Role of nuclear actin filaments in DNA repair dynamics

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Abstract
Actin filaments (F-actin) have well-known functions in the cytoplasm, including generating forces for cell movement and enabling myosin-driven dynamics of vesicles and mRNAs. In addition, the recent development of innovative tools for F-actin detection have revealed dynamic and transient filaments in the nuclei, which form in response to specific stimuli. Here we provide an overview of the functions of F-actin and myosins in nuclei, with a focus on their role in DNA repair and genome stability. We emphasize recent discoveries of nuclear F-actin driving the relocalization of heterochromatic repair sites to the nuclear periphery for ‘safe’ homologous recombination (HR) repair of double-strand breaks (DSBs). F-actin also promotes repair focus clustering and DSB resection in euchromatin, facilitating HR progression. We highlight regulatory mechanisms specialized for actin polymerization during DNA replication and repair, and emphasize recent studies revealing alternative motors for the directional movement of repair sites. Together, these discoveries challenge previous models that actin is substantially monomeric in the nucleus and that DSBs move via Brownian motion, revealing a complex network of dynamic filaments, motors and regulators, coordinating chromatin dynamics with repair progression.
Actin filament structure, regulation, and detection in the nucleus

Actin filaments are major components of the cytoskeleton, where they mediate cell movement and adhesion, or intracellular transport of molecules and vesicles via myosin motors raised. Actin filaments respond dynamically to a variety of stimuli through actin remodelers, including actin nucleators, bundling components, crosslinking proteins, and disassembly factors (Fig. 1). The three major classes of actin nucleators are the Arp2/3 complex, formins, and Spire-family components, and they are characterized by distinct structural properties, regulatory mechanisms, and functions (Fig. 1). The Arp2/3 complex is activated by proteins of the Wiskott-Aldrich Syndrome (WAS) family, including Wash, Wasp, and Scar/Wave, which are specialized for actin nucleation in different contexts. While cytoplasmic roles of actin filaments are well-characterized, nuclear functions have long remained elusive. This is largely because actin filaments are significantly more abundant in the cytoplasm in most cell types, interfering with the detection of nuclear actin filaments when using approaches. Major breakthroughs came with the development of fluorescently tagged F-actin-specific probes with nuclear localization signals (NLS) that enable live imaging of nuclear actin filaments, and the establishment of new genetic approaches that specifically and selectively inactivate actin polymerization and motor functions in the nucleususing tools, recent studies began shedding light on several functions of nuclear actin filaments, revealing a general model where these structures are mostly stimulus-driven and mediate chromatin responses to different types of stress.

Functions of nuclear F-actin

A powerful tool to study nuclear actin filaments has been the germinal vesicle (GV) of the *Xenopus laevis* oocyte, a nucleus several hundred micrometers in diameter with high concentration of nuclear actin due to the lack of the actin export factor Exportin 6. In GVs, actin filaments form a sponge-like mesh for mechanical stability and nuclear organization. Notably, transplantation of somatic cell nuclei into GVs induces transcriptional reprogramming that requires dynamic and prolonged actin polymerization, involving a nuclear function of the actin assembly factor WAVE, which suggests a role for nuclear actin filaments in transcriptional regulation.

In other cell types, nuclear actin filaments form in response to various stimuli, including serum stimulation, cell spreading, T-cell activation, and mitotic exit (recently reviewed in). One of the first observations suggesting dynamic nuclear actin networks came from studies on the cytoplasmic-nuclear shuttling of myocardin-related transcription factor A (MRTF-A), a transcriptional co-activator of serum response factor (SRF). MRTF-A has high affinity for monomeric actin (G-actin, globular actin), and G-actin release from MRTF-A enables MRTF-A accumulation in the nucleus after serum stimulation. Remarkably, this is a consequence of a quick burst (<60 sec) of serum-induced nuclear actin polymerization by formins, which results in lowering the concentration of nuclear G-actin. Similar nuclear actin filament assembly for MRTF-A regulation was observed during cell spreading. However, spreading-induced F-actin structures differ from serum-triggered events, as they appear much shorter and they require a functional LINC complex (Linker of Nucleoskeleton and Cytoskeleton), which
is dispensable for the serum response (Grosse, unpublished observations). It will be interesting to investigate whether nuclear actin structures form during other cell motile events in addition to cell spreading, such as migration in 3D environments when cells squeeze through narrow spaces. Intriguingly, MRTF-A activity is not only dependent on its dissociation from G-actin, but also on its association with the F-actin crosslinking component filamin A\textsuperscript{23}. Actin polymerization is required for this interaction, suggesting an independent and direct role for F-actin in MRTF-A activation\textsuperscript{23}. Actin, actin polymerizing proteins, and myosins also associate with RNA polymerases and transcription sites\textsuperscript{24-28}, and actin dynamics are required for polymerase activity\textsuperscript{27,29-32}. However, RNA polymerase-associated actin appears mostly monomeric\textsuperscript{27}, and whether nuclear actin filaments are specifically and directly involved in these responses is still unclear.

In addition to direct regulation of transcription factors by nuclear actin, a very recent study demonstrated the induction of cytokine expression in response to nuclear F-actin in activated T cells\textsuperscript{33}. This occurs after T-cell receptor engagement in CD4\textsuperscript{+} cells such as during immunological synapse formation, and requires calcium elevations, N-Wasp, and nuclear Arp2/3\textsuperscript{33}. As such, this study provides evidence for the importance of signal-regulated nuclear actin polymerization in immune function.

Nuclear actin polymerization might also contribute to transcriptional regulation by affecting the nuclear positioning of a genomic locus Two parallel studies provided indirect evidence, via live cell imaging of mammalian cells, for active actin-dependent long-range movement during chromosome repositioning, to regulate transcription in interphase nuclei\textsuperscript{34,35}. It would be intriguing to further investigate these responses using the novel tools to visualize and monitor endogenous intranuclear actin dynamics. Interestingly, expression of the non-polymerizable actin mutant G13R-actin robustly inhibits chromosome locus migration\textsuperscript{34}, suggesting F-actin-dependent transport.

Recent studies identified transient nuclear actin polymerization during mitotic exit, which facilitates nuclear volume expansion and chromatin decompaction in early G1\textsuperscript{12}. This process requires the nuclear activity of the severing protein Cofilin-1, as shown with phalloidin proteomics and optogenetics, while the components required for polymerization in this context remain unknown.

Finally, nuclear F-actin is also generated during viral infections to promote viral particle formation and mobilization. For example, the baculovirus Autographa californica multiple nucleopolyhedrovirus hijacks the host nuclear Arp2/3 complex using viral WASP-like proteins to enable actin-based mobilization of the virus and nuclear egress\textsuperscript{36,37}. Similar responses might occur during human cytomegalovirus infection\textsuperscript{38}, although more studies are needed to understand nuclear F-actin functions in this context\textsuperscript{39}. Together, these studies identified several examples of nuclear actin filaments responding to specific stimuli, which are required for transcriptional responses, chromosome positioning and nuclear architecture through distinct regulatory mechanisms.

**Nuclear F-actin is required for DSB repair**

In the past six years, several independent studies identified new and enticing roles of nuclear actin filaments in DNA double-strand break (DSB) repair. The two prominent pathways repairing DSBs are non-homologous end
joining (NHEJ) and homologous recombination (HR). NHEJ involves direct re-joining of the two ends with little processing, and frequently results in mutations at the cut site\textsuperscript{40}. Conversely, HR starts when DSBs are resected to form single-stranded DNA (ssDNA) filaments, which invade ‘donor’ homologous sequences used as templates for DNA synthesis and repair\textsuperscript{41}.

Actin and actin-associated proteins have long been shown to be required for DNA repair. For example, nuclear G-actin complexes participate in DNA repair as components of chromatin remodelers and histone modifiers (reviewed in\textsuperscript{42}); the actin nucleator JMY translocates to the nucleus in response to DNA damage, and promotes the transcription of the repair component p53\textsuperscript{43}; the actin crosslinking protein Filamin-A interacts with the HR proteins Brca1 and Brca2 and promotes repair\textsuperscript{44-46}; the formin-associated protein Suppressor of Cancer cell Invasion (Scal) is recruited to DSBs and required for repair in mammalian cells\textsuperscript{47-49}, and altering actin polymerization or nuclear myosin I (NMI) affects DNA damage responses\textsuperscript{50-54}, including HR repair\textsuperscript{53,54}. In budding yeast, chromatin movements are affected by cytoplasmic actin filaments that transfer forces to the nucleus via nuclear pores, and by nuclear actin associated with chromatin remodelers, both of which influence HR repair\textsuperscript{53}. However, whether those responses are related to the formation of nuclear actin filaments is unknown.

Intriguingly, initial studies from the Hendzel lab revealed that HR and NHEJ components associate with actin filaments \textit{in vitro}, and inactivation of nuclear actin polymerization affects retention of repair proteins at damage sites \textit{in vivo} in human cells\textsuperscript{51}, suggesting a direct role for nuclear actin filaments in DNA repair. In agreement with this, in 2015 the Mullins lab provided the first \textit{in vivo} evidence that treatment of human cells with different damaging agents induces the formation of nuclear actin filaments\textsuperscript{9}. These structures mostly appear as short thick filaments in fixed cells, although >1 um long filaments were also detected\textsuperscript{9}. Inactivation of actin polymerization specifically in the nuclei results in persistent damage foci in cells treated with the DNA damaging agent methyl methanesulfonate (MMS), suggesting defective repair\textsuperscript{9}. Nuclear actin polymerization also occurs in response to laser microirradiation in human cells, where it promotes the recruitment of the checkpoint kinase ATR to repair sites through an unknown mechanism\textsuperscript{55}. Finally, F-actin assembles in the nuclei of mouse oocytes in response to DSBs\textsuperscript{56}. Together, these studies suggested an important role for nuclear actin filaments in the DNA damage response, although the function(s) of these filaments in repair remained poorly understood.

**Nuclear F-actin and myosins relocalize heterochromatic DNA breaks to the nuclear periphery**

Two recent studies identified distinct and direct roles of nuclear actin filaments in the damage response: i) the relocalization of heterochromatic DSBs in mouse and \textit{Drosophila} cells for ‘safe’ homologous recombination repair\textsuperscript{11}, and ii) the clustering of euchromatic DSBs in human and \textit{Drosophila} cells to promote resection and homologous recombination\textsuperscript{11,57,58} (Fig. 2). Pericentromeric heterochromatin is a prominent chromosomal structure that accounts for ~30% of fly and human genomes\textsuperscript{59-61}, and it is absent in budding yeast. Heterochromatin is characterized by ‘silent’ histone marks H3K9me2/3, and associated proteins such as heterochromatin protein 1a (HP1a) in flies\textsuperscript{62,63} and HP1α/β in mammalian cells\textsuperscript{64}. Notably, \textit{Drosophila} and mouse pericentromeric heterochromatin (hereafter referred to as ‘heterochromatin’) is functionally and structurally
distinct from late replicating lamina-associated domains (LADs) distributed along the chromosome arms\textsuperscript{60,65-67}, and in contrast to those, it is not usually associated with the nuclear periphery (see for example\textsuperscript{11,68-73} (reviewed in\textsuperscript{74}).

Heterochromatin is largely composed of repeated DNA sequences\textsuperscript{59-61}. In \textit{Drosophila}, about half of these sequences are ‘satellite’ repeats (predominantly 5-base pair sequences repeated for hundreds of kilobases to megabases) and the rest are transposable elements and other scrambled repeats\textsuperscript{59-61}. The abundance of repeated sequences presents unique challenges to HR repair\textsuperscript{70,74-76}. In single copy sequences, a unique donor is present on the sister chromatid or homologous chromosome, and HR is largely ‘error free’\textsuperscript{41}. In heterochromatin, however, the availability of thousands to millions of identical potential donor sequences on different chromosomes can trigger unequal sister chromatid exchanges, or intra-/inter-chromosomal recombination, leading to deletions, duplications, translocations, and formation of dicentric or acentric chromosomes\textsuperscript{69-71,74-79}. Despite this risk, HR is a primary pathway for heterochromatin repair\textsuperscript{69,71,72,79-81}, and specialized mechanisms enable ‘safe’ HR repair in heterochromatin (reviewed in\textsuperscript{74,76,82}).

Studies in \textit{Drosophila} and mouse cells, where heterochromatin forms distinct nuclear ‘domains’\textsuperscript{63,69,73,83} (called ‘chromocenters’ in mouse cells), revealed that DSB resection starts inside the domains\textsuperscript{69,71,72,81,84,85}, but subsequent HR repair steps are temporarily halted. In flies, this block to HR progression relies on the heterochromatin-enriched complex Smc5/6 and its SUMO E3 ligase activity\textsuperscript{68,71,79} (Fig. 2). Next, the heterochromatin domains expand\textsuperscript{69,86} and DSBs relocalize to outside the domain\textsuperscript{11,69-72,81,84,85}. In mouse cells, repair appears to continue at the edge of the chromocenters\textsuperscript{11,70,72,85}, while in \textit{Drosophila} cells repair sites reach the nuclear periphery before Rad51 recruitment and strand invasion\textsuperscript{11,71}. In both mouse and \textit{Drosophila} cells, relocalization requires resection and the checkpoint kinases ATM and ATR, in addition to Smc5/6\textsuperscript{69,71,72}. Relocalization defects result in aberrant recombination and widespread genomic instability, revealing the importance of these dynamics in genome integrity\textsuperscript{11,69,71,79,87}. Relocalization likely promotes ‘safe’ HR repair while preventing aberrant recombination by isolating the DSBs and the repair templates (on the homologous chromosome or the sister chromatid) away from non-allelic (ectopic) sequences before strand invasion (recently reviewed in\textsuperscript{74,76,82}).

In \textit{Drosophila} cells, recent studies from the Chiolo lab revealed that relocalization relies on a striking network of nuclear actin filaments assembled at repair sites by the actin nucleator Arp2/3, which extend toward the nuclear periphery as branched structures\textsuperscript{11} (Fig. 2). Live imaging reveals repair sites ‘sliding’ along these filaments\textsuperscript{11}, consistent with a role of filaments as ‘highways’ for relocalization. Intriguingly, relocalization of heterochromatic repair sites also relies on three nuclear myosins: Myosin 1A, Myosin 1B, and Myosin V, and on myosin’s ability to translocate along actin filaments\textsuperscript{11}, revealing transport mechanisms similar to those in the cytoplasm\textsuperscript{88}. In agreement, relocalization of heterochromatic DSBs to the nuclear periphery is characterized by directed motions\textsuperscript{11,89}. Arp2/3 and myosin recruitment to repair foci requires the early DSB signaling and processing factor Mre11, and the heterochromatin protein HP1a\textsuperscript{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, but is not required for their recruitment to repair sites\(^1\), suggesting a regulatory role for Smc5/6 in Arp2/3 and myosin function. Smc5/6 is also required for the recruitment of Unc45 to heterochromatic repair sites\(^1\), suggesting this step as a critical switch to promote myosin ‘walk’ along the filaments and DSB relocalization, downstream from Smc5/6. Arp2/3, actin polymerization, and myosins are also required to relocalize and repair heterochromatic DSBs in mouse cells\(^1\), revealing a remarkable conservation of relocalization pathways in multicellular eukaryotes.

**Nuclear F-actin promotes DSB clustering and resection in euchromatin**

In addition to relocalizing heterochromatic DSBs, nuclear actin filaments mediate focus clustering (\textit{i.e.,} the non-elastic collision between repair foci\(^70\)) (Fig. 2). Observed in different organisms, from yeast to mammalian cells\(^1,57,69,70,90-94\), focus clustering might facilitate DSB signaling or repair progression by increasing the local concentration of repair components\(^58,95\). The Gautier lab showed that clustering of DSBs induced by the restriction enzyme AsiSI depends on Wasp, Arp2/3 and actin polymerization; it occurs concurrently with the formation of Arp2/3-dependent nuclear actin filaments that polymerize at and travel with damage sites\(^58\). Notably, AsiSI is blocked by DNA methylation, a typical feature of mammalian heterochromatin, implying that DSBs followed in these studies are largely euchromatic\(^58\). Importantly, Arp2/3 is typically enriched at DSBs undergoing HR repair, and clustering facilitates resection, suggesting a role for actin filaments and nuclear dynamics in HR initiation. Clustering is also promoted by resection, in a positive feedback loop\(^58\). Arp2/3 also promotes clustering of euchromatic DSBs in \textit{Drosophila} cells\(^1\), revealing a conserved pathway. Interestingly, studies in \textit{Drosophila} cells showed that the myosin activator Unc45 is not required for clustering\(^11\). Further, Arp2/3 does not mediate clustering of heterochromatic DSBs\(^1\), revealing that the mechanisms responsible for relocalization of heterochromatic DSBs and for the clustering of euchromatic breaks are genetically distinct.

Together, these studies have revealed two separate functions of nuclear actin filaments in DSB repair. In heterochromatin, actin filaments 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 filaments promote DSB clustering and resection in a myosin-independent fashion (Fig. 2).

**Mechanisms of damage-induced actin nucleation**

Distinct actin nucleators appear to contribute to damage-induced nuclear actin filament formation, likely reflecting differences across repair functions, cell cycle phases, organisms, cell types, and/or chromatin domains (Fig. 3, Table 1).

In \textit{Drosophila}, Arp2/3 (and not formins) mediates the relocalization of heterochromatic DSBs, and inactivation of Arp2/3 blocks nuclear F-actin filament formation\(^11\). Arp2/3 also promotes relocalization of heterochromatic DSBs in mouse cells, and heterochromatin stability in \textit{Drosophila} salivary glands\(^87\), revealing conserved pathways\(^11\). Similarly, Arp2/3 is required for clustering of euchromatic DSBs in both human cells in
S/G2 and in *Drosophila* cells, revealing a major role for Arp2/3 in nuclear actin-driven dynamics during DSB repair\(^{11,58}\). However, relocalization of heterochromatic DSBs in *Drosophila* cells relies on Scar and Wash (and not on Wasp)\(^{11}\), while clustering of human repair sites requires Wasp\(^{58}\), revealing distinct mechanisms for Arp2/3 regulation in these contexts. These studies also identified Arp2/3 and its regulators enriched at repair sites\(^{41,58}\), supporting a direct role for Arp2/3 in nuclear actin filament formation in response to damage.

In addition to Arp2/3, formins have been linked to nuclear actin filament formation and repair focus dynamics. Specifically, early studies in human cells showed that actin filaments responding to MMS require Formin-2 and Spire-1/2\(^9\). An independent study revealed that clustering of euchromatic repair sites in G1 requires Formin-2 activity\(^{57}\). In G1, clustering appears to specifically involve DSBs processed for HR\(^{57}\), suggesting a role for clustering in isolating breaks that cannot be readily repaired. However, G1 clustering also relies on the LINC complex\(^{57}\), and evidence for formin enrichment at repair sites is lacking, leaving the possibility that cytoplasmic forces transferred to the nuclei contribute to focus clustering in this context. Intriguingly, the heterochromatin repair component Scal also interacts with formins in mammalian cells\(^{47-49}\), suggesting that formins might also be required for heterochromatin repair.

While a systematic characterization of actin nucleators and regulators directly regulating chromatin dynamics during DNA repair across different cell cycle phases, chromatin contexts, organisms, or cell types is still missing, it is tempting to speculate that distinct regulators organize different types of nuclear actin filaments, perhaps linked with unique functions, as is the case for cytoplasmic actin networks\(^{96-98}\) (Fig. 3). For example, short cables might be sufficient for local dynamics mediating clustering; while long filaments might be needed for the myosin-dependent, long-range, directional motions of heterochromatic DSBs.

In *Drosophila* cells, most filaments appear as long branched filaments originating from heterochromatic DSBs at the periphery of the heterochromatin domain and reaching the nuclear periphery\(^{11}\). These filaments are highly dynamic, display frequent elongation and shrinking, and largely disassemble at time points that follow relocalization of heterochromatic repair sites\(^{11}\). Human cells undergoing euchromatin repair also assemble highly dynamic actin filaments that frequently merge with each other and travel with repair sites\(^{58}\). These dynamics suggest significant actin remodeling occurs during repair. It is still unclear what signals and components regulate actin filament dynamics during repair, and what is the importance of these dynamics in repair progression. Understanding the mechanisms responsible for filament formation in different 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 actin filaments are not the only structural components promoting nuclear dynamics during DNA repair. Studies in yeast and mammalian cells revealed that disrupting microtubules or kinesins affects repair progression and the dynamics of repair sites\(^{57,99-103}\). These effects appear to be, at least in part, a consequence of disrupting
cytoplasmic microtubules, which influence nuclear dynamics and repair through the LINC complex spanning the nuclear envelope\textsuperscript{57,100,104}.

Intriguingly, a recent study from the Mekhail lab also identified damage-induced nuclear microtubules that ‘capture’ repair foci promoting relocalization of repair sites for break-induced replication (BIR) in budding yeast\textsuperscript{102} (Table 1). Similar to actin-filament-driven motions, nuclear microtubule-induced dynamics are characterized by directed motions\textsuperscript{102}. Notably, the Kar3 kinesin is also required for this movement and for repair\textsuperscript{99,102}. Whether this is a direct consequence of nuclear functions of this motor remains unclear, but an interesting possibility is that kinesin-driven movement along nuclear microtubules contributes to chromatin dynamics for DNA repair. More studies are needed to establish in which organisms and damage conditions nuclear microtubules assemble to promote chromatin dynamics, and the role(s) of kinesins in these pathways.

**Directed and sub-diffusive motion of repair sites**

Nuclear repositioning of repair sites occurs in different contexts\textsuperscript{74,76}, including DSBs in rDNA\textsuperscript{105,106}, damaged telomeric and subtelomeric sequences\textsuperscript{99,107-110}, collapsed replication forks\textsuperscript{111,112}, persistent DSBs\textsuperscript{104,107,111,113,114}, and homology search\textsuperscript{115,116}. Yet, these dynamics have been mostly thought to occur by Brownian motion\textsuperscript{117}.

A traditional approach to distinguish Brownian vs directed motions is the mean-square displacement (MSD) analysis of the positional data for repair sites\textsuperscript{89,118,119} (Fig. 3). When MSD values are plotted at increasing time intervals, curves with progressively increasing slope reflect directed motion, while linear MSD graphs indicate Brownian motion\textsuperscript{89,118,119}. However, given that the chromatin behaves as a polymer, and other constraints to the movement exist (e.g., chromatin compaction, molecular crowding, and anchoring to nuclear structures), chromatin motion is typically sub-diffusive rather than Brownian, resulting in flattened MSD curves\textsuperscript{89,119,120} (Fig. 3). In addition, sub-diffusive (or Brownian) motion occurring in a confined space (e.g., the nucleus or subnuclear domains) are characterized by MSD graphs that reach a plateau, and this is proportional to the radius of confinement (i.e., the radius of the volume explored)\textsuperscript{89}. Given that MSD graphs describing repair focus dynamics typically reach a plateau at increasing time intervals, previous studies concluded that the movement of repair sites is largely confined and sub-diffusive\textsuperscript{117}. However, similar plots also result from averaging MSD curves of asynchronous foci each characterized by mixed types of motions\textsuperscript{11,89,102} (such as an alternation or a combination of Brownian and directed motions), suggesting the need for more sophisticated analyses.

For example, heterochromatic repair foci leave the heterochromatin domain with different kinetics\textsuperscript{11,69,71}, and the movement is largely sub-diffusive and confined before relocalization (Fig. 3), likely because of heterochromatin compaction and limited dynamics associated with this domain before damage and at early time points after IR\textsuperscript{11,69}. Additionally, focus movement is highly confined after relocalization, resulting from anchoring to nuclear pores and inner nuclear membrane proteins at the nuclear periphery\textsuperscript{71} (Fig.3). Consistent with this mixed behavior, MSD analyses of a population of heterochromatic repair foci yields graphs that reach a plateau at increasing time intervals\textsuperscript{11}. However, application of a computational method that identifies tracts of directed motions in a context of mixed types of motions\textsuperscript{89}, un_masks long-lasting directed motions (LDMs) mostly occurring
between the heterochromatin domain periphery and the nuclear periphery\textsuperscript{11} (Fig. 3). LDMs last \~24 min, in agreement with the average duration of nuclear actin filaments. The speed of movement of repair foci is \~0.15 \textmu m/min during LDMs\textsuperscript{11}, which is consistent with the speed of movement of transcription sites that are repositioned in the nucleus in F-actin and myosin-dependent manner\textsuperscript{34,121}.

Similarly, directed motions of repair sites moving along damage-induced nuclear microtubules in yeast cells are not easily detectable by canonical MSD analyses because of two major confounding effects: i) DSB 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\textsuperscript{102}. 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\textsuperscript{102}. At coarser time intervals, this method unmaskes kinesins-dependent directed motions\textsuperscript{102}.

Finally, directed motions occur at telomeres repaired by HR in ALT human cells, and were detected by calculating MSD curves only on time points at which directed motion can be identified by eye\textsuperscript{109}.

These studies revealed that in the context of chromatin dynamics, where directed motions occur non-synchronously for different repair sites, and also concurrently or in alternation with sub-diffusive confined motions, MSD analyses applied to the entire kinetic are insufficient to detect directed motions. In agreement, a re-analysis of the dynamics of persistent DSBs in budding yeast, revealed the presence of directed motions\textsuperscript{102}. Together, these studies suggest that nuclear structures and motors might contribute to the dynamics 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 different contexts.

**Nuclear F-actin in DNA replication**

Interestingly, nuclear architecture and dynamics influence DNA replication at different levels, suggesting roles of nuclear filaments and motors in replication fork initiation, progression, and/or repair. For example, DNA damage occurring during replication of trinucleotide repeats or in the presence of hydroxyurea (HU) or MMS, triggers the relocalization of replication forks to the nuclear periphery to promote replication restart in budding yeast\textsuperscript{111,112}. Further, replication of heterochromatin in mouse cells occurs at the periphery of the chromocenters, suggesting relocalization of the forks prior to or during replication\textsuperscript{122}. Whether these movements rely on nuclear actin filaments and motor components is unknown, but, interestingly, HU treatment stimulates the nuclear import of actin and actin polymerizing proteins in mouse cells\textsuperscript{123}, suggesting a role for nuclear F-actin in replication stress response. In agreement, recent studies from the Cesare lab identified nuclear actin filaments in S-phase of human cells, and suggest a role for these structures in relocalization of damaged replication forks to the nuclear periphery and replication restart\textsuperscript{124}.

Further, actin filaments appear to contribute to replication initiation\textsuperscript{125}. Using human cells and *Xenopus* egg extracts, the Fisher lab identified nuclear actin filaments in G1, and provided evidence for a role of formin-dependent actin polymerization in the loading of the pre-initiation complex (pre-IC) to chromatin\textsuperscript{125}. It is tempting
to speculate that filaments might either increase origin clustering\textsuperscript{126,127}, or pre-IC transport to replication origins\textsuperscript{128}, to stimulate origin firing. Actin polymerization is also required for DNA synthesis\textsuperscript{125}, consistent with additional roles of actin filaments in fork progression. Intriguingly, ATR has been proposed as a mechanosensor for torsional stress at the nuclear membrane, such as during replication of membrane-associated chromatin\textsuperscript{129,130}, and an interesting possibility is that ATR-associated F-actin\textsuperscript{131} mediates this response.

Actin polymerization might also contribute to replication initiation indirectly, by promoting nuclear organization after mitotic exit\textsuperscript{12}. Several studies from yeast to mammalian cells showed that nuclear positioning of replication origins in G1 contributes to establishing origin activation timing throughout S-phase\textsuperscript{132-140}. In budding yeast, for example, the spatiotemporal replication program is at least in part regulated by Forkhead 1 and 2-dependent origin clustering\textsuperscript{126-128,141}, and Rif1-dependent organization of late-replicating lamin-associated domains (LADs) and sub-telomeric regions\textsuperscript{133,135,137,142-144}. Actin filaments might actively participate in this organization, thus contributing to the orchestration of the replication program. Together, these studies reveal the importance of nuclear positioning and dynamics in replication regulation, and more studies are needed to establish how nuclear actin filaments or other structures contribute to replication initiation, progression, and repair.

**Nuclear actin filaments in disease**

The identification of a direct link between nuclear actin polymerization and DSB repair suggests deregulation of these mechanisms as a leading cause of genome instability and tumorigenesis. Accordingly, inactivation of relocalization mechanisms causes genome instability in *Drosophila* and mouse cells\textsuperscript{11}, and HR repair defects in human cells\textsuperscript{58}, revealing the importance of these dynamics to genome integrity. Specifically, defective relocalization results in formation of micronuclei and widespread chromosome rearrangements\textsuperscript{11,71}, both of which are commonly observed in cancer cells and directly contribute to genome instability and cancer progression. In agreement with a role of relocalization pathway components in tumor-suppression, actin, actin-remodeling proteins, and myosins, are frequently mutated in cancer cells (reviewed in\textsuperscript{145}), and deregulation of Arp2/3 activators in WAS also results in HR repair defects in lymphocytes\textsuperscript{58}, and predisposition to non-Hodgkin’s lymphoma and leukemia\textsuperscript{146}.

Defective nuclear actin remodeling has also been linked to Huntington’s disease (HD), a progressive neurodegenerative disorder caused by CAG expansion in the codifying region for the protein huntingtin\textsuperscript{147,148}. Thick stress-induced nuclear actin filaments (actin/Cofilin rods) accumulate in cells derived from HD patients\textsuperscript{149}, with more rods observed as the disease progresses\textsuperscript{149}, revealing abnormal F-actin processing. Intriguingly, huntingtin associates with these nuclear actin rod\textsuperscript{149} and promotes filament disassembly\textsuperscript{149} and DNA damage repair\textsuperscript{150}, suggesting a direct link between disease progression, actin deregulation, and DNA repair defects in HD. It is tempting to speculate that deregulation of nuclear F-actin processing during DNA repair critically contributes to neurodegeneration in HD. Additional studies in budding yeast revealed that replication fork instability at critically long CAG repeats results in relocalization of these sequences to the nuclear periphery for
damage repair and replication progression\textsuperscript{112}. While more studies are needed to understand the role of nuclear actin filaments in this relocalization, and the existence of similar pathways in human cells, these studies suggest that nuclear actin deregulation might not just be 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\textsuperscript{151}, suggesting this decline as a contributor of repair defects and genome instability observed in older organisms\textsuperscript{152-154}. Of note, common mutations of Lamin A responsible for Hutchinson-Gilford Progeria Syndrome (HGPS) disrupt the ability of Lamin A to bundle actin filaments\textsuperscript{155}, raising the possibility that aspects of this premature aging disorder (\textit{e.g.}, DNA damage repair defects and heterochromatin deregulation) also reflect deregulation of nuclear F-actin. 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 deregulated in human diseases, including cancer, neurological disorders, progeria syndromes, and aging-related dysfunctions.

\textbf{Conclusions and perspectives}

Significant efforts in recent years started shedding light on the fascinating roles of nuclear actin filaments in cellular responses, including in nuclear dynamics of genomic sites for DNA transcription and repair. These discoveries challenged the previous conclusions that actin is only monomeric in most nuclei, revealing remarkable filaments of a transient nature with critical cellular functions. Nuclear actin filaments responding to DNA damage appear to have different regulatory mechanisms, likely depending on the cell type, cell cycle phase, types of damages, chromatin domain, and/or repair pathway, suggesting the existence of distinct structures with specialized functions. Filaments serve as ‘highways’ for the myosin-dependent ‘walk’ of repair sites during heterochromatin repair, or as dynamic anchors propelling chromatin movement for focus clustering during euchromatic HR repair. A number of open questions remain. For example, the molecular mechanisms regulating actin nucleators in different contexts remain largely unknown. The fine structure of these filaments requires deeper investigation. Actin remodelers responsible for filament dynamics need to be established, and the importance of these dynamics in repair is unclear. Further, a number of repair pathways across different organisms appear to rely on nuclear dynamics, and recently developed analytical methods will likely uncover more examples of directed motions, and stimulate the investigation of structural/motors components involved. Finally, major efforts started unraveling similarities between relocalization pathways in \textit{Drosophila} and mammalian cells, providing exciting new insights. Understanding these mechanisms is expected to open new avenues for the treatment of cancer, neurodegenerative disorders, other aging-related human diseases and progeria syndromes. We expect many new discoveries in this field in the coming years.

\textbf{Acknowledgements}
We apologize to our colleagues whose work could not be cited owing to space limitations. We thank J. Gautier for sharing raw data for filament quantification. Work supported by NIH R01GM117376 and NSF Career 1751197 to I.C and the DFG GR 2111/7-1 to R.G.
**Table 1**: Structural and motor components linked to different relocalization pathways for genome stability and related studies in different organisms.

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<th>Functions in genome stability</th>
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*Figure legends:*

**Figure 1: Actin remodeling.** A) Spontaneous actin nucleation is characterized by a fast-growing (+) ‘barbed’ end and a slow-growing (-) ‘pointed’ end, with more efficient addition of actin monomers (G-actin) at the (+) end. Actin filament formation and disassembly are regulated by actin remodelers, including actin nucleating proteins and severing factors. B) Actin nucleators include the Arp2/3 complex, Spire and formins. Arp2/3 promotes nucleation at 70 degree angles from preexisting filaments via polymeric actin structural mimicry, and is activated by the WAS family proteins (i.e, Wasp). Spire recruits several actin monomers with its WASp-homology 2 domains (WH2), forming a seeding polymer that promotes polymerization. Formins associate with the (+) end and promotes polymerization by bringing actin monomers in close proximity via formin homology 2 domains (FH2). C) Actin severing proteins include cofilin, which preferentially associates with ADP-actin inside a filament and stimulates filament severing through a steric mechanism.

**Figure 2: Model of repair site dynamics in heterochromatin and euchromatin.** After DSB formation in heterochromatin, initial damage detection and processing (resection) occur inside the heterochromatin domain. Mre11 and HP1a promote association of Arp2/3 and myosins to the repair site; activation of Arp2/3 by Scar and Wash facilitates actin polymerization; filaments grow towards the nuclear periphery; Smc5/6 blocks Rad51 recruitment inside the heterochromatin domain, and recruits Unc45 to activates nuclear myosins. The myosin-Smc5/6-chromatin complex translocates along the actin filament to anchor DSBs to nuclear pores or inner nuclear membrane proteins (INMPs, not shown), where HR repair continues with Rad51 recruitment. Actin filaments are highly dynamic and disassemble after relocalization. In euchromatin, Mre11 and resection promote clustering of repair sites by Arp2/3, Wasp, and F-actin, which in turn facilitate resection and repair by HR or SSA. Actin polymerization results in filaments that travel with repair sites, generating forces that promote clustering.

**Figure 3: Different actin nucleators and motor proteins contribute to DSB dynamics and repair.** A) In *Drosophila* cells (that are mostly in S/G2), directed motions of heterochromatic DSBs to the nuclear periphery requires Arp2/3, the Arp2/3 activators Wash and Scar, F-actin, nuclear myosins and the myosin activator Unc45. Myosins, Unc45 and Arp2/3 are enriched at repair foci, supporting a direct role in chromatin dynamics. Clustering of euchromatic DSBs relies on Arp2/3 and not on Unc45. B) In mouse G2 cells, relocalization of heterochromatistic DSBs also requires Arp2/3, F-actin, and myosins. C) In human S/G2 cells, DSBs prone to HR cluster with each other in a manner dependent on Arp2/3, Wasp, and F-actin. Actin filaments, Wasp and Arp2/3 are associated with repair sites, supporting direct roles in movement and repair of these sites. D) In human cells treated with MMS, F-actin filament form in the nuclei and mediate repair, which also requires Fmn-2 formin and Spire. E) In human G1 cells, clustering of euchromatic DSBs requires Fmn-2 formin. These are likely repaired after S-phase entry suggesting this clustering as a mechanism to halt repair. (*) indicates experimental systems where the nuclear function of the indicated components (and not their cytoplasmic role) has been directly established.
Nuclear actin filaments are also indicated for studies that directly identified those structures. Components that are not required for filament formation or repair in different contexts are also shown in parenthesis. F) MSD curves for different types of motion, as indicated\textsuperscript{11}. G) An example of a 3D reconstruction and tracking with Imaris of a *Drosophila* cell and heterochromatic (HC) or euchromatic (EU) repair foci\textsuperscript{11}, shows mixed types of motion for heterochromatic repair foci that reach the nuclear periphery, as indicated. Time points characterized by directed and sub-diffusive motions were detected with an automated method as described in\textsuperscript{89}, and confirmed by MSD calculation within those time intervals, revealing that they occur between the heterochromatin domain periphery and the nuclear periphery\textsuperscript{11}. Scale bar = 1 µm.
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