Actin' between phase separated domains for heterochromatin repair

Chetan C. Rawal[1], Christopher P. Caridi[1], and Irene Chiolo[1]*
[1]University of Southern California, Molecular and Computational Biology Department, Los Angeles, California 90089, USA

*Correspondence: chiolo@usc.edu
OrcID:
Chetan C. Rawal: 0000-0001-9286-927X
Irene Chiolo: 0000-0002-3080-550X
Christopher P. Caridi: 0000-0002-4866-2631

Summary
DNA double-strand breaks (DSBs) are particularly challenging to repair in pericentromeric heterochromatin because of the increased risk of aberrant recombination in highly repetitive sequences. Recent studies have identified specialized mechanisms enabling 'safe' homologous recombination (HR) repair in heterochromatin. These include striking nuclear actin filaments (F-actin) and myosins that drive the directed motion of repair sites to the nuclear periphery for 'safe' repair. Here, we summarize our current understanding of the mechanisms involved, and propose how they might operate in the context of a phase-separated environment.

Keywords: Homologous recombination, nuclear actin filaments, heterochromatin, phase separation, DSB repair

Abbreviations:

DSBs	Double-strand breaks
HR	Homologous recombination
NHEJ	Non-homologous end joining
TADs	Topologically associated domains
NADs	Nucleolus-associated domains
LADs	Lamina-associated domains
ssDNA	single-stranded DNA
STUbL	SUMO-targeted ubiquitin ligase
SUMO	Small Ubiquitin-like Modifier
MSD	Mean square displacement
IDPs	Intrinsically disordered proteins
BIR	Break-induced replication
FG-nups	Phenylalanine-glycine rich nucleoporins
F-actin	Filamentous, polymeric actin
G-actin	Globular, monomeric actin

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Heterochromatin repair challenges

Studies across different organisms have revealed that genomes are hierarchically organized into distinct domains, from local loops, to higher level topologically-associating domains (TADs), and large chromosome territories (reviewed in[1,2]). On different scales, domains represent regions of higher frequency contacts, while inter-domain interactions are more rare and highly regulated[1,2]. Components maintaining the nuclear organization in domains include: CCCTC-binding factor (CTCF) and cohesins that organize TADs[3-5]; the lamina, which stabilizes specialized TADs named lamina-associated domains (LADs)[6]; and the nucleolus, which organizes nucleolus-associated domains[7,8] (NADs)[9]. Additional interactions are transiently established at transcription or replication 'factories'[10-12]. Biophysical properties of phase-separated domains provide further constraints to the movement of genomic sites, *e.g.,* in pericentromeric heterochromatin[13,14], nucleoli[15], nuclear pores[16], and repair sites[15]. One of the most exciting challenges in recent years has been understanding what forces promote intra- and inter-domain movements for different functions like DNA replication, transcription, and repair.

One of the largest and better described phase-separated nuclear domains is pericentromeric heterochromatin[13,14] (hereafter 'heterochromatin'), which accounts for about 30% of fly and human genomes[17-19], and is absent in budding yeast. Heterochromatin is characterized by 'silent' histone marks (*e.g.,* H3K9me2/3), and associated proteins such as heterochromatin protein 1 (*e.g.* HP1a in flies[20,21] and HP1α/β in mammalian cells[22,23]), which contribute to its compaction and phase separated state[13,14] (reviewed in[24]). Notably, heterochromatin is functionally and structurally distinct from LADs distributed along the chromosome arms, and in contrast to those, it is not usually associated with the nuclear periphery[9] (see for example[9,25-30], reviewed in[31]). Heterochromatin is mostly composed of repeated DNA sequences. In *Drosophila*, about half are 'satellite' repeats (predominantly 5-base pair sequences repeated for hundreds of kilobases to megabases) and the rest are transposable elements, scrambled repeats, and about 250 isolated genes[17-19]. The abundance of repeated sequences in heterochromatin poses unique challenges to DSB repair and genome stability[27,31-33].

The two main pathways for DSB repair are non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is characterized by direct re-joining of the two ends, which frequently generates small mutations at the repair site[34]. HR initiates with resection to form single-stranded DNA (ssDNA), which invade 'donor' homologous templates for DNA synthesis and repair[35]. In single- copy sequences, a unique donor is present on the sister chromatid or the homologous chromosome, and HR is largely 'error free'[35]. In heterochromatin, however, the availability of up to millions of potential donor sequences associated with different chromosomes can initiate unequal sister chromatid exchanges or intra-/inter-chromosomal recombination, leading to deletions, duplications, translocations, release of extra-chromosomal DNA circles (ECCs), and formation of dicentric or acentric chromosomes[28,36-40]. Despite this danger, HR is a primary pathway to repair heterochromatic DSBs in *Drosophila* and mammalian cells[26,28,30,38,39,41-43], and specialized mechanisms enable 'safe' HR in heterochromatin while preventing aberrant recombination.

Choreography of heterochromatin repair mechanisms

Many of the molecular mechanisms responsible for heterochromatin repair have been initially characterized in *Drosophila* cells, where the organization of heterochromatin in a distinct nuclear domain greatly facilitates cytological approaches[9,21,26]. The recruitment of repair components to DSBs also results in cytologically visible foci, which can be tracked in the nucleus using imaging approaches[26,44,45]. These studies have revealed that HR repair is tightly regulated in space and time (Fig.1): proteins required for resection are recruited to repair sites inside the domain[26], while recruitment of strand invasion components is temporarily halted[26,28,38]; next, the heterochromatin domain expands and repair sites move to the nuclear periphery, where HR progresses[26,28,39]. Inactivating this pathway results in defective heterochromatin repair and aberrant recombination among repeated sequences, revealing its importance to genome integrity[26,28,38-40]. Relocalization likely promotes 'safe' repair by isolating DSBs and their repair templates away from ectopic sequences before strand invasion[27,31,33,46]. Notably, *Drosophila* homologous chromosomes are paired in interphase[47], and accordingly both sister chromatids and homologous chromosomes can provide repair templates[39,43]. Similar dynamic responses occur in mouse cells[27,30,39,48,49], where heterochromatin is organized in several 'chromocenters'[50], suggesting conserved mechanisms for heterochromatin repair[31,33]. Here we provide an overview of heterochromatin repair

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

mechanisms in *Drosophila* cells, conserved pathways in mammalian cells, and interesting discoveries in plants. We will also point out some of the most important unanswered questions in the field.

DSB detection and signaling: In response to ionizing radiation (IR), DSB detection and signaling occurs promptly in heterochromatin[26,39,49]. In *Drosophila*, foci of γH2Av (an early mark of DSB formation, corresponding to mammalian γH2AX[51]) and Mdc1/Mu2 (a signaling components that binds to γH2Av[52]) form within seconds to minutes from IR[26,52], and with kinetics surprisingly similar to those in euchromatin[26]. Intriguingly, foci of proteins marking resected DNA (*e.g.* ATRIP and TopBP1) form even faster and appear brighter in heterochromatin than in euchromatin[26], suggesting that either resection or focus clustering (*i.e.*, the non-elastic collision between repair foci[27]) is more efficient in heterochromatin[26]. In mouse cells, damage recognition and processing also occur inside the heterochromatin domain, with the formation of γH2AX and RPA foci[27,30,39,49]. By revealing high efficiency of early repair progression in heterochromatin, these studies reversed the early assumption that silencing or compaction of heterochromatin imposes a barrier to repair initiation.

Notably, focus clustering might facilitate DSB signaling and repair progression by increasing the local concentration of repair components[27,53]. Studies in mouse cells suggest that clustering promotes resection, at least in euchromatin[53]. Focus clustering is also frequently observed inside the heterochromatin domain[26], and might facilitate early HR steps also in this context. Resection is needed for relocalizing heterochromatic DSBs in both *Drosophila* and mouse cells[26,30], and having efficient resection might provide a signal for rapid relocalization of heterochromatic DSBs, preventing accidental strand invasion of ectopic sequences. However, more studies are needed to understand the efficiency of resection in heterochromatin, the mechanisms responsible for focus clustering and resection in this domain, and the importance of both in the spatial and temporal regulation of heterochromatin repair.

Heterochromatin expansion: Resection and checkpoint activation (particularly ATR[26]) are required for global expansion of the heterochromatin domain in *Drosophila* cells, which starts minutes after IR[26]. This corresponds to an increase of up to 50% in domain size[26,40], and is followed by the formation of dynamic protrusions from the domain during focus relocalization[26]. Expansion might reflect global heterochromatin relaxation to facilitate damage processing or dynamics[26]. In agreement, proteins required for expansion also mediate DSB signaling and relocalization[26]. Heterochromatin relaxation also occurs in mammalian cells[30,48,54], where it has been linked to HP1β T51 phosphorylation by casein kinase 2 (CK2)[48]. Blocking this pathway affects H2AX phosphorylation, revealing its importance in DSB signaling[48]. In *Arabidopsis*, expansion following heterochromatic damage generates 'hollow' chromocenters with repair sites in the center, still isolating repair sites from the bulk of repeated sequences[55].

Of note, global heterochromatin expansion likely facilitates relocalization, but is not sufficient for it to proceed. In fact, relocalization defects have even been observed in conditions when expansion is normal (*e.g.,* after Nse2/Qjt RNAi in *Drosophila* cells)[38], genetically separating heterochromatin expansion from relocalization. Together, more studies are needed to understand the functions of global expansion in heterochromatin repair, along with chromatin changes promoting these responses in different organisms.

Block to Rad51 recruitment inside the heterochromatin domain: Recruitment of the strand invasion component Rad51 only occurs after relocalization of heterochromatic repair sites to the nuclear periphery in *Drosophila* cells[26,28]. The initial block to HR progression is dependent on Su(var)3-9 and HP1a[26], revealing the importance of silencing in heterochromatin protection during repair. The block also requires SUMOylation[28,38] by three SUMO E3 ligases: dPIAS and the Smc5/6 subunits Nse2/Qjt and Nse2/Cerv[26,28,38]. Smc5/6 recruitment to heterochromatin relies on HP1a[26], revealing a role for Smc5/6 in heterochromatin protection downstream from HP1a. Removing these components results in aberrant recombination in heterochromatin and widespread chromosome rearrangements[26,28,38]. Rad51 is also recruited outside the chromocenters in mouse cells[30], but relocalization appears to end at the heterochromatin domain periphery[30,39,49], which might provide a functionally isolated environment similar to the nuclear periphery in *Drosophila* cells. Additionally, losing Smc5/6 does not result in Rad51 foci inside mouse chromocenters[30], suggesting alternative or redundant mechanisms to block HR progression in this context. Together, these discoveries revealed the importance of silencing and SUMOylation in

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

blocking Rad51 recruitment inside the heterochromatin domain to prevent aberrant recombination between heterochromatic repeated sequences. The targets of this regulation remain unknown.

Relocalization mechanisms: Smc5/6 and SUMOylation are also required for relocalizing heterochromatic DSBs to the nuclear periphery in *Drosophila* cells, and recent studies revealed some of the components mediating these dynamics. Relocalization relies on a striking network of nuclear actin filaments (F-actin) that start assembling at repair sites *via* Arp2/3 recruitment[39]. Relocalization also requires Myo1A, Myo1B, and MyoV nuclear myosins, and myosin's ability to 'walk' along the filaments[39]. Notably, Arp2/3 and myosins are recruited to DSBs independently from Smc5/6[39]. However, Smc5/6 interacts with these components during repair[39], suggesting a regulatory role for this interaction. Intriguingly, Arp2/3 and actin are known SUMOylation targets[56,57], and it will be important to establish the role of Nse2- and dPIAS-dependent SUMOylation in their activity. Smc5/6 is also required for the recruitment of the myosin activator Unc45 to DSBs, suggesting Unc45 as a molecular switch that activates myosins *via* Smc5/6.

By interacting with both DSBs and myosins[39], Smc5/6 might also provide a physical link between resected DNA and transport mechanisms, translating myosin-driven pulling forces into repair focus movement. Recruitment of Arp2/3 and myosins to repair sites requires the early DSB signaling and processing factor Mre11, and the heterochromatin protein HP1a[39], suggesting the combination of these components as a mechanism for targeting the relocalization machinery specifically to heterochromatic DSBs. Downstream from Mre11, other repair/checkpoint components might mediate Arp2/3 and myosin recruitment, and this still needs to be determined. Together, these data support a model where nuclear F-actin assembles at heterochromatic DSBs to guide their relocalization to the nuclear periphery *via* myosin-driven 'walk' along actin filaments. Arp2/3, actin polymerization, and myosins are also required to relocalize and repair heterochromatic DSBs in mouse cells[39], revealing conserved pathways.

Local chromatin changes: Heterochromatin is characterized by a unique chromatin environment, including high levels of H3K9me2/3, H3K56me3, H4K20me3, and H3K64me3[58-60], which likely influence repair responses in this domain. How this environment contributes to repair and is affected by DSB formation is just starting to emerge. Studies at I-SceI induced site-specific DSBs in the repair cassette *DR-white* in flies, support the model that H3K9me3 and H3K56me3 increase at heterochromatic DSBs to promote HR repair[61]. The histone demethylase Kdm4A counteracts this response by increasing H3K9me1 and H3K56me1, and favoring NHEJ[61]. Kdm4A is also required for relocalization of heterochromatic DSBs in *Drosophila* cells[62], and this function might be independent from its role in NHEJ, given that NHEJ inactivation does not affect focus relocalization[26]. An interesting possibility is that Kdm4A promotes relocalization by increasing local or global chromatin mobility through a local reduction of silencing marks. In agreement with this, imaging studies show low levels of HP1a at HR repair foci[26], suggesting HP1 is removed, or heterochromatin is loosened, to enable repair progression in *Drosophila* cells.

Additional studies in mammalian cells support this local chromatin 'loosening' model. Specifically, 53BP1-dependent recruitment of Kap1pS824 to repair sites promotes Chd3 release from chromatin, chromatin relaxation, and heterochromatin repair downstream from γH2AX[42,63-66]. Notably, blocking Kap1pS824 does not impair relocalization of DSBs but it affects heterochromatin repair[30], consistent with a later function of chromatin relaxation in DSB processing. Kap1pS824 might also play a role in global heterochromatin expansion, given that this modification has been linked to a large-scale increase in chromatin accessibility[67]. *Arabidopsis* does not have Kap1, but the ATM-dependent phosphorylation of the heterochromatin-specific H2A variant H2A.W.7, has been proposed to facilitate chromatin accessibility during repair[68]. While the molecular details remain to be established for Kdm4A, Kap1pS824, and H2A variants in repair pathway choice, repair progression, and dynamics, these studies have begun unraveling heterochromatin-specific changes for DSB repair. A general model is that heterochromatin loosening facilitates early and late heterochromatin repair steps through the regulation of distinct chromatin components.

Nuclear periphery anchoring: In *Drosophila* cells, DSBs move to nuclear pores or inner nuclear membrane proteins (INMPs) of the SUN family Koi and Spag4, where Rad51 is recruited and repair continues[28]. Interaction with the pore is mediated by the 'Y complex' subunit Nup107[28]. In the absence of these anchoring structures, damaged sites continue exploring the nucleoplasm, eventually returning to

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

the heterochromatin domain[28]. This results in defective heterochromatin repair and gross chromosomal rearrangements[28], revealing the importance of DSB anchoring for 'safe' HR progression. Anchoring also appears to be mediated by the SUMO-targeted ubiquitin ligase (STUbL) Dgrn and its partner dRad60 of the RENi (Rad60-Esc2-Nip45) family protein[28], which are enriched at nuclear pores and INMPs[28]. Dgrn and dRad60 also physically interact with Smc5/6 in response to damage, suggesting that the three components establish a docking complex for repair sites at the nuclear periphery[28].

What restarts repair at the nuclear periphery remains unclear, but STUbL proteins might ubiquitinate SUMOylated targets for proteasome-mediated degradation[69-73] or protein activation[74], removing the SUMOylated block to HR progression. This model predicts that the compartmentalization of SUMOylation activities inside the heterochromatin domain and ubiquitination activities at the nuclear periphery are needed for spatial and temporal regulation of repair.

STUbL (and not RENi) is enriched at heterochromatic DSBs even before relocalization[38] suggesting additional, still unidentified, functions of STUbL in early steps of heterochromatin repair. Consistent with this idea, artificial tethering of the STUbL subunit Slx5 to repair sites in budding yeast is sufficient to target a 'persistent/unrepairable' DSB to the nuclear periphery[75], while recruitment of the STUbL RNF4 to repair sites promotes early DSB signaling in human cells[70].

Of note, RNAi depletion of Arp2/3, myosins, Unc45, STUbL/RENi proteins, nuclear pores, or INMPs, affects relocalization without altering the block to HR progression inside the heterochromatin domain, as Rad51 foci do not form inside the domain in these conditions[28,39]. Conversely, losing Smc5/6 or SUMOylation results in Rad51 foci inside the heterochromatin domain[26,28,38], revealing a separation of function between the pathway that blocks HR progression and the mechanism of relocalization. SUMOylation is required for both, but motor/nuclear periphery components only mediate relocalization/anchoring to the nuclear periphery[26,28,38].

These studies also highlighted several distinct functions of silencing histone marks and associated proteins in heterochromatin repair. In *Drosophila* cells, HP1a is required to: i) prevent abnormal Rad51 recruitment inside the domain *via* Smc5/6 and SUMOylation[26,28,38]; ii) promote relocalization of DSBs to the nuclear periphery *via* Arp2/3 and myosin recruitment[39], Smc5/6- and SUMO-dependent Unc45 loading[39], and Kdm4A recruitment[62]; and iii) facilitate nuclear periphery anchoring *via* Smc5/6-associated STUbL-RENi proteins[28]. Additionally, Su(var)3-9-dependent histone methylation facilitates HR repair while Kdm4A-dependent demethylation promotes NHEJ[61]. In mammalian cells, HP1β or Kap1 post-translational modification appears to facilitate heterochromatin loosening, repair, and dynamics[25,48,63]. These studies establish a new paradigm where heterochromatin components promote several steps of heterochromatin repair, rather than interfering with it.

Alternative repair pathways in heterochromatin

Studies in *Drosophila* and mammalian cells reveal that, despite the risks of aberrant recombination, heterochromatin is preferentially repaired by HR when both HR and NHEJ are available (*i.e.* in S and G2 phases of the cell cycle[26,30,41,42]). However, *Drosophila* tissues enriched for G1 cells, and mammalian cells in G1/G0, also largely use NHEJ in heterochromatin[30,41,43]. Surprisingly, single-strand annealing (SSA) that is potentially engaged in repeated sequences[76] does not significantly contribute to heterochromatin repair, at least when repair outcomes are characterized with a DR-*white* repair cassette in flies[43]. Further, NHEJ repair occurs inside the heterochromatin domain in mouse cells[30], suggesting that NHEJ progression does not require relocalization. However, heterochromatic DSBs are frequently detected outside the heterochromatin domains in *Drosophila* tissues, albeit NHEJ prevails in this context[43], suggesting relocalization can occur during NHEJ, at least in flies. Determining how different heterochromatic DSBs are directed toward distinct repair pathways, and relocalization mechanisms linked to them, remain important open questions in the field.

Nuclear F-actin functions and regulation for heterochromatin repair

Actin filaments (F-actin) are major components of the cytoskeleton responsible for cell movement and adhesion, or transport of RNAs and vesicles *via* myosin motors[77-79]. In the nuclei, F-actin functions have long remained elusive because the more abundant cytoplasmic signal interferes with detection of nuclear

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

filaments using traditional staining approaches[80,81]. With recent advances, including the development of nuclear F-actin-specific fluorescent probes[39,80,82-84] and techniques to specifically and selectively inactivate nuclear actin polymerization[39,82-85] (Fig. 2A,B), several nuclear F-actin functions have started to emerge in different cell types. These studies suggest a model where nuclear F-actin is mostly stimulus-driven, is highly dynamic, and mediates chromatin responses to different stresses[46,86]. Functions of nuclear F-actin include transcription regulation[82,84,85,87,88], mitotic exit[85], centromere maintenance[89], replication origin activation[90], replication fork rescue[91], virus mobilization[92-94], and DSB repair[39,53,83,95,96] (reviewed in[46,86]).

During *Drosophila* heterochromatin repair, nuclear F-actin starts polymerizing at repair sites, with most filaments elongating from the heterochromatin domain periphery to the nuclear periphery as branched structures[39] (Fig. 2A). Repair sites 'slide' along the filaments with directed motions[39,97], consistent with a role of filaments as 'highways' for relocalization. Class I and V myosins (including Myo1A, Myo1B, and MyoV) typically move toward the (+) or 'barbed' end of an actin filament[98], corresponding with the nuclear periphery side[39]. While different myosins are involved and whether more than one myosin operates at each repair site remains do be determined.

Actin polymerization and relocalization of heterochromatic repair sites specifically require the actin nucleator Arp2/3, while the nucleators Spire and the formin Dia do not contribute to these dynamics[39]. Additionally, relocalization requires the Arp2/3 activators Scar and Wash, and not Wasp or Whamy[39]. The use of specific nucleators might reflect the ability of the DNA repair machinery to recruit certain components and not others, and relate to the need for filaments with a specific structure. However, more studies are needed to establish the fine structure of these filaments, the significance of 'branches' associated with them, and the regulatory mechanisms coordinating actin polymerization with DSB relocalization and repair. Why polymerization mostly occurs outside the heterochromatin domain is also unknown, particularly given that Arp2/3 is already present at heterochromatic repair sites before relocalization[39].

Damage-induced actin filaments are also highly dynamic. Heterochromatin-associated structures in *Drosophila* frequently elongate and shrink, and disassemble after relocalization of repair sites[39] (Fig. 2A). It is still unclear what signals and actin remodelers regulate these dynamics, and what are their relevance to repair progression, but filament dynamics might enable 'probing' the crowded nuclear space for an efficient relocalization path.

Additionally, release of monomeric actin during filament disassembly might affect repair progression through the contribution of G-actin in chromatin remodeling. Several chromatin modifiers contributing to DSB repair contain monomeric actin (G-actin) (*i.e.,* HDAC1/2, Tip60, INO80, SWR1, SWI/SNF and RSC[100]), which is critical for their assembly, integrity and function[100]. While the roles of these chromatin modifiers in heterochromatin repair remains to be established, it is possible that G-actin release during depolymerization contributes to assembling and engaging these components during repair.

In addition to relocalizing heterochromatic DSBs in *Drosophila* cells, nuclear F-actin has been proposed to drive local dynamics for focus clustering in human cells, promoting HR repair in euchromatin[53] (reviewed in [46]). Arp2/3 is enriched at DSBs and required for repair focus movement[53], and in this context actin assembles short and highly dynamic structures tracking with HR sites[53] (Fig. 2B). Arp2/3 also mediates clustering of euchromatic foci in *Drosophila* cells[39], suggesting conserved pathways. Actin structures might promote clustering by generating propelling forces to move repair sites[53], although more studies are required to understand how F-actin works in this context.

Notably, the dynamic movement of human repair sites requires Wasp[53], revealing a distinct mechanism for Arp2/3 activation than that of *Drosophila* heterochromatin. Additionally, the myosin activator Unc45 is not required for focus clustering in *Drosophila* euchromatin[39], revealing that the mechanisms responsible for relocalization of heterochromatic DSBs and for clustering of euchromatic breaks are genetically distinct.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Formins and Spire proteins have also been identified as actin nucleators in response to different DNA damaging agents[83] (Fig. 2B), and for focus clustering in G1[101], suggesting that the distinct nucleators might contribute to damage-induced F-actin assembly in different contexts of repair, cell cycle phase, chromatin, or cell type (reviewed in[46]).

Together, these studies identified two separate functions of nuclear F-actin in DSB repair. In heterochromatin, F-actin and myosins enable the relocalization of heterochromatic DSBs after resection and Smc5/6 recruitment, to prevent aberrant recombination between repeated sequences and enable 'safe' HR repair at the nuclear periphery. In euchromatin, actin polymerization promotes DSB movement, clustering and resection in a myosin-independent fashion. The structure of F-actin in different contexts might reflect the different functions. For example, short actin polymers might be sufficient for local dynamics mediating clustering; while long filaments might be needed for the myosin-dependent, longer-range, directional motions of heterochromatic DSBs. More work is required to characterize these structures and nucleating mechanisms in different cell types, cell cycle phases, chromatin, and repair contexts.

Directed motion of repair sites

One of the most important discoveries so far from heterochromatin repair studies is that focus movement is characterized by directed motion[97,102], similar to F-actin and myosin-driven movements in the cytoplasm[103]. A traditional approach to distinguishing Brownian versus directed motion is the mean-square displacement (MSD) analysis of the positional data for repair sites[97,102]. When MSD values are plotted at increasing time intervals, graphs with a progressively increasing slope describe directed motion, while graphs showing a linear dependence indicate Brownian motion[97,102] (Fig. 2C). Chromatin is also subject to constraints due to its polymeric nature, compaction, molecular crowding, and anchoring to nuclear structures, resulting in subdiffusive rather than Brownian motion, and flattened MSD curves[97,102,104]. In addition, when subdiffusive motions occur in a confined space (*e.g.,* the nucleus or a phase-separated domain), MSD graphs reach a plateau proportional to the confinement radius[97,102].

However, when directed motions alternate with diffusive motions, and initiate asynchronously in the population of foci, they cannot be detected with a simple MSD analysis[97]. Thus, new analytical methods needed to be developed to uncover tracts of directed motions in the context of mixed types of motion[97]. These analyses revealed that each heterochromatic locus leaving the heterochromatin domain undergoes long-lasting directed motions (LDMs), and those typically last about 24 minutes, consistent with the average duration of nuclear actin filaments[39]. Remarkably, directed motions of heterochromatic repair sites mostly occur between the heterochromatin domain periphery and the nuclear periphery[39], *i.e.,* where most nuclear actin filaments are organized. Inside the heterochromatin domain and until foci reach the periphery of the domain, the movement is largely subdiffusive confined[39] (Fig. 2D,E), likely because heterochromatin compaction[105] and phase separation[13,14] limit dynamics. Similarly, after relocalization, focus movement is highly confined by nuclear periphery anchoring[28,39]. Notably, the average speed of focus motion does not increase during directed motions (Fig. 2), suggesting that actin filaments and motors do not increase motion speed. Rather, they might provide directionality and counteract other forces that limit the release of repair foci from the heterochromatin domain (*e.g.,* chromatin compaction and phase separation).

Application of similar analysis methods[91,106] revealed directed motions associated with subtelomeric DSBs repaired by the HR sub-pathway break-induced replication (BIR) in *S. cerevisiae*[106], and with damaged replication forks in human cells[91] (reviewed in[46]). Additionally, these methods unmasked directed motions[106] for persistent DSBs that move to the nuclear periphery in budding yeast[69,107-111], reverting the initial conclusion that those are characterized by Brownian/diffusive motion[112]. Directed motions have also been detected during homology search for HR repair of telomeres in ALT cells[113]. These studies point to the importance of applying dedicated tools to identifying directed motions, and suggest that nuclear structures and motors might contribute to repositioning repair sites in more contexts than initially thought, including where diffusive motions appear to prevail: DSBs in rDNA[114-117], damaged telomeric and subtelomeric sequences[107,113,118-120], damaged replication forks in yeast[69,91,121], homology search in different contexts[122,123], chromosome territory repositioning[124,125], and focus clustering[26,27,39,53,101,126-130] (reviewed in[31,33]).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Additionally, while studies in *Drosophila* and mammalian cells identified nuclear F-actin and myosins responsible for directed motions[39,91], relocalization of subtelomeric sites for BIR repair in yeast has been linked to nuclear microtubules and the kinesin Kar3[106], suggesting that nuclear architecture and motor components contributing to repair dynamics might be distinct across different cell types and repair pathways. Also in this context, loss of Kar3[106] does not affect the average speed of motion, suggesting a role for filaments and motors in providing a directionality to the repair site rather than affecting speed. More studies are needed to identify repair contexts relying on directed movements and the structural/motor components mediating these dynamics.

HR regulation in phase separated environments

A critical element for successful heterochromatin repair is the ability to separate repair steps in space and time to enable repair progression only at the nuclear periphery. Compartmentalization of repair activities in the nucleus is a likely mechanism to explain this spatial and temporal regulation. For example, the enrichment of HP1a and SUMOylating proteins inside the heterochromatin domain[26,28], and anchoring of SUMO-binding/processing proteins and proteasomes to nuclear pores[28,69,131-133], explain at least some aspects of this regulation. However, how this compartmentalization is achieved is only partially understood, and the recent characterization of the heterochromatin domain as a phase-separated environment provides further insights to understanding this regulation.

Studies in *Drosophila* and mammalian cells revealed that HP1 molecules establish a phase transition compartment through a liquid-like HP1 population that surrounds the chromatin-bound fraction[13,14]. This function is in addition to the ability of HP1 to generate a compact chromatin state through HP1-HP1 interactions of chromatin-bound HP1 molecules[13,14]. Unlike chromatin compaction, phase separation provides a mechanism for selective accessibility of the heterochromatin domain (reviewed in[24]). In the context of DNA repair, a phase-separated environment might selectively retain or exclude repair proteins to influence repair pathway choice and repair progression (Fig. 3). For example, efficient damage processing might rely on high retention of resection components inside the heterochromatin domain, or exclusion of NHEJ proteins from the domain. Accordingly, the early NHEJ component Ku80-GFP is mostly excluded from the HP1a domain in *Drosophila* cells[26], where repair largely occurs by HR[26,28]. Additionally, the heterochromatin domain might retain Smc5/6 and other early repair proteins (*e.g.*, dPIAS, Arp2/3, myosins)[26,28,39], while excluding later repair components (*e.g.*, Rad51, Rad54)[26,38]. In agreement, HP1a loss affects both phase separation and Rad51 exclusion from the domain. Additionally, local HP1a loss at repair sites during a normal repair cycle[26] might enable Rad51 recruitment and repair progression at the nuclear periphery.

A phase separated environment would also facilitate focus clustering inside the heterochromatin domain, promoting early damage processing. Consistent with this hypothesis, repair focus clustering in heterochromatin does not depend on Arp2/3[39], and relocalization of repair sites to outside the domain is frequently concurrent with the splitting of these clusters into smaller foci[26]. Further, exclusion of Arp2/3 activators might promote filament formation only after repair sites have reached the heterochromatin domain periphery.

Phase separation also enables fast regulated changes in the biophysical properties of the domain, which could in turns facilitate repair progression. For example, chromatin modifiers or phosphorylation of heterochromatin components might change the biophysical properties of the heterochromatin domain to promote expansion and facilitate dynamic movements in response to DNA damage.

Intriguingly, other nuclear compartments required for heterochromatin repair are phase separated, including repair foci and nuclear pores. At repair sites, the early recruitment of poly(ADP-Ribose) polymerase 1 (PARP1) promotes poly-ADP-ribosylation, which results in recruitment of intrinsically disordered proteins (IDPs) and phase separation by liquid demixing[15]. While we do not know how these responses operate in heterochromatin, similar biophysical changes might promote the initial exclusion of repair foci from the heterochromatin domain, and their accumulation at the heterochromatin domain periphery where they interact with actin filaments. In agreement with this idea, the initial phase of focus

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

relocalization in *Drosophila* cells is rarely concurrent with directed motions or visible nuclear actin filaments[39], suggesting that independent separating forces contribute to these dynamics.

At nuclear pores, intrinsically disordered phenylalanine-glycine-rich nucleoporins (FG-Nups) generate a phase separated domain that forms a selective permeability barrier[16]. Recent studies further propose that FG-porins organize distinct territories within the pore, maintained by different types of FG motifs[134]. It is tempting to speculate that repair restart at the nuclear pores is influenced by this local environment, which might retain high concentrations of components for strand invasion and further HR progression.

Finally, F-actin and myosin-driven forces might be particularly critical to enabling the formation of protrusions of heterochromatin from the domain and relocalization of repair foci, counteracting surface tension of the phase separated HP1a domain. Thus, phase separation likely influences several aspects of DSB repair in heterochromatin, and understanding how pre-existing biophysical properties and damage-induced changes in these domains contribute to the spatial and temporal regulation of HR repair is an exciting challenge for future studies.

Conclusions and perspectives

Several studies in the past few years have shed light on a number of components that regulate heterochromatin repair in space and time to prevent aberrant recombination and enable 'safe' repair. Repair starts inside the heterochromatin domain, and continues outside with Rad51 recruitment. Nuclear F-actin and myosins generate pulling forces for relocalization, revealing a tight coordination between nuclear architecture and repair progression. These studies have raised many new and exciting questions. How F-actin and myosins are regulated for heterochromatin repair is largely unclear. Targets of SUMOylation and checkpoint kinases remain uncharacterized. How F-actin is disassembled during focus relocalization and the significance of this to repair is also unknown. The mechanisms restarting HR at the nuclear periphery and the role of ubiquitination in this step remain to be defined. The function of local and global chromatin changes in heterochromatin repair still needs to be understood, and the epigenetic targets of this regulation have just started to emerge. Importantly, understanding how the biophysical properties of heterochromatin as a phase separated environment contribute to different repair steps is an exciting direction for further investigation. Additionally, chromatin movement across nuclear domains is not uncommon and an important challenge is to establish the relevance of transient nuclear filaments and motors in nuclear dynamics for different functions. Heterochromatin silencing[135,136], HR repair[137-140], nuclear periphery[141], and actin/myosin components[142] deteriorate with age, suggesting these declines as a contributor to repair defects and genome instability observed in older organisms[143-146] (reviewed in[33,46]). Thus, understanding heterochromatin repair mechanisms is expected to open new opportunities for addressing human disease, and the tools are now in place for exciting new discoveries in the near future.

Acknowledgements

We apologize to our colleagues whose work could not be cited owing to space limitations. We thank S. Keagy for useful comments on the manuscript. Work supported by NIH R01GM117376 and NSF Career 1751197 to I.C.

Competing interests

The authors declare no competing interests.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Figure Legends:

Figure 1: Model of the molecular mechanisms for 'safe' HR repair of *Drosophila* heterochromatin: DSB detection and resection occurs efficiently inside the heterochromatin domain, while Kdm4A and Su(var)3-9 contribute to repair pathway choice. Checkpoint kinases (ATR, ATM) and resection components (Mre11 complex-CtIP, Blm, Exo1/Tosca) facilitate heterochromatin expansion, while Mre11 and HP1a promote the recruitment of Arp2/3 and myosins to repair sites. Smc5/6 subunits Nse2/Qjt and Nse2/Cerv, and dPIAS, block Rad51 recruitment inside the heterochromatin domain, while recruiting the myosin activator Unc45 *via* SUMOylation. Scar and Wash activate Arp2/3, inducing actin polymerization toward the nuclear periphery. The myosin-Smc5/6 complex associated with damaged DNA translocates along actin filaments with directed motions, and anchors repair sites to nuclear pores or INMPs via STUbL-RENi proteins. At the nuclear periphery, STUbL might promote Rad51 recruitment via ubiquitination and proteasome-mediated degradation of SUMOylated proteins, and 'safe' repair with the sister chromatid or the homologous chromosome that relocalized in concert with the damaged DNA. Local chromatin changes might contribute to relocalization and repair progression (not shown). Actin filaments are highly dynamic and disassemble after relocalization.

Figure 2: Damage-induced nuclear actin filaments generate directed motions for relocalization of heterochromatic DSBs. A-B) Examples of damage-induced nuclear F-actin in indicated cell types and damage treatments (adapted from[39,53,83]). The F-actin probe chromobody or utrophin (Utr230-EN) was used as indicated, in either live U2OS[53] and Kc cells[39], or in fixed HeLa cells[83]. In A), times are min after exposure to 5Gy X-rays. 0' is before IR. In B), treatments were: 50 pg/ml neocarzinostatin (NCS) for 2h, 50 J/m^2 UV, or 0.01% methyl methanosulfonate (MMS) for 2h. C) MSD curves for different types of motion (adapted from[97]). D) Example of a 3D reconstruction and tracking with Imaris of a *Drosophila* cell and heterochromatic (HC) or euchromatic (EU) repair foci, shows track intervals characterized by diffusive or directed motion for heterochromatic repair foci that reach the nuclear periphery (adapted from[97]). E) Time points characterized by directed and sub-diffusive motions were detected with an automated method[97], and confirmed by MSD calculations within those time intervals (adapted form[39]). F) Whiskers plot show the quantification of the speed of focus movement before, during, and after LDMs[39], as indicated (average values are shown in red). The average speed for each tract length was calculated using Imaris. Images reproduced with permissions from Springer Nature. Scale bar = 1 µm.

Figure 3: Model for how phase separation might contribute to heterochromatin repair through selective protein accessibility. Liquid-liquid phase separation of heterochromatin, repair sites, and nuclear pores, might contribute to regulating heterochromatin repair in space and time. The heterochromatin domain might be permeable to resection and checkpoint components, while excluding NHEJ proteins (Ku80). The strand invasion component Rad51, and Arp2/3 activators (Scar, Wash), might also be excluded thus enabling resection inside the heterochromatin domain and filament formation at the heterochromatin domain periphery. Heterochromatin expansion might reflect global changes in the biophysical properties of the domain facilitating relocalization. The nuclear pore might provide a favorable environment for ubiquitination of SUMOylated components and for Rad51 recruitment. Finally, phase separation of repair sites might facilitate their diffusion from the core of the heterochromatin domain to its periphery, while in euchromatin it might promote clustering, resection and HR progression.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Reference

1 van Steensel, B. & Belmont, A. S. Lamina-Associated Domains: Links with Chromosome Architecture, Heterochromatin, and Gene Repression. *Cell* **169** (5), 780-791, doi:10.1016/j.cell.2017.04.022 (2017).

2 Rowley, M. J. & Corces, V. G. Organizational principles of 3D genome architecture. *Nat Rev Genet* **19** (12), 789-800, doi:10.1038/s41576-018-0060-8 (2018).

3 Dixon, J. R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J. S. & Ren, B. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* **485** (7398), 376-380, doi:10.1038/nature11082 (2012).

4 Rao, S. S., Huntley, M. H., Durand, N. C., Stamenova, E. K., Bochkov, I. D., Robinson, J. T., Sanborn, A. L., Machol, I., Omer, A. D., Lander, E. S. & Aiden, E. L. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159** (7), 1665-1680, doi:10.1016/j.cell.2014.11.021 (2014).

5 Hansen, A. S., Pustova, I., Cattoglio, C., Tjian, R. & Darzacq, X. CTCF and cohesin regulate chromatin loop stability with distinct dynamics. *Elife* **6**, doi:10.7554/eLife.25776 (2017).

6 Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M. & van Steensel, B. Characterization of the Drosophila melanogaster genome at the nuclear lamina. *Nat Genet* **38** (9), 1005-1014, doi:10.1038/ng1852 (2006).

7 Nemeth, A., Conesa, A., Santoyo-Lopez, J., Medina, I., Montaner, D., Peterfia, B., Solovei, I., Cremer, T., Dopazo, J. & Langst, G. Initial genomics of the human nucleolus. *PLoS Genet* **6** (3), e1000889, doi:10.1371/journal.pgen.1000889 (2010).

8 van Koningsbruggen, S., Gierlinski, M., Schofield, P., Martin, D., Barton, G. J., Ariyurek, Y., den Dunnen, J. T. & Lamond, A. I. High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. *Mol Biol Cell* **21** (21), 3735-3748, doi:10.1091/mbc.E10-06-0508 (2010).

9 Li, Q., Tjong, H., Li, X., Gong, K., Zhou, X. J., Chiolo, I. & Alber, F. The three-dimensional genome organization of Drosophila melanogaster through data integration. *Genome Biol* **18** (1), 145, doi:10.1186/s13059-017-1264-5 (2017).

10 Kitamura, E., Blow, J. J. & Tanaka, T. U. Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories. *Cell* **125** (7), 1297-1308, doi:10.1016/j.cell.2006.04.041 (2006).

11 Edelman, L. B. & Fraser, P. Transcription factories: genetic programming in three dimensions. *Curr Opin Genet Dev* **22** (2), 110-114, doi:10.1016/j.gde.2012.01.010 (2012).

12 Knott, S. R., Peace, J. M., Ostrow, A. Z., Gan, Y., Rex, A. E., Viggiani, C. J., Tavare, S. & Aparicio, O. M. Forkhead transcription factors establish origin timing and long-range clustering in S. cerevisiae. *Cell* **148** (1-2), 99-111, doi:10.1016/j.cell.2011.12.012 (2012).

13 Larson, A. G., Elnatan, D., Keenen, M. M., Trnka, M. J., Johnston, J. B., Burlingame, A. L., Agard, D. A., Redding, S. & Narlikar, G. J. Liquid droplet formation by HP1alpha suggests a role for phase separation in heterochromatin. *Nature* **547** (7662), 236-240, doi:10.1038/nature22822 (2017).

14 Strom, A. R., Emelyanov, A. V., Mir, M., Fyodorov, D. V., Darzacq, X. & Karpen, G. H. Phase separation drives heterochromatin domain formation. *Nature* **547** (7662), 241-245, doi:10.1038/nature22989 (2017).

15 Altmeyer, M., Neelsen, K. J., Teloni, F., Pozdnyakova, I., Pellegrino, S., Grofte, M., Rask, M. D., Streicher, W., Jungmichel, S., Nielsen, M. L. & Lukas, J. Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). *Nat Commun* **6**, 8088, doi:10.1038/ncomms9088 (2015).

16 Hulsmann, B. B., Labokha, A. A. & Gorlich, D. The permeability of reconstituted nuclear pores provides direct evidence for the selective phase model. *Cell* **150** (4), 738-751, doi:10.1016/j.cell.2012.07.019 (2012).

17 Hoskins, R. A., Carlson, J. W., Kennedy, C., Acevedo, D., Evans-Holm, M., Frise, E., Wan, K. H., Park, S., Mendez-Lago, M., Rossi, F., Villasante, A., Dimitri, P., Karpen, G. H. & Celniker, S. E. Sequence Finishing and Mapping of Drosophila melanogaster Heterochromatin. *Science* **316** (5831), 1625-1628, doi:10.1126/science.1139816 (2007).

18 Ho, J. W., Jung, Y. L., Liu, T., Alver, B. H., Lee, S., Ikegami, K., Sohn, K. A., Minoda, A., Tolstorukov, M. Y., Appert, A., Parker, S. C., Gu, T., Kundaje, A., Riddle, N. C., Bishop, E., Egelhofer, T. A., Hu, S. S., Alekseyenko, A. A., Rechtsteiner, A., Asker, D., Belsky, J. A., Bowman, S. K., Chen, Q. B.,

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Chen, R. A., Day, D. S., Dong, Y., Dose, A. C., Duan, X., Epstein, C. B., Ercan, S., Feingold, E. A., Ferrari, F., Garrigues, J. M., Gehlenborg, N., Good, P. J., Haseley, P., He, D., Herrmann, M., Hoffman, M. M., Jeffers, T. E., Kharchenko, P. V., Kolasinska-Zwierz, P., Kotwaliwale, C. V., Kumar, N., Langley, S. A., Larschan, E. N., Latorre, I., Libbrecht, M. W., Lin, X., Park, R., Pazin, M. J., Pham, H. N., Plachetka, A., Qin, B., Schwartz, Y. B., Shoresh, N., Stempor, P., Vielle, A., Wang, C., Whittle, C. M., Xue, H., Kingston, R. E., Kim, J. H., Bernstein, B. E., Dernburg, A. F., Pirrotta, V., Kuroda, M. I., Noble, W. S., Tullius, T. D., Kellis, M., MacAlpine, D. M., Strome, S., Elgin, S. C., Liu, X. S., Lieb, J. D., Ahringer, J., Karpen, G. H. & Park, P. J. Comparative analysis of metazoan chromatin organization. *Nature* **512** (7515), 449-452, doi:10.1038/nature13415 (2014).

19 Hoskins, R. A., Carlson, J. W., Wan, K. H., Park, S., Mendez, I., Galle, S. E., Booth, B. W., Pfeiffer, B. D., George, R. A., Svirskas, R., Krzywinski, M., Schein, J., Accardo, M. C., Damia, E., Messina, G., Mendez-Lago, M., de Pablos, B., Demakova, O. V., Andreyeva, E. N., Boldyreva, L. V., Marra, M., Carvalho, A. B., Dimitri, P., Villasante, A., Zhimulev, I. F., Rubin, G. M., Karpen, G. H. & Celniker, S. E. The Release 6 reference sequence of the Drosophila melanogaster genome. *Genome Res* **25** (3), 445-458, doi:10.1101/gr.185579.114 (2015).

20 James, T. C., Eissenberg, J. C., Craig, C., Dietrich, V., Hobson, A. & Elgin, S. C. Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of Drosophila. *Eur J Cell Biol* **50** (1), 170-180 (1989).

21 Riddle, N. C., Minoda, A., Kharchenko, P. V., Alekseyenko, A. A., Schwartz, Y. B., Tolstorukov, M. Y., Gorchakov, A. A., Jaffe, J. D., Kennedy, C., Linder-Basso, D., Peach, S. E., Shanower, G., Zheng, H., Kuroda, M. I., Pirrotta, V., Park, P. J., Elgin, S. C. & Karpen, G. H. Plasticity in patterns of histone modifications and chromosomal proteins in Drosophila heterochromatin. *Genome Res* **21** (2), 147-163, doi:10.1101/gr.110098.110 (2011).

22 Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. & Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410** (6824), 116-120. (2001).

23 Dialynas, G. K., Terjung, S., Brown, J. P., Aucott, R. L., Baron-Luhr, B., Singh, P. B. & Georgatos, S. D. Plasticity of HP1 proteins in mammalian cells. *J Cell Sci* **120** (Pt 19), 3415-3424, doi:10.1242/jcs.012914 (2007).

24 Janssen, A., Colmenares, S. U. & Karpen, G. H. Heterochromatin: Guardian of the Genome. *Annu Rev Cell Dev Biol* **34**, 265-288, doi:10.1146/annurev-cellbio-100617-062653 (2018).

25 Goodarzi, A. A., Noon, A. T., Deckbar, D., Ziv, Y., Shiloh, Y., Lobrich, M. & Jeggo, P. A. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. *Mol Cell* **31** (2), 167-177, doi:S1097-2765(08)00395-X [pii] 10.1016/j.molcel.2008.05.017 (2008).

26 Chiolo, I., Minoda, A., Colmenares, S. U., Polyzos, A., Costes, S. V. & Karpen, G. H. Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. *Cell* **144** (5), 732-744, doi:10.1016/j.cell.2011.02.012 (2011).

27 Chiolo, I., Tang, J., Georgescu, W. & Costes, S. V. Nuclear dynamics of radiation-induced foci in euchromatin and heterochromatin. *Mutat Res* **750** (1-2), 56-66, doi:10.1016/j.mrfmmm.2013.08.001 (2013).

28 Ryu, T., Spatola, B., Delabaere, L., Bowlin, K., Hopp, H., Kunitake, R., Karpen, G. H. & Chiolo, I. Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. *Nat Cell Biol* **17** (11), 1401-1411, doi:10.1038/ncb3258 (2015).

29 Tjong, H., Li, W., Kalhor, R., Dai, C., Hao, S., Gong, K., Zhou, Y., Li, H., Zhou, X. J., Le Gros, M. A., Larabell, C. A., Chen, L. & Alber, F. Population-based 3D genome structure analysis reveals driving forces in spatial genome organization. *Proc Natl Acad Sci U S A* **113** (12), E1663-1672, doi:10.1073/pnas.1512577113 (2016).

30 Tsouroula, K., Furst, A., Rogier, M., Heyer, V., Maglott-Roth, A., Ferrand, A., Reina-San-Martin, B. & Soutoglou, E. Temporal and Spatial Uncoupling of DNA Double Strand Break Repair Pathways within Mammalian Heterochromatin. *Mol Cell* **63** (2), 293-305, doi:10.1016/j.molcel.2016.06.002 (2016).

31 Caridi, P. C., Delabaere, L., Zapotoczny, G. & Chiolo, I. And yet, it moves: nuclear and chromatin dynamics of a heterochromatic double-strand break. *Philos Trans R Soc Lond B Biol Sci* **372** (1731), doi:10.1098/rstb.2016.0291 (2017).

32 Peng, J. C. & Karpen, G. H. Epigenetic regulation of heterochromatic DNA stability. *Curr Opin Genet Dev* **18** (2), 204-211, doi:S0959-437X(08)00020-8 [pii] 10.1016/j.gde.2008.01.021 (2008).

33 Amaral, N., Ryu, T., Li, X. & Chiolo, I. Nuclear Dynamics of Heterochromatin Repair. *Trends Genet* **33** (2), 86-100, doi:10.1016/j.tig.2016.12.004 (2017).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

34 Chang, H. H. Y., Pannunzio, N. R., Adachi, N. & Lieber, M. R. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat Rev Mol Cell Biol* **18** (8), 495-506, doi:10.1038/nrm.2017.48 (2017).
35 Kowalczykowski, S. C. An Overview of the Molecular Mechanisms of Recombinational DNA Repair. *Cold Spring Harb Perspect Biol* **7** (11), doi:10.1101/cshperspect.a016410 (2015).
36 Peng, J. C. & Karpen, G. H. H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. *Nat Cell Biol* **9** (1), 25-35 (2007).
37 Peng, J. C. & Karpen, G. H. Heterochromatic genome stability requires regulators of histone H3 K9 methylation. *PLoS Genet* **5** (3), e1000435, doi:10.1371/journal.pgen.1000435 (2009).
38 Ryu, T., Bonner, M. R. & Chiolo, I. Cervantes and Quijote protect heterochromatin from aberrant recombination and lead the way to the nuclear periphery. *Nucleus* **7** (5), 485-497, doi:10.1080/19491034.2016.1239683 (2016).
39 Caridi, C. P., D'Agostino, C., Ryu, T., Zapotoczny, G., Delabaere, L., Li, X., Khodaverdian, V. Y., Amaral, N., Lin, E., Rau, A. R. & Chiolo, I. Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature* **559** (7712), 54-60, doi:10.1038/s41586-018-0242-8 (2018).
40 Dialynas, G., Delabaere, L. & Chiolo, I. Arp2/3 and Unc45 maintain heterochromatin stability in Drosophila polytene chromosomes. *Experimental Biology and Medicine* **In press.** (2019).
41 Beucher, A., Birraux, J., Tchouandong, L., Barton, O., Shibata, A., Conrad, S., Goodarzi, A. A., Krempler, A., Jeggo, P. A. & Lobrich, M. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J* **28** (21), 3413-3427, doi:emboj2009276 [pii] 10.1038/emboj.2009.276 (2009).
42 Kakarougkas, A., Ismail, A., Klement, K., Goodarzi, A. A., Conrad, S., Freire, R., Shibata, A., Lobrich, M. & Jeggo, P. A. Opposing roles for 53BP1 during homologous recombination. *Nucleic Acids Res* **41** (21), 9719-9731, doi:gkt729 [pii] 10.1093/nar/gkt729 (2013).
43 Janssen, A., Breuer, G. A., Brinkman, E. K., van der Meulen, A. I., Borden, S. V., van Steensel, B., Bindra, R. S., LaRocque, J. R. & Karpen, G. H. A single double-strand break system reveals repair dynamics and mechanisms in heterochromatin and euchromatin. *Genes Dev* **30** (14), 1645-1657, doi:10.1101/gad.283028.116 (2016).
44 Lisby, M. & Rothstein, R. Choreography of recombination proteins during the DNA damage response. *DNA Repair (Amst)* **8** (9), 1068-1076, doi:10.1016/j.dnarep.2009.04.007 (2009).
45 Costes, S. V., Chiolo, I., Pluth, J. M., Barcellos-Hoff, M. H. & Jakob, B. Spatiotemporal characterization of ionizing radiation induced DNA damage foci and their relation to chromatin organization. *Mutation Research* **704** (1-3), 78-87, doi:10.1016/j.mrrev.2009.12.006 (2010).
46 Caridi, C. P., Plessner, M., Grosse, R. & Chiolo, I. Nuclear actin filaments in DNA repair dynamics. *Nature Cell Biology* **in revision** (2019).
47 McKee, B. D. Homologous pairing and chromosome dynamics in meiosis and mitosis. *Biochim Biophys Acta* **1677** (1-3), 165-180 (2004).
48 Ayoub, N., Jeyasekharan, A. D., Bernal, J. A. & Venkitaraman, A. R. HP1-beta mobilization promotes chromatin changes that initiate the DNA damage response. *Nature* **453** (7195), 682-686, doi:nature06875 [pii] 10.1038/nature06875 (2008).
49 Jakob, B., Splinter, J., Conrad, S., Voss, K. O., Zink, D., Durante, M., Lobrich, M. & Taucher-Scholz, G. DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. *Nucleic Acids Res* **39** (15), 6489-6499, doi:gkr230 [pii] 10.1093/nar/gkr230 (2011).
50 Guenatri, M., Bailly, D., Maison, C. & Almouzni, G. Mouse centric and pericentric satellite repeats form distinct functional heterochromatin. *J Cell Biol* **166** (4), 493-505, doi:10.1083/jcb.200403109 (2004).
51 Madigan, J. P., Chotkowski, H. L. & Glaser, R. L. DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res* **30** (17), 3698-3705 (2002).
52 Dronamraju, R. & Mason, J. M. Recognition of double strand breaks by a mutator protein (MU2) in Drosophila melanogaster. *PLoS Genet* **5** (5), e1000473, doi:10.1371/journal.pgen.1000473 (2009).
53 Schrank, B. R., Aparicio, T., Li, Y., Chang, W., Chait, B. T., Gundersen, G. G., Gottesman, M. E. & Gautier, J. Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. *Nature* **559** (7712), 61-66, doi:10.1038/s41586-018-0237-5 (2018).
54 Natale, F., Rapp, A., Yu, W., Maiser, A., Harz, H., Scholl, A., Grulich, S., Anton, T., Horl, D., Chen, W., Durante, M., Taucher-Scholz, G., Leonhardt, H. & Cardoso, M. C. Identification of the

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

elementary structural units of the DNA damage response. *Nat Commun* **8**, 15760, doi:10.1038/ncomms15760 (2017).
55 Feng, W., Hale, C. J., Over, R. S., Cokus, S. J., Jacobsen, S. E. & Michaels, S. D. Large-scale heterochromatin remodeling linked to overreplication-associated DNA damage. *Proc Natl Acad Sci U S A* **114** (2), 406-411, doi:10.1073/pnas.1619774114 (2017).
56 Hofmann, W. A., Arduini, A., Nicol, S. M., Camacho, C. J., Lessard, J. L., Fuller-Pace, F. V. & de Lanerolle, P. SUMOylation of nuclear actin. *J Cell Biol* **186** (2), 193-200, doi:10.1083/jcb.200905016 (2009).
57 Alonso, A., Greenlee, M., Matts, J., Kline, J., Davis, K. J. & Miller, R. K. Emerging roles of sumoylation in the regulation of actin, microtubules, intermediate filaments, and septins. *Cytoskeleton (Hoboken)* **72** (7), 305-339, doi:10.1002/cm.21226 (2015).
58 Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., Reinberg, D. & Jenuwein, T. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev* **18** (11), 1251-1262 (2004).
59 Jack, A. P., Bussemer, S., Hahn, M., Punzeler, S., Snyder, M., Wells, M., Csankovszki, G., Solovei, I., Schotta, G. & Hake, S. B. H3K56me3 is a novel, conserved heterochromatic mark that largely but not completely overlaps with H3K9me3 in both regulation and localization. *PLoS One* **8** (2), e51765, doi:10.1371/journal.pone.0051765 (2013).
60 Lange, U. C., Siebert, S., Wossidlo, M., Weiss, T., Ziegler-Birling, C., Walter, J., Torres-Padilla, M. E., Daujat, S. & Schneider, R. Dissecting the role of H3K64me3 in mouse pericentromeric heterochromatin. *Nat Commun* **4**, 2233, doi:10.1038/ncomms3233 (2013).
61 Janssen, A., Colmenares, S. U., Lee, T. & Karpen, G. H. Timely double-strand break repair and pathway choice in pericentromeric heterochromatin depend on the histone demethylase dKDM4A. *Genes Dev* **33** (1-2), 103-115, doi:10.1101/gad.317537.118 (2019).
62 Colmenares, S. U., Swenson, J. M., Langley, S. A., Kennedy, C., Costes, S. V. & Karpen, G. H. Drosophila Histone Demethylase KDM4A Has Enzymatic and Non-enzymatic Roles in Controlling Heterochromatin Integrity. *Dev Cell* **42** (2), 156-169 e155, doi:10.1016/j.devcel.2017.06.014 (2017).
63 Goodarzi, A. A., Kurka, T. & Jeggo, P. A. KAP-1 phosphorylation regulates CHD3 nucleosome remodeling during the DNA double-strand break response. *Nat Struct Mol Biol* **18** (7), 831-839, doi:10.1038/nsmb.2077 (2011).
64 Lee, D. H., Goodarzi, A. A., Adelmant, G. O., Pan, Y., Jeggo, P. A., Marto, J. A. & Chowdhury, D. Phosphoproteomic analysis reveals that PP4 dephosphorylates KAP-1 impacting the DNA damage response. *EMBO J* **31** (10), 2403-2415, doi:10.1038/emboj.2012.86 (2012).
65 Klement, K., Luijsterburg, M. S., Pinder, J. B., Cena, C. S., Del Nero, V., Wintersinger, C. M., Dellaire, G., van Attikum, H. & Goodarzi, A. A. Opposing ISWI- and CHD-class chromatin remodeling activities orchestrate heterochromatic DNA repair. *J Cell Biol* **207** (6), 717-733, doi:10.1083/jcb.201405077 (2014).
66 Delabaere, L. & Chiolo, I. ReiNF4rcing repair pathway choice during cell cycle. *Cell Cycle* **15** (9), 1182-1183, doi:10.1080/15384101.2016.1159108 (2016).
67 Ziv, Y., Bielopolski, D., Galanty, Y., Lukas, C., Taya, Y., Schultz, D. C., Lukas, J., Bekker-Jensen, S., Bartek, J. & Shiloh, Y. Chromatin relaxation in response to DNA double-strand breaks is modulated by a novel ATM- and KAP-1 dependent pathway. *Nat Cell Biol* **8** (8), 870-876, doi:10.1038/ncb1446 (2006).
68 Lorkovic, Z. J., Park, C., Goiser, M., Jiang, D., Kurzbauer, M. T., Schlogelhofer, P. & Berger, F. Compartmentalization of DNA Damage Response between Heterochromatin and Euchromatin Is Mediated by Distinct H2A Histone Variants. *Curr Biol* **27** (8), 1192-1199, doi:10.1016/j.cub.2017.03.002 (2017).
69 Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M. B., Roberts, T. M., Brown, G. W., Varela, E., Hediger, F., Gasser, S. M. & Krogan, N. J. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* **322** (5901), 597-602, doi:10.1126/science.1162790 (2008).
70 Galanty, Y., Belotserkovskaya, R., Coates, J. & Jackson, S. P. RNF4, a SUMO-targeted ubiquitin E3 ligase, promotes DNA double-strand break repair. *Genes Dev* **26** (11), 1179-1195, doi:26/11/1179 [pii]10.1101/gad.188284.112 (2012).
71 Luo, K., Zhang, H., Wang, L., Yuan, J. & Lou, Z. Sumoylation of MDC1 is important for proper DNA damage response. *EMBO J* **31** (13), 3008-3019, doi:10.1038/emboj.2012.158 (2012).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

72 Yin, Y., Seifert, A., Chua, J. S., Maure, J. F., Golebiowski, F. & Hay, R. T. SUMO-targeted ubiquitin E3 ligase RNF4 is required for the response of human cells to DNA damage. *Genes Dev* **26** (11), 1196-1208, doi:10.1101/gad.189274.112 (2012).
73 Kuo, C. Y., Li, X., Kong, X. Q., Luo, C., Chang, C. C., Chung, Y., Shih, H. M., Li, K. K. & Ann, D. K. An arginine-rich motif of ring finger protein 4 (RNF4) oversees the recruitment and degradation of the phosphorylated and SUMOylated Kruppel-associated box domain-associated protein 1 (KAP1)/TRIM28 protein during genotoxic stress. *J Biol Chem* **289** (30), 20757-20772, doi:10.1074/jbc.M114.555672 (2014).
74 Guzzo, C. M., Berndsen, C. E., Zhu, J., Gupta, V., Datta, A., Greenberg, R. A., Wolberger, C. & Matunis, M. J. RNF4-dependent hybrid SUMO-ubiquitin chains are signals for RAP80 and thereby mediate the recruitment of BRCA1 to sites of DNA damage. *Sci Signal* **5** (253), ra88, doi:5/253/ra88 [pii] 10.1126/scisignal.2003485 (2012).
75 Horigome, C., Bustard, D. E., Marcomini, I., Delgoshaie, N., Tsai-Pflugfelder, M., Cobb, J. A. & Gasser, S. M. PolySUMOylation by Siz2 and Mms21 triggers relocation of DNA breaks to nuclear pores through the Slx5/Slx8 STUbL. *Genes Dev* **30** (8), 931-945, doi:10.1101/gad.277665.116 (2016).
76 Bhargava, R., Onyango, D. O. & Stark, J. M. Regulation of Single-Strand Annealing and its Role in Genome Maintenance. *Trends Genet* **32** (9), 566-575, doi:10.1016/j.tig.2016.06.007 (2016).
77 Pollard, T. D. Actin and Actin-Binding Proteins. *Cold Spring Harb Perspect Biol* **8** (8), doi:10.1101/cshperspect.a018226 (2016).
78 Rottner, K., Faix, J., Bogdan, S., Linder, S. & Kerkhoff, E. Actin assembly mechanisms at a glance. *J Cell Sci* **130** (20), 3427-3435, doi:10.1242/jcs.206433 (2017).
79 Titus, M. A. Myosin-Driven Intracellular Transport. *Cold Spring Harb Perspect Biol* **10** (3), doi:10.1101/cshperspect.a021972 (2018).
80 Belin, B. J., Cimini, B. A., Blackburn, E. H. & Mullins, R. D. Visualization of actin filaments and monomers in somatic cell nuclei. *Mol Biol Cell* **24** (7), 982-994, doi:10.1091/mbc.E12-09-0685 (2013).
81 Melak, M., Plessner, M. & Grosse, R. Actin visualization at a glance. *J Cell Sci* **130** (3), 525-530, doi:10.1242/jcs.189068 (2017).
82 Baarlink, C., Wang, H. & Grosse, R. Nuclear actin network assembly by formins regulates the SRF coactivator MAL. *Science* **340** (6134), 864-867, doi:science.1235038 [pii]10.1126/science.1235038 (2013).
83 Belin, B. J., Lee, T. & Mullins, R. D. DNA damage induces nuclear actin filament assembly by Formin -2 and Spire-(1/2) that promotes efficient DNA repair. [corrected]. *Elife* **4**, e07735, doi:10.7554/eLife.07735 (2015).
84 Plessner, M., Melak, M., Chinchilla, P., Baarlink, C. & Grosse, R. Nuclear F-actin formation and reorganization upon cell spreading. *J Biol Chem* **290** (18), 11209-11216, doi:10.1074/jbc.M114.627166 (2015).
85 Baarlink, C., Plessner, M., Sherrard, A., Morita, K., Misu, S., Virant, D., Kleinschnitz, E. M., Harniman, R., Alibhai, D., Baumeister, S., Miyamoto, K., Endesfelder, U., Kaidi, A. & Grosse, R. A transient pool of nuclear F-actin at mitotic exit controls chromatin organization. *Nat Cell Biol* **19** (12), 1389-1399, doi:10.1038/ncb3641 (2017).
86 Plessner, M. & Grosse, R. Dynamizing nuclear actin filaments. *Curr Opin Cell Biol* **56**, 1-6, doi:10.1016/j.ceb.2018.08.005 (2019).
87 Kircher, P., Hermanns, C., Nossek, M., Drexler, M. K., Grosse, R., Fischer, M., Sarikas, A., Penkava, J., Lewis, T., Prywes, R., Gudermann, T. & Muehlich, S. Filamin A interacts with the coactivator MKL1 to promote the activity of the transcription factor SRF and cell migration. *Sci Signal* **8** (402), ra112, doi:10.1126/scisignal.aad2959 (2015).
88 Tsopoulidis, N., Kaw, S., Laketa, V., Kutscheidt, S., Baarlink, C., Stolp, B., Grosse, R. & Fackler, O. T. T cell receptor-triggered nuclear actin network formation drives CD4(+) T cell effector functions. *Sci Immunol* **4** (31), doi:10.1126/sciimmunol.aav1987 (2019).
89 Liu, C., Zhu, R. & Mao, Y. Nuclear Actin Polymerized by mDia2 Confines Centromere Movement during CENP-A Loading. *iScience* **9**, 314-327, doi:10.1016/j.isci.2018.10.031 (2018).
90 Parisis, N., Krasinska, L., Harker, B., Urbach, S., Rossignol, M., Camasses, A., Dewar, J., Morin, N. & Fisher, D. Initiation of DNA replication requires actin dynamics and formin activity. *EMBO J* **36** (21), 3212-3231, doi:10.15252/embj.201796585 (2017).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

91 Lamm, N., Masamsetti, V. P., Read, M. N., Biro, M. & Cesare, A. J. ATR and mTOR regulate F-actin to alter nuclear architecture and repair replication stress. *bioRxiv*, 451708, doi:10.1101/451708 (2018).
92 Welch, M. D. & Way, M. Arp2/3-mediated actin-based motility: a tail of pathogen abuse. *Cell Host Microbe* **14** (3), 242-255, doi:10.1016/j.chom.2013.08.011 (2013).
93 Wilkie, A. R., Lawler, J. L. & Coen, D. M. A Role for Nuclear F-Actin Induction in Human Cytomegalovirus Nuclear Egress. *MBio* **7** (4), doi:10.1128/mBio.01254-16 (2016).
94 Ohkawa, T. & Welch, M. D. Baculovirus Actin-Based Motility Drives Nuclear Envelope Disruption and Nuclear Egress. *Curr Biol* **28** (13), 2153-2159 e2154, doi:10.1016/j.cub.2018.05.027 (2018).
95 Andrin, C., McDonald, D., Attwood, K. M., Rodrigue, A., Ghosh, S., Mirzayans, R., Masson, J. Y., Dellaire, G. & Hendzel, M. J. A requirement for polymerized actin in DNA double-strand break repair. *Nucleus* **3** (4), 384-395, doi:10.4161/nucl.21055 (2012).
96 Wang, Y. H., Hariharan, A., Bastianello, G., Toyama, Y., Shivashankar, G. V., Foiani, M. & Sheetz, M. P. DNA damage causes rapid accumulation of phosphoinositides for ATR signaling. *Nat Commun* **8** (1), 2118, doi:10.1038/s41467-017-01805-9 (2017).
97 Caridi, C. P., Delabaere, L., Tjong, H., Hopp, H., Das, D., Alber, F. & Chiolo, I. Quantitative Methods to Investigate the 4D Dynamics of Heterochromatic Repair Sites in Drosophila Cells. *Methods Enzymol* **601**, 359-389, doi:10.1016/bs.mie.2017.11.033 (2018).
98 Homma, K., Yoshimura, M., Saito, J., Ikebe, R. & Ikebe, M. The core of the motor domain determines the direction of myosin movement. *Nature* **412** (6849), 831-834, doi:10.1038/35090597 (2001).
99 Li, J., Lu, Q. & Zhang, M. Structural Basis of Cargo Recognition by Unconventional Myosins in Cellular Trafficking. *Traffic* **17** (8), 822-838, doi:10.1111/tra.12383 (2016).
100 Kapoor, P. & Shen, X. Mechanisms of nuclear actin in chromatin-remodeling complexes. *Trends Cell Biol* **24** (4), 238-246, doi:10.1016/j.tcb.2013.10.007 (2014).
101 Aymard, F., Aguirrebengoa, M., Guillou, E., Javierre, B. M., Bugler, B., Arnould, C., Rocher, V., Iacovoni, J. S., Biernacka, A., Skrzypczak, M., Ginalski, K., Rowicka, M., Fraser, P. & Legube, G. Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. *Nat Struct Mol Biol* **24** (4), 353-361, doi:10.1038/nsmb.3387 (2017).
102 Spichal, M. & Fabre, E. The Emerging Role of the Cytoskeleton in Chromosome Dynamics. *Front Genet* **8**, 60, doi:10.3389/fgene.2017.00060 (2017).
103 Hatakeyama, H., Nakahata, Y., Yarimizu, H. & Kanzaki, M. Live-cell single-molecule labeling and analysis of myosin motors with quantum dots. *Mol Biol Cell* **28** (1), 173-181, doi:10.1091/mbc.E16-06-0413 (2017).
104 Marshall, W. F., Straight, A., Marko, J. F., Swedlow, J., Dernburg, A., Belmont, A., Murray, A. W., Agard, D. A. & Sedat, J. W. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol* **7** (12), 930-939 (1997).
105 Azzaz, A. M., Vitalini, M. W., Thomas, A. S., Price, J. P., Blacketer, M. J., Cryderman, D. E., Zirbel, L. N., Woodcock, C. L., Elcock, A. H., Wallrath, L. L. & Shogren-Knaak, M. A. Human heterochromatin protein 1alpha promotes nucleosome associations that drive chromatin condensation. *J Biol Chem* **289** (10), 6850-6861, doi:10.1074/jbc.M113.512137 (2014).
106 Oshidari, R., Strecker, J., Chung, D. K. C., Abraham, K. J., Chan, J. N. Y., Damaren, C. J. & Mekhail, K. Nuclear microtubule filaments mediate non-linear directional motion of chromatin and promote DNA repair. *Nat Commun* **9** (1), 2567, doi:10.1038/s41467-018-05009-7 (2018).
107 Therizols, P., Fairhead, C., Cabal, G. G., Genovesio, A., Olivo-Marin, J. C., Dujon, B. & Fabre, E. Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. *J Cell Biol* **172** (2), 189-199, doi:10.1083/jcb.200505159 (2006).
108 Kalocsay, M., Hiller, N. J. & Jentsch, S. Chromosome-wide Rad51 spreading and SUMO-H2A.Z-dependent chromosome fixation in response to a persistent DNA double-strand break. *Mol Cell* **33** (3), 335-343, doi:S1097-2765(09)00060-4 [pii] 10.1016/j.molcel.2009.01.016 (2009).
109 Oza, P., Jaspersen, S. L., Miele, A., Dekker, J. & Peterson, C. L. Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. *Genes Dev* **23** (8), 912-927, doi:10.1101/gad.1782209 (2009).
110 Roukos, V., Voss, T. C., Schmidt, C. K., Lee, S., Wangsa, D. & Misteli, T. Spatial dynamics of chromosome translocations in living cells. *Science* **341** (6146), 660-664, doi:10.1126/science.1237150 (2013).

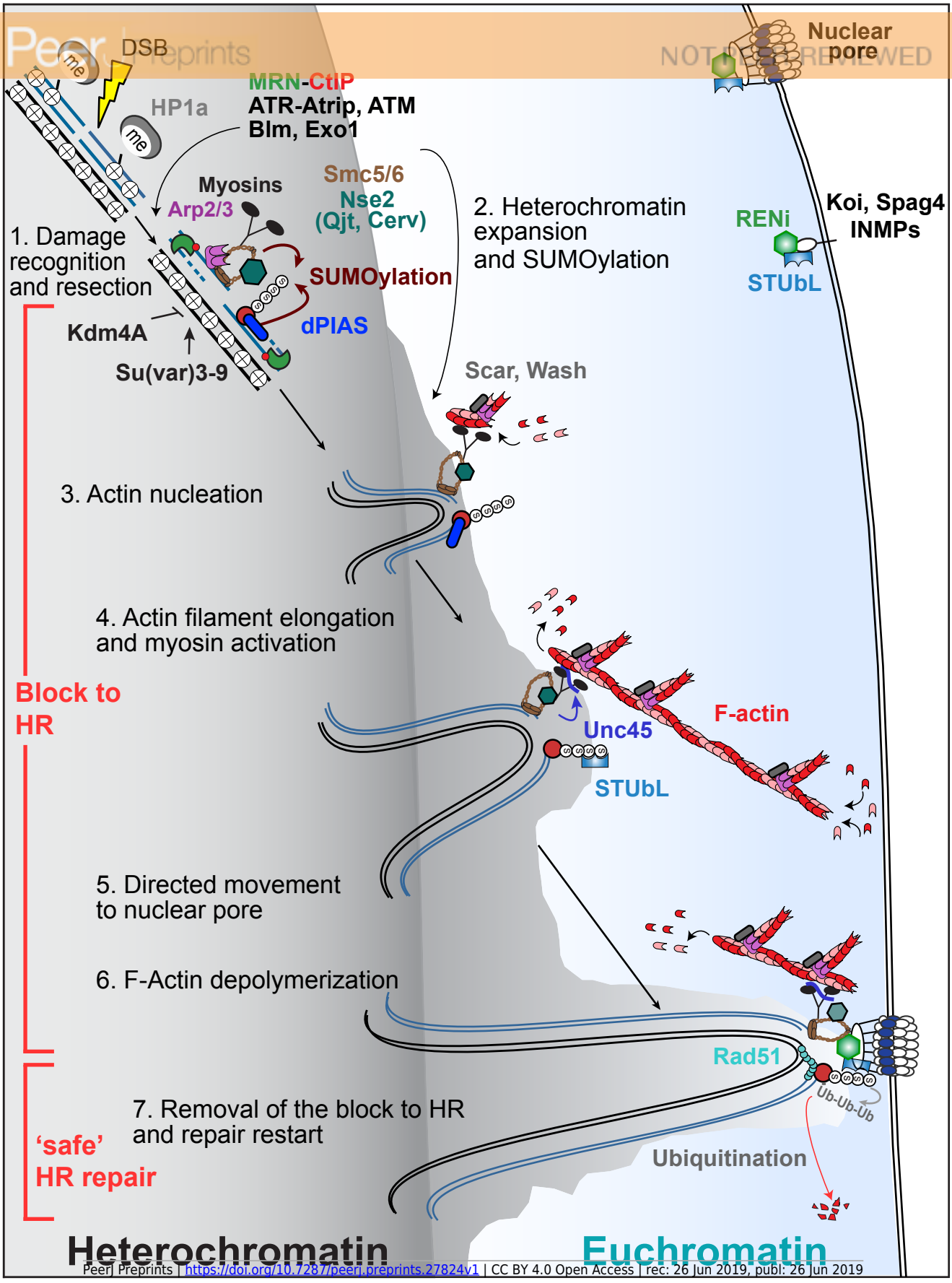
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

111 Swartz, R. K., Rodriguez, E. C. & King, M. C. A role for nuclear envelope-bridging complexes in homology-directed repair. *Mol Biol Cell* **25** (16), 2461-2471, doi:10.1091/mbc.E13-10-0569 (2014).
112 Amitai, A., Seeber, A., Gasser, S. M. & Holcman, D. Visualization of Chromatin Decompaction and Break Site Extrusion as Predicted by Statistical Polymer Modeling of Single-Locus Trajectories. *Cell Rep* **18** (5), 1200-1214, doi:10.1016/j.celrep.2017.01.018 (2017).
113 Cho, N. W., Dilley, R. L., Lampson, M. A. & Greenberg, R. A. Interchromosomal homology searches drive directional ALT telomere movement and synapsis. *Cell* **159** (1), 108-121, doi:10.1016/j.cell.2014.08.030 (2014).
114 Torres-Rosell, J., Sunjevaric, I., De Piccoli, G., Sacher, M., Eckert-Boulet, N., Reid, R., Jentsch, S., Rothstein, R., Aragón, L. & Lisby, M. The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. *Nat Cell Biol* **9** (8), 923-931, doi:10.1038/ncb1619 (2007).
115 Harding, S. M., Boiarsky, J. A. & Greenberg, R. A. ATM Dependent Silencing Links Nucleolar Chromatin Reorganization to DNA Damage Recognition. *Cell Rep* **13** (2), 251-259, doi:10.1016/j.celrep.2015.08.085 (2015).
116 van Sluis, M. & McStay, B. A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes Dev* **29** (11), 1151-1163, doi:10.1101/gad.260703.115 (2015).
117 Horigome, C., Unozawa, E., Ooki, T. & Kobayashi, T. Ribosomal RNA gene repeats associate with the nuclear pore complex for maintenance after DNA damage. *PLoS Genet* **15** (4), e1008103, doi:10.1371/journal.pgen.1008103 (2019).
118 Khadaroo, B., Teixeira, M. T., Luciano, P., Eckert-Boulet, N., Germann, S. M., Simon, M. N., Gallina, I., Abdallah, P., Gilson, E., Géli, V. & Lisby, M. The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. *Nat Cell Biol* **11** (8), 980-987, doi:10.1038/ncb1910 (2009).
119 Chung, D. K., Chan, J. N., Strecker, J., Zhang, W., Ebrahimi-Ardebili, S., Lu, T., Abraham, K. J., Durocher, D. & Mekhail, K. Perinuclear tethers license telomeric DSBs for a broad kinesin- and NPC-dependent DNA repair process. *Nat Commun* **6**, 7742, doi:10.1038/ncomms8742 (2015).
120 Churikov, D., Charifi, F., Eckert-Boulet, N., Silva, S., Simon, M. N., Lisby, M. & Geli, V. SUMO-Dependent Relocalization of Eroded Telomeres to Nuclear Pore Complexes Controls Telomere Recombination. *Cell Rep* **15** (6), 1242-1253, doi:10.1016/j.celrep.2016.04.008 (2016).
121 Su, X. A., Dion, V., Gasser, S. M. & Freudenreich, C. H. Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability. *Genes Dev* **29** (10), 1006-1017, doi:10.1101/gad.256404.114 (2015).
122 Dion, V., Kalck, V., Horigome, C., Towbin, B. D. & Gasser, S. M. Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat Cell Biol* **14** (5), 502-509, doi:10.1038/ncb2465 (2012).
123 Mine-Hattab, J. & Rothstein, R. Increased chromosome mobility facilitates homology search during recombination. *Nat Cell Biol* **14** (5), 510-517, doi:10.1038/ncb2472 (2012).
124 Mehta, I. S., Amira, M., Harvey, A. J. & Bridger, J. M. Rapid chromosome territory relocation by nuclear motor activity in response to serum removal in primary human fibroblasts. *Genome Biol* **11** (1), R5, doi:gb-2010-11-1-r5 [pii] 10.1186/gb-2010-11-1-r5 (2010).
125 Kulashreshtha, M., Mehta, I. S., Kumar, P. & Rao, B. J. Chromosome territory relocation during DNA repair requires nuclear myosin 1 recruitment to chromatin mediated by Upsilon-H2AX signaling. *Nucleic Acids Res* **44** (17), 8272-8291, doi:10.1093/nar/gkw573 (2016).
126 Lisby, M., Mortensen, U. H. & Rothstein, R. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nat Cell Biol* **5** (6), 572-577 (2003).
127 Aten, J. A., Stap, J., Krawczyk, P. M., van Oven, C. H., Hoebe, R. A., Essers, J. & Kanaar, R. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science* **303** (5654), 92-95 (2004).
128 Kruhlak, M. J., Celeste, A., Dellaire, G., Fernandez-Capetillo, O., Muller, W. G., McNally, J. G., Bazett-Jones, D. P. & Nussenzweig, A. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* **172** (6), 823-834, doi:10.1083/jcb.200510015 (2006).
129 Krawczyk, P. M., Borovski, T., Stap, J., Cijsouw, T., ten Cate, R., Medema, J. P., Kanaar, R., Franken, N. A. & Aten, J. A. Chromatin mobility is increased at sites of DNA double-strand breaks. *J Cell Sci* **125** (Pt 9), 2127-2133, doi:10.1242/jcs.089847 (2012).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

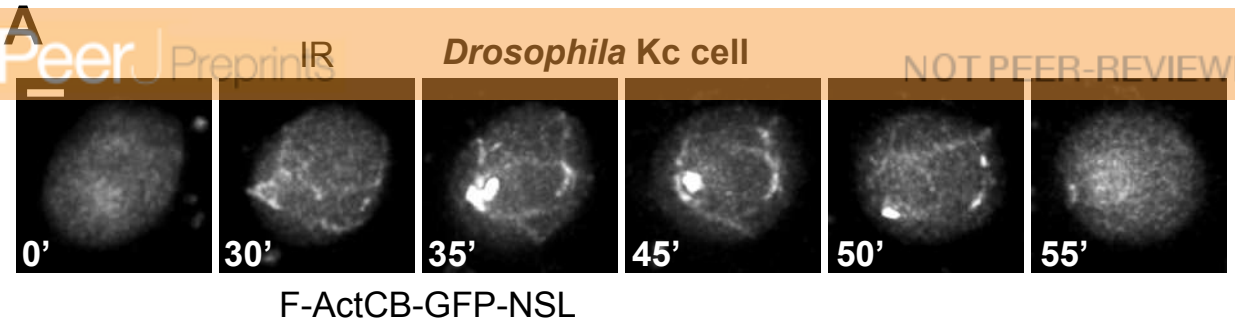
130 Neumaier, T., Swenson, J., Pham, C., Polyzos, A., Lo, A. T., Yang, P., Dyball, J., Asaithamby, A., Chen, D. J., Bissell, M. J., Thalhammer, S. & Costes, S. V. Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. *Proc Natl Acad Sci USA* **109** (2), 443-448, doi:10.1073/pnas.1117849108 (2012).
131 Panse, V. G., Kuster, B., Gerstberger, T. & Hurt, E. Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat Cell Biol* **5** (1), 21-27, doi:10.1038/ncb893 (2003).
132 Smith, M., Bhaskar, V., Fernandez, J. & Courey, A. J. Drosophila Ulp1, a nuclear pore-associated SUMO protease, prevents accumulation of cytoplasmic SUMO conjugates. *J Biol Chem* **279** (42), 43805-43814, doi:10.1074/jbc.M404942200 (2004).
133 Albert, S., Schaffer, M., Beck, F., Mosalaganti, S., Asano, S., Thomas, H. F., Plitzko, J. M., Beck, M., Baumeister, W. & Engel, B. D. Proteasomes tether to two distinct sites at the nuclear pore complex. *Proc Natl Acad Sci U S A* **114** (52), 13726-13731, doi:10.1073/pnas.1716305114 (2017).
134 Huang, K., Tagliazucchi, M., Park, S. H., Rabin, Y. & Szleifer, I. Molecular model of the nuclear pore complex reveals a thermoreversible FG-network with distinct territories occupied by different FG motifs. *bioRxiv*, 568865, doi:10.1101/568865 (2019).
135 Ting, D. T., Lipson, D., Paul, S., Brannigan, B. W., Akhavanfard, S., Coffman, E. J., Contino, G., Deshpande, V., Iafrate, A. J., Letovsky, S., Rivera, M. N., Bardeesy, N., Maheswaran, S. & Haber, D. A. Aberrant overexpression of satellite repeats in pancreatic and other epithelial cancers. *Science* **331** (6017), 593-596, doi:10.1126/science.1200801 (2011).
136 Zhu, Q., Pao, G. M., Huynh, A. M., Suh, H., Tonnu, N., Nederlof, P. M., Gage, F. H. & Verma, I. M. BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. *Nature* **477** (7363), 179-184, doi:10.1038/nature10371 (2011).
137 Hendricks, C. A., Almeida, K. H., Stitt, M. S., Jonnalagadda, V. S., Rugo, R. E., Kerrison, G. F. & Engelward, B. P. Spontaneous mitotic homologous recombination at an enhanced yellow fluorescent protein (EYFP) cDNA direct repeat in transgenic mice. *Proc Natl Acad Sci U S A* **100** (11), 6325-6330, doi:10.1073/pnas.1232231100 (2003).
138 White, R. R., Sung, P., Vestal, C. G., Benedetto, G., Cornelio, N. & Richardson, C. Double-strand break repair by interchromosomal recombination: an in vivo repair mechanism utilized by multiple somatic tissues in mammals. *PLoS ONE* **8** (12), e84379, doi:10.1371/journal.pone.0084379 (2013).
139 Sukup-Jackson, M. R., Kiraly, O., Kay, J. E., Na, L., Rowland, E. A., Winther, K. E., Chow, D. N., Kimoto, T., Matsuguchi, T., Jonnalagadda, V. S., Maklakova, V. I., Singh, V. R., Wadduwage, D. N., Rajapakse, J., So, P. T., Collier, L. S. & Engelward, B. P. Rosa26-GFP direct repeat (RaDR-GFP) mice reveal tissue- and age-dependence of homologous recombination in mammals in vivo. *PLoS Genet* **10** (6), e1004299, doi:10.1371/journal.pgen.1004299 (2014).
140 Delabaere, L., Ertl, H. A., Massey, D. J., Hofley, C. M., Sohail, F., Bienenstock, E. J., Sebastian, H., Chiolo, I. & LaRocque, J. R. Aging impairs double-strand break repair by homologous recombination in Drosophila germ cells. *Aging Cell* **Doi: 10.1111/acel.12556** (2016).
141 Chow, K. H., Factor, R. E. & Ullman, K. S. The nuclear envelope environment and its cancer connections. *Nat Rev Cancer* **12** (3), 196-209, doi:10.1038/nrc3219 (2012).
142 Prochniewicz, E., Thompson, L. V. & Thomas, D. D. Age-related decline in actomyosin structure and function. *Exp Gerontol* **42** (10), 931-938, doi:10.1016/j.exger.2007.06.015 (2007).
143 Scaffidi, P. & Misteli, T. Lamin A-dependent nuclear defects in human aging. *Science* **312** (5776), 1059-1063, doi:10.1126/science.1127168 (2006).
144 Shumaker, D. K., Dechat, T., Kohlmaier, A., Adam, S. A., Bozovsky, M. R., Erdos, M. R., Eriksson, M., Goldman, A. E., Khuon, S., Collins, F. S., Jenuwein, T. & Goldman, R. D. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci U S A* **103** (23), 8703-8708, doi:10.1073/pnas.0602569103 (2006).
145 D'Angelo, M. A., Raices, M., Panowski, S. H. & Hetzer, M. W. Age-dependent deterioration of nuclear pore complexes causes a loss of nuclear integrity in postmitotic cells. *Cell* **136** (2), 284-295, doi:10.1016/j.cell.2008.11.037 (2009).
146 Larson, K., Yan, S. J., Tsurumi, A., Liu, J., Zhou, J., Gaur, K., Guo, D., Eickbush, T. H. & Li, W. X. Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. *PLoS Genet* **8** (1), e1002473, doi:10.1371/journal.pgen.1002473 PGENETICS-D-10-00200 [pii] (2012).

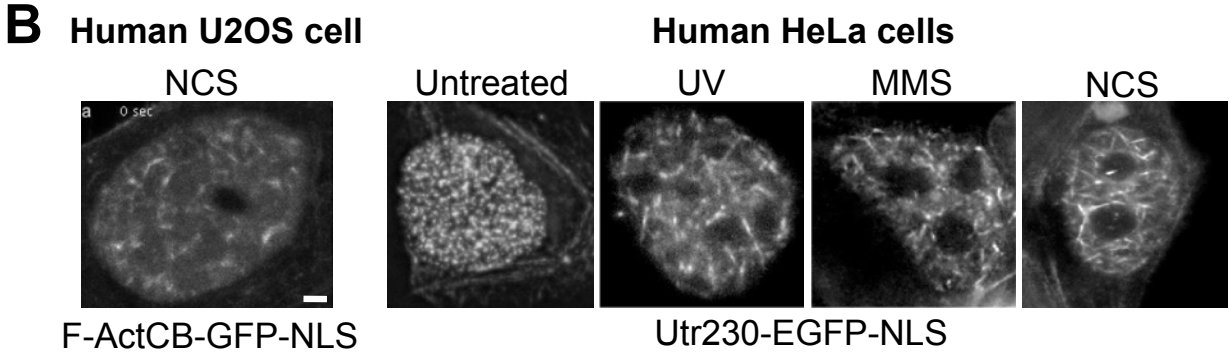
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

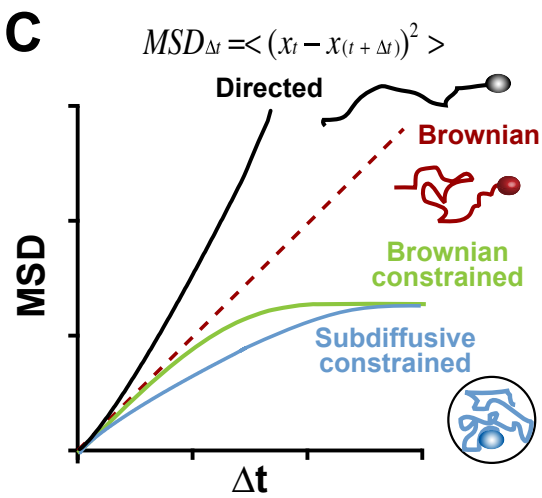


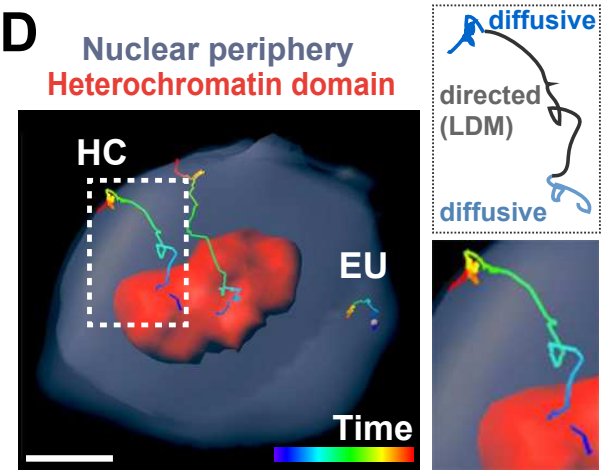
PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

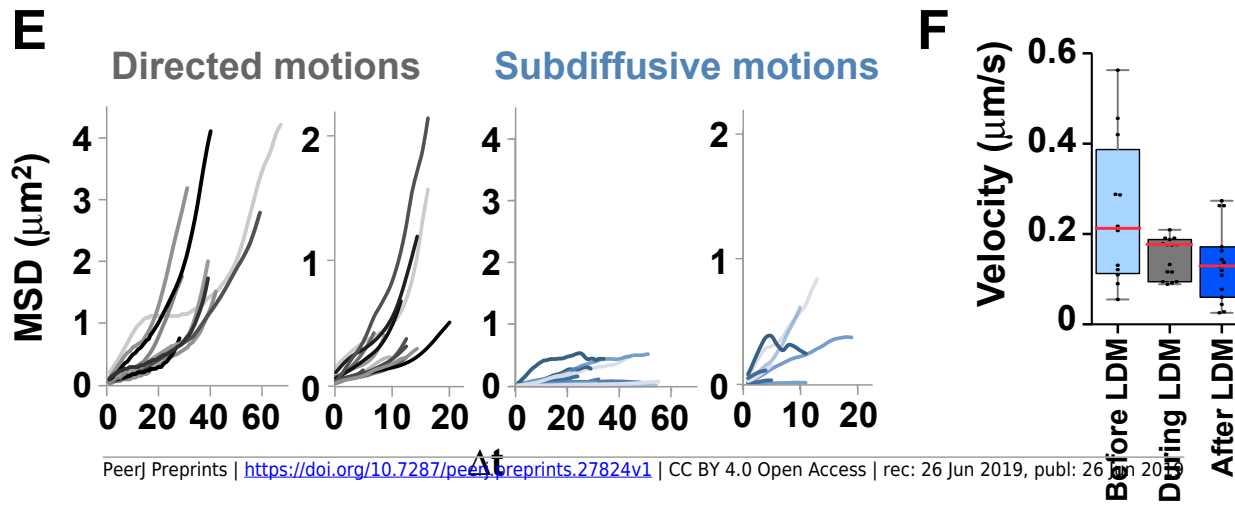
Figure 1











PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019

Figure 2

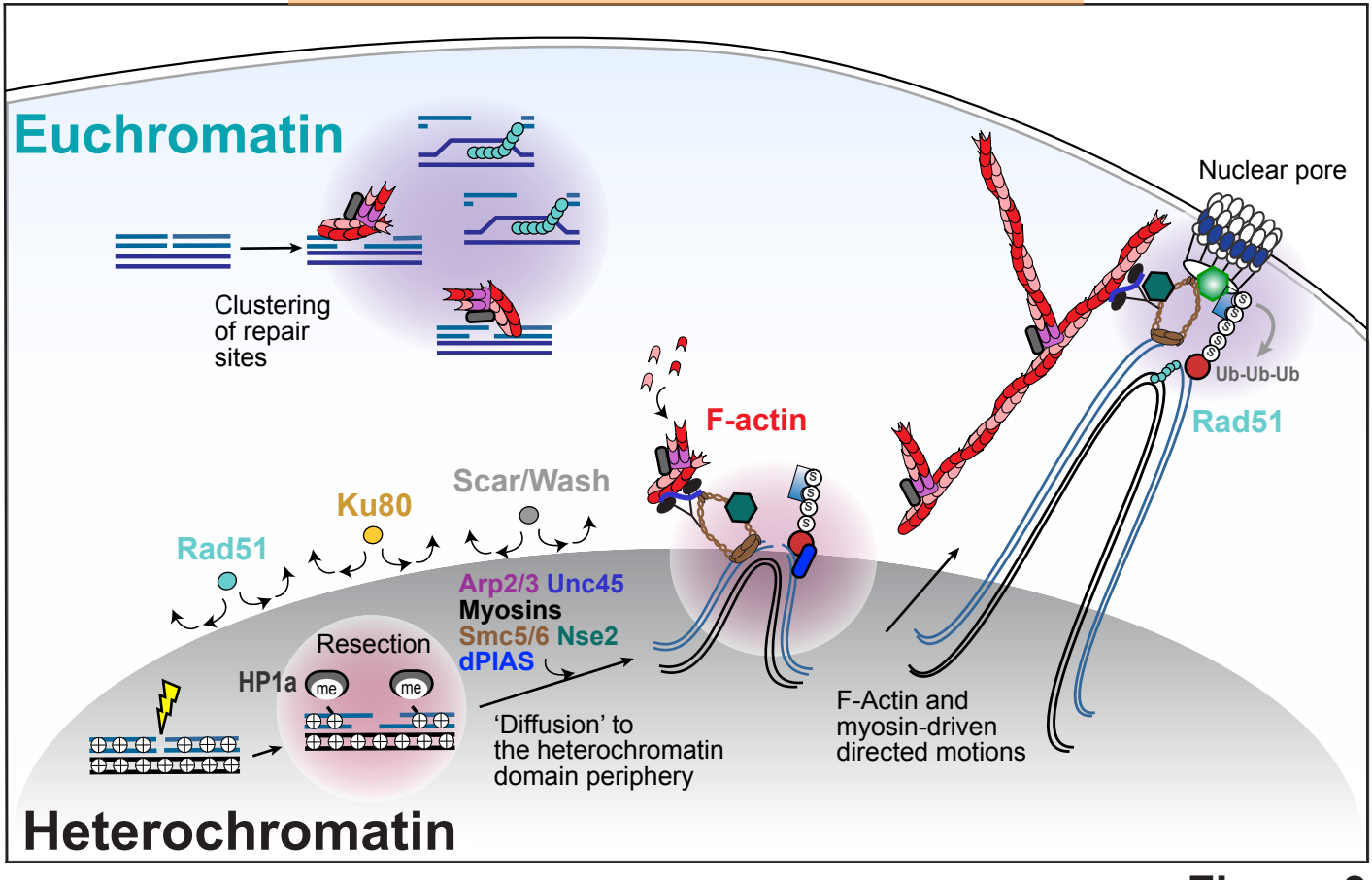


Figure 3

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27824v1 | CC BY 4.0 Open Access | rec: 26 Jun 2019, publ: 26 Jun 2019