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Mechanisms underlying structural variant formation in genomic disorders

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Abstract

With the recent burst of technological developments in genomics, and the clinical implementation of genome-wide assays, our understanding of the molecular basis of genomic disorders, specifically the contribution of structural variation to disease burden, is evolving quickly. Ongoing studies have revealed a ubiquitous role for genome architecture in the formation of structural variants at a given locus, both in DNA recombination-based processes and in replication-based processes. These reports showcase the influence of repeat sequences on genomic stability and structural variant complexity and also highlight the tremendous plasticity and dynamic nature of our genome in evolution, health and disease susceptibility.

Genomic disorders are a group of diseases caused by rearrangements of the human genome due to inherent genomic instability that results in susceptibility to structural variation mutagenesis[1]. In the context of human disease, structural variants include deletions, duplications, triplications, added amplifications (for example, quadruplications) and other large-scale (that is, from ~50–200 bp, the size of an average exon, to megabases of DNA) copy number variants (CNVs) that are not resolved by chromosome karyotype studies (<5 Mb), as well as copy number-neutral inversions, insertions and trans-locations. Structural variants differ from the concept of single nucleotide polymorphisms (SNPs) or single nucleotide variants (SNVs), which only change a single base or a few bases[2], as the former requires the disruption of the sugar–phosphate backbone of DNA and involve many base pairs. The definition of a structural variant can overlap with the concept of small insertions and deletions (indels), which were previously defined as variants of <10,000 bp in length[3]. However, in this Review we consider indels as <50–100 bp in size, that is, a variant size that can be detected within a single next-generation sequencing read.

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Competing interests statement

The authors declare competing interests: see Web version for details.

Structural variants result from different mutational mechanisms, including DNA recombination-, replication- and repair-associated processes. Current approaches to model the formation of structural variants in the human genome include: the use of model organisms[4]; the use of *in vitro* human cells subjected to stress[5]; and the direct observation of human genomic alterations, or rearrangement end products, that convey a disease trait. The manifested trait or genomic disorder enables one to both ascertain the mutational event and distinguish the affected individual from the population.

Studying disease-causing structural variants that have been classified as either extremely rare or *de novo* provides a unique opportunity to glean insights into mutational mechanisms. Such studies have revealed complex exonic, genic and chromosomal rearrangements that can be generated in a single mutagenic event, for example, in disease-associated loci at 17p11.2 and 17p12 (REF. 6), in *MECP2* duplication syndrome (OMIM 300260)[7] and due to chromothripsis-like events in multiple congenital anomalies[8,9]. Other studies have shown that the formation of structural variants can be accompanied by additional genome modification that may result in a disease trait. For example, CNVs and SNVs can be generated concomitantly[10], and SNVs created during mutagenic repair can potentially affect the function of genes that do not map within the CNV. Also, CNVs can be followed by extended regions of absence of heterozygosity (AOH)[11,12]. If the AOH that is generated from template switching between homologues versus sister chromatids occurs at an imprinted locus, disease can result. Alternatively, the AOH region may encompass a variant in a gene for a recessive disease and reduce it to homozygosity when only one parent is a carrier, thus distorting Mendelian expectations.

The accurate detection of the complete mutagenic event at the single-base-pair level requires either the use of Sanger sequencing, along with techniques that enable large-scale CNV identification, or the use of composite pipelines for CNV analysis of next-generation sequencing data[13]. Therefore, using combined molecular analytic tools is necessary to delineate the entire range of variation that is associated with a particular structural variant in an individual personal genome. Methods that can be combined include fluorescence *in situ* hybridization (FISH), array comparative genomic hybridization (aCGH), SNP arrays, next-generation sequencing and Sanger sequencing[13,14]. Potential implications for the evolution of genes and genomes remain to be further explored, but mutational signatures — that is, the ‘scars’ left in the genome by DNA repair and replication mechanisms after structural variant mutagenesis has occurred — can partially explain the complex pattern of polymorphic human structural variants that has been revealed by the 1000 Genomes Project[15].

New mechanistic discoveries in humans are elucidating how the formation of structural variants can re-structure a specific region of the genome to change gene expression either locally or genome-wide[16,17], including through the alteration of chromatin architecture, with pathological consequences for carriers[18]. These discoveries are also paving the way to understanding the molecular basis of human disease, including diseases that result from somatic mutagenesis[19].

In this Review, we first discuss the types of structural variants that cause genomic disorders, before reviewing in detail the recombination and replication-based mechanisms (RBMs) by

which these arise. Our focus is on the pervasive role of the genomic architecture, including DNA sequence repeat structure and orientation, in the mechanism of formation of structural variants that have been implicated in human genomic disorders. We also describe the most common types of complex rearrangements and review current knowledge underlying their formation. We do not review the phenotypic consequences of specific genomic disorders; for a recent review on this topic please see REF. 20.

Repeat sequences in the human genome

The genomic architecture — that is, the local organization of repeat and other sequences, including their relative orientation, size, density and distribution — is a key feature that helps to predict the type of structural variants, rearrangement susceptibility and potential major mechanism that underlie variation at a specific locus. Approximately 50% of the human genome consists of repeat sequences[21], which include mobile elements such as *Alu*-processed pseudogenes, simple sequence repeats, tandemly repeated sequences (which feature at centromeres, telomeres, the short arm of acrocentric chromosomes and ribosomal gene clusters) and low-copy repeats (LCRs) such as segmental duplications (SDs). SDs have been computationally defined as segments of DNA that contain ≥90% of sequence identity and ≥1 kb in length in the reference haploid genome, and they constitute approximately 4–5% of the human genome[22]. One of the surprising results from comparative genomic studies has been the observation that the human genome sequence distinguishes itself from other species genomes, such as the fly or worm, by a greater genome-wide presence of SDs and other LCRs[21], which indicates that these repeat sequences greatly contribute to individual human variation[23,24].

Most large LCRs (>10 kb) consist of a cluster of paralogous sequences of diverse genomic origin. The molecular characterization of such repeated regions in the human genome has revealed a mosaic architecture[25,26]; that is, such repeats are organized in hierarchical groups of direct and inversely orientated sequences as opposed to simple interchromosomal and intrachromosomal SDs[27–29]. The genome-wide distribution patterns of large LCRs show that they overlap with regions that frequently undergo genomic rearrangements that are associated with disease[22]. This finding can be explained by the fact that the large LCRs that flank a unique genomic region negatively affect genome stability[30], making the flanked sequences prone to DNA rearrangements via nonallelic homologous recombination (NAHR; also known as ectopic homologous recombination) or RBMs. These characteristic genomic sequence features can thus be used to identify novel genomic loci that are susceptible to structural variant formation. Indeed, the first definition of genomic disorders[1] arose from the susceptibility of specific variant regions that are associated with LCRs to undergo rearrangement in certain human diseases; for example, a 1.4 Mb duplication of a 17p12 interval causes Charcot–Marie–Tooth disease type 1A (CMT1A; OMIM 118220), and a reciprocal recombination deletion product causes hereditary neuropathy with liability to pressures palsies (HNPP; OMIM 162500).

Recurrent versus nonrecurrent rearrangements

The formation of a CNV depends on the joining of two formerly separated DNA segments; these breakpoint junctions yield insights into the mechanisms that cause the chromosomal structural change. Recurrent rearrangements share the same size and genomic content in unrelated individuals. The breakpoints map within long, highly identical, flanking interspersed paralogous repeats (FIG. 1Aa), which mostly consist of LCRs[28]. One or more dosage-sensitive genes are present in-between the repeats and will be affected by the copy number change. Pathogenic CNVs are preferential rearrangement products at such loci[31]. Recurrent structural variants can also occur within tandem paralogous repeats (FIG. 1Ab) and alter the copy number of a dosage-sensitive gene that is present within[32].

Nonrecurrent rearrangements are rearrangements that have a unique size and genomic content at a given locus in unrelated individuals. A dosage-sensitive gene (or genes) can be identified within the structural variant smallest region of overlap (SRO) among individuals who share overlapping clinical phenotypes. Distinct types of nonrecurrent rearrangements are observed in genomic disorders, some of which are also delimited by LCRs. The formation of complex patterns of rearrangements can frequently be observed in some of these loci. For example, paralogous inverted repeats mediate the formation of a unique end-product pattern that consists of an inverted triplication that is interspersed with duplicated genomic segments. This structure is designated DUP–TRP/INV–DUP (duplication–inverted triplication–duplication)[7,33–36] (FIG. 1Ba). In some cases, the inverted repeat pair consists of small repeats such as *Alu* elements[37], but the majority of DUP–TRP/INV–DUP rearrangements described thus far have been LCR-mediated. Genomic disorders such as *MECP2* duplication syndrome and Pelizaeus–Merzbacher disease (PMD; OMIM 312080) are characterized by nonrecurrent rearrangements, mostly duplications, that present with one breakpoint grouping within an LCR-laden region[38–42] (FIG. 1Bb). By contrast, most of the nonrecurrent rearrangements in genomic disorders seem to be formed without LCR involvement (FIG. 1Bc), although many deletion and duplication variants are mediated by recombination between *Alu* elements[43–45].

Thus, two general types of genomic rearrangement, recurrent and nonrecurrent, are observed and show intrinsically distinct features that reflect their underlying mechanisms of formation. They also have a distinct propensity for the formation of complex rearrangements such as interspersed duplications or triplications, as well as different meiotic versus mitotic risk (discussed below).

Recurrent structural variants: key mechanisms *NAHR*

Recurrent structural variants often result from NAHR between directly-oriented or inverted LCRs that flank unique sequence genomic regions. According to the ectopic synapsis model, the recurrent size and genomic content generated by events that are mediated by NAHR results from the homologous recombination requirement for an ectopic synapsis preceding an ectopic crossing over that is dependent on the distance and the length of the homologous sequence[46]. By contrast, RBMs do not show such a requirement, therefore generating rearrangements that vary in size and genomic content. Ectopic homologous recombination[47] that occurs during meiosis has been known to contribute to human disease for more than 40

years[48] and is perhaps one of the best studied causative mechanisms for genomic disorders[30]. In fact, large LCR pairs located <5 Mb apart can lead to localized genomic instability via NAHR[1,30] (FIG. 2A). Approximately 40 non-overlapping genomic loci are associated with meiotic NAHR that manifest as genomic disorders of microdeletion and microduplication syndromes[49]. Importantly, the duplication of a locus predisposes the region to undergo triplications in subsequent generations by more than 100-fold using the same molecular mechanism[50]. Most NAHR events that are causative of genomic disorders result from crossovers between LCRs located on the same chromosome, that is, intrachromosomal NAHR, or between non-allelic LCRs located in homologous chromosomes, that is, interchromosomal NAHR[30]. Rare cases of recurrent or *de novo* unbalanced translocations[51,52] result from crossovers between repeats located on nonhomologous chromosomes.

The definition of the molecular features of NAHR has been instrumental to uncovering susceptible regions in the human genome[28,53], as well as to discovering new genomic disorders[31]. The reciprocal nature of the NAHR model proposes that interchromatid or interchromosomal crossover will concomitantly generate a deletion and a duplication of the same interval; therefore, some genomic disorders that arise from a deletion CNV have also had a reciprocal duplication counterpart that is associated with a clinical phenotype, whereas other disorders remain to be clinically described[1,30]. Remarkably, reciprocal duplication and deletion syndromes can sometimes manifest mirror image traits for selected phenotypes, for example, weight (overweight and underweight in 17p11.2 microdeletion and microduplication syndromes, respectively), head size (microcephaly versus macrocephaly in 1q21.1 and 16p11.2), microdeletion and microduplication syndromes associated with schizophrenia (del1q21.1 and dup16p11.2) and autism (dup1q21.1 and del16p11.2) (reviewed in REF. 54).

Intrachromatidal NAHR is predicted to exclusively produce deletions, whereas interchromatidal and interchromosomal NAHR can mediate both deletions and duplications[30]. This hypothesis has been confirmed experimentally by analysing the rate of *de novo* NAHR-generated deletions in the germ line, which indicated that deletions occur approximately twice as frequently as duplications[55], suggesting that NAHR has a preference for generating copy-number losses rather than copy-number gains.

NAHR frequency is driven by local genomic architecture

The complex structure of the LCR clusters suggests that more than one repeat pair within a certain cluster can be used as substrates for a *de novo* event. Nevertheless, the frequency of NAHR that occurs within and between LCRs mostly depends on distance, homology and size[1]. The distribution and relative frequencies of NAHR-mediated rearrangements positively correlate with LCR length, whereas the frequency of rearrangement is inversely influenced by inter-LCR distances[46]. Such correlation is even stronger when both LCR length and distance are considered (ln(LCR length/inter-LCR distance))[28,46]. Such coupled dependence suggests that, in meiotic NAHR, an ectopic synapsis occurs as a precursor to ectopic crossing-over[46]. Other LCR features such as DNA sequence identity, GC content and concentration of the PRDM9 homologous recombination hotspot motif 5′-

CCNCCNTNNCCNC-3′ (REFS 56,57) are also positively correlated with the frequency of NAHR[28]. Experimentally, well-studied genomic disorders such as the 17p11.2 microdeletion Smith–Magenis syndrome (SMS; OMIM 182290) and its reciprocal duplication Potocki–Lupski syndrome (PTLS; OMIM 610883) indicate that preferential use of a particular LCR prevails, that is, common and uncommon recurrent CNVs[46,58] (FIG. 2B). Evidence also supports the definition of active (hotspots) and inactive (coldspots) crossover regions or intervals within LCRs[59].

Inter-individual variation in the rate of NAHR-mediated deletions and duplications was observed in sperm-based assay studies[55,60]. One of the potential causes is a personal or population locus-specific structural variation that predisposes individuals who carry particular structural variant haplotypes to present with distinct NAHR susceptibility (FIG. 2C). Nevertheless, the contribution of inter-individual structural variants to NAHR frequency is still not well understood. A recent study revealed that the rate of meiotic NAHR correlated between identical twins and was independent of age[61], which indicates that other as yet unidentified genetic or environmental factors probably have an important role in the regulation of NAHR.

Inversions are particularly challenging to assess in an individual personal genome owing to the lack of high-throughput genome-wide screening techniques to assay such structures. Early evidence suggested that mitotic NAHR-mediated inversions frequently occur in somatic cells and potentially increase with age[62]. Meiotic inversions may also be frequent in the human genome. A study using fosmid paired-end sequencing data from eight human personal genomes from diverse populations identified 50–100 large genomic inversions that are not represented in the human reference genome[63]. Furthermore, genome-wide identification and mapping of inverted LCRs revealed that as much as ~12% of the genome may be susceptible to inversion that is mediated by NAHR[53]; inversions are therefore probably underestimated.

In summary, evidence suggests that paralogous sequence substrate size is important to ectopic synapsis[28,46], and *PRDM9* variation and the PRDM9 hotspot motif facilitate crossing over[56,57].

Homologous versus homeologous recombination substrates

Strand exchange during intrachromosomal homologous recombination in mammalian cells preferentially occurs in regions of uninterrupted sequence identity[64]. A critical target size between consecutive nucleotide mismatches or minimum efficient processing segments (MEPSs) seems to be required. In mammalian genomes, MEPSs were experimentally defined as being between 134 and 232 bases in length; shortening of that segment of identity caused a sharp decrease in the spontaneous recombination rate in mammalian cells[64]. NAHR favours recombination between substrates that share perfect or near-perfect homology, as evidenced by the frequent use of LCRs ≥10 kb in length with ≥97% sequence identity[30]. Nonetheless, disease-causing rearrangements between repeat regions with a lower percentage of sequence identity occurs in some pathogenic CNVs (for example, HERV elements with 94–95% similarity[65–67]). HERV-mediated CNVs show enrichment for PRDM9 hotspot motifs as well as the property of recurrence[65,67], which suggests NAHR as

their underlying mechanism. Other examples of short NAHR substrates generating CNVs include neurofibromatosis type I (NF1; OMIM 162200), where strand exchange occurs between intervals as short as 114 bp (REF. 68), and α-thalassemia, where recombination between shorter intervals (34 bp in length) generates deletions of the human α-globin gene[69].

By contrast, recombination between imperfectly matched substrates, that is, homeologous sequences [70], may be mediated by mechanisms other than NAHR. Homeologous sequences that underlie structural variant formation do not show enrichment for PRDM9 hotspot motifs and are shorter, with a lower percentage homology, than sequences used by NAHR. Structural variant formation events using homeologous sequences mostly generate nonrecurrent rearrangements that can be important contributors to genomic disorders[43,44,71–73]. For example, recombination between retroelements, such as short non-autonomous ~300 bp *Alu* elements, leading to CNVs is a predominant event in some genomic disorders[74], such as autosomal-dominant spastic paraplegia 4 (SPG4; OMIM 182601)[43,72] and alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV; OMIM 265380)[44]. *Alu*-*Alu*-mediated rearrangement events have been historically attributed to NAHR; however, the low nucleotide sequence identity shared between the *Alu* family members, which can be as low as 75%, suggests that alternative recombination mechanisms also take place[43]. A recent report of complex genomic variants such as interspersed duplications and triplications mediated by multiple iterative template switches between *Alu* indicates that DNA synthesis can be involved in *Alu*-*Alu*-mediated events and supports a role for DNA replication in their formation[37].

Nonrecurrent structural variants: key mechanisms

At least 70 genomic disorders are known to be caused by nonrecurrent structural variants[49]. This number is likely to be underestimated, because many of the genomic disorders that are associated with recurrent structural variants co-occur with nonrecurrent events in affected individuals (for selected examples see TABLE 1). Nonrecurrent rearrangements exhibit characteristic genomic features that are distinct from the recurrent structural variants that are mediated by NAHR (FIG. 1), for example, the scattered locations of their breakpoint junctions. Therefore, nonrecurrent rearrangements have a genomic content that is unique to the individual in which the rearrangement was generated (FIG. 1B), which makes the interpretation of potential clinical consequences challenging[75]. Moreover, predicting the occurrence of nonrecurrent events genome-wide is more difficult than that of recurrent structural variants.

The breakpoint junctions of nonrecurrent rearrangements can be characterized by simple blunt ends or microhomologies[76], which is in sharp contrast to the extensive homology provided by LCRs that flank recurrent structural variants. In addition, small insertions have been identified in a number of junctions[77], a characteristic that is not observed in NAHR-mediated events. Such features indicate that nonrecurrent structural variants are formed by molecular mechanisms that are distinct from NAHR, for example, non-homologous end joining (NHEJ) and RBMs such as break-induced replication (BIR), microhomology-mediated break-induced replication (MMBIR), serial replication slippage (SRS) and fork

stalling and template switching (FoSTeS) (reviewed in REF. 78). NHEJ generates mostly simple, blunt CNV end points and, infrequently, short homology (1–3 nucleotides); however, small deletions or the insertion of random nucleotides can also be observed at breakpoint junctions[79]. By contrast, most of the nonrecurrent structural variants that are associated with genomic disorders present 2–33-bp-long microhomologies at breakpoint junctions[80]. In addition, the insertion of short segments (<100 bp) copied from nearby genomic regions is observed in up to 35% of nonrecurrent structural variant junctions[10]. These experimental observations support the notion that the underlying mechanism of formation of nonrecurrent structural variants involves DNA replication in which microhomology is used as a primer to assist replication initiation[77,81].

RBMs

Evidence of DNA synthesis, which implicates RBMs in the formation of structural variants, has been documented in human diseases for almost a decade[77,82]. A key finding was the discovery that complex genomic rearrangements (CGRs), that is, rearrangements that consist of more than two breakpoint junctions[80], can be formed in a single mutational event during DNA repair[77,83]. This contention is strongly supported by observations of a *de novo* nonrecurrent triplication that exclusively involves a single X-chromosome inherited from a non-carrier father[7], and the finding that multiple, interspersed *de novo* copy number-amplified segments can all be stitched together in a single mutational event (chromoanasynthesis)[8,9]. Remarkably, copy number gains are more frequently associated with CGRs than are copy number losses[46]. This phenomenon may reflect the fact that the formation of nonrecurrent copy number gains is particularly likely to be generated by RBMs rather than by any other mechanism, although this observation is potentially biased by the fact that the signatures of DNA synthesis are more likely to remain in rearrangement structures that are associated with copy number gains[46].

The replicative process BIR is a homologous recombination pathway that is conserved from phage to eukaryotes and serves to repair single-ended double-stranded breaks (seDSBs)[84–86] (FIG. 3a). Spontaneous generation of such free DNA ends occurs, for example, during replication fork collapse at stalled forks or during the S phase of the cell cycle, in regions where there is no replication fork coming from the opposite direction (for example, subtelomeric regions). In BIR, the broken chromosome 3′ end invades a homologous template and initiates DNA replication that may extend hundreds of kilobases up to the telomere, in which case homozygosity of all markers distal to the double-stranded breaks (DSBs) can follow[86]. In yeast, BIR can also occur outside of the S phase, for example, in the G2 phase of the cell cycle. BIR requires almost all S phase replication proteins to be initiated, including all three major DNA polymerases (Polα–primase, Polδ and Polε) in addition to the replicative nonessential subunit of Polδ, Pol32 (REFS 87–89).

Recent *in vitro* studies in human cells under replication stress have implicated BIR in the formation of genomic rearrangements, including segmental amplifications[5]. Furthermore, nonrecurrent CNVs that are induced in mammalian cells through the use of replication inhibitors show many similarities to nonrecurrent structural variants that are formed spontaneously in genomic disorders. Similarities include breakpoint junctions that consist of

microhomologies or blunt ends and contain small insertions, as well as the occurrence of complex rearrangements[90]. Importantly, *in vitro* inactivation of *Xrcc4*, which encodes proteins required for canonical NHEJ (c-NHEJ), did not alter the frequency or the features of the resulting structural variants in mouse embryonic stem cells, which suggests that c-NHEJ is unlikely to be the underlying mechanism[90]. Therefore, *in vitro* experiments support the argument that RBMs underlie the formation of nonrecurrent structural variants, a hypothesis that was first proposed almost 10 years ago from the study of rearrangements that are causative for genomic disorders[77,82].

RAD51-dependent and RAD51-independent BIR (that is, MMBIR) seem to have different requirements for homology during DSB repair[91,92]. MMBIR uses microhomology to resume a stalled or collapsed replication fork as opposed to the longer homology tracts that are used in BIR[92] (FIG. 3b). MMBIR has been proposed as the major formative mechanism that underlies nonrecurrent structural variants in genomic disorders[92] on the basis that three main observations could not be readily explained by NAHR, NHEJ or BIR alone: first, the presence of microhomology in most of the breakpoint junctions, which may reflect priming DNA replication; second, template-driven juxtaposition of DNA sequences separated by large genomic distances, that is, long-range template switching; and third, iterative template switches that generate breakpoint complexity and CGRs[77,92].

RBMs are error prone

Microhomology mediates long-range template switching

Template switching refers to a change of the single-stranded DNA template during replication within the same replication fork (short-distance template switch) or between distinct replication forks (long-distance template switch). Long-range template switching that produces tandem amplification was observed in stress-induced *Escherichia coli* between segments sharing microhomology[81]. The presence of microhomology indicates a recombination process that does not require homology for strand invasion and DNA synthesis[81]. In humans, structural variants that are causative of PMD have revealed evidence for long-distance template switching that is mediated by microhomology, which led to the proposal of the FoSTeS mechanistic model in genomic disorders[77]. A key observation during the formation of the structural variants at the PMD locus was the occurrence of multiple iterative template switches (FoSTeSX2, FoSTeSX3, and so on) mediated by microhomology that generated CGR, such as interspersed duplications and triplications[77]. This study also showed that the use of microhomology to assist repair can contribute to the high error rate that is associated with mechanisms that rely on a short stretch of homology to achieve strand transfer[76] (BOX 1).

Template switches can generate complex structural variants

Replicative mechanisms can undergo multiple rounds of strand invasion; microhomology can be used to resume DNA synthesis. If strand dissociation occurs and is followed by template switches it can lead to the generation of complex structural variants[81,92–96]. Intrachromosomal and interchromosomal template switches have the potential to generate different types of CGRs, such as the insertion of short genomic segments at the repair site,

large-scale copy number alterations (for example, duplications, triplications and higher-order amplifications) and inversions (FIG. 4). There seems to be a bias towards intrachromosomal template switching in human rearrangements, although interchromosomal rearrangements do also occur[7,10,97]. Importantly, if nonallelic, homologous segments are used as templates and replication extends for several kilobases[78,94], template switches will not lead to a change in copy number but the resulting genomic segment can show loss of heterozygosity, or observed AOH[94].

The molecular characterization of structural variants in genomic disorders has revealed that apparently 'simple' nonrecurrent rearrangements may actually consist of complex breakpoint junctions. Insertions of short templated segments (<100 nucleotides) can be found in up to 35% of simple CNV junctions[10,98–103]. Most short templated insertions originate from nearby segments (within 300 bp), probably reflecting short-distance template switches that are due to misalignment or to replication slippage within the same replication fork[10,13,93]. Long-distance template switches introducing short template segments at the junctions occurred within <27 kb (REF. 10). By contrast, long-range template switches that produced large-scale CGRs, such as CNVs that are visible by high-resolution microarrays, vary in breakpoint junction distance from a few kilobases to megabases apart[39,77,99,101–105]. These observations may also be relevant for human genomic variability, as 27% of copy number losses analysed in samples from the 1,000 Genomes Project show insertions varying from 1 to 96 nucleotides at their breakpoint junctions; approximately 50% could be characterized as a templated insertion[106].

Complex rearrangements arise due to reduced processivity of DNA polymerases

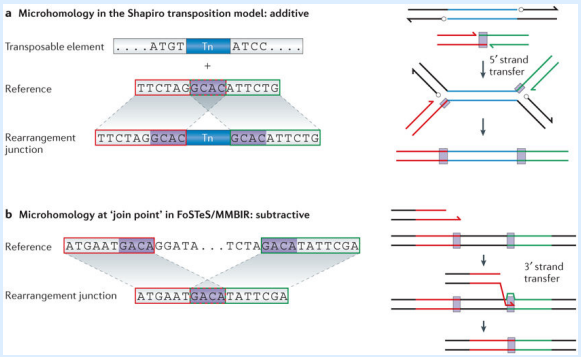
The presence of *de novo* indels, as well as short- and long-distance *trans* and *cis* template switches that occur concomitantly, strongly support a faulty DNA replication process during repair by RBMs[6,10,13,39,77,82,104,105]. In fact, the properties observed from studies of breakpoint junction formation suggest that the polymerase used for RBM has reduced processivity and fidelity relative to intergenerational DNA polymerases[107]. Distinct polymerases may contribute to template switching via different mechanisms. For example, in mammalian cell culture, Polθ introduces short templated insertions that are synthesized from mismatched primer termini during DSB repair by microhomology-mediated end joining (MMEJ)[108]. Long-range template switching, particularly involving the replication of long DNA segments, may involve replicative polymerases; Polδ is a prominent candidate due to its lower processivity compared with Polε. Supporting this argument, BIR microhomology-mediated events depend on the Polδ subunit Pol32 (in yeast) or POLD3 (in humans) to initiate the repair of broken forks; in humans this repair can produce duplications as large as 200 kb (REFS 5,89). The participation of translesion synthesis (TLS) polymerases such as Polζ in short-range template switching is supported by evidence that fork stalling uses Polζ to overcome the impairment in progression[109,110]. Importantly, recent studies in yeast have revealed that the TLS polymerases Polζ and Rev1 mediate template switches in BIR-defective cells using microhomology to prime replication[111]. This finding suggests that MMBIR can actually result from an interrupted BIR[111]. In aggregate, these data support the idea that template switching events that generate CGR may result from the

disruption of replication, dissociation of replicative polymerases and switching to lower processivity polymerases that are error prone.

Box 1

Microhomology can be added or removed during structural variant formation

A replicative mechanism was proposed by James Shapiro in 1979 to explain the semi-conservative transposition of the bacteriophage Mu[149], in which short nucleotide sequences are duplicated flanking the target site of transposition as a result of the replication step that follows the 5′ strand transfer (microhomology additive mode; see the figure, part **a**). By contrast, the fork stalling and template switching (FoSTeS)/microhomology-mediated break-induced replication (MMBIR) model proposes that single-ended, double-stranded DNA (seDNA) is processed by a nuclease. A single strand end of the 3′ tail is then generated upon resection of the 5′ end anneals by virtue of Watson–Crick base pairing to a single-strand DNA sharing microhomology (that is, template switching) and primes DNA replication. This process has the opposite effect to the Shapiro model and results in the deletion of a short nucleotide stretch that is in common or shared between the segments that are involved in generating the rearrangement junction, essentially reducing it from two copies to one (microhomology subtractive mode; see the figure, part **b**).



RBMs show a high mutational rate

In addition to having a high risk of undergoing template switching, DNA synthesis that is associated with repair can also lead to an increased local mutational rate[10,107]. The involvement of DNA synthesis during repair in humans is accompanied by increased *de novo* SNV and indel mutation rates at or near the breakpoint junction of duplicated segments (~2.1×10^{-4} mutations per base pair and ~1.7×10^{-3} events per base pair, respectively)[10]. Furthermore, kataegis, which is frequently associated with genomic rearrangements in some types of cancer such as breast cancer[112,113], is hypothesized to result from susceptible BIR-generated single-stranded DNA (ssDNA)[4].

Consistent with human data, BIR in yeast is accompanied by a 1,000-fold increase in the SNV mutation rate compared with S phase replication[107]. A 5′ extensive resection generates

a 3′ ssDNA end that will precede the strand invasion mediated by Rad51 (REF. 114). In BIR, the combination of an extensive ssDNA in addition to leading and lagging strands that are synthesized in an asynchronous way[115–117] results in the accumulation of ssDNA that is highly susceptible to unrepaired DNA lesions, the formation of secondary structures and cleavage by endonucleases. The conservative mode of DNA synthesis of newly synthesized strands in BIR via a migrating DNA bubble (D-loop) may contribute to the propagation of these orientation-dependent acquired mutations[116,117] (FIG. 3a). Importantly, the extent of replication inaccuracy resulting from BIR seems to be limited by the distance of the break-induced event to an efficient convergent fork as well as to the activity of the endonuclease Mus81. Mus81 cleaves the D-loop and converts conservative DNA synthesis into a canonical replication fork and W-C semi-conservative synthesis[118]. Therefore, Mus81 limits the high mutational rate of RBMs, including template switches, to regions that are near the breakpoint junctions of the structural variants formed by this mechanism[118]. Switching to a semi-conservative synthesis mode is likely to have a selective advantage by limiting the burst of mutations in the long genomic regions that would otherwise follow.

The role of genomic architecture

Formation of nonrecurrent structural variants is non-random

Breakpoint junction mapping of nonrecurrent rearrangements in genomic disorders indicates that the occurrence of structural variants in certain regions of the genome is unlikely to be random. Particular genomic architectures, such as repeated sequences and repetitive elements, with the potential to form non-B DNA structures (for example, A-T rich palindromes, G-quadruplexes, short inverted repeats and retrotransposable elements) are frequently observed in association with the location of a template switch or structural variant breakpoint[45,119–121]. How such elements contribute to structural variant formation is currently being debated: one possibility is that they render certain regions susceptible to the transient formation of secondary DNA structures that can lead to fork collapse and, in some cases, to the formation of DSBs[119,120]. DSBs can contribute to the primary cause of instability in a particular genomic region; however, DSBs may not even be required to trigger genomic stability. For example, common fragile sites (CFSs) that harbour $[A]_n$ and $[TA]_n$ repeats and quasi-palindrome sequences such as short inverted repeats (4–6 nt) have been shown to perturb the progression of S phase Polδ replication upon which the polymerase may pause and dissociate from the replication machinery[122]. This event potentially causes an error-prone polymerase to take over to more efficiently replicate such complex sites, thus leading to genomic instability that is conducive to nonrecurrent genomic rearrangements[122,123].

Nonrandom formation of nonrecurrent rearrangements is supported by analyses of polymorphic structural variants in human populations[15,106] in which distinct signatures of the underlying structural variant mechanism can be associated with the local genomic features where they occurred[106]. For example, DNA replication timing differences across the genome are associated with distinct types of CNVs. Recurrent CNVs are more frequently observed in early replicating regions, whereas nonrecurrent CNVs are more frequently observed in late replicating regions[106,124]. Further analysis has indicated that regions of

reduced rates of replication, which are consistent with polymerase pausing, seem to associate with breakpoint junctions of short homology-mediated CNVs[125], supporting the contention that genomic regions that are prone to polymerase pausing may be more susceptible to genomic instability. Nearby repeated sequences may themselves facilitate replicative repair that can lead to nonrecurrent structural variants.

The role of repeat sequences in the formation of non-recurrent structural variants

Genomic architecture can stimulate the occurrence of template switches near a DNA break. In yeast, the presence of repeats close to an induced break increases the frequency of the formation of CGRs, whereas diverged human-derived *Alu* elements inserted near seDSBs lead to increased template switching[118]. Interestingly, a recent study in budding yeast showed that the position of the strand invasion during repair by RBMs is influenced by 'microhomology islands' that flank the junctions of resulting rearrangements[126]. It remains unclear how frequently microhomology islands are found in nonrecurrent human rearrangements but it is tempting to speculate that repetitive elements such as *Alu*s may provide such microhomology islands that could assist strand transfer or stimulate template switching during repair by RBM. Moreover, a strong association between the locations of the breakpoint junctions of nonrecurrent rearrangements within LCRs has been documented in locus-specific studies (TABLE 1). This association has also been observed genome-wide in polymorphic structural variants[127]; in fact, Kidd *et al.*[128] indicate that ~20% of the breakpoints of certain types of structural variants map within 5 kb of LCRs.

Mechanistically, how LCRs contribute to the formation of nonrecurrent rearrangements is not entirely clear and probably differs in nature from the homologous recombination role that LCRs have in mediating recurrent rearrangements. This is evident from a genome-wide study of polymorphic structural variants that showed that the location of nonrecurrent rearrangements hotspots differs from those of recurrent rearrangements[129]. However, contrary to the challenge of predicting structural variant breakpoints using sequence motifs, LCRs do allow some degree of prediction of the occurrence of nonrecurrent rearrangements and support the hypothesis that the genomic architecture exerts multiple but distinct influences on structural variant formation genome-wide. This is exemplified by specific CGR structures such as DUP–TRP/INV–DUP that can be formed if highly identical inverted LCRs are used as substrates for BIR[7,33,34,130]. The presence of inverted repeats is hypothesized to provide a high degree of nucleotide sequence identity, which renders the region susceptible to undergoing ectopic template switching. Such ectopic template switching during a replicative repair process may result in an inverted segment being formed concomitantly with a copy number gain (FIG. 4e). DUP–TRP/INV–DUP is frequently observed in patients with PMD due to *PLP1* duplication[33] and is also present in ~20% of individuals with *MECP2* duplication syndrome[7,39]. Importantly, DUP–TRP/INV–DUP can cause a much more severe clinical phenotype if the dosage-sensitive gene, that is, *PLP1* or *MECP2*, maps within the triplicated segment[7,33,131,132]. Therefore, the presence of inverted repeats near dosage-sensitive genes can indicate regions in the human genome that are susceptible to DUP–TRP/INV–DUP formation[53], which has been proved to be of clinical relevance at different genomic loci[34,35,130] (TABLE 1).

RBMs underlie some human translocations

Extensive homology present at the telomere and subtelomere of most chromosomes[29] can trigger breakage–fusion–bridge (BFB) cycles[133,134] by forming dicentric chromosomes, intrachromosomal or interchromosomal ectopic recombination[135] and secondary structures that can render these regions prone to breaks[136]. NHEJ is likely to be a predominant mechanism of repair[137], although recurrent NAHR between interchromosomal LCRs also occurs[51,135,138]. Recent data have implicated RBMs in the repair of broken telomeres on which homology, homeology or microhomology is used during repair[37,139–141], including telomeric short inverted repeats[142]. Therefore, LCRs and other types of repeats probably contribute to the rearrangement events involving telomeres, supporting the conclusion that genomic architecture can have multiple roles in the formation of structural variants genome-wide and that RBMs might contribute to increasing an individual's genome complexity.

Conclusions

Studies of structural variation in genomic disorders have resulted in mechanistic findings that apply to model organisms and have wide implications for fields as diverse as human biology, genomics, molecular diagnostics, disease understanding and therapeutic interventions based on gene dosage correction. The emerging picture is a perplexing fractal: large genomic-scale structural variants, that is, alterations involving megabases of DNA, can be associated with additional complexities, such as other structural variants that are generated in a one-off event (chromoanasynthesis and chromothripsis-like events (FIG. 5a))[8,9] or a CGR accompanied by AOH[11,12]. Further alterations might be revealed when these structural variants are scrutinized down to the base-pair sequence level owing to the presence of small-scale changes that can act as mutational signatures, for example, insertions or deletions of short DNA segments and/or *de novo* point mutations that are present adjacent to the junction of a structural variant[10,106,128,143] (FIG. 5b). This picture may further complicate the interpretation of potential functional consequences of a mutagenic event for an a priori apparently simple structural variant.

The genomic architecture at a particular locus contributes to an increase in structural variant complexity, with a prominent role for replicative mechanisms. LCRs can mediate recurrent structural variants by providing substrate sequences for NAHR, and also stimulate non-recurrent structural variants by RBMs, where ectopic repeats can assist DNA repair by providing homology, homeology or microhomology sequences. RBMs underlying the formation of a 'simple' or a 'complex' structural variant have important implications for human disease and transmission genetics. First, the occurrence of triplications can lead to more severe clinical phenotypes; chromoanasynthesis events can cause multiple malformations and congenital diseases, whereas segmental AOH may enable the expression of recessive traits and regional uniparental disomy. Second, point mutations and indels that are derived from error-prone repair that is likely to arise concomitant with a disease-associated structural variant may affect gene expression and contribute to the variability of the associated genomic disorder. Third, RBM-generated nonrecurrent rearrangements are hypothesized to be formed during mitosis. Therefore, these mutations may contribute to diseases that are caused by somatic mutations, including cancer, developmental and

neurological disorders[144] and to an increased probability of parental low-level mosaicism[6,145]. Germline mosaicism can alter the recurrence risk for future pregnancies[19].

Importantly, how genomic architecture contributes to altered gene expression must be broadly investigated. The impact of a particular structural variant on the expression of overlapping genes seems to go beyond simply the direct effect of the altered copy number. For example, lymphoblastoid cells from patients with deletions of the Williams–Beuren region (7q11.23) show dysregulation of flanking genes, reflecting long-range *cis*-regulatory elements within the structural variant[146] that can also potentially change the timing of gene expression[147]. Recently, chromosome conformation capture (4C-seq) and ChIP-seq revealed that modifications of local chromatin were strongly associated with the altered expression of particular genes in 7q11.23 (REF. 16). The contribution of structural variants to the regulation of gene expression in *cis* or in *trans* needs to be further explored as it may help to explain phenotypic variability in patients who carry overlapping nonrecurrent rearrangements[148]. For example, new discoveries of mechanisms for structural variant formation in humans are elucidating how such events can re-structure a specific region of the genome to change the expression of transcripts locally or genome-wide[16,17], including through altering topologically associating domains (TADs), with pathological consequences for carriers[18].

In summary, the contribution of genomic architecture to structural variant formation goes beyond the definition and expansion of a group of human diseases designated as genomic disorders. It implicates particular mechanisms for rearrangement formation and helps to guide studies about the potential causes of human disease, including germline and somatic mutagenesis events.

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Glossary

Genomic disorders	Conditions that result from rearrangements of the genome rather than base pair changes of DNA, and in which genomic instability results from the endogenous genome architecture.
Structural variants	Variants that include copy number variants and copy number neutral inversions, insertions and translocations in a personal genome compared with a reference genome.

Copy number variants (CNVs)	Alteration in copy number (gain or loss) of a locus resulting in deviation from the normal diploid state.
Single nucleotide variants (SNVs)	A single site in a DNA sequence that differs among individuals.
Chromothripsis "Shattering of chromosomes"	A single catastrophic event affecting one chromosome and leading to complex rearrangements in cancer.
Absence of heterozygosity (AOH)	Refers to copy number neutral genomic segments that lack heterozygosity for assayed polymorphic markers.
Template switching	Refers to a transient dissociation of the primer and template followed by a re-association to a distinct template during DNA replication. It can occur within the same replication fork (short-distance template switch) or between distinct replication forks (long-distance template switch).
Array comparative genomic hybridization (aCGH)	Microarray-based technique that measures the relative copy number of DNA segments.
Replication-based mechanisms (RBMs)	Replicative non-homologous DNA repair mechanism of single-ended, double-stranded DNA (seDNA).
Rearrangement susceptibility	Regions of the genome prone to structural variation formation.
Mobile elements	A segment of DNA capable of moving into a new genomic position.
Paralogous sequences	Homologous sequences that arose by duplication.
Nonallelic homologous recombination	Nonallelic pairing of paralogous sequences and crossover leading to deletions, duplications and inversions.
Ectopic synapsis	Chromosomal homologue synapses at a nonallelic position.
Fosmid paired-end sequencing	A clone-based method to sequence the ends of fragments with a known size range.
Homeologous sequences	Imperfectly matched paralogous genomic segments.
Microhomologies	Short stretches of shared nucleotide identity present at the junctions of rearranged genomic segments.
Non-homologous end joining (NHEJ)	Double-stranded break (DSB) mechanism of repair that processes the broken DNA ends and joins non-homologous sequences. It repairs the programmed DSBs created in the immune system.

Break-induced replication (BIR)	Homologous recombination pathway that repairs single-ended double-stranded breaks (seDSBs) through the establishment of a unidirectional replication fork.
Microhomology-mediated break-induced replication (MMBIR)	RAD51-independent break-induced replication that relies on microhomology to resume replication.
Serial replication slippage (SRS)	Multiple rounds of slipped strand mispairing at the replication fork.
Fork stalling and template switching (FoSTeS)	Mechanism of template switching between different replication forks.
Chromoanasynthesis	Chromosome reconstitution or chromosome re-assortment. Constitutive complex rearrangements resulting from multiple template switches.
Microhomology-mediated end joining (MMEJ)	An alternative non-homologous end joining mechanism that repairs broken double-stranded breaks using sequence microhomology to join and stabilize DNA end intermediates.
Kataegis	Somatic single-nucleotide mutation clusters or mutation showers in *cis.*
Breakage–fusion–bridge (BFB) cycles	Processes by which sister chromatids that lack a telomere (breakage) can retrieve them by fusion and the creation of an unstable dicentric chromosome that will be pulled apart during anaphase (bridge). Eventually, the bridge breaks and the cycle starts again until the chromosome is stabilized.

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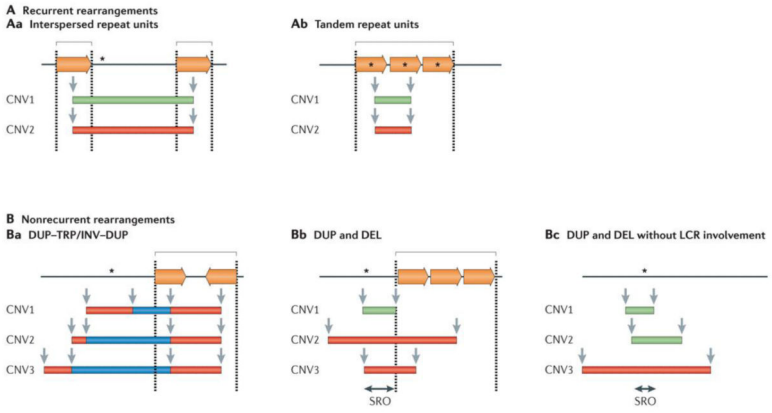

Figure 1. Schematic representation of recurrent and nonrecurrent genomic rearrangements observed in genomic disorders

Black lines represent the genomic segments of a given locus. Dosage-sensitive genes that are involved in the rearrangement are represented by black asterisks. Paralogous repeats or low-copy repeats (LCRs) are represented by inverted and directly oriented horizontal orange arrows. Dashed lines indicate LCR regions. Individual genomic copy number variants (CNVs) are represented by colour-matched rectangles (deleted segments (green); duplicated segments (red); and triplicated segments (blue)) and identified as CNV1, CNV2 and CNV3. Locations of CNV breakpoints are indicated by grey vertical arrows. Square brackets denote regions with clustering or grouping of breakpoint junctions. **A** | Recurrent rearrangements share the same size and genomic content in unrelated individuals. More than 40 non-overlapping genomic disorders are caused by recurrent rearrangements[49]. **Aa** | In these types of structural variants the duplication or deletion breakpoints cluster within long, highly identical flanking interspersed paralogous repeats (represented by directly oriented horizontal orange arrows) that serve as substrates for nonallelic homologous recombination (NAHR). **Ab** | Alternatively, recurrent rearrangements can occur within tandem paralogous repeats and affect the copy number of a dosage-sensitive gene that is present within the repeat. For example, ectopic recombination of a tandem repeat consisting of opsin genes at Xq28 can cause red–green colour blindness (OMIM 303900, OMIM 303800) or it can lead to copy number polymorphism at this locus[32]. **B** | Nonrecurrent rearrangements present a unique size and genomic content at a given locus in unrelated individuals. At least 70 genomic disorders that are caused by nonrecurrent rearrangements have been described[49]. **Ba** | DUP–TRP/INV–DUP (duplication–inverted triplication–duplication) structures are nonrecurrent but, in some cases, have a limited genomic recurrence with two of four breakpoints mapping to an inverted repeat pair[7,33–36]. In these cases, the triplicated segments are inverted in relation to the duplications. **Bb** | Some genomic disorders are characterized by nonrecurrent rearrangements that show breakpoint grouping (rather than clustering as in the recurrent cases discussed above) within paralogous repeats[38–42]. **Bc** | By contrast, some genomic disorders are characterized by nonrecurrent rearrangements without any clustering or grouping of breakpoints[43–45]. Green rectangles represent deletions (DEL); red rectangles represent duplications (DUP); blue rectangles represent triplications (TRP). INV, inversion; SRO, smallest region of overlap.

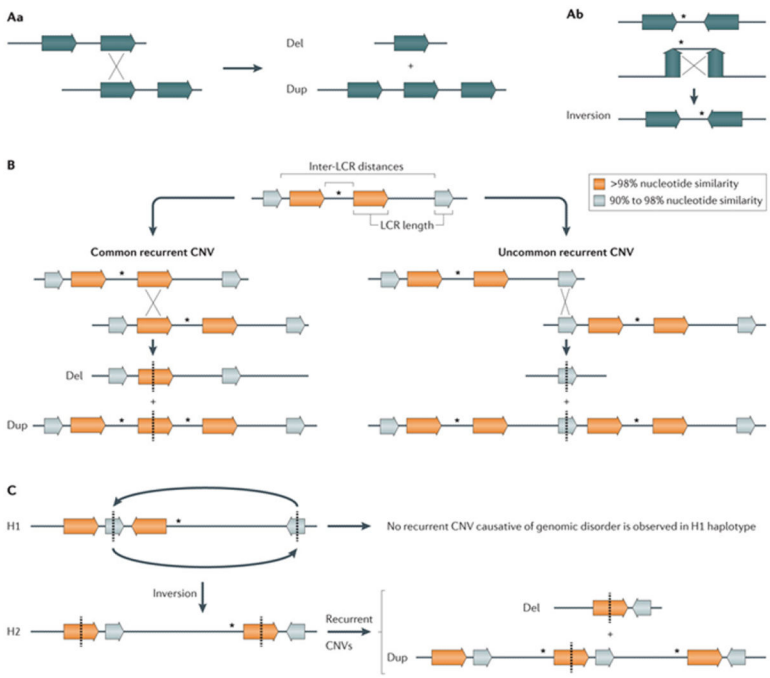

Figure 2. Nonallelic homologous recombination mechanism

A | Nonallelic homologous recombination (NAHR) between directly oriented repeats generates deletions and duplications (part **Aa**), whereas NAHR between inverted repeats generates the inversion of the segment in between the repeats (part **Ab**). **B** | The rate of NAHR may vary within a particular locus owing to the presence of low-copy repeat (LCR) pairs with distinct susceptibility risk. For example, NAHR rate positively correlates with LCR length and inversely correlates with inter-LCR distance, which can lead to common recurrent and uncommon recurrent copy number variants (CNVs) at a given locus identified in different patients with a genomic disorder[30] (for example, in common recurrent and uncommon recurrent deletions and reciprocal duplications in Smith–Magenis syndrome and Potocki–Lupski syndrome, respectively)[46]. **C** | NAHR rate may vary among individuals with distinct genomic architecture in a given locus or a structural variant haplotype that results in directly oriented LCR. This is exemplified by inverted structural haplotypes that present with distinct risks of undergoing NAHR owing to structural variability in LCR content and orientation. For instance, two divergent structural variant haplotypes, H1 and H2, are observed in 17q21.31 region but deletions that cause Koolen-De Vries syndrome (OMIM 610443)[150] occur on chromosomes carrying the H2 haplotype. Dosage-sensitive genes flanked by LCRs are represented by asterisks. LCRs are represented by horizontal colour-matched arrows.

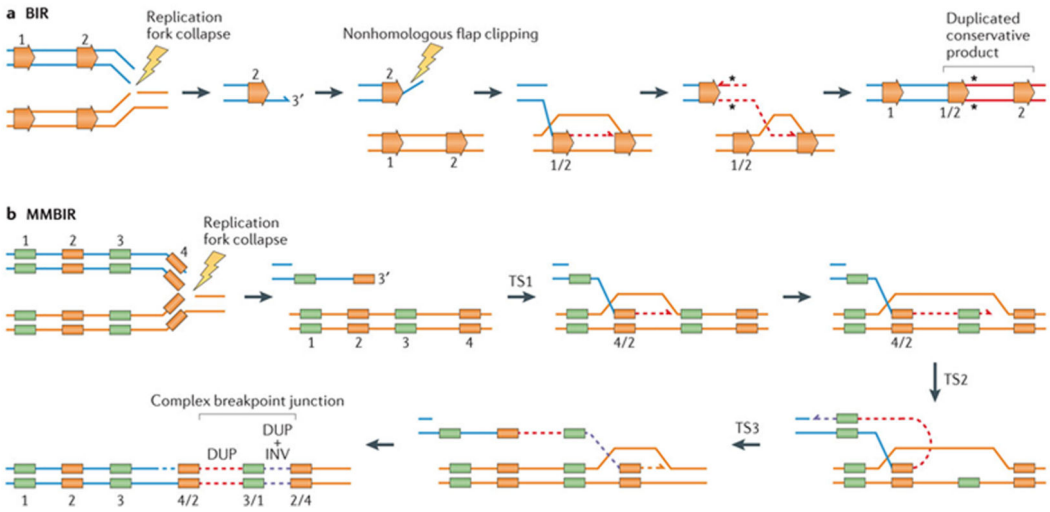

Figure 3. Replication-based mechanisms generate structural variants and single-nucleotide variants

a | Break-induced replication (BIR) can be triggered by a nick on the template strand that may cause stalling or the collapse of the replication fork. Resection of the 5′ end of the broken single-ended, double-stranded DNA (seDNA) molecule exposes a 3′ tail that can invade an allelic (not shown) or a paralogous genomic segment with shared homology (ectopic recombination) to prime replication. Paralogous segments are represented by horizontal orange arrows. The use of ectopic homology to repair broken molecules by BIR can lead to structural variants (for example, duplication (shown here), triplications, deletions and inversions). A conservative mode of repair was recently proposed for BIR that can contribute to perpetuate repair indels or single-nucleotide variant (SNV) (black asterisks) mutations that are acquired during the replicative repair[116,117]. **b** | Microhomology-mediated break-induced replication (MMBIR) can be triggered by a nick on the template strand that may cause stalling or the collapse of the replication fork[92]. Alternatively, MMBIR can also occur by disrupted BIR[111]. Resection of the 5′ end of the broken seDNA molecule exposes a 3′ tail that can anneal to a single-strand DNA sharing microhomology (colour-matched boxes) to prime replication. The initial polymerase extension and replication is carried out by a low processivity polymerase, rendering this repair process prone to undergoing multiple rounds of disengagement and template switches until a fully processive replication fork is established. Short and/or long template switches during repair can generate complexity due to the insertion of templated segments at the rearrangement junction. DUP, duplication; INV, inversion; TS, template switches.

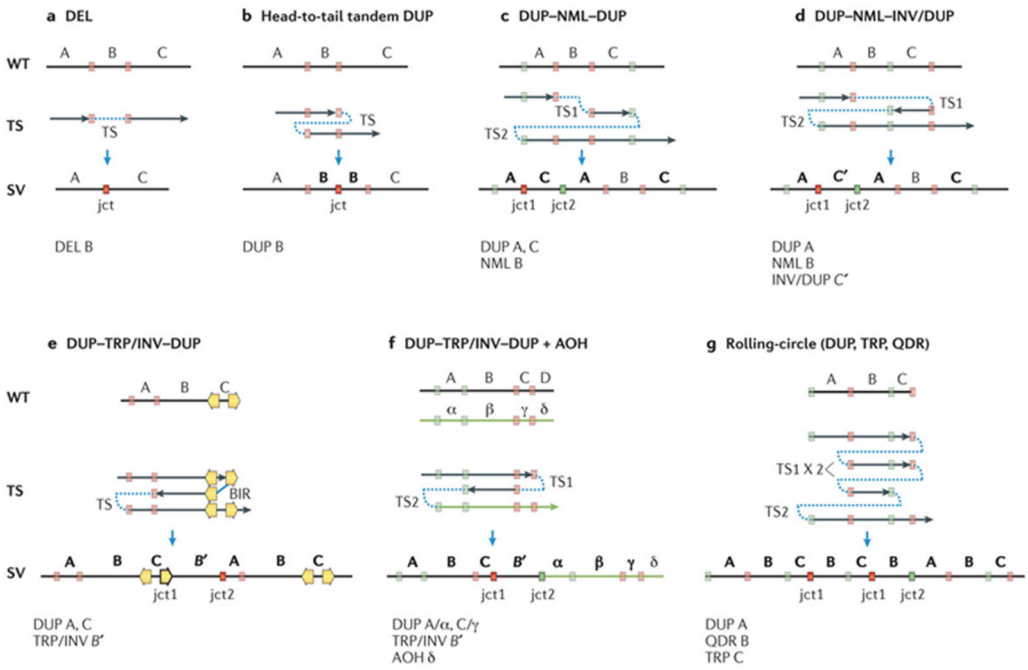

Figure 4. Template switches can generate distinct patterns of 'simple' or 'complex' structural variants

All displayed structural patterns have been identified in one or more genomic disorders (TABLE 1). Top panel: black vertical line indicates a wild-type (WT) genomic segment. Middle panel: black arrows represent segments of DNA generated upon template switches (TSs). Red and green colour-matched boxes represent microhomology regions in WT genomic segment further involved in annealing and resumption of replication during TS. Orange arrows represent inverted low-copy repeats (LCRs) or regions of extended homology that can also be used during TS. Note that formation of any homology/microhomology breakpoint junctions (represented by outlined colour-matched boxes) is accompanied by a relative net reduction of the copy number of those homology/microhomology regions compared to WT regardless of whether the rearrangement results in gain or loss of the flanking genomic material (see microhomology subtractive mode (BOX 1)). Bottom panel: resulting structural variant (SV). Letters (A, B, C, D) represent chromosome alleles. Alleles subject to copy number gains in the resulting SVs are marked as bold letters. Primed letters represent an inverted-oriented genomic segment that originates upon a TS between complementary strands. α, β, γ, δ, represent corresponding homologue alleles. A single TS can generate deletion (DEL) (part **a**) or a head to tail tandem duplication (DUP) with one junction (jct) (part **b**). Two TSs can generate distinct complex end products presenting with two breakpoint junctions (jct1 and jct2). DUP–NML–DUP: copy number neutral segment (normal; NML) interspersed between duplications (DUP) (part **c**). DUP–NML–INV/DUP: inverted interspersed duplications (part **d**). DUP–TRP/INV–DUP: inverted triplication interspersed with duplications (part **e**). DUP–TRP/INV–DUP can be generated by break-induced replication (BIR), if inverted repeats are used as substrates for TS, or microhomology-mediated BIR if microhomology is used for TS. DUP–TRP/INV–DUP (part **f**) can be associated with extended regions of absence of heterozygosity (AOH) if TS occurs

between homologous chromosomes (interchromosomal TS). Rolling-circle amplification (part **g**) can be generated by re-replication (for instance, the generation of two copies of jct1) culminating in the formation of multiple copies of a given segment.

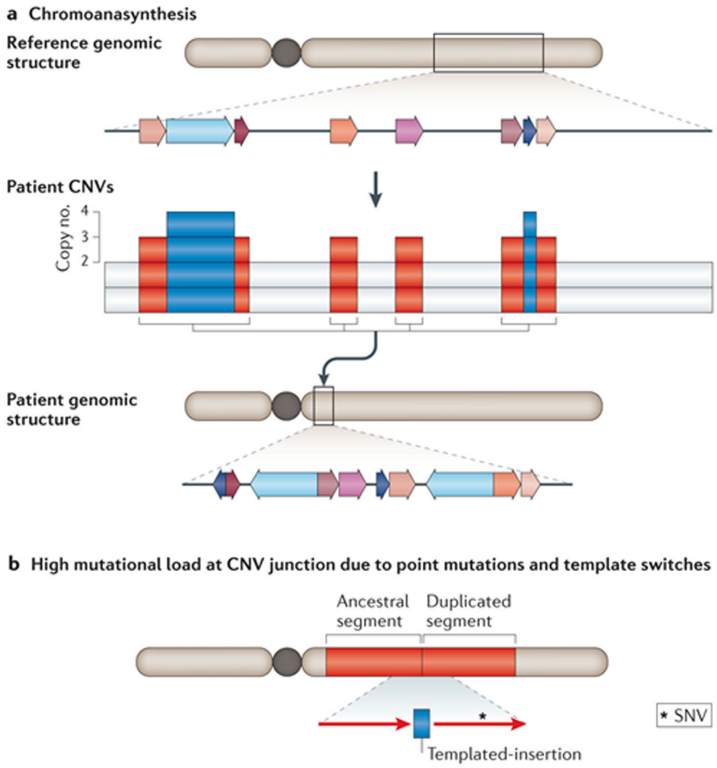

Figure 5. Replication-based mechanisms are error prone

a | Schematic example of copy number variants (CNVs) in multiple, interspersed segments within the same chromosome arm. Chromoanasynthesis in constitutional genomic disorders resembles the phenomenon of chromosome catastrophes (that is, chromothripsis) initially described based on the analyses of hundreds of cancer genome sequences and in different types of cancers[83]. The chromosomal region involved in chromoanasynthesis is denoted by a grey box; local genomic segments involved are represented by coloured arrows. Array comparative genomic hybridization (aCGH) reveals genomic segments with copy number variation. The rearranged chromosome is shown on which the extra copy number segments (represented by colour-matched arrows) are inserted together in a new chromosomal position with respect to the reference genome. This clustering of CNVs in a new position is a hallmark of chromoanasynthesis. **b** | Increased mutational load can be observed at the breakpoint junctions of CNVs generated by replication-based mechanisms (RBMs). SNVs include point mutations and short insertions/short deletions (indels). Red rectangles represent the duplicated segment and red arrows represent the orientation of the duplicated segments.

Table 1

Representative LCRs that mediate recurrent SVs as well as stimulate nonrecurrent genomic disorder associated

LCR	Chromosome	Nonrecurrent SV	Size range (Mb)	Associated clinical phenotype (OMIM)	Refs
A, B, C	3q29	• DEL • DUP	• 1.4–3.2 • 0.2–2.4	• 3q29 microdeletion syndrome (609425) • 3q29 microduplication syndrome (611936)	151
SoS-PREP, SoS-DREP	5q35	• DEL, DUP • DEL–NML–DEL	• 0.37–3.7 • 0.8	• Sotos syndrome (117550)	152,153
A, B, C	7q11.23	• DEL • DUP • TRP	• 0.082–2.4 • 3.6 • 1.25	• Williams–Beuren syndrome (194050) • Williams–Beuren region duplication syndrome (609757)	154 – 156
BP3, BP4, BP5	15q13.1	• DUP–TRP/INV–DUP	• 0.65	• 15q13.1 microdeletion syndrome (612001)	36
1-8 or A-H	22q11.2	• DEL • DEL–NML–DEL	• 0.02–4.0 • 2.5	• Velocardiofacial syndrome (192430) • DiGeorge syndrome (188400) • 22q11.2 distal deletion syndrome (611867) • 22q11.2 microduplication syndrome (608363)	157 – 159
SMS-REPs	17p11.2	• DEL, DUP, Complex	• 0.41–19.6	• Smith–Magenis syndrome (182290) • Potocki–Lupski syndrome (610883)	6,46,160
CMT1A-REPs	17p12	• DEL, DUP, Complex	• 0.009–3.4	• Charcot–Marie–Tooth disease type 1A (118220) • Hereditary neuropathy with liability to pressure palsies (162500)	6,161
NF1-REP-A, B, C	17q12	• DEL	• 0.006–3.0	• Chromosome 17q11.2 deletion syndrome (613675)	162
CRI-S232	Xp22.31	• DUP, Complex	• 0.35–1.9	• X-linked ichthyosis (308100)	105
PMDA-D	Xq22	• DUP, DEL • DUP–TRP/INV–DUP • Complex	• 0.1–11.0	• Pelizaeus–Merzbacher disease (312080) • Spastic paraplegia 2, X-linked (312920)	7,33,40,42,77

LCR	Chromosome	Nonrecurrent SV	Size range (Mb)	Associated clinical phenotype (OMIM)	Refs
JA, JB, JC K1, K2	Xq28	• DUP, DEL • DUP–TRP/INV–DUP • Complex	• 0.040–4.0	• *MECP2* duplication syndrome (300260) • X-linked Emery–Dreifuss muscular dystrophy 1 (310300)	38,39,41,163
L1 (LCR1), L2 (LCR2)	Xq28	• DEL	• 0.005–0.115	• Incontinentia pigmenti (308300)	164

DEL, deletion; DUP, duplication; INV, inversion; LCR, low-copy repeat; NML, normal (copy number neutral region); SV, structural variant; TRP, triplication. *Recurrent SVs mediated by LCRs PMDA-D, K1, K2, L1 and L2 are not causative of diseases. Distinct SV patterns of complex rearrangements, such as DUP–NML–INV/DUP, DUP–NML–DUP, DEL–NML–DEL, are observed at the loci as described.