International Journal of Clinical Science and Medical Research

ISSN(print): 2770-5803, ISSN(online): 2770-582X

Volume 03 Issue 08 August 2023

DOI: https://doi.org/10.55677/IJCSMR/V3I8-01/2023, Impact Factor: 6.967

Page No : 156-162

Causes of DNA Damage and Genomic Instability: A Review

Sixtus, A., Okafor*[1], Luvia, U., Ezeamaku[3], Jovita, A., Daniel[2], Maryjane, C., Onyeugo[1], Augusta, C., Okafor[1], Innocent, C. Ekuma[4], Elizabet, N., Offia-Kalu.[3], Innocent, Ochiagha Eze[3]

[1] Department of Biomedical Engineering, Federal University of Technology, Owerri, Nigeria

[2] Department of Prosthetic and Orthortic, Federal University of Technology, Owerri, Nigeria

[3] Department of Polymer & Textile Engineering, Federal University of Technology, Owerri, Nigeria

[4] Department of Biomedical Engineering, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria

ABSTRACT

Published Online: August 09, 2023

Genomic instability defines all genetic alterations resulting from excessive or high frequency of mutation; base pairs sequence alterations known as microsatellite instability (MSI) and aneuploidy also called chromosome instability (CIN), chromosomal re- arrangements within the genome or the susceptibility of genome to alterations that occur during cell division cycle. The genome of most cancer cells is highly unstable, mostly due to damage to tumour suppressor genes example *tp53* which encodes p53 or other genes coordinating cell division. Telomere is a nucleoprotein complex which extends the physical ends of eukaryotic chromosomes, protecting it from degradation, counteracting sequence loss, protecting genes closer to the chromosomes end and inhibiting cell cycle arrest. Studies have implicated the dysfunction of telomere to the formation of sub-tetraploid aneuploid cells that exhibit tumourigenic capacity and formation of anueploid cells that can elude programmed cell death (apoptosis) and form tumour cells, thus initiating genomic instability. In this review, we presented an insight to the causes of DNA damage and its associated genomic instability. We found that many factors such as fragile sites, end replication problem, DNA double strand breaks, DNA replication defects, deletion, insertion and translocation all are responsible to the fragile nature of the genome.

KEYWORDS:
DNA damage, tDNA, Chromosomes, Telomere, Genomic stability, DNA repair

1.0 INTRODUCTION

1.1 Telomeric DNA (tDNA)

Telomeric DNA (tDNA) is a segment of DNA that occurs at the ends of linear eukaryotic chromosomes in eukaryotic cells (Chow *et al*., 2018). They are made up of repeated segments of DNA (tandem repeats) that consist of the sequence 5′-TTAGGG-3′ [in which T, A, and G are the bases thymine, adenine, and guanine, respectively] (Rodrigues and Lydall 2018a). It plays an essential role in maintaining the integrity and stability of the genome (Dewhurst *et al*., 2021 and Sixtus A. Okafor, *et al*., 2022), as they cap the end sequences of the chromosomes (Maciejowski *et al*., 2021). It is non linear and serves to protect the vulnerable ends of the chromosomes from degradation (Markiewicz-Potoczny *et al*., 2021), functions of the DNA repair system and are susceptible to oxidative DNA damage (Ergünm, and Sahin 2010 and Yang *et al*., 2020). tDNA was discovered by Elizabeth Blackburn and colleagues In 1975–1977. They are non coding, but act as buffer in protecting the coding sequences further behind (Rodrigues, Banks and Lydall 2018), by differentiating them from the DNA double-strand breaks (DSBs) (Schmutz *et al*., 2020) and also protecting them against homologous recombination (HR) and non-homologous end joining (NHEJ) (Guo *et al*., 2011 and Torrance and Lydall 2018).).

Corresponding Author: Sixtus, A., Okafor

****Cite this Article: Sixtus, A., Okafor, Luvia, U., Ezeamaku, Jovita, A., Daniel, Maryjane, C., Onyeugo, Augusta, C., Okafor, Innocent, C. Ekuma , Elizabet, N., Offia-Kalu. and Innocent, Ochiagha Eze (2023). Causes of DNA Damage and Genomic Instability: A Review. International Journal of Clinical Science and Medical Research, 3(8), 150-155***

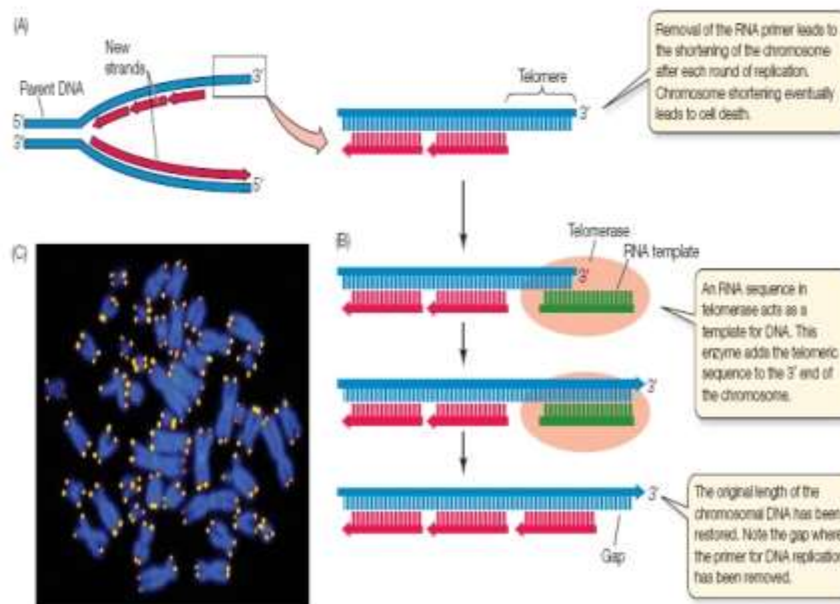

Figure 1: Image A and B, telomere and DNA replication; image C, telomere capping the ends of chromosomes. (Nature Education)

1.2 Genomic instability

Genomic instability is the term used by geneticists to refer to a high frequency or probability of inheritable changes known as mutations occurring within the genome of an organism (Betlem *et al*., 2018). These changes may involve re-arrangement of base pairs, changes in nucleic acid sequences, chromosomal rearrangements or aneuploidy (Wojcicki *et al*., 2018). Although, genome instability is not known to occur in bacteria (Giglia-Mari *et al*., 2011), however, in multicellular organisms and other higher ekaryotes, genome instability is known to play a central role in tumouringenesis and carcinogenesis (Okafor S. A, *et al*, 2022) and in evolution (Takai *et al*, 2003 and Giglia-Mari *et al*., 2011).

In humans it is reported to play major role in some neurodegenerative diseases including amyotrophic lateral sclerosis and the neuromuscular disease called myotonic dystrophy (Betlem *et al*., 2018 and Hanzlikova *et al*., 2020). Genome instability is known to occur as a result of DNA damage and its associated inaccurate translation synthesis past the damages site or errors in DNA repair, leading to mutation (Sandell and Zakian 1993 and Betlem *et al*., 2018). Another major source of genome instability is epigenetic or mutational reductions in expression of DNA repair genes, as endogenos (metabolically-caused) DNA damage is very frequent (Takai *et al*, 2003), occurring on average more than 60,000 times a day in the genomes of human cells, hence, any reduced DNA repair will likely induce genomic instability (Sandell and Zakian 1993and Hanzlikova *et al*., 2020).

Many factors are known to be responsible to the fragile nature of the genome (Hadi *et al*., 2020), research have shown that dysfunction of telomere can lead to the formation of subtetraploid aneuploid cells that exhibit tumourigenic capacity (Sfeir and de Lange, 2012 and Roulet *et al*., 2020). The formation of anueploid cells that can elude programme cell death (apoptosis) and form tumour cells due to loss of telomere has been reported in Drosophila (Blackburn 2000). In most cancer cell genomes, genetic instability has been reported to occur in three forms: (a) intrachromosomal instability occasioned by deletion, insertion and translocation, (b) oligobase pair or point mutation due largely to DNA replication error and (c) anueploidy (Chow *et al*., 2018 and Roulet *et al*., 2020). Instability and decay of the primary structure of the DNA has also been reported to occur as a result of non enzymatic methylation, hydrolysis, reactive oxygen species and general oxidative stress. (Lindahl, 1993 and Platania *et al*., 2021).

2.0 CAUSES OF DNA DAMAGE AND GENOMIC INSTABILITY

DNA damage and genomic instability are known to be induced by:

2.1 DNA replication defects

During cell division, DNA damage usually occur as a result of end replication problem occurring as the replisome tries to navigate obstacles such as tightly wound chromatin (Betlem *et al*., 2018) with bound proteins including single and double strand breaks which can lead to the stalling of the replication fork (Takai *et al*, 2003). Since each protein or enzyme in the replisome must perform its function well so as to result in a perfect copy of the DNA, then mutations of proteins such as DNA polymerase, ligase, can lead to impairment of replication and lead to spontaneous chromosomal exchanges (Rodrigues and Lydall 2018b). Proteins such as TEL1, MEC1 (ATR, ATM in humans) can detect single and double-strand breaks and recruit factors such as Rmr3 helicase to stabilize the replication fork in order to prevent its collapse.

Therefore, mutations in *tel1*, *mec1*, and *rmr*3 helicase is known to result in a significant increase in DNA damage occasioned by chromosomal recombination (Betlem *et al*., 2018 and Rodrigues and Lydall 2018b).

2.2 Fragile sites

There are locations in the genome where DNA sequences are prone to gaps and breaks after inhibition of DNA synthesis such as in the checkpoint arrest (Takai *et al*, 2003 and Demin *et al*., 2021). These sites are called fragile sites, and can occur commonly and naturally. It is present in most mammalian genomes or occurs rarely as a result of mutations, such as DNA-repeat expansion (Garcia and Sanchez-Puerta 2021). These rare fragile sites can lead to genetic disease such as fragile X mental retardation syndrome (Demin *et al*., 2021 and Okafor *et al*., 2021), myotonic dystrophy, Friedrich's ataxia (Bowen and Kolodner 2017), and Huntington's disease, mostly caused by expansion of repeats at the DNA, RNA, or protein level (Garcia and Sanchez-Puerta 2021). Although, seemingly harmful, these common fragile sites are conserved all the way in yeast and bacteria. These ubiquitous sites are characterized by trinucleotide repeats, most commonly CGG, CAG, GAA (Ayra-Plasencia *et al*., 2021). These trinucleotide repeats can form into hairpins, leading to difficulty of replication (Ayra-Plasencia *et al*., 2021 and Olbrich *et al*., 2021).

2.3 DNA double strand breaks (DSBs)

DSBs are a fatal distortion of the DNA architecture (Lovejoy *et al*., 2020), where the double strand helix is severed. It is toxic to the cell and could lead to genomic re-arrangement (Strucko, Lisby and Mortensen 2021). The causes of DSBs include; ionization agents, and radiations, inter-strand cross-link inducing agents [ISCLs] (Wu *et al*., 2021) etc. When a DSBs is joined together at the same point by a cross-linkage, the damage becomes irreparable (Wu *et al*., 2021), as neither of the damaged strand could serve as a template for the repair of the damage (Acharya, 1971), leading to cell death or in rare cases, mutagenesis (Rodrigues and Lydall 2018b and Lovejoy *et al*., 2020). The mechanisms involved in the repair of DSBs include homologous recombination (HR) (Ambjørn *et al*., 2021), Non-homologous end joining (NHEJ) (Lovejoy *et al*., 2020; Ambjørn *et al*., 2021 and Wu *et al*., 2021) and Microhomology-mediated end joining (MMEJ) (Wu *et al*., 2021 and Ambjørn *et al*., 2021).

In MMEJ, short homologous sequences in the single stranded (SS) tail of the DNA are joined (Teixeira-Silva *et al*., 2021). This mediated repair is accurate, when the SS tails in the overhang are compactible (Strucko, Lisby and Mortensen 2021; Wu *et al*., 2021 and Okafor *et al*., 2022). The phase of the cell cycle in which the cell is, prior to the DSBs, determines the mechanism that will be employed for the repair of DSBs. In S and G2 phase of the cell division cycle, the HR mechanism is used to repair damage to the DNA helix, while at the G1 phase, NHEJ mechanism is used (Ambjørn *et al*., 2021and Okafor *et al*., 2022).

During NHEJ, the DSBs are recognized by the Ku70/Ku80, which activates the P13 kinase. This enables the recruitment of Artemis nuclease and the MRN complex (MRE11, RAD50 NBS1) (Charrier-Savournin *et al*., 2001), which co-ordinate the processing of the end of the DNA (Takeda *et al*., 2007; Charrier-Savournin *et al*., 2001and Charifi *et al*., 2021). This end processing is followed closely by ligation, using the XRCC4/Ligase iv complex (Charifi *et al*., 2021 and Okafor *et al*., 2022). In HR, however, the MRN complex recognizes and bound to the DSBs (Charifi *et al*., 2021); this enables the MRN-CtIP complex, along with EXO1, to catalyse the end resection at the DSBs (Takeda *et al*., 2007; Charifi *et al*., 2021 and Olbrich *et al*., 2021).

There are differences in the way and manner various organisms respond to DSBs. In mammal, the 9-1-1 complex (RAD9, HUS1, RAD1) induce the generation of ssDNA (Charifi *et al*., 2021), while in *Saccharomyces cerevisiae* (Okafor *et al.,* 2021), the generation of RPA coated ssDNA is initiated by SGS1/DNA2 and EXO1. In metazoan, though, the MRN- CtIP complex initiates the DSBs processing in analogue to the function of MRX and SAE2 (Charifi *et al*., 2021), the overhangs generated are processed via two nucleases, depending upon the metazoan helicase BLM and the exonuclease 1 (Exo1). Although, metazoan, possess similar checkpoint signalling to *Saccharomyces cerevisiae*, they generally rely on non-homologous end-joining (NHEJ) for the repair of DSBs (Takeda *et al*., 2007; Charrier-Savournin *et al*., 2001 and Charifi *et al*., 2021).

2.4 The 'end replication problem'

During cell division cycle, the DNA is copied and the chromosomes duplicated (Sixtus *et al*., 2022). If a cell's chromosome lacks telomere, the cell will loose its chromosomal end and genomic integrity in a phenomenon called "end replication problem". This end replication problem occurs because the end of linear DNA cannot be replicated completely during replication of the lagging strand at DNA synthesis (Ohki *et al*., 2001 and Russo *et al*., 2021) leading to telomere attrition (Olovnikov, 1973 and Charifi *et al*., 2021). During DNA synthesis, the leading strand is synthesize completely, while the lagging strand is gradually truncated at the ~500-bp with the 3' overhang left behind (Ohki *et al*., 2001and Errichiello *et al*., 2020). Although, the extent of the end replication problem during DNA synthesis is poorly understood, it is thought, that the telomere shortening in telomerase-negative cells are resulted from the end replication problem. The end replication problem has been suggested to occur as a result of the priming failure of the okazaki fragments at the extreme end, and/or the failure of the most distal RNA primer to be replaced by DNA (Ohki *et al*., 2001 and Russo *et al*., 2021).

Unlike prokaryotes, eukaryotes possess linear chromosome, and their DNA is replicated bi-directionally. The inability to replicate completely the terminal region of the lagging strand occupied by the Okazaki fragments (Sixtus *et al*., 2022), could lead to loss of terminal sequences and genetic

information, following each cell division, and can induce loss of cell viability (Ohki *et al*., 2001 and Sixtus *et al*., 2022). The end replication problem is also thought to explain the reason why somatic cells stop replicating after a number of cell replication (Olovnikov, 1973), observe the hayflick effect (Russo *et al*., 2021); undergo senescence and subsequently apoptosis (Olovnikov, 1973 and Jamieson *et al*., 2021).

There are various theoretical models to the end replication problem. The first model suggests that the synthesis of DNA by polymerase from 5' to 3' should have not only a catalytic site, but also a binding site in front of the catalytic site (Russo *et al*., 2021). This site will enable the attachment of enzyme to the parent DNA strand, such that DNA polymerase moving in front of the biding site during DNA replication will dissociate, as it has nowhere to bind, creating the end problem (Olovnikov, 1973 and Jamieson *et al*., 2021). The second model, however, stressed the inability of the polymerase to begin new DNA synthesis itself, rather it is capable of elongating already existing oligonucleotide (Lie *et al*., 2018). However, recent data from the artificially created linear chromosome of the SV40 virus, have shown that *in vitro*, the leading strand was completely synthesised to the 5' end (Jamieson *et al*., 2021), while the lagging strand was truncated at approximately 500bp region, leaving behind, the 3' overhang (Lie *et al*., 2018 and Jamieson *et al*., 2021), termed the end replication problem (Lie *et al*., 2018).

The MRN protein complex (MRE11, RAD50, NBS1) has been implicated in the processing of the 5' end of the parent strand of DNA (Lie *et al*., 2018 and Russo *et al*., 2021) including EXO1 (Shi Y., Hellinga and Beese 2017 and Edera *et al*., 2021) and the Apollo nucleases (Lie *et al*., 2018 and Wu *et al*., 2021). The Apollo nucleases is believed to play a leading role, as a mutation or interference in the gene coding the Apollo nucleases (Edera *et al*., 2021 and Roy *et al*., 2021) and have been shown to lead to loss of the 3' overhang, induce loss of cell viability, senescence and apoptosis (Shi Y., Hellinga and Beese 2017 and Edera *et al*., 2021). The over hangs constitutes the telomeric loops (t-loops), which protects the telomeric DNA architecture from been recognize as double strand breaks (DSBs) which will initiate check point function and DNA repairs (Edera *et al*., 2021 and Roy *et al*., 2021).

The architecture and profile of the end of linear chromosomes such as eukaryotic chromosomes, the overhangs, mimics the DNA double strand breaks (DSB) (Roy *et al*., 2021); and could be recognize as DSB by the DNA repair mechanisms (Sandell and Zakian, 1993 and Roy *et al*., 2021). This recognition could activate checkpoint function and DNA repair pathways, which could degrade the end of the chromosomes, triggering cell arrest and genomic instability (Sandell and Zakian, 1993 and De Lange, 2010). They could also be processed by nucleases for repair either by homologous recombination (HR) or non-homologous end joining (NHEJ) (Sandell and Zakian,1993) thereby, initiating cell cycle arrest, with cell undergoing senescence (Roy *et al*., 2021) and apoptosis (Sandell and Zakian, 1993 and Roy *et al*., 2021). This risk to the physical ends of the chromosomes occasioned by the difficulty of the cell to distinguish it's chromosomes natural ends from the DNA DSBs in the genome is known as the 'end protection problem' (Sandell and Zakian 1993 ; De Lange, T. 2010; Lie *et al*., 2018 and Edera *et al*., 202).

The 'end protection problem', therefore, defines the aggregate DNA damage signalling and repair pathways that require repression at telomere (Sfeir and DeLange, 2012). However, the structure, architecture and composition of the telomeres, enables them to mimic the physical ends of the chromosomes, there by solving the end protection problem (De Lange, 2010). The telomeric shelterin complex also plays a role, in preventing the activation of these pathways (De Lange, 2010 and Morafraile *et al*., 2019) in conjunction with the general DNA damage response factors (Sfeir and DeLange, 2012). Shelterin deficient mouse telomeres have revealed end replication problem, occasioned by nucleotic degradation in the absence of 53BP1tumour suppressor binding protein 1 (Sfeir and DeLange, 2012).

2.5 Transcription-associated instability

In both E. coli and Saccromyces pombe, transcription sites tend to have higher recombination and mutation rates. The coding or non-transcribed strand accumulates more mutations than the template strand (Gandini *et al*., 2019). This is due to the fact that the coding strand is single-stranded during transcription, which is chemically more unstable than double-stranded DNA. During elongation of transcription, supercoiling can occur behind an elongating RNA polymerase, leading to single-stranded breaks (Timashev and De Lange 2020). When the coding strand is single-stranded, it can also hybridize with itself, creating DNA secondary structures that can compromise replication (Garcia *et al*., 2021). In E. coli, when attempting to transcribe GAA triplets such as those found in Friedrich's ataxia, the resulting RNA and template strand can form mismatched loops between different repeats (Gandini *et al*., 2019), leading the complementary segment in the coding-strand available to form its own loops which impede replication.

Furthermore, replication of DNA and transcription of DNA are not temporally independent (Gandini *et al*., 2021); they can occur at the same time and lead to collisions between the replication fork and RNA polymerase complex. In S. cerevisiae, Rrm3 helicase is found at highly transcribed genes in the yeast genome, which is recruited to stabilize a stalling replication fork as described above. This suggests that transcription is an obstacle to replication (Holmes *et al*., 2020), which can lead to increased stress in the chromatin spanning the short distance between the unwound replication fork and transcription start site (Garcia *et al*., 2019), potentially causing single-stranded DNA breaks. In yeast, proteins act as barriers at the 3' of the transcription unit to prevent further travel of the DNA replication fork (Gandini *et al*., 2019 and Garcia *et al*., 2019).

CONCLUSION

We have reviewed the various causes of genomic instability, which include DNA replication defects, DNA fragile site that encourage end joining, DNA double strand breaks, transcription associated instability and end replication problems, all these including hydrolysis, oxidative stress and non enzymatic methylation induce lesion on the DNA architecture, including decay and instability of its primary structure. However, we have also noted the role of telomere in preventing genomic instability caused by non environmental and epigenetic effects, by capping the physical ends of eukaryotic chromosomes and protecting it from degradation.

REFERENCES

1. Ambjørn, S.M., Duxin, J.P., Hertz, E.P.T., ...Lisby, M., Nilsson, J. (2021). A complex of BRCA2 and PP2A-B56 is required for DNA repair by homologous recombination. Nature Communications, 12(1), 5748.
2. Ayra-Plasencia, J., Ramos-Pérez, C., Santana-Sosa, S., ...Lisby, M., Machín, F. (2021).
3. Topoisomerase II deficiency leads to a postreplicative structural shift in all Saccharomyces erevisiae chromosomes. Scientific Reports, 11(1), 14940.
4. Betlem, K., Hoksbergen, S., Mansouri, N., ...Banks, C., Peeters, M. (2018). Real-time analysis of microbial growth by means of the Heat-Transfer Method (HTM) using Saccharomyces cerevisiae as model organism. Physics in Medicine, 6, 1–8.
5. Blackburn, E. H (2000) *Telomere States And Cell Fates*. *Nature* 408, 53–56.
6. Blasco, M. A (2011) *The Telomerase Activator TA-65 Elongates Short Telomeres and Increases Health Span of Adult/Old Mice Without Increasing Cancer Incidence Cell* 10, (4), 604–21
7. Bowen N. Kolodner R. (2017) Reconstitution of *Saccharomyces cerevisiae* DNA polymerase epsilon-dependent mismatch repair with purified proteins. Proc. Natl. Acad. Sci. 114 (28265089): 3607-3612, 10.1073/pnas.1701753114.
8. Charifi, F., Churikov, D., Eckert-Boulet, N., ...Simon, M.-N., Géli, V. (2021) Rad52 SUMOylation functions as a molecular switch that determines a balance between the Rad51- and Rad59-dependent survivors. iScience, 24(3), 102231.
9. Charrier-Savournin, F. B.; Château, M.; Gire, V.; Sedivy, J.; Piette, J.; Dulić, V. (2001). *"p21-Mediated "Nuclear Retention of Cyclin B1-Cdk1 in Response to Genotoxic Stress" Molecular Biology of the Cell* 15 (9), 3965–76.
10. Chow, T. T., Shi, X., Wei, J.-H., ...Huang, B., Blackburn, E.H. (2018) Local Enrichment of HP1alpha at Telomeres alters their Structure and Regulation of Telomere Protection. Nature Communications, 9(1), 3583.
11. De Lange, T. (2010) *How Shelterin Solves The telomere End-protection Problem. Cold Spring Harb Symp Quant Biol.* 75, 167-77.
12. Demin, A. A., Hirota, K., Tsuda, M., ...Takeda, S., Caldecott, K. W. (2021) XRCC1 prevent toxic PARP1 trapping during DNA base excision repair Molecular Cell, 81(14), 3018–3030.e5
13. Dewhurst, S.M., Yao, X., Rosiene, J., ...de Lange, T., Imieliński, M. (2021) Structural variant evolution after telomere crisis, Nature Communications, 12(1), 2093.
14. Edera, A. A., Small, I., Milone, D. H., Sanchez-Puerta, M.V. (2021) Deepred-Mt: Deep representation learning for predicting C-to-U RNA editing in plant mitochondria. Computers in Biology and Medicine. 136, 104682.
15. Ergünm, M and Sahin B (2010) *Telomere Dysfunction Induces Metabolic and Mitochondrial Compromise*. *Nature* 470, 359–65.
16. Errichiello, E., Zagnoli-Vieira, G., Rizzi, R., ...Caldecott, K. W., Zuffardi, O. (2020) Characterization of a novel loss-of-function variant in TDP2 in two adult patients with spinocerebellar ataxia autosomal recessive 23 (SCAR23), Journal of Human Genetics, 65(12), 1135–1141.
17. Gandini, C. L., Garcia, L. E., Abbona, C. C., Sanchez-Puerta, M.V. (2019) The complete organelle genomes of Physochlaina orientalis: Insights into short sequence repeats across seed plant mitochondrial genomes, Molecular Phylogenetics and Evolution, 137, 274–284.
18. Gandini, C.L., Ibañez, V.N., Zubko, M.K., Sanchez-Puerta, M.V. (2021) Complete plastome phylogeny and an update on cox1 intron evolution of Hyoscyameae (Solanaceae). Organisms Diversity and Evolution, 21(3), pp. 521–532.
19. Garcia, L. E., Zubko, M. K., Zubko, E. I., Sanchez-Puerta, M. V.(2019) Elucidating genomic patterns and recombination events in plant cybrid mitochondria Plant Molecular Biology, 100(4-5), 433–450.
20. Garcia, L. E and Sanchez-Puerta, M V. (2021) Transcriptional landscape and splicing efficiency in arabidopsis mitochondria Cells, 10(8), 2054.
21. Garcia, L.E., Edera, A. A., Palmer, J. D., Sato, H., Sanchez-Puerta, M. V.(2021) Horizntal gene transfers dominate the functional mitochondrial gene space of a holoparasitic plant. New Phytologist, 229(3), 1701–1714.
22. Giglia-Mari, G., Zotter, A and Vermeulen, W (2011) *DNA Damage Response Cold Spring Harb Lab*.

23. Guo, N., Parry, E. M., Li, L. S., Kembou, F and Lauder N (2011) *Short Telomeres Compromise β-Cell Signaling and Survival*. PLoS ONE 6(3), e17858.
24. Hadi, K., Yao, X., Behr, J. M., ...Elemento, O., Imielinski, M. (2020) Distinct Classes of Complex Structural Variation Uncovered across Thousands of Cancer Genome Graphs, Cell, 183(1), 197–210.e32.
25. Hanzlikova, H., Prokhorova, E., Krejcikova, K., ...Ahel, I., Caldecott, K. W. (2020) Pathogenic ARH3 mutations result in ADP-ribose chromatin scars during DNA strand break repair, Nature Communications, 11(1), 3391.
26. Holmes, A. C., Zagnoli-Vieira, G., Caldecott, K. W., Semler, B. L. (2020). Effects of TDP2/VPg unlinkase activity on picornavirus infections downstream of virus translation, Viruses, 12(2), v12020166.
27. Jamieson, O., Betlem, K., Mansouri, N., ...Whitehead, K. A., Peeters, M. (2021) Electropolymerised molecularly imprinted polymers for the heat-transfer based detection of microorganisms: A proof-of-concept study using yeast. Thermal Science and Engineering Progress 24, 100956.
28. Lie, S., Banks, P., Lawless, C., Lydall, D., Petersen, J.(2018). The contribution of non-essential schizo saccharomyces pombe genes to fitness in response to altered nutrient supply and targe of rapamycin activity. Open Biology 8(5), 180015.
29. Lindahl, T (1993) *Instability And Decay Of The Primary Structure Of DNA*. *Nature* 362, 709–715.
30. Lovejoy, C.A., Takai, K., Huh, M.S., Picketts, D.J., De Lange, T. (2020) ATRX affects the repair of telomeric DSBs by promoting cohesion and a DAXX-dependent activity, PLoS biology, 18(1), e3000594.
31. Maciejowski, J., Chatzipli, A., Dananberg, A., ...Campbell, P.J., De Lange, T. (2021) APOBEC3-dependent kataegis and TREX1-driven chromothripsis during telomere crisis Nature Genetics, 52(9), 884–890.
32. Markiewicz-Potoczny, M., Lobanova, A., Loeb, A.M., ...Ruiz, S., Lazzerini Denchi, E. (2021). TRF2-mediated telomere protection is dispensable in pluripotent stem cells. Nature, 589(7840), 110–115.
33. Morafraile, E.C., Hänni, C., Allen, G., ...Lydall, D., Zegerman, P. (2019). Checkpoint inhibition of origin firing prevents DNA topological stress. Genes & development, 33(21-22), 1539–1554.
34. Ohki, R., Tsurimoto, T and Ishikawa, F (2001) *In Vitro Reconstitution of the End Replication Problem*. *Mol. Cell. Biol*. 21 (17), 5753-66.
35. Okafor, S. A., Okey-Mbata, C. C., Daniel, J. A. Arukalam, F. M., Daniel-Nwosu E. I. and Okafor, A. L. (2021) Miscellany of Hospital Contact Surfaces Microbiome: A Case Study of Selected Hospitals in Owerri South Eastern Nigeria, Afr. .J Med. Phy., Biomed. Eng. & Sc., (8)2, 48 – 57.
36. Okafor, S.A., Agbasi, P.U., Azeez, O.T., Iwuji, S.C., Ezeamaku, L.U., Arukalam, F.N. and Eziefuna, E.O. (2022). The Frequency of Survivorship in Heterozygous Diploids of Cdc13-1exo1Δ Mutants of S. cerevisiae Is One Survivor Cell in 72 Cells/Generation at 36˚C. Advances in Bioscience and Biotechnology, 13, 469-478.
37. Olbrich, T., Vega-Sendino, M., Tillo, D., .Nussenzweig, A., Ruiz, S. (2021). CTCF is a barrier for 2C-like reprogramming. Nature Communications,12(1), 4856.
38. Olovnikov, A. M (1973) "*A Theory Of Marginotomy. The Incomplete Copying Of Template Margin In Enzymic Synthesis Of Polynucleotides And Biological Significance Of The Phenomenon*". *J. Theor. Biol.* 41 (1), 181–90.
39. Platania, V., Douglas, T.E.L., Zubko, M.K., ...Pietryga, K., Chatzinikolaidou, M. (2021) Phloroglucinol-enhanced whey protein isolate hydrogels with antimicrobial activity for tissue engineering Materials Science and Engineering, 129, 112412.
40. Rodrigues, J., Lydall, D. (2018a).Paf1 and Ctr9, core components of the PAF1 complex, maintain low levels of telomeric repeat containing RNA. Nucleic Acids Research, 46(2), 621–634.
41. Rodrigues, J., Banks, P., Lydall, D. (2018). Vps74 connects the golgi apparatus and telomeres in saccharomyces cerevisiae. G3: Genes, Genomes, Genetics, 8(5), 1807–1816.
42. Rodrigues, J., Lydall, D. (2018b). Cis and trans interactions between genes encoding PAF1 complex and ESCRT machinery components in yeast. Current Genetics, 64(5), 1105–1116.
43. Roulet, M.E., Garcia, L.E., Gandini, C.L., ...Ponce, G., Sanchez-Puerta, M.V. (2020) Multichromosomal structure and foreign tracts in the Ombrophytum subterraneum (Balanophoraceae) mitochondrial genome. Plant Molecular Biology. 103(6), 623 – 638.
44. Roy, U., Kwon, Y., Marie, L., ...Lisby, M., Greene, E.C. (2021) The Rad51 paralog complex Molecular Cell, 81(5), 1043–1057.e8.
45. Russo, L. C., Tomasin, R., Matos, I. A., ...Bruni-Cardoso, A., Hoch, N. C. (2021). The SARS-CoV-2 Nsp3 macrodomain reverses PARP9/DTX3L-

dependent ADP-ribosylation induced by interferon signaling, Journal of Biological Chemistry, 297(3), 101041.

46. Sandell, L. L. and Zakian, V. A. (1993) *Loss Of A Yeast Telomere: Arrest, Recovery, And Chromosome Loss*. *Cel,* 75, 729.
47. Schmutz, I., Mensenkamp, A.R., Takai, K.K., ...Jongmans, M.C., De Lange, T. (2020) Tinf2 is a haploinsufficient tumor suppressor that limits telomere length, eLife, 9, 1–20, e61235.
48. Sfeir, A and de Lange, T (2012) *Removal Of Shelterin Reveals The Telomere End-protection Problem*. *Science* 4; 336(6081), 593-7.
49. Shi Y., Hellinga H. and Beese L. (2017) Interplay of catalysis, fidelity, threading, and processivity in the exo- and endonucleolytic reactions of human exonuclease I. Proc. Natl. Acad. Sci. 114 (28533382): 6010- 6015, 10.1073/pnas.1704845114.
50. Sixtus, A., Okafor, Patrick, U., Agbasi, Oladimeji, T., Azeez, Samuel, C., Iwuji, Luvia, U., Ezeamaku[3], Jovita, A., Daniel, Maryjane, C., Onyeugo, Innocent, C. Ekuma , Elizabet, N., Offia-Kalu. and Henry, C., Okoroego (2022) Thermal Tolerance Phenotype in Cdc13-1exo1Δ Heterozygous Diploids of *S. Cerevisiae* is a Dominant Trait. Engr. & Tech. Jour. 7(12) 1774-79.
51. Strucko, T., Lisby, M., Mortensen, U.H. (2021) DNA Double-Strand Break-Induced Gene Amplification in Yeast. Methods in Molecular Biology, 2153, 239–252.
52. Takai, H., Smogorzewska, A, A. and De Lange, T. (2003) *DNA Damage Loci At Dysfunctional Telomeres*. *Curr Biol,* 13, 1549-56.
53. Takeda, S., Nakamura, K., Taniguchi, Y *and* Paul, T. T (2007) *Ctp1/CtIP And The MRN Complex Collaborate In The Initial Steps Of Homologous Recombination. Mol Cell* 28, 351–52.
54. Teixeira-Silva A., Ait Saada A., Hardy J., Iraqui I., Nocente M.C., Fréon K and Lambert S. (2017). The end-joining factor Ku acts in the end-resection of double strand break-free arrested replication forks. Nat. Commun; 8 (29215009)1982, 10.1038/s41467-017-02144-5.
55. Timashev, L.A., De Lange, T. (2020). Characterization of t-loop formation by TRF2, Nucleus, 11(1), 164–177.
56. Torrance, V., Lydall, D. (2018). Overlapping open reading frames strongly reduce human and yeast STN1 gene expression and affect telomere function. PLoS Genetics, 14(8), e1007523.
57. Wojcicki, J.M., Elwan, D., Lin, J., Blackburn, E., Epel, E. (2018). Chronic obesity and incident hypertension in latina women are associated with accelerated telomere length loss over a 1-year period, Metabolic Syndrome and Related Disorders, 16(6), 262–266.
58. Wu, W., Hill, S. E., Nathan, W. J., ...Ward, M. E., Nussenzweig, A. (2021) Neuronal enhancers are hotspots for DNA single-strand break repair Nature, 593(7859), 440–444.
59. Yang, Z., Takai, K. K., Lovejoy, C. A., de Lange, T.(2020). Break-induced replication promotes fragile telomere formation, Genes and Development, 34(19-20), 1392–1405.