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Established and Novel Mechanisms Leading to de novo Genomic Rearrangements in the Human Germline

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Chromothripsis · Copy number variation · De novo mutation · Genomic rearrangement · Multifocal genomic crisis

Abstract
During gametogenesis, the human genome can acquire various de novo rearrangements. Most constitutional genomic rearrangements are created through 1 of the 4 well-known mechanisms, i.e., nonallelic homologous recombination, erroneous repair after double-strand DNA breaks, replication errors, and retrotransposition. However, recent studies have identified 2 types of extremely complex rearrangements that cannot be simply explained by these mechanisms. The first type consists of chaotic structural changes in 1 or a few chromosomes that result from "chromoanagenesis (an umbrella term that covers chromothripsis, chromoanasynthesis, and chromoplexy)." The other type is large independent rearrangements in multiple chromosomes indicative of "transient multifocal genomic crisis." Germline chromoanagenesis (chromothripsis) likely occurs predominantly during spermatogenesis or postzygotic embryogenesis, while multifocal genomic crisis appears to be limited to a specific time window during oogenesis and early embryogenesis or during spermatogenesis. This review article introduces the current understanding of the molecular basis of de novo rearrangements in the germline.

During gametogenesis, various de novo rearrangements can develop in the human genome [Hastings et al., 2009a]. These genomic alterations are classified into simple and complex rearrangements. Simple rearrangements are defined as genomic changes with 1 or 2 microscopically observable breaks, and consist of deletions of 1 chromosomal segment, tandem duplications, single paracentric or pericentric inversions, and reciprocal translocations [Madan, 2013; Poot and Haaf, 2015]. Any rearrangements accompanied by 3 or more breakpoints are referred to as complex [Poot and Haaf, 2015]. These simple and complex rearrangements jointly account for a substantial percentage of the etiology of intellectual disability and congenital malformation syndromes [Miller et al., 2010].

Recent advances in molecular technologies, such as microarray-based comparative genomic hybridization and whole-genome sequencing, have enabled researchers to characterize the structures of genomic rearrangements

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Table 1. Well-known mechanisms of genomic rearrangements

	Affected process	Breakpoint feature	Typical consequences	Reference
Nonallelic homologous recombination	Recombination	Homologous sequences	Deletion, inversion, amplification or translocation	Lupski, 1998
Nonhomologous end-joining	DNA repair	Blunt end, or 1–4 bp microhomology	Translocation, insertion, deletion, or amplification (rare)	Hastings et al., 2009a
Replication error[a]	Replication	<70 bp microhomology	Duplication or deletion	Lee et al., 2007; Hastings et al., 2009b
Retrotranposition	Transposition of a mobile element	5–20 bp microhomology	Insertion	Kazazian et al., 1988

[a] Fork stalling and template switching or microhomology-mediated break-induced replication.

[van Binsbergen, 2011; Carvalho and Lupski, 2016]. The results of recent studies have provided novel insights into the cellular events that affect chromosomal architecture. In this article, we review the current understanding of the molecular basis of constitutional genomic rearrangements. In particular, we introduce newly proposed mechanisms that lead to catastrophic chromosomal reconstruction and transient multifocal genomic crisis.

Well-Known Mechanisms of Constitutional Genomic Rearrangements

Simple genomic rearrangements arise from 1 of the 4 well-known mechanisms, i.e., nonallelic homologous recombination (NAHR), erroneous repair after double-strand DNA breaks, replication errors, and retrotransposition (Table 1; Fig. 1) [Hastings et al., 2009a; Weckselblatt and Rudd, 2015; Carvalho and Lupski, 2016]. Of these, NAHR represents the major cause of recurrent rearrangements [Hastings et al., 2009a; Liu et al., 2012], while the others usually lead to nonrecurrent structural changes. In addition, these mechanisms can be involved in the development of complex genomic rearrangements.

Nonallelic Homologous Recombination

NAHR refers to the aberrant recombination between 2 highly homologous sequences [Lupski, 1998; Liu et al., 2012]. This mechanism underlies simple deletions, amplifications (duplications, triplications, and more copy-number gains), inversions, and interchromosomal translocations (Fig. 1a) [Liu et al., 2012; Gu et al., 2016]. Typical substrates for NAHR are low copy repeats that are defined as sequences with 95% or higher homology of at least 1 kb in length [Hastings et al., 2009a]. NAHR can also be mediated by retrotransposon-derived sequences such as long interspersed nuclear elements-1 (L1) and *Alu* [Kazazian and Moran, 2017]. Large size, high homology, and short distance of the 2 substrate sequences increase the frequency of NAHR [Liu et al., 2011b; Dittwald et al., 2013].

NAHR predominantly occurs at specific hotspots in the genome [Liu et al., 2012]. An example of NAHR-mediated pathogenic rearrangements is deletions at 15q11.2q13 that result in Prader-Willi syndrome. The 15q11.2q13 region is enriched with low copy repeats. Of note, previous studies have shown that Prader-Willi syndrome patients with deletions on the paternally derived allele were frequently born in autumn [Butler et al., 1985; Ayabe et al., 2013], indicating that the occurrence of NAHR at 15q11.2q13 during spermatogenesis may be regulated by seasonally varying environmental factors.

Erroneous Repair after Double-Strand DNA Breaks

Nonhomologous end-joining (NHEJ) can result in erroneous repair after double-strand DNA breaks (Fig. 1b) [Hastings et al., 2009a]. Such erroneous repair occurs at any position in the genome and often creates nonrecurrent deletions [Gu et al., 2008]. NHEJ-induced deletions were identified in patients with various congenital disorders [Schluth-Bolard et al., 2019]. Also, NHEJ leads to inversions, translocations, and duplications. Indeed, most copy-number neutral nonrecurrent rearrangements, such as inversions and translocations, are predicted to be caused by NHEJ [Chiang et al., 2012; Schluth-Bolard et al., 2019].

The 2 breakpoints of NHEJ are usually blunt-ended, although they occasionally share microhomologies (short

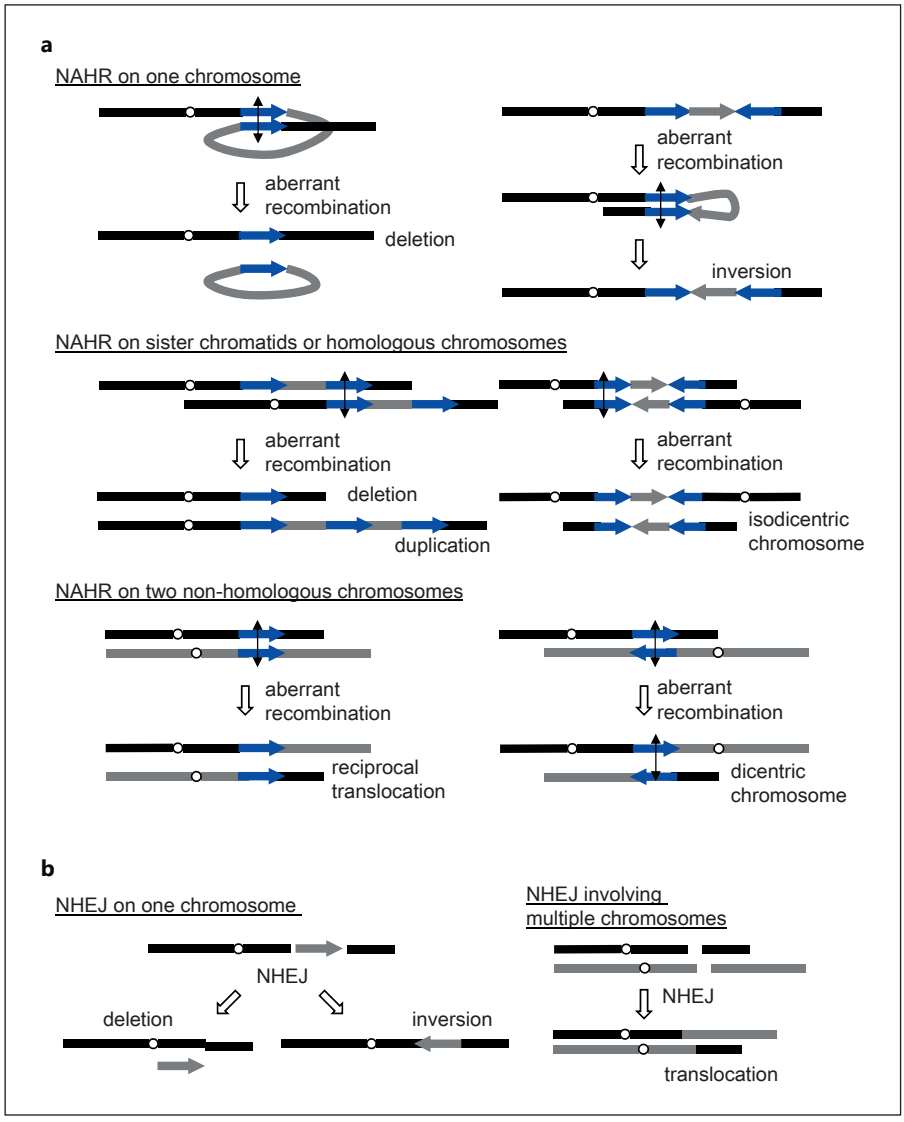

Fig. 1. Schematic representation of the well-known mechanisms of constitutional genomic rearrangements. **a** Nonallelic homologous recombination (NAHR). NAHR is mediated by repetitive sequences (blue arrows) and generates various types of rearrangements [Lupski, 1998]. **b** Erroneous repair. Nonhomologous end-joining (NHEJ) occurs after double-strand DNA breaks and leads to deletions, inversions and translocations [Hastings et al., 2009a]. **c** Replicative microhomology-mediated mechanisms. Fork stalling and template switching and microhomology-mediated break-induced replication are both mediated by microhomology and create simple and complex rearrangements [Lee et al., 2007; Hastings et al., 2009b]. **d** Retrotransposition. DNA cleavage occurs at a consensus sequence, 5′-TTTTA-3′. The poly-A tail of an L1, *Alu*, or SVA transcript anneals to the cleaved poly-T sequence, followed by reverse transcription. The newly synthesized copy of the transposon is integrated into the genome [Kazazian et al., 1988].

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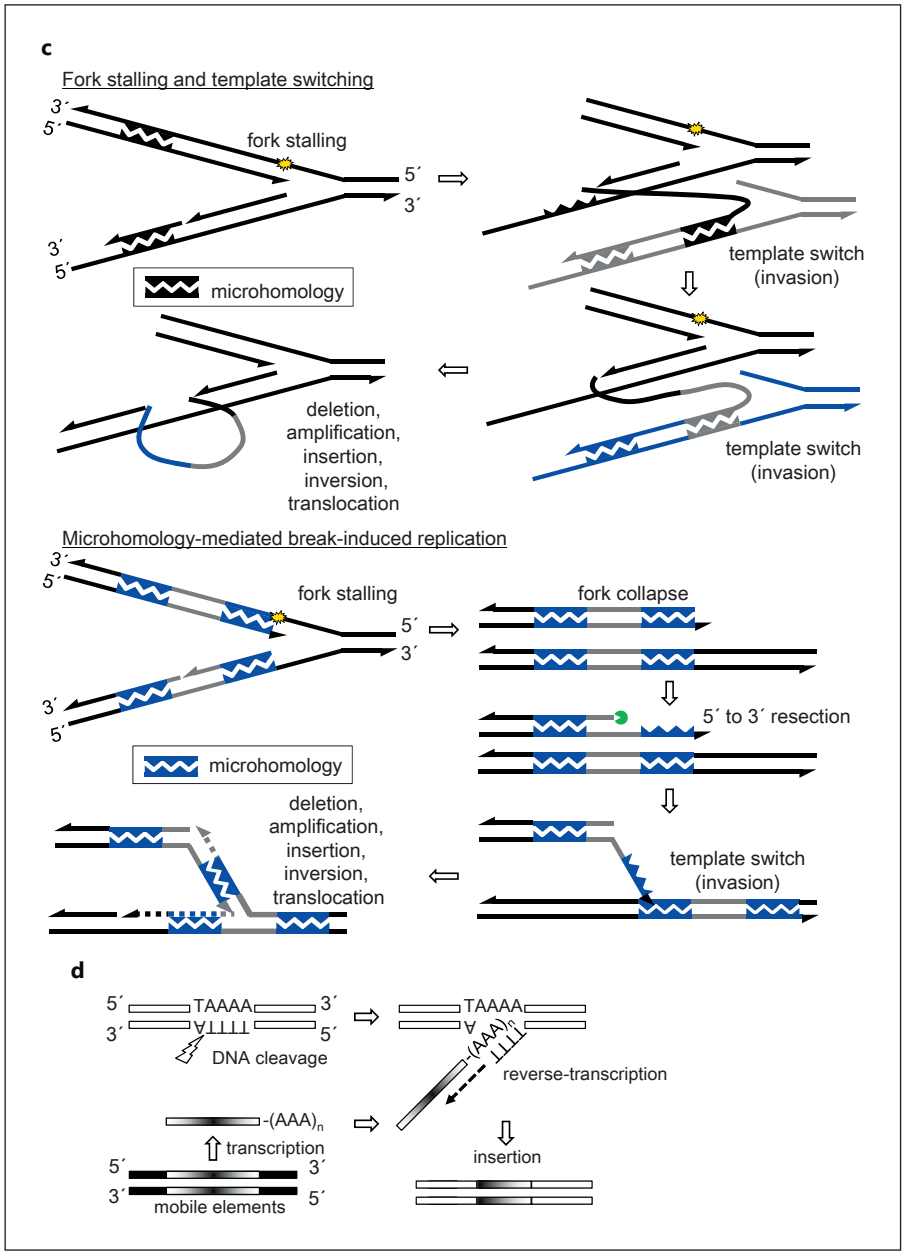
homologous sequences) of 1–4 bp [Pannunzio et al., 2018]. Importantly, NHEJ-mediated rearrangements are often accompanied by short nucleotide insertions at the fusion junction [Pannunzio et al., 2018]. These insertions, known as "information scars," either template their nearby sequences or consist of DNA fragments of origin-unknown [Gu et al. 2008; Onozawa et al, 2014].

Ferguson et al. [2000] proposed an alternative pathway of end-joining. This pathway is more error-prone than canonical NHEJ and can be associated with more than 10-bp microhomologies at the fusion junction [Sallmyr and Tomkinson, 2018]. NHEJ and alternative end-joining are assumed to be mediated by different sets of proteins [Pannunzio et al., 2018; Poot, 2018; Sallmyr and Tomkinson, 2018]. Specifically, NHEJ requires the Ku70/80 heterodimer, the complex of Artemis and DNA-dependent protein kinase catalytic subunit, the Pol X family polymerase, and the DNA ligase IV complex [Pannunzio et al., 2018], whereas alternative end-joining was linked to the meiotic recombination 11-DNA repair protein RAD50-nibrin complex, C-terminal-binding protein interacting protein, DNA polymerase θ, and DNA ligase IIIα/X-ray repair cross-complement 1 [Sallmyr and Tomkinson, 2018].

Replication Errors

DNA replication errors during the S phase create various types of genomic rearrangements including dele-



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tions, amplifications, insertions, inversions, and translocations (Fig. 1c) [Lee et al., 2007; Hastings et al., 2009b]. These rearrangements significantly contribute to the development of congenital disorders [Lee et al., 2007; Hastings et al., 2009b]. For example, deletions, duplications, and inversions due to replication errors at 15q21 were identified in patients with aromatase excess syndrome [Fukami et al., 2013].

Previous studies proposed 2 major models of replication errors, namely, fork stalling and template switching (FoSTeS) and microhomology-mediated break-induced replication (MMBIR) (Fig. 1c) [Lee et al., 2007; Hastings et al., 2009b]. Both FoSTeS and MMBIR are predicted to occur after replication fork collapses. Such collapses have been associated with replication stresses, including DNA-binding proteins, DNA-RNA interaction, DNA damage, secondary DNA structures, and metabolic conditions [Zeman and Cimprich, 2014]. The most characteristic feature of FoSTeS and MMBIR is microhomology at the breakpoints [Lee et al., 2007; Hastings et al., 2009b]. In

Table 2. Characteristics of complex genomic rearrangements

	Number of affected chromosomes	Typical copy number alteration	Typical breakpoint feature	Major mechanisms	Reference
Chromoanagenesis					
Chromothripsis	1–5	Balanced, deletion	Blunt end or small insertions	NHEJ	Stephens et al., 2011; Nazaryan-Peterson and Tommerup, 2016; Poot, 2018
Chromoanasynthesis	Usually 1	Amplification, deletion	Microhomology	Replication error	Liu et al., 2011a
Chromoplexy	Many (usually ≥4)	Balanced, deletion	Blunt end	NHEJ	Baca et al., 2013
Multifocal chromosomal reconstruction					
Organismal mutator phenotype	5–7	Amplification	Microhomology/ microhomeology	Replication error	Liu et al., 2017
Multifocal genomic crisis	5	Balanced, amplification, deletion	Blunt end or microhomology/ microhomeology	NHEJ replication error	Hattori et al., 2019

NHEJ, nonhomologous end-joining.

FoSTeS, a lagging DNA strand of the replication fork disengages from the original template when the fork stalls at a DNA lesion. The disengaged lagging strand invades an ectopic template through annealing of a microhomology and restarts replication. In MMBIR, a DNA strand from the replication fork collapse is resected from the 5′ to 3′ end, leaving a 3′ overhang. The overhang invades an ectopic template through microhomology annealing and restarts replication. FoSTeS and MMBIR generate both deletions and amplifications depending on the genomic position where the disengaged strand invades; invasions into upstream templates of the original position cause amplifications, while invasions into downstream templates result in deletions [Lee et al., 2007; Hastings et al., 2009b]. Significant proportions of nonrecurrent rearrangements in the genome, especially tandem duplications, are likely to be caused by FoSTeS or MMBIR because Mills et al. [2011] showed that 71% of deletions were associated with 2 to 376-bp microhomologies, and 80% of tandem duplications were associated with 2 to 17-bp microhomology. Moreover, a drifting strand in FoSTeS and MMBIR may switch the template again before going back to the original template and thereby generates complex rearrangements [Lee et al., 2007; Hastings et al., 2009b]. Translocations and inversions can also be created by replication errors [Lee et al., 2007; Hastings et al., 2009b].

Retrotransposition of Mobile Elements

The human genome is enriched with mobile elements such as L1, *Alu*, and SINE-VNTR-*Alu* (SVA) [Kazazian and Moran, 2017]. Retrotransposition of such elements generates de novo insertions [Hancks and Kazazian, 2016; Kazazian and Moran, 2017]. Mills et al. [2011] showed that mobile elements account for 98% of insertions with the sizes of 50 bp or longer. More than 120 insertions of mobile elements have been associated with human diseases [Hancks and Kazazian, 2016]. For example, a de novo insertion of L1 into the *F8* gene was reported to cause hemophilia [Kazazian et al., 1988], while an insertion into the androgen receptor gene was linked to abnormal sex development in one family [Batista et al., 2019].

Chromoanagenesis: A New Event that Creates Catastrophic Genomic Rearrangements

The aforementioned 4 mechanisms, particularly replication errors, are known to create both simple and complex rearrangements. For example, FoSTeS and MMBIR have been implicated in the development of various complex copy-number variations (CNVs) at the *PLP1* locus [Lee et al., 2007; Beck et al., 2015]. Presumably, the 4 mechanisms account for the vast majority of complex rearrangements. Nevertheless, recent studies have identified extremely complex rearrangements, which cannot be

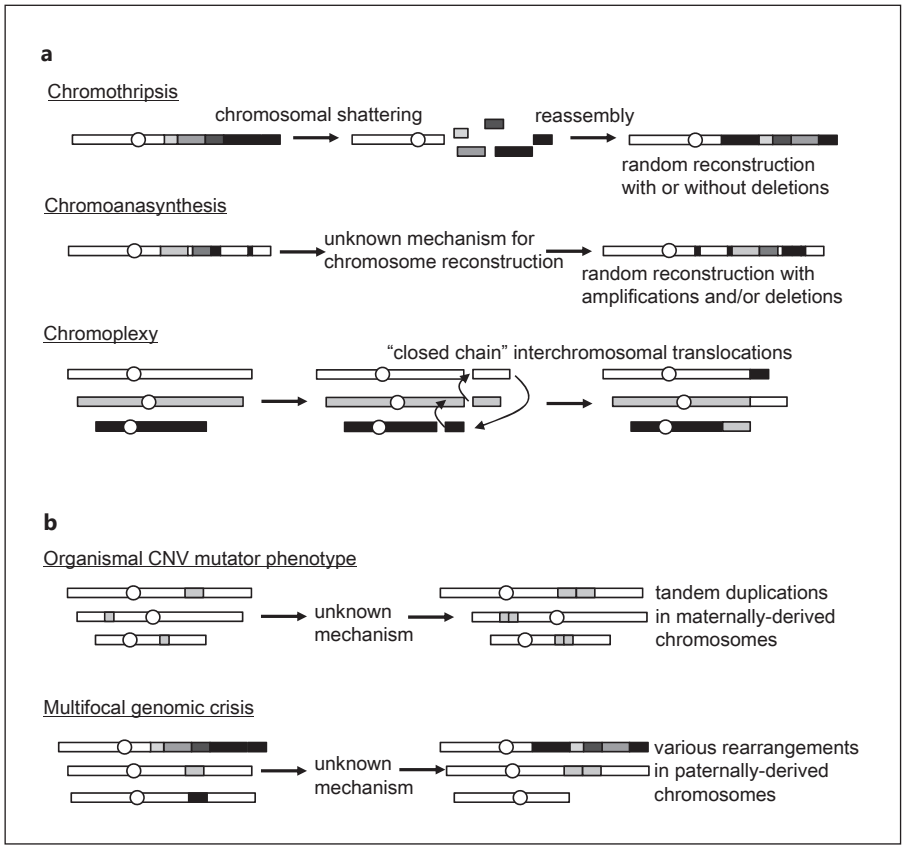

Fig. 2. Schematic representation of newly proposed mechanisms. **a** Chromoanagenesis and its related conditions. Chromoanagenesis is an umbrella term that covers chromothripsis, chromoanasynthesis, and chromoplexy [Liu et al., 2011a; Stephens et al., 2011; Holland and Cleveland, 2012; Baca et al., 2013]. **b** Multifocal chromosomal reconstruction. The organismal CNV mutator phenotype and the transient multifocal genomic crisis were proposed in recent papers [Liu et al., 2017; Hattori et al., 2019].

simply explained by the well-known mechanisms [Holland and Cleveland, 2012; Fukami and Kurahashi, 2018].

Chromothripsis

In 2011, Stephens et al. identified catastrophic chromosomal changes, which are not ascribable to simple NAHR, NHEJ, replication errors, or retrotransposition. A new mechanism "chromothripsis" was proposed to explain these rearrangements (Table 2; Fig. 2a). Chromothripsis is an "all-at-once" event that creates chaotic rearrangements, typically involving 1 chromosome or 1 chromosomal arm. In the report by Stephens et al. [2011], chromothripsis was described as a somatic change in a patient with leukemia. Soon after the report, Kloosterman et al. [2011, 2012] documented chromothripsis in the germline.

Chromothripsis appears to be a unique mutagenic event in which a chromosome(s) is broken into several DNA fragments and subsequently stitched back together in a random manner [Stephens et al., 2011]. Some DNA fragments can be lost during the chromosomal reassembly [Stephens et al., 2011]. Two processes have been proposed to promote chromothripsis. The first one is micronucleus formation [Crasta et al., 2012; Zhang et al., 2015; Hatch and Hetzer, 2015; Ly et al., 2017]. In this case, a missegregated chromosome(s) is encapsulated into a micronucleus. Then, the encapsulated chromosome is pulverized at a certain point after the S phase and randomly reassembled. Finally, the rearranged chromosome is incorporated into the main nucleus. This micronucleus-mediated mechanism is predicted to account for most germline chromothripsis. The other process that causes chromothripsis is telomere crisis [Maciejowski et al., 2015]. In this case, fusion occurs between 2 chromosomal ends with shortened telomere sequences, and creates a dicentric chromosome. When the 2 centromeres of the dicentric chromosome are attached to opposite spindles during mitosis, the dicentric chromosome forms a DNA bridge between 2 daughter cells [Maciejowski et al., 2015]. Such a bridge induces nuclear envelope rupture, digestion of the DNA bridge by cytoplasmic 3′ nuclease TREX1, and subsequent chromosome pulverization and random reassembly [Maciejowski et al., 2015].

Chromothripsis results in massive chromosomal reconstruction with or without deletion. The breakpoints are usually consistent with canonical NHEJ [Kloosterman et al., 2011; Stephens et al., 2011; Marcozzi et al., 2018; Zepeda-Mendoza and Morton, 2019]. In addition, the pulverized DNA fragments may also be reassembled via alternative end-joining or via the co-occurrence of NHEJ and alternative end-joining [Kloosterman et al., 2012; Slamova et al., 2018]. Furthermore, chromothripsis induced by telomere crisis is often indicative of MMBIR [Cleal et al., 2019]. Nazaryan-Petersen et al. [2016] reported a rare case in which chromothripsis-like genomic rearrangements were linked to retrotransposition and NAHR. Thus, multiple mechanisms may be involved in chromosomal reassembly during chromothripsis.

To date, constitutional rearrangements indicative of chromothripsis have been identified in multiple individuals [Kloosterman et al., 2011, 2012; de Pagter et al., 2015; Fukami et al., 2017; Marcozzi et al., 2018]. Most of these individuals exhibited severe developmental delay and congenital malformations, which are ascribable to abnormal expression of genes on the affected chromosomes. However, complex genomic rearrangements, including chromothriptic changes, can also be shared by phenotypically normal individuals [Kloosterman et al., 2012; de Pagter et al. 2015; Bertelsen et al., 2016; Poot, 2020]. It has been suggested that 70% of complex rearrangements are associated with a normal phenotype [Pellestor et al., 2011]. Consistent with this, we identified X chromosomal chromothripsis in a woman who showed no apparent clinical features except for ovarian dysfunction and hyperthyroidism [Suzuki et al., 2016]. The relatively mild phenotype of this woman can be explained by the selective inactivation of the rearranged X chromosome.

It is worth mentioning that, in previously reported cases, germline chromothripsis predominantly affected paternally derived chromosomes [Pellestor et al., 2014; Pellestor and Gatinois, 2018]. Thus far, there is no report suggesting the occurrence of chromothripsis during oogenesis. While Kato et al. [2017] reported a case in which chromothripsis-like rearrangements were detected on the maternally derived chromosomes, these rearrangements were more likely to be created in a postzygotic cell, rather than in an oocyte. Further studies are necessary to clarify whether germline chromothripsis is actually limited to spermatogenesis and postzygotic embryogenesis, or also occurs during oogenesis.

Further Catastrophic Cellular Events

Recent studies documented additional catastrophic cellular events that have some differences from chromothripsis [Berger et al., 2011; Liu et al., 2011a; Baca et al., 2013]. They were designated as "chromoanasynthesis" and "chromoplexy" (Table 2; Fig. 2a). Chromoanasynthesis is characterized by microhomologies around breakpoints. Unlike chromothripsis, chromoanasynthesis is frequently associated with amplifications [Liu et al., 2011a; Marcozzi et al., 2018; Nazaryan-Petersen et al., 2018; Zepeda-Mendoza and Morton, 2019]. Chromoanasynthesis can create multiple CNVs clustered on a single chromosome [Liu et al., 2011a; Nazaryan-Petersen et al., 2018]. Chromoanasynthesis is thought to be mediated by FoSTeS and/or MMBIR induced by DNA replication stresses [Liu et al., 2011a].

Chromoplexy is different from chromothripsis in the following ways: (1) it involves multiple chromosomes, (2) it creates fewer breakpoints in 1 chromosome than those created by chromothripsis, and (3) it is implicated primarily in the translocations of somatic cells [Berger et al, 2011; Baca et al., 2013; Marcozzi et al., 2018; Zepeda-Mendoza and Morton, 2019]. However, chromoplexy has several similar characteristics to chromothripsis, such as copy-number-neutral rearrangements with NHEJ-compatible breakpoint structures. In this regard, Zhang et al. [2013] stated that the case reported by Kloosterman et al. [2011] was actually the first case of chromoplexy, which progressed to chromothripsis. Zhang et al. [2013] designated this case as "translocation-induced chromothripsis."

It remains unclear whether chromothripsis, chromoanasynthesis, and chromoplexy are different phenomena or closely related conditions. Holland and Cleveland [2012] proposed an umbrella term "chromoanagenesis" to cover all cellular events that produce shattering and catastrophic reassembly of a single or a few chromosomes.

New Mechanisms Generating Multifocal Chromosomal Reconstruction

All of the aforementioned mechanisms, including chromoanagenesis, produce structural changes in a specific target region in the genome. Hence, these mechanisms cannot explain the co-occurrence of independent de novo rearrangements on different chromosomes. Here, we describe recently discovered mutagenic events that alter the structures of multiple chromosomes simultaneously, but separately.

The Organismal CNV Mutator Phenotype

In 2017, Liu et al. reported 5 individuals each of whom had 5–9 large de novo CNVs on multiple chromosomes (Table 2; Fig. 2b). These individuals were identified in a genome-wide copy number analysis of approximately 60,000 individuals with developmental disorders. The majority of the de novo CNVs in the 5 individuals were tandem duplications. Somatic mosaicism and interchromosomal translocations were ruled out. Four of the abovementioned individuals (BAB3097, BAB3596, mCNV3, and mCNV4) carried rearrangements predominantly on the maternally derived chromosome. The breakpoints of the rearrangements were mostly associated with microhomologies or microhomeologies (5 bp or more sequences with at least 70% identity), indicating that replication errors were involved in these changes. Based on these results, Liu et al. [2017] proposed "an organismal CNV mutator phenotype" that creates large CNVs on different chromosomes at the same time. This mutagenic event appeared to originate in a primary oocyte and terminated in zygotes before the 4- or 8-cell stages.

The study by Liu et al. [2017] provided the first indication that human oogenesis contains a specific time window permissive to multifocal chromosomal reconstruction. Furthermore, since 1 of the 5 subjects of Liu et al. [2017] (mCNV7) had multiple rearrangements exclusively on the paternally derived chromosomes, spermatogenesis may also be associated with transient genomic instability. However, given the low frequency of cases with the organismal CNV mutator phenotype among patients with developmental defects (5 in ~60,000), this mutatgenic event seems to be an extremely rare phenomenon.

Transient Multifocal Genomic Crisis

More recently, we identified in an infant with multiple developmental defects and neonatal diabetes who harbored large non-mosaic de novo rearrangements on 5 chromosomes (Table 2; Fig. 2b) [Hattori et al., 2019]. This infant was ascertained by analyzing genome data from approximately 2,100 individuals with various types of congenital malformations. The karyotype of the infant was 46,XY,der(6)add(6)(q23.3),der(13)add(13)(q12.1),der(14)add(14)(q31),der(21)del(q11.2)add(q11.2).

Breakpoint characterization suggested that multiple mechanisms were involved in the chromosomal changes of this infant. Two of the 5 chromosomal lesions were chaotic rearrangements indicative of chromothripsis, while another 2 were large simple duplications presumably created by replicative error. The remaining one was a simple paracentric inversion consistent with NHEJ. All of these rearrangements have occurred de novo in paternally derived chromosomes. Importantly, the infant had no interchromosomal translocations, suggesting that his 5 genomic lesions were generated separately. The large duplications on chromosomes 6 and 13 turned out to have occurred during premeiotic mitosis and subsequently underwent physiological meiotic recombination. Postzygotic genomic instability was excluded by repeated genome analyses of the infant. Collectively, the results indicated that a transient multifocal genomic crisis in a prezygotic germ cell can introduce several chromoanagenic and non-chromoanagenic changes into the genome. The same mechanism may also be implicated in a case reported by Liu et al. [2017] (mCNV7).

The underlying mechanism of this transient genomic crisis remains to be clarified. It is possible that several micronuclei concurrently developed in a testicular germ cell and created both simple and catastrophic rearrangements. Consistent with this, Ly et al. [2019] reported that chromosomal missegregation during mitosis can induce various genomic rearrangements including chromothripsis and amplifications. Alternatively, a hitherto unrecognized mechanism may have produced the transient genomic crisis in our case.

The infant exhibited various clinical features including transient neonatal diabetes, cleft palate, iris coloboma, ventricular septal defect, hydronephrosis, umbilical hernia, and clubfoot [Hattori et al., 2019]. Of these, transient neonatal diabetes can be explained by the duplication of *PLAGL1* on the paternally derived chromosome 6 [Mackay and Temple, 2010]. The causative genes for other congenital anomalies remain to be determined. Since the genomic rearrangements caused deletion of 11 genes, disruption of 10 genes, and duplication of 129 genes, altered expression of some of these genes may have exerted negative effects on fetal development. The infant underwent insulin administration as well as various clinical interventions for the congenital anomalies. On the latest visit at 1 year and 11 months of age, he manifested moderate short stature (height, –2.7 SD), but no apparent developmental delay. The parents received genetic counseling and were informed of the etiology-unknown transient chromosomal reconstruction.

Conclusion

Although constitutional rearrangements are usually ascribable to NAHR, NHEJ, replication errors, or retrotransposition, there are some exceptions. Recently,

chromoanagenesis (chromothripsis, chromoanasynthesis, and chromoplexy), the organismal CNV mutator phenotype, and transient multifocal genomic crisis were proposed as novel mechanisms of large constitutional rearrangements. It appears that chromoanagenesis (chromothripsis) occurs predominantly during spermatogenesis or postzygotic embryogenesis, while the organismal CNV mutator phenotype and transient multifocal genomic crisis appeared to be limited to a specific time window during oogenesis and early embryogenesis or during spermatogenesis. There may be further cellular events that alter chromosomal architectures of human embryos.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.H. drafted the manuscript. A.H. and M.F. wrote the paper.

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