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Nuclear actin filaments in DNA repair dynamics

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Abstract

Recent development of innovative tools for live imaging of actin filaments (F-actin) enabled the detection of surprising nuclear structures responding to various stimuli, challenging previous models showing that actin is substantially monomeric in the nucleus. We review these discoveries, focusing on double-strand break (DSB) repair responses, which revealed a remarkable network of nuclear filaments and regulatory mechanisms coordinating chromatin dynamics with repair progression and led to a paradigm shift by uncovering the directed movement of repair sites.

Actin filaments are major components of the cytoskeleton, responsible for cell movement and adhesion or transport via myosin motors[1–3]. F-actin responds dynamically to a variety of stimuli through actin remodellers (e.g., actin nucleators, bundling components, crosslinking proteins, and disassembly factors)[1,4] (Fig. 1). The three major classes of actin nucleators are the Arp2/3 complex, formins, and Spire-family components, each characterized by distinct structural properties, regulatory mechanisms, and functions[2,4]. Arp2/3 is activated by Wiskott-Aldrich Syndrome (WAS) family proteins, including Wash, Wasp, and Scar/Wave, which nucleate actin in different contexts[5]. Whereas cytoplasmic roles and regulations of F-actin are well characterized, nuclear functions have long remained elusive. This is partly because the more abundant cytoplasmic signal interferes with nuclear F-actin detection under traditional staining and imaging approaches[6,7]. Major breakthroughs resulted from the development of fluorescently tagged F-actin-specific probes with nuclear localization signals (NLS) for live imaging of nuclear filaments[6,8–11] and the establishment of genetic

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Competing interests

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approaches that selectively inactivate nuclear actin polymerization[8–12] (see refs.[7,13] for direct comparisons of the pros and cons of different tools to visualize nuclear F-actin). Using these tools, recent studies have illuminated several functions of nuclear F-actin, supporting a general model whereby filaments are mostly stimulus driven and mediate chromatin responses to different stresses[14].

Functions of nuclear F-actin

A powerful system to study nuclear F-actin is the germinal vesicle (GV) of the *Xenopus* oocyte[15], a nucleus several hundred micrometers in diameter that has a high concentration of nuclear actin due to the lack of the actin export factor Exportin 6[16,17]. In GVs, nuclear F-actin forms a sponge-like mesh for mechanical stability[16] and nuclear organization[18,19]. Notably, transplantation of somatic cell nuclei into *Xenopus* oocytes induces transcriptional reprogramming that requires dynamic and prolonged actin polymerization by Wave1[20,21], suggesting a role for nuclear F-actin in transcription regulation.

In other cell types, dynamic nuclear actin filaments form in response to various stimuli, including serum treatment, cell spreading, T-cell activation, mitotic exit, and viral infection[14] (Fig. 1). Serum treatment of human cells induces a quick burst (<60 s) of nuclear actin polymerization by formins[8]. This lowers nuclear G-actin (globular and monomeric) concentration, resulting in G-actin release from the myocardin-related transcription factor (MRTF-A), MTRF-A translocation to the nucleus, and transcriptional co-activation of the serum response factor (SRF)[8,22,23]. Similar MRTF-A regulation occurs during cell spreading[10], although here filaments are shorter and long lasting, and their formation requires a functional LINC (linker of nucleoskeleton and cytoskeleton) complex[10]. Intriguingly, MRTF-A activity also depends on its association with the F-actin crosslinking component Filamin-A[24]. Actin polymerization is required for this interaction, suggesting an independent and direct role for F-actin in MRTF-A activation[24].

Further, a recent study demonstrated a critical role of nuclear F-actin in the induction of cytokine expression after T-cell activation[25]. This occurs after T-cell receptor engagement in $CD4^+$ cells (for example, during immunological synapse formation) and requires calcium elevation, N-Wasp, and nuclear Arp2/3[25], revealing the importance of nuclear F-actin in immune function.

Nuclear F-actin might also contribute to transcriptional regulation by repositioning genomic loci. Two parallel studies provided indirect evidence, via live-cell imaging of mammalian cells, for actin-dependent repositioning of chromosome loci to regulate transcription[26,27]. Expression of the non-polymerizable actin G13R mutant inhibits locus migration[26], consistent with F-actin-dependent transport. Notably, actin, actin-polymerizing proteins, and myosins also interact with RNA polymerases[28–30], are enriched at transcription sites[31,32], and promote polymerase activity[30]. Similarly, actin and the actin-related proteins (ARPs) Arp4-Arp9 are subunits of chromatin remodellers and histone modifiers, affecting transcription locally and globally[17,33–35] (reviewed in ref.[36]). However, here actin appears mostly monomeric, and Arp4-Arp9 do not promote actin nucleation[37,38]; thus, the contribution of F-actin in these contexts remains to be characterized.

Recent studies also identified transient nuclear actin polymerization during mitotic exit, which facilitates nuclear volume expansion and chromatin decompaction in early G_1[12]. This requires the nuclear activity of the severing factor Cofilin 1, as shown with phalloidin proteomics and optogenetics[12]. Notably, formin-dependent nuclear F-actin assembly in G_1 has also been linked to centromere maintenance via CenpA recruitment in human cells[39], as well as to replication initiation via pre-initiation complex (pre-IC) loading in *Xenopus* extracts and human cells[40], suggesting multiple functions of F-actin in G_1. F-actin might also affect replication timing indirectly by promoting nuclear organization and origin positioning upon mitotic exit. Nuclear positioning of replication origins in G_1 affects origin activation timing in S phase from yeast to mammalian cells[41–46]. In budding yeast, for example, the spatiotemporal replication program is at least in part coordinated by Fkh1/2[47–49] and Rif1[50,51], which regulate origin position and dynamics. F-actin might actively participate in this organization, thus contributing to the orchestration of the replication program.

Finally, nuclear F-actin forms during viral infections to promote viral particle mobilization[52–54]. For example, the baculovirus *Autographa californica* M nucleopolyhedrovirus (AcMNPV) hijacks the host nuclear Arp2/3 complex using viral Wasp-like proteins to enable actin-based virus mobilization and nuclear egress[54]. Together, these studies identified exciting examples of nuclear actin filaments responding to different stimuli that regulate transcription, chromosome positioning, and nuclear architecture through distinct regulatory mechanisms.

Nuclear F-actin is required for DSB repair

The two prominent pathways that repair DSBs are non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ promotes direct rejoining of the two DSB ends with little processing and frequent mutations at the break site[55]. HR instead starts with DSB resection to create single-stranded DNA (ssDNA), which invades homologous sequences used as templates (‘donors’) for DNA synthesis and restores the original information[56]. Actin and actin-associated proteins have long been linked to different aspects of DSB repair. For example, the actin nucleator JMY translocates to the nucleus in response to damage and promotes transcription of the p53 repair component[57]; the actin crosslinking protein Filamin-A interacts with Brca1 and Brca2 HR proteins and promotes repair[58–60]; the formin-associated protein suppressor of cancer cell invasion (ScaI) is recruited to DSBs and is required for repair in mammalian cells[61,62]; altering actin polymerization or crosslinking or nuclear myosin I (NMI) affects DNA damage responses[63–67], including HR repair[66,67]. Finally, in budding yeast, chromatin movements during DSB repair are affected by cytoplasmic actin filaments that transfer forces to the nucleus via nuclear pores[66]. Nuclear G-actin and ARPs also participate in DNA repair as components of chromatin remodellers and histone modifiers[36,68], which regulate the chromatin landscape locally and globally[34,35], genome dynamics during DNA repair[69,70], and transcription[34,35,71] in response to damage. However, the role of nuclear F-actin in these responses remains unclear.

Intriguingly, both HR and NHEJ components bind F-actin in vitro, and inactivation of nuclear actin polymerization affects the retention of the Ku[80] NHEJ protein to damage sites

in vivo in human cells[64], suggesting direct roles for nuclear F-actin in DSB repair. In agreement, nuclear actin filaments form in response to different damage treatments in human cells[9], and selective inactivation of actin polymerization in the nucleus results in defective repair after treatment with the damaging agent methyl methanesulfonate (MMS)9. Nuclear F-actin also forms in response to laser microirradiation in human cells, and actin polymerization promotes recruitment of ATR checkpoint kinases to repair sites[72]. Finally, nuclear F-actin assembles in mouse oocytes in response to DSBs[73]. Together, these studies suggest an important yet enigmatic role for nuclear F-actin in DSB repair.

Nuclear F-actin and myosins relocalize heterochromatic DSB to the nuclear periphery

A recent study identified a direct role of nuclear F-actin in the relocalization of heterochromatic DSBs in mouse and *Drosophila* cells for 'safe' HR repair[11] (Fig. 2). Pericentromeric heterochromatin (hereafter called 'heterochromatin') accounts for ~30% of fly and human genomes[74–76], is enriched for 'silent' chromatin marks (e.g., H3K9me2/3 and heterochromatin protein 1 (HP1)[77,78]), and is absent in budding yeast. Notably, heterochromatin has a distinct function and structure compared to lamina-associated domains (LADs) identified along the arms of chromosomes[75,79–81], and, in contrast to those, it is not usually associated with the nuclear periphery[11,82–87] or enriched for H3K27me3[75] (reviewed in ref.[88]).

Heterochromatin mostly comprises repetitive DNA sequences[74–76]. In *Drosophila*, about half of these sequences are 'satellite' repeats (mostly five-base-pair sequences spanning hundreds of kilobases to megabases), and the remaining sequences are transposable elements and other scrambled repeats[74–76]. In single-copy sequences (like most euchromatin), a unique donor is available on the homologous chromosome or the sister chromatid, and HR repair is mostly 'error free'[56]. In heterochromatin, the presence of thousands to millions of potential donor sequences associated with different chromosomes can induce intra- and interchromosomal recombination or unequal sister chromatid exchange, triggering gross chromosomal rearrangements[11,83,85,89–93]. Despite this risk, HR is a primary pathway for heterochromatin repair[83,85,86,92,94,95], and specialized mechanisms exist to mitigate ectopic recombination[88,96,97].

In *Drosophila* and mouse cells, in which heterochromatin forms distinct nuclear 'domains'[78,83,87,98] (named 'chromocenters' in mouse cells), DSB resection starts inside the domains[83,85,86,99], while strand invasion is temporarily halted (Fig. 2). In flies, this block to HR progression relies on SUMOylation by dPIAS and the Smc5/6 subunits Nse2/Cerv and Nse2/Qjt[83,85,92]. Next, the heterochromatin domain expands[83,93,100], and DSBs relocalize to outside the domain[11,83–86,95,99,101]. In *Drosophila* cells, expansion and relocalization require resection and checkpoint kinases (mostly ATR)[83]. Relocalization also requires Kdm4A-dependent histone demethylation[102] and SUMOylation[11,83,85,92]. In mouse cells, the checkpoint kinase ATM and its target Kap1[82,86,103] are required for heterochromatin relaxation. In *Drosophila* cells, repair sites reach the nuclear periphery before Rad51 recruitment and strand invasion[11,85], whereas in mouse cells repair appears to continue at the

chromocenter periphery[11,86,99]. Relocalization defects result in aberrant recombination and widespread genomic instability, revealing the importance of these dynamics for genome integrity[11,83,85,92,93]. Relocalization may prevent aberrant recombination by moving repair sites away from ectopic sequences prior to strand invasion. At the same time, sister-chromatid pairing (along with homologous pairing in *Drosophila*[104]) would guarantee simultaneous relocalization of homologous templates for 'safe' HR progression at the nuclear periphery[11,95].

In *Drosophila* cells, relocalization of heterochromatic DSBs relies on a striking network of nuclear actin filaments assembled at repair sites by Arp2/3 and extending toward the nuclear periphery[11] (Fig. 2). Live imaging revealed repair sites 'sliding' along those filaments[11], consistent with a role of filaments as 'highways' for relocalization. Importantly, filaments were detected with the nuclear F-actin chromobody, which does not alter nuclear actin levels[11], and confirmed using direct F-actin staining with phalloidin[11], ruling out secondary effects of the visualization tool on filament formation or dynamics. Relocalization also relies on three nuclear myosins (Myo1A, Myo1B, and MyoV), as well as on myosin's ability to 'walk' along actin filaments[11,93]. In agreement, relocalization of heterochromatic DSBs is characterized by directed motions[11,105]. Recruitment of Arp2/3 and myosin to repair foci requires the early DSB signalling and processing factor Mre[11] and the heterochromatin protein HP1a[11], suggesting the combination of these components as a mechanism for targeting the relocalization machinery specifically to heterochromatic DSBs. Further, Smc5/6 physically interacts with Arp2/3 and myosins[11], consistent with a regulatory role for Smc5/6 in Arp2/3 and myosin function. Smc5/6 is also required for the loading of Unc[45] to heterochromatic repair sites[11], suggesting that this step is a critical switch for activating myosin and DSB relocalization downstream from Smc5/6. These data support a model in which nuclear F-actin assembles at heterochromatic DSBs to guide their relocalization to the nuclear periphery via a myosin-driven 'walk' along the filaments. In addition to activating myosins by recruiting Unc[45], Smc5/6 might provide a direct link between myosins and resected DNA, translating myosin-driven pulling forces into the movement of repair sites. Arp2/3, myosins, actin polymerization, or myosins' ability to walk along filaments are also required to relocalize and repair heterochromatic DSBs in mouse cells[11], and in for heterochromatin stability in *Drosophila* salivary glands[93], revealing pathway conservation across different cells and tissue types.

Nuclear actin polymerization promotes DSB dynamics and HR in euchromatin

Nuclear actin polymerization has also been proposed to drive local dynamics promoting focus clustering (i.e., the non-elastic collision between repair foci[84]) and HR repair in euchromatin[11,106] (Fig. 2). Observed in various organisms from yeast and mammalian cells[11,83,84,106–111], focus clustering might facilitate repair by increasing the local concentration of damage signalling or repair components[106,112]. In human cells, Arp2/3 is enriched at AsiSI-induced DSBs undergoing HR and is required for repair focus clustering, DSB resection, and HR completion[106]. Intriguingly, resection is also required for the dynamics of repair sites, suggesting a positive feedback loop between focus dynamics and

repair progression[106]. Notably, AsiSI is blocked by DNA methylation, a typical feature of mammalian heterochromatin, implying that the DSBs that occur in response to AsiSI are largely euchromatic[113]. Arp2/3 also mediates the formation of short nuclear actin polymers in response to DSB induction with neocarzinostatin (NCS) in human cells[106]. These structures are highly dynamic and track with HR repair sites[106]. Inactivating nuclear actin polymerization affects HR repair[106], mimicking the loss of Arp2/3[106] and supporting a model in which Arp2/3-induced nuclear actin polymers promote focus movement and HR progression in euchromatin. It has been proposed that actin structures promote clustering by generating forces that move repair sites[106], although more studies are required to understand how F-actin works in this context. Arp2/3 also promotes clustering of euchromatic DSBs in *Drosophila* cells[11], revealing conserved responses. Interestingly, studies in *Drosophila* cells showed that the myosin activator Unc[45] is not required for clustering[11]. Further, Arp2/3 does not mediate clustering of heterochromatic DSBs[11], revealing that the mechanisms responsible for relocalization of heterochromatic DSBs, for clustering of euchromatic breaks, and for clustering of heterochromatic breaks are genetically distinct. Together, these studies unraveled two separate functions of nuclear actin structures in DSB repair. In heterochromatin, F-actin and myosins enable the directed motion 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 (Fig. 2).

Mechanisms of damage-induced actin polymerization

Intriguingly, distinct nucleators appear to contribute to damage-induced nuclear actin polymerization, potentially reflecting differences across repair pathways, cell cycle phases, organisms, cell types, and/or chromatin domains (Fig. 3; Table 1).

Arp2/3 mediates actin nucleation in *Drosophila* and relocalization of heterochromatic DSBs in both *Drosophila* and mouse cells[11,93], whereas Spire and formins do not appear to contribute to these dynamics11. Similarly, Arp2/3 is specifically required for DSB clustering both in human S/G2 cells and in *Drosophila* cells, revealing a major role for Arp2/3 in nuclear actin-driven dynamics during DSB repair[11–106]. However, relocalization of heterochromatic DSBs in *Drosophila* relies on Scar and Wash (but not on Wasp)[11], whereas dynamic movement of human repair sites requires Wasp[106], revealing distinct mechanisms for Arp2/3 activation in these contexts.

In other studies, nucleators other than Arp2/3 appear to promote damage-induced F-actin assembly. In human cells, MMS-induced nuclear F-actin requires Formin 2 (FMN-2) and Spire-1/29, and clustering of euchromatic repair sites in G_1 relies on FMN-2[111]. In G1, clustering specifically involves DSBs processed for HR, suggesting a role for clustering in isolating breaks that cannot be readily repaired[111]. However, clustering in G_1 also requires the LINC complex[111], and evidence for formin enrichment at repair sites is lacking, suggesting that cytoplasmic forces transferred to the nuclei contribute to focus dynamics in this context. Intriguingly, the heterochromatin repair component ScaI also interacts with

formins in mammalian cells[62], suggesting additional roles for formins in heterochromatin repair.

Although a systematic characterization of actin nucleators mediating DNA repair dynamics across different cell cycle phases, chromatin contexts, organisms, or cell types is missing, it is tempting to speculate that distinct regulators organize different types of nuclear actin structures, which are perhaps linked to unique functions (Fig. 3). For example, short actin polymers might be sufficient for local dynamics mediating clustering, whereas long filaments might be needed for the myosin-dependent, longer-range, directional motions of heterochromatic DSBs. Accordingly, in *Drosophila* cells, filaments originating from heterochromatic DSBs appear as long branched structures reaching the nuclear periphery[11]. The importance of branching is also unclear, but it might facilitate relocalization in a 'crowded' environment such as the nucleus by providing alternative paths to the nuclear periphery.

Damage-induced actin filaments are also highly dynamic. Heterochromatin-associated structures in *Drosophila* frequently elongate and shrink, disassembling after relocalization of repair sites[11]. Similarly, short structures detected in human cells continuously fuse and separate[106]. Although it is still unclear which signals and components regulate these dynamics and their relevance to repair progression, actin remodelling is potentially involved, and dynamics might enable 'probing' of the nuclear space for an efficient path for relocalization. Understanding the mechanisms responsible for actin polymerization and disassembly in different repair contexts, and the relationship between structure, dynamics, and function in DSB focus motion and repair, are some of the most exciting open questions in the field.

Other structures and motors for repair focus dynamics

Nuclear F-actin is not the only structural component promoting nuclear dynamics during DNA repair. Studies in yeast and mammalian cells revealed that disrupting microtubules or kinesins affects repair progression and DSB dynamics[111,114–116]. These responses might be at least, in part, dependent on cytoplasmic microtubules, which influence nuclear dynamics through the LINC complex spanning the nuclear envelope[111,114–117].

Intriguingly, recent studies in budding yeast identified damage-induced nuclear microtubules that 'capture' repair foci, promoting relocalization of repair sites for break-induced replication (BIR)[116] (Table 1). Similarly to F-actin-driven motions, nuclear microtubule-induced dynamics are characterized by directed motions[116]. Kar3 kinesin is also required for this movement and for repair[116,118]. Whether this reflects a nuclear function of this motor remains unclear, but an interesting possibility is that kinesin-driven movement along nuclear microtubules drive chromatin dynamics for DNA repair. More studies are needed to establish which organisms and damage conditions nuclear microtubules assemble in to promote chromatin dynamics, as well as the role(s) of kinesins in these pathways.

Nuclear F-actin in replication fork repair

Interestingly, nuclear architecture and dynamics influence not only replication initiation, but also fork progression in the presence of replication challenges. For example, in budding yeast, DNA damage occurring during replication of CAG repeats or in the presence of hydroxyurea (HU) or MMS triggers relocalization of replication forks to the nuclear periphery for fork rescue[119,120]. Further, replication of heterochromatin in mouse cells occurs at the heterochromatin domain periphery, suggesting that fork relocalization facilitates replication through a challenging environment, such as highly repeated satellites[121]. Whether these movements rely on nuclear F-actin and motor components is unknown, but interestingly, HU treatment stimulates the nuclear import of actin and actin-polymerizing proteins in mouse cells[122], and blocking actin polymerization results in sensitivity to replication challenges[106], suggesting the importance of nuclear F-actin in replication stress response. Consistent with this, a recent study in human cells identified ATR-dependent nuclear F-actin in S-phase upon replication stress, suggesting a role for these structures in relocalization of damaged forks to the nuclear periphery for fork restart[123].

Notably, ATR has also been proposed as a mechanosensor for torsional stress at the nuclear membrane (e.g., during replication of membrane-associated chromatin[124,125]), and ATR-associated F-actin might play a role in this response. Together, these studies reveal the importance of nuclear positioning and dynamics in replication regulation. Further investigation is needed to establish how nuclear F-actin or other structures contribute to replication fork rescue and repair.

Directed and subdiffusive motion of repair sites

Nuclear repositioning of repair sites occurs in different contexts[88,96], including DSBs in rDNA[126–129], damaged telomeric and subtelomeric sequences[118,130–133], collapsed replication forks[119,120], persistent DSBs[117,119,130,134–137], and homology search[132,138,139]. However, these dynamics are largely thought to occur by Brownian/subdiffusive motion[140].

A traditional approach to distinguish Brownian versus directed motions is the mean-square displacement (MSD) analysis of the positional data for repair sites[105,141] (Fig. 3). When MSD values are plotted at increasing time intervals, linear MSD graphs reflect Brownian motion, whereas curves characterized by a progressively increasing slope indicate directed motion[105,141]. Notably, chromatin movements are typically subdiffusive rather than Brownian, as chromatin behaves like a polymer and is subject to other constraints (e.g., anchoring to nuclear structure, molecular crowding, and chromatin compaction) that to flattened MSD curves[105,141]. Further, sub-diffusive motions occurring in a confined space (e.g., subnuclear domains or the nucleus) typically yield MSD graphs that reach a plateau proportional to the radius of confinement[105,141]. Given that MSD graphs describing repair focus dynamics typically reach a plateau, previous studies concluded that the movement is subdiffusive and confined[140,142]. However, analogous curves also result from averaging MSD graphs from an asynchronous population of foci, each displaying mixed types of

motions[11,105,116,143] (e.g., the alternation or the simultaneous occurrence of diffusive and directed motions), revealing the need for more sophisticated analyses.

For example, repair foci leave the heterochromatin domain with different kinetics[11,83,85], and the movement is largely subdiffusive confined before relocalization[11,97], possibly because of heterochromatin compaction and limited dynamics. Additionally, focus movement is highly confined after repair-site anchoring to the nuclear periphery[11,85,97]. Consistent with this mixed behavior, MSD analyses of a population of heterochromatic repair foci yielded graphs that reach a plateau at increasing time intervals[11]. However, application of a computational method that identifies tracts of directed motions in a context of mixed types of motions[105] unmasked long-lasting directed motions (LDMs) mostly occurring between the heterochromatin domain periphery and the nuclear periphery[11,97]. A similar approach identified directed motions at replication sites[123]. At heterochromatic DSBs, LDMs last ~24 min, consistent with the average duration of nuclear actin filaments[11]. The speed of movement of repair foci is ~0.15 μm/min during LDMs[11,97], similar to that of transcription sites repositioned in the nucleus in an F-actin and myosin-dependent manner[26,144]. Notably, the speed of focus motion does not increase during directed motions[97], suggesting that actin filaments and motors do not affect the speed of motion. Rather, they might provide directionality and counteract other forces that limit the release of repair foci from the heterochromatin domain (e.g., phase separation[97,145,146] or chromatin compaction[147]).

Directed movements of repair sites along nuclear microtubules in yeast are also affected by two confounding factors: (i) the movement along microtubules is transient; and (ii) microtubules pivot around the microtubule organizing center (MTOC) while MTOCs also translocate along the nuclear periphery, resulting in non-linear directed motions[116]. In this case, directed motions were identified by directional change distribution (DCD) analysis, which measures changes in the angle of a trajectory and can reveal broader motion profiles by increasing the temporal coarse graining[116]. At coarser time intervals, this method unmasks kinesin-dependent directed motions[116]. Notably, removal of the Kar3 kinesin affects relocalization, but not the speed of motion[116], further suggesting a role for filaments in providing directionality rather than increasing velocity.

Finally, directed motions occur at telomeres repaired by HR in ALT human cells and were detected by calculating MSD curves at time points at which directional movements can be identified by eye[132].

These studies revealed that in the context of chromatin dynamics, whereby directed motions occur non-synchronously for different repair sites, and also concurrently or in alternation with subdiffusive confined motions, MSD analyses applied to the entire kinetic are insufficient to detect directed motions. Remarkably, a re-analysis of the dynamics of persistent DSBs in budding yeast revealed the presence of directed motions[116], suggesting that nuclear structures and motors might contribute to repositioning of repair sites in more contexts than initially thought. More studies are needed to identify directed movements and the motor components mediating these dynamics in various contexts.

Nuclear F-actin in disease

The identification of direct functions of nuclear F-actin in DSB repair suggests deregulation of these mechanisms as a contributing factor for genome instability and tumorigenesis. Accordingly, inactivation of relocalization mechanisms causes repair defects and genome instability in *Drosophila* and mouse cells[11,85,92,93] and HR repair defects in human cells[106], revealing the importance of these dynamics for genome integrity. Micronuclei and widespread chromosome rearrangements observed in the absence of relocalization pathways are commonly found in cancer cells and directly contribute to genome instability and cancer progression[148,149]. Consistent with the importance of relocalization pathway components in tumor suppression, actin, actin-remodelling proteins, and myosins are frequently mutated in cancer cells[150], and deregulation of Arp2/3 activators in WAS also results in HR repair defects in lymphocytes[106], as well as predisposition to non-Hodgkin's lymphoma and leukaemia[151]. Given the importance of F-actin in T-cell activation, deregulation of actin polymerization might also contribute to other immune system dysfunctions[25].

Defective nuclear actin remodelling has also been linked to Huntington's disease (HD), a progressive neurodegenerative disorder caused by CAG expansion in the codifying region for the huntingtin protein[152,153]. Thick stress-induced nuclear actin filaments (act in/Cofilin rods) accumulate in cells from patients with HD[154], with more rods observed as the disease progresses[154], revealing abnormal F-actin processing. Intriguingly, huntingtin associates with the rods[154] and promotes filament disassembly[154] and DNA damage repair[155], suggesting a direct link between disease progression, actin deregulation, and DNA repair defects in HD: deregulation of nuclear F-actin processing during DNA repair might critically contribute to neurodegeneration in HD. Independent studies in budding yeast revealed that replication fork instability at critically long CAG repeats is rescued by relocalization of these sequences to the nuclear periphery[120]. Although more studies are needed to understand the role of nuclear actin filaments in this context, and the existence of similar pathways in human cells, this suggests that nuclear actin deregulation might be not only a consequence of huntingtin dysfunction, but also a driving force for repeat expansion and initiation or aggravation of the disease.

Finally, myosins and actin-myosin interaction deteriorates with age[156], and this decline may be a contributor of repair defects and genome instability observed in older organisms[157,159]. Intriguingly, common mutations of Lamin A responsible for Hutchinson-Gilford progeria syndrome (HGPS) disrupt the ability of Lamin A to bundle actin filaments[160], raising the possibility that aspects of this premature aging disorder (e.g., DNA repair defects and heterochromatin deregulation[161,162]) reflect nuclear F-actin deregulation. Additionally, nuclear dynamics contribute to DSB repair in neurons during sleep[163], suggesting a direct link between age-related F-actin deterioration and neurodegeneration. Together, the discovery of critical roles of nuclear F-actin and myosins in DNA repair and genome stability unlocks the door to a better understanding of the molecular mechanisms that are deregulated in human diseases, including cancer, immunological and neurological disorders, progeria, and other aging-related dysfunctions.

Conclusions and perspectives

Significant efforts in recent years have started to shed light on the fascinating roles of nuclear F-actin in cellular responses, including in nuclear dynamics of DNA repair sites. These discoveries challenged the previous conclusions that actin is only monomeric in the nuclei, revealing remarkable filaments of a transient nature with critical cellular roles. Nuclear actin filaments responding to DNA damage appear to have different regulatory mechanisms, suggesting distinct structures with specialized functions. Filaments form 'highways' for the myosin-dependent 'walk' of repair sites during heterochromatin repair, and short structures are linked to focus clustering in euchromatin. These discoveries also opened a number of additional questions. For example, the molecular mechanisms regulating actin nucleation in various contexts are largely unknown. The fine structure of filaments requires deeper investigation. Actin remodellers responsible for filament dynamics need to be established, and the importance of these dynamics in repair is unclear. Further, several repair pathways rely on nuclear dynamics, and recently developed analytical methods[11,105,116,123] will likely uncover more examples of directed motions, stimulating the investigation of structural and motor components involved. Characterizing these mechanisms is expected to broaden our understanding of the molecular causes of a number of diseases, enabling more effective treatments, and the tools are now in place to propel a significant advancement of this field in the near future.

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References

1. Pollard TD Actin and actin-binding proteins. Cold Spring Harb. Perspect. Biol 8, a018226(2016). [PubMed: 26988969]
2. Rottner K, Faix J, Bogdan S, Linder S & Kerkhoff E Actin assembly mechanisms at a glance. J. Cell Sci 130, 3427–3435 (2017). [PubMed: 29032357]
3. Titus MA Myosin-driven intracellular transport. Cold Spring Harb. Perspect. Biol 10, a021972(2018). [PubMed: 29496823]
4. Pollard TD Regulation of actin filament assembly by Arp2/3 complex and formins. Annu. Rev. Biophys. Biom ol. Struct 36, 451–477 (2007).
5. Verboon JM, Sugumar B & Parkhurst SM Wiskott-Aldrich syndrome proteins in the nucleus: aWASH with possibilities. Nucleus 6, 349–359 (2015). [PubMed: 26305109]
6. Belin BJ, Cimini BA, Blackburn EH & Mullins RD Visualization of actin filaments and monomers in somatic cell nuclei. Mol. Biol. Cell 24, 982–994 (2013). [PubMed: 23447706]
7. Melak M, Plessner M & Grosse R Actin visualization at a glance. J. Cell Sci 130, 525–530 (2017). [PubMed: 28082420]
8. Baarlink C, Wang H & Grosse R Nuclear actin network assembly by formins regulates the SRF coactivator MAL. Science 340, 864–867 (2013). [PubMed: 23558171]
9. Belin BJ, Lee T & Mullins RD DNA damage induces nuclear actin filament assembly by Formin −2 and Spire-½ that promotes efficient DNA repair. eLife 4, e07735(2015). [PubMed: 26287480]
10. Plessner M, Melak M, Chinchilla P, Baarlink C & Grosse R Nuclear F-actin formation and reorganization upon cell spreading. J. Biol. Chem 290, 11209–11216 (2015). [PubMed: 25759381]

11. Caridi CP et al. Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. Nature 559, 54–60 (2018). [PubMed: 29925946]
12. Baarlink C et al. A transient pool of nuclear F-actin at mitotic exit controls chromatin organization. Nat. Cell Biol 19, 1389–1399 (2017). [PubMed: 29131140]
13. Spracklen AJ, Fagan TN, Lovander KE & Tootle TL The pros and cons of common actin labeling tools for visualizing actin dynamics during *Drosophila* oogenesis. Dev. Biol 393, 209–226 (2014). [PubMed: 24995797]
14. Plessner M & Grosse R Dynamizing nuclear actin filaments. Curr. Opin. Cell Biol 56, 1–6 (2019). [PubMed: 30193156]
15. Clark TG & Rosenbaum JL An actin filament matrix in hand-isolated nuclei of X. laevis oocytes. Cell 18, 1101–1108 (1979). [PubMed: 574804]
16. Bohnsack MT, Stuven T, Kuhn C, Cordes VC & Gorlich D A selective block of nuclear actin export stabilizes the giant nuclei of *Xenopus* oocytes. Nat. Cell Biol 8, 257–263 (2006). [PubMed: 16489345]
17. Dopie J, Skarp KP, Rajakyla EK, Tanhuanpaa K & Vartiainen MK Active maintenance of nuclear actin by importin 9 supports transcription. Proc. Natl Acad. Sci. USA 109, E544–E552 (2012). [PubMed: 22323606]
18. Feric M & Brangwynne CP A nuclear F-actin scaffold stabilizes ribonucleoprotein droplets against gravity in large cells. Nat. Cell Biol 15, 1253–1259 (2013). [PubMed: 23995731]
19. Oda H, Shirai N, Ura N, Ohsumi K & Iwabuchi M Chromatin tethering to the nuclear envelope by nuclear actin filaments: a novel role of the actin cytoskeleton in the Xenopus blastula. Genes Cells 22, 376–391 (2017). [PubMed: 28318078]
20. Miyamoto K, Pasque V, Jullien J & Gurdon JB Nuclear actin polymerization is required for transcriptional reprogramming of Oct4 by oocytes. Genes Dev. 25, 946–958 (2011). [PubMed: 21536734]
21. Miyamoto K et al. Nuclear Wave1 is required for reprogramming transcription in oocytes and for normal development. Science 341, 1002–1005 (2013). [PubMed: 23990560]
22. Miralles F, Posern G, Zaromytidou AI & Treisman R Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell 113, 329–342 (2003). [PubMed: 12732141]
23. Vartiainen MK, Guettler S, Larijani B & Treisman R Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. Science 316, 1749–1752 (2007). [PubMed: 17588931]
24. Kircher P et al. Filamin A interacts with the coactivator MKL1 to promote the activity of the transcription factor SRF and cell migration. Sci. Signal 8, ra112(2015). [PubMed: 26554816]
25. Tsopoulidis N et al. T cell receptor-triggered nuclear actin network formation drives CD4+ T cell effector functions. Sci. Immunol 4, eaav1987(2019). [PubMed: 30610013]
26. Chuang CH et al. Long-range directional movement of an interphase chromosome site. Curr. Biol 16, 825–831 (2006). [PubMed: 16631592]
27. Dundr M et al. Actin-dependent intranuclear repositioning of an active gene locus in vivo. J. Cell Biol 179, 1095–1103 (2007). [PubMed: 18070915]
28. Philimonenko VV et al. Nuclear actin and myosin I are required for RNA polymerase I transcription. Nat. Cell Biol 6, 1165–1172 (2004). [PubMed: 15558034]
29. Yoo Y, Wu X & Guan JL A novel role of the actin-nucleating Arp2/3 complex in the regulation of RNA polymerase II-dependent transcription. J. Biol. Chem 282, 7616–7623 (2007). [PubMed: 17220302]
30. Serebryannyy LA et al. Persistent nuclear actin filaments inhibit transcription by RNA polymerase II. J. Cell Sci 129, 3412–3425 (2016). [PubMed: 27505898]
31. Söderberg E, Hessle V, von Euler A & Visa N Profilin is associated with transcriptionally active genes. Nucleus 3, 290–299 (2012). [PubMed: 22572953]
32. Sokolova M et al. Nuclear actin is required for transcription during *Drosophila* oogenesis. iScience 9, 63–70 (2018). [PubMed: 30384134]
33. Tondeleir D et al. Cells lacking β-actin are genetically reprogrammed and maintain conditional migratory capacity. Mol. Cell. Proteomics 11, 255–271 (2012). [PubMed: 22448045]

34. Xie X. et al. β-actin-dependent global chromatin organization and gene expression programs control cellular identity. FASEB J. 32, 1296–1314 (2018). [PubMed: 29101221]
35. Xie X, Jankauskas R, Mazari AMA, Drou N & Percipalle P β-actin regulates a heterochromatin landscape essential for optimal induction of neuronal programs during direct reprograming. PLoS Genet. 14, e1007846(2018). [PubMed: 30557298]
36. Klages-Mundt NL, Kumar A, Zhang Y, Kapoor P & Shen X The nature of actin-family proteins in chromatin-modifying complexes. Front. Genet 9, 398(2018). [PubMed: 30319687]
37. Fenn S et al. Structural biochemistry of nuclear actin-related proteins 4 and 8 reveals their interaction with actin. EMBO J. 30, 2153–2166 (2011). [PubMed: 21499228]
38. Cao T et al. Crystal structure of a nuclear actin ternary complex. Proc. Natl Acad. Sci. USA 113, 8985–8990 (2016). [PubMed: 27457955]
39. Liu C, Zhu R & Mao Y Nuclear actin polymerized by mDia2 confines centromere movement during CENP-A loading. iScience 9, 314–327 (2018). [PubMed: 30448731]
40. Parisis N et al. Initiation of DNA replication requires actin dynamics and formin activity. EMBO J. 36, 3212–3231 (2017). [PubMed: 28982779]
41. Raghuraman M. K., Brewer, B. J. & Fangm an, W. L. Cell cycle-dependent establishment of a late replication program. Science 276, 806–809 (1997). [PubMed: 9115207]
42. Jackson DA & Pombo A Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells. J. Cell Biol 140, 1285–1295 (1998). [PubMed: 9508763]
43. Dimitrova DS & Gilbert DM The spatial position and replication timing of chromosomal domains are both established in early G_1 phase. Mol. Cell 4, 983–993 (1999). [PubMed: 10635323]
44. Heun P, Laroche T, Raghuraman MK & Gasser SM The positioning and dynamics of origins of replication in the budding yeast nucleus. J. Cell Biol 152, 385–400 (2001). [PubMed: 11266454]
45. Shermoen AW, McCleland ML & O'Farrell PH Develoμmental control of late replication and S phase length. Curr. Biol 20, 2067–2077 (2010). [PubMed: 21074439]
46. Dileep V et al. Topologically associating domains and their long-range contacts are established during early G_1 coincident with the establishment of the replication-timing program. Genome Res. 25, 1104–1113 (2015). [PubMed: 25995270]
47. Knott SR et al. Forkhead transcription factors establish origin timing and long-range clustering in S. cerevisiae. Cell 148, 99–111 (2012). [PubMed: 22265405]
48. Fang D et al. Dbf4 recruitment by forkhead transcription factors defines an upstream rate-limiting step in determining origin firing timing. Genes Dev. 31, 2405–2415 (2017). [PubMed: 29330352]
49. Zhang H et al. Dynamic relocalization of replication origins by Fkh1 requires execution of DDK function and Cdc45 loading at origins. eLife 8, e45512(2019). [PubMed: 31084713]
50. Peace JM, Ter-Zakarian A & Aparicio OM Rif1 regulates initiation timing of late replication origins throughout the *S. cerevisiae* genome. PLoS One 9, e98501(2014). [PubMed: 24879017]
51. Hafner L et al. Rif1 binding and control of chromosome-internal DNA replication origins is limited by telomere sequestration. Cell Rep. 23, 983–992 (2018). [PubMed: 29694906]
52. Welch MD & Way M Arp2/3-mediated actin-based motility: a tail of pathogen abuse. Cell Host Microbe 14, 242–255 (2013). [PubMed: 24034611]
53. Wilkie AR, Lawler JL & Coen DM A role for nuclear F-actin induction in human cytomegalovirus nuclear egress. MBio 7, e01254–16 (2016). [PubMed: 27555312]
54. Ohkawa T & Welch MD Baculovirus actin-based motility drives nuclear envelope disruption and nuclear egress. Curr. Biol 28, 2153–2159.e4 (2018). [PubMed: 30008331]
55. Chang HHY, Pannunzio NR, Adachi N & Lieber MR Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat. Rev. Mol. Cell Biol 18, 495–506 (2017). [PubMed: 28512351]
56. Kowalczykowski SC An overview of the molecular mechanisms of recombinational DNA repair. Cold Spring Harb. Perspect. Biol 7, a016410(2015). [PubMed: 26525148]
57. Zuchero JB, Coutts AS, Quinlan ME, Thangue NB & Mullins RD p53-cofactor JMY is a multifunctional actin nucleation factor. Nat. Cell Biol 11, 451–459 (2009). [PubMed: 19287377]

58. Yuan Y & Shen Z Interaction with BRCA2 suggests a role for filamin-1 (hsFLNa) in DNA damage response. J. Biol. Chem 276, 48318–48324 (2001). [PubMed: 11602572]
59. Yue J et al. The cytoskeleton protein filamin-A is required for an efficient recombinational DNA double strand break repair. Cancer Res. 69, 7978–7985 (2009). [PubMed: 19808958]
60. Velkova A, Carvalho MA, Johnson JO, Tavtigian SV & Monteiro AN Identification of Filamin A as a BRCA1-interacting protein required for efficient DNA repair. Cell Cycle 9, 1421–1433 (2010). [PubMed: 20305393]
61. Hansen RK et al. SCAI promotes DNA double-strand break repair in distinct chromosomal contexts. Nat. Cell Biol 18, 1357–1366 (2016). [PubMed: 27820601]
62. Isobe SY, Nagao K, Nozaki N, Kimura H & Obuse C Inhibition of RIF1 by SCAI allows BRCA1-mediated repair. Cell Reports 20, 297–307 (2017). [PubMed: 28700933]
63. Sridharan D, Brown M, Lambert WC, McMahon LW & Lambert MW Nonerythroid alphaII spectrin is required for recruitment of FANCA and XPF to nuclear foci induced by DNA interstrand cross-links. J. Cell Sci 116, 823–835 (2003). [PubMed: 12571280]
64. Andrin C et al. A requirement for polymerized actin in DNA double strand break repair. Nucleus 3, 384–395 (2012). [PubMed: 22688650]
65. Kulashreshtha M, Mehta IS, Kumar P & Rao BJ Chromosome territory relocation during DNA repair requires nuclear myosin 1 recruitment to chromatin mediated by ϒ-H2AX signaling. Nucleic Acids Res. 44, 8272–8291 (2016). [PubMed: 27365048]
66. Spichal M et al. Evidence for a dual role of actin in regulating chromosome organization and dynamics in yeast. J. Cell Sci 129, 681–692 (2016). [PubMed: 26763908]
67. Evdokimova VN, Gandhi M, Nikitski AV, Bakkenist CJ & Nikiforov YE Nuclear myosin/actin-motored contact between homologous chromosomes is initiated by ATM kinase and homology-directed repair proteins at double-strand DNA breaks to suppress chromosome rearrangements. Oncotarget 9, 13612–13622 (2018). [PubMed: 29568381]
68. Kapoor P & Shen X. Mechanisms of nuclear actin in chromatin-remodeling complexes. Trends Cell Biol. 24, 238–246 (2014). [PubMed: 24246764]
69. Neumann FR et al. Targeted INO80 enhances subnuclear chromatin movement and ectopic homologous recombination. Genes Dev. 26, 369–383 (2012). [PubMed: 22345518]
70. Horigome C et al. SWR1 and INO80 chromatin remodelers contribute to DNA double-strand break perinuclear anchorage site choice. Mol. Cell 55, 626–639 (2014). [PubMed: 25066231]
71. Park EJ, Hur SK & Kwon J Human INO80 chromatin-remodelling complex contributes to DNA double-strand break repair via the expression of Rad54B and XRCC3 genes. Biochem. J 431, 179–187 (2010). [PubMed: 20687897]
72. Wang YH et al. DNA damage causes rapid accumulation of phosphoinositides for ATR signaling. Nat. Commun 8, 2118(2017). [PubMed: 29242514]
73. Sun MH et al. DNA double-strand breaks induce the nuclear actin filaments formation in cumulus-enclosed oocytes but not in denuded oocytes. PLoS One 12, e0170308(2017). [PubMed: 28099474]
74. Hoskins RA et al. Sequence finishing and mapping of *Drosophila melanogaster* heterochromatin. Science 316, 1625–1628 (2007). [PubMed: 17569867]
75. Ho JW et al. Comparative analysis of metazoan chromatin organization. Nature 512, 449–452 (2014). [PubMed: 25164756]
76. Hoskins RA et al. The Release 6 reference sequence of the *Drosophila melanogaster* genome. Genome Res. 25, 445–458 (2015). [PubMed: 25589440]
77. 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, 116–120 (2001). [PubMed: 11242053]
78. Riddle NC et al. Plasticity in patterns of histone modifications and chromosomal proteins in *Drosophila* heterochromatin. Genome Res. 21, 147–163 (2011). [PubMed: 21177972]
79. Guelen L et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature 453, 948–951 (2008). [PubMed: 18463634]
80. Peric-Hupkes D et al. Molecular maps of the reorganization of genome-nuclear lamina interactions during differentiation. Mol. Cell 38, 603–613 (2010). [PubMed: 20513434]

81. Sexton T et al. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 148, 458–472 (2012). [PubMed: 22265598]
82. Goodarzi AA et al. ATM signaling facilitates repair of DNA double-strand breaks associated with heterochromatin. Mol. Cell 31, 167–177 (2008). [PubMed: 18657500]
83. Chiolo I et al. Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell 144, 732–744 (2011). [PubMed: 21353298]
84. Chiolo I, Tang J, Georgescu W & Costes SV Nuclear dynamics of radiation-induced foci in euchromatin and heterochromatin. Mutat. Res 750, 56–66 (2013). [PubMed: 23958412]
85. Ryu T et al. Heterochromatic breaks move to the nuclear periphery to continue recombinational repair. Nat. Cell Biol 17, 1401–1411 (2015). [PubMed: 26502056]
86. Tsouroula K et al. Temporal and spatial uncoupling of DNA double strand break repair pathways within mammalian heterochromatin. Mol. Cell 63, 293–305 (2016). [PubMed: 27397684]
87. Li Q et al. The three-dimensional genome organization of *Drosophila melanogaster* through data integration. Genome Biol. 18, 145(2017). [PubMed: 28760140]
88. Caridi PC, Delabaere L, Zapotoczny G & Chiolo I And yet, it moves: nuclear and chromatin dynamics of a heterochromatic double-strand break. Phil. Trans. R. Soc. Lond. B 372, 20160291(2017). [PubMed: 28847828]
89. Peng JC & Karpen GH H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. Nat. Cell Biol 9, 25–35 (2007). [PubMed: 17159999]
90. Peng JC & Karpen GH Epigenetic regulation of heterochromatic DNA stability. Curr. Opin. Genet. Dev 18, 204–211 (2008). [PubMed: 18372168]
91. Peng JC & Karpen GH Heterochromatic genome stability requires regulators of histone H3 K9 methylation. PLoS Genet. 5, e1000435(2009). [PubMed: 19325889]
92. Ryu T, Bonner MR & Chiolo I Cervantes and Quijote protect heterochromatin from aberrant recombination and lead the way to the nuclear periphery. Nucleus 7, 485–497 (2016). [PubMed: 27673416]
93. Dialynas G, Delabaere L & Chiolo I Arp2/3 and Unc45 maintain heterochromatin stability in Drosophila polytene chromosomes. bioRxiv preprint at 10.1101/679548 (2019).
94. Beucher A et al. ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G_2. EMBO J. 28, 3413–3427 (2009). [PubMed: 19779458]
95. Janssen A et al. A single double-strand break system reveals repair dynamics and mechanisms in heterochromatin and euchromatin. Genes Dev. 30, 1645–1657 (2016). [PubMed: 27474442]
96. Amaral N, Ryu T, Li X. & Chiolo I Nuclear dynamics of heterochromatin repair. Trends Genet. 33, 86–100 (2017). [PubMed: 28104289]
97. Rawal CPCC & Chiolo I Actin' between phase separated domains for heterochromatin repair. DNA Repair 10.1016/j.dnarep.2019.102646 (2019).
98. Guenatri M, Bailly D, Maison C & Almouzni G Mouse centric and pericentric satellite repeats form distinct functional heterochromatin.J. Cell Biol 166, 493–505 (2004). [PubMed: 15302854]
99. Jakob B et al. DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. Nucleic Acids Res. 39, 6489–6499 (2011). [PubMed: 21511815]
100. Ayoub N, Jeyasekharan AD, Bernal JA & Venkitaraman AR HP1-β mobilization promotes chromatin changes that initiate the DNA damage response. Nature 453, 682–686 (2008). [PubMed: 18438399]
101. Dronamraju R & Mason JM MU2 and HP1a regulate the recognition of double strand breaks in *Drosophila melanogaster*. PLoS One 6, e25439(2011). [PubMed: 21966530]
102. Colmenares SU et al. *Drosophila* histone demethylase KDM4A has enzymatic and non-enzymatic roles in controlling heterochromatin integrity. Dev. Cell 42, 156–169.e5 (2017). [PubMed: 28743002]
103. Goodarzi AA, Kurka T & Jeggo PA KAP-1 phosphorylation regulates CHD3 nucleosome remodeling during the DNA double-strand break response. Nat. Struct. Mol. Biol 18, 831–839 (2011). [PubMed: 21642969]

104. Senaratne TN, Joyce EF, Nguyen SC & Wu CT Investigating the interplay between sister chromatid cohesion and homolog pairing in *Drosophila* nuclei. PLoS Genet. 12, e1006169(2016). [PubMed: 27541002]
105. Caridi CP et al. Quantitative methods to investigate the 4D dynamics of heterochromatic repair sites in *drosophila* cells. Methods Enzymol. 601, 359–389 (2018). [PubMed: 29523239]
106. Schrank BR et al. Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. Nature 559, 61–66 (2018). [PubMed: 29925947]
107. Lisby M, Mortensen UH & Rothstein R Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat. Cell Biol 5, 572–577 (2003). [PubMed: 12766777]
108. Aten JA et al. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science 303, 92–95 (2004). [PubMed: 14704429]
109. Kruhlak MJ et al. Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. J. Cell Biol 172, 823–834 (2006). [PubMed: 16520385]
110. Neumaier T et al. Evidence for formation of DNA repair centers and dose-response nonlinearity in human cells. Proc. Natl Acad. Sci. USA 109, 443–448 (2012). [PubMed: 22184222]
111. Aymard F et al. Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. Nat. Struct. Mol. Biol 24, 353–361 (2017). [PubMed: 28263325]
112. Costes SV, Chiolo I, Pluth JM, Barcellos-Hoff MH & Jakob B Spatiotemporal characterization of ionizing radiation induced DNA damage foci and their relation to chromatin organization. Mutat. Res 704,78–87 (2010). [PubMed: 20060491]
113. Clouaire T et al. Comprehensive mapping of histone modifications at DNA double-strand breaks deciphers repair pathway chromatin signatures. Mol. Cell 72, 250–262.e6 (2018). [PubMed: 30270107]
114. Lottersberger F, Karssemeijer RA, Dimitrova N & de Lange T 53BP1 and the LINC complex promote microtubule-dependent DSB mobility and DNA repair. Cell 163, 880–893 (2015). [PubMed: 26544937]
115. Lawrimore J et al. Microtubule dynamics drive enhanced chromatin motion and mobilize telomeres in response to DNA damage. Mol. Biol. Cell 28, 1701–1711 (2017). [PubMed: 28450453]
116. Oshidari R et al. Nuclear microtubule filaments mediate non-linear directional motion of chromatin and promote DNA repair. Nat. Commun 9, 2567(2018). [PubMed: 29967403]
117. Swartz RK, Rodriguez EC & King MC A role for nuclear envelope-bridging complexes in homology-directed repair. Mol. Biol. Cell 25, 2461–2471 (2014). [PubMed: 24943839]
118. Chung DK et al. Perinuclear tethers license telomeric DSBs for a broad kinesin- and NPC-dependent DNA repair process. Nat. Commun 6, 7742(2015). [PubMed: 26205667]
119. Nagai S et al. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322, 597–602 (2008). [PubMed: 18948542]
120. Su XA, Dion V, Gasser SM & Freudenreich CH Regulation of recombination at yeast nuclear pores controls repair and triplet repeat stability. Genes Dev. 29, 1006–1017 (2015).; [PubMed: 25940904]
121. Quivy J-P, Gérard A, Cook AJ, Roche D & Almouzni G The HP1-p150/CAF-1 interaction is required for pericentric heterochromatin replication and S-phase progression in mouse cells. Nat. Struct. Mol. Biol 15, 972–979 (2008) [PubMed: 19172751]
122. Johnson MA, Sharma M, Mok MT & Henderson BR Stimulation of in vivo nuclear transport dynamics of actin and its co-factors IQGAP1 and Rac1 in response to DNA replication stress. Biochim. Biophys. Acta 1833, 2334–2347 (2013). [PubMed: 23770048]
123. Lamm N, Masamsetti VP, Read MN, Biro M & Cesare AJ ATR and mTOR regulate F-actin to alter nuclear architecture and repair replication stress. bioRxiv preprint at 10.1101/451708 (2018).
124. Bermejo R et al. The replication checkpoint protects fork stability by releasing transcribed genes from nuclear pores. Cell 146, 233–246 (2011). [PubMed: 21784245]
125. Kumar A et al. ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress. Cell 158, 633–646 (2014). [PubMed: 25083873]

126. Torres-Rosell J et al. The Smc5-Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nat. Cell Biol 9, 923–931 (2007). [PubMed: 17643116]

127. Harding SM, Boiarsky JA & Greenberg RA ATM dependent silencing links nucleolar chromatin reorganization to DNA damage recognition. Cell Rep. 13, 251–259 (2015). [PubMed: 26440899]

128. 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, 1151–1163 (2015). [PubMed: 26019174]

129. 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, e1008103(2019). [PubMed: 30998688]

130. Therizols P et al. Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol 172, 189–199 (2006). [PubMed: 16418532]

131. Khadaroo B et al. The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat. Cell Biol 11, 980–987 (2009). [PubMed: 19597487]

132. Cho NW, Dilley RL, Lampson MA & Greenberg RA Interchromosomal homology searches drive directional ALT telomere movement and synapsis. Cell 159, 108–121 (2014). [PubMed: 25259924]

133. Churikov D et al. SUMO-dependent relocalization of eroded telomeres to nuclear pore complexes controls telomere recombination. Cell Rep. 15, 1242–1253 (2016). [PubMed: 27134164]

134. Kalocsay M, Hiller NJ & 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, 335–343 (2009). [PubMed: 19217407]

135. Oza P, Jaspersen SL, Miele A, Dekker J & Peterson CL Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev. 23, 912–927 (2009). [PubMed: 19390086]

136. Roukos V et al. Spatial dynamics of chromosome translocations in living cells. Science 341, 660–664 (2013). [PubMed: 23929981]

137. Mine-Hattab J, Recamier V, Izeddin I, Rothstein R & Darzacq X. in Molecular Biology of the Cell vol. 28 (eds Lidke D, Lippincott-Schwartz J and Mogilner A) 3323–3332 (American Society for Cell Biology, 2017).

138. Dion V, Kalck V, Horigome C, Towbin BD & Gasser SM Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. Nat. Cell Biol 14, 502–509 (2012). [PubMed: 22484486]

139. Mine-Hattab J & Rothstein R Increased chromosome mobility facilitates homology search during recombination. Nat. Cell Biol 14, 510–517 (2012). [PubMed: 22484485]

140. Amitai A, Seeber A, Gasser SM & Holcman D Visualization of chromatin decompaction and break site extrusion as predicted by statistical polymer modeling of single-locus trajectories. Cell Rep. 18, 1200–1214 (2017). [PubMed: 28147275]

141. Spichal M & Fabre E The emerging role of the cytoskeleton in chromosome dynamics. Front. Genet 8, 60(2017). [PubMed: 28580009]

142. Dion V & Gasser SM Chromatin movement in the maintenance of genome stability. Cell 152, 1355–1364 (2013). [PubMed: 23498942]

143. 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, 173–181 (2017). [PubMed: 28035048]

144. Li H, Guo F, Rubinstein B & Li R Actin-driven chromosomal motility leads to symmetry breaking in mammalian meiotic oocytes. Nat. Cell Biol 10, 1301–1308 (2008). [PubMed: 18836438]

145. Larson AG et al. Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin. Nature 547, 236–240 (2017). [PubMed: 28636604]

146. Strom AR et al. Phase separation drives heterochromatin domain formation. Nature 547, 241–245 (2017). [PubMed: 28636597]

147. Azzaz AM et al. Human heterochromatin protein 1α promotes nucleosome associations that drive chromatin condensation. J. Biol. Chem 289, 6850–6861 (2014). [PubMed: 24415761]
148. Cahill DP, Kinzler KW, Vogelstein B & Lengauer C Genetic instability and darwinian selection in tumours. Trends Cell Biol. 9, M57–M60 (1999). [PubMed: 10611684]
149. Zhang CZ et al. Chromothripsis from DNA damage in micronuclei. Nature 522, 179–184 (2015). [PubMed: 26017310]
150. Yang X & Lin Y in Oncol Lett (2017).
151. Buchbinder D, Nugent DJ & Fillipovich AH Wiskott-Aldrich syndrome: diagnosis, current management, and emerging treatments. Appl. Clin. Genet 7, 55–66 (2014). [PubMed: 24817816]
152. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72, 971–983 (1993). [PubMed: 8458085]
153. Mangiarini L et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87, 493–506 (1996). [PubMed: 8898202]
154. Munsie L et al. Mutant huntingtin causes defective actin remodeling during stress: defining a new role for transglutaminase 2 in neurodegenerative disease. Hum. Mol. Genet 20, 1937–1951 (2011). [PubMed: 21355047]
155. Maiuri T et al. Huntingtin is a scaffolding protein in the ATM oxidative DNA damage response complex. Hum. Mol. Genet 26, 395–406 (2017). [PubMed: 28017939]
156. Prochniewicz E, Thompson LV & Thomas DD Age-related decline in actomyosin structure and function. Exp. Gerontol 42, 931–938 (2007). [PubMed: 17706387]
157. White RR et al. Double-strand break repair by interchromosomal recombination: an in vivo repair mechanism utilized by multiple somatic tissues in mammals. PLoS One 8, e84379(2013). [PubMed: 24349572]
158. Sukup-Jackson MR et al. Rosa26-GFP direct repeat (RaDR-GFP) mice reveal tissue- and age-dependence of homologous recombination in mammals in vivo. PLoS Genet. 10, e1004299(2014). [PubMed: 24901438]
159. Delabaere L et al. Aging impairs double-strand break repair by homologous recombination in *Drosophila* germ cells. Aging Cell 16, 320–328 (2017). [PubMed: 28000382]
160. Simon DN, Zastrow MS & Wilson KL Direct actin binding to A- and B-type lamin tails and actin filament bundling by the lamin A tail. Nucleus 1, 264–272 (2010). [PubMed: 21327074]
161. Scaffidi P & Misteli T Reversal of the cellular phenotype in the premature aging disease Hutchinson-Gilford progeria syndrome. Nat. Med 11, 440–445 (2005). [PubMed: 15750600]
162. Scaffidi P & Misteli T Lamin A-dependent nuclear defects in human aging. Science 312, 1059–1063 (2006). [PubMed: 16645051]
163. Zada D, Bronshtein I, Lerer-Goldshtein T, Garini Y & Appelbaum L Sleep increases chromosome dynamics to enable reduction of accumulating DNA damage in single neurons. Nat. Commun 10, 895(2019). [PubMed: 30837464]

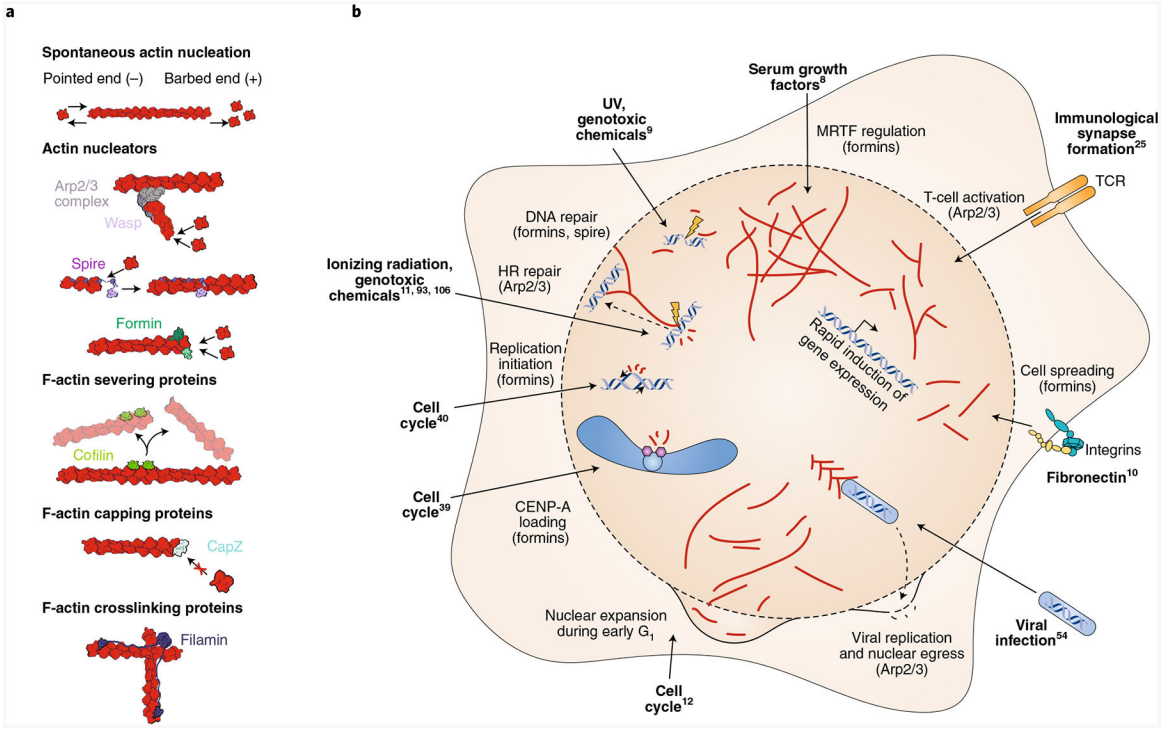

Fig. 1 |. Nuclear actin polymerizes in response to several stimuli.

a, Different actin remodelling pathways are shown. Spontaneous actin nucleation is characterized by a fast-growing (+) 'barbed' end and a slow-growing (−) 'pointed' end, with more efficient addition of G-actin to the (+) end. F-actin formation and disassembly are regulated by actin remodellers, including nucleating, severing, capping, and crosslinking proteins. Arp2/3 promotes nucleation at 70° angles from preexisting filaments and is activated by the WAS family proteins (e.g., Wasp). Spire recruits several actin monomers with its WASP-homology2 domains (WH2), forming a seeding polymer for filament elongation. Formins associate with the (+) end and promote polymerization by bringing actin monomers in close proximity via formin homology 2 domains (FH2). Cofilin stimulates filament severing. CapZ associates with the (+) end, blocking G-actin access and filament elongation. Filamin holds two filaments together, promoting the formation of F-actin networks. **b**, Nuclear F-actin forms in response to different stimuli. DNA damage induces Arp2/3-dependent nuclear actin filament formation and formin-dependent filament formation for relocalization of heterochromatic DSBs and focus clustering, promoting repair[9,11,93,106]. Serum stimulation, fibronectin treatment, or cell spreading, promotes MRTF-A activation through formin-dependent nuclear filaments[8,10]. T-cell-receptor activation results in Arp2/3-dependent nuclear filaments promoting cytokine expression and antibody production[25]. Baculoviruses can hijack the host system to produce Arp2/3-dependent filaments for nuclear egress[54]. Cells entering G_1 experience formin-induced actin polymerization, promoting CenpA recruitment and replication initiation[39,40]. G_1 nuclear filaments also mediate nuclear expansion[12].

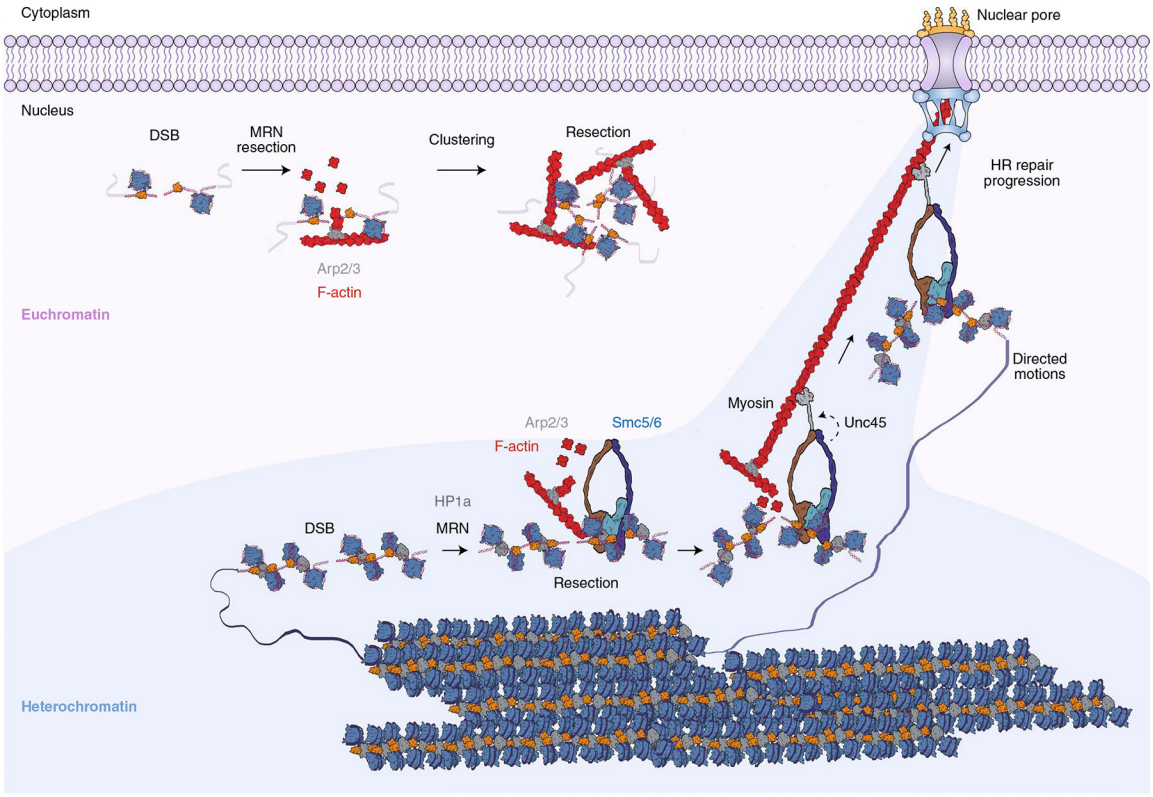

Fig. 2 |. Model for the role of F-actin in DSB repair of heterochromatin and euchromatin.
In heterochromatin, DSB detection and processing (resection) occur inside the heterochromatin domain. Mre[11] (MRN complex) and HP1a promote recruitment of Arp2/3 and myosins to DSBs; Arp2/3 activation by Scar and Wash facilitates actin polymerization and filament growth towards the nuclear periphery; Smc5/6 blocks Rad[51] recruitment inside the heterochromatin domain and recruits Unc[45] to activate nuclear myosins. The myosin-Smc5/6-chromatin complex translocates along actin filaments to anchor DSBs to nuclear pores or inner nuclear membrane proteins (INMPs, not shown), where HR repair continues with Rad51 recruitment and strand invasion. Actin filaments are highly dynamic and start disassembling during relocalization. In euchromatin, Mre11 and resection promote the movement of repair sites via Arp2/3 and F-actin, which in turn facilitate resection and HR repair. Actin polymers travel with euchromatic repair sites, possibly generating propelling forces for clustering.

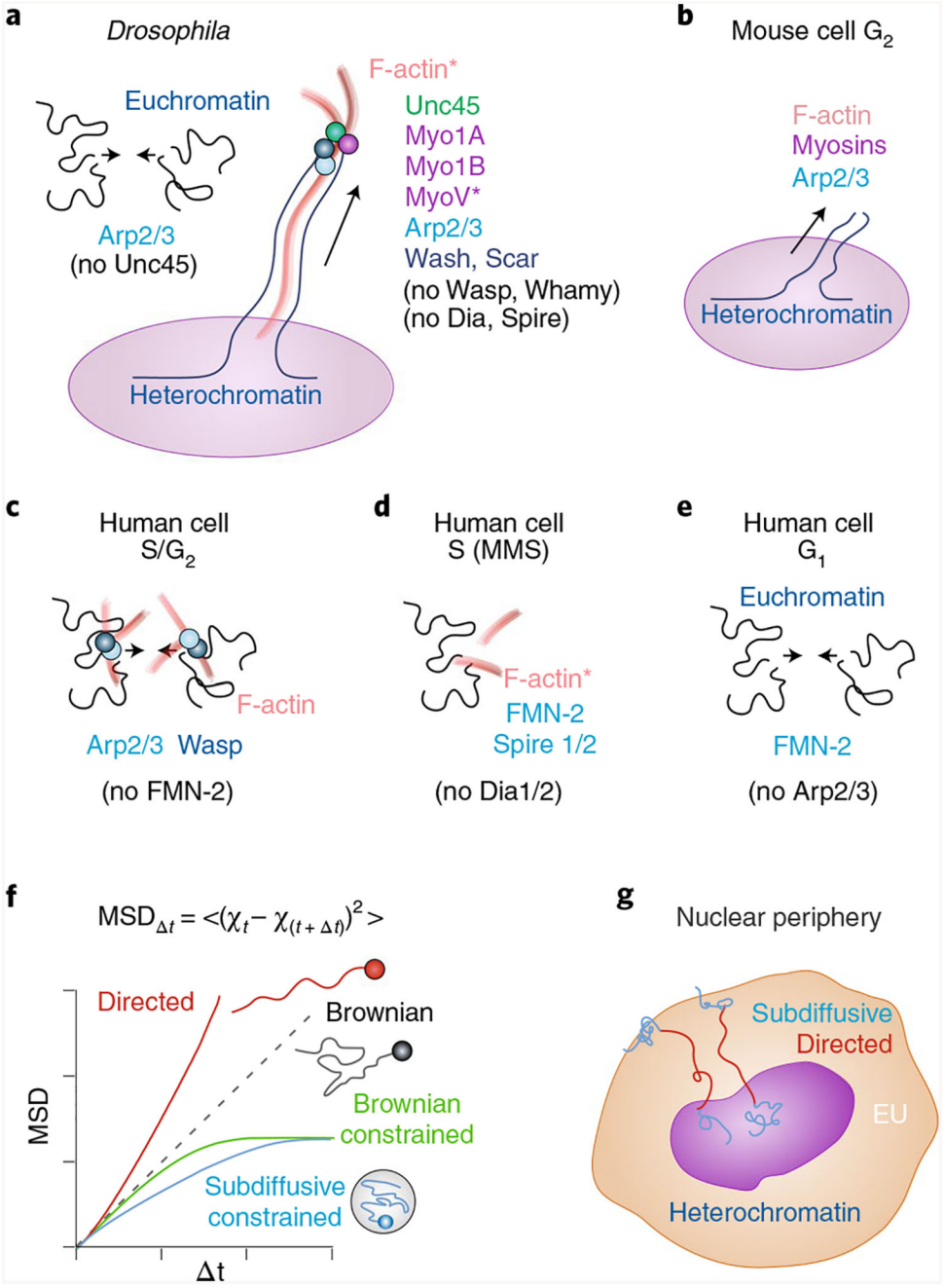

Fig. 3 |. Different actin nucleators and motor proteins contribute to DSB dynamics and repair.

a, In *Drosophila* cells (that are mostly in S/G_2[83]), directed motion of heterochromatic DSBs to the nuclear periphery relies on F-actin, Arp2/3, the Arp2/3 activators Scar and Wash, the myosin activator Unc45, and Myo1A, Myo1B, and MyoV nuclear myosins. Wasp, Whamy, Dia, and Spire are not required. Arp2/3, F-actin, Unc45 and myosins are also enriched at repair foci, consistent with a direct functionin repair[11]. Clustering of euchromatic DSBs relies on Arp2/3 and not on Unc45[11,106]. **b**, In mouse G_2 cells, relocalization of heterochromatic DSBs also requires Arp2/3, actin polymerization, and myosins[11]. **c**, In human S/G_2 cells, dynamics of HR-prone DSBs depend on Arp2/3, Wasp, and F-actin,

which are enriched at repair sites, whereas FMN-2 is not required[106].**d**, In human cells treated with MMS, actin filaments form in the nuclei and mediate repair, which also requires FMN-2 and Spire1/2, but not Dia1/29.**e**, In human G_1 cells, clustering of euchromatic DSBs requires FMN-2[111], and focus movement is not dependent on Arp2/3[106]. (*) refers to experimental systems in which the nuclear function of the indicated components has been directly established. Actin filaments are indicated for studies that directly identified nuclear structures. Components that are not required for filament formation or repair in different contexts are in parenthesis. **f**, Schematic representation of MSD curves for different types of motion, as indicated (adapted with permission from ref.[105]). **g** , Schematic representation of a focus track (adapted from ref.[11]), showing mixed types of motion for heterochromatic repair foci that reach the nuclear periphery. Time points characterized by directed and subdiffusive motions are shown.

Table 1 |

Structural and motor components linked to different relocalization and repair path ways for genome stability, and related studies in different organisms

Functions in genome stability	Nuclear filaments/motors/actin remodellers	Organism (cell cycle phase)	Damage source	References
DSB repair	Actin filaments	Human cells	MMS, UV, NCS, telomere uncapping	9
	Formin 2			
	Spire1/2			
Relocalization of heterochromatic DSBs for HR repair	Actin filaments	*Drosophila* Mouse cells (G_2)	X-rays (DSBs)	11,93
	Arp2/3 complex			
	Wash	*Drosophila*		
	Scar			
	Myosin 1A			
	Myosin 1B			
	Myosin V			
	Myosins	Mouse cells (G_2)		
Clustering of euchromatic DSBs for HR or SSA repair	Actin filaments	Human cells (G_2), *Xenopus* extracts (S)	AsiSI, NCS, IR	106
	Wasp			
	CapZβ			
	Arp2/3 complex	Human cells (G_2), *Drosophila* cells		11,106
	Formin 2	Human cells (G_1)		111
Checkpoint activation (ATR)	mDia2 formin	Human cells	Laser microirradiation	72
Replication initiation and progression	Formins	Human cells, *Xenopus* extracts (S)	Replication and replication stress	40,123
Movement of repair sites for BIR repair	Microtubules	*Saccharomyces cerevisiae*	I-SceI, MMS, Zeocin, Camptothecin	116
	Kinesin 14			

SSA, single-strand annealing.