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A matter of wrapper: Defects in the nuclear envelope of lagging and bridging chromatin threatens genome integrity

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ABSTRACT

The nuclear envelope surrounds the eukaryotic genome and, through the nuclear pore complexes, regulates transport in and out of the nucleus. Correct nucleo-cytoplasm compartmentations are essential for nuclear functions such as DNA replication or repair. During metazoan mitosis, the nuclear envelope disintegrates to allow the segregation of the two copies of DNA between daughter cells. At the end of mitosis, it reforms on each group of chromosomes in the daughter cells. However, nuclear envelope reformation is delayed on lagging chromosomes and DNA bridges. Defects in the coordination between nuclear envelope reformation and chromosome segregation impair the nuclear functions. Mechanical stress to which micronuclei and DNA bridges are subjected to combined with their particular architecture and the altered nuclear functions result in DNA damage. While micronuclei and DNA bridges were considered for more than 100 years as mere indicators of chromosomal instability, rapid technological advances are helping to better understand the biological consequences of these aberrant nuclear morphologies. Recent studies provide interesting evidence that micronuclei and chromatin bridges act as a key platforms for a catastrophic mutational process observed in cancers called chromothripsis and a trigger for the innate immune response. Therefore, they could affect cellular functions by both genetic and non-genetic means.

1. Introduction

Nuclear morphology is not a trivial matter. The nucleus in most normal (non-transformed) cells has a regular and ellipsoid shape, but it is often irregular in cancer cells. Alterations of nuclear structure in tumor cells include changes in nuclear size and shape as well as in the organization of heterochromatin. All these morphological changes are characteristic of certain types of tumors and stages and they have been used in the diagnosis of cancer for many years. For example, the Pap smear developed in the early 20th century by George Papanicolau to simultaneously detect specific nuclear and cytoplasmic features of cancer cells in cervical samples. Changes in the nuclear architecture reveal a characteristic that is transversal to cancer cells: their inability to maintain the integrity of their genome.

Among abnormal nuclear morphologies, micronuclei (MN) have been used for many decades as biomarkers of chromosomal damage, genomic instability and exposure to mutagenic agents [11,21]. Their scoring was considered as the gold standard for detecting exposure to mutagenic agents. However, our perspective on MN has changed dramatically in the last 10 years with recent technological advances for visualizing and recording living cells and massive DNA sequencing. These new approaches have led to relevant progress in our understanding of the biological consequences of MN and have created widespread interest in these nuclear structures. We now know that micronuclei lack structural integrity and they are not fully functional as they cannot properly perform basic nuclear functions such as DNA replication, transcription or DNA damage repair. These new investigations provide evidence that micronucleation can set in motion a deep genome remodelling of cells, thus taking MN to a new dimension: from passive indicators of chromosome instability to sources of DNA damage and karyotypic diversity in cell populations.

Unlike micronuclei, DNA bridges between daughter cell nuclei have long been recognized as a source of DNA damage and intratumoral genetic heterogeneity [14,32]. According to the Breakage-Fusion-Bridge (BFB) model [32], DNA bridges can break and initiate a cycle capable of self-feeding when the broken ends fuse with another broken end. The variety of genome changes caused by the presence of bridges in cells has suddenly spread to unforeseen limits. Classical studies showed that,

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through BFB cycles, chromosomal bridges could generate mutations, chromosome rupture, amplifications and highly rearranged chromosomes. But it was not until recently that we learned that the repertoire of genomic alterations that can be attributed to DNA bridges is much broader, including phenomena capable of dramatically changing the genome of cells such as duplication of the entire genome and chromothripsis.

The evolution in our knowledge of the consequences of micronuclei and DNA bridges derive, to a great extent, from our understanding that these structures are surrounded by a defective nuclear envelope. Defects in the wrapping of micronuclei and chromatin bridges make not only their nuclear functions to be altered, but also their DNA to be inappropriately exposed to the cytoplasmic environment during the interphase, triggering the innate immune response. Taken together, it suggests that MN and DNA bridges could affect cellular functions by both genetic and non-genetic means.

2. Molecular origin of DNA bridges and micronuclei

There are different molecular mechanisms by which DNA bridges and micronuclei can be formed, but for most of them, mitosis is a common key process. This stage of the cell cycle is inherently stressful for cells. During mitosis, actin and tubulin cytoskeleton suffer dramatic changes to reshape the cell. The nuclear envelope is torn apart and the replicated chromosomes condense and move inside the cell; first in a congressional movement towards the equatorial cell plane and then, towards the cell poles for segregating the two copies of the replicated DNA between daughter cells. In this stage of the cell cycle, in which changes in the cellular architecture are of such magnitude, the malfunction of the molecular machinery involved has serious consequences for the cell.

Lagging chromosomes during anaphase are prone to be subsequently incorporated into MN. Laggards can originate from different mechanisms. They frequently arise because of erroneous attachments to microtubules, such as those of merotelic chromosomes in which single kinetochores attach to microtubules emanating from different poles [6]. Laggards also result from unattached acentric fragments, which can be derived from unrepaired DNA double-strand breaks caused by DNA replication stress or as a result of misrepair of DNA breaks [4,11]. Lagging chromosomes can also appear due to the untimely loss of sister chromatid cohesion because of the defective removal of cohesins that tether sister chromatids [39] or from abnormal congress of chromosomes before their segregation due to malfunction of microtubule plus-end directed kinetochore motor proteins [12]. Whatever their origin, lagging chromosomes become MN after their nuclear envelope reassemble independently from the primary nucleus, being spatially separated.

As opposed to laggards, bridging chromatin completely spans the segregating masses of chromosomes during anaphase. Bridges are most often the manifestation of dicentric chromatids in which each centromere of a continuous DNA fibre is pulled towards opposite spindle poles. These rearranged chromosomes with two centromeres in a single DNA molecule are most frequently formed because of misjoined DNA breaks or after fusion of chromosomes with dysfunctional telomeres [13]. Chromatin bridges are also formed during mitosis when the success of complete replication is compromised and it generates chromosomes with non-replicated segments interspersed with other replicated segments [23]. Although to a lesser extent, DNA bridges are also caused by defects in chromatin condensation and sister chromatid cohesion. Chromatin bridges can persist beyond anaphase and form stabilized nucleoplasmic connections between the two daughter cells. At the end of mitosis, the nuclear envelope must reassemble around each group of chromosomes. However, the formation of this envelope on missegregated bridging chromatin, as well as on lagging chromosomes, is usually defective due to the spatial location of these structures in the dividing cell.

3. Defective nuclear envelope on bridging and lagging chromosomes

The nuclear envelope (NE) consists of two lipid bilayer membranes supported by the nuclear lamina and perforated by the nuclear pores. On the nuclear side, the nuclear lamina –a network of intermediate filaments composed of two types of lamin proteins, the B type lamin and the A type (Lmn A/C), and integral membrane proteins– provides mechanical support to the NE and contribute to chromatin organization [16]. Scattered in the envelope, nuclear pores constitute large proteinaceous channels for the selective transport of macromolecules and diffusion of ions and metabolites between the nucleus and the cytoplasm. In eukaryotic cells, the NE has evolved to form a stable physical barrier between the nucleus and the cytoplasm, normally breaking down only during mitosis. NE reassembly is facilitated by the microtubule-mediated accumulation of the DNA cross-bridging barrier-to-autointegration factor (BAF) at the surface of anaphase chromosomes. Relying on its ability to bridge distant DNA sites, BAF shapes a single nucleus from a set of individualized chromosomes at the end of mitosis [38]. Following an initial phase during which BAF localizes on the entire surface of the compact mass of late anaphase chromatin, BAF continues to enrich in central regions of the assembling nuclear rim close to the spindle microtubules. This region of the nuclear envelope near to the pole-to-pole axis of the mitotic spindle is named the “core” domain [17].

To form a functional interphase nucleus, segregating chromosomes have to recruit not only membranes and lamins but also nucleoporins to constitute nuclear pore complexes (NPCs). Therefore nuclear membrane recruitment and NPC assembly must be coordinated to avoid the formation of a closed NE that lacks NPCs [3]. However, the presence of lagging and bridging chromatin in dividing cells compromises the architecture and the function of the NE on missegregated chromatin. The NE of micronuclei typically possess a lower density of nuclear pores relative to primary nuclei and exhibit inefficient transport of nuclear proteins [19,42,7]. Similar to MN, the NE on chromatin bridges is frequently altered. Although positive for the chromatin binding BAF, it is typically depleted for many important proteins, including A/C- and B-type Lamins and NPCs [29]. Alterations in NE composition affect the transport of proteins that need to be shuttled through and as a consequence of it, the nuclear functions become defective. For instance, the DNA replication and DNA repair functions in micronuclei are strikingly impaired [7,42]. Therefore, the nucleo-cytoplasmic compartmentation and transport defects of the NE on missegregated bridging and lagging chromosomes are due to an abnormal assembly of NPCs and other NE components.

What causes the NE on lagging and bridging chromosomes to be defective? Why are MN and DNA bridges depleted of NPCs and other NE components? Studies published in the last decade in the fruit fly *Drosophila melanogaster* pointed to a biochemical model to explain a spatial regulation of NE postmitotic reassembly. According to these studies, NE reassembles around segregating chromosomes only once they have left the mitotic midzone where the concentration of active Aurora B enzyme is high [1,2] (Fig. 1). These observations suggested that a surveillance mechanism would be mediated by localized concentrations of active Aurora B kinase: by keeping key substrates phosphorylated, chromosome decondensation and NE reassembly on late-segregating chromosomes that occupy the spindle midzone would be delayed to facilitate their inclusion in the primary nucleus [46]. However, if the missegregated chromosome fails to incorporate in the primary nucleus, delayed NE reformation will finally have impact on the composition and function of the NE that wraps the missegregated chromatin.

Other recent studies point to mechanical causes for the deficient NE assembly on late segregating chromosomes in human cells (Fig. 1). According to these studies, altered NE assembly on lagging chromosomes is not the consequence of a beneficial checkpoint delay, but rather a

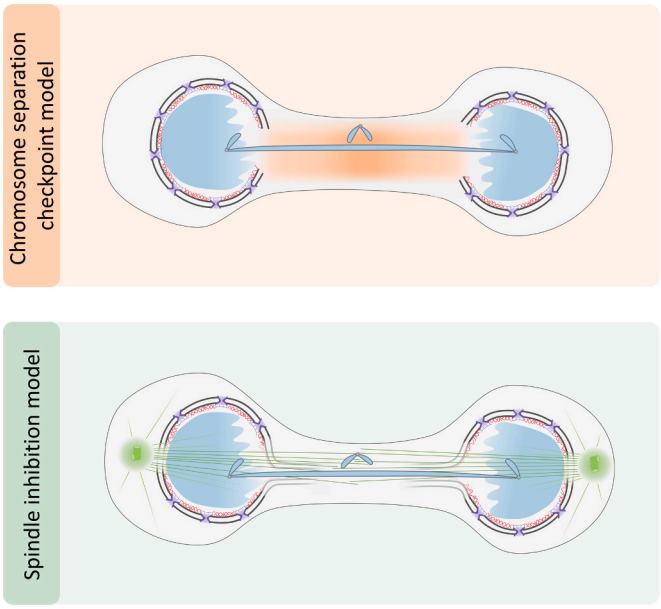


Fig. 1. Two models for nuclear envelope reassembly in telophase. Upper panel: According to the chromosome separation checkpoint model, segregated chromosome masses (light blue) recruit membranes (dark gray) and lamin filaments (red), but NE and NPCs (purple) assembly is locally delayed on chromosome regions close to Aurora B gradient (orange). Under this model, Aurora B activity mediates a surveillance mechanism that prevents chromosome decondensation and NE reassembly until the effective separation of sister chromatids is achieved. Localized delays in nuclear envelope formation have been proposed to facilitate inclusion of late-segregating chromosome fragments. Based on this model, the reassembly of the NE is delayed on lagging chromosomes and DNA bridges. Lower panel: According to the spindle inhibition model, the non-core NE (including NPCs) assembly is inhibited by the mitotic spindle (green) in telophase cells. It is proposed that the chromosome regions in contact with the spindle assemble the core NE (pale gray), whereas the chromosome peripheral regions assemble the non-core NE (dark gray) including NPCs (purple). On the basis of this model, the NE around lagging chromosomes and DNA bridges resembles the core domain of the NE and fails to recruit NPCs and nuclear lamina (red) despite assembling the core membrane. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pathological outcome. NPCs are assembled in two stages, during telophase concomitantly with membrane recruitment on chromatin (hereon referred to as postmitotic assembly) and, later on, during interphase when the nuclear envelope is already sealed [45]. The mechanisms for postmitotic and interphase NPC assembly are fundamentally different and have different kinetics, with the first one being much faster than the second one [10,35]. By using a combination of live-cell imaging and high-resolution 3D electron microscopy, Otsuka and colleagues [36] reported that postmitotic NPC reassembly proceeds by a radial dilation of small membrane openings pre-existing in the endoplasmic reticulum. In contrast to the postmitotic process, the interphase NPC assembly relies on *de novo* membrane fusion as it proceeds by an inside-out evagination of the inner nuclear membrane that grows depth until it fuses with the outer nuclear membrane [35]. Similar to the assembly of other NE components, NPC formation in telophase cells is locally delayed in the so-called "core" NE domains located in the pole-to-pole axis of the mitotic spindle due to dense spindle microtubules on the DNA surface [5] (Fig. 1). In the subsequent interphase, the core domains of the fully sealed interphasic NE progressively assemble new NPCs, but this new wave of NPC assembly during interphase relies on pre-existing NPCs in adjacent regions of the nucleus. An important consequence inferred from this model is that reduced numbers of functional NPC on the envelope of abnormal nuclear structures that emerge at the end of mitosis, such as MN and chromatin bridges, hinder NPC formation throughout interphase. In agreement with this, Liu and colleagues [25] showed that loosening of spindle microtubule bundling by siRNA-mediated depletion of the kinesin KIF4A partially reversed the defect in NPC and other non-core NE proteins on lagging chromosomes.

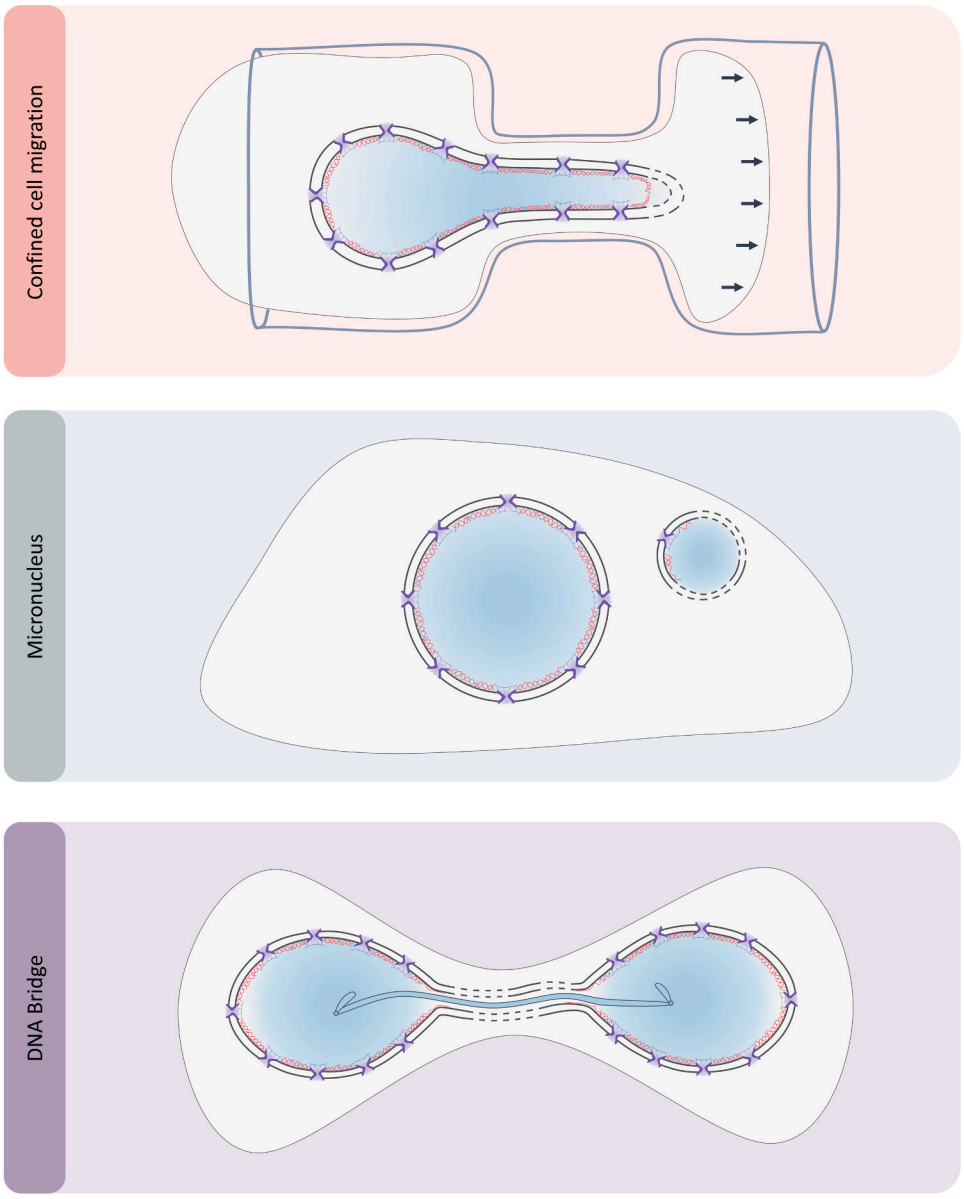
Whatever the causes of poor NE assembly on missegregated chromatin are, whether it is due to mechanical or biochemical causes, NE defects in micronuclei and DNA bridges seem to be linked to the position that the chromatin occupies within the dividing cell. Alteration of the NE composition affects not only basic biological processes, such as DNA replication or repair that requires timely transport of proteins but also affects proper nucleo-cytoplasmic compartmentalization.

4. Nuclear envelope rupture of micronuclei and chromatin bridges

An intriguing phenomenon was reported in 2012 in human cancer cell lines. Using live-cell imaging, Hetzer's laboratory characterized singular episodes wherein the primary nuclei of proliferating cells from different cancer cell lines become temporarily ruptured during interphase [44]. The interphase loss of the nuclear permeability barrier allowed nucleoplasmic proteins to leak out and cytoplasmic proteins to leak in. Still images in U2OS cells revealed that nuclear ruptures initiated from localized deformations of the NE that expanded forming NE herniations, which are eventually ruptured.

Analysis of NE rupture has identified two significant contributors to NE instability: nuclear lamina organization and mechanical stress (Fig. 2). Experiments in cancer cell lines showing that lamin B1 overexpression is sufficient to prevent NE rupture and that lamin depletion promotes envelope rupture revealed the important role of the nuclear lamina in NE integrity [44]. However, external physical forces can also induce NE rupture. Transient rupture at the leading end of the nucleus was visualized in dendritic cells, fibroblasts, and cancer cells forced to migrate through small pores [8,37]. Significantly, NE rupture in cells subjected to mechanical stress relies on the assembly of contractile actin bundles in the cytoplasm that interact with the nucleus via the linker of nucleoskeleton and cytoskeleton (LINC) complex. Experiments showing that inhibition of cytosolic myosin II activity or loss of the LINC complex reduced the occurrence of NE rupture sustained the notion that NE rupture depends on an increase in intranuclear pressure from actin-based nucleus confinement [20]. According to the emerging model, weak membrane areas caused by defects in lamina organization, rupture when mechanical forces on the nucleus, such as actin-based nucleus compression and stretching, increases the internal pressure on the nuclear membrane.

Recent studies have anticipated that nuclear lamina organization defects and mechanical stress may converge in generating NE rupture in relevant pathophysiological processes such as the migration of tumor cells during the process of invasion. Consistent with this concept, a localized detachment of the nuclear membranes from the nuclear lamina in the expanding nuclear blebs of cancer cells migrating through small pores was reported by Denais et al. [8]. Moreover, staining of NE components showed that nuclear pores and lamin B1 were excluded from the ruptured region at the tip of the nucleus [37]. Yet another example of the convergent contribution of mechanical stress and nuclear envelope defects on NE rupture is found in cells born with chromatin bridges. By physically connecting the two nuclei, chromatin bridges exert pulling forces on the nucleus and induce abnormal teardrop morphologies in both daughter nuclei. As a consequence of this, nuclear lamins are lost around the chromatin bridges, which are then prone to rupture [29]. Micronuclei also have a high probability of NE rupture. However, in contrast to chromatin bridges, MN are not subjected to heavy mechanical stress. Research suggests strongly that compositional defects, including nuclear lamina defects that manifest with the appearance of gaps in the lamina, are responsible for MN disruption [19,48]. As discussed in the previous section, the defects in the NE composition of MN are probably linked to the position that the missegregated chromatin



	NE composition defects	Mechanical forces (tension/pressure)	DNA Damage	Reversibility of NE rupture
Confined cell migration	(X)	✓	✓	✓
Micronucleus	✓	(X)	✓	X
DNA Bridge	✓	✓	✓	X

Fig. 2. Mechanisms of nuclear envelope rupture. NE rupture occurs frequently as a result of defects in the organization of the nuclear lamina (red) that give rise to gaps in the lamina meshwork. NE rupture can also occur as a result of increased membrane stress associated with mechanical forces or architectural restrictions. Upper panel: Confined migration of cells leads to the formation of nuclear membrane protrusions at the leading edge of the nucleus as the cell moves through space constrictions. Nuclear blebs form when nuclear membranes (dark gray) detach from the nuclear lamina (red), bulge into the cytoplasm and eventually rupture. DNA double-strand breaks might result from NE openings. Rupture of the primary nucleus during confined cell migration is transient as cells can restore the nuclear membrane integrity during interphase. Middle panel: Micronuclear envelope rupture requires nuclear lamina defects, but the role of mechanical stress is unknown. Disruption of the micronucleus results in DNA damage as a consequence of defects in nuclear functions (deficient DNA replication and DNA repair). Unlike the primary nucleus, micronuclear envelope rupture is irreversible. This is probably a consequence of the high curvature of the micronuclear envelope. Lower panel: nuclear lamina defects and increased mechanical stress is associated with NE rupture of DNA bridges. DNA damage can result from NE rupture of DNA bridges, although mechanical stress on the DNA bridge due to migration of the connected daughter cells also play an important role. Like micronuclei, NE rupture on bridging DNA is also irreversible. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

occupies within the dividing cell.

Transient rupture of the primary nucleus envelope is a source of DNA damage. Intense γH2AX staining, a marker of DNA double-strand breaks (DSBs), was observed at chromatin protrusions of cells that were forced to pass through small pores [8]. The confirmation that DNA damage was caused by migration-induced nuclear deformation and subsequent NE rupture was obtained by live-cell imaging on cells expressing fluorescently labeled 53BP1, another marker of DNA damage [8,22]. Altogether, this suggested that although a transient loss of NE integrity is not fatal for cells, DNA DSBs might arise from it.

Cells are protected against the deleterious consequences of NE rupture by a rapid reestablishment of NE integrity. The envelope of the primary nucleus reseals rapidly during interphase assisted by components of the ESCRT III (endosomal sorting complexes required for transport III) membrane-remodelling machinery [8,37]. However, NE rupture in MN is irreparable as demonstrated with nuclear and cytoplasmic fluorescent reporters [19]. The irreversibility in the loss of compartmentalization of MN potentially arise from its extreme

membrane curvature [28]. Recent evidence revealed that high nuclear curvature imposed by a physical external probe on the primary nucleus promotes NE rupture and favors sustained DNA damage [47]. Once launched, loss of NE integrity is probably also permanent in chromatin bridges. In this case, it is not as a result of the curvature of its NE but of the distance between the nuclei to which the bridge is anchored. In a scenario such as that posed in MNs and DNA bridges, in which the cell is unable to repair collapsed NE, damage to the DNA of these structures is exacerbated.

5. DNA damage associated with micronuclei

Micronuclei have been associated with DNA damage from the late of 1960s when a pioneering study using cytogenetic approaches reported in human cells the presence of pulverized chromosomes presumably derived from MN [33]. Direct evidence for the presence of massive DNA damage in MN was obtained using γH2AX immunolabeling in irradiated human fibroblasts. Uniform intense γH2AX labeling of MN revealed massive fragmentation of the micronuclear chromatin [41]. Although processes involving a selective degradation of the micronuclear DNA could not be excluded, chromothriptic shattering of micronuclear chromatin would also lead to the reported uniform γH2AX labeling pattern.

Chromothripsis, a mutagenic phenomenon relevant in cancer development, consists of a catastrophic shattering of one or more chromosome regions in a single event, followed by a seemingly random repair of the DNA fragments that lead to the generation of highly rearranged chromosomes [40]. Considering that chromothripsis is most frequently restricted to a single chromosome, the physical isolation of missegregated chromosomes within micronuclei offers a mechanistic explanation for this phenomenon. Indeed, Crasta et al. [7] found that acquisition of DNA damage in MN was often associated with DNA replication. MN undergo defective and asynchronous DNA replication — a defect that was associated with “pulverized” appearance of single chromosomes in micronucleated mitotic cells. By using live-cell imaging, the authors tracked the fate of newly generated MN and reported that chromosome aberrations acquired in MN could be incorporated into the genome as the micronuclear chromatin either reincorporated with the primary nucleus after anaphase or persisted as a micronucleus. Confirmation that genomic rearrangements specifically involved the missegregated chromosomes and occurred within one cell cycle came from the elegant combination of live-cell imaging with single-cell DNA sequencing. DNA sequencing of pairs of daughter cells derived from a micronucleated cell progenitor identified a concentration of rearrangements associated with the gained haplotype in one of the daughters [48]. More recently, an experimental approach to induce the formation of MN from a specific chromosome corroborated the association between micronucleation and chromothriptic genome rearrangements. By selectively inactivating the Y centromere, Ly and colleagues showed both at the cytogenetic and the DNA sequencing level that the missegregated Y chromosome exhibit 120-fold higher probability of developing structural rearrangements [26,27]. According to these authors, the cascade of events is initiated by centromere inactivation, followed by missegregation and fragmentation of micronucleated chromatin. In the following interphase, canonical non-homologous end joining, but not homology-dependent repair, facilitates the religation of chromosome fragments if the content of the micronucleus was included in the primary nucleus, but not if it was kept separate after the second mitosis.

While the association between MN and chromothripsis is clear, one question remains to be addressed: why might chromosomes sequestered in MN experience massive fragmentation? Micronucleation of a chromosome can lead to DNA cleavage through different mechanisms. A first type of mechanisms depends on the loss of nuclear functions due to NE defects and NE disruption during interphase. The collapse of the micronuclear envelope upon DNA replication initiation slows or stalls DNA synthesis due to leakage of essential nuclear components [19]. DNA damage associated with replication stress might be exacerbated by deficient DNA damage response signalling. Consistent with this, although MN present significant labeling of the DNA damage marker γH2AX, they show poor recruitment of DNA repair factors such as 53BP1 [41,42]. However, the acquisition of massive DNA damage does not always require NE rupture. Defects in the unruptured NE cause micronuclear DNA replication to be slower, and as a consequence of it proliferating cells enter mitosis when replication of micronuclear DNA is not yet complete. The persistence of a large number of unrepaired DNA breaks and unresolved replication intermediates could be catastrophic upon mitotic breakdown of the micronuclear envelope and condensation of chromosomes [7,25]. According to these models, the micronucleated chromosomes undergo an asynchronous DNA synthesis with the primary nucleus thus suffering from a disturbance in its performance of condensation of not yet fully replicated chromosomes, leading eventually to a phenotype of localized shattering in mitosis.

Yet a second mechanism, which is not associated with DNA replication, might potentially link micronucleation with massive DNA damage. Upon disruption of the micronuclear envelope, not only leakage of nuclear proteins, but also an influx of cytosolic proteins into the micronucleus has been observed. NE collapse leads the MN content exposed to the cytoplasmic environment as evidenced by the rapid accumulation of a fluorescent form of the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS) [31]. Although the downstream consequences of cGAS pathway activation on the micronuclear chromosomes are unknown, upon rupture the micronuclear chromosomes become exposed to harmful cytoplasmic components such as endo- and exonucleases. Taken together, several non-mutually exclusive mechanisms associated with defects in nuclear functions arising from defective nuclear envelope could act to promote massive DNA damage in micronuclei.

6. DNA damage associated with chromatin bridges

Several studies have reported an association between the BFB cycles and chromothripsis [24,34]. While the original BFB model places the resolution of chromatin bridges during the last stages of the cell division, more recent studies indicate that they persist through mitosis and cytokinesis to form stabilized nucleoplasmic connections between the two daughter cells. NE rupture of chromatin bridges during interphase is highly correlated with chromothripsis and it was suggested to be a critical initiating event for the localized shattering of DNA in these abnormal nuclear structures [29]. These authors examined the behavior of dicentric chromosomes using spinning-disk confocal imaging and reported that, after persisting for many hours, resolution of chromatin bridges is facilitated by the cytoplasmic 3′ repair exonuclease 1 (TREX1). It was proposed that this enzyme gains access to the bridging chromatin through NE rupture and it contributes to the resolution of the bridge because the amount of ssDNA in the bridge — a likely key step in this process — was shown to be decreased after inhibiting NE rupture. Whole-genome sequencing of clones derived from cells born with DNA bridges showed rearrangements with spatial clustering of breakpoints, a hallmark of chromothripsis [29,30]. However, even though NE rupture frequently associates with DNA breaks, it is unclear whether this is the primary cause of damage on the bridging DNA as it is not known which is the source of the nicks in the double-stranded DNA that allow the exonuclease TREX1 to initiate resection.

In a recent study, Umbreit et al. [43] reported that deformation of bridging chromatin in migrating cells is sufficient to generate simple breaks and local DNA fragmentation. The authors observed that motile cell lines broke DNA bridges during interphase, whereas less motile cell lines rarely underwent bridge breakage. They concluded that mechanical forces generated by the interphase actomyosin cytoskeleton stretch and break chromosome bridges, promoting local chromosome fragmentation. By using a combination of live-cell imaging with single-cell

whole-genome sequencing, the authors observed that, in a fraction of the cells born with DNA bridges, the immediate genomic consequences of bridge breakage are relatively simple patterns of copy number alterations localized near the sites of breakage. However, highly complex rearrangements suggestive of errors in the replication of bridging DNA were also observed in some cells suggesting that defective DNA replication generates additional DNA damage and chromothripsis [43]. A possibility is that DNA bridge breakage involves both mechanical and biochemical mechanisms as access to DNA of enzymes, such as cytoplasmic nucleases, might be enhanced by mechanical tension through actomyosin-mediated disruption of the NE.

7. Non-genetic effects of the nuclear envelope rupture of micronuclei and chromatin bridges

Two papers published in 2017 established a connection between aberrantly shaped nuclei and activation of the proinflammatory response [18,31]. The papers described a new way of activating the innate immune system through MN surveillance by cGAS, a cytosolic DNA sensor that induces the production of cytokines through the cGAS-STING pathway, thus extending the effects of nuclear atypia on the non-genetic field. Harding et al. [18] observed that inhibiting the c-NHEJ after high doses of radiation decreased the innate immune response instead of activating it. Because there was a strong G2 cell cycle arrest when c-NHEJ was inhibited in irradiated cells, the authors speculated that the activation of the innate immune response required passing through mitosis and subsequent formation of MN. The authors observed that cGAS is concentrated on disrupted MN and reported that inflammatory cytokine production was inhibited by genetic ablation of cGAS. MacKenzie et al. [31] arrived at the same conclusion using a different experimental system. They reported an accumulation of cGAS in MN formed in mouse embryonic fibroblasts lacking RNase H2, an enzyme that has a role in ribonucleotide excision repair. Consistent with the results reported by Harding et al. [18], they also reported a higher degree of proinflammatory gene expression in cells with radiation-induced MN as compared with non-micronucleated cells. The preceding studies support a model whereby loss of micronuclear envelope integrity can provide access of cytoplasmic factors to micronuclear DNA and by extension, to other nuclear shape defects such as DNA bridges. According to this model, NE rupture leads to cGAS accumulation on the micronuclear and bridging chromatin, which activates cGAS to produce cGAMP and signal to the STING adaptor protein, leading to expression of interferons and pro-inflammatory cytokines [9,15].

While the cGAS-STING pathway evolved in eukaryotic cells as a major mechanism for the detection of bacterial and viral infection, activation of cGAS by self DNA has dual opposite consequences. Whereas it represents a threat to the organism as it triggers autoimmunity, it may also act as an immune surveillance mechanism that detects and potentially suppresses a range of neoplasia-inducing processes. Importantly, these processes have a common denominator: they are based on the formation of aberrant nuclear structures that suffer frequent and irreversible rupture of their wrapping.

8. Conclusions

Nuclear architecture defects are now appreciated as sources of phenomena such as chromothripsis, capable of radically changing the genome of cells. Massive DNA damage has been associated with defects in nuclear functions arising from defective nuclear envelope and ectopic access of cytoplasmic nucleases to missegregated chromatin. Also, mechanical stress has been identified as an initiating event for chromothripsis. However, not only the repertoire of genetic consequences has expanded but also the type of consequences. We now understand that micronuclei and DNA bridges can also lead to important non-genetic effects through the cGAS-STING pathway, which activates the innate immune system through secretion of cytokines. Therefore, the direct consequences of micronuclei and DNA bridges do not limit to the cells wearing these abnormal structures but they expand to the whole organism.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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