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► **To cite this version:**

F. Pellestor, Jb Gaillard, A. Schneider, J. Puechberty, V. Gatinois, et al.. Chromoanagenesis, the mechanisms of a genomic chaos. *Seminars in Cell and Developmental Biology*, 2022, 123, pp.90-99. 10.1016/j.semcdb.2021.01.004 . hal-04503066

**HAL Id: hal-04503066**

**<https://hal.science/hal-04503066v1>**

Submitted on 22 Jul 2024

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1 **Chromoanagenesis, the mechanisms of a genomic chaos.**

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1 **Abstract**

2 Designated under the name of chromoanagenesis, the phenomena of chromothripsis,  
3 chromanasythesis and chromoplexy constitute new types of complex rearrangements,  
4 including many genomic alterations localized on a few chromosomal regions, and whose  
5 discovery over the last decade has changed our perception about the formation of  
6 chromosomal abnormalities and their aetiology. Although exhibiting specific features,  
7 these new catastrophic mechanisms generally occur within a single cell cycle and their  
8 emergence is closely linked to genomic instability. Various non-exclusive exogenous or  
9 cellular mechanisms capable of generating chromoanagenesis have been evoked.  
10 However, recent experimental data shed light on 2 major processes, which following a  
11 defect in the mitotic segregation of chromosomes, can generate a cascade of cellular  
12 events leading to chromoanagenesis. These mechanisms are the formation of  
13 micronuclei integrating isolated chromosomal material, and the occurrence of  
14 chromatin bridges around chromosomal material resulting from telomeric fusions. In  
15 both cases, the cellular and molecular mechanisms of fragmentation, repair and  
16 transmission of damaged chromosomal material are consistent with the features of  
17 chromoanagenesis-related complex chromosomal rearrangements. In this review, we  
18 introduce each type of chromoanagenesis, and describe the experimental models that  
19 have allowed to validate the existence of chromoanagenesis events and to better  
20 understand their cellular mechanisms of formation and transmission, as well as their  
21 impact on the stability and the plasticity of the genome.

22

23 **Key-words**

24 Chromoanagenesis, chromothripsis, chromanasythesis, chromoplexy, instability,  
25 micronucleus, chromatin bridges, chromosome missegregation

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## 1 **Introduction**

2 While the identification and classification of complex chromosomal rearrangements, as  
3 well as their mechanisms of formation and transmission seemed to be well analysed and  
4 understood (Pellestor et al., 2011; Madan, 2012), the emergence of innovative genome  
5 sequencing technologies and the advances in computational biology have led to the  
6 identification of new types of chromosome rearrangements that are much more complex  
7 and massive than what had been imagined until then.

8 These unanticipated complex chromosomal phenomena are termed chromothripsis,  
9 chromoanasythesis and chromoplexy. They have been grouped under the name of  
10 chromoanagenesis (Holland and Cleveland, 2012).

11 Accumulation of sequencing data and information drawn from experimental models  
12 have gradually made it possible to better understand the underlying mechanisms of  
13 these phenomena and their effective impact both in cancers and congenital disorders  
14 (Kloosterman and Cuppen, 2013). Thus, in cancer, the paradigm that genome alteration  
15 occurs gradually through the progressive accumulation of mutational events has been  
16 challenged by the observations of chromoanagenesis events in a broad spectrum of  
17 tumour (Kloosterman et al., 2014). In congenital disorders, the notion of inheritance and  
18 viability of massive chromosomal rearrangements had to be reconsidered following the  
19 identification of chromoanagenesis-related rearrangements in numerous patients with  
20 developmental disorders, but also in phenotypically normal subjects (de Pagter et al.,  
21 2015). In addition, the re-analysis of many chromosomal structural abnormalities  
22 considered as “simple” balanced rearrangements, revealed an unsuspected complexity  
23 in connection with chromoanagenesis (Weckselblatt et al., 2015).

24 The discovery of these new form of massive chromosomal rearrangements in human  
25 pathologies has generated a wave of interest for genomic stability and the role of  
26 genome maintenance pathways, especially because similar catastrophic phenomena  
27 have been described in other mammalian species (Meyer et al., 2016), or rodents  
28 (Romanenko et al., 2017), but also in plants (Carbonell-Bejerano et al., 2017; Henry et al.,  
29 2018), in plankton (Blanc-Mathieu et al., 2017), in nematode *Caenorhabditis elegans*  
30 (Itani et al., 2016) and *Saccharomyces cerevisiae* (Anand et al., 2014), indicating that the  
31 cellular pathways responsible for generating such massive patterns of chromosomal  
32 rearrangements are highly conserved.

33

## 34 **Definitions and hallmarks of chromoanagenesis phenomena**

35 Although the biological consequences of these 3 chaotic chromosome phenomena are  
36 close, with the formation of derivative chromosomes highly remodelled, their molecular  
37 mechanisms differ. Specific features have been described, allowing each catastrophic  
38 process to be distinguished from each other and from other types of complex genomic  
39 alterations.

## 40 **Chromothripsis**

41 First described in cancers (Stephens et al., 2011) and then in congenital disorders  
42 (Kloosterman et al., 2011), chromothripsis is defined as a relatively clustered  
43 chromosomal shattering followed by a random restitching of chromosomal fragments,

1 resulting in the formation of complex genomic rearrangements. Since its discovery,  
2 chromothripsis-like-events have been observed in a wide range of cancers (Cai et al.,  
3 2014) and in patients harbouring congenital and developmental disorders (Collins et al.,  
4 2017) or apparently balanced simple rearrangements (Weckselblatt et al., 2015), as well  
5 as in asymptomatic subject (de Pagter et al., 2015). Familial studies showed that  
6 derivative chromothriptic chromosomes can be stably inherited (Bertelsen et al., 2016),  
7 and some reports documented the possible reversibility of chromothripsis (Bassaganyas  
8 et al., 2013) and its potential curative effect (McDermott et al., 2015).

9 Remarkably, the genomic chaotic alterations that characterize chromothripsis appear to  
10 arise in a single cellular event, and all reported chromothripsis-mediated  
11 rearrangements share similar patterns. Consequently, a set of common hallmarks has  
12 been described consistent with the proposition of a single catastrophic event scenario.  
13 These key-features are: the generation of numerous genomic rearrangements clustered  
14 in one or a few chromosomal loci, the restitching of fragments without order and  
15 preferential orientation, the lack of any sequence homology at their breakpoints or only  
16 microhomology of a few nucleotides, the low DNA copy number change and the  
17 preservation of heterozygosity in the rearranged chromosomal segments. Altogether,  
18 these criteria allowed to establish a molecular signature of chromothripsis (Korbel and  
19 Campbell, 2013).

20 In cancer cells, chromothripsis events often involve tens to hundreds of inter- and intra-  
21 chromosomal rearrangements. The phenomenon was observed in 2 to 3% of all human  
22 cancers, with a particularly high incidence in bone cancers and glioblastoma (up to  
23 39%) (Malhotra et al., 2013; Cai et al., 2014). Recent estimates report frequencies in the  
24 order of 45% for all cancers, indicating how the prevalence of chromothripsis in cancers  
25 may have been underestimated (Cortés-Ciriano et al., 2020). In all cases, chromothripsis  
26 is associated with aggressive forms of cancers and poor patient survival (Lee et al.,  
27 2017). A feature of chromothripsis in tumours is the generation of circular, extra  
28 double-minute chromosome markers that often involve amplified oncogenes (Fontana  
29 et al., 2018). Constitutional chromothripsis usually result in less complicated alterations  
30 than in cancers. Accumulative data indicated that chromothripsis occur approximately  
31 in 5% of case with karyotypically identified chromosome rearrangements, and that most  
32 reported *de novo* chromothripsis are of paternal origin, suggesting that chromothripsis  
33 events take place predominantly in male meiosis (Pellestor, 2014; Fukami et al., 2017).

34 Analyses of breakpoint junction sequences indicate that the shattered chromosomal  
35 fragments are reassembled primarily by classical non-homologous end joining (c-NHEJ),  
36 or alternative form of end joining (alt-EJ), both error-prone repair system operating in  
37 all phases of the cell cycle (Willis et al., 2015). Some other processes of DNA repair may  
38 also operate in chromothripsis. Masset et al. (2016) (Masset et al., 2016) described 3  
39 cases of chromothripsis-like-rearrangements with multiple focalised duplications and  
40 insertions mediated by a DNA polymerase Pol $\eta$ -dependent pathway of alternative NHEJ  
41 which may create aberrant end-to-end fusion of multiple chromosomes.

42 High-resolution investigations have revealed additional complexity in chromothripsis  
43 such as the complexification of an initial benign chromosomal alteration in  
44 chromothripsis through unequal crossing-over during meiosis (Pettersson et al., 2018),  
45 or the extension of remodelling events to non-chomothriptic chromosomes wherein  
46 submicroscopic insertions of shattered fragments occur (Kurtas et al., 2019). Also; it is

1 possible that constitutional chromothripsis and multiple *de novo* CNVs (Copy Number  
2 Variations) could occur in the genome as the result of the same multifocal crisis events  
3 (Brás et al., 2020), raising the possibility of “CNV mutator” phenotypes (Liu et al., 2017;  
4 Hattori et al., 2019).

## 5 **Chromoplexy**

6 Chromoplexy constitutes another form of “all-at-one” mechanism of massive genome  
7 reshuffling, initially described in human prostatic cancer (Baca et al., 2013) and  
8 subsequently in bone and soft tissues tumours (Wang et al., 2013).

9 This phenomenon is characterized by the interdependent occurrence of multiple inter-  
10 and intra-translocations and deletions resulting from DSBs with precise junctions.  
11 Chromoplexy events may involve up to 8 chromosomes and this “close-chain” process  
12 lead to the generation of derivative chromosomes that present little or no copy number  
13 alterations. Chromoplexy breakpoints appear to cluster with active DNA replicator or  
14 transcription region and open chromatin configurations. The involvement of the  
15 Tmprss2-ERG gene fusion (EST+) at chromoplexy breakpoints suggests that  
16 chromoplexy events could occur from the same transcriptional mechanism driven by the  
17 androgen receptor (AR) that induce Tmprss2-ERG fusion. The AR-mediated  
18 transcription has been implicated in the occurrence of DSBs through direct interaction  
19 with topoisomerase. By triggering the formation of clustered DSBs, the AR transcription  
20 could promote the creation of chained rearrangements within a restricted nuclear  
21 domain. Chromoplexy could contribute to the aggressive evolution of high-grade  
22 prostate cancers. The description of similar chained rearrangements in melanomas, lung  
23 cancers and neck cancers suggests that chromoplexy can occur in a large spectrum of  
24 cancers (Shen, 2013).

25 Both chromothripsis and chromoplexy processes may occur concurrently or  
26 asynchronously within a same cell, generating different patterns of chromosome  
27 complexity (Zepeda-Mendoza and Morton, 2019).

## 28 **Chromoanasyntesis**

29 The third type of one-time-chaotic cellular event leading to the constitution of massive  
30 chromosomal rearrangement is termed chromoanasyntesis

31 First described by Liu et al. (Liu et al., 2011), chromoanasyntesis is defined as a  
32 replication-based complex rearrangement process. The key-features of  
33 chromoanasyntesis are the localized presence of copy-number gains (duplications and  
34 triplications) in combination with deletions and copy-neutral chromosomal segments.  
35 Such multiple copy number changes cannot be explained by a process of chromosome  
36 shattering and NHEJ-mediated restitching of chromosomal fragments. The breakpoint  
37 junctions of these rearranged segments show micro-homology and template insertions,  
38 consistent with defective DNA replications and suggesting the involvement of error-  
39 prone DNA replication pathways such as fork stalling and template switching (FoSTes)  
40 and micro-homology-mediated break-induced replication (MMBIR) (Lee et al., 2007;  
41 Hastings et al., 2009). In these processes, when a replication fork encounter an obstacle  
42 or a DNA lesion, the lagging strand can serially switch to another intra-chromosomal or  
43 inter-chromosomal area with micro-homology to establish a new active replication fork  
44 and restart the DNA synthesis. The new template strands are not necessarily adjacent to

1 the initial replication fork but in spatial proximity. Multiple fork disengaging and strand  
2 invasions can occur before the resumption of replication on the original template  
3 (Piazza et al., 2017). FoSTes and MMBIR pathways generate hybrid chromosomes with  
4 complex rearrangements involving duplications and triplications with short stretches of  
5 micro-homology. Recent studies have also reported complex intra- and inter-  
6 chromosomal insertions as part of chromoanasythesis events (Gu et al., 2016; Kato et  
7 al., 2017).

8 Numerous exogenous factors and a variety of cellular events can create conditions of  
9 replication stress and chromoanasythesis occurrence by interfering with the  
10 progression of the replication fork (Aguilera and Gómez-González, 2008; Venkatesan,  
11 2015). In particular, interference between transcription and DNA replication represent  
12 an important source of genomic instability because RNA and DNA polymerases operate  
13 on the same template (Lang et al., 2017). A co-directional orientation of the 2 processes  
14 can lead to the displacement of RNA polymerases and the eviction of the pre-formed  
15 transcriptional R-loops (Hamperl et al., 2017). In contrast, when transcription is  
16 convergent to replication, the head-on collision of the 2 systems may increase the R-loop  
17 formation and promote fork collapse and chromoanasythesis events (Lin and Pasero,  
18 2012; García-Muse and Aguilera, 2016).

19 To date, the observation of chromoanasythesis-linked rearrangements has been mostly  
20 limited to constitutional frame (Gudipati et al., 2019a; Zepeda-Mendoza and Morton,  
21 2019). Similar to chromothripsis, developmental delay, autism spectrum disorders,  
22 intellectual disability and dysmorphic features are the mains clinical features described  
23 in patients with chromoanasythesis (Fukami and Kurahashi, 2018; Grochowski et al.,  
24 2018), but phenotypically unaffected carriers have also been reported (Suzuki et al.,  
25 2016; Collins et al., 2017; Sabatini et al., 2018).

26 Chromoanasythesis seems to operate both during gametogenesis and during the  
27 preimplantation period (Plaisancié et al., 2014; Pellestor and Gatinois, 2018). In contrast  
28 to chromothripsis, no preferential paternal origin of chromoanasythesis events has  
29 been reported.

30 Chromoanasythesis process appears to be non-exclusive. It can act in conjunction with  
31 other chromoanagenesis phenomena and various cellular mechanisms leading to  
32 generation of complex structural variants (SVs) patterns (Hattori et al., 2019; Piazza and  
33 Heyer, 2019; Brás et al., 2020).

34

### 35 **Mechanisms for chromanagenesis occurrence**

36 After the discovery and the characterization of these 3 unanticipated catastrophic  
37 phenomena, the mechanisms driving chromoanagenesis emergence have been actively  
38 investigated. To date, several non-exclusive causative mechanisms have been proposed  
39 that could trigger chaotic rearrangements of one or a few chromosomes within a unique  
40 cellular event, and thus explain the diversity and the complexity of chromoanagenesis  
41 phenomena.

42 Various exogenous factors can cause massive chromosomal rearrangements that may  
43 suggest chromoanagenesis events, such as free radicals, environmental toxins or  
44 chemotherapeutic drugs. For instance, ionizing radiations have been shown to induce

1 chromothripsis-like chromosomal rearrangements (Morishita et al., 2016). Another  
2 possible cause of chromoanagenesis could be certain virus infections (Schütze et al.,  
3 2016). Even cannabis exposure has been associated with chromothripsis occurrence  
4 (Reece and Hulse, 2016).

5 However, most of the research on causative factors has focused on endogenous  
6 mechanisms. Among the speculative factors inferring chromoanagenesis-linked  
7 rearrangements, the abortive apoptosis has been regarded as a potential initiating event.  
8 In a population of cells undergoing apoptosis, it is possible that a small portion of these  
9 cells engages in a restricted and incomplete form of apoptosis and thus survive (Tubio  
10 and Estivill, 2011). The subsequent DNA repair might be performed through a fast and  
11 incorrect process leading to the emergence of chromoanagenesis-mediated genomic  
12 alterations. An alternative scenario is that mitotic errors and replication stress could  
13 synergize to promote genome instability and chromoanagenesis occurrence (Jones and  
14 Jallepalli, 2012). In model cell lines, Mardin et al. (Mardin et al., 2015) showed that  
15 chromothripsis arise significantly more often in hyperploid cells than in diploid cells.  
16 Passerini et al. (Passerini et al., 2016) demonstrated that the presence of extra-  
17 chromosome trigger chromosomal instability and replication-related DNA damages and  
18 rearrangements. However, most of these exogenous and endogenous processes affect  
19 the whole genome and not just chromosome regions confined to one or a few  
20 chromosomes, indicating that other cellular mechanisms should participate in the  
21 occurrence of chromoanagenesis.

22 Two alternative cellular mechanisms that can explain the restricted localization of  
23 breakpoints and complex alterations produced by chromoanagenesis phenomena are  
24 the formation of micronuclei incorporating chromosomal materials and the generation  
25 of chromatid bridges during cell division.

## 26 **The micronucleus-mediated model**

27 Micronuclei are frequently observed in tumour cells and they are considered for a long  
28 time as passive indicators of chromosomal instability (Luzhna et al., 2013). Accumulated  
29 data showed that micronucleus is an important source of DNA alterations and genomic  
30 instability, and constitute a key-platform for chromoanagenesis emergence (Russo and  
31 Degrassi, 2018). Recently, it was reported that even the CRISPR-Cas9 genome editing  
32 could induce the formation of micronuclei and then the occurrence of chromoanagenesis  
33 phenomena (Leibowitz et al., 2020).

34 As starting point, there are defects in chromosomal segregation during cell division or  
35 presence of acentric chromosomal fragments (Fig. 1). The underlying pathways that  
36 drive micronuclei formation are multiples and heterogeneous. Various defects in DNA  
37 replication and repair machinery, as well as clastogen agents producing DSBs may lead  
38 to anaphase lag of chromosomal material at anaphase (Ly and Cleveland, 2017). On the  
39 other hand, numerous factors affecting chromosome segregation and cell cycle  
40 checkpoints can result in the spatial isolation of chromosomes or chromatids and the  
41 encapsulation of the lagging material into a micronucleus. These events include spindle  
42 disruption, centromere dysfunction, merotelic kinetochore-microtubule attachment,  
43 centrosome dysfunction, spindle assembly checkpoint (SAC) dysfunction, and chromatid  
44 cohesion defects (for recent review, see Guo et al. (Guo et al., 2019)).



1 Quickly after the identification of chromothripsis and chromoanasythesis phenomena,  
2 experimental studies have demonstrated that micronucleus formation offered an  
3 appealing mechanistic explanation for chromoanagenesis occurrence. By generating  
4 micronuclei in the human cell line HT 1080, Crasta et al. (Crasta et al., 2012) observed  
5 that lagging chromosome trapped in micronuclei underwent defective DNA replications,  
6 asynchronous with the primary nuclei, which led to extensive chromosomal damages  
7 and fragmentations into the micronuclei. Also, these experiments showed that  
8 micronuclei could persist in cells over several generations without degradation and that  
9 micronucleated chromosomal material could be reincorporated into daughter cell nuclei  
10 at a significant frequency (Crasta et al., 2012; Huang et al., 2012).

11 Micronuclei are structurally similar to regular nuclei, with a double-membrane and  
12 nuclear pores (Liu and Pellman, 2020), but ultra-structural studies evidenced important  
13 defects in the micronuclei envelope. The micronucleus envelope has a low density of  
14 nuclear pore complexes and defective nuclear lamina B (Terradas et al., 2010; Hatch et  
15 al., 2013). The lack of lamin B impairs the structural integrity of the micronuclear lamina  
16 and the proper assembly of micronuclear envelope. Liu et al. (Liu et al., 2018) reported  
17 that only the “core” nuclear envelope proteins, and not the “non-core” nuclear proteins,  
18 such as nuclear pore complex proteins, assemble around lagging chromosomal materials.  
19 The authors demonstrated that the high density of microtubules in the area of the main  
20 spindle block the recruitment of non-core proteins on lagging chromosomal material.  
21 This defective constitution leads to a significant defect in the micronuclear recruitment  
22 and retention of essential components for DNA replication, DNA damage response, and  
23 the maintenance of micronucleus envelope integrity.

24 So, the damages to chromosomal material sequestered in the micronucleus could  
25 therefore be primarily mediated by defective DNA replication. This could ultimately lead  
26 to chromoanasythesis-like alterations. On the other hand, it was reported that a  
27 significant proportion of newly formed micronuclei underwent an irreversible  
28 disruption during interphase following their generation, and that almost 50% of  
29 disrupted micronuclear material rejoined the primary nucleus chromatin (Hatch et al.,  
30 2013). The disruption of micronuclei is caused by the micronuclear envelope collapse,  
31 triggered by lamin disorganization (Vargas et al., 2012). This loss of  
32 compartmentalization causes drastic reduction of transcription, replication and  
33 proteasome functions inside the micronucleus (Hatch et al., 2013; Maass et al., 2018;  
34 Soto et al., 2018). The third possibility is that the micronucleus and its content can be  
35 degraded or extruded from the cell (Russo and Degrassi, 2018; Reimann et al., 2020).

36 In order to investigate the timing of DNA damages in micronuclei, Zhang et al. (Zhang et  
37 al., 2015) performed *in vitro* experiments combining live cell imaging and single cell  
38 sequencing technique (referred to as Look-Seq). They showed that damages of  
39 sequestered material do not occur during the G1 phase following the micronucleus  
40 formation, but accumulate as cells progress through the next S and G2 phases, while the  
41 micronuclei have initiated DNA replication. In daughter cells, they subsequently  
42 evidenced the under-replication of DNA and the high degree of chromosomal  
43 rearrangements and fragmentations in micronuclei. Most of these rearrangements  
44 recapitulated the key-features of chromothripsis, but also of chromoanagenesis, with  
45 typical short template-insertions and microhomology. This experimental model  
46 provided the first direct evidence for a cellular mechanism that could explain the

1 occurrence of chromoanagenesis, according to an “all-at-once” catastrophic process. It  
2 also clearly identified mitotic defects as an origin of chromoanagenesis.

3 It was established that the disruption of micronuclear envelope initiated during the  
4 interphase induces the premature condensation of the encapsulated DNA and exposes it  
5 to cytoplasmic endo- and exo-nucleases, such as the exo-nuclease TREX1 which is  
6 activated upon the recognition of intermediate DNA structures, incompletely replicated,  
7 and collapsed replication forks (Piazza et al., 2017). This process induces double  
8 stranded-DNA breaks and the subsequent shattering of micronuclear chromosome  
9 material in acentric fragments, which will be randomly reassembled after their  
10 reintegration into newly formed daughter cell nuclei.

11 Alternatively, the premature condensation of micronuclear DNA induced by mitotic  
12 entry can cause replicative stress, delay active replication fork progression, and then  
13 initiate MMBIR or FoSTes pathways. Subsequently, this leads to the generation of a  
14 derivative chromosome, with localized chromoanagenesis-like rearrangements, and its  
15 potential reintegration into a daughter cell nucleus during the following mitosis  
16 (Terzoudi et al., 2015; Russo and Degrossi, 2018). The collapse or stall of replication  
17 forks initiating the chromoanagenesis process could result from the premature  
18 condensation of the delayed replication regions into micronuclei (Chatron et al., 2020).

19 Once the micronucleus-derived chromosomal material has been reincorporated into a  
20 primary nucleus and reassembled, one can speculate that the newly created derivative  
21 chromosome will be managed by appropriate replication machinery and thus become  
22 stabilized over subsequent cell generations.

23 In 2017, Ly et al. (Ly et al., 2017) described an alternative experimental model, based on  
24 the inducible centromere inactivation of the Y chromosome and the specific  
25 sequestration into micronuclei of missegregated Y chromosome, in order to analyse the  
26 fate of micronucleated chromosomes through several consecutive cell cycles. It was  
27 observed that micronucleated Y chromosome accumulated numerous DNA lesions and  
28 fragmented prior to or during the mitosis of the second cell cycle. Premature  
29 chromosome condensation appeared to be the main cause of chromosome  
30 fragmentation and this PCC-induced fragmentation was dependent on passage through  
31 S-phase. This data confirmed that premature chromosome condensation process  
32 constitutes a leading cause of shattering in micronuclei and an important mechanism  
33 underlying chromoanagenesis initiation (Pantelias et al., 2019). During the next  
34 interphase, the DNA fragments are rapidly repaired through c-NHEJ process and the  
35 resulting re-ligated Y chromosome displays typical hallmark of chromothripsis. The c-  
36 NHEJ pathway appears not to be inefficient in micronuclei. Therefore, c-NHEJ-dependent  
37 re-assembly certainly occurs in the main nucleus, following fragment reintegration. This  
38 elegant model provided new and important insight into the mechanisms driving  
39 chromothripsis events and endorses the idea of a multi-cell cycle process for  
40 chromoanagenesis occurrence. In 2019, Ly et al. (Ly et al., 2019) improved their  
41 experimental system by combining the induced-Y chromosome micronucleation with  
42 the CRISPR-Cas9 technology in order to identify cell carrying a reactivated Y  
43 chromosome centromere. This strategy led to the highlighting of the high frequency and  
44 the diversity of chromosomal abnormalities induced by a single chromosome  
45 missegregation. Ly et al. (Ly et al., 2019) reported a large spectrum of intra-

1 chromosomal abnormalities of the Y chromosome, but also complex patterns of inter-  
2 chromosomal rearrangements, recapitulating the signature of chromothripsis.

3 Accumulating evidence supports that *de novo* alterations frequently occur in  
4 micronuclei. The incidence of DSB in micronuclei is estimated up to 30 times higher than  
5 in primary nuclei (Umbreit et al., 2020). To date, single cell sequencing approaches  
6 established that chromoanasythesis may occur in more than 60% of micronuclei that  
7 are persisted for one cell cycle (Leibowitz et al., 2020), reinforcing the idea that  
8 micronucleus is the most credible hypothesis of chromoanagenesis origin.

9 Understanding the mechanisms of chromoanagenesis phenomena changes traditional  
10 thinking about micronuclei as a passive vector of damaged genetic material to become  
11 an active player in the genesis of DNA alterations and rearrangements. For Guo et al.  
12 (Guo et al., 2020), micronucleus can be compared to an isolated mutation factory in  
13 which single mutation patterns can be exacerbated into chromoanagenesis events.

#### 14 **The chromatin bridge model**

15 Micronucleation is not the only process driving chromoanagenesis phenomena. Because  
16 chromothripsis-linked rearrangements are often localized within the telomeric regions,  
17 an alternative mechanism for chromoanagenesis occurrence has been proposed (Fig. 1),  
18 based on the telomere crisis.

19 Telomere crisis encompasses various alterations or dysfunctions of telomeres, such as  
20 replication-mediated shortening, defective telomere-associated proteins usually  
21 generating chromosomal instability (Mathieu et al., 2004; Dewhurst, 2020). Due to such  
22 events, an end-to-end fusion between 2 uncapped or broken sister chromatids or 2 non-  
23 homologous chromosomes may occur and give rise to the formation of a dicentric  
24 chromosome (Cleal and Baird, 2020). During mitosis, when the 2 centromeres of a  
25 dicentric chromosome are pulled to opposite poles, an anaphase bridge is created. At the  
26 end of mitosis, such chromatid bridge may break and cause the formation of fragments  
27 detached from the spindle microtubule and eventually sequestered into a micronucleus,  
28 after the new nuclear envelope formation. After replication, these broken chromosome  
29 fragments can fuse again together, giving rise to new dicentric chromosome and thus  
30 undergo repeated cycle of breakage-fusion-bridge (BFB) over several cycles, leading to  
31 regional amplification or reorganization of small chromosome segment potentially with  
32 chromoanagenesis-like rearrangements (Maciejowski and de Lange, 2017). Also, in case  
33 of deficiencies in nuclear lamin proteins, some fragments may be involved in the  
34 formation of nuclear blebs, kinds of protusion of the nuclear envelope (Broedersz and  
35 Brangwynne, 2013; Chen et al., 2018).

36 However, the chromatin bridge may also remains unbroken after cytokinesis, leading to  
37 the formation of a nucleocytoplasmic bridge connecting the 2 newly formed daughter  
38 cells. As in the case of micronucleus, this chromatin bridge structure loss nuclear  
39 envelope integrity during interphase (Terradas et al., 2016).

40 After inducing telomere fusion and creation of anaphase chromatin, Maciejowski et al.  
41 (Maciejowski et al., 2015) observed a significant depletion of lamin components and  
42 nuclear pore complexes (NPCs) as the bridge extended. Localized ruptures and loss of  
43 compartmentalization in the bridge allow access of cytoplasmic exonuclease TREX1 to  
44 the bridging chromatin. The digestive action of these enzymes on the stretched

1 chromatin will result in the generation of single-stranded DNA (ssDNA). This also  
2 initiates the resolution of the bridge and the fragmentation of the chromatin it contains,  
3 before the primary nucleus entered S phase. In contrast with rearrangement resulting  
4 from micronuclei, the chromatin alteration generated chromatin-bridge breakages do  
5 not require ongoing DNA synthesis. The damages are localized in the short area of the  
6 TREX1-mediated DNA fragmentation and bridge resolution. These sites are often  
7 associated with clusters of point mutation, termed kataegis, exhibiting cytosine  
8 substitution in TpC dinucleotides due to the activity of the apolipoprotein B mRNA-  
9 editing catalytic subunit (APOBEC) family of enzymes (Petljak and Maciejowski, 2020).  
10 These data support the hypothesis of a close mechanistic relation between hypermutations  
11 and chromothripsis events.

12 The multiple chromatin fragments can be involved in the formation of micronuclei, in  
13 one or both daughter cell at the end of mitosis, with the possibility of subsequent  
14 chromoanagenesis occurrence. The damaged bridging chromatin can also retract and  
15 join the daughter cell nuclei, then undergo illegitimate DNA repair and result in a  
16 reassembled chromosome potentially with chromothripsis-like rearrangements  
17 (Terradas et al., 2016).

18 By sequencing the post-telomere crisis cells, Maciejowski et al. (Maciejowski et al.,  
19 2015) established that 50% of them exhibit cluster of genome alterations with  
20 hallmarks of chromothripsis. Most of these chromothripsis derived from 2  
21 chromosomes and included terminal deletions, which is consistent with the hypothesis  
22 of an end-to-end fusion-mediated chromothripsis process. In order to analyse these  
23 different hypothesis and to follow the fate of chromosome bridges and their DNA  
24 content, Umbreit et al. (Umbreit et al., 2020) developed an experimental system of  
25 chromosome bridge induction and combined live cell imaging with single-cell whole  
26 genome sequencing (Look-Seq) technique. They observed severe disturbances in the  
27 DNA replication process, leading to template-switching errors (Cleal et al., 2019) and the  
28 generation, in a few daughter cells, of distinct chains of template short insertions,  
29 termed Tandem Short Template (TST) jumps and originated from various distant  
30 breakpoint hotspots. This finding is to be compared to the observation of short sequence  
31 insertions throughout rearranged chromothriptic chromosomes (Collins et al., 2017;  
32 Slamova et al., 2018) and the description of short genomic area (rearrangement hubs) in  
33 different loops of chromothripsis-like rearrangements (Chatron et al., 2020). More  
34 complex rearrangements were then identified in fragmented chromosome from broken  
35 bridges during the next mitosis, due to aberrant mitotic DNA replication of this  
36 incompletely replicated chromatin. More than 50% of division resulted in micronuclei  
37 formation in the second generation cells, promoting the occurrence of additional  
38 chromosome bridge formation or chromoanagenesis events in the following cell cycles.  
39 These data provide clear evidence that telomere dysfunction and BFB process can  
40 trigger chromoanagenesis events.

41 Strong analogies exist between micronucleus- and chromatin bridge-mediated  
42 processes with in both cases defective nuclear envelope assembly around  
43 chromosomal material and severe alterations in DNA replication. These studies  
44 evidenced that the 2 processes are non-mutually exclusive but can coexist in a same  
45 context of cellular crisis. Altogether, these data provide a novel insight into the cellular  
46 mechanisms of chromoanagenesis formation.

## 1 **Factor promoting the emergence of chromoanagenesis**

2 The phenomena of chromoanagenesis are perceptible reflections of the complex and  
3 multiple cellular processes of managing genomic instability. So, it is important to ask  
4 what are the cellular and genomic factors that promote genome instability and  
5 contribute to triggering the development of chromoanagenesis.

6 Defective mitosis progression is increasingly recognized as an essential cause of  
7 chromoanagenesis in mammals (Hattori and Fukami, 2020) and plants (Comai and  
8 Tan, 2019). Previous studies have highlighted the link between errors in chromosomal  
9 segregation and the formation of structural chromosomal abnormalities.

10 The correct assembly of the mitotic or meiotic spindle is crucial for the efficient  
11 segregation of chromosomes during cell divisions. The spindle is a dynamic self-  
12 organized bipolar array of microtubules whose assembly mobilizes several hundred  
13 proteins and several control systems such as the spindle assembly checkpoint (SAC) or  
14 the chromosome passenger complex (CPC) (Prosser and Pelletier, 2017). All alterations  
15 and disturbances in the pathways that contribute to spindle assembly and the timely  
16 completion of mitosis or in the gene controlling spindle formation and cell division may  
17 potentially give rise to instability and potentially to chromoanagenesis occurrence. Thus,  
18 abnormal centromere duplication, maturation or separation (Pihan, 2013) as well as the  
19 presence of supernumerary centrosomes trigger chromothripsis by promoting  
20 kinetochore mis-attachment and anaphasic chromosome lagging (Ganem et al., 2009;  
21 Nam et al., 2015). The sister chromatin cohesion is also crucial for the bi-orientation of  
22 chromosomes on the mitotic and the meiotic spindle, and for the repair of damaged DNA  
23 (Skibbens, 2019). Defects in chromosome cohesion and its control compromise the  
24 fidelity of chromosome segregation and trigger premature loss of cohesion, anaphase  
25 lagging and aneuploidy (Barbero, 2011). The existence of a spindle matrix  
26 corresponding to a network of nuclear lamina components involved in the spindle  
27 assembly has been suggested (Schweizer et al., 2014). The lamin B, whose role has been  
28 evoked both in the micronucleus formation and the chromatin bridge process, could be  
29 included in this spindle matrix since the depletion of lamin B was found to impede  
30 spindle assembly (Shi et al., 2014).

31 To date, numerous studies have shown that chromosome segregation defects and  
32 aneuploidy may induce replication damages and lead to the accumulation of  
33 chromosomal rearrangements in aneuploid cells (Thompson and Compton, 2008).  
34 Basically, replication stress and perturbations of DNA repair process are mainly  
35 associated with chromosomal instability (Wilhelm et al., 2020). The replication stress  
36 may alter the cell cycle process by slowing or blocking the progression of the  
37 “missegregating” cells through S-phase or G2/M phase, thus leading to slowed DNA  
38 replication and potentially to the formation of chromatin bridges or micronucleation.  
39 Depending on the cell-cycle position, DNA repair mechanisms will differ. Chromosomal  
40 instability is more likely to occur if the DNA repair efficiency is suboptimal (Streffer,  
41 2010). The NHEJ pathway is operational throughout the cell cycle whereas replicative  
42 repair mechanisms such as FoSTes and MMBIR can only work during S-phase and  
43 eventually in G2-phase (Kass et al., 2016). Another determining factors can also be the  
44 number of DSBs to repair. A large number of breaks can quickly saturate the capacity of  
45 cellular DNA repair pathways. Gudjonsson et al. (Gudjonsson et al., 2012) evidenced that  
46 more than 20 DSBs could alter standard error-free DNA repair mechanism such as

1 homologous recombination (HR) pathway, in favour of faster but error-prone repair  
2 processes such as NHEJ and alt-EJ. The aneuploidy-linked genome instability could also  
3 be explained by a reduced expression of replication factors such as replicative helicase  
4 MCM and ORC (Passerini et al., 2016).

5 Given the essential role of the genome organization in driving and modulating genome  
6 functions, various specific genomic features must be taken into account for a clear  
7 understanding of the sudden onset of chromoanagenesis phenomena. Repetitive  
8 sequences are known to facilitate genomic rearrangements. Sequence analyses of  
9 chromoanagenesis-mediated rearrangements have evidenced the frequent presence of  
10 low-copy repeats (LCR) sequences or tandem repetitive sequences such as minisatellite  
11 or ALU sequences, in the vicinity of breakpoint junctions (Kloosterman et al., 2011;  
12 Weckselblatt et al., 2015; Nazaryan-Petersen et al., 2018). These repeat and repetitive  
13 sequences create area of genomic instability and potentially facilitate template  
14 switching and the subsequent occurrence of intra- and inter-chromosomal  
15 rearrangements. Fragile sites are other DNA sequences frequently associated with  
16 rearrangements and genetic diseases. They contribute to genome instability and  
17 replication impairment (Barlow et al., 2013) and consequently a plausible link between  
18 chromoanagenesis events and fragile sites have been considered, since they can be the  
19 sites of multiple chromoanagenesis-mediated breakage events (Mackinnon and  
20 Campbell, 2013). Fragile sites, Alu sequences, microsatellites and LCR as well as other  
21 particular motifs like palindromic sequences can also promote instability by inducing  
22 the formation of unusual chromatin secondary structures such as hairpins, cruciforms  
23 or DNA triplexes, which are able to disrupt replication and cause DSBs (Cooper et al.,  
24 2011; Liu et al., 2011). Also, several recent studies have documented the contribution of  
25 transposable elements to the development of chromoanagenesis (Hancks, 2018).  
26 Nazaryan-Petersen et al. (Nazaryan-Petersen et al., 2016) reported a retrotransposition-  
27 mediated chromothripsis displaying the insertion of an SVA (SINE\_VNTE-Alu) element  
28 at one pair of breakpoints and several other DNA breaks produced by L1-endonuclease  
29 activity. The L1-endonuclease activity could be an important contributory factor to  
30 chromosome shattering. In the gibbon genome, the insertion of the retrotransposon  
31 LAVA is at the origin of a high rate of chromothripsis-mediated rearrangements leading  
32 to the accelerated evolution of the gibbon karyotype and the emergence of different  
33 gibbon lineages (Meyer et al., 2016). Approximately 45% of the human genome derives  
34 from transposable elements (Pace and Feschotte, 2007). Only a small proportions of  
35 transposable elements, in particular the retrotransposons LINE and SINE, retain the  
36 capacity to change their position within the genome by transposition (Kawakami et al.,  
37 2017). They can act as mutagen and consequently they are frequently associated with  
38 genetic disorders (Burns, 2017). They also can induce DSBs leading to genome  
39 instability (Gasior et al., 2006). The abundance of retrotransposons in the human  
40 genome provides numerous potential substrates for mitotic or meiotic recombination  
41 structural variations and rearrangements. Consequently, transposable elements are  
42 potential threats to genome stability because they can initiate ectopic recombination  
43 between non-homologous regions or non-homologous chromosomes, and thus lead to  
44 structural variations and large chromosomal rearrangements (Song et al., 2018).

45 Beyond these characteristics linked to the DNA sequence, these data suggest that the  
46 conformation of the chromatin and the dynamic architecture of the nucleus are key-  
47 factors in the emergence of chromoanagenesis. The modulatory role of chromatin is  
48 evident throughout the cell cycle for essential functions such as transcription,

1 replication and repair (Misteli, 2020). It has been proposed that the chromatin looping  
2 can regulate DSBs repair through histone modifications and nucleosome remodelling  
3 within approximately 50kb on each side of DSBs, in order to induce the formation of  
4 open and relaxed chromatin conformation and thus to facilitate loading of DNA repair  
5 proteins (Price and D'Andrea, 2013). In accordance with this chromatin regulation,  
6 disturbances in epigenetic mechanisms could alter the dynamics of the organization of  
7 chromatin and the replication initiation, for example in gametes or during early  
8 embryonic development, and thus promote the occurrence of more or less complex  
9 rearrangements (Åsenius et al., 2020). The ultimate level of nuclear organization and  
10 regulation is the spatial partitioning of the genome into chromatin domains and  
11 chromosome territories, referred to as Topologically Associating Domains (TADs),  
12 Lamina Associated Domains (LADs), or *cis*-Regulatory Domains (CRDs) (Luperchio et al.,  
13 2014; Lupiáñez et al., 2016; Delaneau et al., 2019). It has been established that the  
14 occurrence of complex chromosomal rearrangements disrupts the conserved  
15 organization of these territories, transforming their architecture and their interaction,  
16 but also the regulation of the genes (Ye et al., 2019). In addition to the 3D genome  
17 architecture, it is important to take into account the mobility of chromatin within the  
18 nucleus. An increase in chromatin mobility has been observed in response to DSBs  
19 (Dion and Gasser, 2013). In the vicinity of DSBs, the damaged site is found to be more  
20 mobile. The nuclear response triggered by a DSB is not limited to the damaged site and it  
21 is the dynamics of the whole genome that increases in proportion to the number of DSBs  
22 (Miné-Hattab et al., 2017). This chromatin mobility promotes the recombination of  
23 homologous chromosomal regions. The Rad5 protein, which plays an essential role in  
24 the recombination process, is directly involved in chromatin movements (Dion et al.,  
25 2012). It can therefore be speculated that increased chromatin mobility facilitates the  
26 search for homologous template sequences in the replication-based DNA repair  
27 processes such as FoSTes and MMBIR.

28 All these data reinforce the notion that chromoanagenesis events occur in cellular and  
29 genomic context which compromise genomic stability. Genome instability is a broad  
30 concept of which chromosome instability is the most prevalent form (Geigl et al., 2008).  
31 Growing literature indicates that this instability is linked to a large spectrum of genomic  
32 and non-genomic factors and mechanisms (Venkatesan, 2015).

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1 **Conclusion**

2 To date, the mechanisms of chromoanagenesis are among the most fascinating cellular  
3 events evidenced over the past decade. It has been a controversial notion that these  
4 massive and complex rearrangement phenomena usually occur through a single cell  
5 cycle. The experimental data obtained have allowed to prove the existence of  
6 chromoanagenesis and to shed light on their mechanisms of formation. In particular, the  
7 description of micronucleation and chromatid bridges formation highlighted the close  
8 links that may exist between apparently simple errors in chromosome segregation and  
9 the cellular stress and instability processes inducing chromoanagenesis. In the light of  
10 these data, it appears that the human genome can tolerate important modifications of its  
11 conformation, and that chromonagenesis pathways are mechanisms of rapid and  
12 profound restructuring of the genome. Their discovery has renewed interest in  
13 questions of genome plasticity, the role of nuclear topology and the capacity of cells to  
14 manage or not such crises and cellular chaos. Even if DNA remains the support of  
15 heredity, a new framework more focused on the concept of genome must be taken into  
16 consideration to better understand the cellular, clinical and evolutionary impact of the  
17 chromoanagenesis phenomena.

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38

1 **Legend of figure**

2 **Figure 1: Cellular mechanisms for chromoanagenesis occurrence:**

3 **The micronucleus-mediated model.** Following the anaphasic loss of a chromosome  
4 (or of chromosomal fragments), a micronucleus sequestering this chromosomal material  
5 is formed at the exit of mitosis. In this micronuclei involving a defective envelope, the  
6 chromosomal material will undergo premature condensation, and then rupture of the  
7 envelope allowing cytoplasmic exonucleases such as TREX1 to fragment DNA. The repair  
8 and inefficient replication of this DNA is accompanied by fragmentation of chromosomal  
9 material. The micronucleus can persist over several cell cycles, but frequently the  
10 micronucleus is reincorporated into the primary nucleus of a daughter cell where the  
11 repair, replication, and reassembly of chromosomal fragments results in the creation of  
12 a highly rearranged chromosome.

13 **The chromatin bridge-mediated model.** The formation of dicentric chromosomes due  
14 to the end-to-end telomere fusion leads to the formation of chromatin bridges between  
15 daughter cells, at the exit of mitosis. In this chromatin bridge, surrounded by a defective  
16 envelope, the chromatin segment is stretched. The rupture of the envelope allows the  
17 resection of the DNA and its fragmentation by cytoplasmic exonucleases such as TREX1.  
18 The chromatin fragments are reintegrated into the primary nucleus of daughter cells  
19 where they can be repaired, replicated and reassembled to generate a  
20 chromoanagenesis (+/- kataegis) event. New end-to-end telomere fusions can occur,  
21 giving rise to Breakage-Fusion-Bridge (BFB) cycles. The rearranged chromosomal  
22 material can also be missegregated during a new mitosis and lead to the formation of a  
23 micronucleus, and then to chromoanagenesis.

24

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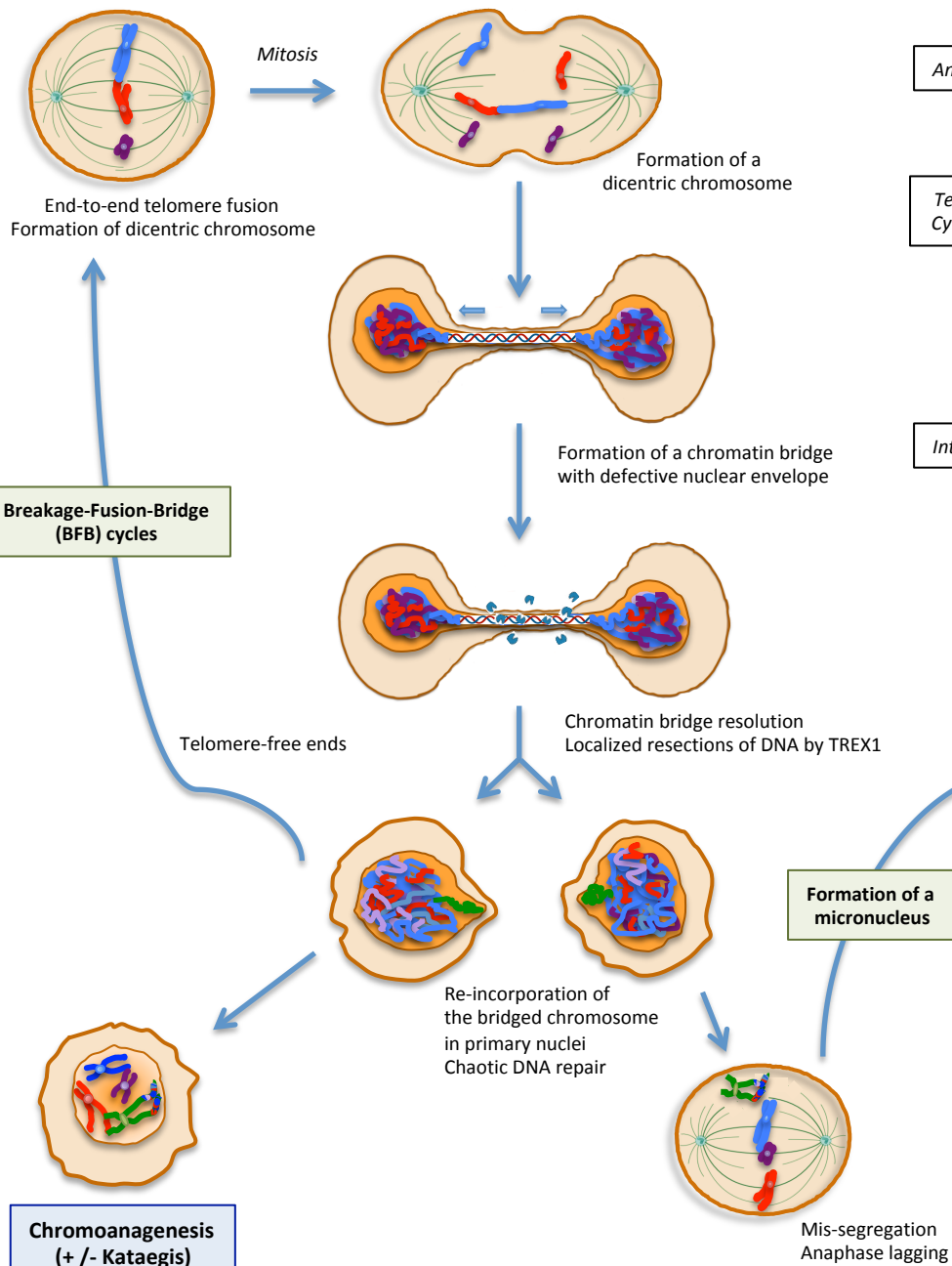
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29 **Acknowledgements**

30 Work in the unit of Chromosomal Genetics is supported by the CHU research platform  
31 CHROMOSTEM

**The chromatin bridge-mediated model**



**The micronucleus-mediated model**

