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Rebuilding Chromosomes After Catastrophe: Emerging Mechanisms of Chromothripsis

Peter Ly and **Don W. Cleveland**

Ludwig Institute for Cancer Research; Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA 92093

Abstract

Cancer genome sequencing has identified chromothripsis, a complex class of structural genomic rearrangements involving the apparent shattering of an individual chromosome into tens to hundreds of fragments. An initial error during mitosis, producing either chromosome missegregation into a micronucleus or chromatin bridge interconnecting two daughter cells, can trigger the catastrophic pulverization of the spatially isolated chromosome. The resultant chromosomal fragments are re-ligated in random order by DNA double-strand break repair during the subsequent interphase. Chromothripsis scars the cancer genome with localized DNA rearrangements that frequently generate extensive copy number alterations, oncogenic gene fusion products, and/or tumor suppressor gene inactivation. Here we review emerging mechanisms underlying chromothripsis with a focus on the contribution of cell division errors caused by centromere dysfunction.

Keywords

chromothripsis; genomic instability; chromosome rearrangements; mitosis; micronuclei; DNA repair

Hidden in Plain Sight: Chromothripsis in the Cancer Genome

The karyotypes of cancer cells are often remarkably complex – littered not only with mutations but also small- and large-scale changes in both chromosome number and architecture. Copy number alterations in the form of whole-chromosome or segmental aneuploidy are present in the vast majority of tumors, yet its role as a cause or consequence of cancer development remains under debate [1, 2]. Structural aberrations and gross rearrangements alter the linear organization of chromosomes, and in some instances can directly drive tumorigenesis. The Philadelphia chromosome in chronic myelogenous

Correspondence: Peter Ly (p3ly@ucsd.edu); Don W. Cleveland (dcleveland@ucsd.edu).

Conflicts of Interest

The authors declare no conflicts of interest.

leukemia is the classic example [3] involving a translocation between chromosomes 9 and 22 to generate an oncogenic gene fusion product that can be effectively targeted by clinical therapeutics [4].

Although it appeared firmly established that tumorigenesis develops through the gradual and sequential accumulation of genetic and/or epigenetic changes [5], recent cancer genome sequencing efforts challenged this dogmatic view by identifying several new classes of mutational processes that form simultaneously in a single burst. Adapted from the age-old theory in evolution, the paradigm-shifting concept of **punctuated equilibrium** (see "Glossary") has now been applied toward our understanding of cancer progression models, as recently described in the renewed context of pancreatic cancer [6]. These punctuated events currently include alterations such as **kataegis** (regions of clustered hypermutation) [7, 8] and **chromoplexy** (serial rearrangements linking multiple chromosomes) [9] – the underlying bases of which are not well understood.

The most striking example of punctuated equilibrium is **chromothripsis** (a Greek neologism for 'chromosome shattering'), in which tens to hundreds of structural rearrangements are rapidly acquired within a short timeframe [10]. These complex rearrangements are curiously restricted to one or a few chromosome(s) with breakpoints scattered across an entire chromosomal axis or clustered within a specific arm or region. Such alterations were predicted to form *de novo* from massive DNA damage and repair events, leading to a derivative chromosome that shares little resemblance to its original configuration. The characteristic mutation signature for chromothripsis [11] has now been detected in a broad spectrum of cancers, and of particular interest, at high frequency in specific tumor types – including bone, blood, and brain cancers [6, 12–17]. Although the majority of chromothripsis cases occur somatically, inherited forms of germline chromothripsis have also been documented [18]. Additionally, similar types of complex rearrangements have been reported in individuals with developmental and/or cognitive disorders, which appear to be caused by DNA replication errors [19] in a process termed **chromoanasynthesis**. The catchall phrase **chromoanagenesis** (Greek for 'to be reborn') has been coined [20] to encompass all the possible types of localized and complex chromosomal rearrangements in human genomes irrespective of their underlying mechanism of formation.

If chromothripsis causes such drastic changes in chromosome structure and occurs frequently in some cancers, why had it not been discovered prior to 2011? Several types of large-scale structural rearrangements, such as gross translocations, can be easily identified by routine cytogenetic methodologies involving chromosome-banding patterns or fluorescent *in situ* hybridization. However, submicroscopic-scale structural variations in the range of kilobase- to even megabase-sized rearrangements or more complex abnormalities, such as chromothripsis, can easily escape recognition. Thus, initially missed by microscopy-based approaches, the recent advent of higher resolution next-generation DNA sequencing technologies enabled Stephens *et al.* [10] to identify and resolve the complexity of rearrangements characterizing chromothripsis in cancer genomes.

The highly localized and complex nature of chromothripsis initially puzzled both cell biologists and cancer geneticists, leading to a spectrum of proposed hypotheses for the

underlying mechanism(s) [10, 20–23]. Considering that the rearrangements are often restricted to a single chromosome, it was strongly suspected that the affected chromosome must have been spatially isolated from the remaining genome, even if only for a transient period. DNA damage in the form of double-strand breaks (DSBs) resulting in chromosome breakage is also likely involved followed by one or more mechanism(s) of error-prone DNA repair to produce the resulting rearrangements [10]. Many key aspects regarding the cellular mechanisms have emerged over the last five years, including evidence supporting a role of cell division errors in the shattering of an initially missegregated chromosome [24–26]. In this review, we cover recent insights into the mechanisms of chromothripsis with a particular focus on the role of mitotic errors driven by centromere dysfunction. We also highlight a number of outstanding questions.

Chromothripsis Driving Tumorigenesis

How does chromothripsis contribute to cancer development? The simultaneous formation of a multiple alterations through chromothripsis can lead to the acquisition of one or more selective advantages (Figure 1A). Because chromothripsis can result in both the loss of DNA segments and the formation of *de novo* rearrangements, two obvious culprits are the disruption of tumor suppressor genes and the formation of oncogenic fusion products, respectively. Rearrangements formed between two normally distant loci may also juxtapose an active regulatory element (e.g., a promoter) adjacent or in close proximity to an otherwise repressed oncogene. Cancer genome sequencing efforts have indeed documented numerous examples of tumor suppressor loss [10], gene fusion events [14], and perturbed regulatory elements [15, 27] associated with chromothripsis in human malignancies (Box 1).

In certain cases [10, 13, 28], there is also an association between chromothripsis and gene amplification. These can be manifested as extrachromosomal DNA elements in the form of **double minute (DM) chromosomes**, suggesting that the ends of one or more fragments can ligate and circularize [25] into self-propagating entities. In the initial example from small-cell lung cancer, chromothripsis of chromosome 8 produced a megabase-long DM through the stitching of fifteen fragments that led to the amplification of the *MYC* oncogene [10]. These regions were lost from the reassembled derivative chromosome 8. Remarkably, such DMs have recently been detected in nearly half of all human cancers and can lead to exceptionally high expression of the corresponding DM-located genes [29] – typically oncogenes and/or genes conferring resistance to therapy [30]. That said, the frequency at which chromothripsis directly contributes to the formation of DMs across varying cancer types is not established.

Loss of the *TP53* tumor suppressor gene, which can halt cell cycle progression in response to DNA damage, also appears to be a prerequisite event for chromothripsis [13]. Current experimental models for studying chromothripsis in human cells have required depletion or inactivating mutations of p53 [24–26, 31, 32]. Bypassing the p53 checkpoint is therefore a critical step towards tolerating and overcoming the damage accompanying chromothripsis. Although the vast majority of non-transformed cells experiencing chromothripsis do not survive, the cases exemplified in cancer genomes are likely rare exceptions that underwent clonal selection and expansion towards cancer. Overall, one or a combination of these

rearrangements, which collectively produce a hallmark mutation signature [11] for chromothripsis (Figure 1B), could fuel cancer development and tumor evolution through selective processes.

The Micronucleus Revisited

At the exit of mitosis, nuclear lamins and pore complexes redeposit around newly segregated chromosomal masses to encapsulate the genome within the nuclear envelope (NE), ultimately forming the cell nucleus. A chromosome that fails to correctly segregate to either of the two mitotic spindle poles, perhaps due to improper kinetochore–microtubule attachments, will produce a micronuclear envelope (micro-NE) assembled around the lagging chromosome. The resultant **micronucleus** spatially isolates one or sometimes few missegregated chromosome(s) into a small nucleus-like structure that is positioned outside of the adjacent primary nucleus (Figure 2). Micronuclei are therefore a consequence of chromosome segregation errors during mitosis.

Accumulating evidence has established micronuclei as a unique source of genomic instability and DNA damage. The initial proposal for this emerged in 1968 when Kato and Sandberg determined that chromosomes in micronuclei undergo pulverization in mitosis after failing to complete DNA replication prior to mitotic entry [33]. Subsequent cell fusion studies between cells in S-phase and mitosis demonstrated that actively replicating chromosomes become fragmented upon premature chromosome condensation caused by exposure to a mitotic cytoplasm [34].

More than 40-years later, the discovery of chromothripsis in 2011 [10] has reawakened widespread interest in what is now recognized as the distinct biology of micronuclei. Consistent with the proposal by Kato and Sandberg, more recent evidence supports that micronuclear chromosomes do in fact acquire DNA damage and/or exhibit delayed replication kinetics compared to the main nucleus [24, 35–37]. Additionally, elegant use of live-cell imaging combined with single-cell DNA sequencing (a technique called ‘Look-Seq’) detected the presence of multiple, aberrantly ligated DNA fragments in the daughter cell(s) that arise from a previously micronucleated chromosome in a dividing mother cell [25]. These ligation events produced complex patterns of localized rearrangements that are highly reminiscent of cancer-associated chromothripsis, although whether an actual derivative chromosome was produced has not been established. Indeed, the experimental creation of a micronucleus-derived chromothriptic chromosome capable of stable transmission in subsequent cell cycles and that include the defining features of a fully functional chromosome has not yet been accomplished and represents a critical next step forward.

Similar mechanisms of chromosome shattering and re-ligation also appear to be present in genetic plant [38] and fission yeast [39] models of chromosome missegregation. In *Arabidopsis* models, apparent micronuclei have been reported to produce complex rearrangements [38] that are strikingly similar to chromothripsis observed in human cancer genomes, implicating chromothripsis as a potentially conserved consequence of mitotic errors across evolution. Interestingly and along similar lines, protozoan ciliates such as

Tetrahymena carry both a macronucleus and a micronucleus, the latter containing germline chromosomes that undergo deliberate DNA fragmentation and extensive rearrangements for subsequent conversion into the macronucleus [40].

Sources of Micronuclear DNA Damage

In mammalian cells, why might micronuclear chromosomes acquire DNA damage in the form of DSBs? As micronucleated cells progress through interphase (Figure 3), the micro-NE has a substantial tendency to undergo disruption that causes abrupt loss of nuclear contents (as detected by loss of GFP fused to nuclear import sequences) [41]. Correspondingly, the sequestered chromatin becomes exposed to normally cytoplasmic-localized components that diffuse into the micronucleus [41]. Thus, disruption of the micro-NE impairs proper nucleocytoplasmic transport and its exclusion of normally cytoplasmically localized proteins. The frequency of disruption is remarkably high, occurring in approximately half of micronuclei in an asynchronously cycling population, and accumulates with cell cycle progression through interphase [41]. Why micronuclei are prone to envelope disruption has not been solved, but could potentially arise from *1)* differences in the stoichiometry of nuclear lamin or pore components assembled into the micro-NE during mitotic exit, *2)* the more extreme membrane curvature of the micro-NE, and/or *3)* cytoskeletal or external compressive forces. The main nucleus can also rupture, although transiently as it rapidly reseals [42] through repair by the membrane remodeling ESCRT machinery [43, 44]. In contrast, disruption of the micro-NE is irreversible, partially or completely terminating normal nuclear function [41].

Two non-mutually exclusive models have been raised to reconcile how micronuclear DSBs are generated after micro-NE disruption. In the first model, disruption during interphase can cause nuclear components of the micronucleus to become diluted through leakage into the cytoplasm. Disruption prior to or during S-phase can delay active replication fork progression, slowing or completely stalling DNA synthesis [24, 33, 37, 41], which itself may act as a source of DNA damage owing to replication stress [45]. Disruption has been suggested to be a critical step for chromothriptic-like rearrangement signatures arising from micronuclei [25], which accumulate to peak frequency during S and G2 phases of the cell cycle [41]. The persistence of a large number of unrepaired DNA DSBs and/or unresolved replication intermediates could be catastrophic upon the dramatic remodeling of chromatin organization that occurs during mitosis, in particular the condensation of chromosomes that is required to facilitate the spatial separation of DNA.

The second model involves aberrant exposure of the micronuclear chromosome to one or more potentially damaging component(s) from the cytoplasm. Upon disruption of the micro-NE, influxes of cytosolic-localized proteins into the micronucleus have been observed (as detected by the inclusion of GFP fused to nuclear export sequences). Recent evidence [43, 44] suggest that transient NE rupture of the main nucleus triggers the recruitment of the cytosolic DNA sensor cGAS [46], which evolved to detect foreign and pathogenic DNAs in the cytoplasm to activate the innate immune response [47]. During interphase, cGAS can be rapidly detected at sites of NE rupture at the nuclear periphery, which is subsequently followed by the focal acquisition of DNA damage [43, 44]. Similar rupturing events were

reported to promote genomic copy number aberrations [48], and cGAS has been shown to associate with condensed chromosomes after NE breakdown during mitosis [49]. Consistent with this, recent work confirmed that cGAS could indeed sense micronuclear DNAs exposed to the cytoplasm following micro-NE disruption as cytosolic self-DNA [50], although the downstream consequences on the micronuclear chromosome itself are unknown.

Harmful cytoplasmic components could include endo- or exonucleases whose localization is tightly regulated or that are activated upon recognition of specific DNA intermediate structures arising from replication stress. MUS81 is one potential nuclease given its role in inducing DSBs at replication stress-induced late-replicating loci, which activates POLD3-dependent mitotic DNA synthesis to safeguard against the formation of ultra-fine anaphase bridges [51]. DNA synthesis errors during mitosis could be one source of chromosomal rearrangements arising from micronuclei that undergo mitotic entry with partially replicated DNA. Altogether, several mechanisms acting in parallel likely converges to promote the massive DNA damage associated with chromothripsis in micronuclei.

One Centromere Too Few: Chromothripsis Driven by Centromere Inactivation

Several experimental approaches have been used to investigate the properties of micronuclei and the eventual fate of the encapsulated chromosome [52]. One widely employed method to generate micronuclei is through prolonged mitotic arrest using microtubule inhibitors, such as nocodazole, followed by release and subsequent missegregation of one or few random chromosomes. An alternative experimental approach was recently developed involving the inactivation of a specific **centromere** (Box 2) to induce micronuclei containing a defined chromosome-of-interest (the human Y chromosome) – a strategy that has identified the temporal sequence of chromothriptic events over several consecutive cell cycles [26].

Centromere inactivation initiates a series of events beginning with chromosome missegregation into micronuclei at the end of the first cell cycle. DNA damage accumulates within micronuclei, triggering chromosome shattering during mitosis of the second cell cycle. Chromosome fragments are subsequently reassembled throughout interphase of the third cell cycle. Analyses of metaphase spreads by fluorescent *in situ* hybridization provided direct evidence supporting micronucleus-mediated chromosome shattering, revealing >50 microscopic chromosomal fragments dispersed across the mitotic cytoplasm – all but one or a few of which lacked centromeric DNA sequences [26]. Chromosome shattering is likely an intermediate stage for chromothripsis (Figure 1A) as the vast majority of **acentric** fragments would be unstable long-term and lost. With exception of circularized extrachromosomal DMs, reassembly would be required to stabilize acentric fragments to at least one fragment containing an active centromere to form a derivative chromosome that can be genetically inherited.

Bringing It All Back Home: Reassembly through DNA Repair

The overwhelming proportion of DNA fragments produced by chromosome shattering are acentric [26], which alone are incapable of attaching to the mitotic spindle. The resulting

fragments are therefore – at best – passively distributed, with likely asymmetric partitioning into newly formed daughter cells and reintegration into the main nucleus if they are in close proximity to either of the poleward-segregating chromosome masses (Figure 4A). The ends of these fragments are presumably recognized as DNA DSBs in the subsequent G1, thereby activating the DNA damage response (Figure 4B). Indeed, suppressing classical non-homologous end joining (c-NHEJ) (Box 3) by depleting or inhibiting the activity of LIG4 or DNA-PKcs prevented micronuclei-derived fragments to undergo repair [26], evidence supporting that c-NHEJ is the predominant mechanism for reassembling chromosomal fragments. LIG4 also appears to be required for repairing missegregated chromosomes in genetic plant models [38]. Chromosomal translocations in human cells are mediated primarily through c-NHEJ, although murine cells appear to favor alt-EJ [53, 54]. Sequence analyses of the breakpoint junctions from chromothriptic tumors [10] and experimental models of chromothripsis [25, 38] has revealed that a large proportion of breakpoints (but not all, as discussed below) lack significant homology or microhomology – consistent with the repair signature of c-NHEJ.

Although DNA DSBs accumulate in micronuclei following rupture in interphase, the resultant breaks are likely not subjected to DNA repair as micro-NE disruption would cause dilution of components involved in the DNA damage response [41]. Micronuclei with phosphorylated histone γH2AX, a marker of DNA DSBs, often failed to recruit or retain detectable levels of the DNA repair factor 53BP1 [35]. As micronucleated cells enter mitosis, a fraction of micronuclei also seemingly fail to disassemble the micro-NE and persist into the next interphase [24, 55]. However, high-throughput sequencing of purified micronuclear DNAs demonstrated that extensive rearrangements do not accumulate to detectable levels within micronuclei [26], further indicating that most reassembly events occur in the main nucleus following fragment reintegration.

Several lines of evidence suggest that nuclear ligation of fragmented DNA ensues rapidly with efficient repair kinetics. Analysis by Look-Seq of daughter cell pairs revealed that a high proportion of the fragments were ligated within ~4 hours after mitotic exit [25], most likely during G1. In 3/8 examples, fragments that were apparently lost from one daughter cell were found ligated within the paired daughter cell [25], which reflects the oscillating regions of copy number loss characteristic of chromothripsis [10, 11]. Additionally, use of the Y centromere-specific inactivation strategy demonstrated that inhibiting c-NHEJ produced chromosome fragments that persists into the next mitosis [26], indicating that the duration of a single cell cycle is sufficient for complete (or nearly complete) fragment reassembly.

How chromosomal fragments are inherited between daughter cells during mitosis remains a key question. Spindle forces and motor proteins drive chromosome movement during mitosis, but these do not engage chromosome fragments lacking a centromere. In *Drosophila* neuroblasts, acentric chromosomal fragments have been reported to partially segregate poleward [56] through kinetochore-independent microtubules and the chromokinesin Klp3a [57], which shares similarity to human KIF4A. Alternative models include the topological linkage or “tethering” of chromosome fragments to each other, as suggested by 5/8 Look-Seq examples in which the majority of fragments were unequally distributed to a single

daughter [25], or perhaps onto other chromosomes, a mechanism analogous to the proposal that extrachromosomal DNAs (including DMs or viral episomes) could tether and segregate in *trans* with centromere-containing chromosomes [58–60]. Recent evidence suggests that the c-NHEJ components XRCC4-XLF can physically bridge two DSB ends prior to ligation [61]. Although DNA repair is normally suppressed throughout mitosis to prevent **telomere** fusions [62, 63], whether this or other tethering mechanisms are involved in maintaining chromosome fragments in close spatial proximity until segregation and/or ligation remains unsolved.

One Centromere Too Many: Chromothripsis Driven by Dicentric Chromosomes

In certain instances, a single chromosome can harbor two active centromeres, both of which are capable of attaching to the mitotic spindle. These **dicentric** chromosomes can be formed through several mechanisms. A neocentromere can spontaneously form at a non-centromeric region on the chromosome arm, an event that naturally occurs at a rare frequency through poorly defined mechanisms (Box 2). Most often and perhaps by telomere shortening, a dicentric can be produced by an end-to-end fusion event between the telomeres of two non-homologous chromosomes (chromosome-type fusion) or between sister chromatids (chromatid-type fusion). In the latter, a pseudodicentric chromosome is formed in which the sister centromeres are properly attached to the opposing spindle poles in mitosis but remain linked at the fused end during chromatid separation at anaphase. Lastly, dicentric chromosomes can also result from the fusion of two chromosome fragments that each contains an active centromere [64]. Regardless of the mechanism of dicentric chromosome formation, a chromatin bridge is usually formed during mitosis that can persist until, or even long after, cytokinesis (Figure 5A).

Recent efforts identified that dicentric chromosomes developing into chromatin bridges during late mitosis could act as another source of chromothripsis [32]. Anaphase chromatin bridges were created using an established method to induce chromosome-type telomere fusions [65] through expression of a dominant-negative mutant of the telomere-associated shelterin component TRF2 [66]. Following the completion of an apparently normal cytokinesis, the chromatin bridge remains intact and interconnected between the two newly formed daughter cells throughout early interphase. As the daughters migrate away from one another, the NE surrounding the bridged DNA undergoes rupturing during G1 that is accompanied by the acquisition of the single-stranded DNA binding protein RPA. This rupture mediates access of the cytoplasmic 3′ exonuclease TREX1 to the exposed and stretched DNA, driving cleavage and ultimate resolution of the chromatin bridge (Figure 5B). Homozygous deletions of TREX1, however, partially delayed but did not completely prevent eventual bridge resolution, suggesting possible roles of other nucleases and/or physical mechanisms responsible for the breakage of chromatin bridges. Sequencing of clones revealed chromothriptic-like rearrangements involving the fused chromosomes [32] through an unidentified DNA repair mechanism. Consistent with a role for telomere fusions in chromothripsis, depletion of TRF2 to uncap telomeric ends in non-cancerous cells followed by selection for partially transformed cells also generated chromothriptic-like

signatures [31]. Combinations of chromothripsis with breakage–fusion– bridge cycles (Figure 5C) can add another layer of complexity to the mutation signatures associated with cancer genomes [6, 64, 67].

How do chromothripsis and rearrangement patterns generated by chromatin bridges differ from those arising through micronuclei? Whereas replication defects have been proposed to be an important component of the DNA damage associated with the micronucleus model, chromothripsis from telomere fusions likely do not require ongoing DNA synthesis. Rather, the fragmentation of the bridged DNA is dependent on its resolution by TREX1, which frequently occurs prior to S-phase entry [32]. Additionally, multiple examples from cancer genomes have been documented in which chromothriptic rearrangements were focally restricted to a single arm or localized to a terminal region of a given chromosome rather than the entire chromosome [10]. Therefore, in contrast to the rearrangements produced from micronuclei that are distributed across an entire chromosome, dicentric bridge formation could explain how focal chromothripsis occurs. Analyses of chromothriptic examples generated from dicentrics revealed that many of the rearrangements were indeed clustered within a focal chromosomal region [32] that is probably the site of TREX1-mediated bridge resolution. Unexpectedly, these regions were often associated with hotspots of local hypermutation preferentially affecting cytosine nucleotides (kataegis) [7], implicating a potential mechanistic linkage between chromothripsis and hypermutation.

Alternative Mechanisms and Forms of Chromothripsis

A fraction of chromothriptic breakpoint junctions contain microhomology [10], suggesting potential repair by alternative end joining (Box 3) or the involvement of other mechanisms for localized rearrangements that are independent of chromosome shattering events, such as chromoanasynthesis. Mechanisms that have been proposed to contribute to the complex structural rearrangements defined by chromoanasynthesis include errors in DNA replication, most notably aberrant DNA template switching at stalled forks (called fork stalling and template switching, or FoSTeS) or collapsed/broken forks (called microhomology-mediated break-induced replication, or MMBIR) [68, 69]. In individuals with inherited genomic disorders, iterative rounds of such replication-based events could produce catastrophic rearrangements that are accompanied by templated insertions, duplications, and/or microhomology at the sequence junctions [19]. Such complex rearrangements can be reminiscent of, but distinct from, chromothripsis through chromosome shattering and fragment re-ligation [70, 71]. Interestingly, a few examples of micronuclei-derived rearrangements also exhibited sequence features at junctions that share similarities with chromoanasynthesis, in particular small insertions and the presence of microhomology [25], suggesting possible overlapping mechanisms between chromothripsis and chromoanasynthesis.

Concluding Remarks

The discovery of chromothripsis has advanced our understanding of the complexities associated with cancer genomes, as well as opened exciting new avenues for research. The development of novel cell biological tools combined with computational methods to

examine the fate and sequence characteristics of missegregated chromosomes has recently contributed to the mechanisms underlying chromothripsis. Much remains to be determined (see ‘Outstanding Questions’), in particular the exact causes of NE disruption and DNA damage in micronuclei. Key among these questions is what does it take to assemble a fully functional and heritable human chromosome, and how is this achieved following catastrophic processes such as the shattering of an individual chromosome? Whether other unidentified types of punctuated equilibrium-driven chromosomal alterations are present in cancer or disease remains to be seen, but advancements in DNA sequencing technologies will likely enable further discovery of more unexpected and hidden features of the human genome.

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Glossary

Acentric
a chromosome, or fragment of a chromosome, that lacks an active centromere

Chromothripsis
complex rearrangements arising from the catastrophic shattering of a single or few chromosome(s)

Chromoanasynthesis
complex rearrangements arising from the defective replication of a single or few chromosome(s)

Chromoanagenesis
a catchall term that encompasses catastrophic mutational processes involving one or a few chromosomes, independent of the precise mechanism(s); included here are chromothripsis and chromoanasynthesis, which arise through distinct mechanisms

Chromoplexy
a series of chained, complex rearrangements frequently involving five or more chromosomes

Centromere
a specialized region on each chromosome designated for assembly of the kinetochore and whose unique position is identified and maintained epigenetically

Dicentric
a chromosome harboring two active centromeres

Double minute (DM) chromosomes
circular and replication-competent extrachromosomal DNA elements that accumulate and amplify multiple gene copies that drive high levels of expression

Kataegis
clusters of focal hypermutation preferentially favoring cytosine substitutions

Micronucleus
small, nuclear structures that encapsulate missegregated chromosomes and are spatially isolated from the main nucleus

Micronuclear envelope disruption
irreversible rupture of the nuclear envelope surrounding a micronucleus that causes abrupt loss of nucleocytoplasmic partitioning and terminates micronuclear function

Non-homologous end joining
a major form of DNA double-strand break repair whereby two damaged ends undergoes direct ligation

Punctuated equilibrium
a long-standing theory in evolutionary biology with recent implications for cancer development, in particular the rapid acquisition of a large number of oncogenic alterations over a short timescale

Telomeres
repetitive sequences that protect each of the terminal ends of chromosomes from shortening during replication and detection by the DNA damage response

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Box 1

Three case examples of chromothripsis driving genomic changes associated with cancer and disease

Pancreatic cancer is thought to develop from an initiating clone that gradually accumulates oncogenic mutations over relatively long timescales to eventually acquire the capacity to disseminate to distant organs and become metastatic. However, the majority of pancreatic cancer patients are asymptomatic and not diagnosed until the tumor has reached the metastatic stage – an endpoint with extremely poor clinical outcome. This observation has challenged the conventional stepwise paradigm of pancreatic cancer progression and instead raises an alternative model: the rapid acceleration of early pancreatic lesions toward metastatic disease occurs through punctuated events that simultaneously drive multiple oncogenic changes. Sequencing of 107 pancreatic cancer genomes revealed that, along with genome duplication events (polyploidy), chromothripsis affecting at least one chromosome was evident in at least two-thirds of examined cases [6]. Multiple chromosomes characterized by the signatures of chromothripsis were also found, which concurrently inactivated several classic pancreatic driver genes, including *CDKN2A*, *SMAD4*, and *TP53*. Parallel events involving presumptive breakage-fusion-bridge cycles also triggered further genomic complexity, including the amplification of mutated *KRAS* alleles.

Whole-genome sequencing analyses of nine supratentorial ependymomas, a type of brain and spinal cord tumor, strikingly revealed chromothripsis affecting chromosome 11 in all nine cases examined [14]. In eight cases, complex rearrangements generated a fusion of the oncogenic *RELA* gene with an uncharacterized *C11orf95* gene, which are normally located ~2 megabases apart and separated by 73 genes on chromosome 11. Expression of *RELA-C11orf95* fusions in neural stem cells implanted into the cerebellum of mice resulted in a marked increase in brain tumors, providing evidence that gene fusion products created through chromothripsis can be highly oncogenic in nature.

In one truly remarkable medical case, a patient with an extremely rare congenital immunodeficiency disorder called WHIM syndrome was serendipitously cured through somatic chromothripsis of chromosome 2 [72]. These rearrangements resulted in the loss of a disease-causative, gain-of-function mutant allele of the *CXCR4* gene within a hematopoietic stem cell clone that was capable of repopulating the myeloid lineage and restoring normal neutrophil count.

Box 2

Epigenetic maintenance and function of the centromere

Centromeres are unique chromosomal loci that establish assembly of the kinetochore, a large multi-protein complex that directly facilitates chromosome movement and segregation by attachment to spindle microtubules during mitosis. Although the overwhelming majority of human centromeres are found on repetitive alpha-satellite DNA sequences, these sequences are neither sufficient nor necessary for centromere formation, maintenance, or function, as evident by the discovery of mitotically-stable neocentromeres formed at distinct loci located on chromosome arms [73–75]. Instead, the position of each centromere is specified epigenetically by the histone H3 variant CENP-A [76], which self-templates its own propagation every cell cycle (reviewed in detail [77]). These epigenetic mechanisms act to ensure that one – and strictly one – centromere is active per chromosome to safeguard against genomic instability.

Box 3

Repair mechanisms for DNA double-stranded breaks

DNA DSBs are repaired by two primary mechanisms in mammalian cells. In the first, homologous recombination (HR) utilizes long tracts of homologous sequence as a template to repair DSBs and is most active during S and G2 phases of the cell cycle. In the second, **non-homologous end joining** (NHEJ) directly joins two DSBs together without use of long sequence homology and is therefore a more error-prone repair pathway.

There are at least two recognized subtypes of NHEJ that mechanistically operates through distinct components and pathways: classical NHEJ (c-NHEJ) and the less characterized alternative end joining (alt-EJ) pathway (recently reviewed [78, 79]). c-NHEJ functions through a signaling cascade involving DNA-PK, a heterotrimeric complex composed of DNA-PKcs, Ku70, and Ku80 subunits, with direct end joining activity mediated by DNA Ligase 4 (LIG4)–XRCC4. Because end processing is minimal, repair by c-NHEJ can produce between 0–4 bp of microhomology at junction sequences, the majority of which occur by chance. In contrast, repair by alt-EJ does not require the components involved in c-NHEJ.

Microhomology-mediated end joining (MMEJ) is the major form of alt-EJ (reviewed in detail [80, 81]). MMEJ requires an initial resection step mediated by the MRN complex and CtIP (a step shared with HR) followed by a search for microhomologous sequences between the two resected ends. Once aligned, the ends are subjected to fill-in synthesis by the DNA polymerase Polθ and ligation by DNA Ligase 3. Breakpoint junctions repaired by MMEJ contain scars of 3–8 bp of microhomology (and up to 20 bp) that are usually accompanied by small deletions.

Trends

- Chromothripsis is a catastrophic event in which one or a few chromosome(s) are shattered and stitched back together in random order, producing a derivative chromosome with complex rearrangements within a few cell cycles.
- Chromosome missegregation during cell division frequently produces small nuclear structures called micronuclei, which are prone to irreversible nuclear envelope disruption during interphase and impaired nucleocytoplasmic compartmentalization.
- Micronucleated chromosomes accumulate extensive DNA damage and are susceptible to shattering during the next mitosis, generating multiple, distinct DNA fragments.
- Chromosome fragments are reassembled by DNA double-strand break repair to form a derivative chromosome.
- Chromatin bridges trapped between daughter cells are attacked by a cytoplasmic nuclease (TREX1) during interphase to generate DNA breaks and focal chromothripsis.

Outstanding Questions

- What are the contributing factors that predispose micronuclei to undergo nuclear envelope disruption during interphase?
- What are the underlying sources of DNA damage in a micronucleus following the disruption of its nuclear envelope?
- How are chromosomal fragments segregated between nascent daughter cells during mitosis, and are these fragments topologically or physically tethered to facilitate segregation *en masse?*
- Does the nuclear reintegration of chromosomal fragments activate the DNA damage response to engage DNA repair, and if so, how are these fragments spatially positioned within the interphase nucleus to promote efficient reassembly?
- At what frequency do reassembled fragments form a fully functional chromosome that is capable of long-term inheritance?
- Do chromothripsis and chromoanasynthesis share similar underlying mechanisms?
- What other types of complex DNA alterations caused by “punctuated” events exist in the genomes of individuals suffering from diseases or disorders?

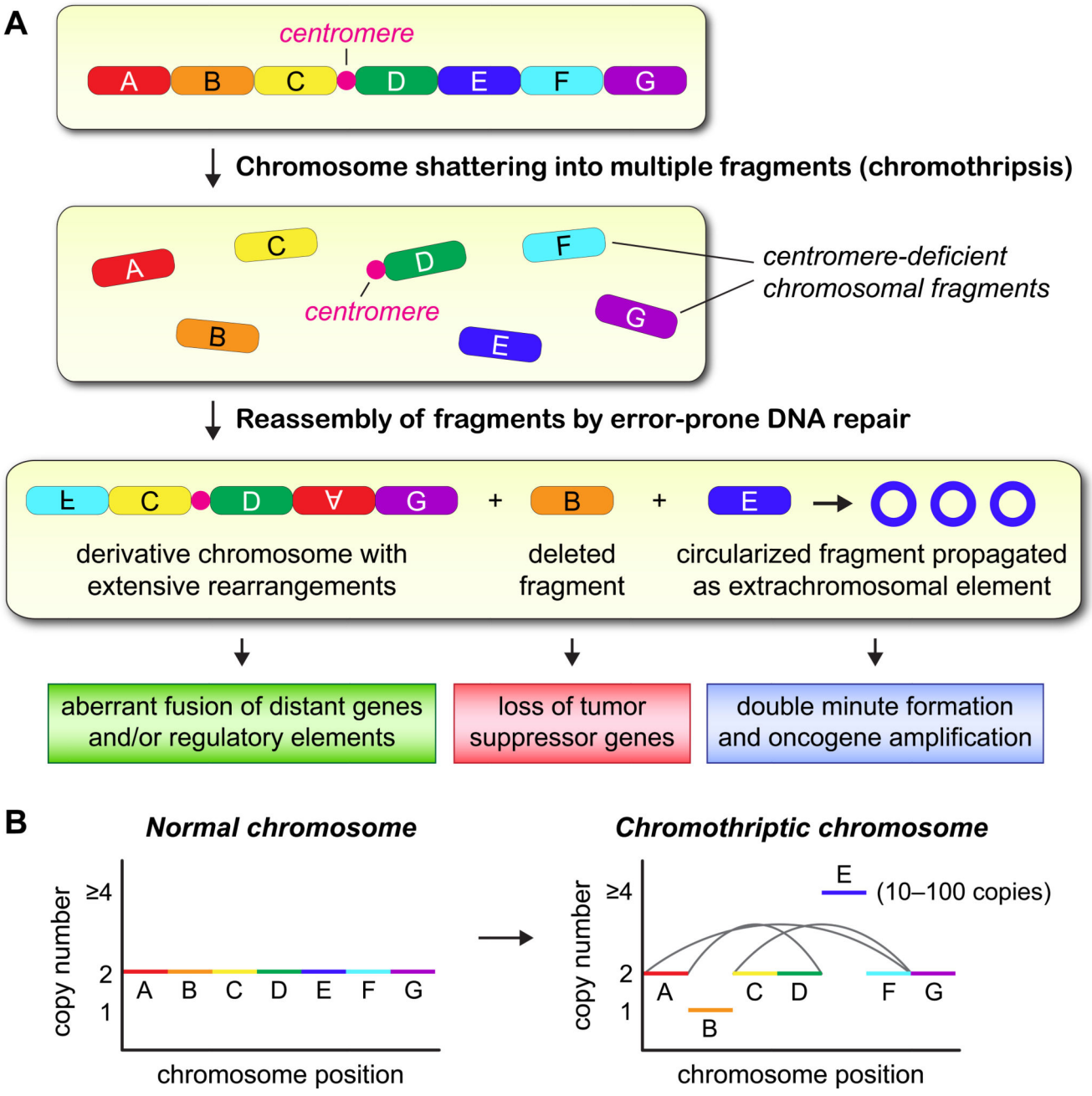

Figure 1. Genomic and tumorigenic consequences of chromothripsis

(A) The shattering of an individual chromosome can produce tens to hundreds of acentric DNA fragments that persist as intermediates until they are re-ligated and stabilized by intrinsic DNA repair mechanisms. These fragments reassemble to form a scrambled, derivative chromosome containing multiple rearrangements (chromothripsis), become lost, and/or self-ligate into circular DNA structures called double minutes. **(B)** Chromothriptic events can give rise to a characteristic mutation signature that has been detected in a broad range of cancer genomes, including oscillating copy number states and complex patterns of intrachromosomal rearrangements in apparently random fashion.

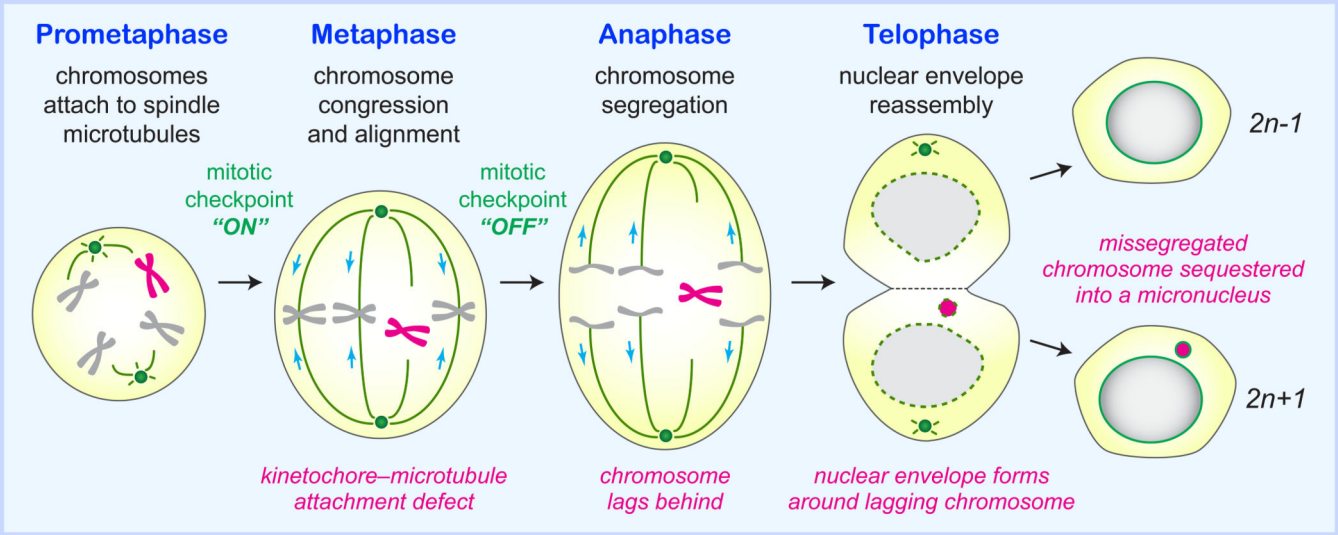

Figure 2. Chromosome segregation errors during mitotic cell division can entrap DNA within a micronucleus

During mitosis, a chromosome that fails to congress, align, and/or form proper bipolar spindle microtubule–kinetochore attachments prior to anaphase onset can be left behind during the physical separation of the duplicated genome. A nuclear envelope assembles around the missegregated chromosome, subsequently forming a micronucleus at the exit of mitosis.

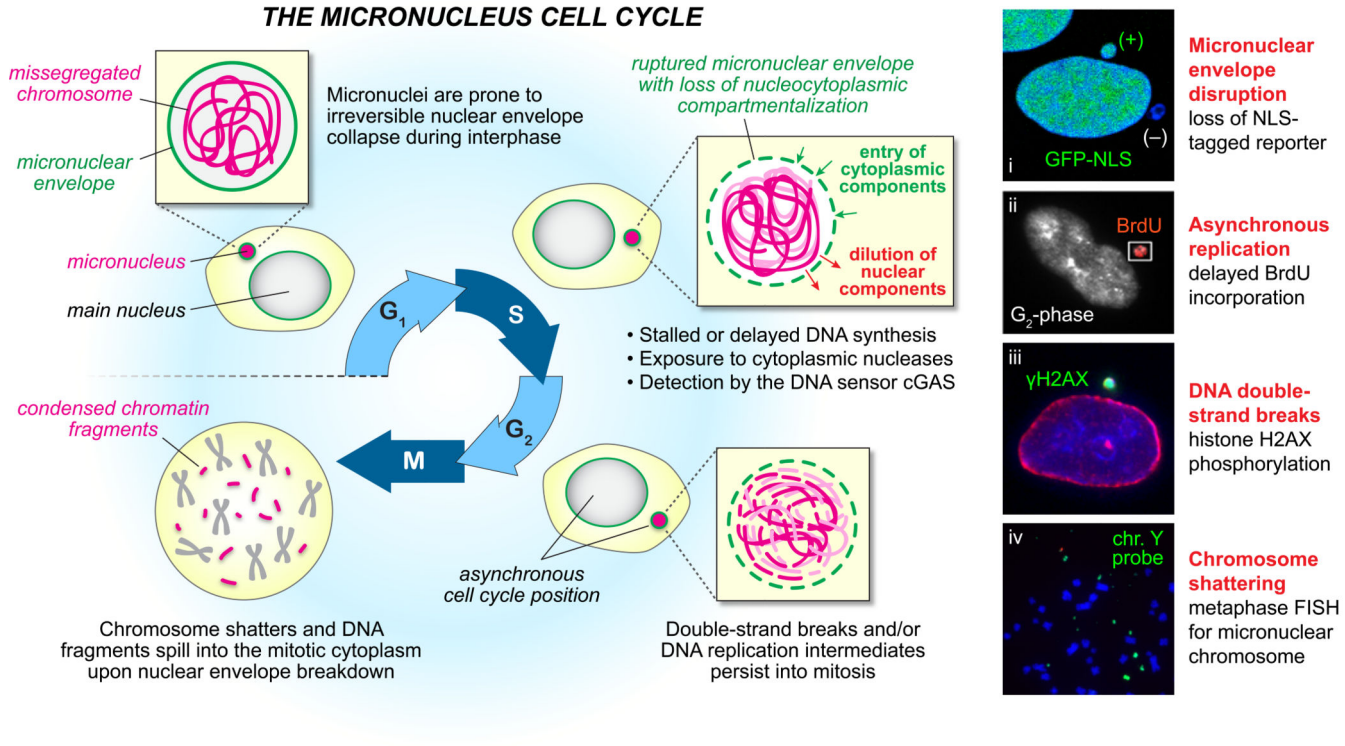

Figure 3. DNA damage in micronuclei triggers in the catastrophic shattering of individual chromosomes

(A) Chromosomes isolated in micronuclei are sequestered by a highly unstable nuclear envelope, which are susceptible to disruption throughout interphase that interferes with normal nucleocytoplasmic transport and compartmentalization *(i)* [41]. Disruption can cause DNA replication asynchrony between the main nucleus and micronucleus *(ii)* [24], as well as permit exposure of micronuclear DNAs to damaging cytoplasmic components such as nucleases. In turn, DNA damage restricted to the micronucleated chromosome *(iii)* [26] persists throughout the cell cycle and into mitosis. Nuclear envelope breakdown and chromatin condensation initiated by mitotic entry subsequently causes the micronuclear chromosome harboring multiple double-stranded DNA breaks to undergo shattering that is accompanied by the spatial separation of chromosomal fragments *(iv)* [26]. Data images were modified and reproduced with permission from Elsevier *(i)* and Nature Publishing Group *(ii–vi)*. NLS, nuclear localization signal.

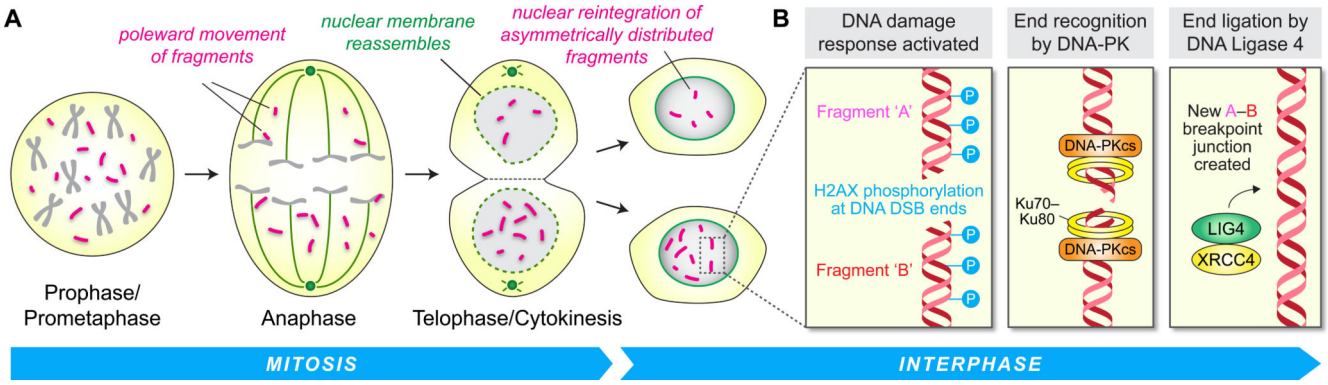

Figure 4. DNA damage repair mechanisms contributing to the reassembly of fragmented chromosomes

(A) Chromosome fragments produced by chromothripsis spill into the mitotic cytoplasm and are subsequently incorporated into newly formed daughter cell nuclei at the exit of mitosis, possibly through the physical tethering between fragments and/or onto intact, centromere-containing chromosomes. **(B)** In the next interphase, reintegrated fragments activate the DNA damage response. In the absence of functional p53, DNA double-strand break repair ensues through error-prone non-homologous end joining, which directly links multiple fragments together in a haphazard manner by ligation. The reassembled chromosome is characterized by extensive DNA rearrangements harboring *de novo* breakpoint junctions that carry the signatures of the underlying DNA repair mechanism.

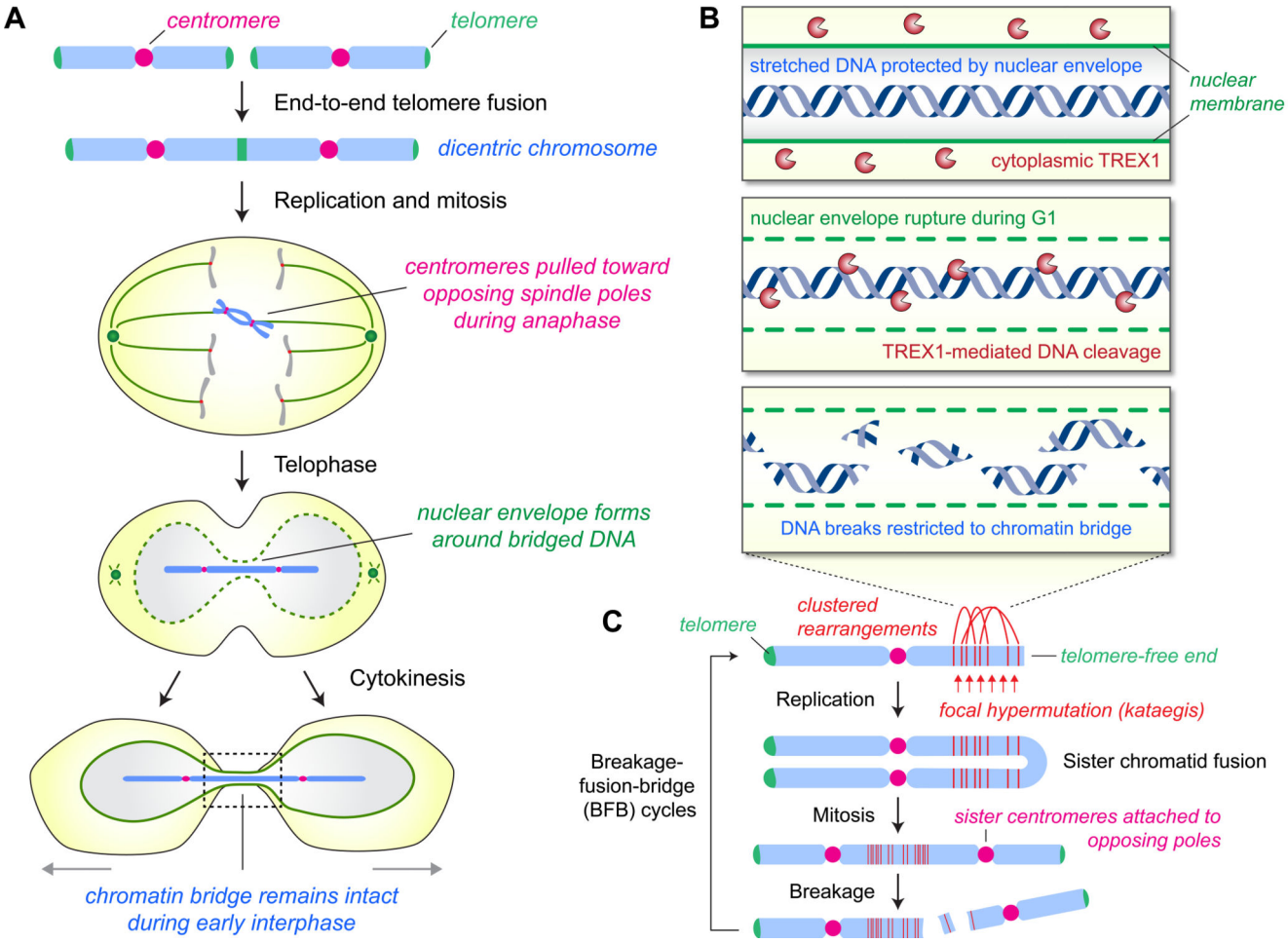

Figure 5. Chromatin bridges act as a source of focal chromothripsis

(A) Telomere fusion events can create dicentric chromosomes that harbor two active centromeres, both of which are capable of forming kinetochore–microtubule attachments during mitosis and segregation toward opposite spindle poles. Nuclear envelope reassembly at the exit of mitosis produces a chromatin bridge (dotted box, magnified in **B**) that persists into interphase connecting two nascent daughter cells. **(B)** Rupture of the nuclear envelope surrounding the chromatin bridge enables access of the normally cytoplasmic-localized TREX1 exonuclease to the underlying DNA, causing chromosome breaks restricted to the bridge that are likely repaired during the same or subsequent interphase. **(C)** Clustered rearrangements and hypermutation localized to a specific chromosome arm or region are common outcomes for fragmented bridges. Subsequent fusion events between telomere-free ends can facilitate further genomic instability through repeated cycles of breakage-fusion-bridge