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Deciphering the Code of the Cancer Genome: Mechanisms of Chromosome Rearrangement

Nicholas A. Willis, **Emilie Rass**, and **Ralph Scully**

Department of Medicine, Division of Hematology Oncology and Cancer Research Institute, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston MA 02215

Abstract

Chromosome rearrangement plays a causal role in tumorigenesis by contributing to the inactivation of tumor suppressor genes, the dysregulated expression or amplification of oncogenes and the generation of novel gene fusions. Chromosome breaks are important intermediates in this process. How, when and where these breaks arise and the specific mechanisms engaged in their repair strongly influence the resulting patterns of chromosome rearrangement. Here, we review recent progress in understanding how certain distinctive features of the cancer genome, including clustered mutagenesis, tandem segmental duplications, complex breakpoints, chromothripsis, chromoplexy and chromoanasynthesis may arise.

Genomic instability and the evolution of a cancer

Cancers evolve by natural selection. Mutations that confer increased fitness on a daughter cell contribute proportionately higher numbers to subsequent generations of cancer cells. In the context of the evolving cancer, “fitness” embraces accelerated growth, suppression of cell death, acquisition of metastatic capabilities and the other recognized hallmarks of cancer [1]. Unlike normal cells, which have evolved complex regulatory networks in support of multicellular organismal viability, the cancer cell is bound by no such constraints and is free to occupy any niche that its physiology and the host environment will allow. In this regard, the cancer cell resembles a parasitic microorganism. A certain level of genomic instability may increase the robustness of a population of microorganisms living under varying selective conditions. Similarly, genomic instability in the cancer cell, accompanied by waves of selection, may enable the cancer cell population to adapt rapidly to changing host environments during tumor growth, dissemination and metastasis [2]. Indeed, the high frequency of genomic instability in certain cancers, notably in solid tumors, suggests that this process plays a key role in the development of a mature metastatic cancer.

Recent advances in genome sequencing have revolutionized our understanding of the cancer genome and have shown that cancer-associated chromosome rearrangements are more

Corresponding author: Scully, R. (rscully@bidmc.harvard.edu).

numerous and more complex than was previously imagined [3]. Research into how these rearrangements arise is yielding exciting new insights relevant to both basic and translational arenas. First, it has revealed interesting parallels between defective double strand break (DSB) repair in cancer and model organisms. Second, it has unearthed new mechanisms connecting defective cell cycle progression with genomic instability. Third, it is expanding the universe of molecular targets and biomarkers for cancer therapy. Central to all of these areas is the formation and repair of DSBs in tumorigenesis. Here, we review these important new discoveries and discuss how they influence our understanding of cancer.

Double strand breaks: drivers of chromosome rearrangement

An unsheltered DNA end is not stable for an extended period within the cell. DSB repair mechanisms will force its interaction with other DNA molecules (Box 1). A major cause of DSBs in replicating cells is the stalling and/or collapse of the replication fork following collision with transcription complexes or at sites of abnormal DNA structure, often combined with the action of nucleases on the stalled fork [4, 5, 6]. Nuclease action can cause chromosome breakage in numerous additional ways. In normal physiology, programmed, site-specific DSBs mediate chromosome rearrangement during meiosis [7] and initiate V(D)J recombination and class switch recombination (CSR) during immunological development [8]. Other intrinsic sources of free DNA ends include the deprotection of telomeres, such as occurs during telomere attrition during aging or in cells lacking a functional shelterin complex [9]. Extrinsic causes of chromosome breakage include oxidative stress, ionizing radiation, radiomimetic chemicals and hyperosmolality [10, 11]. A host of other genotoxins can cause DSBs indirectly, *via* replication fork stalling or collapse [5]. Thus, DSB formation is a fairly frequent event in normal physiology. A number of elegant model systems have been employed to study chromosome translocations induced in mammalian cells in response to defined DSBs [12, 13, 14, 15, 16]. These studies suggest that spatial proximity strongly influences the likelihood that two genomically remote DNA ends will be joined.

Why might a cancer cell respond to DSBs differently from a normal cell? The answers are many-layered. Replication fork stalling (“replication stress”) is prevalent throughout tumorigenesis, due to the action of oncogenes, loss of cell cycle checkpoints and imbalances in cancer cell metabolism, and agents that stall replication induce copy number variations in human cells [17, 18]. Stalled replication forks and defective mitotic progression present specific challenges to the DSB repair system, as discussed in more detail below. The level of chemical damage to the cancer genome may also be elevated, for example, as a consequence of altered cancer cell metabolism. A final critical element is the configuration of the DSB repair system. Hereditary cancer syndromes provide a powerful example of the impact of DSB repair defects on cancer predisposition, by far the most prevalent examples in the human population being mutations in the hereditary breast/ovarian cancer predisposition genes *BRCA1* and *BRCA2*. However, much of the genomic instability observed in sporadic cancers may reflect somatic inactivation of DSB repair genes, either by *de novo* mutation or by promoter methylation. Defective DSB repair has also emerged as a vital “Achilles’ heel” of some cancers that can be exploited for cancer therapy, as discussed below.

Pathways of DSB repair

DSB repair is commonly divided into distinct pathways of classical non-homologous end joining (C-NHEJ), alternative end joining (A-EJ), homologous recombination (HR) and single strand annealing (SSA). Box1 and Figure 1 provide an overview of these pathways in somatic cells. ***C-NHEJ*** is a high flux, rapid rejoining mechanism that results in the ligation of DNA ends without reference to the specific DNA sequence [19, 20]. A critical first step in C-NHEJ is the binding of the Ku heterodimer to the DNA ends. Ku binds avidly to double stranded (ds)DNA ends but less well to single stranded (ss)DNA tails [21]. Thus, a long ssDNA tail, if unprocessed by nucleases, may be an inefficient substrate for C-NHEJ.

A-EJ is a non-homologous end joining mechanism that does not require C-NHEJ genes [22, 23, 24, 25]. A-EJ-mediated rejoining is strongly biased towards the use of microhomology (MH)-mediated end joining (MMEJ), in which one or more complementary base pairs at the breakpoint are shared by the two DNA ends (Box 1) [26]. However A-EJ and MMEJ are not synonymous. For example, immune V(D)J recombination is strongly C-NHEJ-dependent but a proportion of V(D)J breakpoints in wild type cells are microhomologous. Rejoining can also be accompanied by nucleotide insertions between the two DNA ends. These insertions, which are often templated, eradicate information on breakpoint MH and have been considered to be examples of cryptic MH [27]. MH at cancer rearrangement breakpoints shows an interesting variation between different cancer types, being more prevalent in breast than prostate cancer [3, 28, 29]. The presence of MH might appear to suggest that A-EJ is the major mechanism of rejoining in cancer genome rearrangement. However, given that MH can also be a feature of C-NHEJ, additional criteria are needed to determine the mechanism. In this regard, the *PolQ* gene has provided tantalizing clues regarding the possible role of A-EJ in genome rearrangement.

PolQ encodes the error-prone DNA polymerase Θ. Research in model organisms has revealed a role for PolΘ in joining of DNA ends that contain extensive ssDNA tails, where rejoining is largely C-NHEJ-independent [30, 31, 32]. *POLQ* null mice reveal spontaneous genomic instability and fail to insert nucleotides at repair junctions during class switch recombination [30, 33]. An *in vitro* study showed that purified PolΘ stabilizes minimal MH by using the second end of the break as a template in *trans* for repair synthesis [34]. In other words, PolΘ can “broker the deal” between two DNA ends that are poor substrates for C-NHEJ and lack the extensive homology needed for homology-directed repair. Indeed, *PolQ*-mediated rejoining has been implicated as a mediator of chromosome rearrangement, including the fusion of dysfunctional telomeres and chromosome translocations induced by CRISPR/Cas9 breaks [35]. Cancer cells frequently reveal elevated levels of PolΘ and growth of *BRCA* mutant cancers is impaired by loss of PolΘ, raising the possibility that it might be a useful target for cancer therapy [35, 36].

Although deletion of key genes involved in either C-NHEJ or A-EJ promotes genomic instability [33, 37], both pathways are also implicated as mediators of pathological chromosome rearrangement [35, 38, 39]. Presumably, the availability of both C-NHEJ and A-EJ broadens the spectrum of DNA ends that can be efficiently rejoined, but these

normally genome protective mechanisms can also be co-opted to mediate chromosome rearrangement in cancer.

SSA is a mechanism of joining two DNA ends that are single stranded and share extensive homology (Box 1). SSA in yeast is dependent on the HR gene *RAD52* but is independent of *RAD51* (the mammalian *RecA* homolog and central mitotic recombinase – see Figure 1), since it does not entail strand exchange [40]. The significance of SSA to cancer genome rearrangement is not well understood, since homologous breakpoints could potentially arise from either of the two "homology-directed repair" pathways of SSA or HR.

HR in somatic cells is primarily a non-crossover repair mechanism (Figure 1A). Crossing over—when it does occur—can cause loss of heterozygosity, with potential to inactivate tumor suppressor genes. During somatic HR of a two-ended DSB, mediated by the "synthesis-dependent strand annealing" (SDSA) pathway, displacement of the nascent strand and its annealing to the second DNA end normally limit gene conversion to a short tract (typically ≤100 bp) [41, 42, 43, 44]. HR also resolves "daughter strand gaps"— ssDNA gaps left in the wake of the fork [45]. When considered in the idealized case of a two-ended DSB, HR is a potentially error-free process. However, the major trigger to HR in cycling somatic cells—including cancer cells—is not an isolated two-ended DSB but a stalled or collapsed replication fork. Breakage of the stalled fork can generate a "one-ended" DSB (Figure 1B)—a DNA end that lacks a second end for the completion of DSB repair. In HR-defective cancers, this may be compounded by specific instability of the stalled replication fork. BRCA2, together with Rad51, BRCA1 and Fanconi anemia proteins protect newly synthesized DNA strands at the stalled fork from degradation by the MRE11 nuclease [46, 47]. This suggests that an HR-defective tumor suffers a "double whammy" of increased fragility of the stressed fork, in addition to the underlying DSB repair defect. In this regard, it is notable that HR is the DSB repair pathway most frequently implicated in cancer predisposition in the human population—through loss-of-function mutations of *BRCA1*, *BRCA2*, Fanconi anemia genes or a number of other HR genes [48, 49]. Indeed, a recent study identified instability of stalled forks in human cells that are haploinsufficient for *BRCA1* [50], raising the exciting possibility that stalled fork instability contributes to very early stages of *BRCA1*-linked tumorigenesis, prior to loss of the wild type *BRCA1* allele. To understand how such a defect might contribute to genomic instability, we need to consider the hazards posed by a one-ended DSB.

Replicative responses to DNA breaks: break-induced replication

A natural solution to the problem posed by a one-ended break is to reinvade the neighboring sister chromatid at the stalled replication fork and reinitiate conventional replication. Indeed, prokaryotes such as *Escherichia coli* achieve exactly this *via* an adaptor protein called PriA, which reassembles the replisome at sites of fork collapse [51]. Indeed, since *E. coli* have only one specified origin of replication, this process is essential for survival in the face of fork collapse. In eukaryotes, there are multiple origins of replication dispersed across the linear chromosomes and a current view is that new origins are not normally established once S phase has been initiated, even at sites of breakage and recombination. To date, no eukaryotic homolog of PriA has been identified. Thus, eukaryotes appear to lack a simple

mechanism to process one-ended breaks at collapsed forks by restarting conventional replication [52].

In yeasts, one-ended breaks can trigger “break-induced replication” (BIR), a highly error-prone HR-mediated replicative response that can generate gene conversions of >100 kb [53, 54, 55]. Mapping of gene conversion tracts of spontaneous HR in *Saccharomyces cerevisiae* revealed a bimodal distribution of tract lengths, with median peaks at ~6 kb and >50 kb [56]. This suggests that extensive replicative responses to DSBs are common in yeast HR during normal cell growth. BIR in *S. cerevisiae* is mediated by *POL32*—a gene encoding a non-essential subunit of DNA polymerase ∂ [57, 58] and by the Pif1 helicase [59, 60]. Importantly, BIR in *S. cerevisiae* does not entail formation of a *bona fide* replication fork [60, 61]. Direct structural analysis revealed that BIR generates long tracts of single stranded DNA through a bubble migration mechanism [60]. This process is highly error-prone, introducing mutations into the newly synthesized strand at a much higher rate than during conventional replication (Figure 1B). BIR can be established following several “long tract” gene conversions (LTGCs), punctuated by template switches between homologs of the donor chromosome [62]. In the context of breaks formed at stalled/collapsed replication forks, BIR is normally limited by encounter with the adjacent replication fork (derived from the neighboring origin of replication) or by the action of the nuclease Mus81 [63]. Thus, work in model organisms has provided a framework for understanding the deleterious consequences of a one-ended break and has begun to reveal mechanisms that limit its mutational impact.

Mammalian cells can also mount extensive replicative responses during HR—experimentally measured as “long tract” gene conversion (LTGC), a process that may be analogous to BIR in yeast [64, 65, 66, 67]. Where measured, mammalian LTGC appears thus far to be limited to ~10 kb—shorter than the typical BIR tracts observed in yeast. At a conventional DSB in mammalian cells, a proportion of “one-ended” HR invasions are terminated after ~1kb of copying [66]. Thus, classical BIR is not an obligatory outcome of a one-ended HR invasion in mammalian cells. In our work on HR triggered by a site-specific replication fork barrier, loss of *BRCA1* or *BRCA2* paradoxically elevated the frequency of LTGC/BIR at sites of replication fork arrest, identifying the stalled fork as a significant source of aberrant replicative HR responses in mammalian cells [68]. By analogy with the work in yeast discussed above, this raises the possibility that one-ended breaks are generated more frequently at stalled forks in *BRCA* mutant mammalian cells [68]. Additional evidence of BIR-type copying in mammalian HR comes from analysis of copy number variation in human genomic disorders, where a BIR model was proposed to explain the formation of inverted repeats of up to ~500 kb [69].

A potential connection between mutagenesis in cancer and mammalian BIR is suggested by the phenomenon of clustered mutation or “kataegis” (thunderstorm) in cancer [70, 71]. Described simultaneously in studies of human cancer and of yeast grown under exposure to chronic alkylating damage, kataegis entails clustered mutations caused by deamination of cytosine in TpC dinucleotides (generating C->T transitions), colocalized with sites of chromosome rearrangement. Importantly, kataegis reveals “strand coordination”, whereby a switch in polarity is observed within the DNA strand from C coordination (C->T) to G

coordination (G->A). These observations suggest that kataegis is organized around extensive ssDNA tracts formed with opposite polarities at sites of chromosome breakage. Consistent with this, kataegis in cancer reflects the action of APOBEC family ssDNA cytidine deaminases [72, 73, 74]. This raises the question: what is the source of ssDNA tracts that underlie kataegis? The tract length of individual kataegic clusters (up to ~200 kb) seems much greater than would be predicted to arise from conventional DNA end resection. Genetic analysis in yeast implicated the stalled replication fork as a cause of kataegis, potentially implicating one-ended breaks and BIR as an underlying mechanism. In support of this, BIR in *S. cerevisiae*, which entails the production of multi-kilobase tracts of ssDNA, was recently shown to reproduce kataegis-like patterns of clustered mutagenesis [75] (Figure 1B). However, the above noted switch in the polarity of strand coordination in kataegis is difficult to explain by a simple BIR model. It may be that additional mechanisms can contribute to kataegis in cancer cells.

Microhomology-mediated template switching and complex breakpoints

Although BIR is strongly *RAD51*-dependent in yeast [76, 77], a *RAD51*-independent BIR mechanism can act on short tracts of homology [78, 79]. Work in yeast [80] and in *E. coli* [81] identified MH-triggered replicative mechanisms for forming tandem segmental duplications (SDs). In the yeast system, generation of all SDs was dependent on *POL32*, suggesting that a replicative mechanism generated the SDs. SDs with homologous breakpoints were dependent on the HR gene *RAD52* while those with MH breakpoints were *RAD52*-independent [82]. Interestingly, SD frequencies were elevated >7-fold in a *rad51*Δ strain. These observations are relevant to understanding the widespread tandem SDs observed in breast and ovarian cancer genomes [3, 70, 83]. Either rejoining (sister chromatid breakage/fusion) or replicative mechanisms could explain tandem SDs observed in human cancers (Figure 2).

Genetic analysis in *E. coli* implicated MH-mediated template switching of nascent daughter strands at stalled replication forks as a trigger to SD formation [81]. MH-mediated template switching is also observed in *S. cerevisiae* [84]. A MH-driven template switch mechanism was proposed to explain complex breakpoints observed in the formation of *PLP1* gene duplications in the human genomic disorder, Pelizaeus-Merzbacher disease [85]. The authors proposed a fork stalling and template switching model, in which a 3′ ssDNA tail undergoes template switching into the lagging strand of a spatially proximate but genomically distant replication fork [85]. This process has also been termed “microhomology-mediated break-induced replication” (MMBIR) [86], although the term “BIR” here encompasses very short tracts of copying (~hundreds of base pairs) that might be only distantly related to classical BIR as described in yeast.

These patterns of genomic instability invite direct comparison with the cancer genome. Cancer genomes frequently contain complex breakpoints at sites of rearrangement, containing arrays of short sequences derived from distinct genomic loci. Although a MH-mediated template switch mechanism (Figure 3) could explain some of these breakpoints, one study reported that complex breakpoints in cancer exhibit less MH than simple breakpoints, perhaps suggesting a simple rejoining mechanism (C-NHEJ or A-EJ) as the

underlying cause [87]. However, the patterns of complex breakpoints in this study closely match those observed during A-EJ-mediated translocations induced in mammalian cells in response to two site-specific DSBs, in which a MH-mediated template switch mechanism best fits the observed data [12]. A more definitive understanding might require the development of new tools to study complex breakpoint formation in cancer.

Connections to cancer therapy

Many effective, well established cancer chemotherapeutic agents work by stressing the replication fork. For example, the agent cisplatin forms chemical adducts on DNA, including interstrand DNA crosslinks (ICLs). ICLs present an absolute barrier to replication fork progression and HR-defective cells (*BRCA* mutant, Fanconi anemia mutant etc.) are hypersensitive to these agents. Thus, the largely empirical development of cancer chemotherapeutics over decades of clinical practice could be seen as an experiment in provoking intolerable levels of replication stress and lethal mitotic defects in the already overburdened cancer cell. A recent example of this is the development of inhibitors of poly(ADP-ribose) polymerase (PARP) for the treatment of HR-defective cancers. Cells lacking *BRCA1*, *BRCA2* or other core HR genes exhibit a ~1000-fold increase in sensitivity to PARP inhibitors in comparison to wild-type cells [88, 89]. Approximately 10 years after the first demonstration of this phenomenon, PARP inhibitors were approved by the US Food and Drug Administration for the treatment of advanced *BRCA*-linked ovarian cancer. PARP inhibition affects multiple repair pathways and the targets that are critical for killing HR-defective cancers are not settled. However, several models invoke a mechanism that targets replication fork stability in HR-defective cells. These advances provide a strong rationale for ongoing efforts to identify the genes that normally maintain stalled fork stability, since some of these genes may be new targets for cancer therapy. A second area of translation is based on the idea that certain genomic instability "signatures" might be useful as biomarkers for cancer therapy. In this regard, the prevalence of defective HR in cancer (often termed "*BRCA*ness") is a particularly important phenotype. At present, there is no single biomarker of *BRCA*ness, because of the multiplicity of genes that control HR and the frequent down-regulation of HR genes by promoter methylation rather than by mutation. Conceivably, if a specific pattern of genomic instability were to correlate with *BRCA* mutation status, the pattern itself might serve as a biomarker of *BRCA*ness for cancer therapy.

Mechanisms of complex cancer genome rearrangements

Patterns of localized chromosome rearrangement in cancer cells have long been recognized as mediators of copy number variation and oncogene amplification. The classical mechanism of sequential sister chromatid breakage-fusion-bridge (BFB) cycles [90] exemplifies how complex, localized chromosome rearrangements can arise by repeated breakage and rejoining at a single locus over the course of several cell cycles, for example as a result of telomere attrition [91]. However, cancer genome rearrangements suggestive of a sudden, catastrophic event have recently been identified—termed "chromothripsis" (chromosome shattering), "chromoplexy" (braid of chromosomes) or "chromoanasynthesis" (chromosome reconstitution or chromosome reassortment) [92, 93, 94, 95, 96]. Each process might lead to sudden changes in genotype and phenotype—as exemplified by a recent cure

of WHIM immunodeficiency syndrome by chromothriptic inactivation of the disease-causing dominant mutant chemokine receptor gene *CXCR4* [97].

Chromothripsis was originally described in a case of chronic lymphocytic leukemia, which displayed tightly focused rearrangements on a single chromosome, with highly constrained copy-number oscillations between only two copy-number states [92, 98]. This pattern was proposed to derive from chromosome shattering, followed by the formation of tens to hundreds of locally clustered DNA rearrangements through a single event, with deletion of intervening chromosome fragments. Chromothripsis therefore is the prototype of catastrophic genome rearrangement and of punctuated genome evolution. It has since been observed in numerous cancers, often disrupting functionally important cancer genes, with gliomas showing the highest frequency of up to 39% in one study [87]. The reason for the variation in frequency of chromothripsis between different tumor types is unknown. Chromothripsis has been associated with the formation of small circular “double-minute” chromosomes, which may play a critical role in oncogene amplification [92, 99].

An intriguing insight into the mechanism underlying chromothripsis has emerged from work on micronuclei—small cytoplasmic DNA-containing bodies derived from lagging mitotic chromosomes (Figure 4A). Rupture of the micronuclear membrane could expose the micronucleated chromosome(s) to cytoplasmic endonucleases, leading to chromosome shattering [100]. Direct support for this mechanism came from single cell whole genome sequencing of two daughter cells derived from a micronucleated cell [101]. Fragments of the lagging chromosome were found reincorporated into the genomes of each daughter cell, in some cases generating complementary patterns of chromothripsis in each of the progeny cells (Figure 4A). Other rearrangements included translocations and circular derivative chromosomes—potential precursors of double minutes that carry amplified oncogenes in some cancers. Some breakpoints contained multiple short insertions (<500 bp) derived from other loci on the rearranged chromosome, suggestive of MH-mediated template switching. Work in *Arabidopsis thalania* has also pointed to defective mitotic progression as a cause of chromothripsis [102].

Copy number neutral “constitutional chromothripsis”, generating balanced chromosome rearrangements, can cause human developmental disorders in the subsequent generation and is typically caused by *de novo* genome rearrangements inherited from the father [103, 104, 105]. The mechanisms of constitutional chromothripsis are currently unclear, but the copy number neutrality argues against a replication-related process. It presumably reflects extensive chromosome breakage and rejoining in the spermatid or sperm, occurring between the second meiotic division and fertilization.

Chromoplexy provides a second example of coordinated, catastrophic genome rearrangement in cancer. Like chromothripsis, chromoplexy primarily results from deletion/rejoining mechanisms. Originally described in prostate cancers, where it affects up to 40% of cases, chromoplexy entails linked rearrangements between a number of heterologous chromosomes [94]. This linkage strongly suggests that the translocations occurred in a spatially and temporally constrained fashion. The involvement of classical *TMPRSS-ERG* fusions at chromoplexy breakpoints suggests an underlying transcription-related mechanism.

Intriguingly, androgen receptor (AR)-mediated transcription has been implicated in the formation of localized DSBs through interaction with the topoisomerase TOP2B [106]. This raises the possibility that AR transcription coordinates the induction of breaks at remote genomic loci, effectively providing the temporal and spatial linkage implied by the phenomenon of chromoplexy (Figure 4B).

Chromoanasynthesis was described in human developmental genomic disorders as complex chromosome rearrangements with variable copy number gains at a restricted genomic locus [93]. This differs from chromothripsis and chromoplexy, which are primarily deletional/rejoining processes. Chromoanasynthesis may be part of a continuum of segmental amplification mechanisms, the tandem segmental duplication serving as the simplest element (Figure 2). A critical question is whether the copy number gains associated with chromoanasynthesis occur as a temporally discrete catastrophic event (involving BIR or aberrant re-replication of specific chromosome regions), or whether they accumulate over several cell cycles, in which case they could be explained by repeated rounds of replication with breakage-fusion cycles. The locus-specific nature of the phenomenon appears to favor the “catastrophe” model. However, an unrepaired persistent inter-strand crosslink (ICL) could cause localized copy number gains cumulatively over several cell cycles (Figure 5).

Intriguing insights into the mechanisms underlying chromoanasynthesis came from mutagenesis analysis in *C. elegans* exposed to ICL-inducing agents mechlorethamine or cisplatin [91]. In contrast to other genotoxins tested, exposure of wild type hermaphrodite parents to ICLs gave rise to offspring containing localized copy number gains. Some patterns (e.g., copy number increase from 2 to 3) could reflect simple reintegration of a retained sister chromatid fragment (Figure 5). Other patterns entailed up to 5-fold copy number increases of clustered chromosome regions 5-10 kb in size. Importantly, the timing of exposure to the genotoxins did not allow replication to occur within the hermaphrodite parent following ICL formation. Therefore, any replication-coupled ICL-induced mutagenesis must have occurred in the offspring. Since the offspring in question were not mosaics, these copy number gains likely occurred during a single S phase—the first zygotic S phase of the affected offspring. These amplifications may have arisen by repeated LTGCs of up to 10 kb at the site of the ICL. Interestingly, some examples of mammalian LTGC entail localized copy number increases with concatemer formation indicative of multiple rounds of copying of the recombining segment [65]. Perhaps intermediates of the type shown in Figure 5 could serve as templates for rolling circle replication in chromoanasynthesis.

Concluding remarks

The identification of sudden crises of chromosome rearrangement implies a potential for rapid evolution of organisms and of cancer. These processes could facilitate rapid adaptation within a species or in a population of cancer cells exposed to sudden new selective pressures. In model organisms, the capacity to undergo such rearrangements can be stress-induced [81] and similar stress-inducibility may apply to genomic catastrophes in cancer. Aberrant mitosis is increasingly recognized as a prominent cause of genomic catastrophes. Given the known relationships between replication stress—a near universal feature of cancer

cells—and disordered mitotic progression, replication stress may be an underlying driving force behind both replication-associated and mitosis-associated chromosome rearrangement. The application of next generation sequencing to appropriate model systems promises to reveal these mechanisms in greater detail. As outlined in the Outstanding Questions Box, we expect that this rapidly developing field will reveal new biomarkers and new therapeutic targets in cancer.

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Glossary

APOBEC	"Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like." A family of cytidine deaminases that act on cytosine in single stranded DNA.
Break-induced replication (BIR)	a gene conversion in which up to hundreds of kilobases are copied from the donor molecule.
Chromoanasynthesis	A chromosome rearrangement characterized by highly localized but variable increases in copy number.
Chromoplexy	Linked translocations observed in cancer genome, suggestive of spatially and temporally coordinated break induction on multiple different chromosomes.
Chromosome rearrangement	A type of chromosome abnormality involving a change in the structure of the native chromosome.
Chromothripsis	A catastrophic rearrangement, often localized to one chromosome, caused by chromosome shattering. In cancer genomes, it leads to oscillations between two copy number states along the affected chromosome.
Clustered mutagenesis	An extreme form of nonrandom distribution of mutations in the genome.
Complex breakpoint	Breakpoints of chromosome rearrangement involving multiple loci in the reference genome rearranged into a single contiguous region of the test genome.
Constitutional chromothripsis	Complex chromosome rearrangements carried in the germline, typically copy number neutral.
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats adapted to guide the Cas9 nuclease to introduce site-specific DSBs.

Deletion	A rearrangement characterized by loss of a segment of a chromosome.
Double-minute chromosomes	Circular fragments of DNA up to only a few megabases in size that can mediate gene amplification in cancer.
Gene conversion	A process by which one DNA sequence replaces a homologous sequence, usually in the context of homologous recombination.
Inter-strand crosslink (ICL)	A covalent connection of the complementary strands of DNA that prevents DNA strand separation, blocking replication and transcription.
Kataegis	clustered hypermutation identified in some cancer genomes, mediated by the action of APOBEC cytidine deaminases on tracts of single stranded DNA.
Long tract gene conversion (LTGC)	A gene conversion in which up to tens of kilobases are copied from the donor molecule.
Microhomology (MH)	a type of breakpoint in which a small number of complementary base pairs at the breakpoint are shared by the two DNA ends.
Microhomology-mediated end joining (MMEJ)	An end joining event in which the breakpoint reveals microhomology between the two DNA ends.
Micronucleus	a small extranuclear body that contains a chromosome that was not incorporated into one of the daughter nuclei during the previous cell division.
Segmental duplication	Generation of a second copy of a segment of DNA, located elsewhere in the genome.
Shelterin	A complex of telomere-associated proteins that maintains normal telomere homeostasis.
Sister chromatid	one of the two identical copies (chromatids) formed by the replication of a single chromosome.
Synthesis-dependent strand annealing" (SDSA)	A non-crossover mechanism of homologious recombination in somatic cells.
Tandem segmental duplication	a segmental duplication that abuts the original DNA segment "head to toe".
Translocation	A chromosome rearrangement between nonhomologous chromosomes.

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Box 1

DSB repair pathways

The major pathways of DSB repair are classical non-homologous end joining (C-NHEJ), alternative end joining (A-EJ), single strand annealing (SSA) and homologous recombination (HR). HR, A-EJ and SSA share common initial nuclease/helicase-mediated DNA end-processing steps, generating a 3′ ssDNA intermediate, which becomes coated with the ssDNA-binding RPA heterotrimer. **C-NHEJ** is a rapid, high flux pathway in mammalian cells that is active throughout the cell cycle. A critical initial step is the binding of the Ku70/Ku80 heterodimer to the DNA end. A third component of this complex, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), together with additional c-NHEJ factors, mediates synapsis between the two DNA ends. The DNA ligase IV/XRCC4 heterodimer, in conjunction with additional scaffolding proteins, ligates the DNA ends, sometimes introducing small insertions or deletions at the ligation site (shown in red). Ku binds less well to single stranded (ss)DNA tails than to blunt or minimally recessed ends. Thus, the structure of the DNA end may influence whether CNHEJ is efficiently engaged. **A-EJ** can rejoin two DNA ends in the absence of C-NHEJ factors. A-EJ frequently uses microhomology (MH; typically 1-5 bp) between the two DNA ends to achieve ligation. Repair synthesis (red half-arrow) can be mediated by DNA polymerase Θ. The **SSA** pathway mediates annealing between two ssDNA ends containing homologous direct repeats. The major features of **HR** are outlined in Figure 1.

Outstanding questions

• Are there as yet undiscovered new types of catastrophic genome rearrangement in cancer?

• What are the genetic and mechanistic underpinnings of specific cancer genome rearrangements?

• What explains the cancer type-specific character of some chromosome rearrangements?

• Do any types of genome rearrangement in cancer correlate with tumor genotype and have potential as biomarkers in cancer? Is there a specific genome rearrangement that could serve as a biomarker of "*BRCA*ness" across a range of different cancers?

• To what extent do rejoining *vs*. replicative DSB repair mechanisms contribute to cancer genome rearrangement? Do these different mechanisms vary with cancer type and do they have predictive power as biomarkers for personalized and targeted cancer therapy?

• Does the balance of DSB repair functions vary as a function of the differentiation state of the cell? Do stem cells follow established "rules" of DSB repair?

• Will the set of genes that normally suppress or promote cancer genome rearrangement reveal new targets for cancer therapy? How many new cancer therapies that exploit stalled fork instability or defective mitotic progression are awaiting discovery?

- Advances in whole genome sequencing have provided new insight into the complexity of cancer genome rearrangements.
- The discovery of catastrophic genome rearrangements in cancer establishes that genome evolution can occur in a punctuated fashion, involving temporally coordinated alterations that affect multiple distinct genetic loci.
- Decoding the patterns of cancer genome rearrangement has pointed to error-prone pathways of double strand break (DSB) repair and defective mitotic progression as critical mediators of this process.
- Recent advances in understanding DSB repair control provide new clues as to how specific types of cancer genome rearrangement might have arisen.
- Promising new therapeutics exploit these defective DSB repair processes in the clinic.

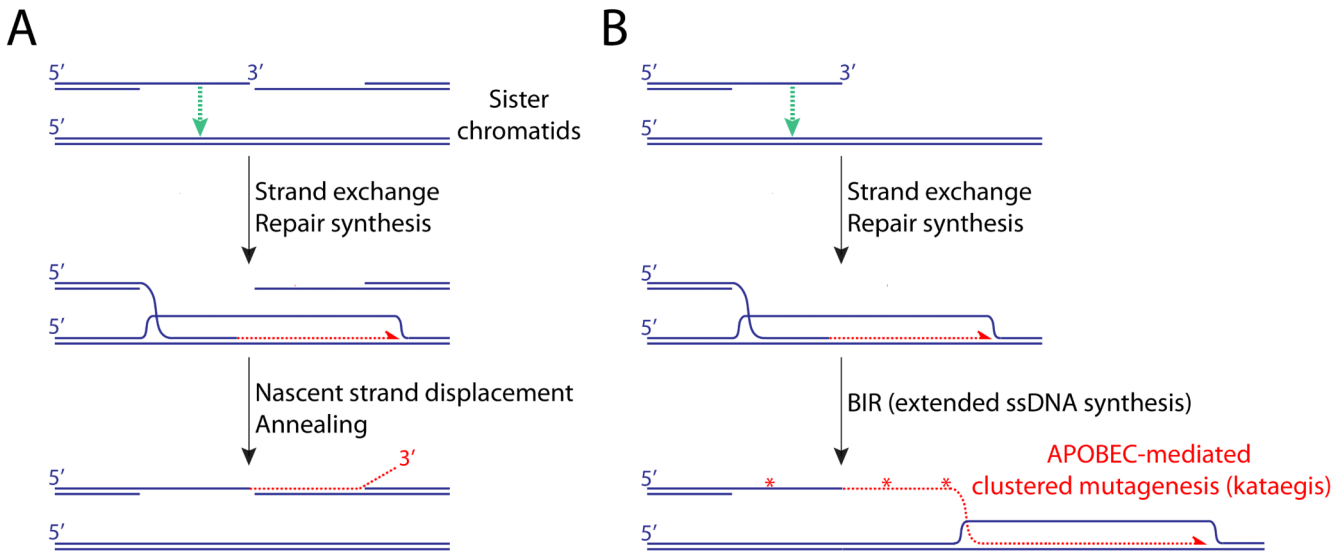

Figure 1. Homologous recombination in somatic cells

HR mediators (primarily BRCA2 in mammalian cells and Rad52 in yeast) displace RPA from ssDNA on the resected DNA end and load Rad51, forming a nucleoprotein filament. The Rad51 filament performs a homology-seeking invasion of neighboring double stranded (ds)DNA molecules and, if a high degree of homology is detected (typically ≥100bp), a DNA polymerase extends the 3′ end of the invading ("nascent") strand. Sequence differences copied from the donor DNA alter the sequence of the repaired DNA molecule ("gene conversion"). In S/G2 phase, the neighboring sister chromatid is the preferred donor for recombination, with potential for error-free DSB repair. However, the Rad51 filament can also detect homology at distant loci, potentially contributing to genome rearrangement. A. "Synthesis-dependent strand annealing" (SDSA) pathway of HR. Rad51-mediated strand exchange (green arrow) enables repair synthesis (red half-arrow), using the donor as template. In somatic cells, HR termination entails helicase-driven displacement of the nascent strand from the donor template followed by annealing (homologous pairing) with complementary ssDNA of the second end of the DSB. This termination mechanism does not lead to crossing over. **B. Break-induced replication and kataegis.** Typically triggered by a one-ended invasion, BIR is mediated by a "migrating bubble" mechanisms of leading strand synthesis (red half-arrow). The extensive tracts of ssDNA generated by BIR are potential targets of cytidine deamination (red asterisks) by APOBEC family enzymes, leading to patterns of clustered mutagenesis (kataegis).

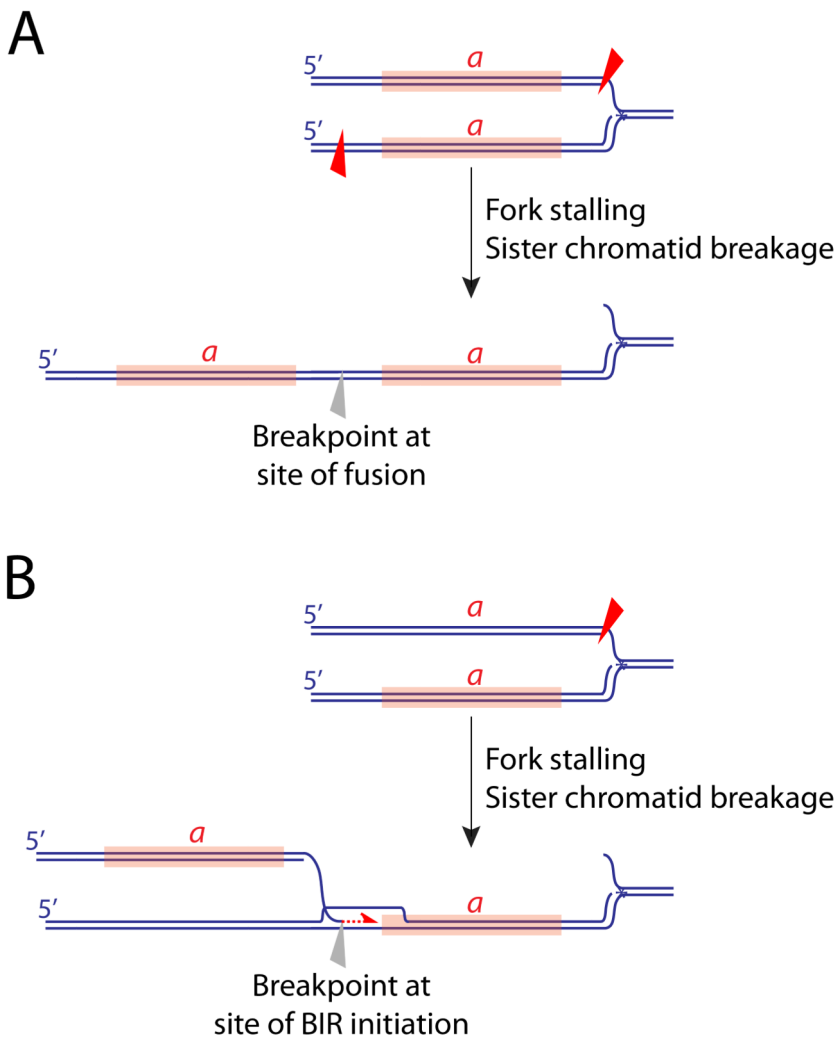

Figure 2. Mechanisms of tandem segmental duplication

Aberrant processing of a stalled fork, perhaps augmented by an HR defect, may produce unscheduled breakage of sister chromatids in the vicinity of the stalled fork. Tandem duplication of segment *a* (marked in orange) could arise by asymmetrical breakage (red triangles) and fusion of sister chromatids (**A**), or by break-induced replication following breakage of one sister chromatid (**B**).

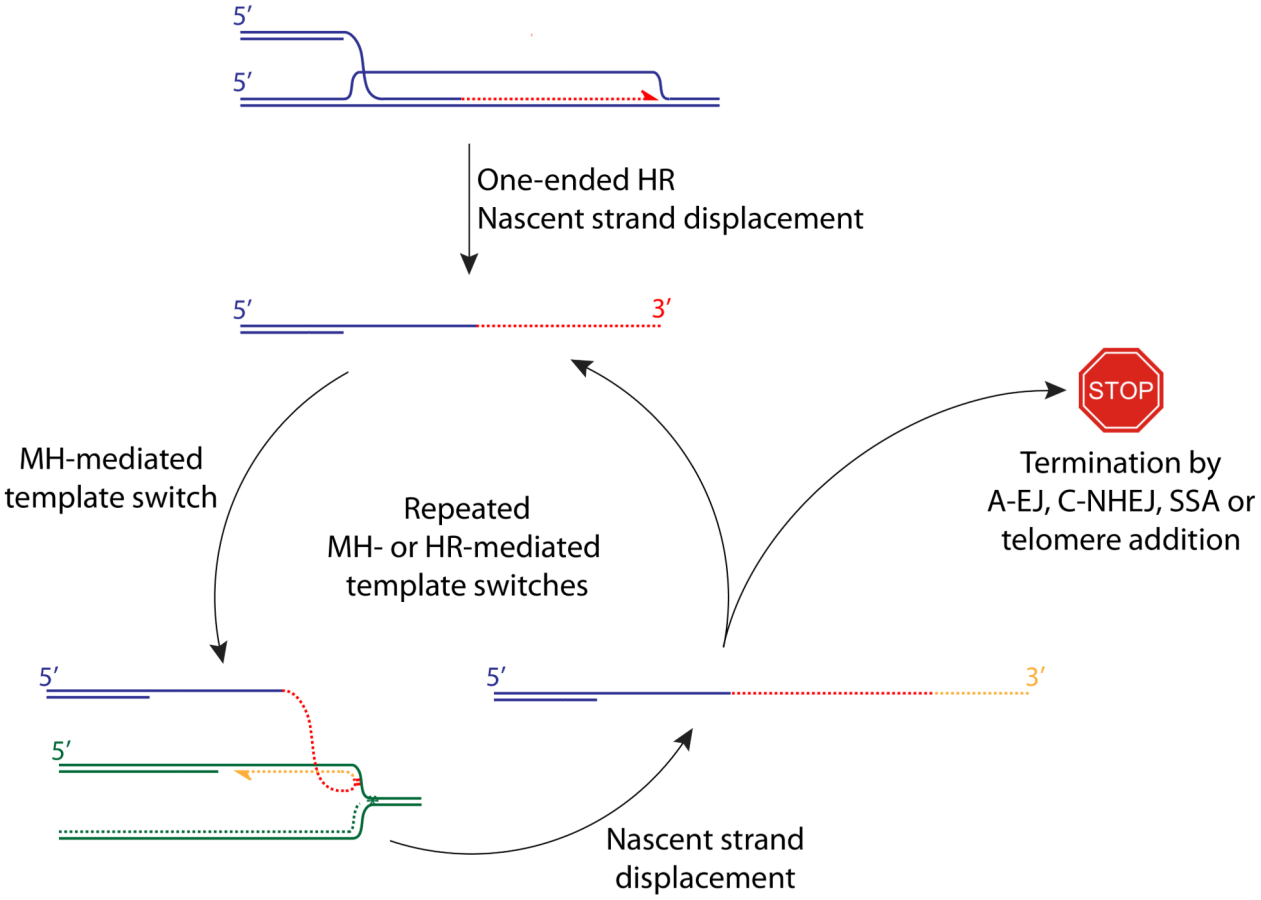

Figure 3. Origins of complex breakpoints in cancer: one-ended breaks and MH-mediated template switching

The displaced 3′ ssDNA tail produced following a “one-ended” HR invasion may undergo MH-mediated template switching—shown here into the lagging strand of a neighboring stalled fork. Repeated rounds of MH- or HR-mediated template switches could generate the complex breakpoints observed in cancer. Termination of a template switch cycle must involve either joining to a second DNA end or telomere addition.

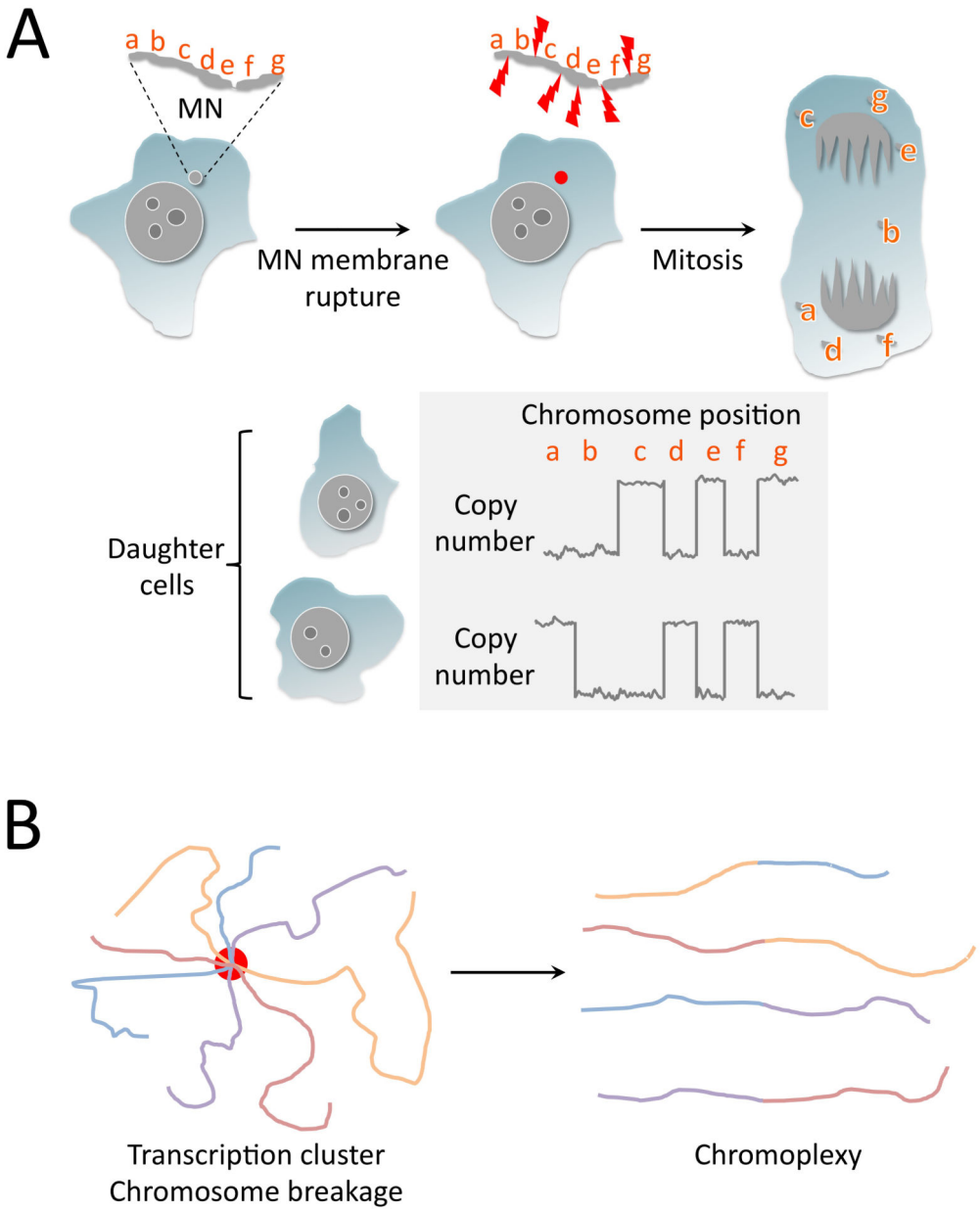

Figure 4. Chromothripsis and chromoplexy mediate catastrophic genome rearrangements *via* distinct mechanisms

A. Chromothripsis can arise following mitotic damage to micronucleated chromosome(s). A micronucleated chromosome ("MN") containing loci "a-g" is exposed to cytoplasmic nucleases when the MN membrane ruptures (red dot) and undergoes fragmentation. During the subsequent mitosis, the chromothriptic fragments segregate randomly into the two daughter cells, or are lost (e.g., fragment b shown here). The daughter cell genomes reveal constrained copy number oscillations characteristic of chromothripsis. **B.** A model of chromoplexy in prostate cancer. Clustering of transcriptional elements (red circle), for example, at androgen receptor (AR) responsive loci, coupled with AR-associated

chromosome breakage, leads to rejoining of broken chromosoms with the production of linked translocations characteristic of chromoplexy.

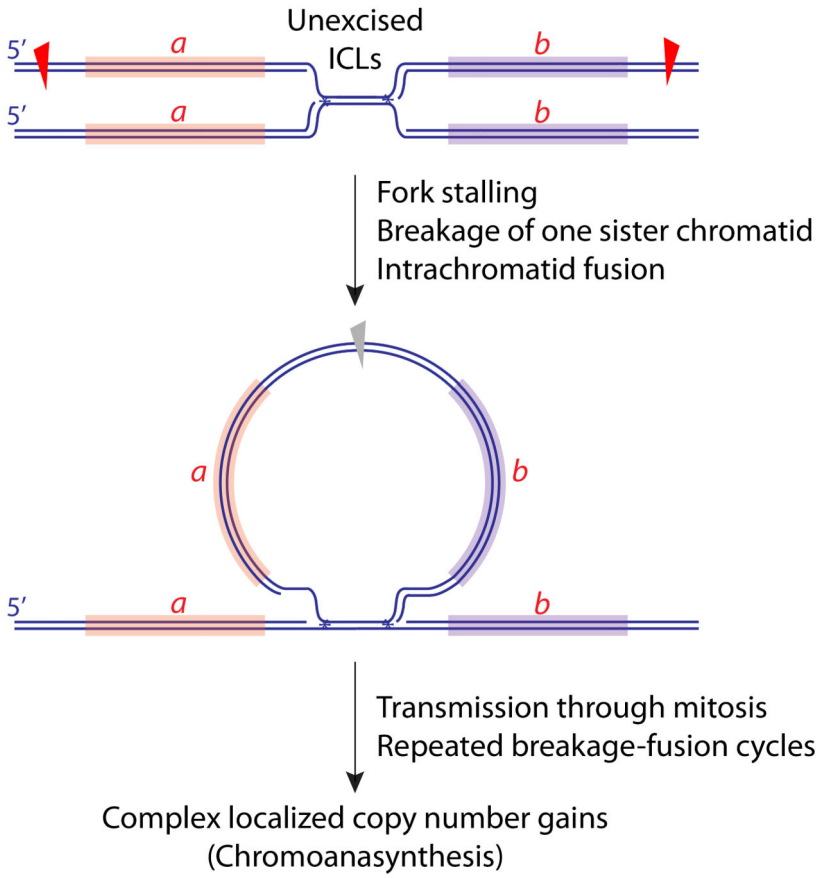

Figure 5. A model of chromoanasynthesis

An unexcised ICL could lead to breakage of one sister chromatid, with circularization of a retained fragment, containing loci *a* and *b*, as shown. Provided the retained circular fragment lacks a centromere, it will not be a barrier to mitosis and will carry additional copies of loci *a* and *b* into the subsequent S phase. Scheduled replication, rearrangement and integration of the fragment into the gemome could contribute to the localized copy number gains characteristic of chromoanasynthesis. Notably, if the circularized fragment were to contain an origin of replication (not shown), this could further increase the opportunity for rapid copy number gains *via* rolling circle replication (not shown).

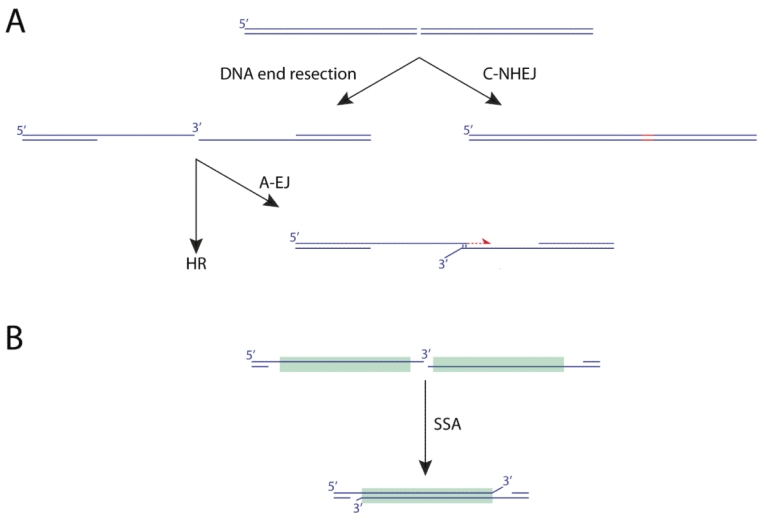

Figure I.

(associated with Box1): Diagram of major pathways involved in the repair of DNA double strand breaks (HR, C-NHEJ, A-EJ, SSA).