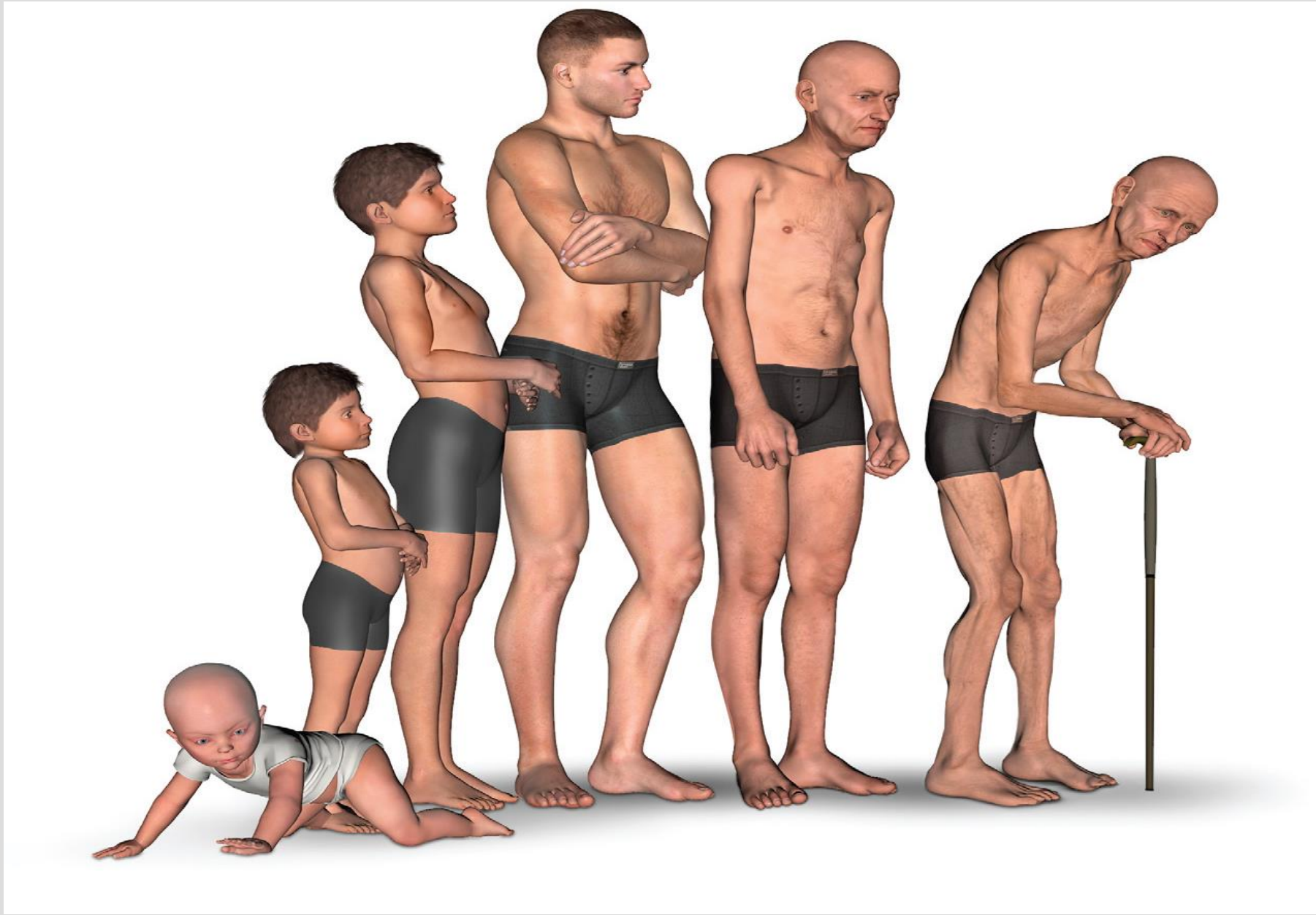
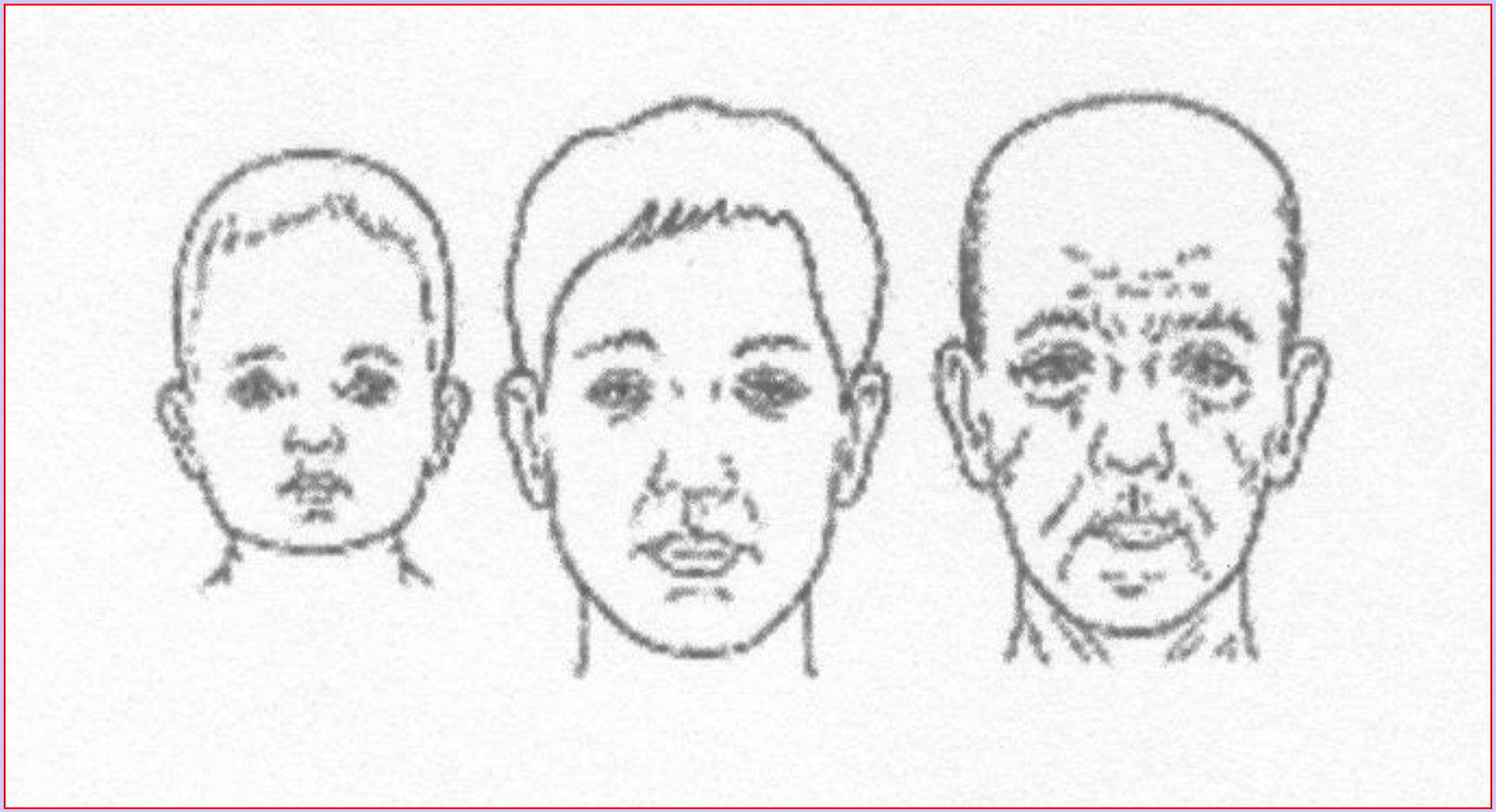
DNA damage, DNA Repair and Aging

Prof. Kalluri Subba Rao
Indian National Science Academy Hon.Scientist

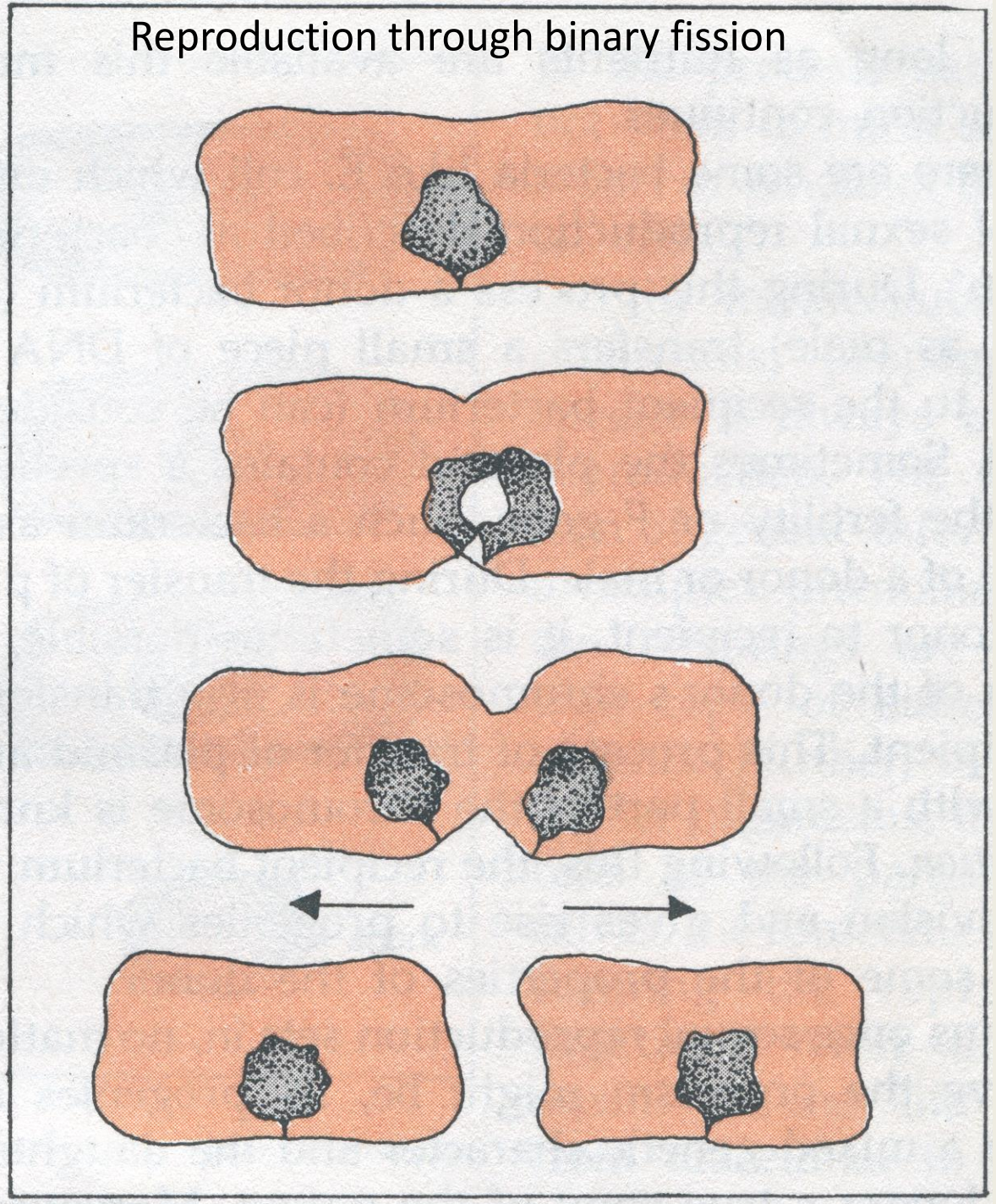
School of Medical Sciences
University of Hyderabad
Hyderabad – 500 046. India

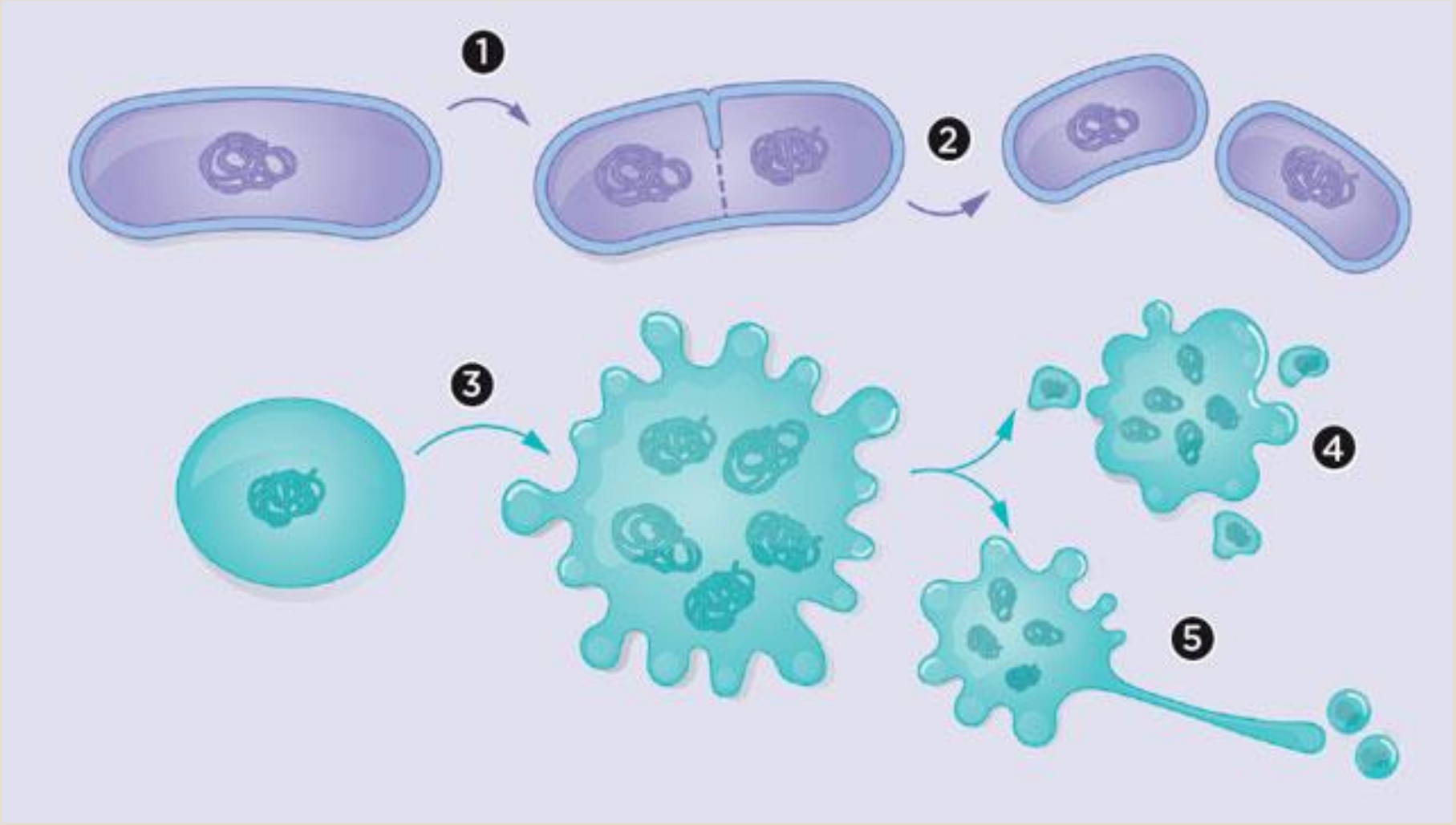




WHY DO WE BECOME OLD ?

Reproduction through binary fission




1
2
3
4
5

Longevity & Time taken for Reproductive Maturity

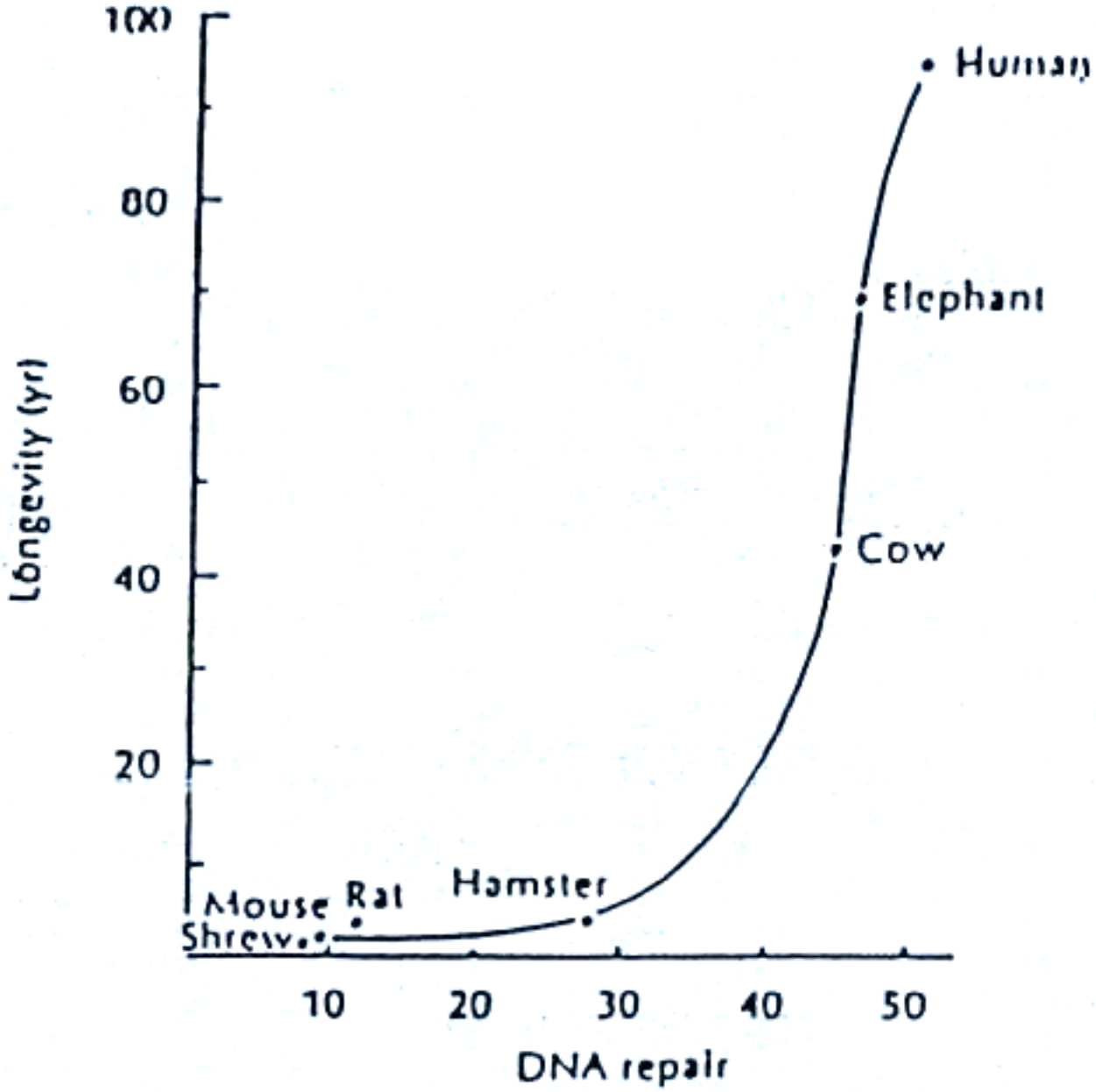
Species	Longevity (Years)	Age of Puberty (Years)
HUMANS	100	12-14
ELEPHANT	70	12-14
CHIMPANZEE	40	10
DOG	30	1
RHESUS MONKEY	25	3
CAT	25	1.5
RAT	3	0.25
MICE	3	0.20

Aging is genetically Programed

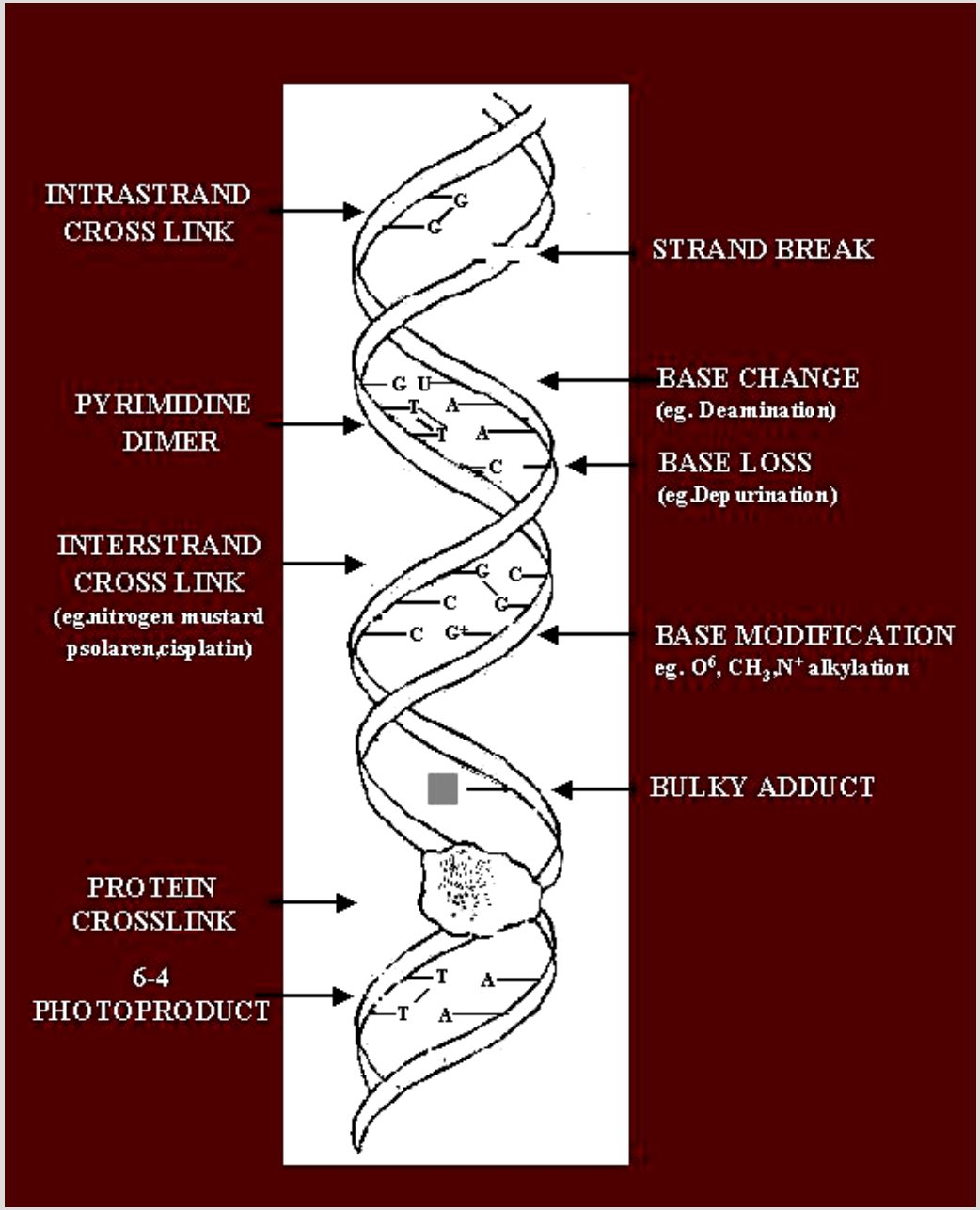
- Hayflick's limit for in vitro grown cells
- Fixed Life Span of Species
- Aging Begins at discrete stage of Life
- Premature Aging Syndromes
- Gerontogenes (Genes affecting life span)
- Immortalization of cells in Laboratory

LONGEVITY GENES

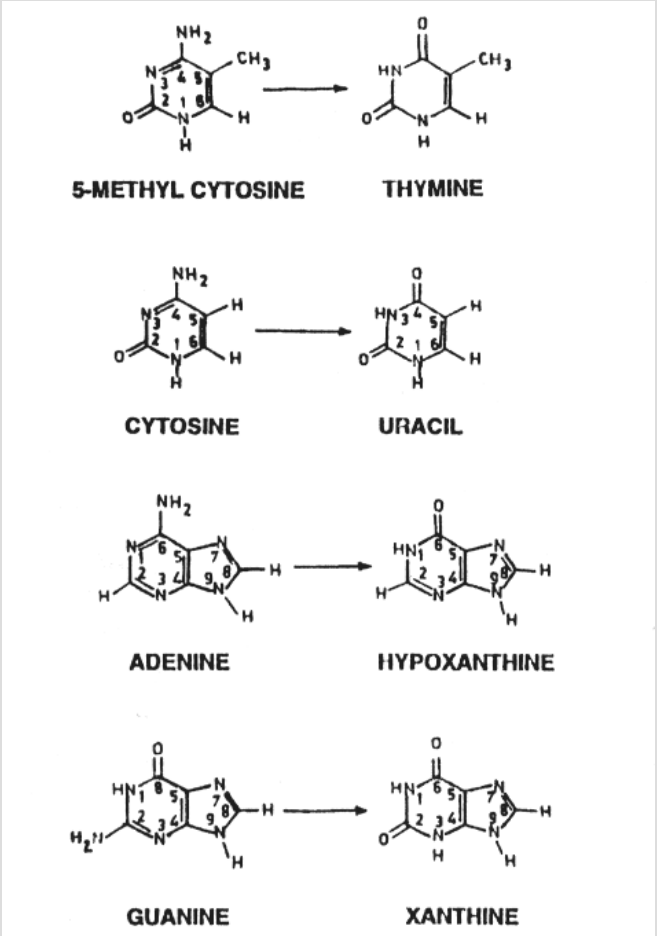
Caenorhabditis elegans	Drosophila melanogaster	Saccharomyces cerevisiae	Mice/Humans
Age-1 ↕			PI3-kinase
Daf-2 ↕ Clk-1 ↓	Methuselah gene (G-protein coupled receptor)	SIR2 gene Histone deacetylase in chromatin	IGF-like Receptor
Daf-16 ↓			HNF3 (Tr.Factor)
WRN ↓			WRN(Werner Syndrome)Helicase
Catalase ↓			Catalase

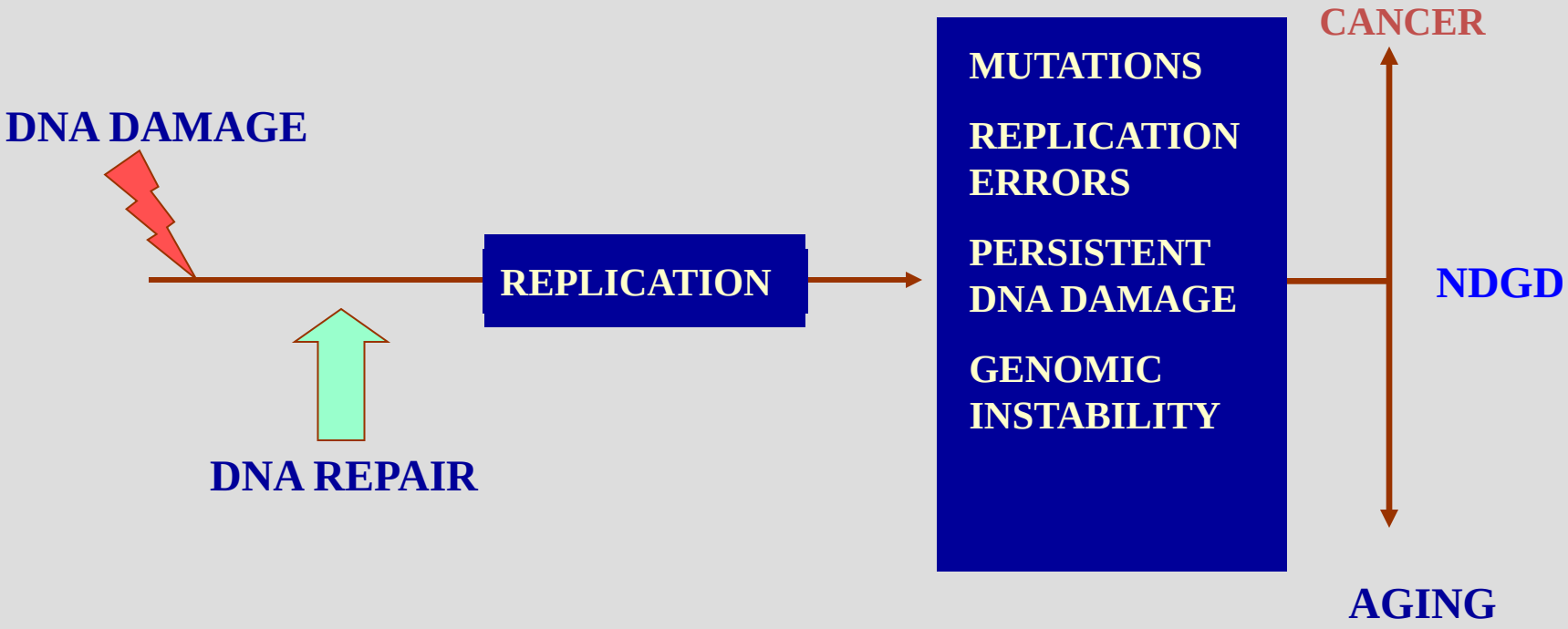

100
80
60
40
20
Longevity (yr)
Human
Elephant
Cow
Hamster
Rat
Mouse
Shrew
10
20
30
40
50
DNA repair

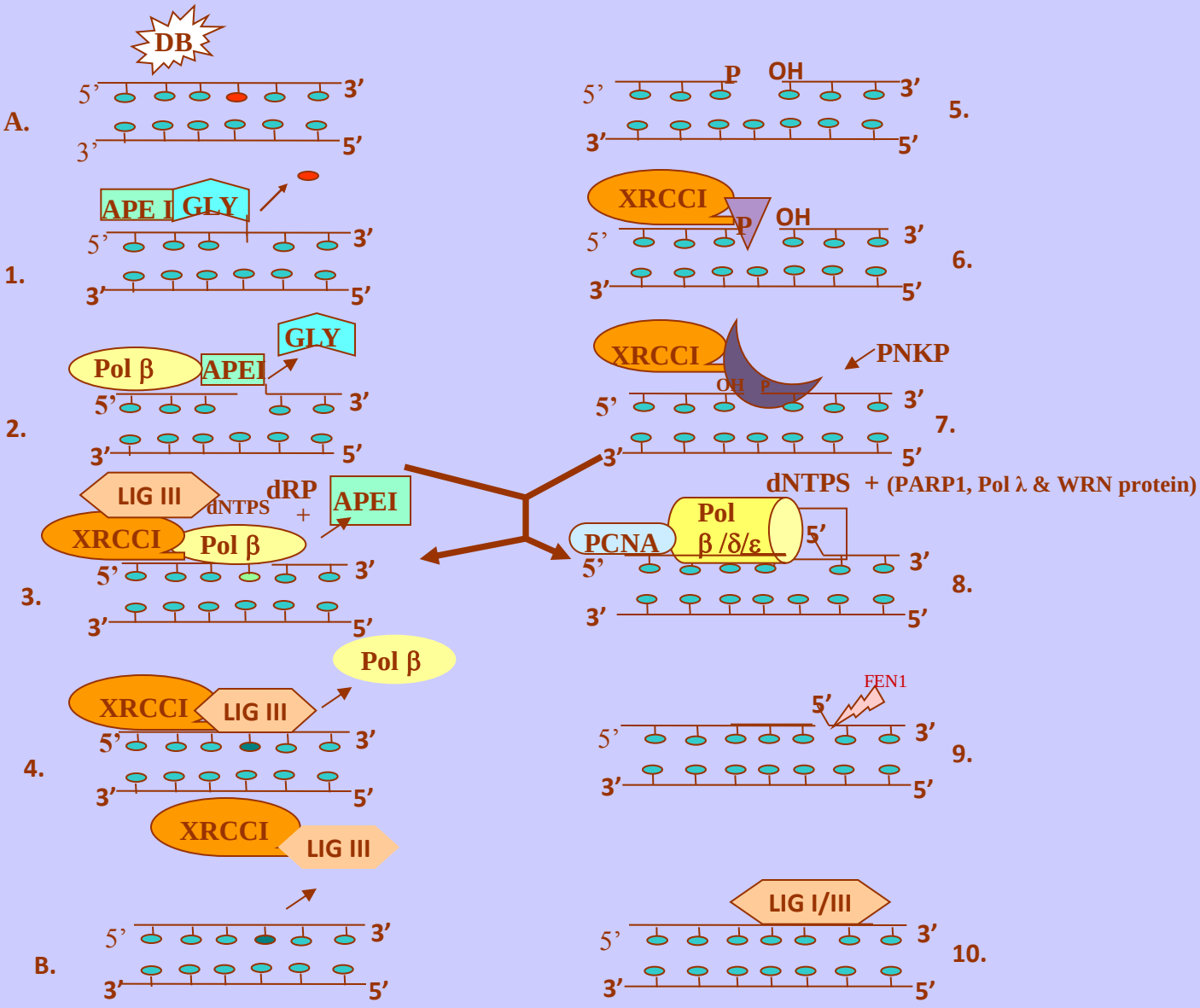
Different forms of DNA damages due to various endogenous and exogenous sources



Deamination products of DNA bases



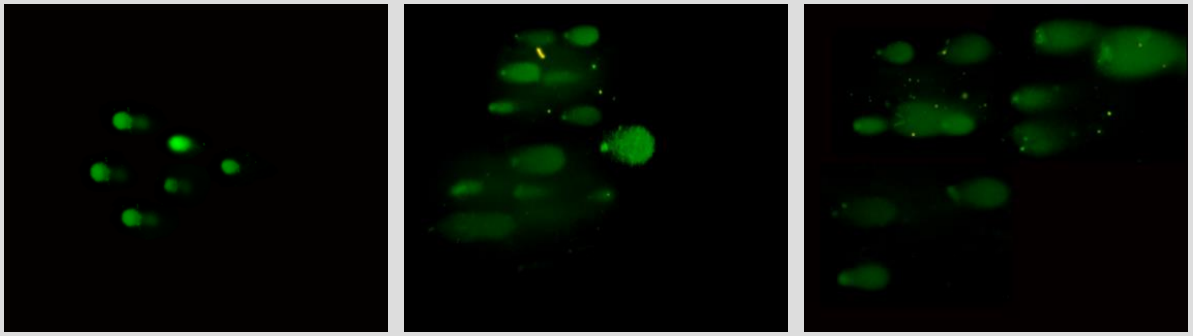

DNA DAMAGE
DNA REPAIR
REPLICATION
MUTATIONS
REPLICATION ERRORS
PERSISTENT DNA DAMAGE
GENOMIC INSTABILITY
CANCER
NDGD
AGING



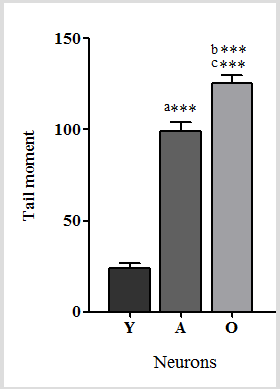
BASE EXCISION REPAIR PATHWAY

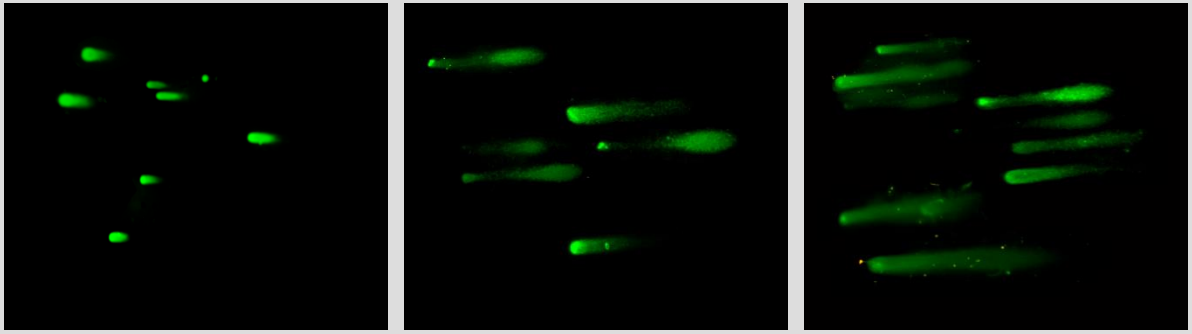
Different types of DNA damage accumulate with age (Alkaline and Neutral conditions of Comet Assay)

Young Adult Old

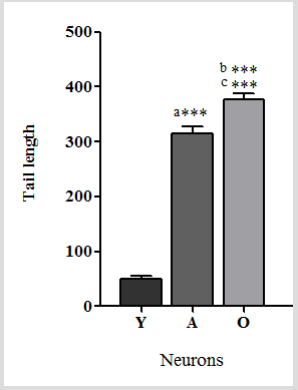


Alkaline condition

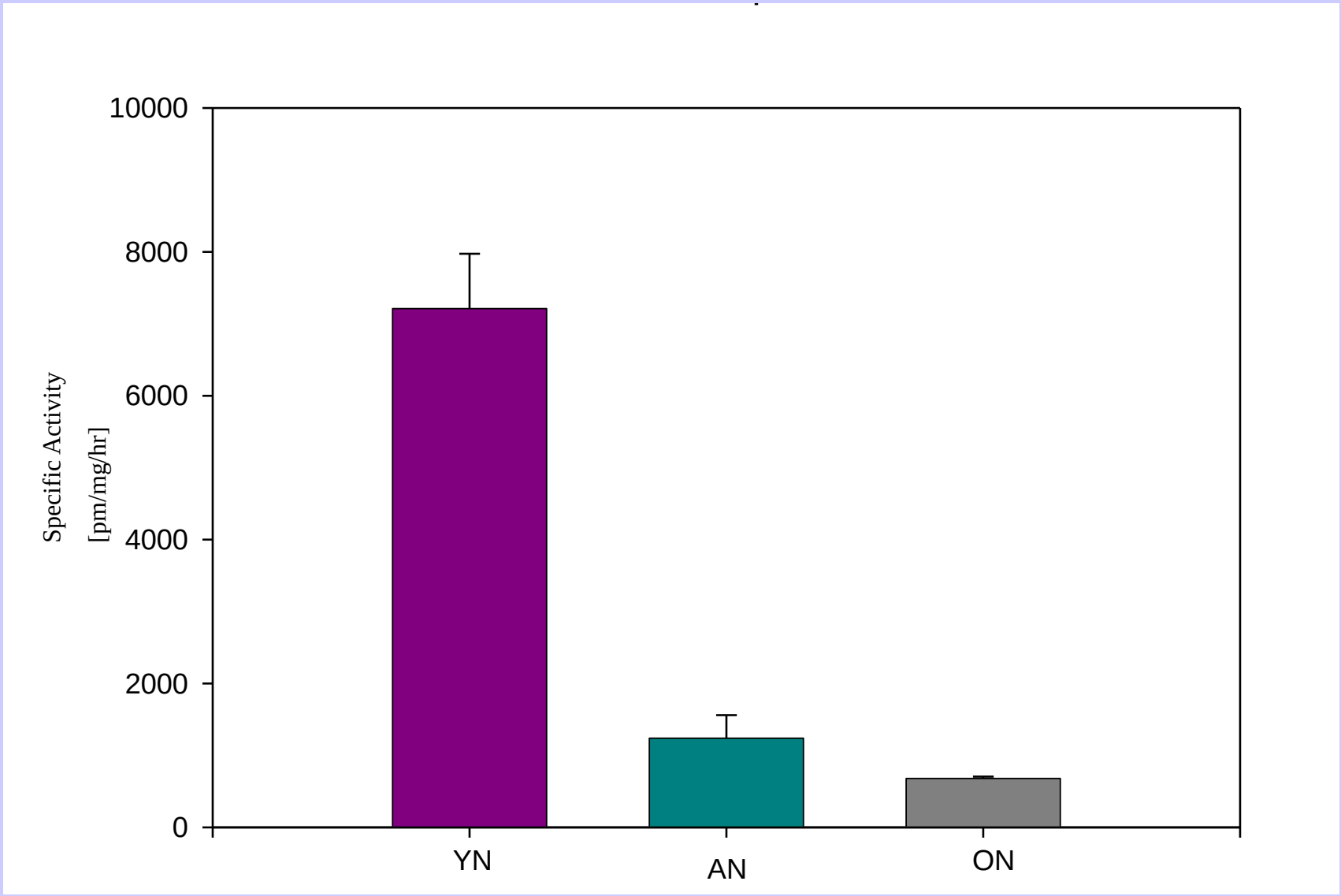




Neutral condition



DNA polymerase β activity of Young,Adult,Old Rat Neuronal extracts using 'Activated'DNA as substrate.



Oligonucleotides used in this study.

G-U oligo duplex

8 th

^{32}P 5’-g c c a t t g U g c t a c c g a t c g c g- 3’(Oligo-1)
3’-c g g t a a c G c g a t g g c t a g c g c- 5’(Oligo-2)

Where U is Uracil

AP oligo duplex

14 th

^{32}P5’-C G C G A T C G G T A G C F C A A T G G C-3’ (Oligo-3)

3’-G C G C T A G C C A T C G C G T T A C C G-5’ (Oligo-4)

Where F is THF analog of AP SITE

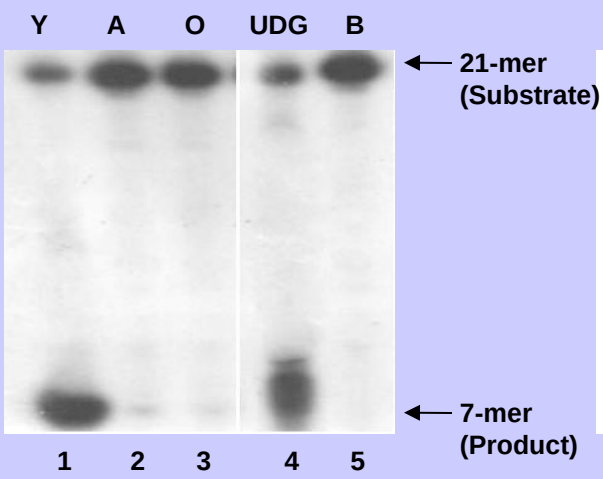
8-oxoG-C oligo duplex

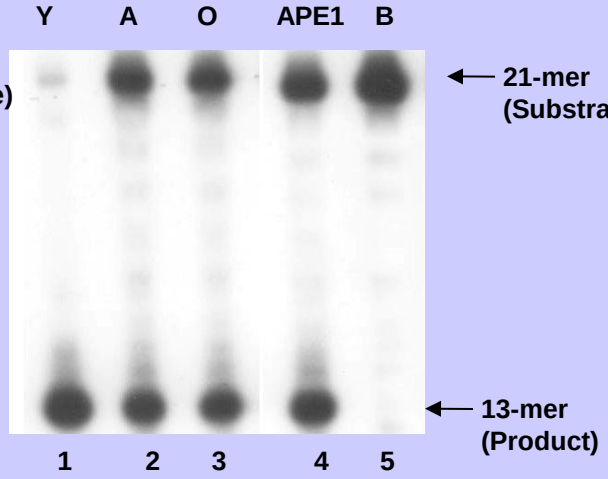
10 th

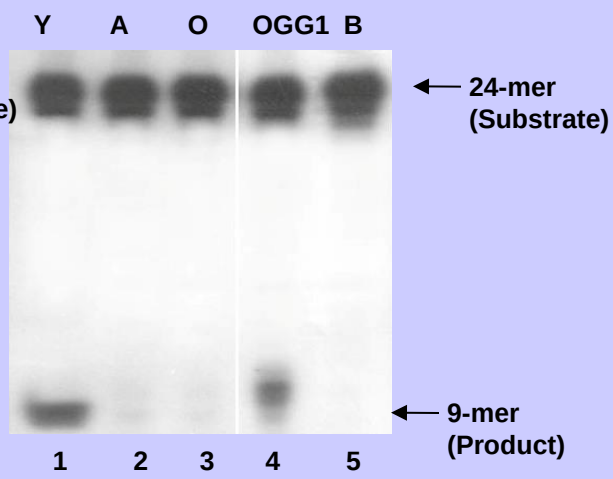
P^{32}5’ GAACTAGTGOATCCCCCGGGCTGC 3’ (Oligo-5)
3’CTT GATCACCTAGGGGGCCCGACG 5’ (Oligo-6)

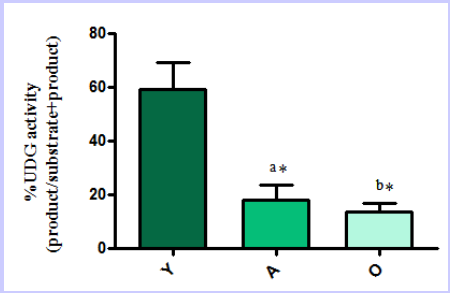
where O is 8-oxoguanine.

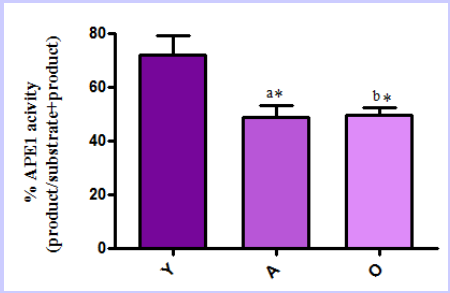
UDG,APE1 and OGG1 activities in young, adult and old neurons.











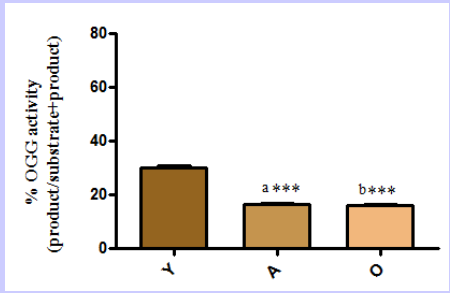
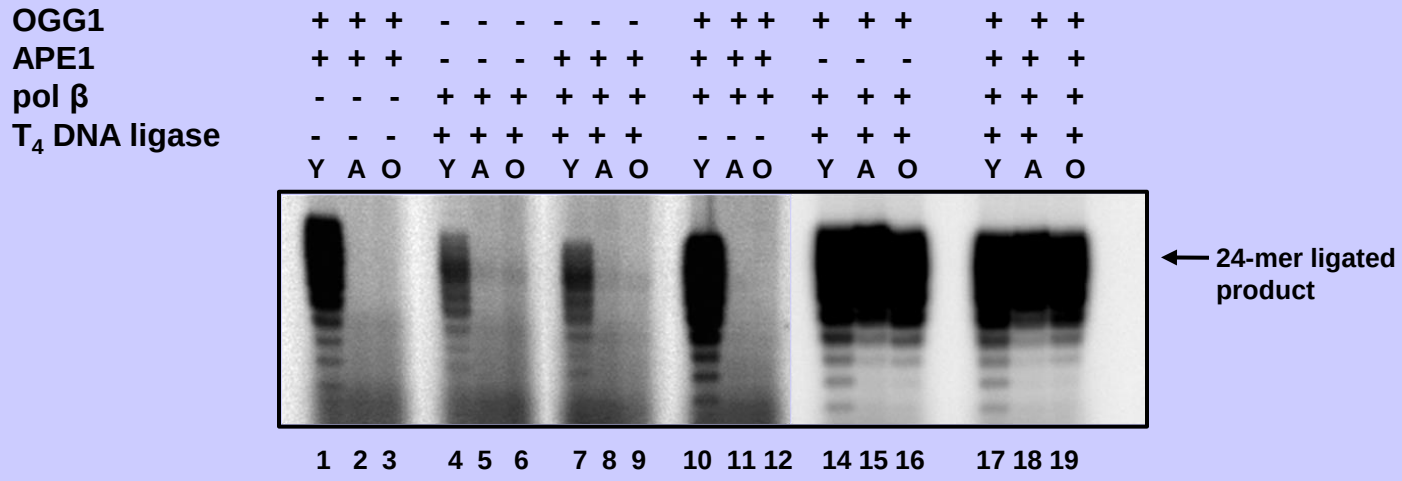


Fig.7

(A)


OGG1 + + + - - - - - - + + + + + + + + +
APE1 + + + - - - + + + + + + - - - + + +
pol β - - - + + + + + + + + + + + + + + +
T4 DNA ligase - - - + + + + + + - - - + + + + + +
Y A O Y A O Y A O Y A O Y A O Y A O
1 2 3 4 5 6 7 8 9 10 11 12 14 15 16 17 18 19
24-mer ligated product

(B)

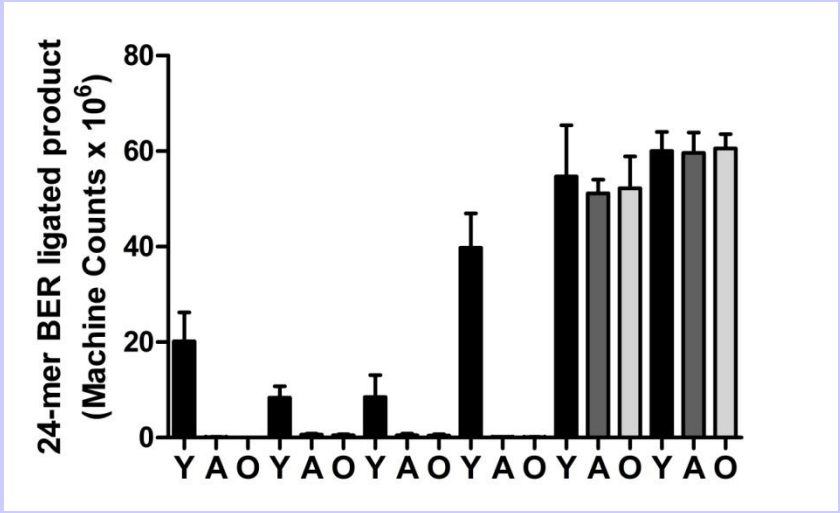
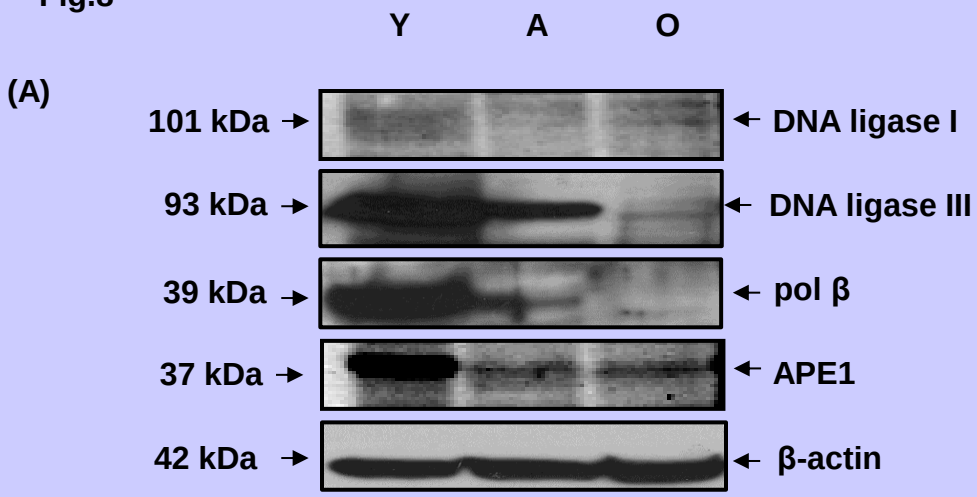
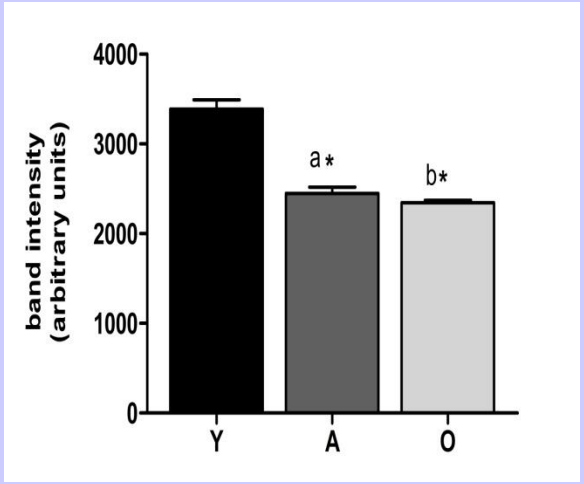
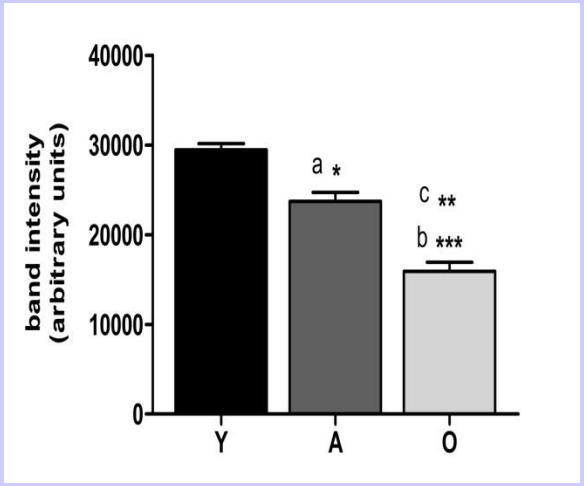
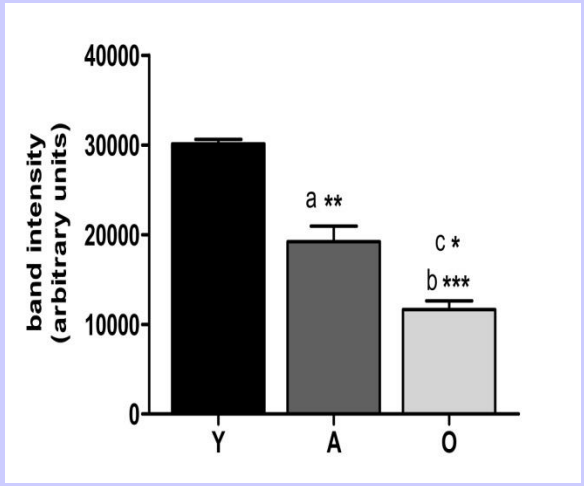

24-mer BER ligated product (Machine Counts x 10^6)
80
60
40
20
0
Y A O Y A O Y A O Y A O Y A O Y A O
OGG1 + + + - - - - - - + + + + + + + + +
APE1 + + + - - - + + + + + + - - - + + +
pol β - - - + + + + + + + + + + + + + + +
T4 DNA ligase - - - + + + + + + - - - + + + + + +

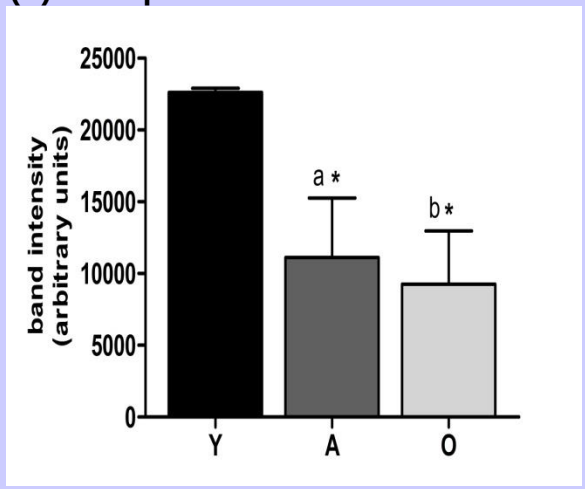
Fig.8

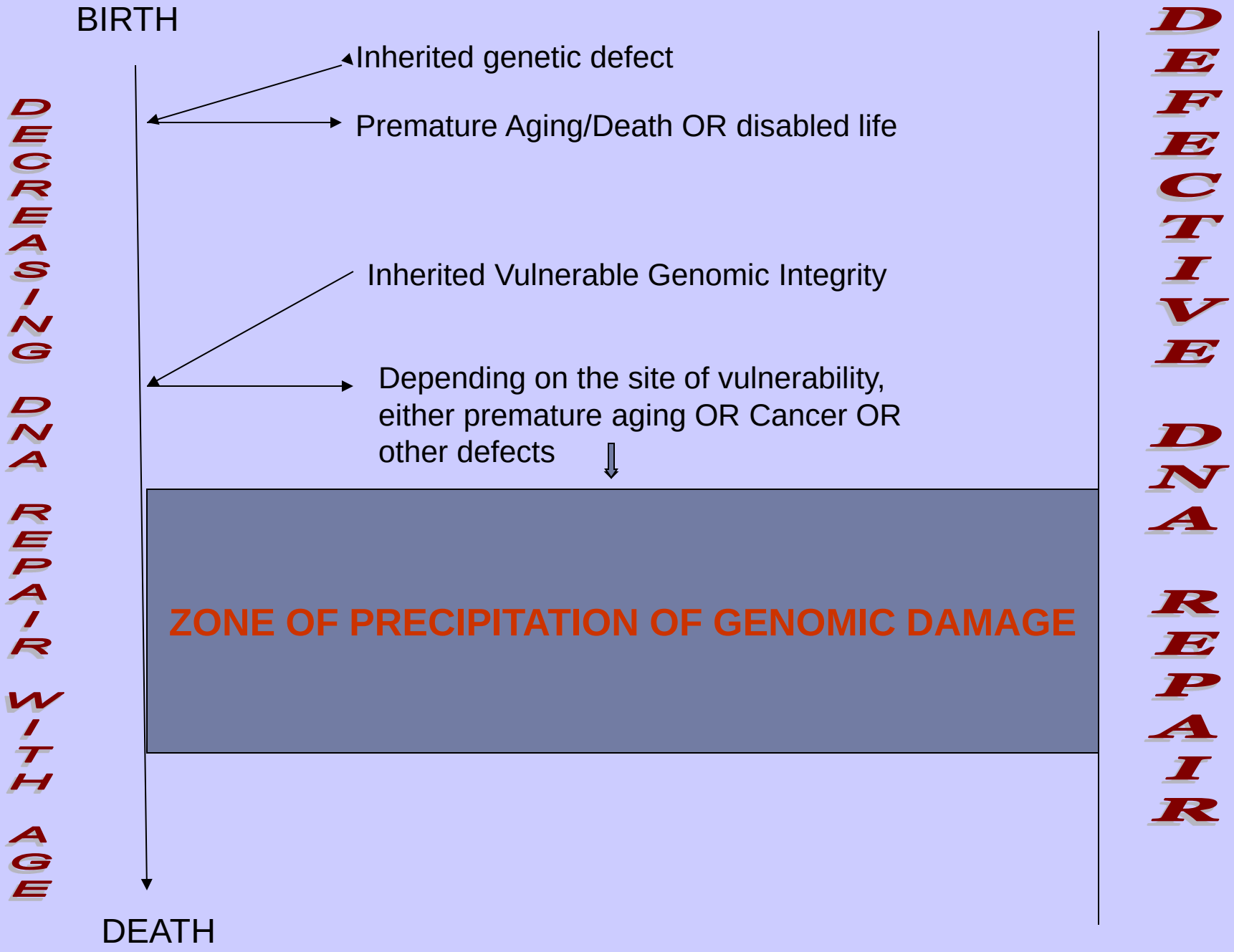

Y
A
O
(A)
101 kDa
DNA ligase I
93 kDa
DNA ligase III
39 kDa
pol β
37 kDa
APE1
42 kDa
β-actin


(B) DNA ligase I protein level
band intensity (arbitrary units)
4000
3000
2000
1000
0
a*
b*
Y
A
O


(C) DNA ligase III protein level
band intensity (arbitrary units)
40000
30000
20000
10000
0
a *
c **
b ***
Y
A
O


(D) pol β protein level
band intensity (arbitrary units)
40000
30000
20000
10000
0
a **
c *
b ***
Y
A
O


(E) APE1protein level
band intensity (arbitrary units)
25000
20000
15000
10000
5000
0
a *
b *
Y
A
O


BIRTH
Inherited genetic defect
Premature Aging/Death OR disabled life
Inherited Vulnerable Genomic Integrity
Depending on the site of vulnerability, either premature aging OR Cancer OR other defects
ZONE OF PRECIPITATION OF GENOMIC DAMAGE
DEATH
DECREASING DNA REPAIR WITH AGE
DEFECTIVE DNA REPAIR

DNA gap oligo duplex model

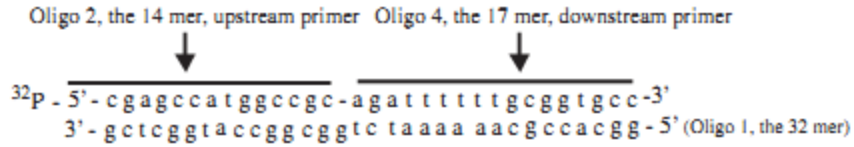


Fig. 1 A model oligo duplex having a 1-nucleotide gap in one of the strands used as substrate for assaying 1-gap repair. It may be noted that the strand with 1 nuceotide gap also has a ^{32}P label on 5′ side.

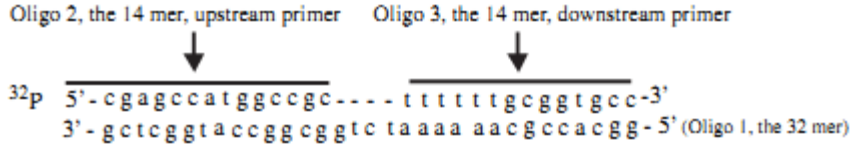
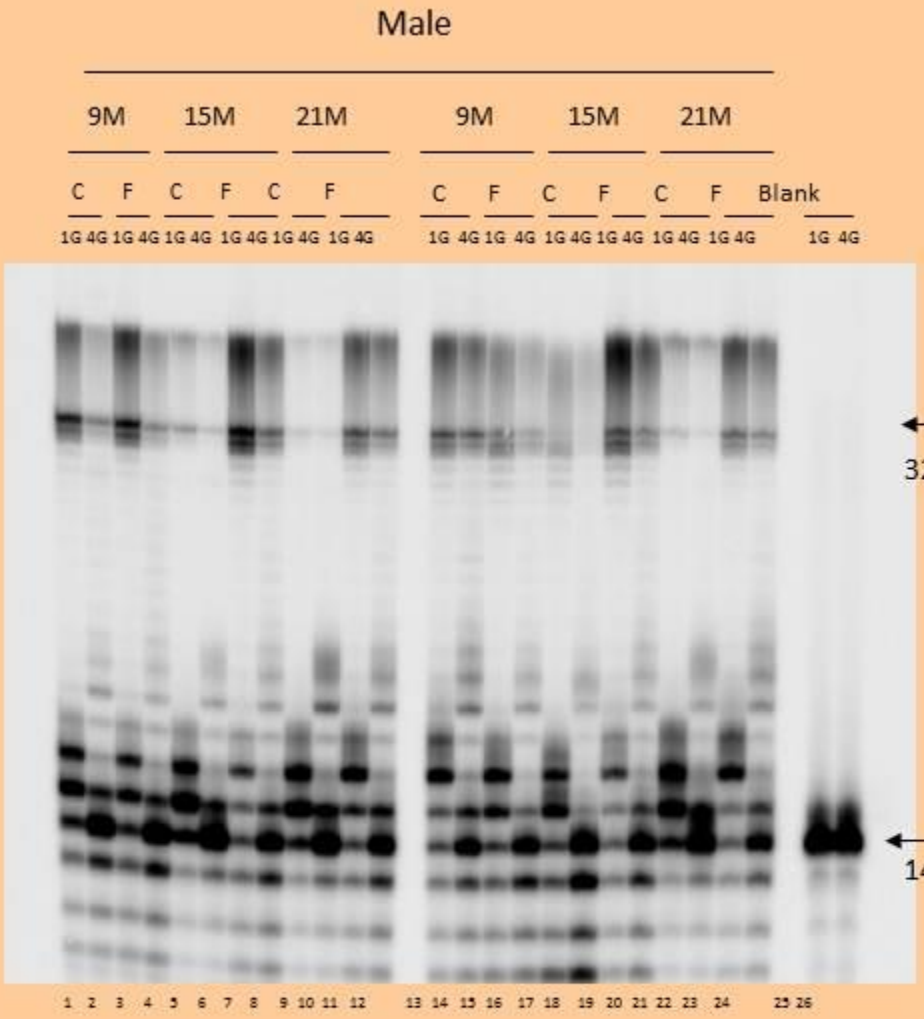
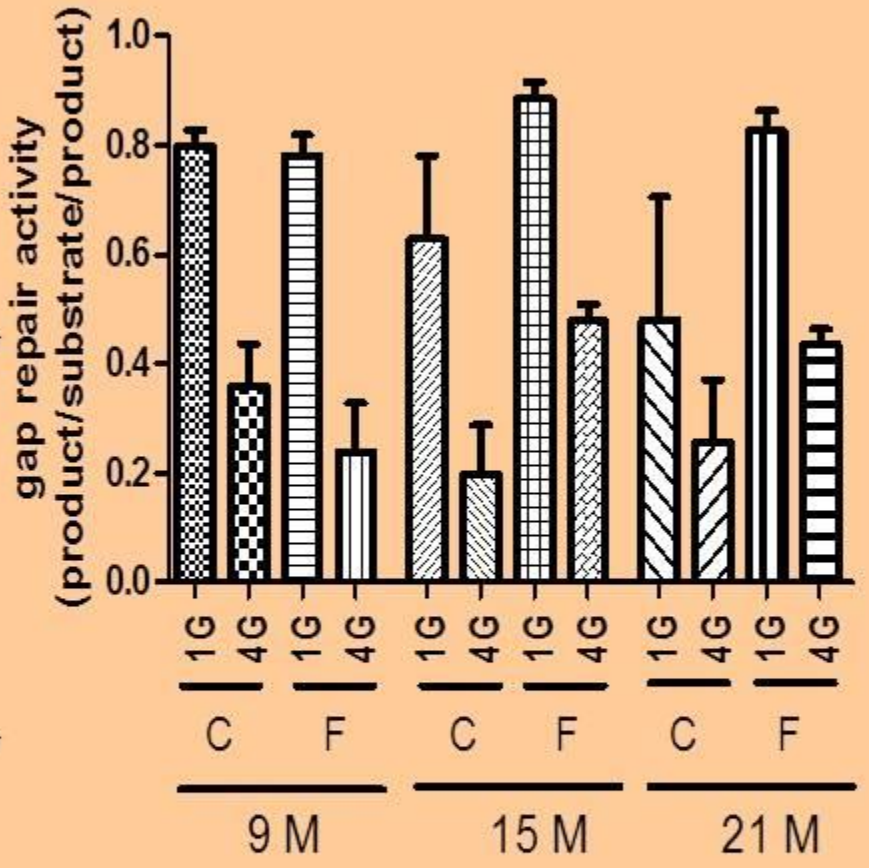


Fig. 2 A model oligo duplex having a 4-nucleotide gap in one of the strands used as substrate for assaying 4-gap repair. It may be noted that the strand with 4-nucleotide gap also has a ^{32}P label on 5′ side.

Krishna et al.2005, Journal of Neurochemistry 92(4): 818-823.

DNA gap repair activity in control and Amalakirasayana fed rat **Testis** with age





“ Take two of these and call me a century from now.”