



Genome maintenance meets mechanobiology

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Abstract

Genome stability is key for healthy cells in healthy organisms, and deregulated maintenance of genome integrity is a hallmark of aging and of age-associated diseases including cancer and neurodegeneration. To maintain a stable genome, genome surveillance and repair pathways are closely intertwined with cell cycle regulation and with DNA transactions that occur during transcription and DNA replication. Coordination of these processes across different time and length scales involves dynamic changes of chromatin topology, clustering of fragile genomic regions and repair factors into nuclear repair centers, mobilization of the nuclear cytoskeleton, and activation of cell cycle checkpoints. Here, we provide a general overview of cell cycle regulation and of the processes involved in genome duplication in human cells, followed by an introduction to replication stress and to the cellular responses elicited by perturbed DNA synthesis. We discuss fragile genomic regions that experience high levels of replication stress, with a particular focus on telomere fragility caused by replication stress at the ends of linear chromosomes. Using alternative lengthening of telomeres (ALT) in cancer cells and ALT-associated PML bodies (APBs) as examples of replication stress-associated clustered DNA damage, we discuss compartmentalization of DNA repair reactions and the role of protein properties implicated in phase separation. Finally, we highlight emerging connections between DNA repair and mechanobiology and discuss how biomolecular condensates, components of the nuclear cytoskeleton, and interfaces between membrane-bound organelles and membraneless macromolecular condensates may cooperate to coordinate genome maintenance in space and time.

Keywords Genome stability · Replication stress · DNA repair · Biomolecular condensates · Telomere maintenance · Mechanobiology

Introduction to cell cycle regulation

The eukaryotic cell cycle comprises a series of tightly controlled events that culminate in cell division and in the generation of two new daughter cells (Figure 1). It can be divided into the two main stages mitosis (M-phase) and interphase. While mitosis refers to the process of chromosome segregation followed by cell division, interphase separates two M-phases and provides the time needed for genome duplication and to prepare for cell division. Interphase can be subdivided further into three consecutive cell cycle phases, which are called Gap 1 (G1), Synthesis (S), and Gap 2 (G2). During G1, cells grow and produce proteins and organelles

for cellular metabolism and to prepare for later cell cycle phases. G1 length varies depending on growth conditions and intracellular and extracellular cues. In unfavorable conditions, cells may exit the cell cycle in G1 and enter a non-proliferative state known as quiescence or Gap 0 (G0). Following S-phase commitment in favorable growth conditions, cells duplicate their genome by DNA synthesis. After two identical copies of the genetic material have been generated through semi-conservative DNA replication, the ensuing G2-phase serves as an additional gap phase for protein synthesis and cell growth in preparation for mitosis. Finally, in M-phase, the two sets of chromosomes are first condensed and aligned at the equatorial metaphase plate and then segregated as cells divide into two newly emerging daughter cells. M-phase can be subdivided morphologically and functionally into prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis (Figure 1).

The central regulators of the cell cycle are cyclin-dependent kinases (CDKs). CDKs are serin/threonine kinases

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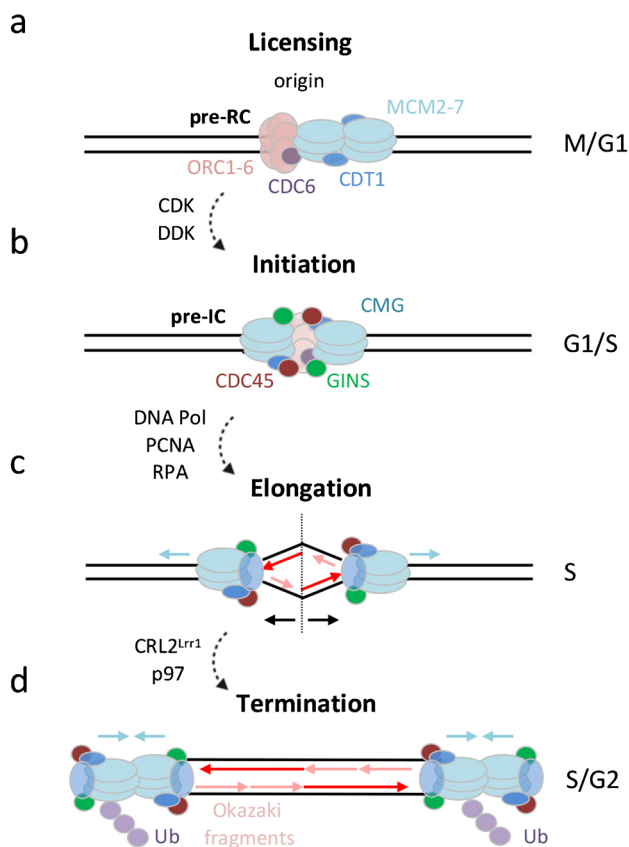


Fig. 2 Overview of DNA replication. Indicated are the four phases of DNA replication and their timing during the cell cycle. (a) Origin licensing in late M and early G1. Formation of the pre-replication complex (pre-RC) by the assembly of ORC1-6, CDC6, CDT1, and MCM2-7 at the origin of replication. (b) Replication initiation at the G1/S transition. CDK- and DDK-mediated phosphorylation of the MCM complex leads to the recruitment of CDC45 and the GINS complex, which together with the MCM proteins form the CDC45-MCM-GINS (CMG) helicase, thereby setting up the pre-initiation complex (pre-IC). (c) Elongation during S-phase progression. Recruitment of additional replication proteins, including PCNA, RPA, and DNA polymerases (DNA Pol), leads to the transition from the pre-IC into two active replisomes moving in opposite directions. (d) Termination of replication in S/G2 when two replisomes converge, followed by CRL2^{Lrr1}-dependent ubiquitylation of MCM7 and p97-mediated extraction from the DNA. M, mitosis; G1, Gap 1; S, S-phase; G2, Gap 2; CDK, cyclin-dependent kinase; DDK, DBF4-dependent kinase; Ub, ubiquitin

Origin licensing (Figure 2a) occurs before S-phase in late M and early G1 phase of the cell cycle, when the origin recognition complex (ORC), a hexameric protein complex consisting of the subunits ORC1-6, binds to replication origins (Masai et al. 2010). ORC binding leads to the recruitment of the licensing factors CDC6 and CDT1, which facilitates the recruitment of inactive hexameric MCM protein complexes consisting of the proteins MCM2-7 (Nishitani & Lygerou 2002). The origin-associated multiprotein complex formed by ORC, CDC6, CDT1, and MCM2-7 is called

pre-replication complex (pre-RC). Two MCM complexes are loaded onto the chromatin head-to-head and form the core of the replicative helicase (Bleichert 2019; Deegan & Diffley 2016; Evrin et al. 2009; Miller et al. 2019; Remus et al. 2009).

Cells load an excess number of pre-RCs and only a subset of licensed origins is used for replication initiation (Masai et al. 2010). Origins have therefore been classified into constitutive, flexible, and dormant origins (Blow et al. 2011; Fragkos et al. 2015). The excess of licensed origins can serve as a backup to complete DNA replication under conditions of replication stress (Blow et al. 2011; Courtot et al. 2018; Fragkos et al. 2015).

The restricted time window for origin licensing in late M and early G1 prevents re-licensing during S-phase. Re-licensing in S-phase could cause re-replication of already copied DNA, leading to amplification of DNA sequences and increased genome instability (Blow & Gillespie 2008; Fragkos et al. 2015; Limas & Cook 2019; Neelsen et al. 2013). The negative regulator of origin licensing Geminin, which is destabilized in G1/M by the APC/C ubiquitin ligase complex, accumulates after APC/C inactivation at the G1/S transition and prevents re-licensing (Machida et al. 2005; Petropoulos et al. 2019). Geminin interacts with the licensing factor CDT1 and blocks loading of the MCM complex, thereby inhibiting the formation of new pre-RCs in S-phase (Lee et al. 2004; Wohlschlegel et al. 2000; Yanagi et al. 2002). A second way of controlling pre-RC formation is mediated by the ubiquitin ligases SCF-Skp2 and DDB1-Cul4, which ubiquitinate CDT1 and ORC1 in S-phase, leading to their proteasomal degradation (Li et al. 2003; Méndez et al. 2002; Nishitani et al. 2006). Finally, in G2 and early M-phase, high CDK activity inactivates pre-RC components, ensuring that licensing of new origins occurs only after chromosome segregation (Machida et al. 2005; Petropoulos et al. 2019).

Initiation

Licensed origins with pre-RCs are converted to pre-initiation complexes (pre-ICs) upon S-phase entry. This step is regulated by the recruitment of CyclinE-CDK2, DDK (DBF4/CDC7), and CyclinB-CDK1 (Suski et al. 2022), which phosphorylate the MCM complex (Figure 2b). MCM phosphorylation leads to the recruitment of CDC45 and the GINS protein complex, thereby forming the CDC45-MCM-GINS (CMG) helicase (Lewis et al. 2022). CMG complex formation is a prerequisite to activate the MCM helicase activity (Ilves et al. 2010; Zou & Stillman 2000). The protein TopBP1 and its interaction partner Treslin are also part of the pre-IC and bind to the MCM complex in a CDK phosphorylation-dependent manner. Recruitment of TopBP1-Treslin to the MCM complex is required for activation of the CMG helicase

(Kumagai et al. 2010, 2011). As a result, two inactive MCM complexes get remodeled into two active CMG complexes, which start to unwind the parental DNA in a bidirectional manner, thereby allowing the recruitment of additional replication proteins, including replication factor c (RFC), proliferating cell nuclear antigen (PCNA), replication protein a (RPA), and DNA polymerases (Limas & Cook 2019; Parker et al. 2017). Together they mediate the transition from a pre-IC into two active replisomes that move in opposite directions and generate the replication bubble (Douglas et al. 2018; Fragkos et al. 2015).

Elongation

Once DNA replication has been initiated by origin firing, replisomes move away from the replication origin to copy the parental DNA (Figure 2c). Replicative DNA polymerases are incapable of initiating DNA synthesis de novo, but instead need a start site, or primer, to begin DNA synthesis. This primer is synthesized by DNA Pol α -primase, which generates a short stretch of ribonucleic acid (RNA) (Arezi & Kuchta 2000). The primer is recognized by RFC, which loads the replication sliding clamp PCNA and displaces DNA Pol α -primase with replicative DNA Pol δ/ϵ (Moldovan et al. 2007; O'Donnell et al. 2013). As new deoxyribonucleotides can only be added at the 5'-phosphate ends of nascent DNA strands, one of the two daughter strands is synthesized in a continuous manner in the same direction as the moving replication fork (leading strand synthesis), while the opposite daughter strand must be synthesized away from the replication fork and hence in a discontinuous manner (lagging strand synthesis). For leading strand synthesis DNA Pol ϵ needs only one primer formed at the origin, whereas for lagging strand synthesis the process of primer synthesis followed by extension must be repeated periodically. The newly synthesized fragments of the lagging strand are termed Okazaki fragments (Okazaki et al. 1968). Through leading and lagging strand synthesis in conjunction with Okazaki fragment ligation, both parental DNA strands are copied, and continuous stretches of newly synthesized DNA are formed within each replication unit.

Termination

DNA replication is terminated when converging replication forks coming from two replication units encounter each other (Figure 2d). When converging forks meet, the replisomes disassemble leaving a ssDNA gap between the 3'-end of the leading strand and the downstream Okazaki fragment of the opposing fork. The remaining gap is filled by extension of the leading strand resulting in a continuous DNA molecule (Dewar & Walter 2017). Unloading of the CMG helicase from termination sites was recently shown to require the

ubiquitin-selective segregase p97 after polyubiquitylation of MCM7 by CRL2^{Lrr1} (Dewar & Walter 2017; Fan et al. 2021). A backup mechanism seems to exist in mitosis to trigger global replisome disassembly through MCM7 polyubiquitylation by the ubiquitin E3 ligase TRAIIP, followed by p97-mediated extraction from the chromatin (Deng et al. 2019; Priego Moreno et al. 2019; Sonnevile et al. 2019; Villa et al. 2021). Failure to terminate replication, e.g., due to obstacles that impair replication fork speed, can undermine the faithful propagation of the genetic information to the next cell generation and is associated with DNA replication stress.

Replication stress

During the process of DNA replication, the replication machinery is confronted with a variety of obstacles that can interfere with DNA synthesis and jeopardize timely completion of genome duplication (Mazouzi et al. 2014; Saxena & Zou 2022). Conditions that lead to replication fork slowing or stalling and perturb DNA synthesis are generally referred to as replication stress (Gaillard et al. 2015; Zeman & Cimprich 2014). Replication stress can be caused by exogenous sources such as DNA-modifying chemicals and alterations of the DNA structure, e.g., through ionizing radiation (IR) or ultraviolet (UV) light (Figure 3a). Endogenous sources of replication stress include depleted deoxyribonucleotide pools, ribonucleotide incorporation into DNA, DNA lesions caused by metabolic byproducts such as reactive oxygen species (ROS), interstrand crosslinks (ICLs), DNA secondary structures such as hairpins and G4-quadruplexes, repetitive DNA sequences, transcription-replication conflicts, and RNA-DNA hybrids (Brickner et al. 2022; Garcia-Muse & Aguilera 2016; Petermann et al. 2022; Saxena & Zou 2022; Zeman & Cimprich 2014). Replication stress has emerged as major cause of genome instability and is a hallmark of most cancers (Macheret & Halazonetis 2015). Considering the elevated levels of replication stress in cancer cells, enzymes involved in the response to replication stress are promising targets for cancer therapy (Cybulla & Vindigni 2023; da Costa et al. 2023; Dobbstein & Sorensen 2015).

Replication stress response

Obstacles that lead to stalling of DNA polymerases on the lagging strand are generally well tolerated. Due to the discontinuous nature of Okazaki fragment synthesis, such lesions can be efficiently bypassed, leaving behind short ssDNA gaps, which are repaired post-replicatively (Berti & Vindigni 2016; Marians 2018; Yeeles et al. 2013). On the other hand, stalling of DNA polymerases during leading strand synthesis can cause helicase-polymerase uncoupling and challenge fork stability (Taylor & Yeeles 2018; Taylor & Yeeles 2019).

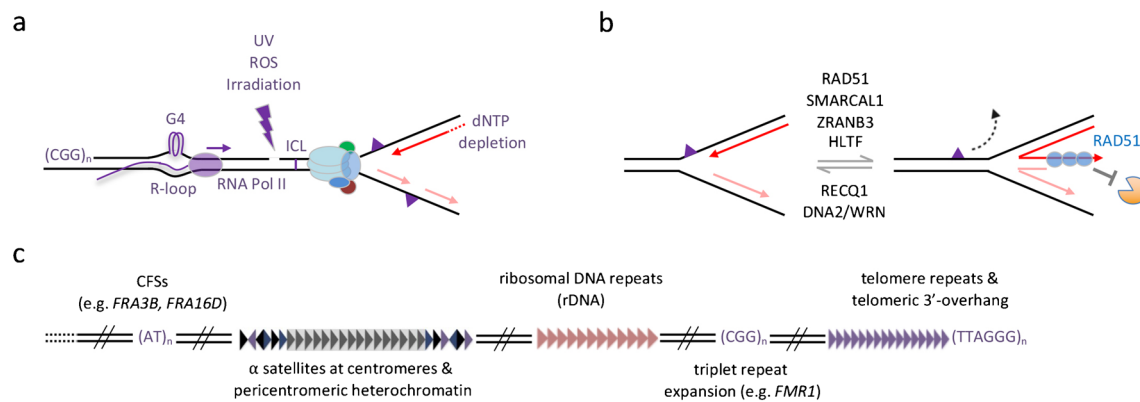


Fig. 3 Replication stress and fragile genomic regions. (a) Sources of replication stress that can lead to replication fork stalling and fork collapse. Indicated are exogenous sources of replication stress (e.g., UV light, irradiation) and endogenous sources of replication stress (e.g., repetitive sequences, secondary structures such as G4-quadruplexes and R-loops, reactive oxygen species (ROS), interstrand crosslinks (ICLs), dNTP depletion). (b) Replication fork reversal as

a protective mechanism against replication stress. Indicated is the dynamic process of fork reversal and re-reversal for fork restart, as well as main regulators of fork reversal and of reversed fork protection. (c) Fragile genomic regions that are prone to experiencing replication stress. Indicated are common fragile sites (CFSs), centromeric and pericentromeric regions, ribosomal DNA, rare fragile sites of triplet repeat expansion, and telomere repeats

Replication fork uncoupling can lead to extensive stretches of RPA-bound ssDNA, which recruits the central replication stress response kinase ATR through its interaction partner ATRIP (MacDougall et al. 2007; Zou & Elledge 2003). Once recruited, ATR gets activated through two specific activator proteins, namely TopBP1 and ETAA1 (Bass et al. 2016; Haahr et al. 2016; Kumagai et al. 2006; Lee et al. 2016). The ssDNA-bound RPA also serves as a recognition site for the Timeless-Tipin complex, which stimulates claspin-mediated phosphorylation of checkpoint kinase 1 (CHK1) by ATR (Kemp et al. 2010). ATR/CHK1 activation leads to fork stabilization and inhibits origin firing, thereby controlling the number of active replisomes. This is important to protect already active forks against irreversible breakage, which can occur upon exhaustion of limiting replication factors such as dNTPs and RPA (Buisson et al. 2015; Toledo et al. 2013). On the other hand, dormant origins in the vicinity of fork-stalling lesions escape from ATR/CHK1-mediated suppression and can rescue stalled forks to ensure completion of DNA synthesis (Ge & Blow 2010; Saldivar et al. 2017).

Fork protection and restart

To resolve replication stress-inducing problems, multiple mechanisms have evolved to stabilize stalled replication forks and promote their later restart. The choice of fork remodeling and repair pathways is dependent on the type of problem that triggered replication stress as well as the duration of the replication block itself (Berti et al. 2020; Panagopoulos & Altmeyer 2021).

One way to stabilize stalled replication forks is a process known as fork reversal, also referred to as fork regression (Figure 3b). During fork reversal, standard three-way replication forks are converted into four-way junctions by unwinding of the newly synthesized DNA strands and subsequent annealing of the two nascent strands and reannealing of the parental strands (Berti, Cortez, & Lopes 2020). Fork reversal can fine-tune fork speed and pause DNA synthesis in response to a variety of genotoxic treatments, upon oncogene-induced replication stress, and when endogenous replication obstacles are encountered (Berti et al. 2013; Berti, Cortez, & Lopes 2020; Follonier et al. 2013; Neelsen & Lopes 2015; Quinet et al. 2017). After fork uncoupling, RPA-coated ssDNA leads to the recruitment of RAD51, which initiates fork reversal (Bhat & Cortez 2018). Different from the known functions of RAD51 in DNA double-strand break (DSB) repair, RAD51-mediated fork remodeling does not require its classical loading factor BRCA2, nor the formation of stable RAD51 filaments (Mijic et al. 2017; Scully et al. 2019). RAD51-mediated fork reversal is regulated by RADX and the homologous recombination (HR) co-factors RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 (Berti et al. 2020; Bhat et al. 2018; Halder et al. 2022; Krishnamoorthy et al. 2021). Other proteins involved in fork reversal include SMARCAL1, HLTF, and ZRANB3 (Bai et al. 2020; Kolinjivadi et al. 2017; Poole & Cortez 2017; Tagliatela et al. 2017). SMARCAL1 catalyzes fork regression and Holliday junction migration, thereby promoting efficient fork repair (Bétous et al. 2012). HLTF is a DNA translocase and ubiquitin E3 ligase that gets recruited to the 3'-ssDNA-end of the leading strand where it polyubiquitylates PCNA (Bai et al. 2020; Kile et al. 2015). ZRANB3

interacts with polyubiquitylated PCNA and assists replication fork remodeling through its DNA translocase activity (Ciccia et al. 2012; Vujanovic et al. 2017; Weston et al. 2012). While SMARCAL1 and ZRANB3 guide the initial annealing of the displaced daughter strands, ZRANB3 and HLTf catalyze branch migration (Halder et al. 2022). Upon reversal, the regressed fork resembles a one-ended DSB that must be protected from nucleolytic degradation. Important factors mediating this protection include BRCA1 and RAD51, whose function in fork protection, contrary to the role in initiating fork reversal, is dependent on BRCA2 (Berti, Cortez, & Lopes 2020; Lemacon et al. 2017; Mijic et al. 2017; Tarsounas & Sung 2020).

Stalled and reversed forks can be restarted in multiple ways. Fork restart can be mediated by the helicase RECQ1, which promotes branch migration and converts four-way junctions back into replication-competent three-way junctions. The activity of RECQ1 is regulated by PARP1-dependent poly(ADP-ribosyl)ation (PARylation), which ensures that forks restart only upon repaired damage (Berti et al. 2013). Additionally, re-establishment of three-way junctions can be mediated through fork processing by the Werner syndrome helicase WRN and the DNA2 nuclease (Datta et al. 2021; Thangavel et al. 2015).

Replication obstacles on the leading strand can also be overcome by fork repriming (Bianchi et al. 2013; García-Gómez et al. 2013; Guillian et al. 2017; Quinet et al. 2021). For repriming, new primers are placed downstream of the obstacles to continue DNA replication, at the cost of leaving behind unreplicated ssDNA gaps that have to be repaired after replication (Mourón et al. 2013). The key enzyme involved in repriming is primase and DNA-directed polymerase (PrimPol), which gets recruited to ssDNA via direct interaction with RPA (González-Acosta et al. 2021; Guillian et al. 2017). PrimPol not only has primase activity, but also acts as DNA polymerase with low processivity and fidelity (Bianchi et al. 2013; García-Gómez et al. 2013; Guillian & Doherty 2017; Tirman et al. 2021). Gaps that are left behind the fork can be repaired post-replicatively by either DNA translesion synthesis (TLS), in which specific TLS polymerases mediate replication across the lesion, or by template switching (TS), a process in which the intact sister strand is used for homologous recombination repair (Piberger et al. 2020; Tirman et al. 2021; Wong et al. 2021).

Fragile sites in the human genome

Certain regions in the genome are particularly vulnerable to endogenous replication stress due to their inherent difficulty to be replicated (Glover et al. 2017; Lezaja & Altmeyer

2021). These difficult-to-replicate regions include chromosomal fragile sites as well as repetitive sequences at ribosomal DNA, centromeres, and telomeres (Figure 3c). Fragile sites are prone to form gaps and breaks visible on metaphase chromosomes, often referred to as fragile site expression (Özer & Hickson 2018). Rare fragile sites are caused by pathological expansion of trinucleotide repeat sequences and are only present in a small percentage of the human population. One example is CGG triplet expansion in the *fragile X messenger ribonucleoprotein 1 (FMR1)* gene causing fragile X syndrome (Zhou et al. 2016). Common fragile sites (CFSs) on the other hand are present in all individuals, e.g., the fragile sites *FRA16D* and *FRA3B*, which harbor the tumor suppressor genes *fragile histidine triad diadenosine triphosphatase (FHIT)* and *WW domain containing oxidoreductase (WWOX)*, respectively (Durkin & Glover 2007; Özer & Hickson 2018). CFSs in cancer cells are often associated with breakpoints of genomic rearrangements, micro-deletions, and copy number variations (Glover et al. 2017; Sarni & Kerem 2016). The sensitivity of CFSs to replication stress is caused by their tendency to have AT-rich sequences, which are prone to form secondary structures, sparsity of replication origins, association with very long genes that can take more than one cell cycle to be fully transcribed, and their late replication timing, typically being the last regions of the genome to be replicated (Brison et al. 2019; Kaushal et al. 2019). These features challenge faithful and complete replication during S-phase and can lead to under-replicated DNA and CFS instability (Debatisse & Rosselli 2019).

Ribosomal DNA (rDNA) consists of DNA tandem repeats that encode ribosomal RNAs (rRNAs) required for ribosome biosynthesis. Their high rate of transcription makes replication-transcription conflicts almost inevitable. Although replication fork barriers positioned within each rDNA unit were shown to coordinate progression of replication with transcription in eukaryotic cells (Akamatsu & Kobayashi 2015; Gadaleta & Noguchi 2017), R-loops form at transcribed rDNA repeats and cause replication-transcription conflicts in the nucleolus that undermine rDNA stability (El Hage et al. 2010; Lezaja & Altmeyer 2021; Özer & Hickson 2018; Salvi et al. 2014; Tsekrekou et al. 2017; Warmerdam & Wolthuis 2019). Additionally, non-transcribed rDNA repeats cluster in heterochromatic regions at the nucleolar periphery and show, unlike actively transcribed repeats, late replication timing, which makes them prone to form under-replicated DNA and breaks late in the cell cycle (Lezaja & Altmeyer 2021; Warmerdam & Wolthuis 2019).

Centromeres are chromosomal domains needed for faithful transmission of duplicated chromosomes to daughter cells during cell division by assembling the kinetochore and mitotic spindle microtubules for sister chromatid separation. They are composed of a series of 171 nt long AT-rich DNA tandem repeats, named alpha satellites (Barra & Fachinetti

2018). The surrounding pericentromeric heterochromatin is also organized in short tandem repeats. Due to the repetitive nature of centromeric and pericentromeric DNA, secondary structures like DNA loops and catenates are being formed, giving rise to target sites for DNA topoisomerases and the DNA recombination machinery (Barra & Fachinetti 2018). These secondary structures, together with the heterochromatic environment and the late replication timing, contribute to the fragility of centromeric and pericentromeric repeats (Lezaja & Altmeyer 2021; Mitrentsi et al. 2020), and make centromeres hotspots of DNA damage and recombination (Saayman et al. 2023; Yilmaz et al. 2021).

A fourth important class of fragile regions is represented by telomeres, constitutive heterochromatic regions at chromosome ends that determine replicative (im)mortality. Replication stress at telomeres is primarily driven by their composition of terminal tracts of tandem repeats, the presence of secondary DNA structures including G-quadruplexes, R-loops, and telomere loops (t-loops) formed by the G-rich 3'-telomeric ssDNA overhang, and the fact that stalled replication forks downstream of the most distal origin cannot be rescued by dormant origin firing (Lezaja & Altmeyer 2021; Lu & Pickett 2022). Telomeres are protected by the shelterin complex, consisting of the subunits TRF1, TRF2, RAP1, POT1, TPP1, and TIN2 (de Lange 2005). At intact telomeres, the shelterin complex competes with RPA and promotes t-loop formation, thereby suppressing DNA damage response signaling from

chromosome ends and unwanted DNA repair reactions that could lead to telomere fusions (Kratz & de Lange 2018). A telomeric long non-coding RNA termed telomeric repeat-containing RNA (TERRA) comprised of G-rich telomere repeats is important for the regulation of telomeric chromatin structure and telomere stability (Azzalin et al. 2007). Binding of TERRA to telomeric DNA leads to the formation R-loops by displacement of the G-rich DNA strand, which are stabilized when telomeres experience elevated levels of replication stress (Feretzaki et al. 2020; Fernandes et al. 2021; Lu & Pickett 2022; Niehrs & Luke 2020).

Telomere elongation in cancer

Due to the end-replication problem associated with telomeric lagging strand DNA synthesis, telomeres shorten in each cell cycle (Figure 4a). In somatic cells, when critically short telomeres accumulate, cellular senescence, apoptosis, or a permanent cell cycle arrest is triggered (D'Souza et al. 2013; Koliada et al. 2015). In contrast to most somatic cells, stem cells and progenitor cells express low levels of the enzyme telomerase, which extends telomere repeats and contributes to prolonged proliferative capacity. Telomerase is a ribonucleoprotein complex consisting of the enzyme telomerase reverse transcriptase (TERT) and the telomere-sequence containing non-coding human telomerase RNA, which binds to the telomeric 3'-ssDNA overhang (Roake &

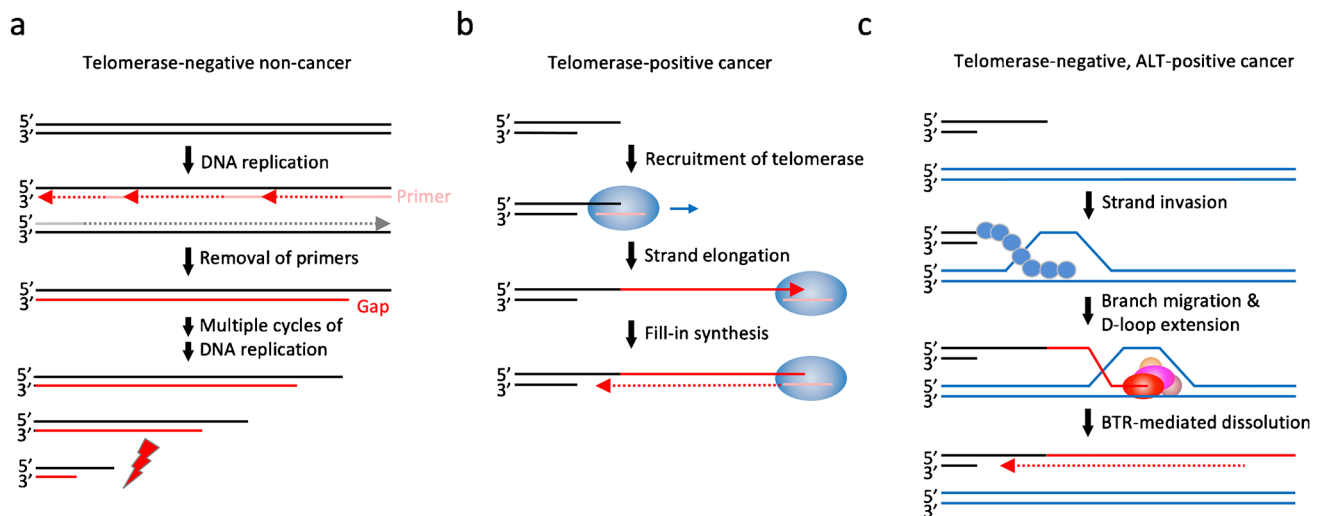


Fig. 4 Telomere maintenance in cancer. (a) End-replication problem and successive telomere shortening in somatic cells with inactivated telomerase. Terminal gaps at the lagging strand cause telomere shortening, which can lead to senescence or cell death when telomeres become critically short. (b) Reactivation of telomerase in cancer cells enables replicative immortality. The ribonucleoprotein complex telomerase uses its reverse transcriptase activity and a telomere sequence-containing non-coding RNA for elongation of the telomeric

3'-ssDNA overhang. Successive shortening of telomeres caused by the end-replication problem is countered by telomerase activity. (c) Alternative lengthening of telomeres (ALT) in telomerase-negative cancer cells. ALT-positive cancer cells use recombination-based mechanisms for telomere elongation. For simplicity, productive BTR complex-mediated D-loop dissolution is indicated, although unproductive non-crossover and crossover resolution leading to aborted telomere extension can also occur

Artandi 2020). The telomeric 3'-ssDNA overhang is then extended by the reverse transcriptase activity of TERT, using the telomerase RNA as template (Figure 4b). While TERT is usually silenced in somatic cells, most cancer cells show reconstituted expression of the enzyme, thereby achieving replicative immortality (Shay & Wright 2019).

Although expression of telomerase was long seen as a general cancer marker, around 10–15% of tumors, predominantly of mesenchymal origin, lack this enzyme (Bhargava et al. 2022; Lu & Pickett 2022; Shay & Wright 2019). These cancer cells use a homologous recombination-based pathway to extend and maintain their telomeres (Figure 4c), known as alternative lengthening of telomeres (ALT) (Barthel et al. 2017; Dilley & Greenberg 2015; Heaphy et al. 2011). Typically, they display several hallmarks of ALT, including long and heterogeneous telomere length, abundant extrachromosomal circular telomere repeats (ECTR), incorporation of non-telomeric sequences, high levels of telomere sister chromatid exchange, and the formation of ALT-associated PML bodies (APBs) (Bhargava et al. 2022; Bryan et al. 1995; Cesare & Griffith 2004; Londoño-Vallejo et al. 2004; Silva et al. 2019; Yeager et al. 1999a; Zhang & Zou 2020).

APBs are membraneless nuclear condensates that contain the promyelocytic leukemia (PML) protein, clustered telomeres, and several proteins involved in DNA repair, recombination, and replication, thereby generating a local hub for telomere recombination and telomere DNA synthesis (Draskovic et al. 2009; Grobelyny et al. 2000; Nabetani et al. 2004; Stavropoulos et al. 2002; Wu et al. 2000; Yeager et al. 1999b). APB formation was suggested to involve phase separation properties of APB-associated proteins, including multivalent SUMO-SIM interactions (Min et al. 2019; Spegg & Altmeyer 2021; Zhang et al. 2020). Of note, the shelterin complex components TRF1 and TRF2 also show features of phase separation and form condensates at telomere repeats (Jack et al. 2022; Soranno et al. 2022). The interplay between TRF1/2-driven telomere condensates and APB condensates in ALT-positive cancer cells remains to be determined.

ALT is induced at stalled and collapsed replication forks, suggesting that replication stress at telomeres and the associated telomere fragility are a driving force behind this process (Amato et al. 2020; Pan et al. 2019; Silva et al. 2021; Turkalo et al. 2023). The ALT pathway was initially characterized in budding yeast mutants lacking functional telomerase, where two distinct subpathways were described: Rad51- and Rad52-mediated amplification of repetitive subtelomeric sequences, and Rad52-dependent but Rad51-independent telomere maintenance by expanding telomeric repeats (Kockler et al. 2021; Le et al. 1999; McEachern & Haber 2006; Zhang & Zou 2020). Also in human cancer cells both RAD51 and RAD52 seem to be involved in ALT-dependent

telomere maintenance, either directly or indirectly (Cho et al. 2014; Dilley et al. 2016; Lu & Pickett 2022; Min et al. 2019; Zhang et al. 2019). ALT utilizes break-induced replication (BIR), a recombination process initiated by one-ended DSBs, which results in sequence extension by conservative DNA replication using a homologous template (Anand et al. 2013; Kramara et al. 2018; Zhang & Zou 2020). Resection of one-ended DSBs might be involved, mediated by BLM and DNA2/EXO1 (Nimonkar et al. 2011; Sturzenegger et al. 2014), which can lead to the formation of longer 3'-ssDNA overhangs to facilitate strand invasion of homologous templates. The ssDNA overhang is first bound by RPA before being handed over to RAD52, which promotes annealing of the broken telomere end with a homologous template to form a D-loop (Verma et al. 2019; Zhang et al. 2019). Extension of the D-loop is then mediated by DNA Pol δ and its subunits POLD3 and POLD4 (O'Rourke et al. 2019; Zhou et al. 2012). A RAD52-independent ALT pathway seems to exist as well, because RAD52 loss leads to BLM- and POLD3/4-dependent ALT DNA synthesis associated with increased c-circle formation (Epum & Haber 2022; Zhang et al. 2019).

A central positive regulator of ALT is the DNA helicase BLM, which functions in a complex together with TOP3A and RMI1/2, thereby forming the BLM-TOP3A-RMI1/2 (BTR) complex (Bhargava, Lynskey, & O'Sullivan 2022; Manthei & Keck 2013). BLM is critical for ALT-associated DNA synthesis upon telomere clustering, for mitotic DNA synthesis (MiDAS) at telomeres, and for functional APB formation (Min et al. 2019; O'Sullivan et al. 2014; Shorrocks et al. 2021; Sobinoff et al. 2017; Stavropoulos et al. 2002; Zhang et al. 2019). The BLM-containing BTR complex processes recombination intermediates formed during strand invasion and initiates POLD3/4-dependent telomere synthesis. Upon completion of the replicative process, the BTR complex dissolves Holliday junctions, hence its alias *dissolvasome*, thereby preventing the exchange of telomeric sequences between sister chromatids (Sobinoff et al. 2017).

RAD51 associated protein 1 (RAD51AP1) was also shown to be important for telomere clustering and break-induced telomere synthesis (BITS). Loss of RAD51AP1 leads to decreased ALT activity, reduction of APBs, defective recruitment of RAD52 and POLD3 to telomeres, and causes telomere shortening (Barroso-González et al. 2019; Kaminski et al. 2022; Yadav et al. 2022).

Negative regulators of ALT include the ATRX/DAXX histone chaperone complex and the histone variant H3.3, which are often mutated in ALT-positive cancers (Heaphy et al. 2011; Kannan et al. 2012; Minasi et al. 2021; Schwartztruber et al. 2012). H3.3 mutations deregulate H3K9 methylation and heterochromatin formation at telomeres (Udugama et al. 2022). The ATRX/DAXX histone chaperone complex is involved in H3.3 deposition and chromatin compaction, thereby regulating expression of TERRA

and the formation of TERRA R-loops (Bhargava, Lynskey, & O'Sullivan 2022; Clynes et al. 2015; Flynn et al. 2015; Law et al. 2010). The annealing helicase SMARCAL1 was also found to harbor inactivating mutations in ALT-positive cancers (Brosnan-Cashman et al. 2021; Diplas et al. 2018). SMARCAL1 counteracts replication stress at telomeres by promoting fork reversal and fork restart, thereby suppressing ALT (Bétous et al. 2012; Cox et al. 2016; Poole et al. 2015).

The endonuclease SLX4, in complex with SLX1 and ERCC4, plays an opposing role to the BTR complex. While the complex around BLM supports non-crossover dissolution of replication intermediates and productive telomere extension, the complex around SLX4 counteracts dissolution by crossover and non-crossover resolution with aborted telomere extension (Sobinoff et al. 2017). Therefore, a tightly regulated balance between BLM and SLX4 seems to determine ALT productivity and telomere maintenance. An important regulator of this balance is the SLX4 interacting protein SLX4IP. SLX4IP favors SLX4-mediated resolution by antagonizing BLM's dissolution activity. Loss of SLX4IP leads to an increase in ALT-related phenotypes, and in conjunction with loss of SLX4 to a synthetic growth defect (Panier et al. 2019). While overexpression of SLX4 reduces APB formation, its depletion leads to an increase in APBs, elevated c-circles and ALT telomere extension, and reduces telomeric MiDAS (Özer et al. 2018; Sobinoff et al. 2017). Combined depletion of SLX4 and RAD52 results in increased telomere loss, unresolved telomere recombination intermediates, and mitotic infidelity, representing a synthetic lethal effect (Panier et al. 2019; Verma et al. 2019).

Two proteins of the Fanconi anemia pathway were also shown to control ALT activity: FANCD2 counteracts BLM-mediated resection and strand exchange, which promotes intramolecular resolution of stalled replication forks during ALT. Loss of FANCD2, similar to the loss of SLX4IP, leads to hyperactivation of ALT with increased extrachromosomal telomeric DNA and recombinational byproducts (Root et al. 2016). The ATPase and DNA translocase FANCM controls ALT at multiple levels: Similar to SMARCAL1, FANCM promotes remodeling of stalled replication forks and fork reversal, thereby counteracting replication stress at telomeres (Gari et al. 2008). FANCM also counteracts replication stress prior to fork stalling by controlling TERRA levels and regulating telomeric R-loop formation (Silva et al. 2019). Additionally, FANCM interacts with the BTR complex and regulates its branch migration activity (Lu et al. 2019; Silva et al. 2019).

Telomeric DNA synthesis during ALT occurs in S/G2 and in mitosis, indicating that telomere elongation and maintenance are not completed during S-phase. Recent findings suggest that telomere replication and recombination intermediates in ALT-positive cancer cells are even transmitted to the next cell cycle, where they are shielded by RPA to

prevent excessive telomere damage and promote a process termed post-mitotic DNA synthesis (post-MiDAS) in G1 cells (Lezaja et al. 2021). Thus, telomere maintenance by ALT seems uncoupled from the general cell cycle-embedded principle of temporally separating genome duplication and maintenance from cell division. Clustering of telomere repeats from multiple chromosomes may allow telomere recombination irrespective of cell cycle phase, and in the next paragraphs we discuss emerging principles of telomere clustering in ALT-positive cancer cells in connection with RPA condensation.

DNA repair condensates and ALT

Biomolecular condensates that support DNA repair reactions, their molecular compositions, mechanisms of assembly, and functions are manifold (Alghoul et al. 2023; Dall'Agnese et al. 2023; Laflamme & Mekhail 2020; Mine-Hattab et al. 2022; Spegg & Altmeyer 2021). Their formation typically follows a multi-step process, in which several types of associative interactions cooperate to build functional compartments (Spegg & Altmeyer 2021). This multi-step process, once initiated through specific interactions at sites of DNA damage, can be seen as a self-perpetuating assembly process, promoted in part by self-association of the recruiting factors. In addition to such feed-forward amplification, negative feedback regulation is typically also involved to avoid excessive (in space and/or time) recruitment (Altmeyer & Lukas 2013). Interestingly, ALT itself is a self-perpetuating process: ALT activity promotes replication stress, which in turn induces a BIR-driven feedforward loop of SUMO-dependent repair protein recruitment and ALT telomere synthesis in APB condensates (Zhang et al. 2021). Disruption of this feedforward loop results in reduced replication stress at telomeres and reduced RPA recruitment. RPA protects telomeric ssDNA in ALT-positive cancer cells not only in S/G2 but also at post-MiDAS sites in G1 (Lezaja et al. 2021). Despite its ultra-high affinity for ssDNA, the RPA complex readily phase separates in solution through associative interactions to form ssDNA-containing liquid droplets (Spegg et al. 2023). An excess of free RPA was previously shown to facilitate rapid exchange of RPA on ssDNA (Gibb et al. 2014; Ma et al. 2016). Consistently, sub-stoichiometric amounts of ssDNA were most effective in triggering dynamic RPA condensates (Spegg et al. 2023). Taken together, these findings suggest that RPA condensation generates a reservoir of highly concentrated RPA around ssDNA to promote rapid exchange between the free and bound state and allow handover to downstream ssDNA-binding proteins such as RAD51/RAD52 (Spegg et al. 2023). This model implies non-stoichiometric assembly of RPA on ssDNA, with the surplus of RPA around ssDNA facilitating continuous RPA exchange. RPA condensation

properties are modulated by phosphorylation-induced negative charges on an intrinsically disordered region (IDR), and phosphomimetic mutants of RPA fail to form liquid droplets *in vitro* and light-induced condensates in cells (Spegg et al. 2023). Charge blockiness, rather than specific target site phosphorylation, was recently shown to regulate cell cycle-specific phase separation (Yamazaki et al. 2022) and, consistently, multisite phosphorylation of the IDR in RPA cooperatively affects RPA clustering (Spegg et al. 2023). Cells expressing phosphomimetic RPA show altered ALT activity, with reduced telomere clustering, elevated ssDNA at telomeres, impaired RAD52 recruitment, and increased telomere loss (Spegg et al. 2023). As telomere clustering is a hallmark of ALT, defective clustering may cause unproductive telomere synthesis and exacerbated telomeric DNA damage. Although the exact mechanism of impaired telomere clustering in RPA phosphomimetic mutant cells remains to be fully elucidated, several observations suggest an emerging connection between RPA condensation at fragile genomic regions, including ALT telomeres, and activation of the nuclear cytoskeleton for enhanced chromatin mobilization.

Emerging links between repair condensates and the nuclear cytoskeleton

Using optogenetic tools for controlled light-inducible Cry2-dependent protein condensation (Kilic et al. 2019; Shin et al. 2017) coupled to sensitive TurboID proximity labeling proteomics (Alghoul et al. 2021; Frattini et al. 2021) revealed that RPA condensation not only results in selective partitioning of RAD52 and the ALT-promoting BTR complex, but also in the selective enrichment of several components of the actin- and myosin network (Spegg et al. 2023). Considering that the light-induced clustering of RPA and the simultaneous TurboID-mediated proximity labeling were performed for a comparatively short duration of only 15 minutes, the identified proteins likely represent the first responders to RPA condensation. Among them were ACTN4, MYO1C, and MYH9, which have known nuclear functions in chromatin organization, transcription, and post-mitotic nuclear expansion (Almuzzaini et al. 2015; Krippner et al. 2020; Kumeta et al. 2010; Sarshad et al. 2013; Ye et al. 2020). As central component of the cytoskeleton, actin plays fundamental roles in cell division, cell movement, cell signaling, and organelle transport across species (Boldogh et al. 2001; Chakrabarti et al. 2018; Grosse et al. 2003). Actin exists in a monomeric globular form (G-actin) or as polymerized multimers forming the filamentous actin (F-actin) network (Dominguez & Holmes 2011; Gunning et al. 2015). Myosins, on the other hand, are ATP-dependent motor proteins that move along

actin filaments and transport cargo (Minozzo & Rassier 2013; Woolner & Bement 2009).

The actinomyosin network is not only involved in cytoplasmic transport processes, but was more recently also shown to participate in nuclear processes such as chromatin decondensation and nuclear volume expansion after cell division, initiation of DNA replication, and in the regulation of transcription by enhancing RNA polymerase II clustering (Baarlink et al. 2017; Krippner et al. 2020; Parisis et al. 2017; Plessner & Grosse 2019; Ulferts et al. 2021; Wei et al. 2020). Intriguingly, nuclear actin filaments were also shown to form upon treatment with different DNA damaging agents including UV-radiation, methylmethanesulfonate (MMS), and neocarzinostatin (NCS), and are increasingly recognized to play a role in DNA repair (Andrin et al. 2012; Belin et al. 2015; Hurst et al. 2019). The actin regulating ARP2/3 protein complex and its associated factor WASP were found to localize to sites of DNA damage in mammalian cells where they nucleate actin filament formation. This promotes DSB mobility and clustering for repair by HR (Schrack et al. 2018; Schrack & Gautier 2019). Consistently, nuclear actin polymerization and myosin are required for the directed movement of DSBs within heterochromatin towards the nuclear periphery for error-free HR repair in *Drosophila* and mammalian cells (Caridi et al. 2018; Caridi et al. 2019; Merigliano & Chiolo 2021; Rawal et al. 2019), and damaged rDNA relocalizes to the nucleolar periphery in an ATM-, ARP2/3-, and myosin-dependent manner (Harding et al. 2015; Marnef et al. 2019). Similarly, rDNA breaks in yeast transiently move to extranucleolar regions for recombinational repair (Torres-Rosell et al. 2007), and breaks in pericentric heterochromatin of mouse cells relocate to the periphery of heterochromatin domains after resection (Tsouroula et al. 2016). More recently, nuclear F-actin was also found to play a role in response to replication stress in mammalian cells, where it counteracts nuclear deformation and promotes myosin-dependent re-localization of stressed replication forks to the nuclear periphery in an ATR- and WASP-ARP2/3-dependent manner (Lamm et al. 2020; Lamm et al. 2021). Interestingly, WASP associates with RPA at stressed replication forks and promotes RPA binding to ssDNA (Han et al. 2022), and actin nucleators regulate RPA availability under conditions of replication stress (Nieminuszczy 2023).

Reciprocally, RPA condensates may concentrate monomeric G-actin and thereby trigger nucleation and growth of actin filaments at sites of DNA damage (Figure 5a). Previous work has shown that self-assembled polypeptide condensates serve as hub for actin enrichment and polymerization *in vitro* (Graham et al. 2023; McCall et al. 2018). Furthermore, condensation of actin by intrinsically disordered regions of actin-associated proteins was proposed as general mechanism for actin network organization (Billault-Chaumartin et al. 2022).

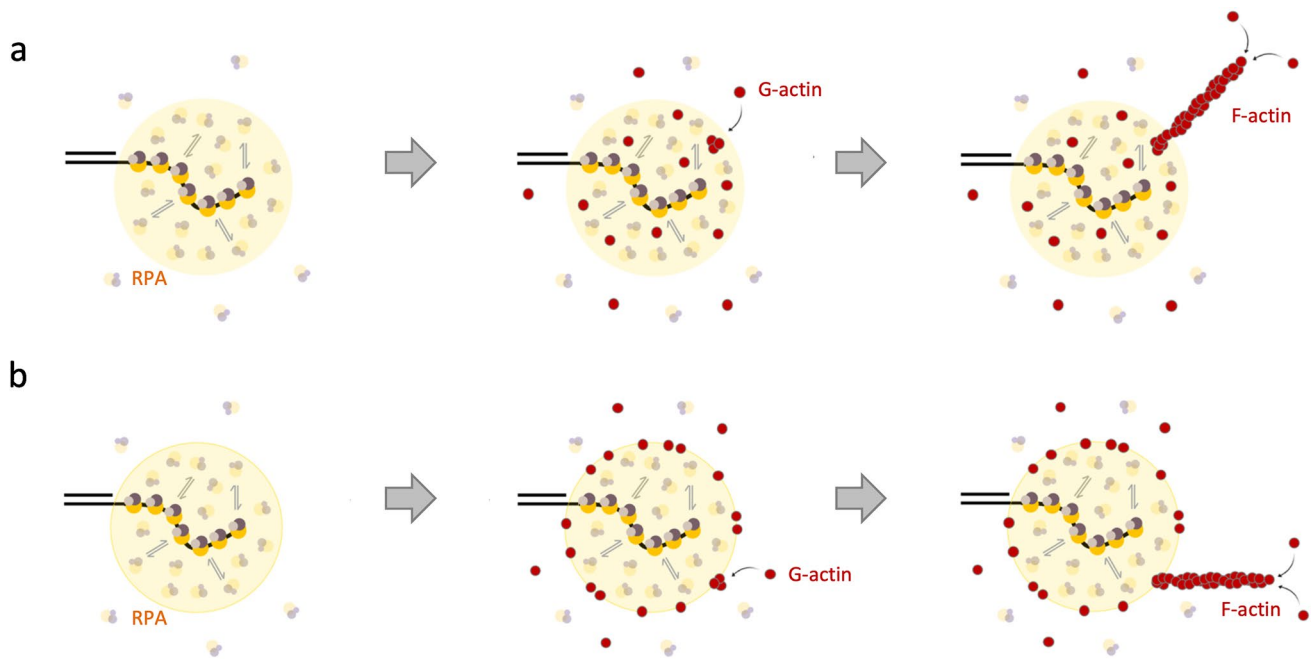


Fig. 5 Emerging links between biomolecular condensates and activation of the nucleoskeleton. (a) Model of actin filament formation through G-actin concentration in DNA repair condensates formed by

RPA and associated proteins. (b) Model of actin filament formation through G-actin concentration on the surface of DNA repair condensates formed by RPA and associated proteins

Consistently, during oocyte development, actomyosin cortex activation is promoted by the emergence of thousands of short-lived protein condensates enriched in actin, WASP, and ARP2/3 forming an active micro-emulsion (Yan et al. 2022). Phase separation of actin regulatory factors was shown to increase the dwell time of nucleators to initiate F-actin formation, as demonstrated for WASP and ARP2/3 at the cell membrane (Case et al. 2019). Reducing dynamic interaction landscapes from a 3D environment to a 2D interface may generally help to concentrate molecules and promote their activation. Along these lines, actin and actin nucleation factors might get selectively enriched on the surface of RPA condensates through interfacial affinity, rather than in the interior (Figure 5b). Similar interactions have been observed between microtubule subunits and stress granules (Böddeker et al. 2022).

In yeast, Rad52-dependent DNA repair condensates were previously shown to induce nuclear microtubule filaments, which is required for moving the damaged DNA compartment to the nuclear periphery for repair (Oshidari et al. 2020). Microtubule-dependent DNA damage mobility was also observed in mouse cells with unprotected, dysfunctional telomeres (Lottersberger et al. 2015). In human cancer cells that use ALT-dependent recombination at fragile telomeres, nuclear actin filaments might serve as molecular highways for the directed movement of RPA-enriched repair condensates, with the condensate surface or co-condensing adaptor molecules serving as anchoring points for myosin

(Figure 6a). A conceptually related mechanism seems to be at work during neuronal long-distance transport of RNAs, where phase-separated RNA granules hitchhike on lysosomes through a low complexity domain-containing tether protein (Liao et al. 2019).

Both in yeast and in human cells, stressed telomeres relocate to the nuclear periphery and this process is driven by nuclear F-actin polymerization and involves RPA and SUMOylation (Churikov et al. 2016; Pinzaru et al. 2020; Spichal et al. 2016). Interestingly, RPA contains a SIM motif, through which it can interact with SUMOylated proteins (Zhu et al. 2023), and RPA itself is SUMOylated when collapsed replication forks are relocated to the nuclear periphery in yeast (Whalen et al. 2020). Whether RPA SUMOylation and RPA-SUMO interactions are involved in RPA condensation and actin polymerization remains to be addressed.

Similar to actin, also myosin is increasingly recognized to play important roles in the nucleus for transcription and in the DNA damage response, and was recently shown to regulate phase separation by promoting condensate coalescence (Cook & Toseland 2021; Feng et al. 2022; Hari-Gupta et al. 2022). The actomyosin network appears to be more important for homology-directed repair and RAD52-mediated single-strand annealing (SSA), compared to DNA repair by non-homologous end-joining (NHEJ) or alternative end-joining (alt-EJ) (Pfitzer et al. 2019), consistent with a predominant role during homology-directed repair of fragile repetitive

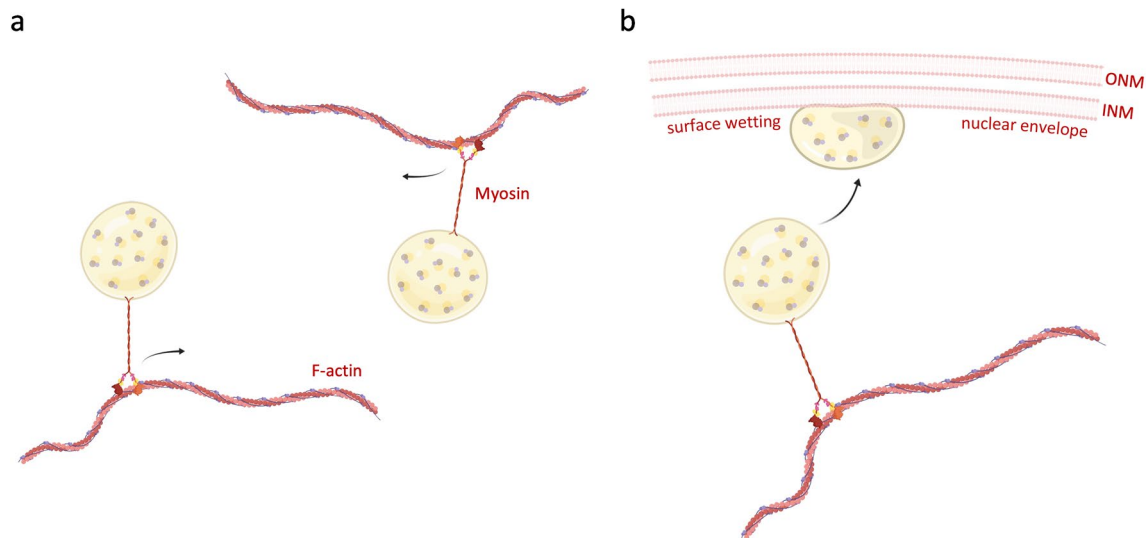


Fig. 6 DNA repair condensate mobilization and surface wetting at the nuclear envelope. (a) Model of actinomyosin-mediated movement of DNA repair condensates through the nuclear space. (b) Model of

surface wetting-assisted anchoring of DNA repair condensates at the nuclear periphery. INM, inner nuclear membrane; ONM, outer nuclear membrane

sequences such as telomeres. Moreover, formation of nuclear F-actin in response to genotoxic stress was recently shown to serve as scaffold for PML nuclear bodies (Cobb et al. 2022). Whether the same holds true for APBs at telomeres remains to be shown. Nevertheless, several connections between self-assembly features of repair condensates and the nuclear cytoskeleton have started to emerge, spurring considerations about the mechanobiology of genome maintenance.

Connecting DNA repair condensates to mechanobiology

Cells are exposed to dynamically changing extrinsic mechanical forces, including shear stress, stretching, stiffening, and compression, and these forces are counteracted by cell-intrinsic force generation through the cytoskeleton and through modulation of the viscoelastic properties of the cellular interior (Bertillot et al. 2022; Dupont & Wickström 2022). With the intensified research on material properties of biomolecular condensates, new connections between mechanobiology and viscoelastic polymer networks in membraneless organelles are being revealed (Lee et al. 2022; Wiegand & Hyman 2020). For instance, recent work demonstrated that cytoplasmic forces tune nuclear condensate coalescence and molecular kinetics within condensates (Al Jord et al. 2022). Mechanical force experienced by the nucleus modulates permeability across nuclear pores, indicating that transport of proteins through the hydrogel-like meshwork of FG-rich repeats inside nuclear pore complexes is mechanosensitive (Andreu

et al. 2022). Moreover, mechanosensing of cell density by adhesion forces was shown to control cell cycle progression at the G2/M transition through activation of the WEE1 checkpoint kinase (Donker et al. 2022). Alterations in cell tension also affects cell cycle progression from G1 to S-phase (Perez-Gonzalez et al. 2019), and the mechanosensing Hippo pathway with its mechanotransducers YAP/TAZ and LATS1/2 has also been implicated in DNA damage signaling and repair (Pefani et al. 2014; Pefani & O'Neill 2016).

Nuclear condensates are not only scaffolds for biochemical reactions, but also mechanic entities with viscoelastic properties that generate and respond to mechanical force (Spagnol et al. 2016). Similarly, the chromatin polymer itself is a main factor in defining nuclear mechanical properties (Barbieri et al. 2013; Nicodemi & Pombo 2014; Stephens et al. 2019). Depending on the time and length scale, chromatin behaves both as an elastic solid and as a viscous liquid (Zidovska 2020), a rheological behavior that is also observed for the cytoskeleton (Wiegand & Hyman 2020). The viscoelastic properties of the nuclear environment is regulated by several factors, including external cytoskeletal forces that change nuclear morphology, the nuclear lamina, chromatin compaction and structural organization, and the formation and regulation of biomolecular condensates (dos Santos & Toseland 2021). As physical properties and dynamic forces are often altered in human disease, which can deregulate gene expression programs and challenge genome stability, understanding the interplay between genome organization and mechanobiology may have clinical implications.

Nuclear deformation in migrating immune and metastatic cancer cells causes nuclear blebbing and rupture and

is associated with increased DNA damage, depletion of DNA repair factors, and cell cycle checkpoint activation (Denais et al. 2016; Irianto et al. 2016; Irianto et al. 2017; Isermann & Lammerding 2017; Pfeifer et al. 2018; Raab et al. 2016; Xia et al. 2018; Xia et al. 2019; Xie et al. 2020). Moreover, external mechanical stimuli from the cytoplasm can modulate processes inside the nucleus by transmitting force through connections between the cytoskeleton and the nucleoskeleton (Dupont & Wickström 2022; Goelzer et al. 2021). Such connections are provided by linker of nucleoskeleton and cytoskeleton (LINC) complexes (Alam et al. 2016; Crisp et al. 2005; Leno 1992; Mammoto et al. 2012; Wang et al. 2018). LINC complexes, embedded in the nuclear envelope, connect cytoplasmic intermediary filaments, microtubules, and actin filaments with the nuclear lamina and with silenced heterochromatin regions in lamina-associated domains (LADs) (dos Santos & Toseland 2021; Spichal & Fabre 2017). Defects in the nuclear lamina are associated with diseases like Hutchinson-Gilford progeria, muscular dystrophy, and cardiomyopathies, and can lead to changes in chromatin structure as well as deregulated DNA replication, repair, and gene expression (Cho et al. 2019; dos Santos & Toseland 2021; Schreiber & Kennedy 2013). Interestingly, LINC complex components are involved in DNA damage relocation and clustering and promote homologous recombination repair (Aymard et al. 2017; Bozec et al. 2023; Lawrence et al. 2016; Lottersberger et al. 2015; Marnef et al. 2019; Swartz et al. 2014). Moreover, the LINC complex proteins SUN1 and SUN2, together with dynamic microtubules and nuclear pore proteins, drive the formation of DSB-capturing nuclear envelope tubules (dsbNETs) to support repair in the interior of the nucleus (Shokrollahi et al. 2023).

Condensed chromatin is a barrier for the DNA repair machinery (Mitrentsi et al. 2022), and the local viscoelasticity of the nucleus varies by compartment and degree of chromatin condensation (Lee et al. 2022). While nucleosomes and nucleosome clusters on the nanoscale are mobile and have liquid-/gel-like properties, condensed chromatin polymers on the mesoscale seem physically constrained and more immobile, with soluble chromatin-binding proteins coalescing on the solid chromatin scaffold (Hansen et al. 2021; Strickfaden et al. 2020; Tortora et al. 2022). Chromatin decompaction is needed to improve the efficiency of DNA repair upon damage (Polo & Almouzni 2015), and induced chromatin decompaction reduces nuclear stiffness by ~35–50% (Hobson et al. 2020; Krause et al. 2013; Shimamoto et al. 2017; Stephens et al. 2017). The change from a locally stiffer to a softer chromatin environment may energetically favor nucleation and growth of DNA repair condensates. Nuclear stiffness is indeed reduced upon DNA damage and the reduction in nuclear tension promotes repair (dos Santos et al. 2021). Moreover, nuclear softening upon severe nuclear

deformation, driven by Piezo1-triggered reduction of lamina-associated H3K9me3-marked heterochromatin to insulate the genetic material from mechanical force, promotes genome stability (Nava et al. 2020).

The size to which biomolecular condensates can grow in a viscoelastic environment is limited, because with increasing growth more energy is required to deform the surrounding stiffer matrix (Lee et al. 2022; Wiegand & Hyman 2020). Indeed, the chromatin polymer may mechanically suppress droplet coalescence and ripening and control condensate number, size, and positioning (Zhang et al. 2021). Such considerations could also apply to DNA repair condensates and might affect their mobility and growth. Possibly, repair condensates redirected to the nuclear periphery for error-free repair could wetten the inner nuclear membrane (Mangiarotti et al. 2022; Oshidari et al. 2020; Strom et al. 2023), which might be a mechanism to anchor repair compartments to the nuclear envelope by means of adsorption (Figure 6b). Upon membrane wetting, biochemical reactions at the 2D membrane-condensate interface may be accelerated compared to the 3D volume of a non-membrane-tethered condensate, potentially providing an additional advantage for genome repair.

Conclusions and perspectives

Recent work has started to unveil intriguing connections between nuclear condensates involved in the cellular response to replication stress and DNA damage and the nuclear cytoskeleton. Moreover, material properties and the mechanobiology of chromatin, nuclear condensates, and the nuclear cytoskeleton are receiving increasing attention. While it is becoming clear that genome functions and cellular responses to stress, including genotoxic stress, are tightly linked to nuclear architecture and to the dynamically changing material properties of the nuclear interior, the varying length- and timescales at which different nuclear processes and macromolecular assemblies occur complicate their analysis and interpretation. Many open questions remain about whether and how nuclear condensates sense mechanical stimuli and if they regulate genome functions (e.g. chromatin organization, epigenetic states, replication timing, DNA repair) in response to external forces. Conversely, how changes in chromatin structure and genome stability affect nuclear mechanobiology is currently not well understood.

Fragile telomeres, which per se exhibit strong subdiffusive motion (Lee et al. 2022) yet become mobilized upon telomeric replication stress and DNA damage (Lamm et al. 2021), may represent a paradigm for emerging connections between viscoelastic repair condensates formed around damaged genomic regions and nuclear mechanobiology. Further

insights into the material properties of chromatin domains and nuclear compartments and how they are linked to material properties of the cytoskeleton and of the surrounding membranes are going to benefit a mechanistic understanding of genome functioning and its deregulation in disease. Elucidating the interplay between material properties and biochemical reactions in cells may also enable their targeted modulation, e.g., by shifting material properties from liquid/gel-like to stiffening, and vice versa, or by inducing local stirring of molecules. Considering that material properties of cellular components, including chromatin compartments, biomolecular condensates in- and outside the nucleus, membranes, and the cytoskeleton, may age and experience fatigue in diseases such as cancer and neurodegeneration, integrating concepts from soft matter physics and polymer mechanics, from material science and engineering, and from theoretical modelling and computer simulations, may reveal new biology and open new avenues for biomedical research.

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Declarations

Competing interests The authors declare no competing interests.

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