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Telomeres in cancer: tumour suppression and genome instability

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Abstract

The shortening of human telomeres has two opposing effects during cancer development. On the one hand, telomere shortening can exert a tumour-suppressive effect through the proliferation arrest induced by activating the kinases ATM and ATR at unprotected chromosome ends. On the other hand, loss of telomere protection can lead to telomere crisis, which is a state of extensive genome instability that can promote cancer progression. Recent data, reviewed here, provide new evidence for the telomere tumour suppressor pathway and has revealed that telomere crisis can induce numerous cancer-relevant changes, including chromothripsis, kataegis and tetraploidization.

Telomeres have an essential role in ensuring that the natural ends of chromosomes are not mistaken for sites of DNA damage. Telomere function depends on three factors: telomeric DNA, the shelterin complex and the telomerase complex (FIG. 1). Human and mouse telomeres are composed of a long double-stranded array of TTAGGG repeats bound by the six-subunit shelterin complex (BOX 1; FIG. 1). Shelterin represses the DNA damage response (DDR) at telomeres, thereby preventing the activation of the kinases ATM and ATR that can induce cell cycle arrest in response to DNA double- strand breaks (DSBs) and other types of DNA damage. In addition, shelterin ensures that telomeres are not processed by several DSB repair pathways, including the non-homologous end joining (NHEJ) pathway that could lead to chromosome end fusions. Shelterin partially protects telomeres by forming the t-loop structure by which the telomere terminus is hidden (FIG. 1). T-loops are formed through strand invasion of the long 3′ overhang at the telomere end into the double-stranded telomeric DNA. This 3′ overhang is recreated after DNA replication through exonucleolytic degradation of the 5’ ends of the telomeres (FIG. 2a). As a result of this processing and the inability of DNA polymerases to duplicate the ends of linear DNA molecules, human telomeres shorten by ~50 bps per cell division. This telomere attrition can be counteracted by telomerase reverse transcriptase (TERT) (FIG. 1), which adds GGTTAG repeats to the chromosomal 3′ DNA terminus at the end of the chromosome.

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Competing interests statement

The authors declare no competing interests.

During human development, telomerase activity is downregulated through the silencing of *TERT*, which encodes the reverse transcriptase subunit of the complex. As a result, most human somatic cells (with the exception of certain stem cells) undergo programmed telomere shortening. Eventually, the loss of telomeric DNA leads to insufficient chromosome end protection and to the activation of the DDR, which will arrest cell proliferation and can induce senescence or apoptosis. The repression of telomerase in somatic cells and the resulting telomere proliferation barrier have the hallmarks of a tumour suppressor pathway that limits tumour cell outgrowth after a delay. New evidence, reviewed below, argues that telomere shortening indeed protects against tumour development. Eventually, however, in incipient cancer cells that lack the pathways necessary for cell cycle arrest, mounting telomere dysfunction becomes a source of genomic instability in a stage referred to as telomere crisis[1–3]. The escape from telomere crisis requires the activation of telomerase, which reconstitutes telomere function and restores proliferative capacity. The outcome of this scenario is a telomerase-positive, transformed cell with a heavily rearranged, but stabilized, genome that has attained new and potentially tumorigenic genetic mutations.

Cancer genomes are characterized by extensive chromosome rearrangements that facilitate oncogenic progression[4]. The unexpected extent and staggering complexity of these rearrangements, which include deletions and amplifications, translocations, chromothripsis, kataegis and tetraploidization, has only been appreciated in recent years[5]. Although there are many potential mechanisms underlying these rearrangements[6], new data have linked telomere dysfunction to a near- comprehensive list of cancer-relevant genome alterations[2,3,7–9], suggesting that telomere crisis contributes to the genetic disorder that is typical of cancer[10]. Here, we review these new data on the role of telomeres in genome instability in cancer and discuss new findings pertaining to the role of telomere shortening in tumour suppression.

Tumour suppression by short telomeres

Telomere shortening in human cells has long been thought to represent a tumour suppressor mechanism. Although mouse models have previously illustrated this potentially advantageous aspect of telomere attrition, recent data now provide evidence for this proliferative barrier in human cancer cells.

Silencing of telomerase and telomere shortening

Telomerase is a reverse transcriptase that synthesizes telomeric DNA *de novo* using integral RNA as the template and the 3′ end of the chromosome as the primer[11–17] (FIG. 1). The core components of telomerase are the reverse transcriptase TERT and telomerase RNA component (TERC), which provides the template for the synthesis of telomeric DNA. Telomerase is associated with a set of accessory proteins, including dyskerin, nucleolar protein 10 (NOP10), non-histone protein 2 (NHP2), GAR1 and telomerase Cajal body protein 1 (TCAB1), that contribute to the biogenesis and trafficking of telomerase inside the nucleus[18,19] (for reviews, see REFS 17,20,21).

TERT silencing downregulates telomerase activity in human somatic cells (reviewed in REF. 22). The other components of telomerase, including TERC, are expressed widely; thus, the

expression of exogenous TERT is sufficient to activate telomerase in many primary human cells[23]. However, *TERC* expression can still be a limiting factor and the co-expression of *TERT* and *TERC* is needed for robust telomerase catalytic activity in some cell types[21,24,25]. Telomerase activation and the resulting telomere-length maintenance leads to the bypass of senescence and ultimately to cell immortalization[23,26,27].

The programmed silencing of *TERT*, loss of telomerase activity and the resulting shortening of telomeres is not a universal phenomenon in mammals. Apparently, this tumour suppressor pathway is restricted to large animals with a reproductive strategy that requires a long lifespan[28]. For example, telomerase activity is repressed in somatic cells of elephants but not in mice.

In the absence of telomerase, each human telomere shortens at a rate of 50–100 bps per population doubling[29]. The rate of telomere attrition is partly due to the inability of DNA polymerases to copy the end of linear DNA (FIG. 2a). The 5' end resection that generates the telomeric 3′ overhang contributes substantially to the rate of telomere shortening[30] (FIG. 2a). Shelterin governs this processing and the formation of the correct structure of the telomere terminus in mouse cells and most likely in human cells. The process involves the initiation of resection by the shelterin-bound Apollo nuclease, further resection by exonuclease 1 (EXO1) and finally a fill-in synthesis step mediated by the shelterin-bound CST (CTC1–STN1–TEN1) complex[31–38] (FIG. 2a). Owing to this regulated terminal sequence loss, the proliferative lifespan of primary human cells (known as the `Hayflick limit`) is partly determined by how shelterin controls resection and fill-in at telomeres. It will be interesting to determine whether the lifespan of human cells can be extended by diminishing the extent of 5' end resection.

Telomere-induced senescence

Loss of telomere function at a few chromosome ends in a cell is sufficient to induce replicative arrest[9,39,40] (FIG. 2b). The point at which telomere attrition results in the loss of telomere protection at one or a few chromosome ends is dependent on the rate of telomere shortening, the initial telomere length and, importantly, the length of the shortest telomeres in the cells[41]. Because human telomeres are heterogeneously sized, several very short telomeres can be present in cells with an apparently ample telomere reserve, which makes measurements of bulk telomere length an imprecise predictor of cellular proliferative potential.

Senescent human fibroblasts display the molecular hallmarks of an activated DDR[39], including ATM and ATR signalling, and nuclear foci containing DNA damage markers, such as γ-H2AX, p53-binding protein 1 (53BP1) and mediator of DNA damage checkpoint protein 1 (MDC1). Upregulation of p53 and induction of the cyclin-dependent kinase (CDK) inhibitors p21 and p16 are also indicators of an activated DDR[42,43]. The inactivation of shelterin similarly activates DNA damage signalling pathways, results in the upregulation of p21 and p16, and leads to the accumulation of DDR factors at telomeres, thus linking telomere dysfunction and senescence[39,42]. Proof that replicative senescence is due to telomere shortening came from the bypass of senescence upon *TERT* expression[23]. Furthermore, over-expression of the shelterin subunit telomeric repeat-binding factor 2

(*TRF2*; also known as *TERF2*) can delay the onset of senescence[44], arguing that the DDR in senescence is due to an insufficient loading of shelterin at the shortened telomeres.

Senescent cells are usually in G1 phase, consistent with p53 activation and induction of the CDK inhibitors p21 and p16. The upregulation of p16 and the accompanying hypophosphorylation of the tumour suppressor RB can contribute to telomere-induced senescence. Moreover, inactivation of both the RB and the p53 responses to dysfunctional telomeres is needed to completely circumvent this block to proliferation[43]. Because the complete bypass of telomere shortening-induced senescence in human cells requires the inactivation of multiple pathways, this mechanism of curbing the proliferation of transformed cells is likely to be robust[43,45–48]. By contrast, p53 inactivation alone is sufficient to avoid telomere-induced senescence and apoptosis in mice[49,50].

Telomere shortening, telomerase downregulation and cancer prevention

Experiments in genetically altered mice support the view that telomere shortening can act as a strong barrier to tumorigenesis (FIG. 2b). Crosses of telomerase-deficient mice with various tumour model mice have demonstrated that critically short telomeres limit tumour formation when the p53 pathway is functional[51–55]. In addition, the original demonstration that telomerase is active in most human cancers, whereas the enzyme is undetectable in normal tissues, suggested that the telomere tumour suppressor pathway may operate in most cancer types[56]. However, the upregulation of telomerase expression could be an irrelevant consequence of transcriptional rewiring during tumorigenesis, perhaps reflecting a stem cell phenotype in cancer. The recent identification of activating mutations in the *TERT* promoter in several cancer types argues strongly that these tumours had undergone selection for the activation of telomerase[57,58]. Similarly, the amplification of *TERT* and other mutations that increase telomerase activity in some cancers argue in favour of a selected phenotype[59–61].

A strong argument in support of the telomere tumour suppressor pathway emerged recently from a study of a large family with a predisposition to melanoma[58]. A linkage analysis and high-throughput sequencing identified an activating mutation in the *TERT* promoter that co-segregates with disease predisposition. This mutation (T>G; 57 bp upstream of the transcription start site) creates a binding motif for ETS (E26 transformation-specific) transcription factors. Thus, tissues that express ETS transcription factors are predicted to maintain telomerase activity, presumably resulting in the maintenance of telomere length and the consequent disruption of the telomere tumour suppressor pathway.

A similar example is provided by the recently identified melanoma-predisposing mutations in the gene encoding the protection of telomeres 1 (POT1) sub unit of shelterin[62,63]. These mutations alter *POT1* mRNA splicing or compromise the oligonucleotide and/or oligosaccharide-binding folds in the single-stranded DNA-binding domains of POT1. As a consequence, the ability of POT1 to bind to single-stranded telomeric DNA is diminished. Carriers of these mutations have longer telomeres, presumably owing to the loss of POT1-mediated inhibition of telomerase (BOX 1). Because the increased telomere length is present at birth, these mutations are likely to push the onset of senescence to later population doublings, thus postponing the tumour-suppressive effects of telomere attrition to a point of

irrelevance. However, whether the diminished POT1 function also promotes genome instability is currently unknown[64].

Another observation in support of the telomere tumour suppressor pathway is that longer telomere length has been associated with an increased risk of B cell lymphoma and chronic lymphocytic leukaemia (CLL)[65–67]. In a recent study, single-nucleotide polymorphisms in telomere maintenance genes that are associated with telomere length[68,69] were examined to determine the cancer risk of 95,568 individuals from the general population[70]. This analysis found that genetic determinants of long telomeres are associated with an increased overall cancer risk, especially lung cancer and melanoma. Collectively, these data are consistent with telomere shortening functioning as a tumour suppressor pathway.

Telomere crisis and genome instability

Although the telomere tumour suppressor pathway may be a powerful mechanism to limit cancer development, failure of transformed cells to undergo senescence can produce telomere crisis, during which the cell population does not expand. In telomere crisis, cells struggle with a high level of genome instability owing to the presence of many dysfunctional telomeres. Activation of telomerase provides a path out of telomere crisis, ultimately leading to the formation of a cancer clone with a heavily rearranged genome (FIG. 3).

Continued growth past the senescence barrier can occur in cells that lack the p53 and RB tumour suppressor pathways, rendering their cell cycle transitions impervious to inhibition through ATM and ATR signalling. Continued telomere shortening eventually leads to cells with numerous dysfunctional telomeres, thereby increasing the chance that one dysfunctional telomere becomes fused to another. Consequently, cells in telomere crisis have end-to-end fused `dicentric chromosomes`, which lead to mitotic mis-segregation and genomic instability. Cells in telomere crisis undergo frequent cell death. A common assumption is that this loss of viability is driven by chromosome breakage and mis-segregation, although it may also involve additional telomere deprotection during an extended mitotic arrest that occurs in some of the cells[71,72].

Mammalian cells can use two types of end-joining pathways to repair DSBs: classical NHEJ (c-NHEJ) and alternative NHEJ (alt-NHEJ)[73,74]. c-NHEJ relies on the Ku70–Ku80 heterodimer and DNA ligase 4 and can either be accurate or result in small deletions. By contrast, alt-NHEJ, which is mediated by poly(ADP- ribose) polymerase 1 (PARP1) and DNA ligase 3, creates insertions and more extensive deletions. Telomere fusions formed during telomere crisis in cultured cells are mediated by alt-NHEJ and exhibit insertion of new sequences at the fusion point[75,76]. Similarly, alt-NHEJ has been implicated in telomere fusion in human cancer[77,78] and in telomere fusions in mouse models[79]. By contrast, when telomeres are compromised through the loss of TRF2, their repair is carried out by c-NHEJ[80–82]. The reason for this difference is not yet clear.

Genome instability in cells undergoing telomere crisis was initially found to give rise to chromosome gains and losses (aneuploidy), translocations, gene loss (manifested as loss of heterozygosity (LOH)) and regional amplification through breakage–fusion–bridge (BFB)

cycles[1,80,83]. However, it has recently become clear that the repertoire of genomic alterations that can be ascribed to telomere crisis is more extensive and includes whole-genome reduplication, chromothripsis and kataegis[3,8,9].

BFB cycles and their associated chromosomal rearrangements

BFB cycles, first observed more than half a century ago by Barbara McClintock[84], can occur when dicentric chromosomes (including those formed by telomere fusion) break, followed by a second fusion of the broken ends in the daughter cell[85] (FIG. 4a). Telomere fusions can occur between different chromosomes or between sister chromatids after DNA replication, thus leading to different outcomes[86]. Collectively, BFB cycles can lead to three outcomes that are pertinent to cancer: LOH, non-reciprocal translocations and gene amplification.

LOH, which is frequent at cancer-relevant loci, can occur when a dicentric chromosome breaks and one of the daughter cells inherits a chromosome with a terminal deletion (FIG. 4b). Non-reciprocal trans-locations could arise when the DNA end of a broken chromosome invades another chromosome and copies part of this chromosome through a process called `break-induced replication`[87,88]. Non-reciprocal trans-locations occur during tumorigenesis in mice with shortening telomeres[1] and are a frequent class of rearrangements in cancer[89]. Sequence analysis of more than 1,000 telomere fusion events has shown that a chromosome end lacking telomere protection can recombine with diverse chromosome-internal loci[90].

Gene amplification can result when the telomere fusion event involves sister chromatids, thus creating a large palindrome (FIG. 4b). Subsequent asymmetric breakage of such an isochromosome and multiple BFB cycles can then generate amplicons that are organized in inverted repeats[91]. BFB cycles have been demonstrated to initiate gene amplification in human cancer cells and in hamster cells[92–94]. Moreover, the inverted amplicon arrangements that are typical of BFB cycles have been observed in many cancer types, including pancreatic cancer, oesophageal cancer, breast cancer and leukaemias[91,95–98].

Chromothripsis

Recently, chromothripsis was shown to be one of the outcomes of experimentally induced telomere crisis. Chromothripsis is a mutagenic process whereby one or more chromosomal regions undergo catastrophic shattering in a single event, followed by an apparently haphazard repair of the DNA fragments. This process results in genomes in which one or few chromosome segments are affected by tens to hundreds of genomic rearrangements[99]. Chromothripsis has been observed in diverse tumour types, especially those with p53 loss[100,101], and several studies have noted an association between BFB cycles and chromothripsis[91,98,102]. Consistent with these associations, chromothripsis was demonstrated to be the result of telomere crisis induced by the inactivation of the shelterin subunit TRF2 in p53-deficient and RB-deficient epithelial cells[3]. This study used live-cell imaging to determine the fate of dicentric chromosomes formed during telomere crisis and showed that dicentric chromosomes do not break during mitosis. This finding was in agreement with work in yeast cells and a subsequent analysis in human cells[3,103,104] (FIG. 5a). These dicentric chromosomes invariably persist through mitosis and form long

chromatin bridges that connect the daughter cells well into the next G1 phase[3]. These chromatin bridges contain a nuclear envelope that is contiguous with the nuclear envelop of the connected nuclei. However, when chromatin bridges are formed, there is frequent rupture of the nuclear envelope of the connected nuclei, resulting in mixing of the nuclear and cytoplasmic contents. Spontaneous nuclear envelope rupture has been observed in cancer cell lines and is frequent in micronuclei[105,106]. It is not clear how chromatin bridges induce nuclear envelope rupturing, but lamin depletion from the nuclear envelope may have a role as lamin B1 overexpression suppressed the ruptures, and lamin depletion can promote envelope rupture in cancer cell lines[3,105]. A second source of nuclear envelope rupture may be the deformation of the two nuclei connected by the stretching chromatin bridge. The dicentric chromosome in the bridge seems to exert pulling forces on the nuclear envelope, perhaps because it is attached to the nuclear lamins[107]. In support of this view, two recent studies have shown that cell migration through tight constrictions induces nuclear envelope rupture in the squeezed nuclei[108,109].

After persisting for many hours, the chromatin bridges are resolved by 3′ repair exonuclease 1 (TREX1) (FIG. 5b), a highly abundant and widely expressed 3′ exonuclease that degrades DNA species in the cytoplasm[110–113]. TREX1 seems to gain access to the chromatin bridge during nuclear envelope rupture[3]. The enzyme may preferentially attack the DNA in the chromatin bridges because they lack the nucleosomes that normally repress TREX1 activity[3]. The exonuclease creates extensive single-stranded DNA in chromatin bridges, leading to an accumulation of the single-stranded DNA-binding protein replication protein A (RPA) on the bridges (FIG. 5b). The nicks in the double-stranded DNA that allow TREX1 to initiate resection were shown to exist in chromatin bridges[114], but their source is unknown. Eventually, TREX1 digestion is thought to resolve the chromatin bridge once the resection of the Watson and Crick strands of the DNA converges.

Following chromatin bridge resolution, the remnants of the dicentric chromosome are reincorporated back into the nuclear genome, but continue to be marked by the presence of RPA, indicating that single-stranded DNA persists[3]. As the RPA mark usually dissipates within one cell cycle, repair of the fragmented dicentric chromosome is presumed to occur during this period[3]. The exact repair pathways that are responsible for generating the chromothriptic product have not yet been determined.

Fragmentation of chromatin bridge DNA by TREX1 could explain the regional DNA breaks that are typical of chromothripsis because only the portion of the dicentric chromosome residing inside the chromatin bridge is attacked by TREX1. Moreover, the repair of these fragments in the primary nucleus is consistent with the catastrophic, but localized, rearrangements that are observed in chromothripsis (FIG. 5c).

The observation that dicentric chromosomes persist through mitosis intact, suffer extensive fragmentation and give rise to chromothripsis is not in conflict with a telomeric origin of BFB cycles. Chromatin bridges can be resolved even in the absence of TREX1, indicating that other mechanisms are at work[3]. Potentially a TREX1-independent pathway for bridge resolution could involve a nuclease that makes a single DSB and does not fragment the chromatin in the bridge. Such a broken dicentric chromosome could initiate BFB cycles by

fusing the broken end with another broken end or, after DNA replication, by fusing with the sister chromatids. The genome rearrangements induced through this pathway may become clear from the analysis of genome instability in TREX1-deficient cells progressing through telomere crisis.

Another source of chromothripsis in cancer may be chromosomes that mis-segregate into micronuclei. The chromosome in a micronucleus experiences DNA damage, extensive fragmentation and subsequent repair, leading to shattering of the entire chromosome[115,116]. In addition, it was recently shown that simultaneous TRF2 depletion and inhibition of the spindle assembly checkpoint kinase MPS1 can also result in chromothripsis, but the precise molecular pathway is not clear[117,118].

Kataegis

Chromothripsis induced by experimental telomere crisis is often accompanied by kataegis (FIG. 5c). Kataegis is a hypermutation pattern of clustered C>T and C>G changes at TpC dinucleotides[119]. Kataegis is thought to result from the activity of the apolipoprotein B mRNA-editing catalytic subunit (APOBEC) family of enzymes[120–122], which can deaminate cytosine residues to generate uracil, and therefore act as mutators[123]. Many APOBECs are active in the cytoplasm, where they restrict RNA and DNA virus infection, most notably HIV, and other parasitic genomes, thereby contributing to an innate retroviral defence[124]. APOBECs preferentially target single-stranded DNA, and can produce a cluster of strand-coordinated mutations that affect cytosine bases in the same (Watson or Crick) strand. Consistent with APOBEC activity, kataegis is found at the breakpoints of chromothriptic rearrangements created by telomere crisis[3]. A possible explanation for this observation is that the extensive single-stranded DNA that accumulates following TREX1-mediated resection serves as a substrate for APOBEC deaminases.

Telomere-driven tetraploidy

Finally, telomere crisis can induce tetraploidization (doubling the set of chromosomes)[8,9], which is inferred to be a frequent event during the development of human cancers[125]. Many human tumour cell lines have a near-tetraploid or `hyper-triploid karyotype`, which is indicative of past tetraploidization[125]. Tetraploidization can promote tumorigenesis[9,125–133], and tetraploid cells have a high tolerance of chromosome mis-segregation and resilience to chromosomal instability[134].

Tetraploidization can be induced in cells that lack the p53 and RB pathways, which have a high load of dysfunctional telomeres[8,9] (FIG. 6). The mechanism of tetraploidization involves persistent ATM-dependent and/or ATR-dependent signalling induced by irreparably damaged telomeres. This signalling leads to a prolonged G2 phase and ultimately a bypass of mitosis and entry into a G1-like state. A second S phase then results in whole-genome reduplication and tetraploidy. Tetraploidization is observed following experimental inactivation of shelterin and in p53-deficient and RB-deficient human cells undergoing telomere crisis.

The prevalence of telomere crisis in cancer

Telomere crisis may be a frequent event during the development of human epithelial cancers, which initially lack telomerase. Shorter telomeres are frequently observed in cancer relative to their adjacent normal tissue[135–142]. Anaphase bridges, which can be formed by telomere– telomere fusion, have been observed in human cancer samples, including in early-stage colorectal tumours[52]. However, a consideration of anaphase bridges may overestimate the level of telomere dysfunction because they could also result from other defects, including errors in DNA decatenation[143] and cohesin resolution[144].

Telomere crisis in breast cancer has been particularly well documented. An analysis of genome instability and other features associated with telomere crisis, including anaphase and chromatin bridges, suggests that transition through telomere crisis in breast cancer occurs during progression from usual ductal hyperplasia (UDH) to ductal carcinoma *in situ* (DCIS)[145]. This phenomenon is consistent with the higher rate of chromosome aberrations in DCIS than in UDH[146], the shortened telomeres found in DCIS[147] and the activation of telomerase in DCIS[148].

Methods to directly detect the scars of prior telomere crisis in cancer genomes have now been developed[75,149,150]. The telomere–telomere fusions that are typical of telomere crisis can be detected with a PCR-based assay that uses correctly oriented primers situated in the subtelomeric DNA of two (or more) chromosome ends[75,150]. Using this approach, evidence for past telomere crisis has been obtained in CLL as well as in breast cancer, colorectal adenomas and other solid tumours[75,77,78]. In colorectal cancer, telomere fusion occurs during the adenoma–carcinoma transition and may also be present before the occurrence of most somatic mutations[78]. These studies have also revealed a prognostic value to stratifying patients according to the length of the shortest telomeres — as determined by a PCR assay that measures individual telomere lengths — and the likelihood that telomere fusions will take place[151,152]. In CLL and invasive ductal carcinoma of the breast, overall survival is shorter when the telomeres are in a size range expected to result in telomere fusions[151,152].

Telomerase activation

Telomerase activation is often accomplished through mutations in the *TERT* promoter[57,58]. These mutations are the most common mutations in non-coding sequences in cancer and are found in a long, and almost certainly growing, list of cancers[153–158]. Similar to the inherited *TERT* promoter mutations, the sporadic mutations (–57A>C, –124C>T and –146C>T) occur near the transcription start site where they create *de novo* binding sites for ETS transcription factors. An analysis of these mutations in urothelial cancers showed that they are correlated with higher levels of TERT mRNA and protein levels and enzymatic activity and with greater telomere length[159]. In glioblastomas, sporadic *TERT* mutations were shown to activate transcription by enabling the recruitment of the transcription factor GA-binding protein α-chain (GABPA)[160]. Introduction of these mutations into embryonic stem cells prevented *TERT* silencing upon differentiation and resulted in increased telomerase activity that counters telomere shortening[25].

TERT promoter mutations are not the only mechanism by which telomerase activity can be restored or enhanced. In neuroblastoma, telomerase activity is increased by recurrent genomic rearrangements that pair the *TERT* coding sequence with strong enhancer elements, thereby defining a subgroup of patients with poor prognoses[60]. However, in many human cancers, the mechanism by which telomerase is upregulated is yet to be determined. Furthermore, although most human cancers (~90%) escape telomere crisis by activating telomerase[56], a significant minority of cancers use an alternative telomere maintenance system, referred to as `alternative lengthening of telomeres` (ALT)[161]. ALT is associated with mutations in the chromatin remodeller α-thalassaemia/mental retardation syndrome X-linked (*ATRX*) both *in vitro* and in several human cancers, including glioblastomas[162–165]. The observations that *TERT* promoter mutations are usually mutually exclusive with mutations in *ATRX* support the notion that ALT may provide a telomerase-independent escape from telomere crisis[166,167].

Telomere dysfunction following telomerase activation

The types and severity of genome instability induced by dysfunctional telomeres can vary between transient and persistent telomere dysfunction. In mouse models, continued telomere dysfunction can constrain cancer progression, whereas telomerase reactivation alleviates intratumoural DNA damage and leads to more aggressive tumour progression and metastasis[168].

However, some telomere dysfunction may persist even after telomerase activation and exit from telomere crisis. Changes in telomere length can occur owing to stochastic telomere loss, as has been demonstrated in squamous cell and bladder cell carcinoma cell lines[169,170], and ongoing telomere dysfunction has been found in cancer cells with ALT[162,171]. Telomere loss at individual chromosome ends is sufficient to produce many of the rearrangements seen in telomere crisis and in cancer, including amplifications, LOH, translocations, chromosome non-disjunction during mitosis, and the formation of isochromosomes and ring chromosomes[170,172]. Even limited telomere dysfunction can wreak havoc on the genome because instability at individual chromosome ends can be transferred to other chromosomes through non-reciprocal translocations[172,173]. Telomeres in human cancer are often shorter than in normal tissues[135]. It is possible that this setting of short telomere length reflects selection for a telomere length distribution that affords a low level of genome instability without diminishing cell viability.

Perspectives

An attractive feature of telomere crisis as a source of genome instability in cancer is its transient nature. A mutator phenotype is favoured when extrinsic or intrinsic forces demand the generation of variants. This process can enable cancer cell populations to adapt rapidly to new challenges presented by shifting environments. However, persistence of a mutator phenotype comes at a cost because most mutations are deleterious to cellular fitness. The brief, or at least transient, episode of genomic instability offered by dysfunctional telomeres avoids a persistent mutator phenotype that might hamper cell proliferation[10]. Ultimately,

telomerase reactivation provides a route out of telomere crisis, stabilizing the genome and rescuing cellular fitness.

Although much has been learned in recent years about the role of telomere crisis in cancer development, many basic questions remain. The current list of known genome rearrangements in cancer that follow telomere crisis is probably not comprehensive. For example, telomere crisis may contribute to `chromoplexy`, in which chains of translocations link several chromosomes in a temporally constrained event[174]. Bioinformatic methods to detect the remnants of telomere–telomere fusions in entire cancer genome sequencing data sets need to be developed to fully understand the relationship between chromosome fusion events and consequent chromosome rearrangements. Future studies will help to reveal the mechanisms underlying the complexity of the cancer genome, and with continued investment, these insights may be translated into valuable prognostic indicators and more effective treatments.

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Glossary

ATM
A PI3K–related protein kinase that initiates the response to double-strand breaks, with crucial roles in cell cycle regulation and DNA repair.

ATR
A PI3K–related protein kinase that responds to the formation of single-stranded DNA, with a crucial role in the response to replication stress and double-strand breaks.

Non-homologous end joining
A major double-strand break repair pathway that does not rely on sequence homology and can result in small insertions and deletions at the site of repair.

Hayflick limit
The finite proliferation potential of primary human cells.

Dicentric chromosomes
Abnormal chromosomes with two centromeres that can result from telomere–telomere fusion.

Break-induced replication
An origin of replication-independent replication restart that is initiated by the invasion of resected DNA into homologous sequences.

Micronuclei
Abnormal, small nuclei containing one or more chromosome (fragments);. often formed as a result of mitotic chromosome segregation defects.

Lamin
An intermediate filament protein that imparts structural rigidity to the nucleus by assembling into a meshwork at the inner nuclear membrane.

Hyper-triploid karyotype
A genome that contains more than three (3N) but less than four (4N) sets of chromosomes.

Anaphase bridges
DNA bridges that connect chromatin masses undergoing separation during anaphase and can be observed with conventional DNA staining techniques.

Usual ductal hyperplasia
A benign overgrowth of cells that line the ducts or milk glands and is associated with an elevated risk of breast cancer.

Ductal carcinoma *in situ*
A noninvasive, early form of breast cancer characterized by proliferative, malignant cells that are confined to the milk duct.

Alternative lengthening of telomeres
A telomere lengthening mechanism that relies on homologous recombination-mediated DNA copying to counteract telomere shortening.

Chromoplexy
A class of complex DNA rearrangements frequently observed in prostate cancer, which is characterized by multiple chromatin rearrangements that arise in a highly interdependent manner.

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Box 1 | The shelterin complex and its functions

Shelterin is a complex comprising the following six subunits: telomeric repeat-binding factor 1 (TRF1), TRF2, repressor/activator protein 1 (RAP1), TRF1-interacting nuclear factor 2 (TIN2), TPP1 (also known as adrenocortical dysplasia protein homologue) and protection of telomeres 1 (POT1) (see the figure). Shelterin associates with mammalian telomeres, where it regulates various aspects of telomere function[175–177]. Shelterin is recruited to telomeres through TRF1 and TRF2, which bind to double-stranded telomeric DNA and to TIN2. POT1 binds to single-stranded telomeric DNA and is linked to TRF1 and TRF2 through its binding partner TPP1, which associates with TIN2. RAP1 associates with TRF2.

Shelterin maintains telomere length and preserves genome integrity by regulating the access of telomerase to chromosome ends by controlling end-resection at newly replicated telomeres, and by masking telomeres from the DNA damage response (DDR). Specifically, TRF2 represses ATM-dependent DNA damage signalling and classical non-homologous end joining (c-NHEJ), whereas POT1 is responsible for repressing ATR signalling and cooperates with RAP1 in suppressing homologous recombination. Avoiding the DDR is partially mediated by TRF2-dependent t-loop formation. T-loops are formed through the invasion of the 3′ overhang at the telomere end into double-stranded telomeric DNA (FIG. 1), and is thought to prevent ATM activation by masking the chromosome end from the double-strand breaks sensor complex MRE11-RAD50-NBS1 (MRN) and by blocking c-NHEJ induction by preventing the loading of the Ku70-Ku80 heterodimer on the chromosome end. Repression of ATR signalling by POT1 involves occlusion of the single-stranded DNA sensor replication protein A (RPA). Importantly, this repression depends on the association of POT1 with the rest of shelterin via its interaction with TPP1. Telomere protection is compromised when telomeres become too short to support sufficient shelterin binding.

Shelterin also functions to facilitate telomere maintenance by the reverse transcriptase complex telomerase (FIG. 1), which is recruited to telomeres by the shelterin components TPP1 (REFS 178–181) and TIN2 (REFS 182,183). Shelterin also has a role in the regulation of telomerase-mediated telomere length maintenance (reviewed in REF. 184). Several shelterin subunits are negative regulators of telomere length, suggesting that shelterin subunits ‘count’ telomeric repeats to regulate telomerase activity and limit telomere length as part of a *cis*-acting negative-feedback loop (reviewed in REF. 185). This regulatory pathway may be important in the germ line, in which telomere length needs to be maintained within a narrow range to provide offspring with telomeres that are sufficiently long for normal development and tissue homeostasis, whereas at the same time are sufficiently short to suppress cell transformation by inducing replicative senescence.

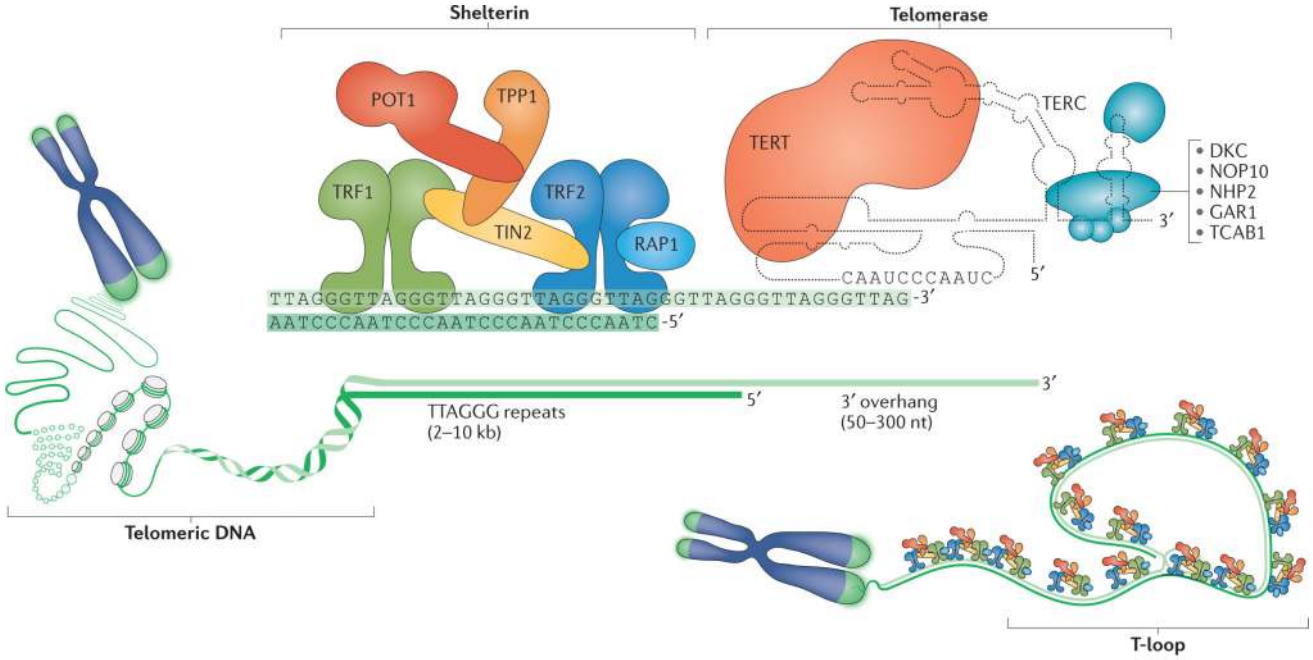

Figure 1. Composition and structure of the human telomere system

Human telomeres comprise three components: telomeric DNA, the shelterin complex and the telomerase complex. Telomeric DNA consists of a long array of double-stranded TTAGGG repeats that culminates in a 50–300 nucleotide (nt) single-stranded 3′ overhang. This 3′ overhang invades double-stranded telomeric repeats to form a t-loop structure that is crucial for telomere function. Telomeric DNA protects chromosome ends through its association with the six-subunit shelterin complex. The length of telomeric repeats can be maintained by telomerase, which is composed of telomerase reverse transcriptase (TERT), telomerase RNA template component (TERC) and several accessory proteins (blue). TERT synthesizes telomeric DNA *de novo* using TERC as a template, whereas the accessory factors contribute to the biogenesis and nuclear trafficking of telomerase. DKC, dyskerin; NHP2, non-histone protein 2; NOP10, nucleolar protein 10; POT1, protection of telomeres 1; RAP1, repressor/activator protein 1; TCAB1, telomerase Cajal body protein 1; TIN2, TRF1-interacting nuclear factor 2; TRF, telomeric repeat-binding factor.

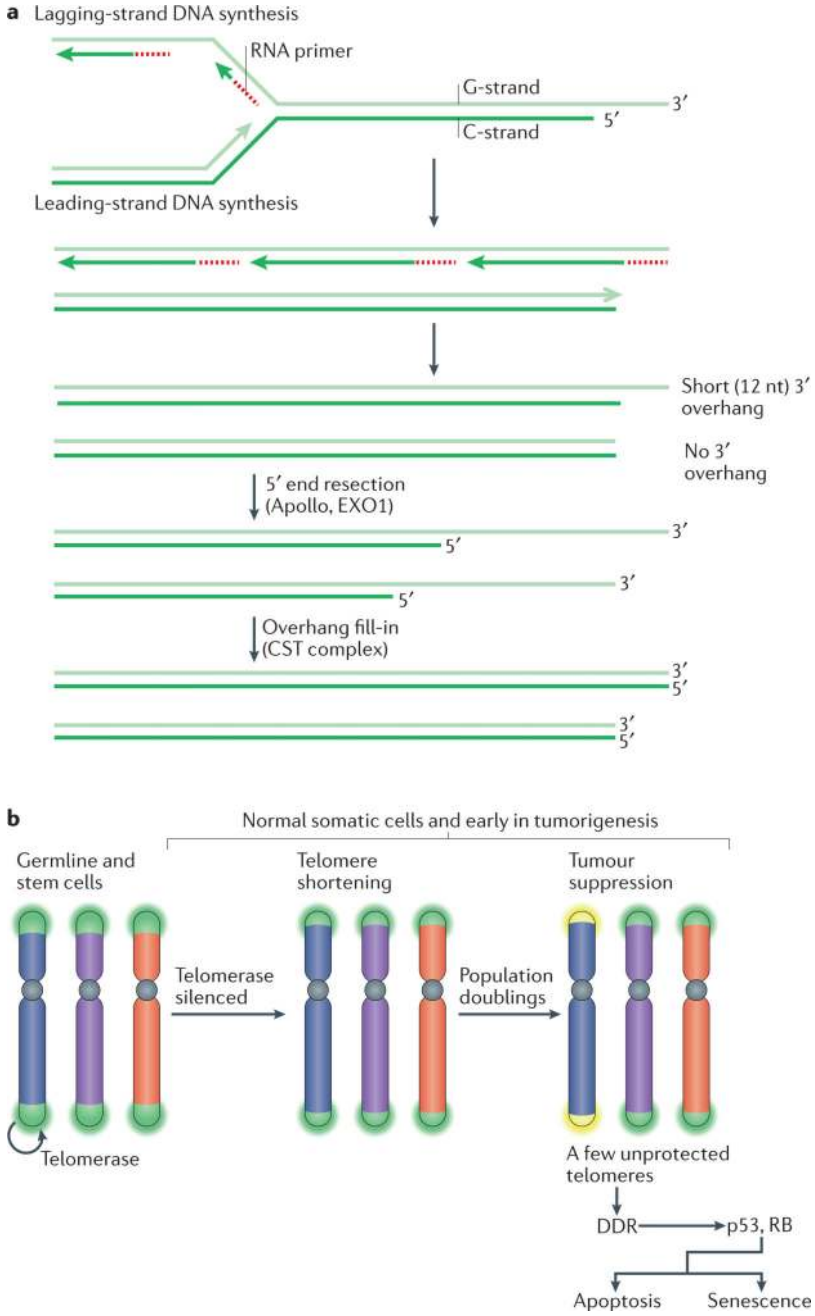

Figure 2. Telomere shortening as a barrier to tumorigenesis

a | The molecular basis of telomere shortening. Incomplete DNA synthesis at the end of the lagging strand (at the site of the terminal RNA primer) leaves a short 3′ overhang. Additional loss of telomeric DNA occurs through the processing of the leading-strand ends of telomeres to regenerate the 3′ overhang, which is necessary for t-loop formation and the structural integrity of the telomere. This process is carried out by the nuclease Apollo, which is bound to telomeric repeat-binding factor 2 (TRF2). Both the leading end and the lagging end of telomeres are further resected by exonuclease 1 (EXO1) to generate transient long overhangs. The CST (CTC1–STN1–TEN1) complex then binds to shelterin and mediates

fill-in synthesis of the cytosine-rich strand (C-strand) at both ends. **b** | During development, telomerase is switched off through telomerase reverse transcriptase (*TERT*) silencing. As a result, telomeres experience the gradual attrition described in part **a**. After numerous population doublings, a few telomeres become too short (yellow) and lose their protective function. As a result, the kinases ATM and ATR are activated at the unprotected chromosome ends and this DNA damage response (DDR) signalling induces replicative arrest and senescence or apoptosis. This process limits the proliferative capacity of incipient cancer cells, thus functioning as a tumour suppressor pathway. Cells lacking p53 and RB function can avoid this replicative arrest.

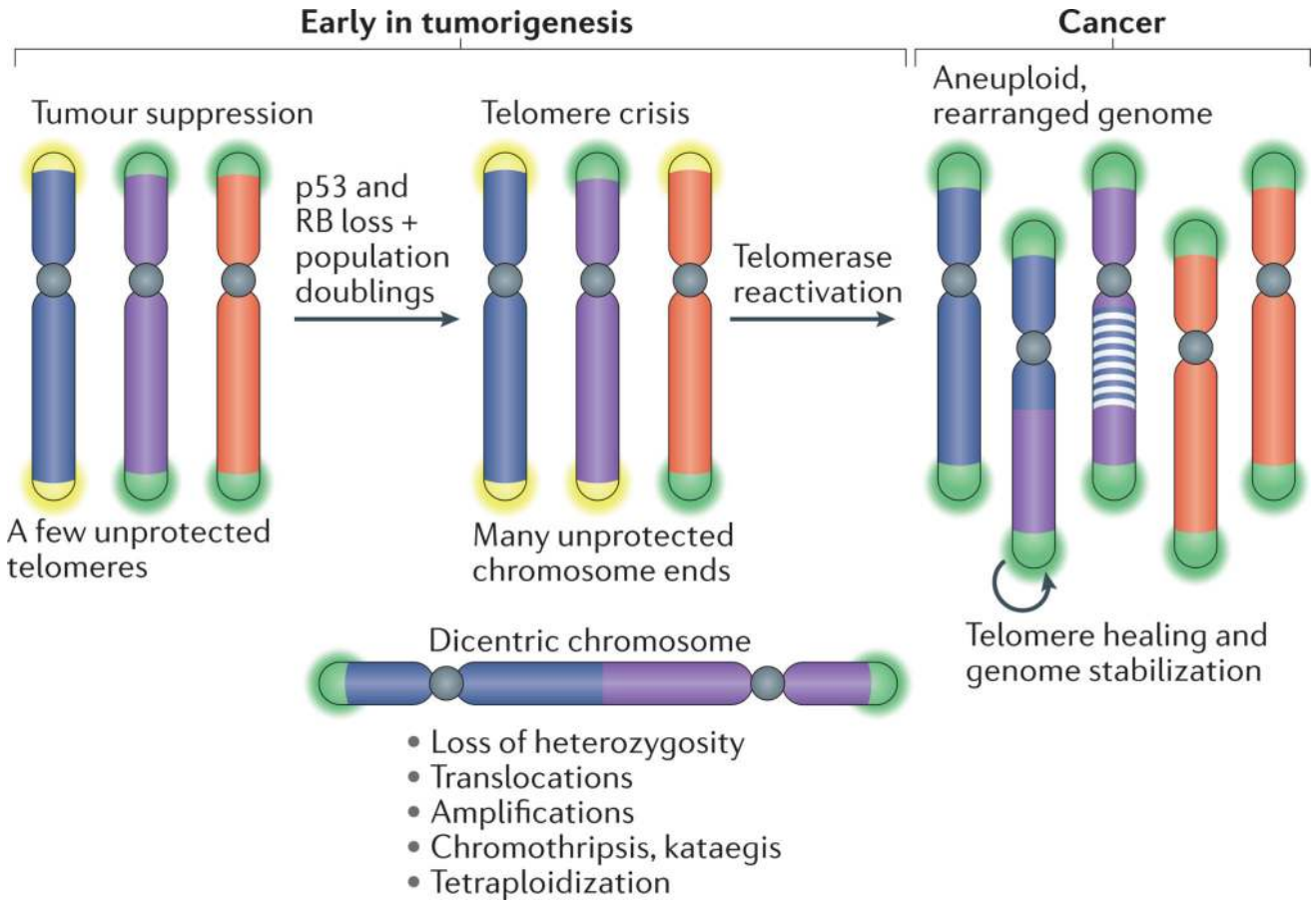

Figure 3. Telomere crisis

Loss of the RB and p53 tumour suppressor pathways disables the ability of cells to respond with cell cycle arrest to ATR and ATM signalling. As the cells continue to divide, their telomeres continue to shorten. Once many telomeres become too short to function, the unprotected chromosome ends generate end-to-end fusions and dicentric chromosomes, leading to many forms of genome instability. Ultimately, telomerase reactivation provides a route out of telomere crisis by healing critically shortened telomeres and improving genomic stability, thereby increasing cell viability. The resulting tumour will have active telomerase and a heavily rearranged genome.

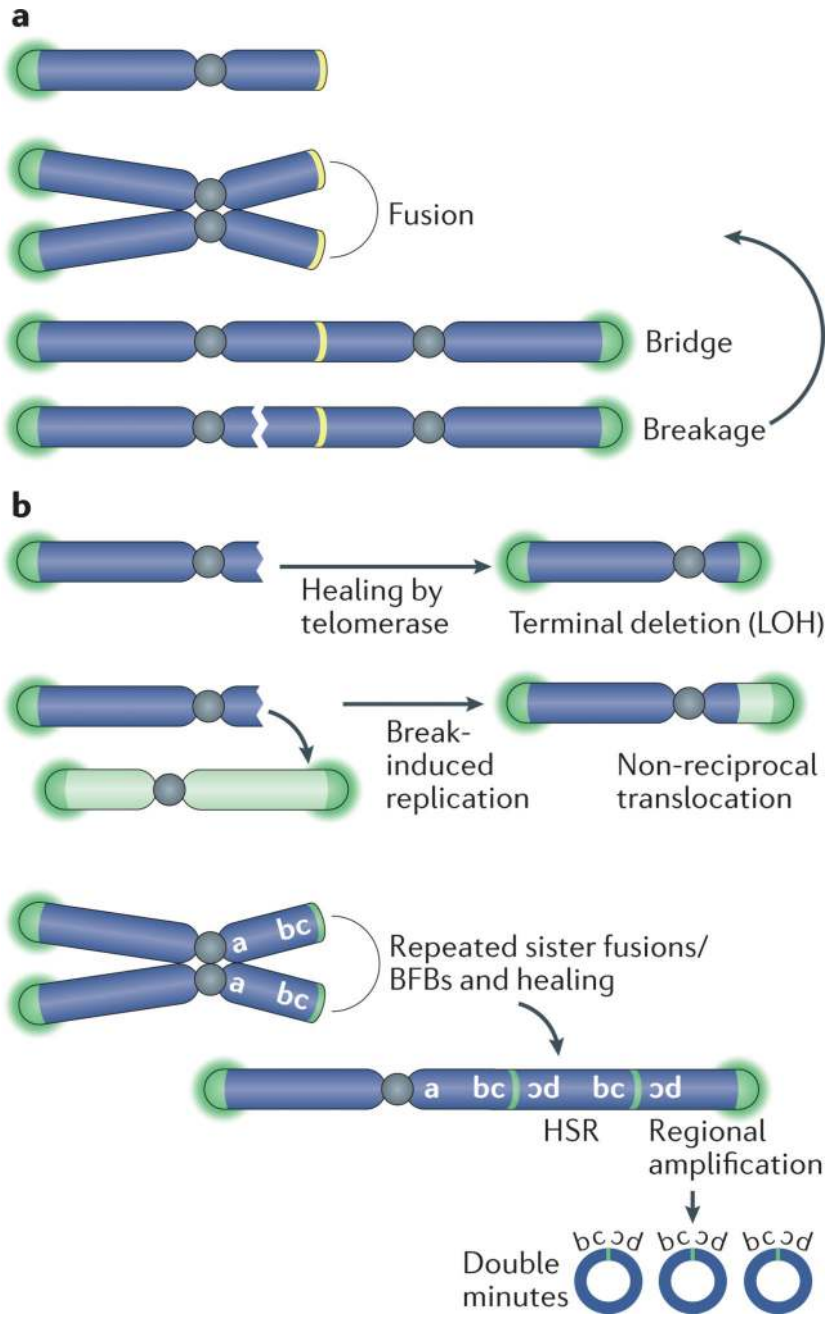

Figure 4. BFB cycles and chromosomal rearrangements during telomere crisis

a | Breakage–fusion–bridge (BFB) cycles can occur when telomere fusion generates a dicentric chromosome. During anaphase, the mitotic spindle pulls this dicentric chromosome towards opposite spindle poles, thereby generating the widely observed anaphase bridges. During cell division, the dicentric chromosome undergoes breakage and the broken ends fuse again, giving rise to another dicentric chromosome. **b** | BFB cycles can be interrupted by telomerase-mediated telomere healing. If this process occurs following breakage, it can result in the formation of a terminal chromosome deletion and loss of heterozygosity (LOH). Alternatively, broken chromosomes can be repaired by break-induced replication, yielding a

non-reciprocal translocation. Repeated cycles of BFB that occur between sister chromatids can result in regional amplification and the generation of a homogeneously staining region (HSR) following chromosome staining. This HSR consists of multiple amplicons of inverted repeats. Excision of the amplified sequences out of the chromosome will generate circular double-minute chromosomes.

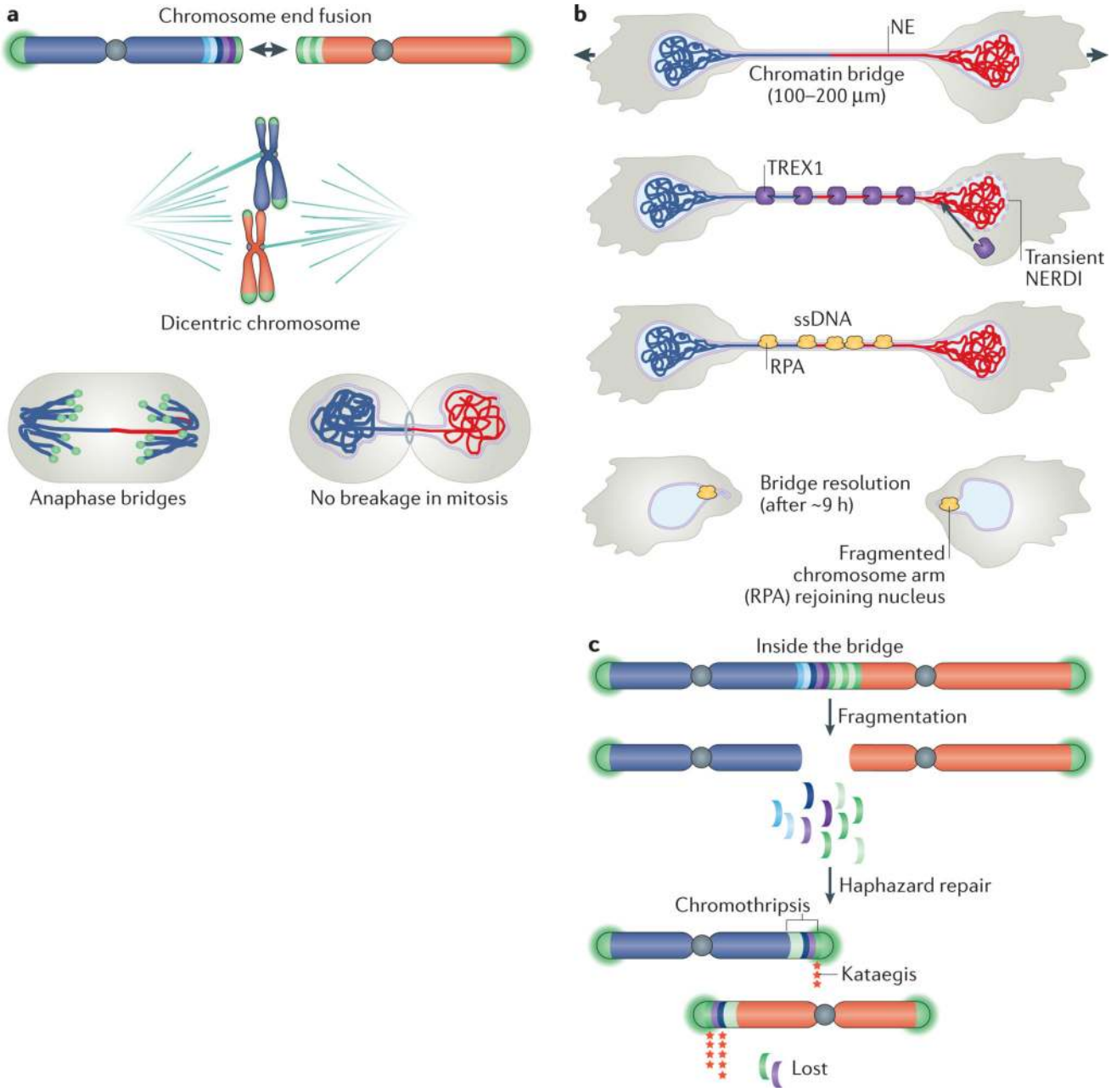

Figure 5. Chromothripsis and kataegis in telomere crisis

a | Dicentric chromosomes formed by telomere fusion rarely, if ever, break during mitosis and instead form chromatin bridges. **b** | Daughter nuclei connected by chromatin bridges undergo frequent nuclear envelope (NE) rupture in interphase (NERDI), resulting in the accumulation of 3′ repair exonuclease 1 (TREX1) on bridge DNA. TREX1-mediated resection of DNA leads to the formation of single-stranded DNA (ssDNA), which is bound by replication protein A (RPA), and bridge resolution. Bridge fragments are internalized into the nucleus where they remain associated with RPA for approximately 24 hours. **c** | Part of the dicentric chromosome that is present in the chromatin bridge undergoes extensive fragmentation followed by haphazard repair, which yields a chromothriptic chromosome in

which many original chromosome fragments are lost and retained fragments are present in seemingly random order and orientation. Chromothriptic breakpoints are frequently associated with kataegis mutation clusters.

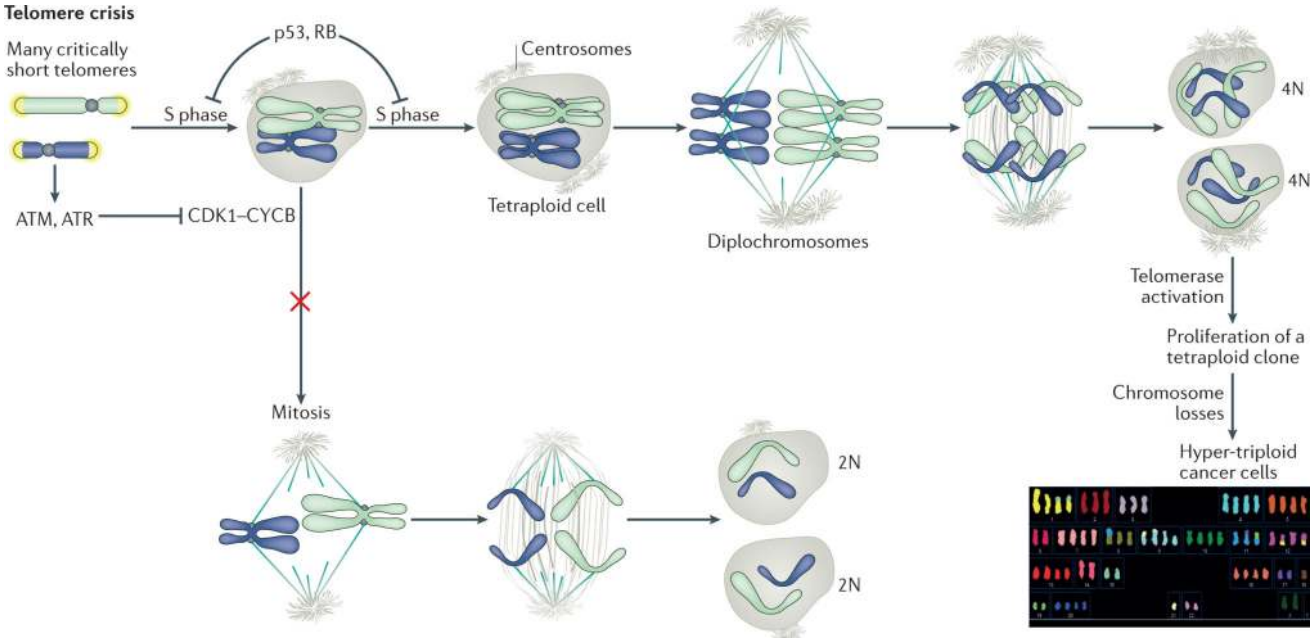

Figure 6. Tetraploidization during telomere crisis

Telomere crisis can lead to persistent DNA damage signalling when repair fails to join all the unprotected ends and dysfunctional telomeres persist. The persistent ATM and ATR signalling and activation of their downstream effector kinases checkpoint kinase 2 (CHK2) and CHK1, respectively, results in prolonged inhibition of cyclin-dependent kinase 1 (CDK1)–cyclin B (CYCB), thus blocking entry into mitosis. Eventually, cells bypass mitosis, enter a G1-like state and then undergo a second S phase. The resulting tetraploid cells have diplochromosomes in the first mitosis following endoreduplication. Subsequently, the cells undergo frequent chromosome losses, leading to the hyper-triploid cells that are frequently observed in cancer. The example karyotype shown is from Capan-2, a hyper-triploid pancreatic cancer cell line (http://www.pawefish.path.cam.ac.uk/PancCellLineDescriptions/Capan-2.html), courtesy of Vorapan Sirivatanauksorn and Paul Edwards.