., 2017); however, it is worth noting that clustering can also happen with a low number of DSBs (Lisby et al., 2003; Caridi et al., 2018).

Enhanced motion by Arp2/3 specifically targets HDR breaks, suggesting that DSBs undergoing minimal resection like NHEJ or MMEJ need not cluster. In line with this notion, Arp2/3 inactivation does not affect the repair kinetics of AsiSI-induced DSBs in G1 cells, which use NHEJ for repair or the efficiency of NHEJ and MMEJ repair in reporter cell lines (Schrank et al., 2018). However, Arp2/3 activity is required for the long-range resection and repair of DSBs in G2 AsiSI cells. In addition, etoposide-induced breaks that require end-processing for resolution move faster than simpler DSB lesions (Krawczyk et al., 2012). Thus, DSB clustering may reflect an effort to resolve bulky adducts that require resection. Notably, Arp2/3 is still required for efficient HDR at a single locus such as restoring GFP in a DR-GFP assay (Schrank et al., 2018). Thus, it is also conceivable that Arp2/3 directly stimulates resection by transiting exonucleases along a scaffold.

It has also been proposed that DSB clustering could shield DSBs from misrepair. For example, DSBs in highly transcribed regions (Aymard et al., 2017) or DSBs requiring processing occurring in G1 phase might temporarily cluster to avoid end-joining until they can be fixed by HDR in S/G2 phases (Fig. 3); however, bringing DSBs in close proximity within repair factories could also be risky, as it could favor chromosome translocations and rearrangements. A recent study monitored translocations in senataxin-depleted cells. Senataxin is a RNA-DNA helicase that unwinds R-loops. Translocations between DSBs known to cluster occurred at a higher frequency in senataxin-depleted cells (Cohen et al., 2018). The impact of disrupting repair domain formation in this setting has not been documented.

Impairment in nuclear repair domain formation may have significant implications for human disease. The genes encoding the Arp2/3 complex are frequently amplified in colorectal, breast, ovarian, bladder, and esophageal tumors analyzed in large patient cohorts (Molinie and Gautreau, 2018). Furthermore, two recent large studies (analyzing 10,000 tumor samples each) both identified the WAS gene as a pathogenic germline variant predisposing for cancer and a putative cancer driver (Bailey et al., 2018; Huang et al., 2018). Deregulation of the Arp2/3 regulatory system has been proposed to promote tumor cell invadopodia leading to metastasis (Yamaguchi and Condeelis, 2007). In contrast, mutations that completely abrogate WASP expression cause X-linked recessive WAS. WAS male children exhibit microthrombocytopenia, T cell lymphopenia, and autoimmunity and are particularly predisposed to lymphoma and leukemia. In addition to the documented roles of WASP/Arp2/3 in the cytoplasm, the DNA repair function of the nuclear cytoskeleton is novel and should be considered in future studies. Indeed, cells derived from patients with WAS have defects in DSB end resection and repair (Schrank et al., 2018). Finally, WASP was recently identified as a tumor suppressor in T cell lymphoma (Menotti et al., 2019). Thus, by driving the formation of nuclear repair domains, WASP may serve an essential tumor suppressor role. Alternatively, Arp2/3-driven DSB movement and clustering might affect chromosomal translocations that drive oncogenesis. Indeed, direct observation of translocating DSBs reveal they are highly mobile relative to breaks undergoing NHEJ (Roukos et al., 2013). Thus, the impact of nuclear actin dynamics on translocation is ripe for investigation (Fig. 3).

Connecting HDR domains with other nuclear factories: Crosstalk

DSBs that fall within transcriptionally active genes load Rad51 in G2 and repair by HDR (Aymard et al., 2014). These HDR breaks migrate into clusters, whereas NHEJ-prone DSBs do not (Aymard et al., 2017). Intriguingly, DSBs arise proximate to transcription start sites in highly transcribed genes that are frequent substrates for oncogenic translocations (Klein et al., 2011; Schmitz et al., 2014; Schwer et al., 2016). For example, IgH, IgK, and IgL loci are known to translocate with c-myc in the development of Burkitt's lymphoma (Schmitz et al., 2014). Transcribed genes are brought into close proximity via their association with a limited number of specialized transcription factories (Schoenfelder et al., 2010). The basis for the clustering of actively transcribed genes is unknown; however, the formation of transcription factories and repair foci both require IDPs, which aggregate and phase separate into liquid droplets (Altmeyer et al., 2015; Chong et al., 2018; Sabari et al., 2018). It is tempting to speculate that some of the factors that drive DSB motion are shared with transcriptional machinery; however, whether these breaks cluster (or translocate) because of their transcriptional status or repair pathway choice remains to be elucidated. Generation of membrane-less nuclear compartments often requires "crowding" of IDPs, RNA-binding proteins, and RNAs. There is increasing evidence that, in the case of DNA repair, liquid–liquid phase separation is mediated in part by PAR, which could mimic RNA (Altmeyer et al., 2015; Singatulina et al., 2019). It is conceivable that Arp2/3-dependent random movements of DSBs facilitate clustering, thereby increasing PAR-dependent interactions of IDPs within repair foci, eventually triggering the reversible formation of liquid droplets (Fig. 4). Finally, a recent report confirmed that damaged telomeres in ALT cells cluster in phase-separated nuclear condensates (Min et al., 2019).

Finally, the chromatin remodeling machinery is also shared between transcription and repair pathways, suggesting potential crosstalk between the two processes. Chromatin decompaction following DSB generation increases the movement of chromatin both locally at the break site and globally in *S. cerevisiae* (Neumann et al., 2012; Seeber et al., 2013). Thus, DNA decompaction by chromatin remodeling complexes

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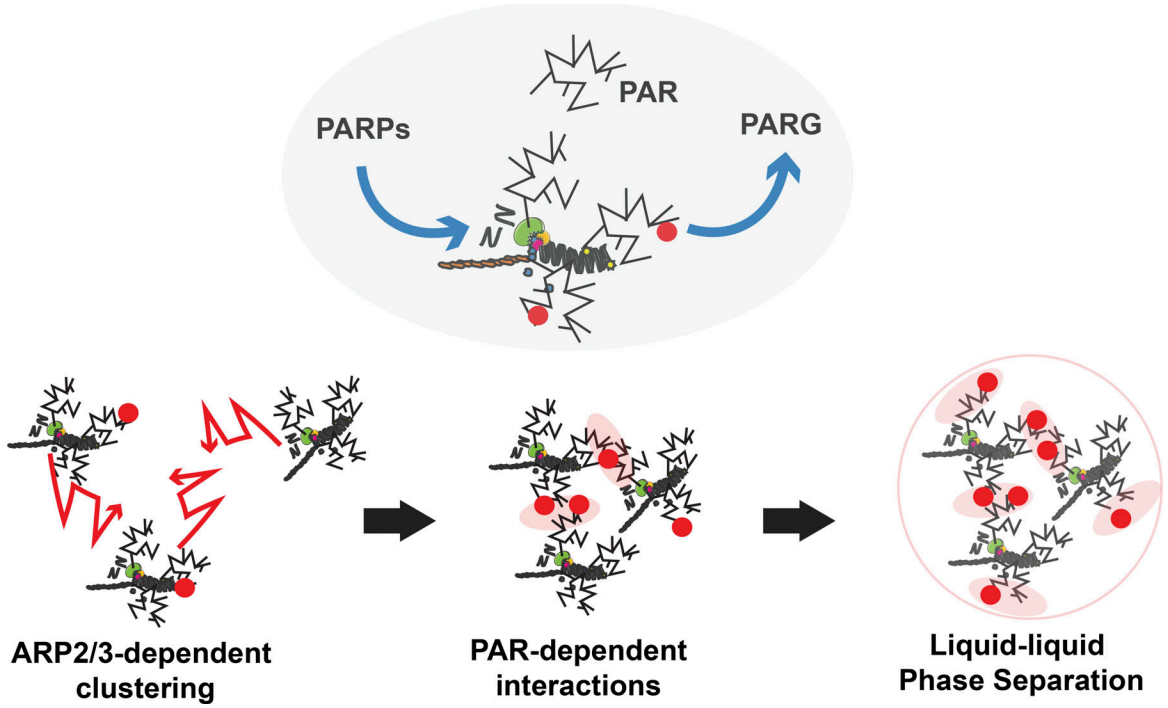

Figure 4. **Model connecting ARP2/3-dependent movements and PAR-dependent liquid–liquid phase separation.** Several classes of proteins are dynamically parylated at sites of DNA damage (top) through the balanced actions of poly(ADP-ribose) polymerases (PARPs) and poly(ADP-ribose) glycohydrolase (PARG). Red circles indicate proteins interacting with PAR, such as BRCT-containing proteins (BRCA1/2), parylated proteins (hnRNPs/FUS), or IDPs that associate via PAR. We propose that "random" movements generated by Arp2/3-dependent actin polymerization facilitate interactions among parylated proteins, PAR-interacting proteins, and IDPs, which together facilitate liquid–liquid phase separation of DSB repair domains surrounding multiple DSBs.

during transcription and repair releases genomic sites from nucleosomal constraints. This genome fluidity may then allow cytoskeletal forces to more efficiently move transcribed loci into domains. Similar to DSB repair, the Arp2/3 complex, WASP, and F-actin localize upstream of promoters of actively transcribed genes (Taylor et al., 2010). Moreover, actin has been found to drive the movement of transcribed genes (Tumbar and Belmont, 2001; Chuang et al., 2006; Percipalle and Visa, 2006; Dundr et al., 2007). Knockdown of N-WASP or Arp2/3 inhibits RNA polymerase II–dependent transcription in HeLa cells, suggesting that actin-driven nuclear domain formation mediates transcription efficiency (Wu et al., 2006; Yoo et al., 2007). Thus, we propose that chromatin remodeling complexes relax chromatin thereby potentiating cytoskeletal driven movements. In turn, phase separation at sites of transcription and repair maintain nuclear domain integrity via electrostatic interactions.

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