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Roscoff (France), 14-18 novembre 2016

Transcription-Replication Crosstalk and Genome Instability

Couplage transcription-réplication et instabilité du génome

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Rapport sur la Conférence

Conference Report

The Jacques Monod Conference "**Transcription-Replication Crosstalk and Genome Instability**" was held in Roscoff, France on November 14-18, 2016. This conference, organized by Philippe Pasero (president) and Andrés Aguilera (vice-president) brought together 102 participants from France (39%), Europe (41%) and from the rest of the world (20%). Besides the invited speakers, 37% of the participants were PIs, which illustrates the attractiveness of the conference.

Aim of the Conference. Cells are continuously exposed to events threatening the integrity of their genomes and adopt a wide range of strategies to protect it. During the past 25 years, tremendous progress has been made in elucidating the cellular response to exogenous genotoxic events. However, cells must also deal with endogenous molecular processes that undermine genome integrity. Recent advances strongly suggest that RNA metabolism is perhaps the most relevant of all the endogenous cellular processes that impact on the integrity of the genome and the epigenome, presumably by interfering with DNA replication. However, the mechanisms involved remain poorly understood. The main goal of this conference was to foster discussions and collaborations between scientists working in the fields of DNA replication, RNA metabolism and chromatin. These fields are in continuous expansion but have developed separately and there is a need to create synergies between them and stimulate the interest for common scientific views. This conference came at the right time, as new concepts placing replication forks at the center of key processes such as chromatin silencing and genomic instability are currently emerging.

Conference overview.

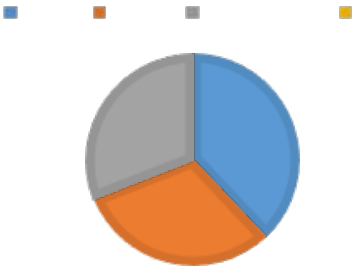
The conference started with the plenary lecture on Monday, November 14th after welcome drinks and a dinner at the Gulf Stream Hotel. Lectures were given in the auditorium of the Station Biologique CNRS. Part of the Thursday afternoon was devoted to an excursion to the Batz Island or free time. The conference ended on Friday, October 18th at 10am.

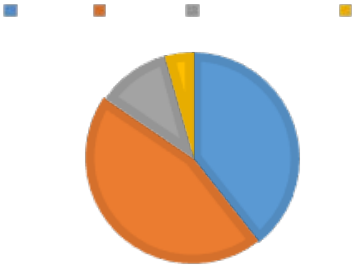
- Plenary lecture (60 min)
- Seven sessions with a dedicated chair chosen among the invited speakers:
  - Session 1 - Replication-transcription conflicts
  - Session 2 - R-loops and pervasive transcription
  - Session 3 - Replication stress
  - Session 4 - Transcription and genomic instability
  - Session 5 - DNA Repair and human diseases I
  - Session 6 - Maintenance of epigenetic information
  - Session 7 - DNA Repair and human diseases II
- 29 presentations by invited speakers (25 min + 5 min for questions)
- 13 short presentations selected from abstracts (15 min + 5 min for questions)
- Two poster sessions (2 hours each)

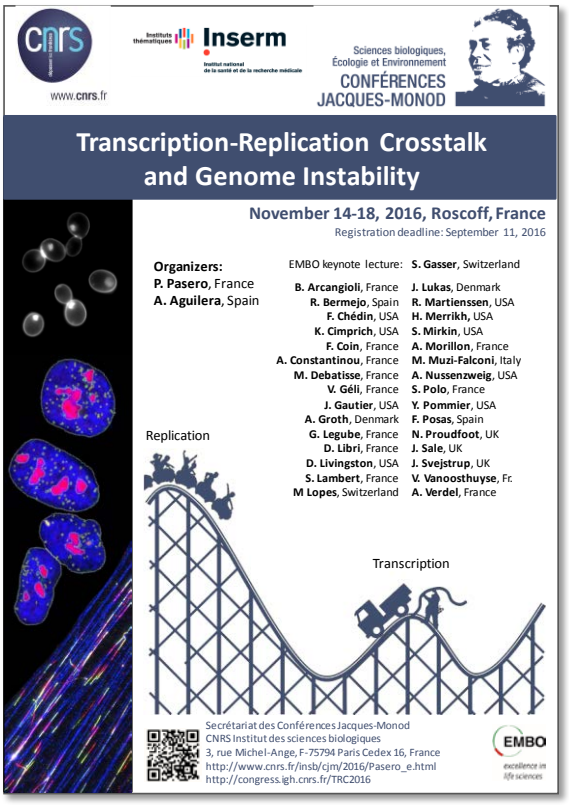
All aspects of management of the conference and the food were more than satisfactory, thanks to the expert care of Nathalie Babic and her colleagues. Overall, the conference was a big

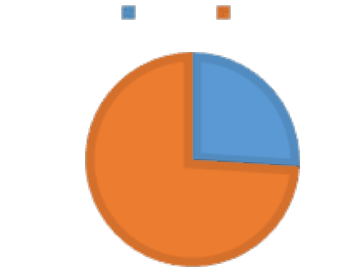
success. 90% of the participants who replied to the questionnaire evaluated the talks as excellent and all of them asked for its renewal.

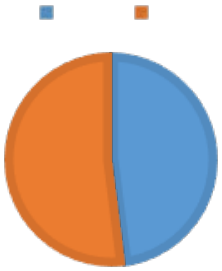
Conference statistics

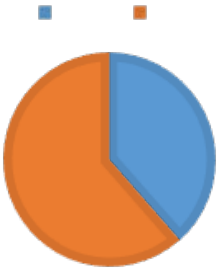


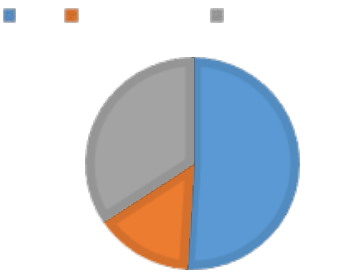


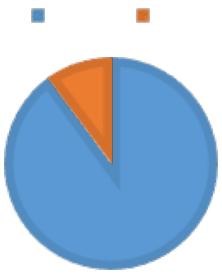


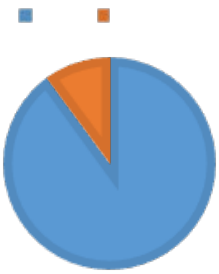












Scientific program

Scientific context

The accurate and complete duplication of the genome and maintenance of its primary sequence is crucial for normal cellular and organismal function. Changes in DNA sequence can ultimately lead to changes in the structure, function and/or expression of different proteins, altering numerous phenotypes. Indeed, genome instability contributes to many diseases, including cancer, neurodegenerative disease and developmental disorders. Significant efforts over the past twenty years have focused on understanding how genome instability can arise. DNA damage is a significant source of genome instability, and cells are constantly exposed to genotoxic agents from the environment as well as to endogenous metabolic byproducts that can damage the genome. It is generally accepted that the deregulation of cellular processes such as DNA replication can also lead to DNA damage. Failures in DNA replication represent therefore major sources of genomic instability. Importantly, high levels of spontaneous replication defects are detected in precancerous lesions as a consequence of oncogene-induced replication stress and play a central role in the cancer process. However, the origin and the consequences of this chronic replication stress remain poorly understood.

The human genome is replicated by thousands of replication forks that progress at high speed along the DNA and pause when they encounter obstacles such as DNA lesions, tightly-bound protein complexes, highly-expressed genes and co-transcriptional R-loops. Stalled forks are highly-recombinogenic structures that are repaired and restarted by a variety of error-free and error-prone pathways. These mechanisms have been extensively studied in the context of the acute exposure to exogenous DNA damaging agents, but their function in unchallenged growth conditions has remained largely unexplored. A growing body of evidence indicates that replication defects not only induce mutations and chromosome rearrangements, but can also impair the inheritance of chromatin marks and therefore modify the epigenetic information at specific loci. Finally, chromatin changes associated with replication fork pausing have been recently associated with the establishment of RNAi-mediated silencing in yeasts and the process of senescence in human cells.

Altogether, these data place the replication fork at the center of a complex interplay between DNA synthesis, transcription, RNA processing and epigenetics, with major consequences for genomic instability. The aim of this Jacques Monod conference is to bring together the best experts from these different fields in order to discuss these novel and important issues.

Plenary lecture (Chair: Philippe Pasero)

The conference started with a plenary lecture from **Susan GASSER** (FMI, Basel), who has made major contributions to the fields of DNA replication, DNA damage, nuclear organization and chromatin in budding yeast and more recently in *C. elegans*. During her lecture, Susan Gasser presented compelling evidence that histone H3K9 methylation ensures the stability of a repetitive elements in *C. elegans* by suppressing promiscuous transcription of repeats.

Mutations that eliminates the two H3K9 histone methyltransferases, SET-25 and MET-2, did not affect development but promoted the formation of R-loops at repeated elements and induced replication fork stalling and genomic instability at these loci. Histone H3K9 methylation plays therefore a key role in *C. elegans* by preventing conflicts between replication and transcription at repetitive elements.

Session 1 - Replication-transcription conflicts (Chair: Andrés Aguilera)

This session addressed the mechanisms by which transcription interferes with DNA replication in eukaryotes. DNA replication occurs on chromatin, where transcription also occurs. The existence of these processes on the same template requires spatial and temporal coordination to minimize potentially dangerous interactions between the processes, yet there are situations in which interactions between DNA replication and RNA processing are unavoidable. These include frontal collisions between polymerases and collisions between DNA polymerases and RNA-DNA hybrids formed during transcription, called co-transcriptional R-loops. **Karlene CIMPRICH** (Stanford University, CA) presented an original episomal system containing a unidirectional replication origin next to an inducible reporter gene in an HO (head on) or CD (codirectional) orientation. Importantly, the reporter gene contained or not sequences prone to form RNA-DNA hybrids, to monitor the contribution of R-loops without overexpressing RNase H. She found that HO conflicts increase plasmid instability in human cells and induced a checkpoint response, but only in the presence of R-loops. Remarkably, she also found that HO collisions increased R-loop formation, whereas the CD orientation favored the clearance of R-loops. Along the same line, **Houra MERRIKH** (University of Washington, WA) engineered a conflict system consisting of inducible reporter genes inserted in both orientations on the circular chromosome of B. subtilis. She showed that, as in human cells, genes in HO orientation are particularly disruptive to DNA replication because they form stable R-loops that block fork progression and prevent restart. This replication-dependent formation of R-loops at HO genes also blocked transcription. Moreover, replication resumption and cell viability was exquisitely dependent on RNase HIII, at the expense of increased mutagenesis in HO genes. **Jacqueline BARLOW** (UC Davis, CA) presented the characterization of a novel class of recurrent DNA lesions at early replicating sites, termed Early Replication Fragile Sites (ERFS), which occur within transcriptionally active chromatin regions. While ERFS associate with marks of active and elongating transcription, there appears to be no enrichment of paused or poised RNA Pol2 at these sites. These data suggest that active, elongating transcription is associated with ERFS fragility, while nonmoving poised or paused Pol2 does not present a major hindrance to replication progression. **Giordano LIBERI** (Pavia, Italy) addressed mechanisms of replication-transcription collisions in budding yeast and showed that Sen1, the yeast homolog of human Senataxin, interacts genetically with the MRX complex and prevents replication fork arrest at a specific gene organized in a HO orientation. In sen1 mutants, rescue of stalled forks depends on the fork protection complex (FPC) and on the activation of a dormant origin. **Francesc POSAS** (Barcelona, Spain) presented evidence that the stress-activated protein kinase Hog1 regulates replication-transcription conflicts induced by osmostress in budding yeast. Hog1 executes this

important function by phosphorylating the FPC component Mrc1, which also integrates the signal from other stress-activated kinases. **Michelle DEBATISSE** (IGR, Villejuif) addressed the respected roles of replication and transcription in the fragility of Common fragile sites (CFSs) are loci that recurrently display chromosome breaks upon replication stress. Since most CFS overlap with very long genes, she asked whether transcription interferes with DNA replication at these loci. Inhibition of transcription at active CFS or induction of transcription at inactive CFS affected fragility without altering the frequency of fork stalling, indicating that the interplay between replication and transcription at these sites is more complex than initially anticipated. **Jesper SVEJSTRUP** (Francis Crick Institute, London) reported that UV light induces a global reduction of transcription rate and affects the splicing of specific genes by favoring short isoform over long ones. In particular, short isoforms of the ASCC3 gene promote transcription restart whereas a long isoform helps maintain transcription repression. Finally, **Kevin HIOM** (University of Dundee, UK) reported that the mRNA processing factor SFPQ promotes normal DNA replication by minimizing the accumulation of replication-blocking R-loops and promoting repair through BRCA2-mediated homologous recombination.

Session 2 - R-loops and pervasive transcription (Chair: Nick Proudfoot)

This session specifically addressed the impact of R-loops and pervasive transcription on DNA replication and genome integrity. **Frédéric CHEDIN** (UC Davis, CA) presented the most recent technologies developed by his group to map R-loops in the human genome. He also presented results on the differential modulation of R-loop formation by DNA Topoisomerase I across long genes and at human replication origins. **David LIVINGSTON** (Harvard Medical School, MA) reported evidence that BRCA1 is involved in the repair of R-loop driven DNA damage, together with SETX. Remarkably, BRCA1 acts by recruiting the RNAi machinery (Ago1/2, Dicer) to chromatin. In the absence of BRCA1, SETX or Dicer, ssDNA and γ-H2AX accumulate at specific sites associated with genomic instability. **Vincent VANOOSTHUYSE** (ENS, Lyon) reported his attempts to monitor R-Loop formation at the single molecule resolution using Atomic Force Microscopy (AFM). Using a plasmid bearing a sequence prone to form RNA:DNA hybrids, he showed that compact structures are formed in a transcription-dependent manner. He also provided evidence that the S9.6 antibody used to detect RNA:DNA hybrids can also detect RNA:RNA hybrids. **Domenico LIBRI** (Institut J. Monod, Paris) addressed the question of how cells prevent pervasive transcription from interfering with other DNA-associated processes such as DNA replication. In particular, he presented evidence that roadblock termination mediated by Rap1 and other transcription factors protects replication origins from pervasive transcription in budding yeast. **Antonin MORILLON** (Institut Curie, Paris) presented an overview of the structure and the regulation of antisense long non-coding RNAs and their impact on the epigenetic landscape in yeast and human cells. Finally, **Benoit PALANCADE** (Institut J. Monod, Paris) revealed that introns protect eukaryotic genomes from transcription-associated genetic instability by counteracting the accumulation of genotoxic R-loops. Deletion of endogenous introns increases R-loop formation, while insertion of an intron into an intron-less gene

suppresses R-loop accumulation and its deleterious impact on transcription and recombination in budding yeast.

Session 3 - Replication stress (Chair: Michelle Debatisse)

This session covered general aspects of the replication stress response and replication fork restart, with a focus on oncogene-induced replication stress and its consequences on cancer development. **Jiri LUKAS** (Copenhagen, Denmark) reported his attempts to decipher the mechanisms by which replication stress is transmitted through mitosis to the next cell cycle. Using high-content imaging, his team identified several new genes involved in this process and leading to a wide variety of mitotic defects when inactivated. **André NUSSENZWEIG** (NIH, Bethesda) presented striking evidence that DNA double strand breaks occur at high frequency in gene-rich regions as a consequence of transcription, using a novel method called END-seq. **María GOMEZ** (Madrid, Spain) reported that HMGB1 competes with histone H1 for chromatin binding and that inactivation of either HMGB1 of 50% of the histone H1 genes differentially affected replication landscapes by altering fork progression and/or origin usage. **Domenico MAIORANO** (Montpellier, France) presented evidence that Ddx19 prevents R-loop mediated genomic instability by promoting mRNA export from the nucleus and resolving RNA:DNA hybrids. Moreover, Ddx19 translocates into the nucleus in a CHK1-dependent manner in response to replication stress. **Massimo LOPES** (University of Zürich, Switzerland) showed that fork reversal is not a pathological process induced by replication stress. In contrast, this remodeling of fork architecture appears to protect normal and cancer cells from chromosomal breakage and represents a promising target to potentiate anticancer treatments. He also presented AFM images of R-loop decorated with S9.6 antibodies. **Sarah LAMBERT** (Institut Curie, Orsay) reported that a single terminally arrested fork, with no apparent un-replicated parental DNA, can be converted into an anaphase bridge in fission yeast. Using a separation-of-function rad51 mutant, she showed that Rad51 binding is sufficient to safeguard terminally arrested forks and avoid mitotic sister chromatid non-disjunction. **Rodrigo BERMEJO** (CIB-CSIC, Madrid) presented evidence that a novel yeast protein called Bul2 acts at stalled forks to stimulate the ubiquitylation and the extraction of cohesin by Cdc48 and Wpl1. Cohesin mobilization likely contributes to poise cohesin for sister chromatids entrapment and support a protective stalled fork-replisome architecture. Finally, **Aparna GORTHI** (San Antonio, USA) addressed the mechanism by which fusions involving the RNA binding protein EWSR1 and the transcription factor FLI1 affects HR in Ewing Sarcoma. She showed that the EWSR1-FLI1 fusion and the loss of EWSR1 lead to an accumulation of R-loops, replication stress and impaired homologous recombination, recapitulating BRCA1 deficiency. She proposed a model in which R-loops titrate BRCA1 away from break sites.

Session 4 - Transcription and genomic instability (Chair: Karlene Cimprich)

This session focused on the impact of transcription on genome rearrangements. **Nick PROUDFOOT** (Oxford, United Kingdom) reported that R-loops can act as intrinsic promoters for RNA pol II and that that many antisense transcripts associated with transcribed protein coding genes derive from this R-loop promoter activity. He also showed that the RNA helicase Ddx1 is required to

unravel G4 RNAs encoded by a repetitive sequence region involved in antibody gene class switch recombination. This promotes the formation of R-loops over the switch regions and initiates class switching through AID dependent DNA deamination. **Angelos CONSTANTINOU** (Montpellier, France) revealed that Fanconi Anemia proteins FANCD2/I regulate the dynamics of splicing factors in the nucleus in an ATR-dependent manner. Suppression of FANCI/D2 interferes with the dynamics of splicing factors induced by HU, causes the accumulation of post-catalytic splicing lariats and delays the eviction of splicing factors from condensed chromosomes in mitosis. **Sergei MIRKIN** (Tufts University, MA) reported that R-loops are involved in the large-scale expansions of triplet DNA repeats in yeast in the absence of RNase H. Using a novel method for the genome-wide identification of trans-modifiers for repeat expansion, he also found that mutations in the endoribonuclease subunit of the mRNA cleavage and polyadenylation complex (YSH1) dramatically increase the rate of expansions of triplet DNA repeats, but only when they are actively transcribed. Finally, **Jean GAUTIER** (Columbia University, NY) performed a genome-wide screen for genes which loss is synthetic lethal with MYC overexpression. He found that proteins involved in transcription-coupled repair are essential to cope with MYC overexpression. This suggests that MYC drives genome instability not only through DNA replication stress, but also through transcription stress.

Session 5 - DNA Repair and human diseases I (Chair: David Livingston)

In this session, a variety of DNA repair mechanisms relevant to a human diseases were addressed. **Yves POMMIER** (NIH, Bethesda) presented evidence that Topoisomerase I is a DNA ribonuclease that readily processes rNMPs incorporated in the genome by DNA polymerases. TOP1 efficiently converts ribonucleotides into DNA nicks that can lead to DSBs when cleavage by TOP1 also occurs on the other strand. **Marco MUZI-FALCONI** (University of Milano, Italy) reported that the accumulation of rNMPs in the absence of RNase H interferes with DNA replication and that the duplication of rNMP- containing chromosomes require the intervention of specialized translesion DNA polymerases (TLS) and result in chronic activation of the post replication repair pathway and of the DNA damage checkpoint. **Natalia GROMAK** (University of Oxford, UK) developed an affinity purification approach, followed by mass-spectrometry to identify R-loop binding factors in HeLa cells. She showed that the R-loop interactome consists of known R-loop factors (senataxin, SRSF1 and topoisomerase I) and yet uncharacterized interactors. Among them, she found that DHX9 is required to remove R-loops after CPT exposure. **Sreerama Chaitanya SRIDHARA** (University of Lisbon, Portugal) showed that pausing of RNA pol II drives the phosphorylation of the DDX23 helicase by SRPK2 and the suppression of R-loops. In the absence of either SRPK2 or DDX23, accumulation of R-loops leads to massive genomic instability. **Gaelle LEGUBE** (LBCMP, Toulouse) used a cell line called DIvA (for DSB Inducible via AsiSI), developed by her group, to show that DSBs induced on the genome by AsiSI undergo clustering in an ATM-dependent manner. She also showed by capture Hi-C that clustered DSBs are those that are repaired by HR. Clustering occurs also in G1 cells to delay repair and depends on MRN, Formin and on the LINC complex. **Vincent GELI** (CRCM, Marseille) presented evidence that in budding yeast, the nuclear pore complex is involved in the regulation of telomere

recombination in the absence of telomerase. **Frédéric COIN** (IGBMC, Illkirch) used small molecules targeting TFIIH to reveal new functions of XPB in transcription and DNA repair. In particular, he showed that TFIIH is still recruited to promoters and that transcription is still active in the absence of XPB while nucleotide excision repair is severely affected. In contrast, contrast, inhibition of the ATPase activity of XPB affects both transcription and DNA repair. Finally, **Jonathan HOUSELEY** (Babraham Institute, Cambridge) showed that environmental change drives accelerated adaptation through stimulated copy number variation. In particular, he showed that CNVs at the yeast *CUP1* locus are promoted by transcription. These data demonstrate the existence of a site-specific mechanism that accelerates the acquisition of useful genetic changes beyond that achievable through random mutation.

Session 6 - Maintenance of epigenetic information (Chair: Frederic Chedin)

In this session, different aspects of the interplay between replication, transcription and repair in the maintenance of epigenetic information. **Constance ALABERT** (BRIC, Copenhagen) reported the use of Nascent Chromatin Capture (NCC), an explorative proteomic approach to understand epigenome maintenance during chromatin replication. She also presented evidence of a histone reader based mechanism that recognizes the post-replicative chromatin state in order to promote HR when a sister chromatid is present. **Sophie POLO** (Epigenetics & Cell Fate Centre, Paris) described histone deposition pathways involved in restoring chromatin structure and transcriptional activity in response to genotoxic stress. She also reported the use of an innovative system allowing simultaneous visualization of new and parental histone dynamics at sites of DNA damage in live cells. **Julian SALE** (MRC Cambridge, UK) reported the use of the Bu-Ia locus in DT40 cells to monitor the loss of epigenetic information induced by fork arrest at G4 structures and GAA repeats. In particular, he discussed the role of PrimPol in repriming of DNA synthesis downstream of the block. **Rob MARTIENSSEN** (Cold Spring Harbor, NY) revealed that RNAi promotes heterochromatic silencing at peri-centromeric regions in fission yeast through replication-coupled release of RNA polymerase II. At these regions, collision of RNA polymerase with the replication machinery are resolved by co-transcriptional RNAi, which releases PolII, allows replication to complete and couples the spreading of heterochromatin with fork progression. **André VERDEL** (Institute for Advanced Biosciences, La Tronche) presented additional evidence for an RNAi-dependent control of genome integrity in fission yeast, involving physical interactions between the RNAi and DNA replication/repair machineries. Finally, **Mikel ZARATIEGUI** (Piscataway, USA) provided new insights into the mechanisms by which transposable elements promote mitotic recombination in *S. pombe*. He showed that the long terminal repeat (LTR) of the Tf2 transposon contains a polar replication fork barrier that causes fork collapse and increased recombination. Stable fork arrest depends on Sap1 and on proteins of the CENP-B family of DNA binding factors, which silence the promoter present at the LTR.

Session 7 - DNA Repair and human diseases II (Chair: Philippe Pasero)

Finally, additional DNA replication/repair mechanisms relevant for human diseases were discussed in this last session. **Benoit ARCANGIOLI** (Institut Pasteur, Paris) addressed the

mechanism by which the lysine specific demethylases Lsd1 and Lsd2 control replication fork blocks at the rDNA and at the mating-type locus in fission yeast. These results uncover novel links between epigenetic marks and fork arrest in the regulation of stress-responsive copy number variations. **Jutta ZIMMER** (Oxford Institute for Radiation Oncology, UK) reported the characterization of a novel MRE11 inhibitor and its potential therapeutic application for the treatment of BRCA2-deficient tumors. Finally, **Bjoern SCHWER** (San Francisco, USA) concluded the conference by showing striking evidence that long neural genes harbor recurrent DNA break clusters in neural stem/progenitor cells. Using an unbiased high throughput approach to map recurrent DSBs, he identified 27 recurrent DSB clusters within gene bodies of long genes replicated late in S phase. This study reveals a basis of gene fragility and suggests potential impacts of DNA breaks on neurodevelopment and neural functions.

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