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Cancer Genomic Rearrangements and Copy Number Alterations from Errors in Cell Division

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Abstract

Analysis of cancer genomes has shown that a large fraction of chromosomal changes originate from catastrophic events including whole-genome duplication, chromothripsis, breakage-fusion-bridge cycles, and chromoplexy. Through sophisticated computational analysis of cancer genomes and experimental recapitulation of these catastrophic alterations, we have gained significant insights into the origin, mechanism, and evolutionary dynamics of cancer genome complexity. In this review, we summarize this progress and survey the major unresolved questions, with particular emphasis on the relative contributions of chromosome fragmentation and DNA replication errors to complex chromosomal alterations.

1. INTRODUCTION

Numerical and structural aberrations of chromosomes are a well-known hallmark of cancer. The hypothesis that these aberrations originate from cancer-associated abnormalities in mitosis dates back more than 100 years, shortly after the formulation of the chromosome theory of inheritance. Details about these early studies were reviewed by Wunderlich (2007) and Bignold et al. (2009). Despite the appreciation of the correlation between chromosome abnormalities and cancer (Boveri 1902, 1914, 2008), the extent to which large-scale chromosome alterations (aneuploidy) drive cancer development has been debated for more than a century and remains an important, incompletely resolved issue (Ben-David & Amon 2020, Gordon et al. 2012, Holland & Cleveland 2009, Santaguida & Amon 2015). Also not fully understood are the biological mechanisms leading to structural chromosome abnormalities (Hastings et al. 2009, Holland & Cleveland 2012, Leibowitz et al. 2015), particularly the complex chromosome rearrangements that are now recognized as a prevalent feature in most solid tumors (Cortes-Ciriano et al. 2020, Li et al. 2020).

Genome sequencing has significantly advanced our understanding of chromosome alterations in cancer, especially small segmental copy number changes and recombination patterns. The expanding knowledge of cancer genome complexity, enabled by technological advances and consortium-based approaches (Garraway & Lander 2013, ICGC/TCGA PCAWG Consort. 2020, Vogelstein et al. 2013), has revealed a variety of recurrent patterns of DNA rearrangements. We review the most prevalent patterns and discuss how they relate to underlying mechanisms of chromosomal instability. We specifically focus on how cell division and chromosome segregation errors (**Figure 1*a*–*c***), including both short- and long-term genomic consequences of these errors, may explain a large fraction of cancer genomic complexity.

2. WHOLE-GENOME DUPLICATION AND ARM-LEVEL COPY NUMBER EVOLUTION

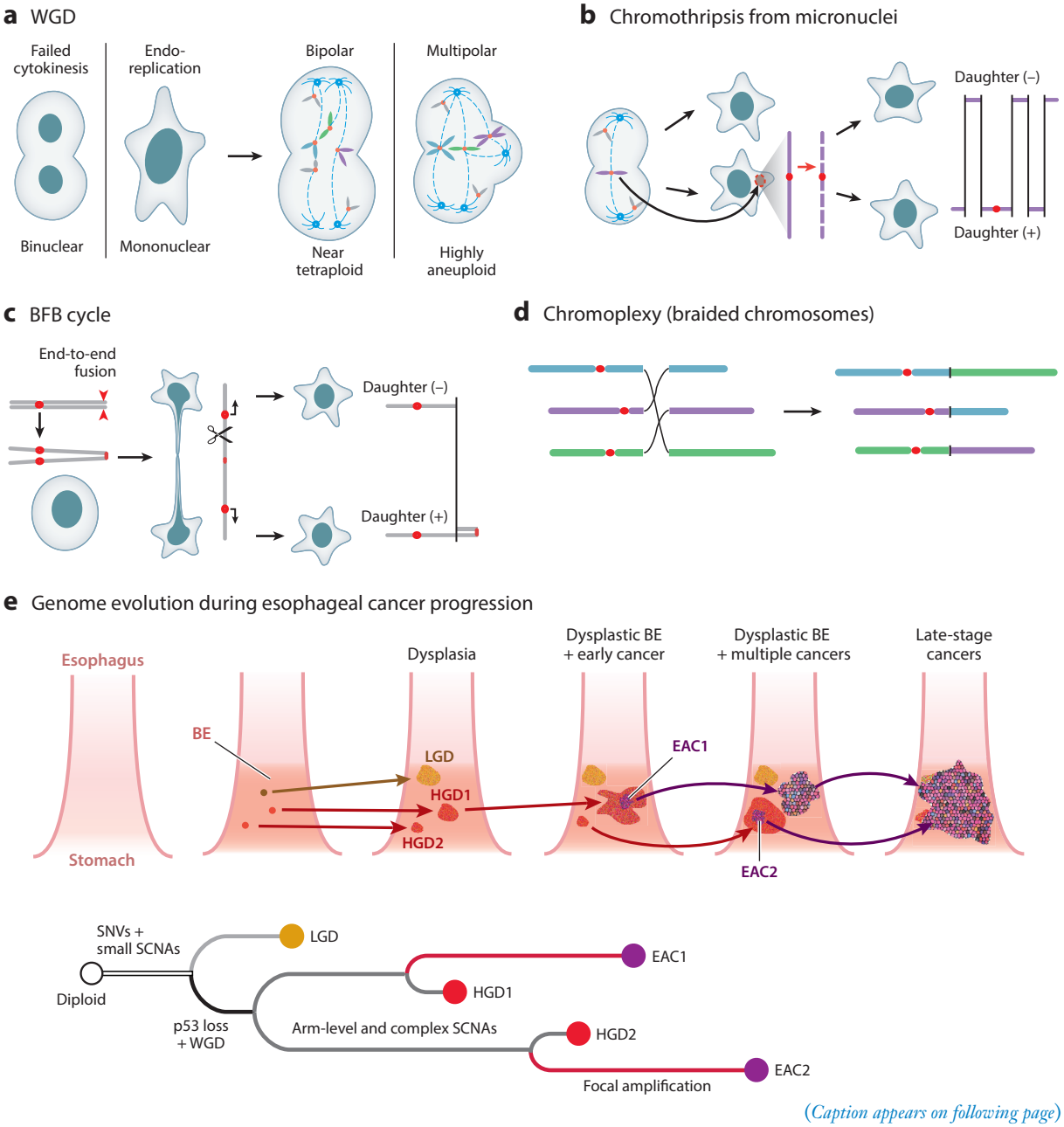
The observation that aneuploid cancer genomes often have a nearly doubled chromosome number of near-tetraploid genomes (Carter et al. 2012, Zack et al. 2013) is consistent with the proposal that they have undergone whole-genome duplication (WGD) (Barrett et al. 1999; Boveri 1914, 2008; Fujiwara et al. 2005; Shackney et al. 1989). The inference that aneuploidy with frequent chromosome duplication results from episodic WGD events followed by chromosome loss, rather than from progressive chromosome gain from a diploid state, is supported both by statistical modeling (see the **Supplemental Appendix**) and by two earlier observations (Storchova & Pellman 2004). First, many cancer cells contain extra centrosomes, which can originate from cytokinesis failure or endoreduplication. Second, tetraploid cells, whose division leads to highly aneuploid cells, is observed in precancerous lesions (Barrett et al. 1999; Galipeau et al. 1996; Reid et al. 1987, 1992) and cell culture models (Lens & Medema 2019) (see the sidebar titled Accumulation of Chromosomal Abnormalities During Progression from Barrett's Esophagus to Esophageal Adenocarcinoma). Below, we discuss links between WGD and tumorigenesis and relate WGD-related evolutionary patterns in cancer genomes to in vitro studies on the consequences of WGD.

Supplemental Material >

2.1. How Prevalent Is Whole-Genome Duplication in Advanced Cancers?

The inference of WGD from cancer sequencing data requires knowledge of the allele-specific chromosomal copy number as determined by copy number imbalance between different chromosomes or allelic imbalance between homologous chromosomes. (For technical details on how to infer WGD from cancer sequencing, readers are referred to the **Supplemental Appendix**.) Both copy number and allelic imbalance at the chromosomal level can be assessed accurately

with sufficient sequence coverage from microarray data (Taylor et al. 2018, Zack et al. 2013) or even from targeted sequencing data (Bielski et al. 2018). An early analysis of genome-wide SNP (single-nucleotide polymorphism) array copy number data suggested WGD to be highly prevalent (>50%) in common epithelial cancers including colorectal, breast, lung, and ovarian cancers, with esophageal adenocarcinomas (EACs) displaying the highest frequency (>60%) (Zack et al. 2013). The high prevalence of WGD was later confirmed in larger cohorts (Bielski



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Major mutational processes driving rapid genome evolution during tumorigenesis. (*a*) WGD originates from either failed cytokinesis (yielding a binucleated cell) or endoreplication (yielding a mononucleated cell). WGD doubles centrosome number, leading to either a pseudo-bipolar spindle and small-scale aneuploidy or multipolar spindles and extensive aneuploidy. (*b*) Chromothripsis from micronuclei. Lagging mitotic chromosomes (*purple*) or acentric chromosome fragments (not shown) generate micronuclei after cell division and the micronuclear chromosomes undergo fragmentation. Fragments are distributed to daughter cells (+ and −) and generate chromothripsis after the second cell division. (*c*) Chromosome BFB cycles are initiated by dicentric chromosome bridge formation. Shown is a bridge resulting from end-to-end fusion of sister chromatids whose breakage leads to reciprocal terminal gain and loss in daughter cells. See **Figure 3** for the outcomes of different types of dicentric bridges. Bridge breakage can also generate chromothripsis near the site of breakage (not shown). (*d*) Chromoplexy is a braiding pattern of translocations involving multiple chromosomes. (*e*) Genome evolution during the progression from BE to LGD, HGD, and, eventually, EAC. (*Top*) Sequential clonal expansions of dysplastic lesions and cancers within a BE field. (*Bottom*) Phylogeny and evolutionary history of dysplastic BE and early EAC samples. Loss of p53 and WGD are often early events preceding the acquisition of arm-level and complex SCNAs, including chromothripsis and terminal segmental copy number changes due to BFB cycles. Continuous evolution of unstable chromosomes eventually leads to focal amplification of oncogenes that drive transformation. Abbreviations: BFB, breakage-fusion-bridge; BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; HGD, high-grade dysplasia; LGD, low-grade dysplasia; SCNA, somatic copy number alteration; SNV, single-nucleotide variant; WGD, whole-genome duplication.

et al. 2018, Quinton et al. 2021, Taylor et al. 2018). Indeed, ~90% of highly aneuploid cancers in the Cancer Cell Line Encyclopedia were inferred to have gone through at least one WGD (Quinton et al. 2021). The overall frequency of WGD across all types of primary cancers is estimated to be >30% (Bielski et al. 2018, Taylor et al. 2018), making it the second most prevalent alteration behind *TP53* mutations (39%) (Donehower et al. 2019). Metastatic cancers display higher WGD prevalence (56%) (Priestley et al. 2019). Moreover, a few rare cancers, such as uterine carcinosarcomas and testicular germ cell carcinomas, show exceptionally high WGD prevalence (>90%) (Bielski et al. 2018, Quinton et al. 2021). The mechanistic basis for cancer-specific WGD rates is an important open question.

2.2. What Genomic Features Are Associated with Whole-Genome Duplication?

Genetic alterations associated with WGD include *TP53* inactivation and *CCNE1* amplification (Zack et al. 2013), mutations that disrupt G1/S cell cycle arrest (Bielski et al. 2018), and inactivating mutations in the regulatory subunits of protein phosphatase 2A (PP2A) (Quinton et al. 2021, Zack et al. 2013) that may promote centrosome clustering (Antao et al. 2019). WGD genomes display more arm-level and segmental copy number alterations than near-diploid genomes (Taylor et al. 2018, Watkins et al. 2020, Zack et al. 2013) and a somewhat higher burden of point mutations even after correction for ploidy (Quinton et al. 2021). Moreover, there are significantly more arm-level DNA losses after WGD than before WGD after ploidy correction (Zack et al. 2013), suggesting that WGD may actively promote arm-level alterations. In esophageal cancers, WGD is also associated with frequent focal amplification events (Stachler et al. 2015). Finally, WGD is rare in hypermutated cancers (Bielski et al. 2018, Quinton et al. 2021), consistent with the hypothesis that high point mutation rates obviate the need for WGD and chromosomal instability during tumorigenesis (Lengauer et al. 1997).

2.3. How Does Whole-Genome Duplication Arise and How Do Duplicated Genomes Subsequently Evolve?

WGD is a frequent event during organismal evolution (Comai 2005, Otto 2007, Ramsey & Ramsey 2014, Van de Peer et al. 2017). It is quite common in plants (Stull et al. 2021,

ACCUMULATION OF CHROMOSOMAL ABNORMALITIES DURING PROGRESSION FROM BARRETT'S ESOPHAGUS TO ESOPHAGEAL ADENOCARCINOMA

Barrett's esophagus (BE), a pathological condition of the esophagus associated with gastroesophageal reflux, was first described in the 1950s (Barrett 1950, Cook & Thrift 2021) and is the only known precursor to esophageal adenocarcinoma (EAC) (Peters et al. 2019, Reid et al. 2010). BE is present in 1–2% of adults (Cook & Thrift 2021). Although the rate of BE-to-EAC progression is low (~0.5%), the presence of BE in about 50% of EAC patients (Cook & Thrift 2021), together with shared molecular and genetic features between paired BE and EAC cells (Contino et al. 2017), indicates that BE is a common precursor of EAC.

The evolution from BE to EAC can be tracked because BE patients undergo routine surveillance endoscopy. Early flow cytometry studies (Reid et al. 1987, 1992) identified tetraploid cells as precursors to aneuploidy and malignant progression. Subsequently, tetraploidization was found to be preceded by p53 loss (Galipeau et al. 1996), enabling the survival of aneuploid cells generated by chromosome loss (Barrett et al. 1999).

The history of EAC evolution can also be inferred by analyzing paired BE and EAC samples both taken at the time of surgery from the same patient (Ross-Innes et al. 2015, Stachler et al. 2015). EAC genomes not only harbor extensive chromosome duplications and aneuploidy but also display a significant burden of segmental copy number alterations, including frequent oncogene amplification alterations. The observation that few segmental copy number alterations are shared between paired EAC and BE genomes supports a model of branching evolution driven by chromosomal instability.

To gain further information on segmental copy number evolution during BE progression, one of us (C.-Z. Zhang) recently performed whole-genome sequencing analyses of advanced BE and early EAC using two complementary strategies (Stachler et al. 2021). First, bulk whole-genome sequencing was performed on BEs with progressive histological aberrations and early EACs (Peters et al. 2019) identified in endoscopic mucosal resections (**Figure 1*e***). Second, whole-genome sequencing was performed on single cells collected by endoscopic brushing of a high-grade BE lesion. Both strategies enabled direct sampling of genetic heterogeneity in BE cells or subclones. We used the genetic variation information to determine the phylogeny of individual genomes and then inferred the evolutionary sequence of chromosomal changes.

These analyses revealed patterns of segmental copy number alteration and evolution during BE progression that suggest multigenerational evolution starting from dicentric chromosome formation (Umbreit et al. 2020). These patterns include both single-copy segmental gain or loss that can be attributed to a single breakage-fusion-bridge (BFB) cycle and multicopy segmental duplications or amplifications that can arise from multiple BFB cycles. Two classes of chromothripsis events were further identified: Those that are localized to a subchromosome arm region are consistent with the direct consequence of dicentric chromosome breakage (Umbreit et al. 2020), and those spanning whole-chromosome arms are consistent with secondary events after dicentric chromosome breakage (Li et al. 2014, Maciejowski et al. 2015, Umbreit et al. 2020), most likely micronucleation (Umbreit et al. 2020, Zhang et al. 2015). These results suggest that many complex chromosomal rearrangement patterns in EAC genomes, including both chromothripsis (Nones et al. 2014) and focal gene amplifications (Stachler et al. 2015), may have originated from the formation and evolution of dicentric chromosomes. The insights into the evolution of complex EAC genomes illustrate the power of the integration of high-resolution (e.g., single-cell) sequencing of precancer conditions with mechanistic knowledge that constrains the phylogenetic inference.

Yang et al. 2021) and is also inferred to have occurred multiple times during the evolution of animals (Berthelot et al. 2014, Lien et al. 2016, Session et al. 2016, Shingate et al. 2020, Van de Peer et al. 2017). WGD or tetraploidization can also be developmentally programmed in normal tissues and arise by cell fusion, endoreplication (DNA replication without mitosis), or tissue-specific cell cycles lacking cytokinesis (Davoli & de Lange 2011, Orr-Weaver 2015). Normal polyploid cells are

generally terminally differentiated, nondividing cells that may be advantageous as tissue barriers (e.g., trophoblast giant cells of the placenta) or factories of biosynthesis (e.g., megakaryocytes producing platelets). A notable exception is polyploid hepatocytes, whose ability to divide after stress or injury suggests a possible connection between polyploidy and either damage tolerance or possibly tissue regeneration (Donne et al. 2020, Duncan et al. 2010, Knouse et al. 2018, Zhang et al. 2018). Pathological WGDs in cancer likely arise from similar mechanisms. Cell fusion can be induced by viral infection (Duelli & Lazebnik 2007) or result from processes similar to phagocytosis leading to cell-in-cell structures termed entosis (Fais & Overholtzer 2018). Prolonged G2/M cell cycle arrest due to persistent DNA damage can cause mitotic bypass, triggering endoreplication (Davoli et al. 2010). Finally, cytokinesis failure is the final common outcome of many cell division errors (Lens & Medema 2019).

Although various processes can give rise to WGD, the evolutionary consequences of WGD may be similar. As WGD is usually accompanied by centrosome duplication, tetraploid cells are prone to varying degrees of chromosome missegregation due to multipolar cell divisions or chromosome attachment errors from defective bipolar spindles (Ganem et al. 2009, Holland & Cleveland 2009, Lens & Medema 2019, Storchova & Pellman 2004). Multipolar division of a tetraploid cell may give rise to three or four highly aneuploid daughters, whereas bipolar mitosis with chromosome missegregation will lead to significant but less dramatic aneuploidy (**Figure 1*a***). The missegregated chromosomes often "lag" at anaphase and can be partitioned into abnormal nuclear structures called micronuclei (Krupina et al. 2021). As discussed below in Section 4.1, micronuclei are themselves a source of extensive DNA damage and chromosome rearrangement (**Figure 1*b***). These downstream events after WGD provide mechanistic explanations for elevated rates of both arm-level and segmental copy number alterations after WGD seen in cancer genomes (Minussi et al. 2021, Stachler et al. 2021). Although fitness effects will contribute to the final chromosome composition in a cancer genome, the similarity of cancer genomes to the outcomes of in vitro WGD without strong selection supports the conclusion that one-off tetraploidization events make a major contribution to cancer genome architecture.

2.4. When Do Whole-Genome Duplication Events Occur During Tumorigenesis?

WGD may occur at any time during somatic genome evolution, but only those WGD events that have undergone clonal fixation can be detected in bulk cancer genome sequencing data. Interestingly, clonal fixation of WGD is inferred to occur quite early during cancer evolution despite its deleterious short-term fitness effects (see Section 2.5).

The timing of clonal fixation of WGD during cancer evolution can be inferred based on three features: (*a*) the clonality of WGD compared to other alterations, (*b*) the number of somatic mutations acquired before WGD (present on all duplicated homologs) and after WGD (present on a subset of duplicated homologs), and (*c*) the presence of WGD in longitudinal or multiregional cancer samples. Based on the clonality of WGD relative to other cancer gene alterations, Bielski et al. (2018) found that clonal fixation of WGD usually occurred prior to the acquisition of subclonal drivers but after the truncal oncogenic point mutations (e.g., activated RAS) and after *TP53* inactivation. By analyzing the burden of pre- and post-WGD CpG > TpG mutations that are proposed to accumulate steadily like a molecular clock (Alexandrov et al. 2015), Gerstung et al. (2020) estimated that WGD events often occurred years, even more than a decade, prior to tumor diagnosis. The early incidence of WGD and subsequent copy number evolution is further supported by multiregional (Watkins et al. 2020) and single-cell sequencing (Minussi et al. 2021) of primary cancers.

A related question is the timing of WGD fixation during the course of disease progression. The evolutionary sequence of p53 loss followed by WGD, and then aneuploidy, has been well established in the development of EAC from the premalignant condition known as Barrett's esophagus (BE) (Barrett et al. 1999, Galipeau et al. 1996, Reid et al. 1992) (see the sidebar titled Accumulation of Chromosomal Abnormalities During Progression from Barrett's Esophagus to Esophageal Adenocarcinoma). The analysis of EAC and adjacent BE cells at different stages based on histopathology confirmed the high prevalence of WGD in both EACs and high-grade BE cells (Stachler et al. 2021). The observation of frequent branching evolution of genomically divergent high-grade dysplastic or cancerous lesions, either from independent WGD events or from independent copy number evolution following a truncal WGD event (Stachler et al. 2021), provides direct evidence of WGD as a frequent event that can occur throughout cancer progression and fuel genomic diversity.

2.5. What Are the Evolutionary Consequences of Whole-Genome Duplication?

Cells undergoing WGD are under immediate negative selection due, in part, to p53 dependent G1 arrest if cytokinesis failure occurs after prolonged mitosis (Margolis 2005, Uetake & Sluder 2010). (This provides selective pressure for WGD clones to emerge in p53-deficient cells.) The evolution of aneuploid genomes due to post-WGD genome instability also causes cells to be less proliferative (Siegel & Amon 2012). Given the immediate negative fitness effects of WGD (Ganem & Pellman 2007), it seems counterintuitive that WGD events are frequently fixed in the founding tumor clone.

The short-term negative fitness effects of WGD may be counterbalanced by at least three sources of long-term evolutionary advantage (Bakhoum & Landau 2017, Gordon et al. 2012, Otto 2007, Sansregret & Swanton 2017, Van de Peer et al. 2017, Vendramin et al. 2021). The first is increased genetic diversity due to post-WGD instability that is demonstrated both in vitro (Bollen et al. 2021, Ganem et al. 2009) and in vivo (Stachler et al. 2021, Watkins et al. 2020). The second is faster acquisition of dominant mutations simply because doubling chromosome content doubles the chances of acquiring any given mutation. This model is supported by (*a*) an overall positive correlation between the burden of point mutations and aneuploidy (Davoli et al. 2017, Quinton et al. 2021, Taylor et al. 2018) and (*b*) the presence of many subclonal oncogenic drivers acquired after WGD (Bielski et al. 2018, Gerstung et al. 2020, Watkins et al. 2020). Finally, extra chromosomes buffer the phenotypic consequences of deleterious recessive mutations or chromosome aneuploidies (Dewhurst et al. 2014, Lopez et al. 2020, Mayer & Aguilera 1990, Storchova et al. 2006).

The evolutionary benefits of WGD are perhaps best demonstrated in the acquisition of oncogenic gene amplification. In a study from one of us (D. Pellman), it was shown that tetraploidization of p53-null mammary epithelial cells can promote the development of mammary cancers after transplantation into nude mice (Fujiwara et al. 2005). All of these tumors acquired focal gene amplifications, including recurrent amplifications spanning the *Yap1* gene that was later shown to be a recurrent target of amplification in liver cancer (Zender et al. 2006) and in brain metastases (Shih et al. 2020). DNA amplification is often initiated by the formation of unstable chromosomes and is accompanied by extensive DNA loss (Shoshani et al. 2021, Zhang 2021). Therefore, WGD promotes DNA amplification both by causing chromosomal instability and by buffering the effects of DNA loss that can occur during the course of gene amplification.

In summary, WGD is a unique evolutionary event that has the potential to confer a long-term adaptive advantage, despite its immediate fitness cost. The negative immediate fitness cost of WGD implies that clonal fixation of WGD should be rare. These considerations suggest that we can think of WGD like any other mutation (albeit one with large and complex fitness effects): In

the absence of selection its effects are generally deleterious, but under selection rare outcomes of WGD can be adaptive (Comai 2005, Otto 2007, Selmecki et al. 2015, Van de Peer et al. 2017).

2.6. What Is the Rate and Evolutionary Pattern of In Vivo Whole-Genome Duplication?

Despite its profound impact on cancer genome evolution, there have been relatively few experimental studies of WGD and many open questions remain to be addressed. How frequently do post-WGD cells undergo multipolar cell division? How quickly do cells restore karyotype stasis? How diverse is the post-WGD progeny population and how much does this vary by cell type? Addressing these questions will advance our understanding of both the dynamics of cancer evolution (including the rate and timing of WGD incidence) and the emergence of tumor heterogeneity. It will be important to understand whether and how WGD incidence and post-WGD evolution depend on the tissue architecture (Knouse et al. 2018), especially in pathological conditions such as liver cirrhosis (Brunner et al. 2019) or chronic inflammation (Olafsson et al. 2020). Due to the dynamic nature of post-WGD evolution, addressing these questions will necessitate single-cell analysis (Knouse et al. 2014).

3. AN EVOLVING LANDSCAPE OF SEGMENTAL COPY NUMBER COMPLEXITY IN CANCER GENOMES

DNA copy number changes were initially assessed by microarrays and whole-exome sequencing (Beroukhim et al. 2010, Carter et al. 2012, Zack et al. 2013). At that time, individual copy number alterations were generally assumed to be independent events that accrue over many generations. For arm-level changes, WGD is a clear example of a single catastrophic event that generates many chromosome duplications all at once (Carter et al. 2012). For segmental changes, there is no easy way to distinguish alterations that arise independently from repetitions of a single process (e.g., simple tandem duplications or deletions) from those generated by all-at-once or multigenerational catastrophes (Hicks et al. 2006).

The treatment of individual somatic copy number alterations (SCNAs) as independent events led to an appealingly simple model of segmental SCNA formation that was largely determined by chromatin conformation and replication timing (Beroukhim et al. 2010, De & Michor 2011, Fudenberg et al. 2011). This model was partially consistent with the mechanisms of recurrent chromosomal translocations (Chiarle et al. 2011, Hakim et al. 2012, Klein et al. 2011) or DNA deletions (Debatisse et al. 2012, Durkin & Glover 2007). However, the high resolution afforded by whole-genome sequencing changed this view. Even the early whole-genome sequencing studies indicated that somatic chromosomal rearrangements display significant complexity (Campbell et al. 2008, Lee et al. 2010, Pleasance et al. 2010, Stephens et al. 2009) that typically cannot be deconvoluted into independent duplications or deletions.

Several studies focusing on sequence rearrangements accompanying DNA amplification (Bignell et al. 2007, Campbell et al. 2010, Storlazzi et al. 2010) suggested a possible connection to breakage-fusion-bridge (BFB) cycles (**Figure 1*c***), a well-known form of chromosomal instability in cancer cells (Gisselsson et al. 2000). BFB cycles were first described in maize by Barbara McClintock in the late 1930s (McClintock 1939, 1941, 1951). Unlike multigenerational BFB cycles, the discovery of chromothripsis (**Figure 1*b*** and **2*a***) (Kloosterman et al. 2011a,b; Rausch et al. 2012; Stephens et al. 2011) suggested that massive chromosome rearrangement can occur all at once in one generation. Other hypothesized all-at-once rearrangement patterns include chromoplexy (Baca et al. 2013), characterized by a braided joining pattern between paired break ends (**Figure 1*d***), and chromoanasynthesis (**Figure 2*b***) (Liu et al. 2011), characterized by

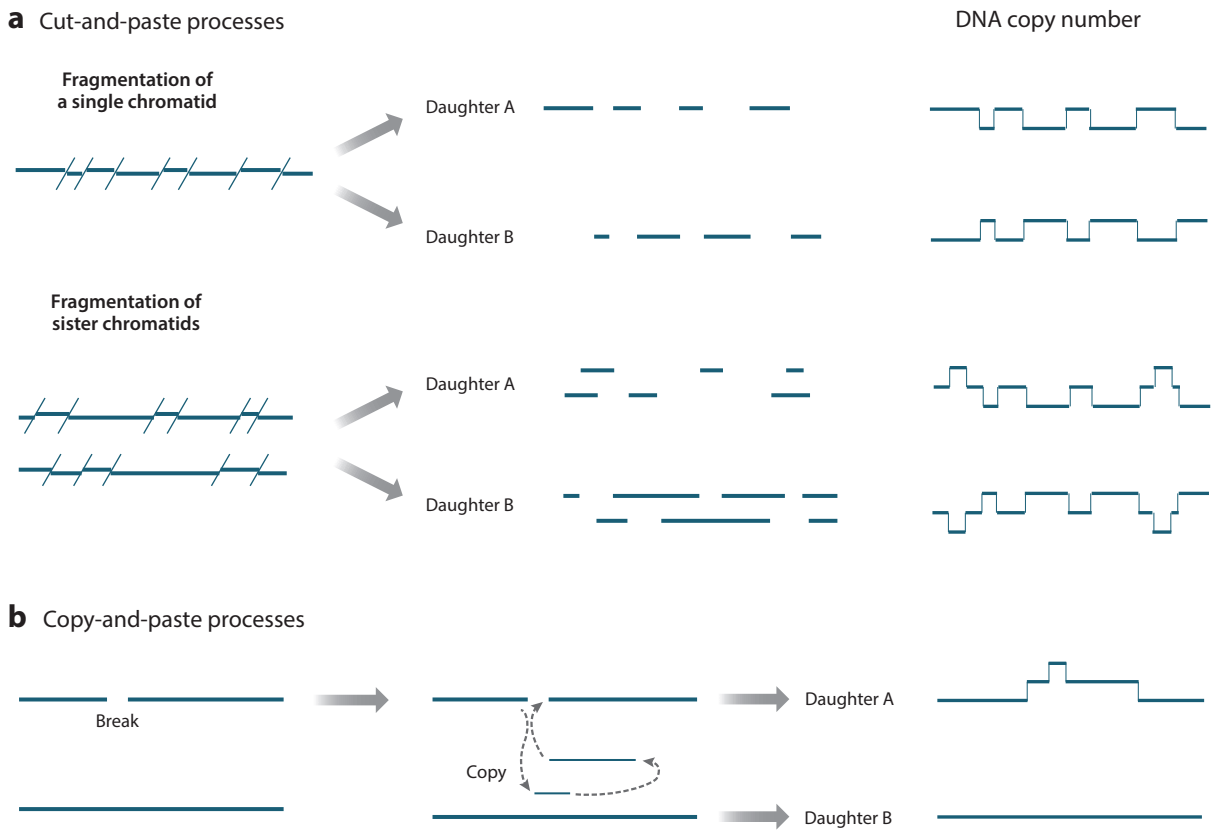


Figure 2

Cut-and-paste versus copy-and-paste processes of chromosome rearrangements. (*a*) Cut-and-paste rearrangements arise from chromosome fragmentation. (*Top*) If fragments of a single chromatid are distributed to both daughters, DNA retention and loss result in two-state oscillating copy number patterns. (*Bottom*) If both sister chromatids are fragmented, there can be three copy number states reflecting loss (copy number 0), the retention of one sister (copy number 1), and the retention of both sisters (copy number 2) (first proposed by Li et al. 2014). (*b*) Copy-and-paste rearrangements arise from aberrant DNA replication with template-switching events. Aberrantly copied DNA (*thin lines*) does not originate from or necessarily lead to breaks in the donor DNA (the template; *bottom thick line*); the rearranged chromosome is inherited by one daughter (A), whereas the other daughter (B) will inherit an intact chromosome. By contrast with cut-and-paste processes, copy-and-paste processes result in a net gain of DNA (combining copy number in both daughters).

interspersed segmental gain and loss, postulated to arise from aberrant DNA replication (Hastings et al. 2009).

While complex rearrangements were initially thought to be rare or associated with specific tumor types, subsequent large-scale cancer whole-genome sequencing studies showed that these events contribute a large fraction of genomic rearrangements in human cancers (Li et al. 2020), including oncogenic alterations (Rheinbay et al. 2020) derived from recurrent translocations (Anderson et al. 2018, Lee et al. 2019), tumor suppressor loss (Yang et al. 2013), and the formation of extrachromosomal DNA circles/double minute chromosomes (Rausch et al. 2012, Stephens et al. 2011, Verhaak et al. 2019). The prevalence of chromothripsis was initially estimated to be 2–3% based on the detection of oscillating DNA copy number (Stephens et al. 2011). However, subsequent analyses of cancer whole-genome sequencing data suggest that chromothripsis is

present in ~30% of all cancers (Cortes-Ciriano et al. 2020), with especially high prevalence in liposarcomas (~100%), osteosarcomas (~90%), glioblastomas (~75%), and EACs (50–70%). Chromoplexy was originally identified in prostate cancers (Baca et al. 2013) as a frequent mechanism leading to recurrent *TMPRSS2-ERG* fusions; it is now also appreciated to be a frequent source of recurrent fusions in sarcomas (Anderson et al. 2018) and lung cancers (Lee et al. 2019). Chromoanasynthesis, originally identified in congenital disorders (Liu et al. 2011), is now thought to be a special case of complex rearrangements due to template-switching events in aberrant replication processes (Li et al. 2020, Liu et al. 2012, Meier et al. 2014).

The discovery of complex rearrangement patterns described above challenged the view of cancer genome evolution as a gradual accumulation of chromosome alterations (Bignell et al. 2010, Fearon & Vogelstein 1990, Nowak et al. 2002). The quest for mechanisms underlying these patterns has led to a series of discoveries relating mechanisms of chromosome instability to patterns of DNA rearrangement and evolution. In the remaining sections of this review, we focus on recent progress linking complex rearrangement patterns to chromosome segregation errors that has been enabled by a combination of DNA sequencing and experimental cell biology.

4. SEGMENTAL COPY NUMBER ALTERATIONS AND REARRANGEMENTS DUE TO CHROMOSOME MISSEGREGATION

4.1. Chromothripsis from DNA Fragmentation in Micronuclei

Chromothripsis (from the Greek for "shattered chromosomes") has two distinguishing features: a scrambled pattern of sequence rearrangements that are concentrated to one or a few chromosomes, and an oscillating pattern of DNA copy number (**Figure 2*a***). These two features are poorly explained by a gradual evolution model of sequential rearrangement acquisition both because of the local concentration of rearrangement breakpoints and because progressive sequence rearrangements are expected to lead to more variation in copy number states than simple oscillations (Stephens et al. 2011). The copy number and rearrangement features of chromothripsis led Peter Campbell and colleagues to hypothesize that chromothripsis resulted from extensive fragmentation of individual chromosomes. The chromosome fragmentation model offers a parsimonious explanation of the concentration of alterations to single chromosomes and the picture of segmental DNA rearrangements dominated by fragment loss. However, because the underlying mechanism was unknown, and because it challenged the gradualism view, the chromothripsis model was initially not universally accepted (Kinsella et al. 2014, Righolt & Mai 2012).

The concentration of alterations to single chromosomes suggested a possible connection between chromothripsis and missegregated chromosomes (Meyerson & Pellman 2011, Stephens et al. 2011). Coincidentally, one of us (D. Pellman) was investigating the biology of micronuclei because they are a frequent outcome of mitotic errors that generate lagging chromosomes, commonly from so-called merotelic kinetochore attachments (a kinetochore is caught in a tug-of-war between microtubules from opposite spindle poles) (Cimini 2007, Cimini et al. 2001). Micronuclei are also a well-known feature of the abnormal nuclear architecture of cancer cells known as nuclear atypia (de las Heras & Schirmer 2014, Fischer et al. 2010, Zink et al. 2004). The discovery that micronuclei show a variety of deficiencies of nuclear import, gene transcription, and DNA metabolism, together with a striking observation of a pulverized appearance of micronuclear chromosomes at metaphase, raised an appealing model that DNA damage in micronuclei might lead to chromosome fragmentation (Crasta et al. 2012; Kato & Sandberg 1967; Obe & Beek 1975, 1982) and potentially chromothripsis (Crasta et al. 2012).

To definitively test this model, we combined live-cell imaging with single-cell whole-genome sequencing (termed Look-Seq) and directly demonstrated that chromosomes partitioned into

micronuclei can, within a single generation, undergo fragmentation and ligation to generate chromothripsis (Leibowitz et al. 2021, Zhang et al. 2015). In the study by Zhang et al. (2015), micronuclei were induced by random chromosome missegregation, and the identity of the micronuclear chromosome was inferred based on its odd DNA copy number state due to deficient DNA replication in micronuclei (Obe & Beek 1982). The underreplication feature as a hallmark of the micronuclear chromosome was further validated by our recent study where micronuclei were generated by DNA breakage induced by CRISPR-Cas9 and the identity of the underreplicated acentric chromosome fragment was known a priori (Leibowitz et al. 2021). Using a different approach targeting Y chromosomes (chrY) to micronuclei (Ly et al. 2017) followed by bulk DNA sequencing of clonal populations, Ly et al. (2019) identified Y chromothripsis and other complex alterations that may arise from downstream events in the progeny populations derived from cells with missegregated chrY. This and other studies (Kneissig et al. 2019) independently confirmed that micronuclei can cause chromothripsis and demonstrated that at least a fraction of cells with fragmented chromosomes from micronuclei can expand into clonal populations as seen in cancer. The discovery of chromosome missegregation events (Vanneste et al. 2009), including micronucleation (Daughtry et al. 2019, Vazquez-Diez et al. 2016), during early mammalian development, or in certain circumstances in plants (Tan et al. 2015), suggests that chromothripsis in congenital disorders (Kloosterman et al. 2011a) may also arise from micronuclei (Papathanasiou et al. 2021). The finding of congenital chromothripsis that can pass through the germline (de Pagter et al. 2015) also raises the possibility that chromothripsis could contribute to karyotype alterations during organismal evolution (McClintock 1984, Shapiro 2021).

A unique advantage of single-cell analysis is that the distribution of chromosome fragments between the two daughter cells can be determined from the mirror image copy number pattern between daughters, which was used to clearly establish that chromosome fragmentation is a major component of chromothripsis (**Figure 1*b***) (Zhang et al. 2015). As cancer cells with chromothripsis would most commonly originate by clonal expansion of only one of the two daughter cells, the DNA partitioned into the daughter cell that failed to expand was lost in the expanded clone. Therefore, chromothripsis from micronuclei is fundamentally a cut-and-paste process due to the uneven distribution of chromosome fragments (**Figure 2*a***).

As is discussed below, Liu et al. (2011) suggested an alternative model (termed chromoanasynthesis) that the oscillating DNA copy number pattern results from sequential copy-and-paste of DNA sequences through a series of template-switching events (**Figure 2*b***) during a process called microhomology-mediated break-induced replication (MMBIR) (Kockler et al. 2021). Although the molecular processes of MMBIR are distinct from chromosome fragmentation in micronuclei, the copy number outcomes of these processes may appear similar or indistinguishable in a single cell. Definitively distinguishing between copy-and-paste and cut-and-paste processes requires complementary information from both daughter cells.

4.2. Genomic Rearrangements Resulting from Breakage-Fusion-Bridge Cycles

The BFB cycle was originally discovered by Barbara McClintock as a form of ongoing evolution of dicentric ring chromosomes (McClintock 1939) or linear chromosomes (McClintock 1941) in maize. Chromosome bridges arise from a tug-of-war between the centromeres of dicentric chromosomes pulled toward opposite spindle poles. The initial dicentric chromosome may be generated by end-to-end chromosome fusion due to telomere erosion or illegitimate DNA recombination, or from linked or entangled sister chromatids due to incomplete DNA replication or decatenation (Umbreit et al. 2020). Additional BFB cycles occur because of one or two unresolved broken chromatids. A single dicentric chromatid initiates the chromatid-type BFB cycle

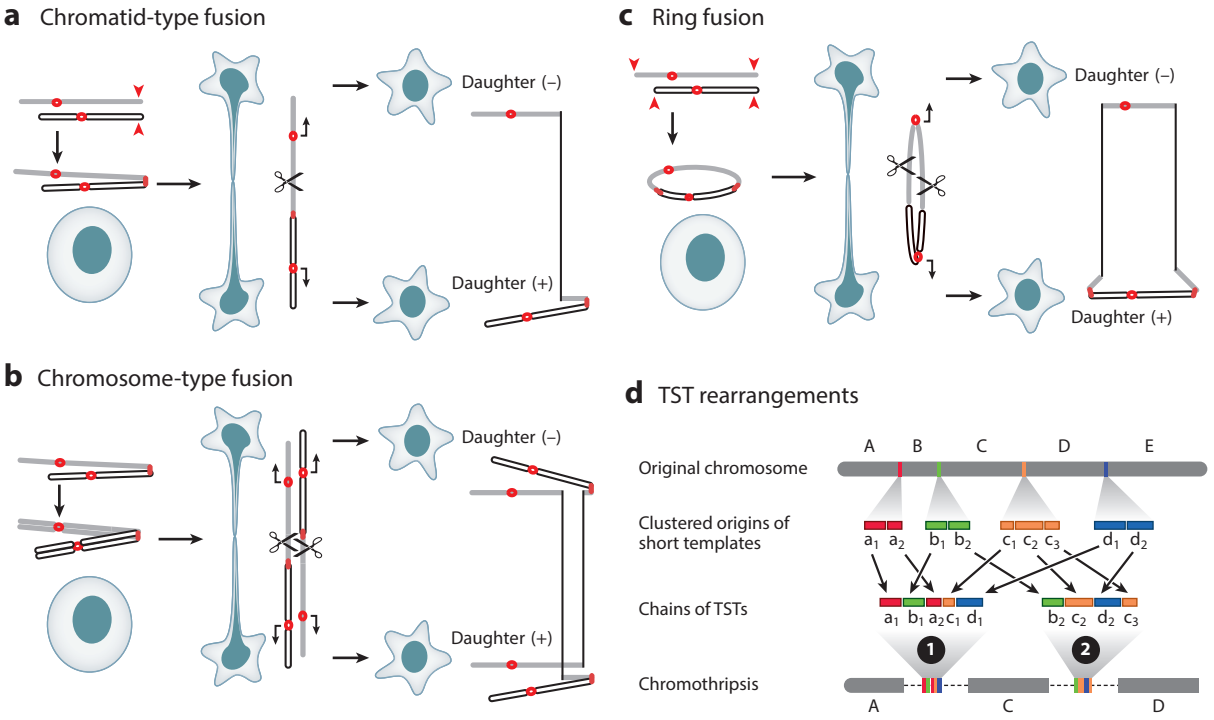


Figure 3

Rearrangement outcomes from the breakage of dicentric chromosome bridges. (*a*) Asymmetric breakage of a single dicentric chromosome (chromatid-type fusion in G2 phase) generates terminal segmental gain and loss in the daughters. The open and filled chromatids can be either sister chromatids or chromatids from different chromosomes. (*b*) Fusion between two different chromosomes in G1 phase creates a dicentric chromosome that is replicated to become a pair of dicentric chromatids. If the kinetochores of both centromeres attach to the same spindle pole, a dicentric chromatid will segregate to each daughter and there is no bridge (not shown). If the kinetochores of both centromeres of each chromatid are attached to opposite spindle poles, both dicentric chromatids form a bridge. If the two dicentrics orient along the mitotic spindle in an antiparallel fashion, the copy number outcome from breakage of both dicentrics is a reciprocal gain and loss of an internal segment (see Umbreit et al. 2020, figure 2C). (*c*) If both telomeres from a chromatid are lost and undergo fusion, either a multicentric (not shown) or a ring chromosome can be generated. Breakage of these chromosomes can generate reciprocal gain or loss of centromere-containing internal segments. (*d*) Tandem short template (TST) rearrangements consist of a series of short DNA (~200–400 bp) sequences (termed templates) placed in tandem. TST rearrangements are identified in both experimentally induced chromothripsis and primary cancers. In experimentally induced chromothripsis, the templates (a_1, a_2, b_1, b_2, etc.) typically originate from regions near the ends (*red*, *green*, *orange*, and *blue*) of broken DNA (*gray*; A, B, C, etc.). The templates are joined randomly and inserted into the long-range junctions (❶ and ❷) between broken DNA.

mediated by the fusion between sister broken chromatids after DNA replication (**Figure 3*a***); a pair of dicentric chromatids initiate the chromosome-type BFB cycle that is mediated by the fusion between the broken chromatids (**Figure 3*b***). Both types of BFB cycles cause an asymmetric distribution of sister DNA into daughter cells in every generation (gain in one daughter and reciprocal loss in the other) and give rise to progeny populations with significant copy number heterogeneity (Gisselsson et al. 2000) and nonreciprocal translocations (Artandi et al. 2000).

Given that critical telomere shortening is implicated in tumorigenesis (Artandi & DePinho 2010, De Lange 2005), it is natural to expect that end-to-end chromosome fusion can initiate BFB cycles and shape cancer genome evolution. The best-known rearrangement signature associated with BFB cycles is nested foldback rearrangements (inverted duplications) that are attributed to sister chromatid fusion in BFB cycles (Bignell et al. 2007, Campbell et al. 2010, McClintock 1941).

However, foldback rearrangements are neither the exclusive outcome nor an exclusive feature of BFBs. First, sister chromatid fusion only occurs in the chromatid-type BFB cycles, but not in the chromosome-type BFB cycles that generate interchromosomal translocations (**Figure 3**) (McClintock 1951, Umbreit et al. 2020). In situations of gross genome instability (Artandi et al. 2000, Cleal et al. 2019, Maciejowski et al. 2015), we expect chromosome-type fusions to be at least as frequent as, if not more frequent than, sister chromatid fusions in S/G2 cells. Second, as each BFB cycle is expected to generate reciprocal DNA loss and gain in daughter cells, foldback rearrangements reflecting copy number gains are only propagated to a subclonal population and may not be detected from sequencing the dominant clone. Finally, nested foldback rearrangements are not only caused by sister chromatid fusion but also can result from replication-coupled recombination of sister DNA [e.g., near inverted repeats (Haber & Debatisse 2006)].

To directly determine the genomic outcomes of multigenerational BFB evolution, we have extended our Look-Seq approach to incorporate both short- and long-term population growth, combining direct single-cell sequencing with bulk sequencing of subclones derived from a single cell (Umbreit et al. 2020). This analysis revealed a spectrum of DNA copy number and rearrangement outcomes that extend results from earlier studies using bulk DNA sequencing of post–telomere crisis clones (Cleal et al. 2019, Maciejowski et al. 2015).

The most common outcome of the BFB cycle is reciprocal gain and loss of large chromosome segments (>5 Mb) between daughter cells due to an asymmetric breakage of the bridge chromosome (**Figure 3**). We identified both terminal gain and loss due to the chromatid-type BFB cycle (**Figure 3*a***) and a previously unreported pattern of internal gain and loss due to the chromosome-type BFB cycle (**Figure 3*b***) (Umbreit et al. 2020). In addition to these two outcomes, we further envision a scenario where both telomeric ends of a chromatid are fused to other chromosome ends, generating either a ring chromosome or multicentric chromosomes (**Figure 3*c***): Breakage of these chromosomes may generate reciprocal gain and loss of an internal segment spanning the centromere, as seen in primary cancer genomes (Stachler et al. 2021) and complex ring chromosomes in soft tissue sarcomas (Garsed et al. 2014).

In addition to reciprocal large SCNAs due to asymmetric chromosome breakage, we also observed reciprocal retention and loss of small DNA segments (0.1–1 Mb) near the site of inferred bridge breakage (because it was within the larger SCNA) (Umbreit et al. 2020). As these small-scale DNA copy number changes were observed even when chromosome bridges were mechanically broken with glass micropipettes (Umbreit et al. 2020, figures 4A and S8), we attributed these changes to local chromatin fragmentation related to bridge extension and breakage that depend on actin contractility or tensile force. The retention of small DNA fragments near large segmental loss is similar to the so-called local-jump rearrangement pattern identified in cancer genomes (Li et al. 2020). However, the reciprocal pattern of retention and loss enabled by the analysis of both daughters suggests that these rearrangements result from the uneven distribution of chromosome fragments (i.e., cut-and-paste), rather than aberrant replication processes (copy-and-paste), as previously proposed (Li et al. 2020).

Finally, in about 20% of cases, we observed a unique pattern of sequence rearrangement consisting of multiple short DNA sequences (<1 kb) inserted in tandem between large DNA segments (**Figure 3*d***). We refer to this pattern as tandem short templates (TSTs). The TST rearrangement pattern was first described in our analysis of micronuclei (Zhang et al. 2015) but appeared to be more prevalent in cells with broken bridges (with the caveat that the sample sizes of these experiments are small: 10–20 cell pairs). The inserted sequences in TST rearrangements were almost exclusively derived from 1–10-kb breakpoint hotspots on the bridge chromosome. The TST rearrangement pattern resembles tandem insertion rearrangements (chains, bridges, or cycles of templated insertions) seen in cancer genomes (Li et al. 2020). Short insertions have

also been observed in both human (Lee et al. 2007, Liu et al. 2011) and cancer (Lee et al. 2019) genomes, as well as in experimental models (Cleal & Baird 2020, Cleal et al. 2019). Although short insertions are often assumed to reflect copied sequences during aberrant replication (Hastings et al. 2009, Kockler et al. 2021), the observations of insertions related to chromothripsis (Cortes-Ciriano et al. 2020, Stephens et al. 2011, Umbreit et al. 2020, Zhang et al. 2015) suggest that other mechanisms involving local fragmentation may be possible. Therefore, future efforts to determine the origin of the TST signature are expected to identify important new mutational processes common during tumorigenesis.

4.3. Footprints of Breakage-Fusion-Bridge Cycles in Primary Cancers

As one cannot identify reciprocal copy number changes in a single cancer clone expanded from a single ancestral cell, it is impossible to discern the most obvious signature of BFB cycles in cancer genomes. However, the footprints of multigenerational BFB evolution in cancer genomes may be identified from the compound copy number outcomes of BFB cycles. The analysis of copy-number evolution in esophageal cancer progression provides multiple lines of evidence indicating that BFB cycles directly generate or indirectly contribute to a large fraction of segmental SCNAs (Stachler et al. 2021).

Through haplotype-specific DNA copy number analysis of late-stage BE lesions and adjacent early EACs isolated from biopsies from the same patient (see the sidebar titled Accumulation of Chromosomal Abnormalities During Progression from Barrett's Esophagus to Esophageal Adenocarcinoma), one of us (C.-Z. Zhang) has identified two patterns of branching SCNA evolution during EAC progression that are most simply explained by BFBs. First, examples of reciprocal DNA copy number changes in related BE/EAC subclones were observed that exactly recapitulate the reciprocal distribution of broken dicentric chromosomes into daughter cells in a single BFB cycle as detected in a single-cell analysis (Umbreit et al. 2020). Second, many examples of divergent DNA copy number patterns in related BE/EAC subclones were observed that have shared ancestral terminal or internal SCNAs, consistent with multigenerational BFB evolution. Two rearrangement patterns were further identified that strongly indicate bridge breakage as the underlying mechanism. The first pattern consists of multiple interchromosomal rearrangements joining breakpoints associated with internal or terminal SCNAs; these patterns recapitulate observations from postcrisis clones (Maciejowski et al. 2015) and may result from unbalanced interchromosomal translocations between multiple broken dicentric chromatids (Umbreit et al. 2020). The second pattern is chromothripsis localized either near the boundaries or spanning the region of terminal/internal SCNAs; this pattern is consistent with local fragmentation of the bridge chromatin (Umbreit et al. 2020).

5. FUTURE DIRECTIONS

5.1. What Are the Rearrangement Signatures of Aberrant DNA Replication Processes?

Single-ended DNA breaks generated during replication can initiate break-induced replication (BIR) (Anand et al. 2013, Kockler et al. 2021). BIR is most extensively studied in budding yeast and has been implicated in various repair processes in mammalian cells (Costantino et al. 2014, Dilley et al. 2016, Li et al. 2021, Minocherhomji et al. 2015, Scully et al. 2019). Hastings et al. (2009) further proposed a model of MMBIR, a replication process with frequent template-switching events (**Figure 2*b***), to explain complex genomic rearrangements observed in some

human patients with congenital disorders (Beck et al. 2019; Lee et al. 2007; Liu et al. 2011, 2017). MMBIR is thought to proceed by a similar mechanism as BIR but is initiated by short stretches of microhomology (as few as 2 bps) rather than large sequence homology characteristic of BIR.

MMBIR was proposed to be a copy-and-paste mechanism that could give rise to duplications of varying sizes (Lee et al. 2007, Li et al. 2020, Liu et al. 2011); the presence of short insertions (<1 kb) at rearrangement junctions is usually regarded as the most distinguishing feature of template-switching events (Cleal et al. 2019, Lee et al. 2019, Li et al. 2020). However, the observation of frequent short insertions in rearrangements generated by chromosome fragmentation from micronuclei (Zhang et al. 2015) or chromosome bridges (Cleal et al. 2019, Umbreit et al. 2020) motivates reconsidering the possibility that the insertions may originate from chromosome fragmentation/cut-and-paste mechanisms.

The possibility that aberrant DNA replication could itself cause DNA fragmentation is, in fact, not surprising, given the abnormal nucleoplasm and defective DNA replication in micronuclei and chromosome bridges (Obe & Beek 1982, Umbreit et al. 2020). Although the lack of extensive junctional homology has led to classical nonhomologous end-joining (c-NHEJ) being proposed as the major repair process in chromothripsis (Ly et al. 2017, Stephens et al. 2011), the presence of short insertions (10–100 bp) that are not expected for c-NHEJ is consistently observed in chromothripsis both in vivo (Cortes-Ciriano et al. 2020, Stephens et al. 2011) and in vitro (Cleal et al. 2019, Umbreit et al. 2020, Zhang et al. 2015; also see Ly et al. 2019, supplemental figures 8 and 11). Moreover, both chromothripsis and insertion rearrangements were observed in cells lacking c-NHEJ (Cleal et al. 2019, Ratnaparkhe et al. 2018). These observations raise the question of how short insertions at the junctions of long-range rearrangements are generated. If these short sequences originate from hotspots of DNA fragmentation (e.g., breakage sites in a broken bridge chromosome), the mechanism leading to extreme local DNA fragmentation needs to be determined. If these short sequences reflect low-processivity DNA copying in MMBIR, it remains to be explained how this process is initiated and why aberrantly copied DNA sequences are clustered in hotspot breakage regions.

5.2. What Processes Give Rise to Chromoplexy?

A recent pan-cancer analysis reported chromoplexy in ~18% of cancers (Li et al. 2020). Chromoplexy essentially represents balanced translocations involving more than two chromosomes (**Figure 1*d***). Both chromoplexy and reciprocal balanced translocations between two chromosomes are thought to result from end-joining between paired break ends generated by double-strand DNA breakage. It is however unclear how these breaks are generated and why they form complex chains instead of two-way reciprocal translocations. An intriguing observation about chromoplexy and balanced translocations is that they are frequently involved in the generation of recurrent fusions, such as the *TMPRSS2-ERG* fusion in prostate cancers (Baca et al. 2013) or *EWSR* fusions in Ewing sarcomas (Anderson et al. 2018). Prior studies on recurrent translocations in lymphocytes suggested that spatial proximity and DNA breakage frequency are major mechanistic factors influencing the frequency of translocation between two loci (Hakim et al. 2012, Zhang et al. 2012). Based on this model, one would expect that the multiple loci involved in chromoplexy would have to be actively colocalized. Otherwise, the frequency of oncogenic translocations arising from chromoplexy would be orders of magnitude lower than those arising from simple balanced translocations between two loci. Alternatively, the chromoplexy translocations might not occur synchronously, as originally postulated (Baca et al. 2013), but instead occur by a multigenerational process akin to BFB cycles.

5.3. What Are the Initiating Events of Gene Amplification?

Gene amplification is a common mechanism of oncogenic activation. The generation of tens or dozens of copies of amplified DNA may involve both DNA rereplication and uneven sister DNA distribution during cell division (Cowell 1982, Stark & Wahl 1984, Stark et al. 1989). The connection between gene amplification and chromosomal instability has been well known, and the evolution of amplified DNA from unstable chromosomes was partially recapitulated in two recent studies by Ly et al. (2019) and Shoshani et al. (2021) that validated the prediction that extrachromosomally amplified DNA can originate from chromothripsis (Rausch et al. 2012, Stephens et al. 2011, Zhang et al. 2015).

Despite these advances, we still do not have the full picture of the gene amplification process because of several experimental limitations. Single-cell analysis can provide insights into the generation of broken DNA that initiates amplification, but not their downstream evolution. By contrast, analysis of clonal populations derived from cells under selection provides only inferential information about the initiating events. Neither approach can provide information on either the rate of DNA amplification in individual cells or the frequency of cells with amplified DNA. Therefore, a deeper understanding of multigenerational gene amplification requires better visualization of the intermediate steps, especially the ability to distinguish cut-and-paste and copy-and-paste events based on sister DNA information. As gene amplification is frequently associated with therapy resistance or metastasis, answering these questions will have significant clinical relevance.

6. SUMMARY

The picture of cancer genome complexity has come full circle (Weinberg 2014). We have gone from an initial simplifying classification of chromosomal abnormalities based on DNA copy number changes (Beroukhim et al. 2010) to an expanding list of highly complex DNA rearrangement patterns (Bao et al. 2021, Hadi et al. 2020, Li et al. 2020, Steele et al. 2021). Yet mechanistic insights are again bringing us back to simplifying themes involving chromosome fragmentation (cut-and-paste) or aberrant DNA replication (copy-and-paste).

Given that we are just starting to understand the origin of large-scale chromosomal alterations that have been recognized and analyzed by cytogenetic methods for decades, dissecting the origin of segmental copy number complexity, with many nuanced features only recently revealed by whole-genome sequencing, remains a daunting challenge. We are nonetheless optimistic about further progress in understanding the origins of cancer genome complexity because of two promising developments. The first is the growing ability to identify rearrangement signatures through computational analysis of cancer genomes (Garsed et al. 2014; Li et al. 2014, 2020; Stephens et al. 2011) and relate these signatures to the rearrangement outcomes of chromosomal instability (Bollen et al. 2021, Kneissig et al. 2019, Ly et al. 2019, Maciejowski et al. 2015, Mardin et al. 2015, Mazzagatti et al. 2020, Meier et al. 2014, Shoshani et al. 2021, Sidiropoulos et al. 2021, Umbreit et al. 2020, Willis et al. 2017, Zhang et al. 2015). The second is the ability to explain very complex rearrangement patterns in cancer genomes as the compound outcomes of multigenerational evolution of unstable chromosomes (Stachler et al. 2021, Umbreit et al. 2020). This raises the possibility that the seemingly endless puzzles of complex rearrangement patterns may ultimately be reduced to different combinations of chromosomal alterations arising from specific classes of cell division errors (Leibowitz et al. 2015, Lens & Medema 2019, Zhang et al. 2013) or errors in DNA replication and repair (Berti et al. 2020, Scully et al. 2019). The interplay between computational cancer genome analysis and in vitro studies of genome instability has already generated important insights. The adoption of new techniques of genomic analysis such

as long-read DNA sequencing, single-cell manipulation (Bollen et al. 2021, Yan et al. 2021), and high-throughput single-cell sequencing (Laks et al. 2019) should further accelerate this progress.

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