Catastrophic endgames: emerging mechanisms of telomere-driven genomic instability

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Abstract

When cells progress to malignancy, they must overcome a final telomere-mediated proliferative lifespan barrier called replicative crisis. Crisis is characterized by extensive telomere fusion that drives widespread genomic instability, mitotic arrest, hyperactivation of autophagy via the cGAS–STING pathway and cell death. Recently it has become apparent that that the resolution of dicentric chromosomes, that arise from telomere fusions during crisis, can initiate a sequence of events that leads to chromothripsis, a form of extreme genomic catastrophe. Chromothripsis is characterized by localized genomic regions containing a few tens to thousands of rearrangements, and it is becoming increasing apparent that chromothripsis occurs widely across tumor types and has a clinical impact. Here we discuss how telomere dysfunction can initiate genomic complexity and the emerging mechanisms of chromothripsis.

Telomere crisis

Telomeres (see glossary) were originally defined for the essential role they play in governing genome stability [1]. Telomeres are subjected to end-replication losses, that arise due to the inability of semi-conservative DNA replication to fully replicate the **lagging strand,** coupled with the functional requirement for the presence of a terminal 3′ single-stranded G-rich over-hang generated by nucleolytic processing [2]. Consequently, in the absence of **telomerase**, telomeres in human cells gradually erode with ongoing cell division which leads to a well-defined decrease in telomere length [3-5] (Figure 1A). Superimposed on gradual end-replication losses, human telomeres can suffer more substantial stochastic deletion events that result in single dysfunctional telomeres [4, 6-8]. Telomere erosion can continue until one or more telomeres in a cell [9, 10] have eroded to a length at which they lose their end-capping function and are recognized as a double stranded DNA break (DSB) [11]. Which elicits a TP53/p21 (tumor suppressor/cyclin-dependent kinase inhibitor) dependent G1/S cell cycle arrest [11, 12]. Importantly, the DNA damage checkpoint response is not accompanied by DSB repair. Instead these cells can remain in this state of replicative senescence, with unrepaired telomeres, persisting for many years [7, 13]. Telomere dependent replicative senescence is therefore considered to be a key tumour suppressive mechanism that limits cellular replicative capacity in long-lived species [14]. However, perturbations in the TP53 response can allow cells to continue to divide past the point of senescence [15, 16] where telomeres continue to erode, ultimately reaching a point at which single telomeres are completely denuded of telomere repeats [7, 17].

The loss of telomeres during this extended replicative period is accompanied by a progressive increase in the frequency of telomere fusion events [17, 18]. Ultimately cells enter a state of **replicative crisis** during which extensive telomere fusion drives widespread genomic instability, mitotic arrest, and cell death. Furthermore, telomere crisis was recently associated

with the **cGAS-STING** (cyclin GMP-AMP synthase – Stimulator of interferon genes) pathway, which is involved in sensing of cytosolic DNA fragments and induction of an interferon inflammatory response [19]. The widespread genome instability in crisis led to a sequence of events that culminated in hyperactivation of autophagy via the cGAS–STING pathway and cell death, underlining the role of autophagy in the tumour suppressive crisis mechanisms [20, 21].

Replicative crisis occurs early in the progression to malignancy and is associated with increased genomic complexity [22-28]. Cancers with short telomere length profiles, consistent with telomere dysfunction, confer a poorer prognosis and response to treatment [29-33]. It is therefore important to understand the underlying mechanisms of telomere-driven genomic instability, with a view to providing prognostic, predictive and diagnostic clinical tools, as well as potential therapeutic interventions.

Mutagenic consequences of telomere dysfunction

Telomere fusion occurs between sister chromatids, telomeres of heterologous chromosomes, and non-telomeric DSBs across the genome [7, 8, 34, 35] (Figure 1B). Fusion is primarily mediated by both the classical and alternative non-homologous end-joining pathways (c-, a-NHEJ), with Ligase4 (LIG4) dependent c-NHEJ driving predominantly inter-chromosomal fusion, and Ligase1/Ligase3 (LIG1/LIG3) dependent a-NHEJ driving fusion between sister-chromatids [7, 8, 35-39]. Consistent with a-NHEJ activity, microhomology is observed at telomere fusion junctions, and is accompanied by deletion into telomere-adjacent sequences, which is presumably a consequence of nucleoytic processing prior to fusion [7, 18, 36]. The full extent of sub-telomeric deletion that accompanies telomere fusion has not been defined, although early reports in human cells suggest these events may be extensive [18, 40, 41]. Studies in yeast models demonstrate telomeric resection of many 10s of kbs [42, 43],

which raises the possibility that telomere fusion-associated deletions may lead to the loss of terminal coding sequences.

During cell division, the centromeres of dicentric chromosomes arising from telomere fusion are pulled to opposite poles at anaphase (Figure 2A). Instead of breaking, dicentric chromosomes give rise to persistent DNA bridges that can survive mitosis. These bridges break during the subsequent interphase, leaving daughter cells with broken chromosomes that have lost or gained DNA [44] (Figure 2B-C). Broken chromosomes can be subjected to further fusion events, that can initiate cycles of **breakage, fusion and anaphase bridging (BFB)**[1]. BFB cycles can be halted by ring-chromosome formation, centromeric inactivation or the acquisition of a telomere maintenance mechanism that seeds telomeres *de novo* on DSBs and stabilises the genome. BFB cycling is thought to generate all manner of structural diversity with potentially high copy number states reached after extended cycling.

Inter-chromosomal fusions resulting in dicentric chromosomes are expected to yield terminal deletions or non-reciprocal translocations resulting in amplification. Fusion between sister-chromatids can likewise result in end deletions or produce terminal inverted repeats that can become amplified in sub-sequent rounds of BFB, with large copy number gains possible [45-49]. Thus, BFB cycles are often invoked to explain the large terminal deletions and inverted, amplified repeats commonly observed in cancers.

Chromothripsis and genome catastrophe

The potential of BFB cycling to propagate genome instability and generate genome complexity has long been appreciated [1]. However, over the past decade, the introduction of whole genome sequencing technology has allowed genomic rearrangement patterns to be studied at a genome-wide level with base-pair resolution. This led to a new wave of discoveries revealing that rearrangement patterns in cancer and some congenital disorders can be

astonishingly complex, presenting difficulties in trying to discriminate underlying mechanisms and classes of rearrangement pattern.

In a landmark paper [50], a chronic lymphocytic leukaemia patient was documented with a large network of structural variants, consisting of 42 rearrangements found on the long arm of chromosome 4, but also 9 translocations linking chromosomes 1, 12 and 15. The breakpoints showed clustering on the reference genome, and conspicuous deletions were identified between neighbouring breaks giving an alternating copy number profile between one or two copies. Additionally, the authors found evidence of similar patterns in bone cancer patients, previously published pancreatic cancer data [51], and several cancer cell lines [50]. In one of the analysed cell lines (SCLC-21H), highly amplified double minute chromosomes were found with 50 – 200 copies per cell which occurred along-side highly rearranged normal chromosomes. Double minute chromosomes are circularized DNA fragments that can harbor highly amplified oncogenes, and are common in certain forms of cancer such as glioblastoma [52]. To explain their findings, the authors suggested the term **chromothripsis** (from the Greek "*thripsis*", to shatter) with the hypothesis that affected chromosomes undergo fragmentation or shattering, followed by random re-ligation into the derivative chromosomal arrangement [50]. It was argued that a one-off shattering event could explain the observed copy number states and the seemingly random end-joining patterns, although others have pointed out that a multi-step process, involving progressive rearrangements over time, could also plausibly generate such arrangements and should not be ruled out [53].

In a separate study of patients with congenital deficiencies, rearrangement patterns were found that consisted of a mixture of duplications and triplications, with complex microhomology and insertions at the joins [54]. The authors argued that chromosome shattering was insufficient to explain these features and that a replicative mechanism involving template switching could better accounts for the findings. Thus, the **fork-stalling and template**

switching (**FoSTeS**) and **microhomology-mediated break-induced replication (MMBIR**; Figure 2C) models of genome rearrangement were invoked as the possible underlying mechanism, and the observed pattern was given the name **chromoanasynthesis** to reflect this etiology [54-56].

Building on this work, a separate group found that a high proportion of prostate cancer patients typically harboured chains of structural rearrangements that linked multiple chromosomes, with 88 % of tumours showing a chain of 5 or more rearrangements [57]. The authors argued that the frequency or translocations and the number of chromosomes involved was too high to be compatible with chromothripsis, and so the term "**chromoplexy**" (from the Greek "*pleko*", to weave or braid) was coined. Chromoplexy is thought to result from simultaneous breaks in multiple chromosomes, and random repair gives rise to balanced chains or cycles of breakpoints. The terms chromothripsis, chromoanasynthesis and chromoplexy have also been grouped under the name of chromoanagenesis as a catch-all moniker that is agnostic of the underlying mechanism, although other phrases such as "**genome catastrophe**" have been used to the same effect [58].

Although some progress has been made in understanding these ostensibly differing processes, the underlying mechanisms are incompletely understood and there is yet to be a systematic comparison between categories. Guidelines for the inference of chromothripsis have been proposed which are based on quantifying patterns seen in NGS data such as the density of breakpoint clustering, loss of heterozygosity, randomness of fragment joining and fluctuations in copy number, among other properties [59]. However, especially in cancer samples, multiple genome catastrophe processes may occur in tandem, potentially alongside events such as BFB cycling, chromosomal instability and aneuploidy, making inference of individual processes from sequencing data challenging [54, 57-61]. Recent studies increasingly support the idea that there are several categories of genome catastrophe, although the

underlying mechanisms and end-joining pathways of these events are still being elucidated. Without a clearer understanding of the underlying biology, and to simplify our discussion, we will here use the term, chromothripsis or "genome catastrophe", to refer to these complex events.

Outcomes and prevalence of genome catastrophe

In contrast to the gradual accrual of mutations described by traditional Darwinian evolution, genome catastrophe processes are thought to occur within only a few or perhaps a single cell division. These large-scale events have the potential to result in massive functional alterations to the genome via gene dosage effects from deletions or copy number gains, or changes in the epigenetic and regulatory landscape. Consequently, genome catastrophe events may have important roles in evolutionary processes shaping the germline and somatic tissues. Genome catastrophe occurring in the germline or early during development can lead to acquired or potentially inheritable developmental and congenital conditions, and the incidence of such events may be more common than previously thought [62-64]. There is also the astonishing case of a patient with WHIM syndrome, whose condition was cured by chromothripsis. WHIM syndrome is a dominant combined immunodeficiency disease that is caused by a gain-of-function mutation in the chemokine receptor CXCR4. Chromothripsis in this patient occurred in a hematapoietic stem cell, resulting in deletion of the disease allele, as well as 163 other genes from the same chromosome. This founding cell subsequently repopulated the myeloid lineage, and thus cured the disease [65].

In somatic tissues, genome catastrophe processes can generate clonal diversity and heterogeneity which is the currency of tumour development, and there is a possibility that fragmented chromosomes arising during chromothripsis may trigger inflammatory processes through cGAS signalling [66]. As genome catastrophe may alter genomic structure and

function, most events are probably deleterious to the cell, and consequently survival from such an event may be associated with deficits in cell death pathways, or genome surveillance. A recent large-scale survey of 2658 cancers found that chromothripsis was pervasive, with frequencies above 50 % in certain cancer types [61]. The study also highlighted that chromothripsis exists on a continuum of complexity and can contribute to oncogene amplification and inactivation of tumour suppressors.

Chromothripsis has been associated with poorer prognosis in several cancers including neuroblastoma and acute myeloid leukaemia [61, 62, 67, 68]. However, this was not apparent in a pan-cancer analysis following stratification of patients based on the presence or absence of chromothripsis [61] The association of genome catastrophe with clinical outcomes in cancer is likely to be nuanced and multifaceted, as these processes can potentially be both a cause and an effect of cancer, arising from cancer related genome instability. The size of cancer cohorts with whole genome sequencing data, limits the power to detect clinical associations, however as these datasets expand the full clinical implications of tumours exhibiting genome catastrophe will become apparent.

Emerging mechanisms of chromothripsis and genome catastrophe

Chromothripsis and micronuclei

Micronuclei are extra-nuclear bodies containing large DNA fragments or whole chromosomes that can serve as markers of genome instability and genotoxicity [69]. In cancer, a commonly observed mitotic error is the occurrence of lagging chromosomes which can fail to segregate properly and become partitioned into micronuclei [69-71] (Figure 3). Recent studies have shown that micronuclei play a major role in the generation of chromothripsis and genome instability [70, 72-74].

The occurrence of chromothripsis in micronuclei has been linked to deficits in the micronuclear envelope. Shortages of 'non-core' proteins and nuclear pore complexes (NPCs) potentially leads to DNA breaks through aberrant DNA replication and repair [70, 75, 76]. Additionally, as a proportion of micronuclei replicate asynchronously with the main nuclear compartment, the cell may enter mitosis before micronuclear DNA has finished replicating, which may lead to premature chromosome compaction and DNA breakage [72, 73, 77, 78]. The physical isolation of chromosomes in micronuclei, combined with DNA compaction-driven breakage, offers an attractive mechanism to explain the chromosome "shattering" thought to underlie chromothripsis.

Recent studies show that fragmented chromosomes in micronuclei can sometimes be partitioned evenly into daughter cells, so lost fragments in one daughter cell correspond to gains in the other, which supports a fragmentation model of chromothripsis [72, 79]. Micronuclear-derived chromosomes also show defective kinetochore assembly which interferes with proper segregation, and can lead to the chromosome being maintained within a micronucleus over several generations [80, 81]. Chromosomes trapped within micronuclei may therefore be subject to multiple rounds of pulverisation [73, 80, 81]. Additionally, micronuclei-associated chromosomes can sometimes undergo successful segregation, resulting in reincorporation and retention within the primary nuclear compartment [72, 74, 80], which may explain how chromothriptic chromosomes may sometimes appear as stable entities over multiple cell divisions, or be constitutional in nature [50, 82].

An interesting possibility raised recently, is that repair of fragmented chromosomes within micronuclei is only achieved during the following cell cycle when micronuclei DNA fragments are exposed to repair factors in the primary nucleus [78]. Using a model system of Y-chromosome centromere inactivation to induce miss-segregation into micronuclei, fragmented micronuclear chromosomes, visible by microscopy, could only be detected after one complete

cell cycle, post micronuclei formation. Fragmentation appeared to coincide with re-entry into mitosis, suggesting that premature condensation may either precede, or directly drive fragmentation. The generated chromosomal fragments then persisted until the following cell cycle, and siRNA knockdown experiments suggested fragments were reassembled by the c-NHEJ pathway (Figure 1C) [74, 78]. Recent studies have also inferred the activities of c-NHEJ repair in chromothripsis from the occurrence of blunt end joins or random levels of microhomology at breaks [83].

However, the model of chromosome-shattering in micronuclei is unlikely to account for all genome catastrophe patterns. Difficulties arise when trying to explain the occurrence of more than a few copy number states, complex repair signatures, or the observation that multiple chromosomes are sometimes involved in chromothriptic rearrangement networks, with sometimes only a handful of segments derived from minor chromosomes. A multistage process has been suggested, involving the inclusion of multiple chromosomes in a single micronucleus, potentially combined with multiple rounds of shattering and mixing of fragments to explain more complex rearrangement patterns [74].

Using irradiation models of micronuclei formation, other studies have reported that DNA repair factors may be identified within micronuclei, such as RAD51 (homologous recombination factor), or NBS1 (DSB break repair factor), BRCA1 (tumor suppressor), TP53 and phosphorylated RPA (replication protein A) which binds single stranded DNA [74, 84-86]. The presence of these factors suggest DNA repair in micronuclei may be possible via recombination or replication based pathways such as MMBIR, which could plausibly generate complex join types and copy states associated with some chromothripsis events (Figure 1 C) [72]. There have been several reports showing complex microhomology and templated insertions in chromothripsis, suggesting this mechanism may occur in a subset of micronuclei-associated chromothripsis cases [54, 58, 72].

Genome catastrophe and telomere dysfunction

Alongside progress in understanding the role of micronuclei in chromothripsis, other groups have investigated the possibility that telomere dysfunction is involved. Indeed, it was originally suggested in the first report of chromothripsis that dysfunctional telomeres and BFB cycling may play a role [50]. Several early sequencing studies provided circumstantial evidence that this may be the case, describing the occurrence of "fold-back" inversions – genomic rearrangements that are thought to represent the footprint of a BFB cycle, that often occurred alongside complex genome rearrangements in cancers such as leukaemia, breast, oesophageal and pancreatic [45, 47, 50, 51, 87-89]. In acute lymphoblastic leukaemia patients with large-scale amplification of chromosome 21 (iAMP21 ALL), a remarkable association was uncovered that illustrates how dicentric chromosomes and chromothripsis can contribute to disease [45]. Patients born with a rare constitutional Robertsonian translocation between chromosome 15 and 21, that resulted in a dicentric chromosome, showed a 2,700-fold higher risk for developing iAMP21 ALL. Furthermore, the iAMP21 amplification appeared to result from a chromothripsis event. In sporadic cases of iAMP21 however, copy number profiling and cytogenetic analysis was used to infer that a BFB cycle was typically the initiating event, and this was often followed by chromothripsis to produce the derivative iAMP21 pattern. This study highlights how dicentric chromosomes precipitate genome instability and chromothripsis, resulting in large scale changes in gene dosage and selection of disease traits [45].

In 2015, two studies provided experimental evidence linking telomere dysfunction to chromothripsis [44, 90]. Using an inducible TRF2 (Telomeric Repeat-Binding Factor 2) knockdown model of telomere crisis, these studies identified chromothriptic rearrangements in post-crisis cells. By studying copy number joining patterns, BFB cycling appeared to precede

chromothripsis, and a propensity of chromothripsis to occur in hyperploid cells was identified [90]. Long dicentric chromatin bridges were identified in dividing cells and these sometimes persisted beyond cytokinesis before their eventual rupture [44]. Post-crisis clones were screened for abnormal karyotypes and subsequent whole genome sequence analysis found a high incidence of chromothriptic rearrangements and the phenomenon of kataegis near breaksites [44]. Kataegis refers to the clustering of single nucleotide mutations that are produced by APOBEC (cytidine deaminase) enzymes acting on single-stranded DNA [44, 91]. Furthermore, the authors found evidence that TREX1 (Three Prime Repair Exonuclease 1), a cytoplasmic nuclease, may be responsible for dicentric bridge resolution, offering a plausible mechanism for the chromosome fragmentation thought to occur in chromothripsis [44, 92] (Figure 3B, C). TREX1 was found to localise to chromatin bridges, and knockout cells showed slower bridge resolution and reduced genomic complexity following escape from telomere crisis [44, 79, 92-94]. Interestingly, RPA-coated single stranded DNA was detected at chromatin bridges, and there appeared to be deficiencies in certain nuclear envelope proteins such as NPCs (Nuclear Pore Complexes), Lamins (components of intermediate filaments) A/C and B1 [44]. These observations draw similarities with the nuclear envelope defects associated with micronuclei, and potentially provides a mechanistic link between these processes, where nuclear envelope dysfunction, and perhaps rupture, triggers or is associated with complex genome rearrangement [44, 70].

Following this work, a recent study investigated the consequences of a telomere crisis on the structural integrity of the genome, using a model system based on the expression of DN-hTERT and extended cell culture, to induce telomere erosion and crisis [58]. Sequencing of post-crisis samples again identified large scale rearrangement patterns that were characteristic of previously reported chromothripsis cases. Additionally, fold-back inversions were found near chromothriptic rearrangements further suggesting a role for telomere involvement.

However, contrary to expectations, genome catastrophe occurred when NHEJ pathways were compromised, occurring in knockout lines defective in c-NHEJ (*LIG4*$^{-/-}$), a-NHEJ (*LIG3*$^{-/-}$:*TP53*$^{-/-}$), a double a-NHEJ: c-NHEJ knockout line (*LIG3*$^{-/-}$: *LIG4*$^{-/-}$), and in a LIG3 overexpressing line.

These findings are in line with a recent study that used a *TP53*-deficient murine tumour model, where inactivation of c-NHEJ components (*LIG4* or *XRCC4*), but also in *BRCA2* mice, resulted in the appearance of complex genome rearrangements [95]. Analysing murine tumours, in addition to human glioblastoma, breast cancer and melanoma, the authors found an association between DNA repair deficiency and complex genome rearrangements. Interestingly, mutational "signature 3" was frequently identified in these mouse models, which was also a pattern identified in a separate study in association with chained rearrangements [58, 95]. Signature 3 has previously been associated with abnormal homologous recombination-based repair, and might result from a backup mutagenic repair pathway during genome catastrophe [58, 95, 96]. However, based on analysis of breakpoint microhomologies and insertion profiles, the authors suggested that joining of these complex events was performed by a-NHEJ [95]. The finding that genome catastrophe can occur in the absence of c-NHEJ and a-NHEJ [58] would suggest otherwise, although flexibility in which end-joining pathways are employed is also likely, as is the possibility that complex rearrangements might arise by multiple mechanisms. Together these studies indicate that genome catastrophe can sometimes occur in the absence of NHEJ and raise the possibility that NHEJ may even be protective against certain forms of genome catastrophe (Figure 2).

Also using a TRF2 knockdown model of telomere dysfunction, as well as single telomere-targeted double strand DNA breaks (DSB), a recent study elegantly demonstrated that telomere fusions can lead to a cascade of genome instability and chromothripsis [79]. Using the Look-Seq protocol, cells with dicentric bridges were monitored by microscopy and following bridge

resolution, daughter cells were individually sequenced to investigate genomic rearrangements. Rather than TREX1 being responsible for bridge resolution, actomyosin forces appeared to be the main driver of chromosome breakage (Figure 3B, C). Bridge resolution was initially associated with simple breakage patterns, but also more complex rearrangement patterns were found which were indicative of replicative repair [79] (Figure 3D - F). In the following mitoses, the broken chromosomes were subjected to mitotic DNA replication and further chromothriptic events. Also, dicentric bridge formation was often associated with centromere dysfunction, which increased formation of micronuclei over subsequent cell divisions, further demonstrating how telomere dysfunction leads to progressing genome instability. Further work is required to clarify the apparent discrepancy between the TREX and the actomyosin force mediated bridge resolution mechanisms, and to establish whether these are mutually exclusive, or a continuum of processes by which these aberrant structures are resolved.

Recombination based pathways and genome catastrophe

Several observations support the concept that end-joining in genome catastrophe can occur via recombination-based pathways. These include the apparent independence from NHEJ in certain cases, complex microhomology and insertion profiles at joins, patterns of copy number gains, and the stitching together of short fragments that could arise from template-switching [54, 58, 72, 79, 97]. Recent studies have also reported “bursts” of mitotic DNA replication at the stubs of broken chromosome bridges or damaged telomeres, which may be the de facto event giving rise to some of these rearrangement signatures, potentially occurring in a micronuclei-independent manner [79, 94].

Several distinct HR pathways are recognised and can result in extensive (homologous) or near identical (homeologous) copying of duplex DNA at breaksites [98]. In chromothripsis, DSBs arising from dicentric rupture or breakage in micronuclei, may be incompatible with the

lowest-error HR pathways such as synthesis-depended strand annealing (SDSA). As proposed by several studies, these substrates may instead be processed by error-prone sub pathways such as BIR [54, 58, 72, 79, 97]. These pathways initially proceed through the formation of a Rad51-ssDNA filament which performs homology searching at multiple loci [98]. A DNA strand-invasion reaction then occurs, resulting in the formation of a D-Loop that can have a number of fates depending on the nature of the D-Loop and interactions with various DNA joint molecules [98]. Single-ended DNA that can arise from dicentric bridge rupture, can be processed by the BIR pathway resulting in long-range displacement DNA synthesis, only stopping at the telomere or merging with a replication fork [99]. BIR can be prone to template switching and error prone DNA synthesis, which can be common near the initial break [56, 99, 100], and kataegis has additionally been associated with BIR [101]. Recent work has also described other HR pathways capable of generating complex genome rearrangements such as the **Multi-Invasion-Induced Rearrangements** (**MIR**) pathway, which involves invasion of the ssDNA end into multiple donor DNA molecules to produce simultaneous translocations [102]. MIR is independent of LIG4, the main NHEJ ligase, and is stimulated by the length of homology and physical proximity of the partners. Thus, there may be several recombination-based pathways capable of generating the patterns of genome catastrophe.

In a recent study, complex genome rearrangements arising during telomere crisis were assembled into longer contigs, which revealed a surprising level of complexity in the organisation of rearrangements [58]. Sometimes more than 10 rearrangements were identified within 1-2 kb of sequence, and often showed complex microhomology and insertion profiles, and higher mutation rates than background [58]. The patterns of rearrangement over the reference genome showed deviations from complete randomness, with a tendency for joining to occur within the same genomic locus, or sometimes to occur in coordination with some distant loci, whilst at the exclusion of others. These findings were interpreted as evidence of

either a multistep process, or plausibly as a signature of template switching within localised genomic regions, dictated by the spatial organisation of the genome [58]. Consistent with these findings recent reports have described rearrangement signatures referred to as "Tandem Short Template jumps", or templated-insertions, which were considered to arise via a replicative mechanism [79, 103]. Similarly, complex patterns have been described in patient data, with studies highlighting that very short fragments, complex joins, and copy number gains, are difficult to explain by chromosome shattering alone [61, 79, 97, 100, 103, 104]. Thus, accumulating evidence suggests that replicative repair pathways play an important role in generating complex genome rearrangements.

Concluding Remarks and Future Perspectives

Recent studies employing whole genome sequencing have highlighted the central role of telomere dysfunction in driving genome instability and generating structural diversity. The extent to which telomere dysfunction underlies genome catastrophe is also becoming clearer. Telomere fusion is directly mutagenic, acting across the genome, and can be accompanied by exonucleolytic resection. The resulting dicentric chromosomes may be resolved in several ways leading to the large terminal deletions and inverted, amplified repeats observed in cancers. Importantly, dicentric chromosome formation and resolution is increasingly recognized as a driver of extreme genetic complexity through the process of chromothripsis.

The underlying mechanisms by which telomere dysfunction propagates genome instability and leads to genome catastrophe are starting to be revealed. Future studies will fully dissect out the sequence of events by which just a single dysfunctional telomere can lead to such dramatic mutational events, and what specific repair pathways are involved (see also Outsanding Questions). With the increasing availability of sequencing data from a broad range of cancers it will be of interest to identify signatures of telomere-driven crisis and genome

catastrophe, and evaluate how telomere dysfunction impacts the evolving cancer genome. Additionally, the clinical implications of genome catastrophe need to be refined with larger cohort studies together with long-term clinical follow-up, to understand whether measures of genomic complexity at diagnosis can inform on individual patient prognosis and response to treatment. A further aspect will be to understand the interplay between genomic complexity during crisis and inflammatory signaling via the cGAS-STING pathway *in vivo*.

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Glossary

BFB cycle
A breakage-fusion-bridge cycle begins when a dysfunctional telomere or uncapped chromosome end fuses with another genomic loci to create a dientric chromosome. Dicentric chromosomes can cause problems during cell division, leading to the formation of a chromatin bridge. Rupture of the bridge creates additional free chromosome ends which can undergo further cycles of fusion and bridging.

Replicative Crisis
Cellular state induced by short dysfunctional telomeres, where widespread DNA damage induces autophagic cell death via the cGAS/STING pathway.

cGAS-STING pathway
A component of the innate immune system for sensing cytosolic DNA fragments. Activation of this pathway results in the expression of inflammatory genes.

Chromothripsis
Large-scale genome rearrangement pattern affecting one or more chromosomes, characterized by an alternating copy number profile, and randomized end joining.

Chromoanasynthesis
Used to describe a genome rearrangement pattern with a mixture of copy number states, and joining involving templated insertions and short fragments.

Chromoplexy
Genome rearrangement pattern consisting of cycles of rearrangements that span multiple chromosomes, often showing 'deletion bridges' between breaks on the reference genome.

FoSTeS
Fork stalling and template switching, describes a replicative mechanism for causing complex structural rearrangements.

Genome catastrophe
A colloquialism used to describe the chaotic genome rearrangement patterns seen in cancer and some congenital disorders, which typically consist of dozens to hundreds of breakpoints affecting one or more chromosomes.

Lagging strand
One of two DNA strands found at the replication fork, requiring a slight delay before undergoing replication. Replication of the lagging strand occurs discontinuously in small fragments.

Microhomology mediated break induced replication
A DNA replication-based mechanism which can generate complex genome rearrangements via template switching.

Multi-Invasion-Induced Rearrangements pathway
A pathway involved with processing homologous-recombination by-products, where a single DNA end can invade multiple donors to produce complex rearrangements.

Non-Homologous End Joining
A DNA double strand break repair pathway that joins DNA ends without a requirement for homology.

Telomere
The structure that caps the ends of linear eukaryotic chromosomes.

Telomerase
Enzyme complex that syntheses telomere repeats at the chromosomal terminus to counteract the loss of terminal DNA sequences during replication.

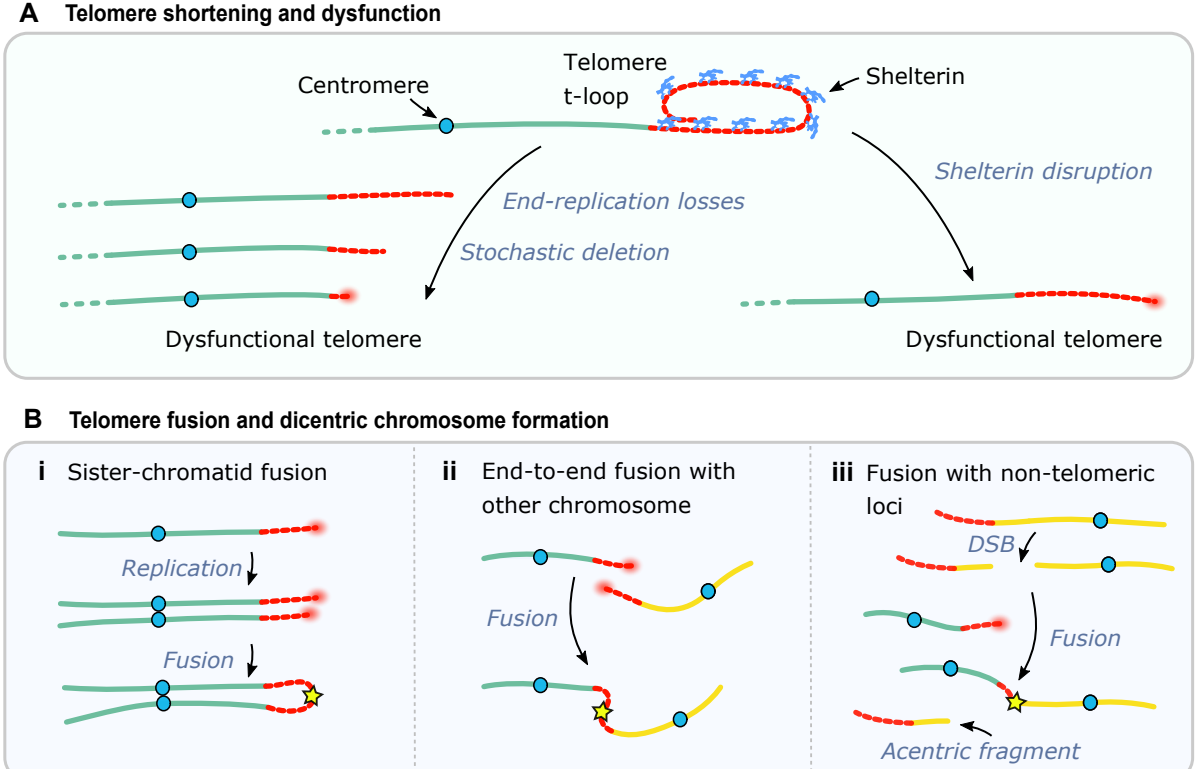
Figure Legends

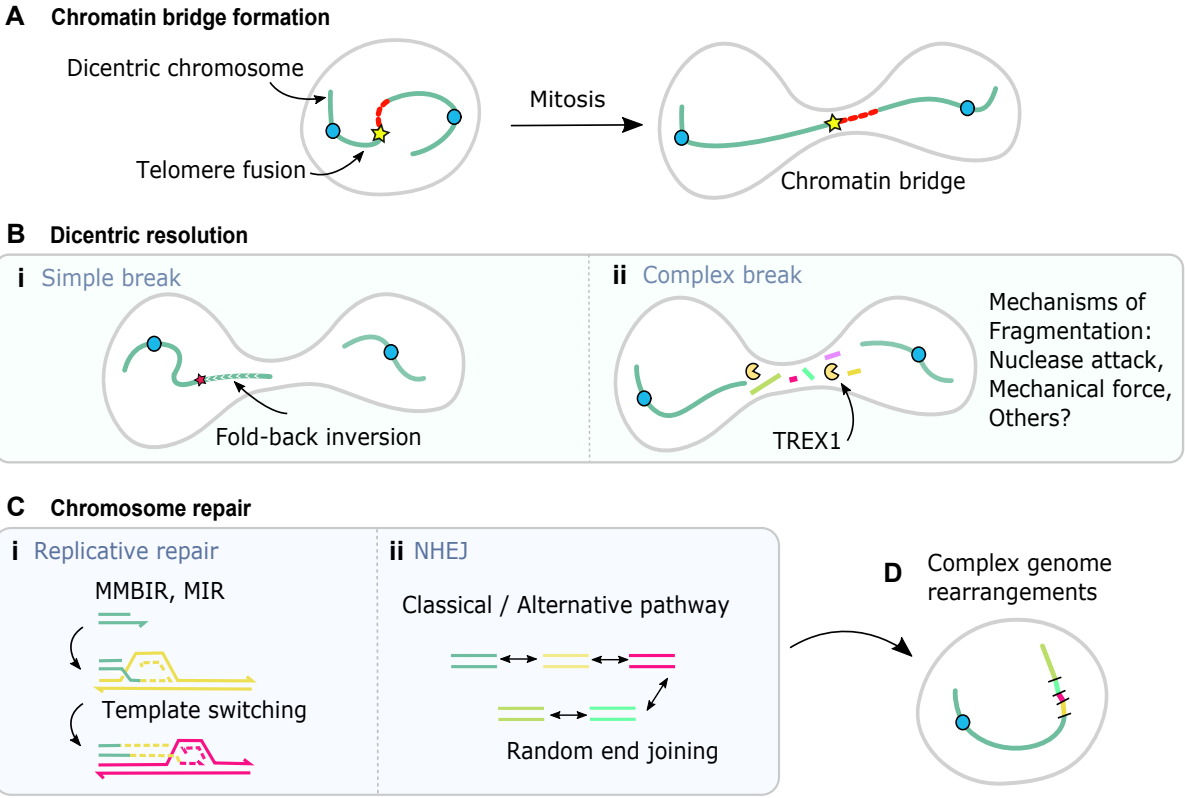
Figure 1. Telomere dysfunction and fusion. (A) Chromosome ends are capped by specialized chromatin structures called telomeres, which consist of a telomere repeat array (labelled red) that is bound by the shelterin complex (blue), assembling into a higher order structure known as a t-loop. Telomeres undergo shortening from progressive end-replication losses or from stochastic deletion events, eventually leading to telomere dysfunction. Alternatively, telomere dysfunction can be experimentally induced by disrupting the shelterin complex. (B) Dysfunctional telomeres can be targeted by DNA repair pathways leading to telomere fusions with a number outcomes. (Bi) Telomere fusions that occur following DNA replication can result in sister-chromtid events. (Bii) Fusions with other dysfunctional telomeres result in end-to-end joining of homologous or non-homologous chromosomes. (Biii) Dysfunctional telomeres can also be joined with non-telomeric loci following an additional DSB, which also generates an acentric chromosome fragment.

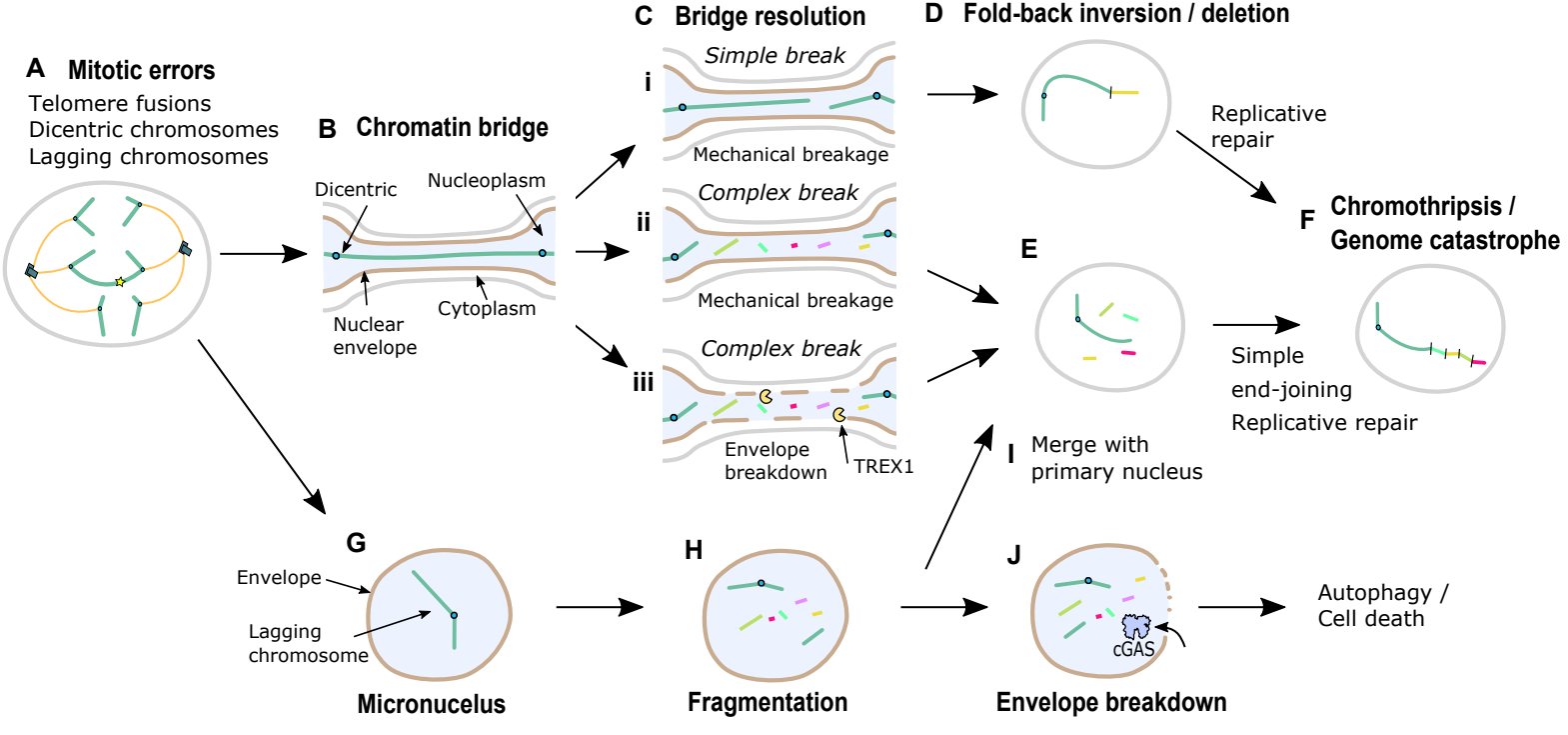
Figure 2. The genomic consequences of telomere dysfunction. (A) Dysfunctional telomeres can fuse with other genomic loci creating dicentric chromosomes. During cell division, migration of the connected centromeres to opposite poles creates a chromatin bridge that can survive mitosis. (B) Chromatin bridges can be resolved by simple DNA breakage leading to a fold-back inversion which is essentially a large inverted duplication (Bi). Alternatively, multiple DNA breaks may be induced simultaneously giving rise to several fragments (Bii). The mechanism of these ‘complex’ breakages may involve the exonuclease TREX1, or be induced by mechanical fragmentation, or other yet to be discovered mechanisms (Bii). Subsequently, resolved chromosomal ends are repaired into a new structure (C). Replicative repair pathways such as MMBIR involve template switching and the inclusion of short templated insertions or duplications (Ci), whereas NHEJ pathways typically result in simpler

joins (Cii). The result of repair is often a series of complex genome rearrangements (D), which are typically analysed by whole genome sequencing.

Figure 3. The emerging pathways and consequences of genome catastrophe. (A) During mitosis, a variety of errors and complications may arise such as the occurrence of dicentric chromosomes from telomere fusions, or lagging chromosomes. (B) Dicentric chromosomes result in the formation of a chromatin bridge which can be resolved in several ways. Mechanical forces from actomyosin may be sufficient to resolve the structure, resulting in simple DNA breakage (Bi), or leading to more extensive chromosome fragmentation (Bii). A competing model argues that the 3′-to-5′ exonuclease TREX1 is involved in bridge resolution, requiring transient nuclear envelope breakdown to mediate resolution (Biii). (D) Simple breakage results in the formation of a fold-back inversion or large terminal deletion, whilst complex breakages leave daughter cells with fragmented chromosomes (E). The broken ends of chromosomes or chromosomal fragments may be targeted by different DNA repair pathways. (E) Simple end joining of fragments by NHEJ pathways may lead to the observed patterns of chromothripsis (F). Alternatively, replicative repair mechanisms acting at the stubs of broken chromosomes, may also result in the complex genome rearrangements associated with chromothripsis (F). (G) Dicentric chromosomes or lagging chromosomes can be partitioned into micronuclei. (H) These structures exhibit various deficiencies that can lead to fragmentation of the entrapped chromosome, either through premature DNA compaction during mitosis, or defective DNA replication and repair. Broken chromosomes within micronuclei can have several fates including subsequent rounds of micronuclei entrapment and DNA breakage in granddaughter cells, or potentially reincorporation and retention within the primary nucleus (I). (J) Alternatively, rupture of the micronuclear envelope can lead to cGAS localization and the activation of autophagy and cell death.


A
Telomere shortening and dysfunction
Centromere
Telomere
t-loop
Shelterin
Shelterin disruption
End-replication losses
Stochastic deletion
Dysfunctional telomere
Dysfunctional telomere
B
Telomere fusion and dicentric chromosome formation
i
Sister-chromatid fusion
Replication
Fusion
ii
End-to-end fusion with other chromosome
Fusion
iii
Fusion with non-telomeric loci
DSB
Fusion
Acentric fragment


A Chromatin bridge formation
Dicentric chromosome
Telomere fusion
Mitosis
Chromatin bridge
B Dicentric resolution
i Simple break
Fold-back inversion
ii Complex break
TREX1
Mechanisms of Fragmentation: Nuclease attack, Mechanical force, Others?
C Chromosome repair
i Replicative repair
MMBIR, MIR
Template switching
ii NHEJ
Classical / Alternative pathway
Random end joining
D Complex genome rearrangements


A Mitotic errors
Telomere fusions
Dicentric chromosomes
Lagging chromosomes
B Chromatin bridge
Dicentric
Nucleoplasm
Nuclear envelope
Cytoplasm
C Bridge resolution
i
Simple break
Mechanical breakage
ii
Complex break
Mechanical breakage
iii
Complex break
Envelope breakdown
TREX1
D Fold-back inversion / deletion
Replicative repair
F Chromothripsis / Genome catastrophe
E
Simple end-joining
Replicative repair
I Merge with primary nucleus
G
Envelope
Lagging chromosome
Micronucleus
H
Fragmentation
J
cGAS
Envelope breakdown
Autophagy / Cell death