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Multi-scale dynamics of heterochromatin repair

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Studies across different organisms show that nuclear architecture and dynamics play central roles in different aspects of homologous recombination (HR) repair. Here we review the most recent discoveries in this field, ranging from directed motions mediating relocalization pathways, to global chromatin mobilization, local DNA looping, and changes in repair focus properties associated with clustering and phase separation. We discuss how these dynamics work in different contexts, including molecular mechanisms and regulatory pathways involved. We specifically highlight how they function in pericentromeric heterochromatin, which presents a unique environment for HR repair given the abundance of repeated DNA sequences prone to aberrant recombination, the 'silent' chromatin state, and the phase separation characterizing this domain.

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Introduction

Organisms are constantly exposed to various sources of DNA double-strand breaks (DSBs), and repairing these lesions is essential for cell survival and genome integrity. DSB repair mostly relies on non-homologous end joining (NHEJ) and homologous recombination (HR) pathways. NHEJ directly re-joins the broken ends with no or little homology required, and it is frequently error-prone. HR relies on extensive DSB resection to generate single strand DNA (ssDNA), which invades 'donor' homologous templates for DNA synthesis, and repair is typically 'error-free'. However, when DSBs occur in repeated DNA sequences, a multitude of ectopic templates is available for repair and their use can lead to chromosome rearrangements and genome instability (reviewed in Ref. [1]).

Studies in recent years also identified the importance of nuclear dynamics for HR repair. A first level of dynamics relates to the 'homology search' step, where the Rad51-coated nucleofilament finds its template in the genome. When the sister chromatid is used, it is typically in close proximity due to cohesion, resulting in a local search and minimal dynamics. However, when the homologous chromosome is used, the nucleofilament might need to travel for a long distance (reviewed in Ref. [2]). Additionally, 'safe' HR repair of repeated sequences at high risk of ectopic recombination, including pericentromeric heterochromatin (hereafter 'heterochromatin'), requires the isolation of repair sites to new nuclear locations, resulting in extensive dynamics (reviewed in Refs. [3,4]). This review focuses on emerging concepts in the field of nuclear dynamics of repair foci undergoing HR, including: i) directed motions of certain repair sites to distant locations; ii) local and global changes in chromatin compaction contributing to dynamics; and iii) local changes in dynamics related to DNA looping, focus clustering, and phase separation. We specifically emphasize our current understanding of how these dynamics contribute to the repair and stability of heterochromatin. Notably, components required for the dynamics of repair sites are frequently misregulated in cancer cells, cancer-prone genetic disorders, neurodegenerative diseases, neuromuscular disorders, and deteriorate with age (reviewed in Refs. [3–5]). Thus, understanding the molecular mechanisms driving these dynamics is expected to have a major impact on the future development of therapeutic strategies for a variety of human disorders.

Relocalization of heterochromatic DSBs and other repeated sequences

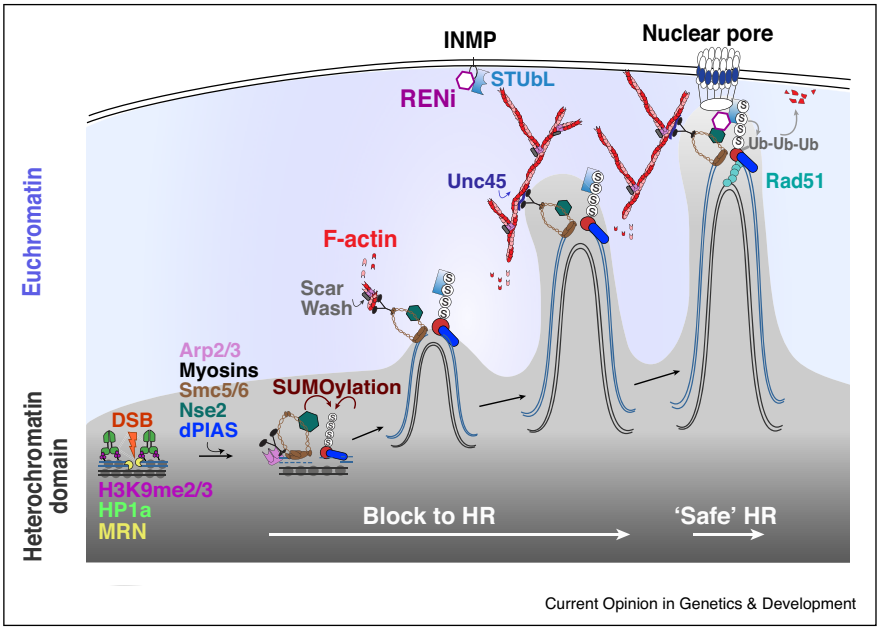
Heterochromatin presents unique challenges to HR repair and is an interesting case study for understanding nuclear dynamics. Heterochromatin occupies about 30% of fly and human genomes, is enriched for silencing epigenetic marks, and is mostly composed of repeated DNA sequences (reviewed in Ref. [4]). In *Drosophila*, about half of these sequences are 'satellite' repeats (predominantly 5-base pair sequences repeated for hundreds of kilobases to megabases) and the rest are transposable elements and other scrambled repeats (reviewed in Ref. [1,4]). Up to millions of identical repeated sequences associated with pericentromeric regions of different chromosomes exacerbate the risk of ectopic recombination in heterochromatin. Importantly, heterochromatin is functionally and structurally distinct from other silenced regions of the genome, such as lamina-associated domains (LADs), and unlike LADs, it is not typically associated with the nuclear periphery [6–11].

Studies in *Drosophila* cells, where heterochromatin forms a distinct nuclear domain [7,10] and dynamics have been characterized in depth, revealed that 'safe' HR repair of heterochromatic DSBs requires relocalization of repair sites to the nuclear periphery before strand invasion [8,11] (reviewed in Ref. [1,5]) (Figure 1). In this context, relocalization is driven by a striking network of transient nuclear actin filaments (F-actin) assembled at heterochromatic DSBs by the actin nucleator Arp2/3, and extending toward the nuclear periphery [11]. Relocalization also requires nuclear myosins (i.e.,Myo1A, Myo1B and MyoV) that interact with the heterochromatin repair component Smc5/6, and are activated by Unc45 downstream from Smc5/6, to promote the directed motion of repair sites along actin filaments [11]. Of note, relocalization is coordinated with HR progression to prevent aberrant recombination. While DSB resection occurs inside the domain [7], Rad51 recruitment is initially halted by Smc5/6- and dPIAS-dependent SUMOylation [7,8,12], and restarts after relocalization of repair sites to nuclear pores and inner nuclear membrane proteins (e.g. the SUN proteins of the LINC complex Koi and Spag4 in *Drosophila*). A SUMO-targeted Ubiquitin ligase (STUbL)/RENi complex, enriched at the nuclear periphery, likely mediates repair restart through ubiquitination of the SUMOylated proteins, followed by their degradation or reactivation [8], but the targets of this modification remain unknown.

Notably, also in mouse cells actin polymerization and myosins mediate relocalization of heterochromatic DSBs to the periphery of heterochromatic domains (or 'chromocenters'), where Rad51 is recruited [9,11,13]. Thus, while the final destination of this movement appears distinct between fly and mouse cells, relocalization pathways are conserved. Relocalization defects result in unrepaired heterochromatic breaks and widespread chromosome rearrangements in *Drosophila* cells and tissues [7,8,11,12,14], and in genome instability in mouse cells [11], revealing the conserved and fundamental importance of these dynamics to genome integrity.

Similar relocalization pathways contribute to HR repair of other repeated sequences, including rDNA, CAG repeats, and telomeres. In all these contexts, relocalization likely facilitates 'safe' repair by isolating DSBs and their repair

Figure 1



Model of heterochromatic DSBs relocalization pathway.
DSB detection and resection occur inside the *Drosophila* heterochromatin domain, where the Mre11 complex (MRN) and HP1a promote the loading of Arp2/3 and nuclear myosins. After resection, the Smc5/6 complex, its Nse2 SUMO E3 ligase subunits (Cerv and Qjt) and the SUMO E3 ligase dPIAS are also recruited to heterochromatic DSBs to generate a block to HR progression (i.e., by halting Rad51 recruitment). Repair foci move to the heterochromatin domain periphery, while Scar and Wash activate Arp2/3, inducing actin polymerization at the repair site. Next, Unc45 recruitment by Smc5/6 activates nuclear myosins that 'walk' along actin filaments, while Smc5/6 bridges the interaction between myosins and damaged DNA. This activation drives the directed motion of repair sites to nuclear pores or inner nuclear membrane proteins (INMPs) where a SUMO-targeted Ubiquitin Ligase (STUbL)/RENi complex stabilizes the association of repair sites with the nuclear periphery. STUbL also ubiquitylates SUMOylated proteins, inducing their proteasome-mediated degradation or activation (not shown), thus enabling Rad51 recruitment and HR progression. Relocalization prevents ectopic recombination and promotes 'safe' repair, by isolating damaged sites and their homologous templates (grey lines) from undamaged heterochromatic repeats before strand invasion.

templates away from ectopic sequences before strand invasion, or by promoting unconventional repair pathways (reviewed in Refs. [1,3,4]). For example, DSBs in yeast nucleoli relocalize to nuclear pores for HR repair, which requires Smc5/6 and Rad52 SUMOylation [15,16]. In human cells, damaged rDNA relocalize to the nucleolar periphery, with Arp2/3, myosins and the LINC complex mediating these dynamics [17]. Further, CAG repeats damaged during replication relocalize to nuclear pores in yeast, and this movement relies on the STUbL Slx5/8 and Smc5/6-dependent SUMOylation of the break-induced replication (BIR) components required for fork restart (i.e ., Rad52, Rad59, and RPA) [18]. Eroded telomeres also relocalize to nuclear pore complexes in budding yeast, through a process involving STUbL and SUMOylation of telomeric proteins, including RPA [19]. Finally, in human cells, replication stressed telomeres relocalize to the nuclear periphery in a nuclear F-actin and nuclear pore-dependent manner [20]. Together, these studies point to conserved pathways for relocalization of DNA breaks in repeated sequences, albeit with differences in terms of SUMOylation targets and final destination of the movement.

A critical discovery from heterochromatin repair studies in *Drosophila* is that relocalization occurs through directed motions [11]. These mostly occur between the heterochromatin domain periphery and the nuclear periphery (i.e.,where actin filaments assemble), and are consistent with the observation that repair sites slide along the filaments [11]. In contrast, repair focus motion is mostly subdiffusive inside the heterochromatin domain and at the nuclear periphery [1,11], where other constraints to the motion prevail (e.g., phase separation and compaction inside the heterochromatin domain and anchoring structures at the nuclear periphery [1,8,11]) (reviewed in Ref. [1,2]). These studies and computer simulations further highlight that in a context of mixed trajectories (*e.g.* alternating subdiffusive and directed motions), traditional mean-square displacement (MSD) analyses are insufficient to detect directed motions, and more sophisticated methods need to be applied [11,21] (reviewed in Ref. [2]). Application of such methods also revealed directed motions in other contexts, such as for BIR repair of subtelomeric DSBs in yeast, where dynamics are driven by nuclear microtubules and Kar3 kinesin [22], or for repair of stressed replication forks at the nuclear periphery in human cells, where relocalization requires nuclear F-actin and myosin II [23••]. Additionally, these methods unmasked directed motions for persistent DSBs that move to the nuclear periphery in budding yeast [22], reversing the initial conclusion that these occur by Brownian/subdiffusive motion [24]. These studies point to the importance of applying dedicated tools to identifying directed motions, and suggest that nuclear filaments and motors might contribute to repositioning repair sites in more contexts than initially thought.

Recent studies also revealed that different features of repair focus dynamics are detected at distinct time scales of imaging [25] (reviewed in Ref. [2]). More frequent imaging (*e.g.* in the millisecond-scale) detects local chromatin dynamics, such as those driven by Rad51 and homology search, while coarser imaging kinetics (*e.g.* in the second to minute-scale) detect longer range motions, including directed motions for heterochromatic breaks [11,25]. Thus, choosing the right imaging regimen is critical for identifying different types of motion.

Local and global chromatin responses for heterochromatin repair

It is a common misconception that heterochromatin is refractory to protein access and repair because of its silent and compact state. On the contrary, heterochromatin is accessible to large macromolecules and protein complexes, including chromatin remodelers and histone modifiers (reviewed in Ref. [4]). Heterochromatin is also organized as a phase separated domain (see next section), characterized by selective permeability for certain molecules, high diffusion of molecules within the domain, and quick response to post-translational modifications that alter the biophysical properties of the domain [26,27 ,71••,72]. Consistent with an accessible environment, early DNA damage detection and signaling occur efficiently in heterochromatin [7,28,29], with foci of proteins responding to resection appearing even faster than in euchromatin [7].

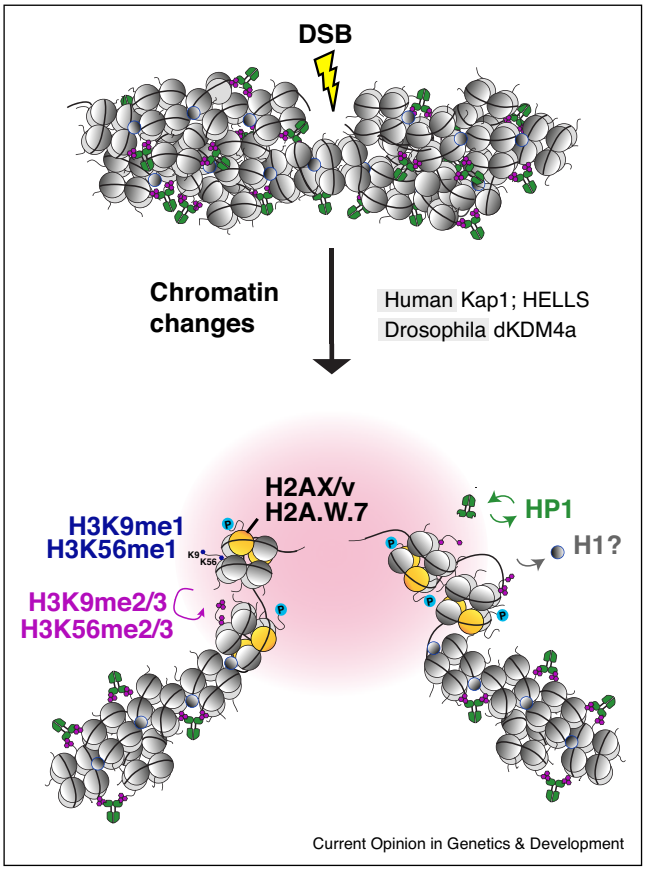
In addition to providing accessibility *per se*, heterochromatin responds dynamically to damage. First, the entire domain expands in mouse and *Drosophila* cells [7,30], and dynamic protrusions emerge during focus relocalization [7]. Expansion of mouse chromocenters has been linked to HP1β phosphorylation by Casein Kinase 2 [30], which increases HP1β turnover on the chromatin and promotes DSB signaling [30]. In *Drosophila* cells, heterochromatin domain expansion is dependent on early DSB processing (*i.e.* resection and ATR-dependent checkpoint activation) [7], although the targets remain unknown, and the significance of expansion to repair still needs to be understood. Expansion might reflect chromatin loosening and/or changes in phase separation properties, promoting new protein accessibility, focus dynamics, or repair progression.

Intriguingly, studies in yeast linked increased nuclear dynamics during HR repair to global histone loss [31,32] and enhanced chromatin stiffness [25,33]. The conservation of these responses across different organisms and their role in heterochromatin repair remains to be established.

Second, local chromatin changes occur at heterochromatic DSBs and influence repair, similar to observations in other genomic regions [34,35,36•] (reviewed in [1,4])

(Figure 2). For example, in mammalian cells, the transcriptional repressor Kap1 is phosphorylated at DSBs by the checkpoint kinase ATM to promote HP1β mobilization, chromatin 'loosening' and repair progression in heterochromatin [6,37]. In *Arabidopsis* the ATM-dependent phosphorylation of the H2A variant H2A.W.7 has been suggested to increase chromatin accessibility specifically at heterochromatic DSBs [38]. Recent studies also indicate that, in human cells, the Snf2-like chromatin remodeler HELLS facilitates HR repair in heterochromatin by promoting the recruitment of the resection component CtIP [39]. Repair defects after HELLS RNAi can be rescued by treatment with chloroquine, which relaxes the chromatin, consistent with a role for HELLS in heterochromatin relaxation during repair [39]. In line with a role for local heterochromatin loosening to facilitate DNA repair, decompaction of mouse chromocenters has also been detected after 405 nm [29] or UV [40] laser treatments, and has been linked to histone H1 displacement in response to UV damage [40]. Of note, damage-induced Kap1 phosphorylation or H1 displacement are not limited to the heterochromatin domain [35,41], suggesting a broader function of these responses in chromatin relaxation for repair. However, these chromatin changes might be particularly important in heterochromatin given the higher initial compaction.

Figure 2



Local chromatin changes at heterochromaticDSBs**.** Heterochromatin undergoes local changes that facilitate DSB repair. Chromatin remodeling and relaxation are orchestrated by chromatin modifiers, including Kap1 and HELLS in human cells, and Kdm4A in *Drosophila*. Histone modifications occurring in heterochromatin include: i) phosphorylation of H2A variants by checkpoint kinases, including the heterochromatin-specific H2A.W.7 variant in *Arabidopsis*; and ii) increased H3K9me1 and H3K56me1. H1 histone loss occurs in response to 405 nm or UV-laser treatments, and might similarly promote DSB repair. HP1 reduction at the repair sites could also facilitate chromatin loosening and repair progression. These local changes might facilitate HR repair by promoting the recruitment of repair factors, affecting repair pathway choice, and increasing dynamics, thus promoting repair progression.

Whether increasing heterochromatin accessibility in response to damage also requires the removal of 'silent' chromatin marks is controversial. Super resolution imaging of heterochromatic regions and studies in response to laser-induced DSBs suggest that H3K9me3 is largely retained during chromatin relaxation at IR or Cas9-induced DSBs targeting the major satellite, in mammalian cells [30,42]. Similarly, UV-induced heterochromatin relaxation occurs in conditions that maintain H3K9me3 and HP1α [40]. However, HP1a appears to be locally displaced in response to IR or laser-induced damage in *Drosophila* cells [7]. Additionally, ChIP analysis identified a Kdm4A-dependent increase in H3K9me1 and H3K56me1 at site-specific heterochromatic DSBs in *Drosophila* [43•], and Kdm4A is required for relocalization of heterochromatic DSBs [43•,44], suggesting a local reduction of silencing during focus dynamics. This H3K9me1 and H3K56me1 increase might be a transient response, as H3K9me3 and H3K56me2 also increase at heterochromatic DSBs in this context, which promotes HR over NHEJ [43•].

Together, these studies suggest that global and local heterochromatin decompaction might be coordinated by different mechanisms, including histone modifications, histone loss, post-translational modification of chromatin-associated proteins, and/or chromatin remodeling. The mechanisms responsible remain to be clearly defined in different model systems and repair pathways, and transient responses might require high resolution techniques to be detected.

Other contributors to HR focus dynamics: looping, clustering, and phase separation

Recent studies have shown that spatial organization of chromatin in nuclear subdomains, and damage-induced changes of this organization, are important contributors to local repair dynamics. Here, we highlight recent advances in our understanding of these responses and point out how they might operate in the unique heterochromatin context.

DNA looping

The genome is organized in a higher order structure defined by topologically associating domains (TADs), whose boundaries are demarcated by CTCF and organized as DNA loops (reviewed in Ref. [45]). Recent studies have established the importance of this organization in regulating γH2AX spreading, which is the phosphorylation of the histone variant H2AX that spans Kb to Mb-sized domains on each side of the DSB, and contributes to DSB signaling. Hi-C and super resolution imaging of human cells revealed that γH2AX mostly spreads within TAD boundaries, identifying TADs as functional units for DSB signaling and repair [42,46]. Hi-C and ChIP-Seq studies further established that γH2AX spreading relies on cohesins that induce a one-sided loop extrusion from each side of the break to the nearest CTCF [47••], and this response appears to be conserved from yeast to humans [47••,48,49]. Notably, increased chromatin contacts detected within the TAD during γH2AX spreading are consistent with the increased mobility of DNA ends observed by live imaging in multiple studies (reviewed in Ref. [2]). Super resolution imaging also identified examples of γH2AX spanning across multiple TADs. These are organized in 3D circular structures by 53BP1 and Rif1, which maintain local chromatin compaction while preventing DSB hyper-resection [50].

How the pre-existing topological organization of the genome and damage-induced looping participate in heterochromatin repair is unknown. TADs have been described in pericentromeric regions, at least in single copy sequences [51,52], and cohesins, Rif1 and CTCF are enriched in heterochromatin [53,54], consistent with the organization of 3D sub-structures. However, the presence or organization of TADs in highly repeated DNA sequences, which constitute most pericentric heterochromatin, is not known. Cohesin recruitment to DSBs, γH2AX spreading mechanisms, and TAD re-organization in response to damage, are also unexplored in this domain. Importantly, looping could facilitate relocalization of heterochromatic DSBs to outside the domain and local chromatin changes, while maintaining silencing and compaction in nearby regions. Thus, understanding these mechanisms is important to establish the role of nuclear architecture in heterochromatin dynamics and repair progression.

Focus clustering

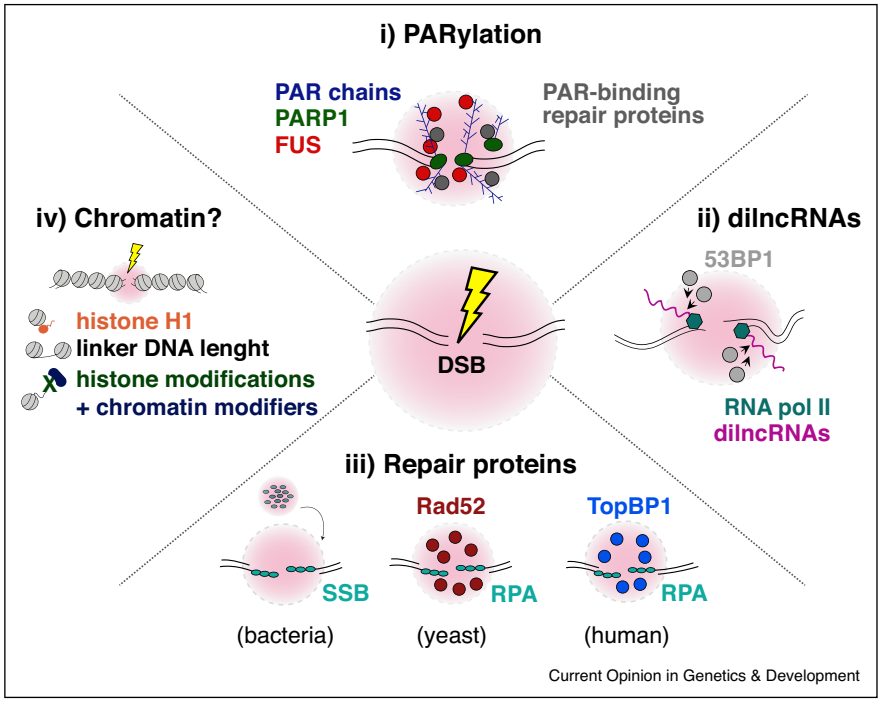
Another component contributing to the dynamics of repair foci undergoing HR is focus clustering (i.e., the non-elastic collision of DSB repair sites), which has been detected across different chromatin contexts and model systems (reviewed in Ref. [13,55]). Focus clustering might facilitate DSB signaling and repair progression by increasing the local concentration of repair components, and studies in human cells revealed the importance of clustering in promoting resection of euchromatic DSBs [56]. Clustering is also observed in HR-prone DSBs in G1 cells, in the absence of a sister chromatid, where it might induce the sequestration of DSBs in a paused state for subsequent repair in S-phase [57]. Clustering requires actin nucleation in both *Drosophila* and human cells [11,56,57], although the underlying mechanism likely differs from that driving the directed motion of heterochromatic repair foci. In fact, the myosin activator Unc45 is not required for focus clustering in *Drosophila* euchromatin, while it is necessary for directed motion of heterochromatic DSBs [11]. Actin filaments might promote clustering by generating propelling forces to move repair sites [56], or by creating structures along which foci concentrate due to the 'wetting' behavior of phase separated structures [58•] (see next section). Alternatively, they might create nucleoplasmic flow dynamics that increase the probability of collision between repair foci, similar to the role proposed for short nuclear microtubules in Rad52 focus clustering in budding yeast [58•].

Notably, repair focus clustering is also frequently observed inside the heterochromatin domain [7], where it might facilitate early HR steps, including resection [7,9]. However, in this context, F-actin and myosins do not seem to be required to promote clustering, and other properties of phase separated domains might be responsible for this response.

Phase separation

Once foci are positioned in close proximity, their fusion can be promoted by the phase separating properties of these structures. Liquid–liquid phase separation (LLPS) is typically established by intrinsically disordered regions (IDRs) of proteins interacting with each other through multivalent weak interactions, which create a local environment with distinct biophysical properties from its surroundings (reviewed in Refs. [59–61]). LLPS of repair foci appears to be promoted by multiple components, including (Figure 3): i) the nucleic acid-mimicking biopolymer poly(ADP-ribose) (PAR), and its associated protein FUS [62–64]; ii) damage-induced long non-coding RNAs, which promote the molecular crowding of the largely unstructured DNA damage response protein 53BP1 [65,66••]; and iii) repair proteins, like yeast Rad52, bacterial SSB (the homolog of RPA), and human TopBP1 [58•,67•,68,69•]. Chromatin also phase separates *in vitro*,and this is dependent on the structurally disordered histone tails, histone H1, linker DNA length, histone modifications, and chromatin-associated proteins [70••,71••,72], suggesting a role of chromatin in modulating phase separation of repair sites (Figure 3). Phase separation can also be induced by bridging molecules (also called 'bridging-induced phase separation', or BIPS) [73], and this could be mediated by cohesins [73] at DSBs, although the role of BIPS in damage responses remains unclear.

Figure 3

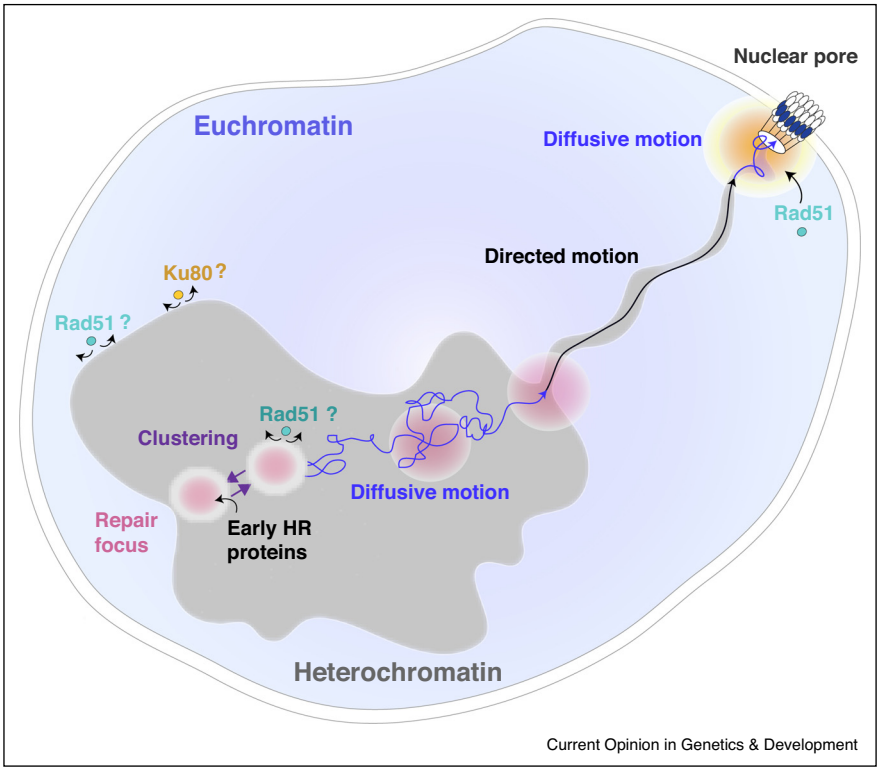


Mechanisms promoting phase separation of repair foci.
Components that contribute to inducing phase separation at DSBs include: i) PAR chains deposited by the Poly [ADP-ribose] polymerase 1 (PARP1), and their binding partner FUS; ii) damage-induced long non-coding RNAs (dilncRNAs) synthesized by RNA pol II, which promote the molecular crowding of 53BP1; and iii) repair proteins, like Rad52, SSB/RPA, and TopBP1. Bacteria cells store SSB condensates at the cell membrane, which are rapidly recruited to repair sites in response to damage. Yeast Rad52 assembles liquid droplets at DSBs, and human TopBP1-induced condensates that promote ATR activation at damaged replication forks. iv) Several chromatin features that alter LLPS also affect the DSB response, suggesting a role for chromatin in damage-induced phase separation of repair foci.

How phase separation of repair foci affects repair in pericentromeric heterochromatin is unknown. LLPS of repair foci might facilitate clustering by increasing the affinity between foci relative to the surrounding environment (Figure 4). In this view, the increased dynamics of repair foci, promoted by either nuclear structures or cytoplasmic forces transmitted to the nucleus through the LINC complex that spans the nuclear membrane, would also promote clustering by increasing the contact probability between repair foci. Notably, phase separated environments are characterized by selective permeability. Thus, in addition to facilitating interactions with other repair foci or nuclear structures, LLPS of repair sites can influence repair pathway choice and promote repair progression by increasing the local concentration of certain repair components while excluding others (reviewed in Ref. [1]) (Figure 4). Condensate formation also correlates with a reduction in chromatin density, at least at transcription centers [74] and synthetically induced chromatin-associated condensates [75]. Thus, LLPS could also promote local chromatin decondensation at heterochromatic repair sites to facilitate HR progression.

Of note, the heterochromatin domain is, *per se*, phase separated [26,27,71••72], which likely influences repair at different levels (Figure 4). First, it might contribute to regulating repair progression inside the domain through selective permeability for DNA repair proteins [1]. Accordingly, HP1a, which mediates phase separation of *Drosophila* heterochromatin, is required for Smc5/6 enrichment inside the domain and for Rad51 exclusion from the domain [7]. Also, the early NHEJ component Ku80 is not detectable in the domain [7], suggesting broad exclusion that might help promote HR [7,8] (Figure 4). Similarly, a phase separated environment could facilitate focus clustering inside the heterochromatin domain by compartmentalizing repair foci and increasing their contact probability. Consistent with this hypothesis, repair focus clustering inside the domain does not depend on Arp2/3 [11], and relocalization of repair sites to outside the domain is frequently concurrent with the splitting of these clusters into smaller foci [7]. In addition, the phase separated heterochromatin domain could facilitate free diffusion of repair foci toward the edge of the domain, where directed motion starts (Figure 4). The diffusion of a liquid phase to the surface of another liquid

Figure 4



Model for clustering and phase separation of heterochromatin repair foci.
Phase separation of the heterochromatin domain and repair foci might influence repair at different levels, by: i) increasing the concentration of early HR repair components in the domain (i.e. damage signaling proteins and resection factors), while excluding others (i.e. Rad51, Ku80); ii) promoting repair focus (pink sphere) clustering inside the domain; iii) promoting chromatin decondensation (white shade underneath repair foci); and iv) facilitating the subdiffusive motion of repair foci to the edge of the heterochromatin domain, where directed motion along actin filaments starts. Phase separation of nuclear pores (yellow sphere) might also create an environment with different biophysical properties, compatible with Rad51 access and repair progression.

phase is a typical behavior of coexisting, immiscible, liquids with similar surface tensions (reviewed in Ref. [59]). Similar properties of immiscible liquids have been suggested to organize nucleolar caps at the periphery of mammalian nucleoli in response to DSBs (reviewed in Ref. [76]). In agreement with a role for LLPS in relocalizing repair foci to the heterochromatin domain periphery, these early dynamics are rarely concurrent with directed motions or visible nuclear actin filaments [1,11], suggesting that different forces contribute to this movement.

Notably, the biophysical properties of phase separated domains can be quickly altered by post-translational modifications (PTMs) (reviewed in Ref. [77]). Thus, SUMOylation, phosphorylation, and demethylation, which promote the relocalization of heterochromatic repair sites [7,12,44,8], could act by altering LLPS properties of the heterochromatin domain or repair foci. For example, PTM of heterochromatin components could promote accessibility to new repair proteins and, in turn, facilitate relocalization of repair foci.

Finally, LLPS might also contribute to heterochromatin repair at nuclear pores, where intrinsically disordered phenylalanine-glycine-rich nucleoporins (FG-Nups) establish a heterogeneous phase separated environment that contributes to the selective permeability of the pore [78]. It is tempting to speculate that repair restart at the pores is influenced by such a local environment, which might for example retain a high concentration of components required to remove the SUMOylated block to HR progression and promote strand invasion. Thus, phase separation likely influences several aspects of heterochromatic DSB repair, and understanding how pre-existing biophysical properties and damage-induced changes in these properties contribute to the spatio-temporal regulation of HR repair is an important goal for future studies.

Conclusions and perspectives

Recent studies have revealed that nuclear and chromatin organization and dynamics play crucial roles in HR repair, in even more diverse contexts than initially thought. Understanding the biophysical properties of these

motions, how they are regulated, and their impact on repair are important goals and exciting challenges. The complex nature of this motion demands multi-scale tracking, simulations, mathematical modeling, and computational tools applied to mixed trajectories, to better describe these dynamics and their regulation in different contexts. Pericentromeric heterochromatin and other repeated DNA sequences are particularly reliant on repair dynamics for their stability. While much has recently been learned about the nature of these dynamics, many of the mechanisms involved await further exploration. Which chromatin changes occur at global *versus* local scales also requires a deeper investigation. Future work should also clarify how DNA looping and phase separation affect repair dynamics in heterochromatin and other repeated sequences, identify post-translational modifications modulating these responses, establish conserved pathways across different organisms, and define how misregulation of these pathways contributes to human diseases.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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