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Evaluating the Evidence of the Role of DNA Polymerase in Apoptosis and Aging

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Abstract

We conducted a study to examine the role of DNA Polymerase on aging in humans. 12 human blood/body fluid samples from subjects aged between 6 weeks and 101 years were used for the experiment, which was conducted at the Kenyatta University's School of Pure and Applied Sciences, Nairobi, Kenya. The samples for the experiments were obtained from public hospitals within Nairobi. A synthesized DNA single strand of known sequence with DNA polymerase was added into each of the 12 samples to act as template for the replication of a copy. The DNA copies were isolated from the media and their base sequences compared with that of the original known strand.The trend in the errors made varied significantly with age of sample. Many errors in DNA replication were noted with DNA Polymerase of old age. The results show that DNA Polymerase makes more errors the older the person is. Aging is due to the inability of DNA Polymerase to correct errors it makes during DNA synthesis.

Keywords: DNA Polymerase, Aging, Bases, DNA Replication.

1. Introduction

Normal human aging is a gradual, cumulative process that spans decades and most likely involves multiple mechanisms. Information on the specific contribution of DNA Polymerase activity to human aging can be inferred through the analysis of disorders associated with increased mutations or frequency during DNA replication. Tissues

most affected by disorders associated with inherited DNA mutations are the same tissues markedly affected by normal aging; these include the brain, heart, skeletal muscle, kidney and the endocrine system. Disorders associated with increased levels of DNA mutations generally fall into two classes: those associated with specific, maternally-inherited DNA mutations; and, those associated with mutations in nucleus-encoded genes important for maintaining the fidelity of DNA replication and DNA stability, Zhang et ale (2000). Because disorders in the latter category result in random accumulation of many different DNA mutations and deletions, they may better represent the potential consequences of age-related DNA mutation accumulation in humans, Melov (1995).

Although most molecular damage is reversible through repair or molecular turnover mechanisms, unrepaired DNA damage may lead to mutations that accumulate as a function of age. The accumulation of mutations ultimately leads to permanent age-related mitochondrial dysfunction, which contributes to the aging phenotype, Melov et ale (1999).

Every time a human cell divides and its DNA replicates, it has to copy and transmit the exact same sequence of 3 billion nucleotides to its daughter cells, Melov (1997). In life, nothing is perfect. While most DNA replicates with fairly high fidelity, mistakes do happen, with polymerase enzymes sometimes inserting the wrong nucleotide or too many or too few nucleotides into a sequence. Fortunately, most of these mistakes are fixed through various DNA repair processes. Repair enzymes, notably DNA Polymerase, recognize structural imperfections between improperly paired nucleotides, cutting out the wrong ones and putting the right ones in their place Zhang, (2003). But some replication errors make it past these mechanisms, thus becoming permanent mutations. These altered nucleotide sequences can then be passed down from one cellular generation to the next, and if they occur in cells that give rise to gametes, they can even be transmitted to subsequent organismal generations. Moreover, when the genes for the DNA repair enzymes themselves become mutated, mistakes begin accumulating at a much higher rate. In eukaryotes, such mutations can lead to diseases especially cancer, aging and death Michikawa, (1999).

DNA polymerase enzymes are amazingly particular with respect to their choice of nucleotides during DNA synthesis, ensuring that the bases added to a growing strand are correctly paired with their complements on the template strand (i.e., A's with T's, and C's with G's) Nekhaeva, (2002). Nonetheless, these enzymes do make mistakes at a rate of about 1 per every 100,000 nucleotides. That might not seem like much, until you consider how much DNA a cell has. In humans, with our 6 billion base pairs in each diploid cell, that would amount to about 120,000 mistakes every time a cell divides, Michikawa, et ale (1999).

Fortunately, cells have evolved highly sophisticated means of fixing most, but not all, of those mistakes. Some of the mistakes are corrected immediately during replication through a process known as proofreading, and some are corrected after replication in a process called mismatch repair Lin, (2002). When an incorrect nucleotide is added to the growing strand, replication is stalled by the fact that the

nucleotide's exposed 3′-OH group is in the "wrong" position. During proofreading, DNA polymerase enzymes recognize this and replace the incorrectly inserted nucleotide so that replication can continue. Proofreading fixes about 99% of these types of errors, but that's still not good enough for normal cell functioning, Wanagat, (2001). After replication, mismatch repair reduces the final error rate even further. Incorrectly paired nucleotides cause deformities in the secondary structure of the final DNA molecule. During mismatch repair, enzymes recognize and fix these deformities by removing the incorrectly paired nucleotide and replacing it with the correct nucleotide.

2. Materials and Methods

Synthesis of DNA: the DNA of known sequence was synthesized using the following reagents:

i. DNA bases
ii. DNA Polymerase
iii. Cations; magnesium, calcium
iv. ATP
v. DNA ligase

These reagents were mixed at a pH of 7.2 and temperature of 35-37 degrees centigrade for 45 minutes. These conditions allowed synthesis strand DNA by adding labled bases ATGC. We extracted DNA Polymerase from the blood of the following 12 humans blood specimens in 12 test tubes correctly labled;

1. Aborted foetus of 6 weeks old
2. Aborted foetus of 6 months old
3. Newborn infant of one day
4. 6 year old child
5. 15 year old
6. 25 year old
7. 40 year old
8. 65 year old
9. 78 year old
10. 85 year old
11. 92 year old
12. 101 year old

The synthesized DNA single strand of known sequence with DNA polymerase was added into each of the 12 test tubes. New DNA strand was allowed to be synthesized for 30 minutes.

The DNA was isolated from the media.
Next the DNA was annealed, heated at 39 degrees centigrade. The two strands were separated, and the new strand from each of the 12 test tubes were sequenced using restriction enzymes; Figures 1,2 and 3.

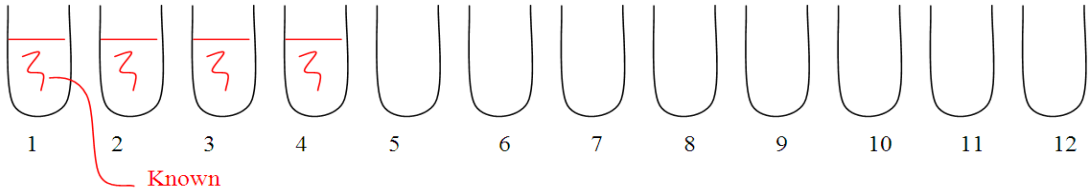

Fig. 1

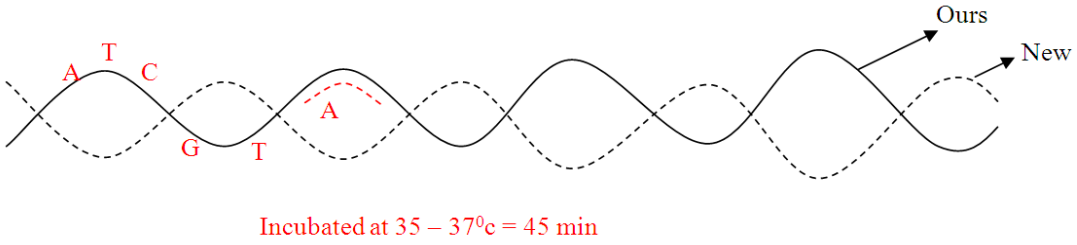

Fig. 2

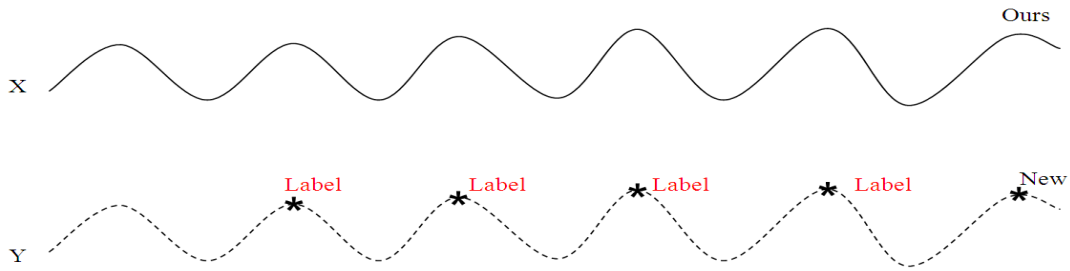

Fig. 3

3. Results

The aim of this investigation was to find how many errors in the insertion of bases were made by the 12 samples by DNA Polymerase according to age. The amount of errors made during DNA replication varied greately depending on age. These results are summarized graphically in Fig. 4

We know that DNA Polymerase edits errors it makes during replication. The results show that DNA Polymerase makes more errors the older the person is.

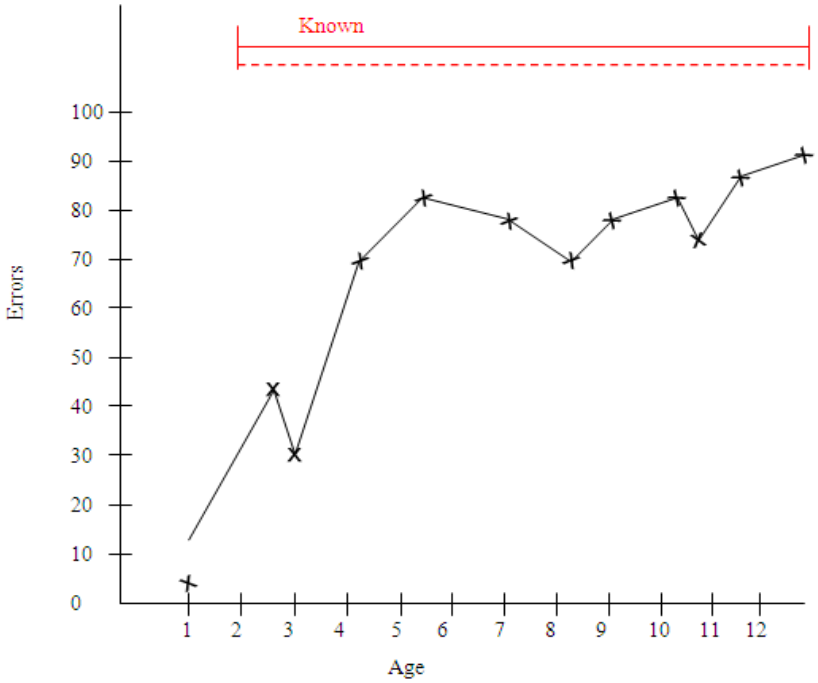

Fig. 4: Errors made by DNA Polymerase with age in humans.

4. Conclusion

Aging is due to the inability of DNA Polymerase to correct errors it makes during DNA synthesis. This results in cell and tissue deterioration, and humans it is characterized with: grey hair, of teeth, joint pains, skin wrinkles, immobilization, low immune response, heart attack, kidney failure, liver failure, high blood pressure.

These results should help uncover the relevance of DNA Polymerase's role to animal aging, and allow the rational design of therapeutic interventions.

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