Chromothripsis: how does such a catastrophic event impact human reproduction?

Franck Pellestor

1Laboratory of Chromosomal Genetics, Arnaud de Villeneuve Hospital, Montpellier CHRU, Montpellier, France
2Inserm Unit ‘Plasticity of the Genome and Aging’, Institute of Functional Genomics, Montpellier, France

*Correspondence address. E-mail: f-pellestor@chu-montpellier.fr

Submitted on November 1, 2013; resubmitted on December 27, 2013; accepted on January 6, 2014

ABSTRACT: The recent discovery of a new kind of massive chromosomal rearrangement, baptized chromothripsis (chromo for chromosomes, thripsis for shattering into pieces), greatly modifies our understanding of molecular mechanisms implicated in the repair of DNA damage and the genesis of complex chromosomal rearrangements. Initially described in cancers, and then in constitutional rearrangements, chromothripsis is characterized by the shattering of one (or a few) chromosome(s) segments followed by a chaotic reassembly of the chromosomal fragments, occurring during one unique cellular event. The diversity and the high complexity of chromothripsis events raise questions about their origin, their ties to chromosome instability and their impact in pathology. Several causative mechanisms, involving abortive apoptosis, telomere erosion, mitotic errors, micronuclei formation and p53 inactivation, have been proposed. The remarkable point is that all these mechanisms have been identified in the field of human reproduction as causal factors for reproductive failures and chromosomal abnormalities. Consequently, it seems important to consider this unexpected catastrophic phenomenon in the context of fertilization and early embryonic development in order to discuss its potential impact on human reproduction.

Key words: chromothripsis / chromosomal rearrangement / genomic instability / fertilization / embryo

Introduction

Chromosomal abnormalities account for the majority of pre- and post-implantation embryo wastages and make a major contribution to pregnancy loss, birth defects and genetic disorders found in humans (Gardner et al., 2012).

Most of the abnormalities are de novo, resulting from meiotic errors during gametogenesis in subjects with normal karyotypes or occurring post-fertilization during early cleavage divisions. Consequently, investigating human gametes and embryos has become an important field contributing to the understanding of the formation, the transmission and the etiology of chromosomal abnormalities. The introduction of techniques for in situ chromosomal analysis and rapid genomic investigation has led to the advent of efficient procedures for cytogenetic examination of human gametes and the development of preimplantation genetic diagnosis (PGD), which offer an alternative to prenatal diagnosis and pregnancy termination to carriers of chromosomal abnormalities (for review see Martin, 2005; Delhanty and Pellestor, 2011).

Among the chromosomal abnormalities, structural aberrations are of particular interest since they involve a multitude of rearrangements which can induce a great variety of syndromes. The rearrangements involving at least three chromosomal breakpoints on two or more chromosomes are named complex chromosomal rearrangements (CCRs) (Pellestor et al., 2011a). They are usually considered to be the most complicated form of chromosomal rearrangements. Because of a high risk for abnormal reproduction, CCRs constitute a difficult challenge for genetic and reproductive counseling. To date, only a few laboratories have been successful in performing segregation analysis of these abnormalities and developing PGD procedures for couples with CCRs (Escuder et al., 2008; Lim et al., 2008; Loup et al., 2010; Pellestor et al., 2011b; Scriven et al., 2013).

The recent discovery of a new type of massive chromosomal rearrangement has radically revisited the view of how de novo structural rearrangements are generated.

Definition and key features of chromothripsis

The term ‘chromothripsis’ (from chromo for chromosome and thripsis for breaking into small pieces) has been chosen to designate this newly recognized phenomenon, first observed in tumors (Stephens et al., 2011) and then rapidly described in congenital disorders (Kloosterman et al., 2011). The hallmark of chromothripsis is the occurrence of tens
to hundreds of genomic rearrangements confined to one or a few chromosomes, through a unique cataclysmic cellular event, leading to chaotically reassembled chromosomes (Fig. 1). Only the novel technique of mate-pair sequencing presently allows the identification of chromothripsis and the complete assessment of the complexity of the generated genomic rearrangements. A recent re-analysis by paired-end sequencing of 45 reciprocal translocations and 7 inversions previously defined as balanced has revealed extensive and yet balanced rearrangements due to chromothripsis in 20% of them (Chiang et al., 2012). These findings validated the occurrence of chromothripsis in the human germline or in early embryonic development, and also confirmed its compatibility with viability and transmission.

Several features common to all chromothripsis rearrangements distinguish this phenomenon from other complex structural aberrations. (i) Chromothripsis always occurs in a unique catastrophic genomic event. (ii) This cataclysmic event leads to the generation of tens to hundreds of rearrangements, locally clustered on one single chromosome or a few chromosomes rather than scattered throughout the whole genome. (iii) Multiple copy numbers and structural aberrations resulting from double-strand breaks (DSB) are found in derivative chromosomes, including translocations, inversions, insertions, deletions, duplications and triplications. (iv) Most of the reassembled DNA fragments are randomly joined and they do not display any sequence homology in their breakpoints or only micro-homology of a few nucleotides. (v) In the reassembled segments, copy-number profiles show oscillating states between loss and retention of heterozygosity, which would be unlikely in a scenario of gradually acquired rearrangements. (vi) Finally, congenital chromothripsis rearrangements are characterized by their low copy-number changes and their relatively balanced status. This does not signify that unbalanced chromothripsis does not occur in germline or during post-zygotic divisions, but it strongly emphasizes the effect of selection as a bias factor in the assessment of congenital chromothripsis, since only balanced chromothripsis outcomes compatible with life have been found in individuals to date.

Altogether, these key features define the molecular signature of chromothripsis (Maher and Wilson, 2012; Korbel and Campbell, 2013). The definition of these features characterizing chromothripsis is an important step towards our understanding of how and where chromothripsis arises, and if it arises alone or in association with other cellular mechanisms (Sorzano et al., 2013; Wyatt and Collins, 2013).

### Potential causes of chromothripsis in human reproduction

Analyses of breakpoint junction sequences have revealed that the re-assembly of DNA fragments is driven either by template-independent double-strand break (DSB) repair mechanisms such as non-homologous end-joining (NHEJ), or by replicative processes such as fork stalling template switching (FoSTeS) and micro-homology-mediated break-induced repair (MMBIR). NHEJ mechanism may explain the lack of sequence homology or micro-homology at breakpoint junctions as well as insertions or deletions of nucleotides, whereas replicative processes offer an explanation for the generation of duplications and triplications in chromothripsis events (Liu et al., 2011a; Kloosterman et al., 2012).

Although the molecular mechanisms for chromosomal segment stitching are well understood, the causes of chromothripsis and its mechanistic basis are still under discussion. What exogenous or endogenous processes can induce such a highly localized chromosomal pulverization and how can cells manage such a cataclysmic crisis on a short time scale? A striking noteworthy point is that all the mechanisms which are now being suggested to trigger chromothripsis were previously identified as...
Chromothripsis in germlines

Existing data show that constitutional chromothripsis occurs in the male germline (Kloosterman et al., 2011; Chiang et al., 2012). This finding is consistent with the preferential paternal origin of the vast majority of de novo chromosomal structural aberrations found at term (Pellestor et al., 2011a). This confirms the great vulnerability of spermatogenesis to DNA damage and its limited or less efficient DNA repair capacity when compared with somatic tissue cells.

In the course of spermatogenesis and according to the availability of DSB repair systems, chromothripsis initiation can occur either during the pre-meiotic division of diploid spermatogonia through the first meiotic division involving recombination events, or during the differentiation of round spermatids into spermatozoa (i.e. spermiogenesis). During each of the three steps, environmental stimuli such as ionizing radiations or free radicals may act as triggers for chromothripsis. DNA replication stress can also serve as an endogenous stimulus. Various factors such as inhibition of DNA polymerase function or defective topoisomerase activity may induce a DNA replication stress through replication fork collapse and template-switching events, leading to genomic instability and induction of chromothripsis (Liu et al., 2012).

The frequent localization of chromothripsis rearrangements on a unique chromosomal region suggests that pulverization might occur when chromosomes are condensed, i.e. during mitosis. Since spermatogonia can undergo hundreds of mitotic divisions before entering into meiotic prophase, these cells seem to be particularly prone to chromothripsis. However, examples involving several chromosomes indicate that the phenomenon may also occur during interphase when chromosomes are relaxed throughout the nucleus. This notion implies either a spatial proximity of chromosomes during the chromothripsis, with reference to the well-known spatial organization of chromosomes in nuclear territories (Bickmore and van Steensel, 2013) and/or the movement of chromatin within the nucleus which recent studies have correlated with DSB repair process (Dion and Gasser, 2013).

In this context, various triggers have been suggested for chromothripsis initiation. Tubio and Estivill (2011) proposed that chromothripsis might be caused by abortive apoptosis. During spermatogenesis, apoptosis is a major mechanism controlled by Sertoli cells for the elimination of defective germ cells. Among the cells undergoing apoptosis, a few could undergo a restricted form of apoptosis or escape the process, and subsequently survive after performing a rapid and chaotic DNA repair to maintain their genome integrity. This could induce multiple and complex chromosome rearrangements. Another view is that chromothripsis is caused by the combination of mitotic errors due to various mechanisms (centrosome overduplication, chromatin cohesion deficiency . . .) and replication stress (Jones and Jallepalli, 2012). This can occur through alterations of DNA replication checkpoints or deficient replication mechanisms, generating genomic instability. Since many examples of chromothripsis rearrangements affect chromosome ends, telomere attrition could also induce chromothripsis through end-to-end chromosome fusion and generation of multiple chromosome breaks and reassociations during the resolution of anaphase bridges (Stephens et al., 2011).

An attractive explanation to link all these causal processes with the confined nature of the damage created during chromothripsis is that the damaged chromosome(s) is (are) incorporated into a micronucleus which can persist in cells over several generations (Crasta et al., 2012). In micronuclei, chromosomal material can undergo defective and asynchronous DNA replication as well as aberrant chromatin compaction. Thus, chromosomal pulverization and reassembly are restricted to the chromosome trapped in the micronuclei. The rearranged chromosome can be re-integrated into the nucleus of the daughter cell and be stably maintained through subsequent cell divisions.

During meiosis, the chromatin of germ cells sustains programmed structural changes (meiotic recombination) which require the formation and the repair of DSB. Deficiencies in the recombination machinery linked to exogenous agents or intrinsic causes such as gene mutations might result in ectopic synopsis and erroneous resolution of physiological DSB by non-homologous pathways (Hassold and Hunt, 2001; Liu et al., 2011b). Variations in some repeat sequence arrays, such as PRDM9, can strongly affect meiotic recombination activity and stimulate intra-chromosomal homologous recombinations (Berg et al., 2010). Heavy loads of DSB probably saturate the error-free repair machinery and stimulate DSB resolution by error-prone systems such as multiple NHEJ, leading then to the random chromosome reassembly characteristic of chromothripsis (Gudjonsson et al., 2012). Also, the possibility of chromothripsis occurrence in female meiosis should not be neglected since female meiosis is the major source of aneuploidy (Pellestor et al., 2005; Fragouli et al., 2011) and displays permissive checkpoints (Steurwald, 2005). Chromosome mal-segregation or premature separation of sister chromatids occurring during the two female meiotic divisions can induce genome instability, micronuclei formation and subsequent chromothripsis events. The particular cytokinesis operating in female meiosis between the large-sized oocyte and the small polar bodies could facilitate the initiation of chaotic rearrangements.

Finally, during spermiogenesis, round spermatids have a slow and moderate repair capacity. At this stage, DSB cannot be repaired by homologous recombination since spermatids lack sister chromatids for homologous repair. Consequently, repair is processed by homology-independent and error-prone mechanisms such as NHEJ or less well-defined repair pathways (Ahmed et al., 2010). This can also be associated with the abortive apoptosis process. In late spermiogenesis, maturing cells also undergo a histone-to-protamine transition. This extensive chromatin remodeling requires the creation and ligation of DSB. Alterations in this process may lead to DNA fragmentation and unrepaired DNA breaks that make spermatozoa more susceptible to post-testicular assaults, especially during transport through epididymis where the generation of reactive oxygen species and exogenous factors might induce additional sperm DNA breaks (Oliva, 2006).

Chromothripsis in zygotes and preimplantation embryos

During the fertilization process, the paternal nucleus decondenses and undergoes extensive chromatin remodeling and DNA replication. This new modification of chromatin architecture certainly has an impact on DNA repair mechanisms since modulation of the chromatin structure itself constitutes a precondition for the recruitment and action of DNA repair proteins. DNA breaks generated during spermiogenesis and sperm transport need to be repaired before the first round of
DNA replication in the zygote, since extensive DNA damage is virtually incompatible with embryonic development. This phase of DNA repair is entirely dependent on the repair capacities of the oocyte and the zygote. The ability of human oocytes to repair DNA damage in the fertilizing sperm nucleus is limited to 8–10% of the haploid genome (Sakkas and Alvarez, 2010), and this is probably highly variable among oocytes according to the genetic background, the maturity status and the women’s age. Thus, when confronted with extensive damage, the repair machinery could be inefficient and the zygote could die. However, one can speculate that in good quality oocytes and zygotes, the cell would be able to rapidly repair chromatic damage localized on one chromosomal segment or on a few closed chromosome domains in order to maintain a relatively complete and balanced genome. This kind of repair could be performed through fast and error-prone repair pathways leading to the chaotic reassembling of chromatic segments. This repair scenario could be facilitated by relaxed cell-cycle checkpoints in oocytes and in early cleavage embryos (Harrison et al., 2000).

Another phenomenon that may initiate chromothripsis during fertilization is the premature chromosome condensation (PCC) of sperm nuclei due to the asynchrony between the female nucleus blocked in the metaphase II stage and the male nucleus in S-phase and the abnormal activity of persistent mitotic promoting factors. Thus, incompletely replicated male chromosomes can be partially pulverized. In the vast majority of cases, zygotes with such extensive damage will die through an apoptotic process. However, the induction of PCC might subsequently corrupt the G2-checkpoint. Thus, the zygote with partially under-replicated and fragmented DNA might prematurely enter into mitosis (Stevens et al., 2010) and generate the formation of micronuclei in which fragmented chromosomes can undergo random repair and independent replication before being reincorporated into the nucleus of a daughter embryonic cell.

All the alterations evoked can also occur during early cleavage divisions. Accumulating data show that human preimplantation embryos have a high rate of chromosomal abnormalities, including frequent chromosomal mosaicsisms and chaotic chromosomal patterns (Mantzouratou and Delhanty, 2011; Mertzaniou et al., 2013). It has also been shown that good quality human preimplantation embryos display a remarkably high incidence of structural abnormalities involving complex segmental rearrangements (Vanneste et al., 2009) and frequent chromosomal instability (Voet et al., 2011, Fragouli et al., 2013). Thus, a high incidence of chromosome defects in rapidly dividing blastomeres with relaxed cell-cycle and mitotic checkpoints supports the assumption that chromothripsis events may also arise during early human embryogenesis. It is probable that the combination of chaotic chromosomal rearrangements and gene dosage alterations preferentially lead to non-viable embryos which are lost around the time of implantation. Only chromothripsis resulting in balanced and stable chromosomal rearrangements would be viable. According to the embryonic stage where the catastrophic event occurs, chromothripsis will be confined to extra-embryonic tissues or be present as full chromothripsis or mosaic chromothripsis in the embryonic lineage and have pathogenic consequences (Lebedev, 2011).

When in vitro conceived embryos are morphologically assessed, blastomere fragmentation, presence of micronuclei or asynchronous cleavage is often noted (Chavez et al., 2012). These observations may constitute arguments supporting the assumption of chromothripsis occurrence at early cleavage stages. They also raise questions about the implication of in vitro manipulations in the initiation of chromothripsis. Several studies have speculated that the in vitro culture conditions and handling of gametes and embryos could alter the behavior of cells and possibly affect the efficiency of checkpoints and the ability to repair DNA damage (Ledbetter, 2008). This stresses the need to better understand how zygotes and embryos in culture experience changes in the capacity to manage DNA repair.

### Link between chromothripsis and p53 family proteins in human reproduction

Finally, the occurrence of chromothripsis has been strongly associated with dysregulation of the p53 tumor suppressor gene (Rausch et al., 2012). Known as the guardian of the genome, p53 plays a major role in maintaining genome stability by mediating cell-cycle arrest, apoptosis and cell senescence in response to DNA damage (Vogelstein et al., 2000). Alterations to the p53 pathways could support or initiate chromothripsis by disrupting these fundamental mechanisms of cell-cycle control and then facilitating cell survival after chromosome shattering and massive rearrangements (Kloosterman and Cuppen, 2013). The p53 family proteins, involving p53, p63 and p73, play important roles in reproduction. p53 is highly expressed in testes, in particular in early spermatocytes where it mediates DNA damage-induced apoptosis (Odorioso et al., 1998). In p53 null mice, a high incidence of multinucleated cells and limited apoptosis are observed. Recently, p53 has also emerged as a fertility regulator controlling embryo implantation (Corbo et al., 2012). In female meiosis, the p53 homologue p63 is essential for the process of eliminating damaged oocytes (Suh et al., 2006). Oocytes without p63 are resistant to DNA damage-induced apoptosis. The third transcription type p73 is also involved in the regulation of oocyte quality by interacting with the spindle assembly checkpoint complex. Oocytes that are deficient in p73 exhibit spindle abnormalities and an increasing rate of chromosomal defects (Hu et al., 2011). All these data seem to be consistent with the hypothesis of an association between the occurrence of constitutional chromothripsis and alterations of p53 family proteins. Further understanding of the functions of these proteins in reproduction may shed light on the molecular mechanisms governing the process of chromothripsis.

### Conclusion

Chromothripsis is an unexpected phenomenon whose discovery has deeply modified our perception of the genesis of genome rearrangements and their impact on human development and congenital disorders. Multiple lines of evidence support the concept that chromothripsis originates through a single catastrophic event. Although the causes of chromothripsis are still being debated, various procedures have been proposed. The present short review shows that all the causative mechanisms suggested may occur in germelines or during early embryonic development. Clearly, more work is needed to define the contribution of chromothripsis to altered human development and congenital disorders. However, it seems important to take into consideration this new process for the study of reproductive disorders, and particularly for the investigation of the causes of low success rates in natural and assisted reproduction.
Acknowledgements
The author thanks Dr G. Lefort and Dr J. Puechberty for critically reading the manuscript.

Authors’ roles
F.P. was responsible of the writing of the manuscript, reference search and figure execution.

Funding
This manuscript did not have any specific funding.

Conflict of interest
The author declares no conflict of interest.

References


