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COMMENTARY

Mechanism of complex gross chromosomal rearrangements: A commentary on Concomitant microduplications of *MECP2* and *ATRX* in male patients with severe mental retardation

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In previous issue of the *Journal of Human Genetics*, Honda *et al.*[1] have reported on a family with X-linked mental retardation cosegregating with two concomitant duplications on X chromosome. A region including the *ATRX* gene at Xq21.1 was duplicated and inserted into the Xq28 region containing the *MECP2* gene, which was also tandemly duplicated. As a potential mechanism for this complex gross chromosomal rearrangement (GCR), these authors discuss the possibility of fork-stalling and template switching (FoSTeS). Recent trends in utilizing cytogenetic microarrays to detect disease-causing submicroscopic chromosomal abnormalities have also enabled the fine localization of GCR breakpoints. A substantial number of disease-related GCRs and benign copy number variations have now been identified in this way and the breakpoints have been analyzed. As a consequence, a subset of GCRs has been found to have more complex breakpoints than was previously thought.

To date, the mechanism underlying recurrent GCR has been thought to be non-allelic homologous recombination (NAHR) characterized by unequal crossover between low-copy repeats (LCRs), in other words, segmental duplications that extend for several hundred base-pairs with a high sequence homology (often >97%). Multiple lines of evidence now indicate that the NAHR that occurs during meiosis I, whether as an inter- or intra-chromosomal event.[2] In contrast to this NAHR, FoSTeS has been proposed as a mechanism for non-recurrent GCR. Pelizaeus–Merzbacher disease is one of the hereditary neurodegenerative disorders resulting from the deletion/duplication of the chromosomal region that includes the dosage-sensitive *PLP1* gene on Xq22. In contrast to other deletion/duplication syndromes, analysis of the breakpoints in this disorder has revealed no LCR-like genomic structures at either ends of these deletions or duplications. Instead, the junctions are characterized by complex structures harboring smaller deletions and duplications in either orientation that are joined via 2–5 nucleotides of microhomology. On the basis of the finding that sequence refractory fragments that preclude the progression of DNA polymerases have been consistently identified at these rearrangement junctions, a unique mechanism related to DNA replication-stalling was proposed.[3] The emergence of the concept of FoSTeS was somewhat of a sensation in this field, and a considerable number of case studies were subsequently published describing the involvement of FoSTeS in the mechanism of GCR. However, most of these reports discussed the possible involvement of FoSTeS only in terms of the two main features of the junction without any evidence of replication stalling.

The basics of the replication-mediated mechanism underlying the generation of complex GCRs had been proposed earlier using another term, serial replication slippage.[4] FoSTeS encompasses a much narrower definition in which specific sequences that potentially form a non-B DNA replication barrier, such as hairpin DNA, are identified at the breakpoints.[5] However, as most GCRs have non-recurrent breakpoints with no such characteristic sequence, the same group that proposed the FoSTeS definition created another term, microhomology-mediated break-induced replication (MMBIR), to explain similar types of GCR.[6] The MMBIR hypothesis is based on the possibility that DNA breakages presenting before replication may induce a similar process to FoSTeS, but without any replication barrier sequence. On the other hand, in somatic cells such as leukemia/lymphoma cells, microhomology at the GCR junction has implicated microhomology-mediated end joining or alternative end joining as a mechanism for repair of the DNA breakage.[7] Recent genome-wide analysis of structural variations indicates that nearly 50% of breakpoints are generated via microhomology-mediated mechanisms.[8] Hence, it is becoming important to understand the processes that lead to GCRs, although the terminology is becoming confusing and chaotic.

In my view, GCRs are fundamentally dependent on two distinct processes: the generation of DNA breaks and repair of these breaks.[2] DNA breaks can result from exogenous agents such as ionizing radiation and chemotherapeutic drugs, and also from endogenously generated reactive oxygen species and mechanical stress at the chromosomes. Replication stalling by potential replication barrier sequences or DNA-protein complexes can also trigger replication fork collapse leading to DNA breaks. DNA breaks can then activate cell cycle checkpoints and eventually cause cell death by triggering apoptosis unless they are adequately repaired. DNA repair is executed via various repair pathways that are

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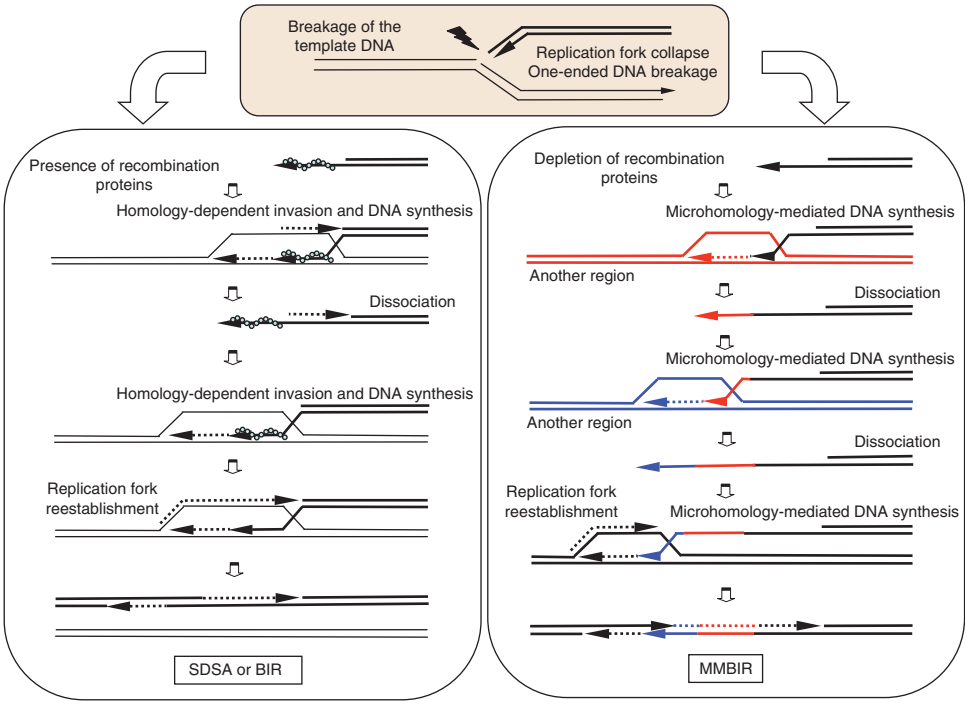

Figure 1 Proposed mechanism for the formation of the complex junction observed in non-recurrent gross chromosomal rearrangements (GCRs). When a replication fork encounters a DNA break such as nick in the template strand, a replication fork collapse occurs leading to formation of a one-ended double-strand-break (DSB). (Left) With the aid of RAD51 and RAD52, the DNA end produces a nucleofilament complex (blue circles), which facilitates homology scanning for template DNA, which would generally be a sister chromatid (thin lines) or occasionally a homologous chromosome. The single-stranded DNA end next invades the homologous duplex DNA and forms a D-loop structure. The nascent strand (dotted lines) is subsequently dissociated and searches for the second DNA end that should be formed by a standard DSB. The DNA end that cannot anneal to the second end invades the template duplex again and DNA extension resumes. This cycle will repeat until the nascent strand captures the second end. (Right) When the recombination proteins are depleted, DNA synthesis resumes by annealing via micro-homology to the single-stranded region in another replication fork that is in physical proximity (red or blue lines). The cycle (invasion, extension and dissociation) might be repeated several times until the replication fork is re-established.

dependent on the cell cycle stage. In S/G2 phase, when a sister chromatid can be used as a template for DNA synthesis, DNA breaks are generally repaired via error-free systems; that is, homologous recombination (HR). However, in the G1 phase when no template is available other than the homologous chromosome, GCRs occasionally arise as the result of an illegitimate error-prone repair system; that is, non-homologous end joining. In a subset of GCRs, the presence of long duplicated segments without the involvement of any LCRs at the breakpoints implicates a mechanism that is unrelated to HR but initiated by DNA replication. Indeed, the administration of DNA polymerase inhibitors in cell cultures induces deletions and duplications that have junctions with microhomology.[9]

In this context, MMBIR appears to provide a sophisticated explanation for the DNA repair pathway that leads to the complex junctions observed in GCRs. Double-strand-breaks during replication are generally repaired via a homology-dependent pathway whereby DNA ends seek out homologous sequences and then invades the corresponding DNA duplexes with the aid of the RAD51 and RAD52 and commence DNA synthesis (Figure 1, left). The nascent strand is then dissociated and looks for the second DNA end. The end of the nascent strand that cannot anneal to the second end invades the template duplex again and DNA extension resumes. This cycle will repeat until the nascent strand captures the second end (synthesis-dependent strand annealing). In the case of a one-ended DNA break arising when the replication fork encounters a replication barrier; for example, non-B DNAs such as a haipin structure or a DNA breakage in the template strand, the extension continues to another replicon or chromosome end and is called break-induced replication.[10] Fundamentally, all of these processes are conducted via an error-free DNA repair system. However, in certain situations where the recombination proteins are depleted, DNA synthesis resumes via microhomology annealing (Figure 1, right).[6] As the DNA end cannot find a homologous region within the replication fork in this case, the free DNA end might anneal to a single strand DNA in another replication fork within physical proximity in the nucleus via microhomology; that is, template-switching. The cycle (invasion, extension and dissociation) might be repeated several times until the free end reestablishes a replication fork that is fused to another replicon, yielding a complex junction structure that is occasionally observed in non-recurrent GCRs.

The MMBIR hypothesis is thus established and a subset of non-recurrent GCRs can be readily explained by this proposed mechanism. However, this hypothesis is based only on the junction structure obtained by sequence analysis and has not yet been experimentally validated. More evidence, including the identification of molecules that participate in the process, is required to validate MMBIR. In this area of research also, a new paradigm referred to as chromothripsis, which represents chromosome shattering followed by random reassembly, was recently proposed from the findings obtained through whole-genome sequencing. Surprisingly, it has been reported also that chromothripsis is generated via a replication-based mechanism.[11] However, this conclusion may be somewhat premature at present as the long duplication segment; that is, characteristic of GCRs via replication-based mechanisms has

not been observed in chromothripsis. Regardless, a more thorough analysis of chromothripsis will likely reinforce this hypothesis.

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1 Honda, S., Satomura, S., Hayashi, S., Imoto, I., Nakagawa, E., Goto, Y.-i. *et al.* Concomitant microduplications of *MECP2* and *ATRX* in male patients with severe mental retardation. *J. Hum. Genet.* **57,** 73–77 (2012).
2 Kurahashi, H., Bolor, H., Kato, T., Kogo, H., Tsutsumi, M., Inagaki, H. *et al.* Recent advance in our understanding of the molecular nature of chromosomal abnormalities. *J. Hum. Genet.* **54,** 253–260 (2009).
3 Lee, J. A., Carvalho, C. M. & Lupski, J. R. A DNA replication mechanism for generating nonrecurrent rearrangements associated with genomic disorders. *Cell* **131,** 1235–1247 (2007).
4 Chen, J. M., Chuzhanova, N., Stenson, P. D., Férec, C. & Cooper, D. N. Complex gene rearrangements caused by serial replication slippage. *Hum. Mutat.* **26,** 125–134 (2005).
5 Kurahashi, H., Inagaki, H., Ohye, T., Kogo, H., Kato, T. & Emanuel, B. S. Palindrome-mediated chromosomal translocations in humans. *DNA Repair. (Amst.)* **5,** 1136–1145 (2006).
6 Hastings, P. J., Ira, G. & Lupski, J. R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* **5,** e1000327 (2009).
7 Lieber, M. R. NHEJ and its backup pathways in chromosomal translocations. *Nat. Struct. Mol. Biol.* **17,** 393–395 (2010).
8 Conrad, D. F., Bird, C., Blackburne, B., Lindsay, S., Mamanova, L., Lee, C. *et al.* Mutation spectrum revealed by breakpoint sequencing of human germline CNVs. *Nat. Genet.* **42,** 385–391 (2010).
9 Arlt, M. F., Mulle, J. G., Schaibley, V. M., Ragland, R. L., Durkin, S. G., Warren, S. T. *et al.* Replication stress induces genome-wide copy number changes in human cells that resemble polymorphic and pathogenic variants. *Am. J. Hum. Genet.* **84,** 339–350 (2009).
10 Smith, C. E., Llorente, B. & Symington, L. S. Template switching during break-induced replication. *Nature* **447,** 102–105 (2007).
11 Liu, P., Erez, A., Nagamani, S. C., Dhar, S. U., Kołodziejska, K. E., Dharmadhikari, A. V. *et al.* Chromosome catastrophes involve replication mechanisms generating complex genomic rearrangements. *Cell* **146,** 889–903 (2011).