TA-65:

Telomere maintenance

Epigenetic regulation

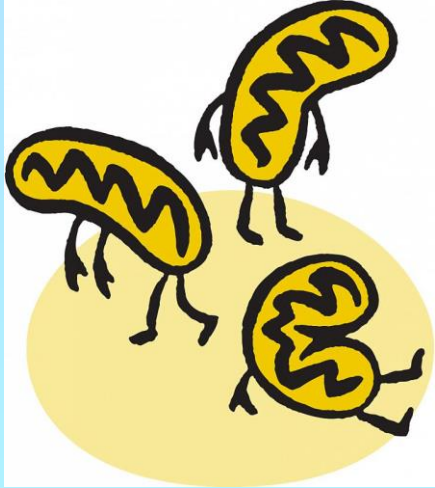
Mitochondrial Protection

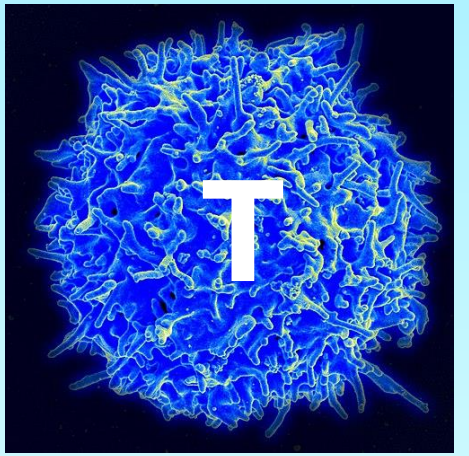
Genome Protection

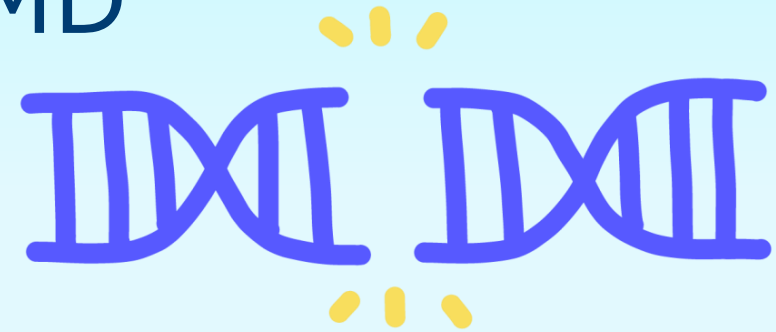
Immune optimization

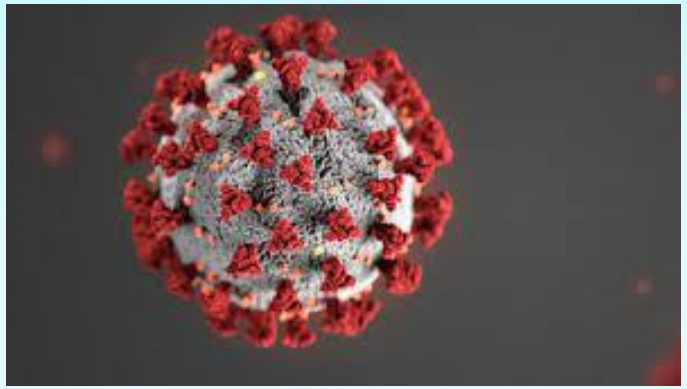
What's new?

Ron Rothenberg MD









TA-65

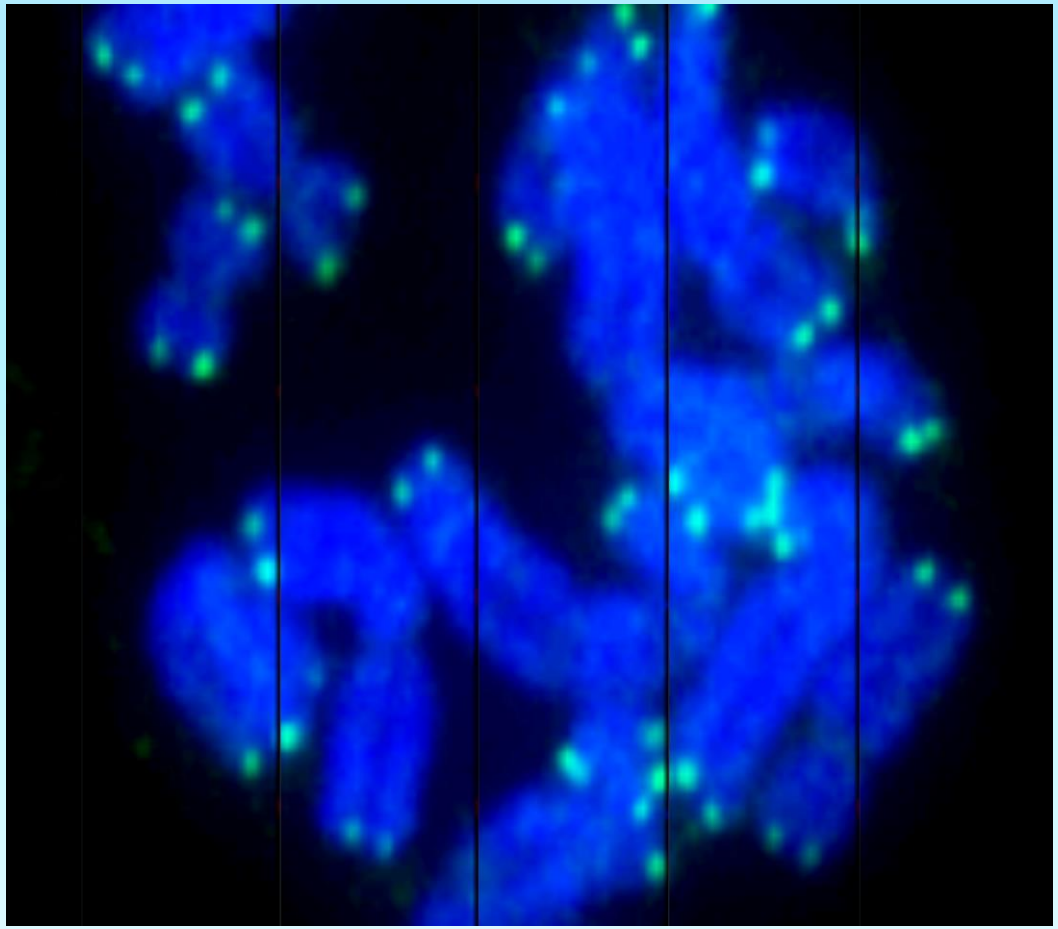
- You probably already know that…
- Telomere loss causes the Hayflick limit
- TA-65 transiently activates telomerase and lengthens telomeres
- TA-65 effectiveness and safety demonstrated in many PEER reviewed studies in humans over the past 20 years

TA-65

- Did you know that ?…
- Non-canonical effects are as important as telomere extension
- TA-65 optimizes epigenetics through TERT
- TA-65 protects mitochondria and is an “intelligent antioxidant”
- TA-65 is a potential cardio and neuro treatment
- TA-65 improves immune senescence
- Patients keep taking TA-65 because they feel good and their telomere testing, and immune status improves

Telomeres

- Region of repetitive nucleotide sequences (TTAGGG) at each end of the chromatid.
- Telomeres act as the cellular aging clock.
- Blackburn et al. Nobel Prize
- Telomere loss is a Major Cause of Cellular Aging, Inflammation and Mutation



Telomere Length Determines Cellular Age

Somatic cells

- Make up > 99% of the cells in the adult body
- Have little or no telomerase and telomeres shorten as we get older.

Telomere Length Shortening:

- **Conception**: Telomeres start out **15,000** base pairs (bp) long.
- By **Birth** the embryo has divided so many times that telomere length is down to **10,000 bp.**
- Over the rest of our lifetime, we lose another **5,000** to **7,000** bp**.**
- When telomere length gets to **3-5,000 bp,** the genome is no longer protected from mutations, the cell can no longer divide, becomes senescent, metabolism slows down, and the cell dies.
- Senescence-Associated Secretory Phenotype - inflammation

Telomeres are the Biological Clock of Aging

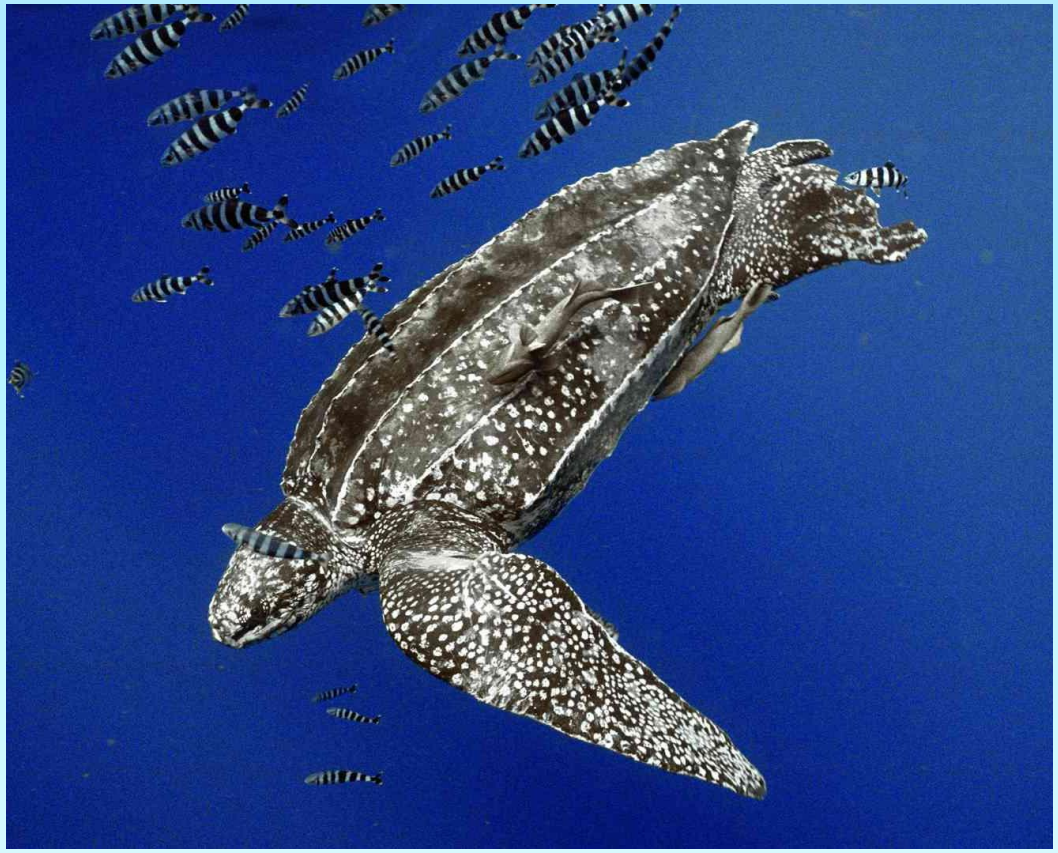
- Organs deteriorate as more and more of their cells die off or enter cellular senescence.
- Shortened telomeres impair immune function that might also increase cancer susceptibility
- Telomere length represents biological age as opposed to chronological age
- Eisenberg DT. An evolutionary review of human telomere biology: the thrifty telomere hypothesis and notes on potential adaptive paternal effects*. Am J Hum Biol.2011. 2, 149-67*

Why is a hydra immortal? Expresses Telomerase

Schaible R. et al. Aging and Potential for Self-Renewal: Hydra Living in the Age of Aging. *Gerontology* 2014;60:548–556

Dermochelys coriacea

Leatherback Sea Turtle



- Virginia Plot et al. Telomeres, Age and Reproduction in a Long-Lived Reptile. *Plos One*. July 2012.Vol 7, Issue 7

What shortens telomeres?

- Aging (except in immortal animals)
- Rapid cellular division in response to infection
- Oxidative stress
- Inflammation
- Lifestyle factors
- Homocysteine
- Hormone deficiencies

To make telomeres longer…

- Activate ***Telomerase***
- Endogenous enzyme
- Stabilizes telomere length
- Adds DNA repeats (TTAGGG) onto the telomeric ends of the chromosomes
- Compensates for the erosion of telomeres when cells divide

Telomerase = TERT +TERC + Shelterin
TERT exerts anti-aging activity

- Preserves telomere integrity
- Modulates gene expression and cellular signaling pathways
- Governs cellular survival, senescence, neurogenesis, and stress resistance

Telomerase

E

Reverse

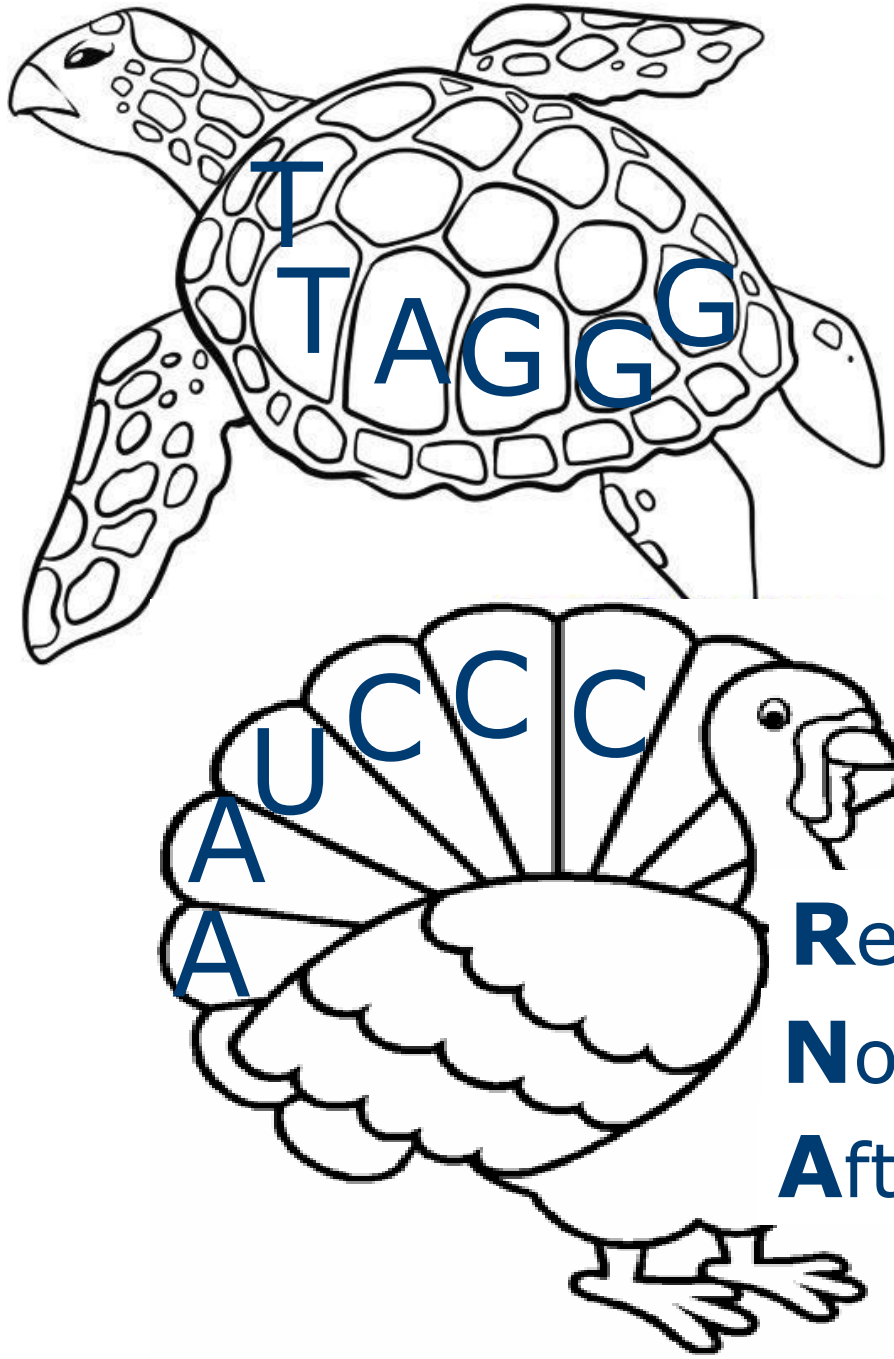
Transcriptase

Telomerase

E

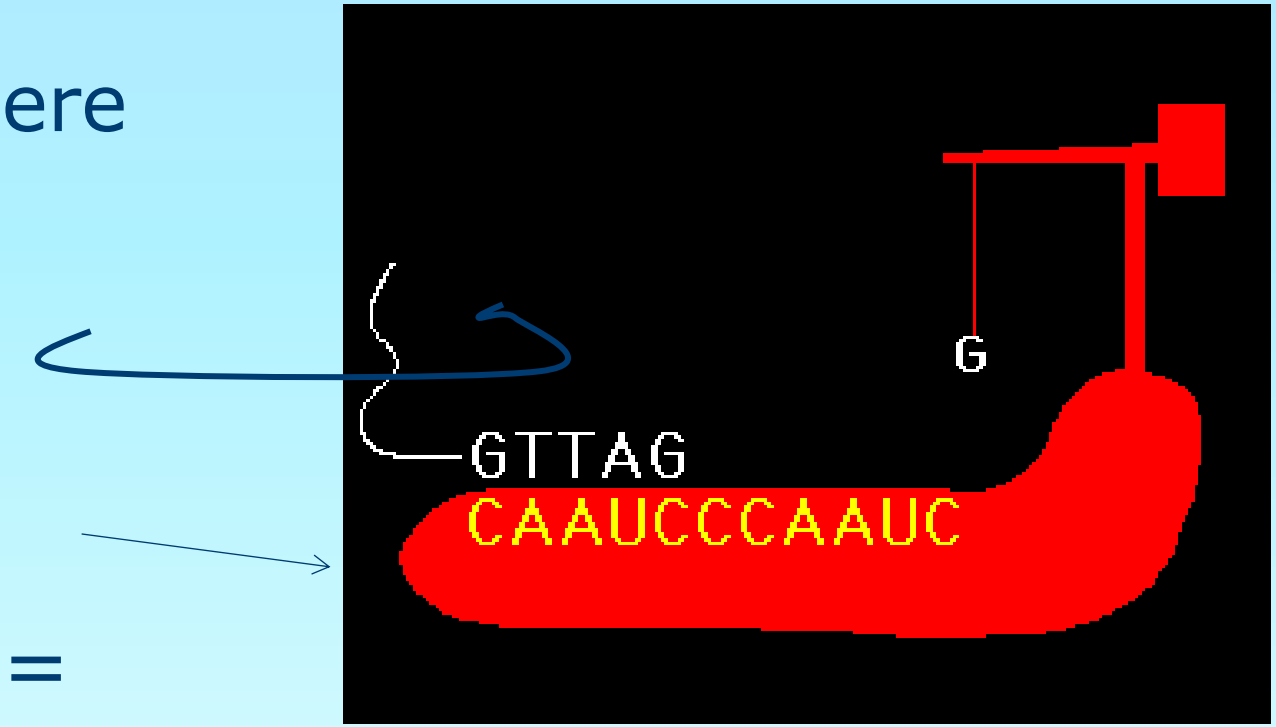
RNA

Component



Telomerase = TERT + TERC

Telomere



Telomerase reverse transcriptase- hTERT

hTERT

hTR = Template = *hTERC*

Telomerase is a “molecular motor” that adds new DNA bases (TTAGGG) onto the ends of telomeres

Telomerase

- Maintains the telomere length
- Promotes genomic integrity, proliferation, and lifespan
- Protects the mitochondria from oxidative stress
- Confers resistance to apoptosis
- **Important for the survival of non-mitotic, highly active cells such as neurons**

- Grin Y et al. Telomerase activity in the various regions of mouse brain: non-radioactive telomerase repeat amplification protocol (TRAP) assay**.** *J Vis Exp.* 2014 Sep 2

Telomeres are the Biological Clock of Aging

- Organs deteriorate as more and more of their cells die off or enter cellular senescence.
- Shortened telomeres impair immune function that might also increase cancer susceptibility
- Telomere length represents biological age as opposed to chronological age
- Eisenberg DT. An evolutionary review of human telomere biology: the thrifty telomere hypothesis and notes on potential adaptive paternal effects. *Am J Hum Biol. 2011. 2, 149-67*

Telomeres and Aging

- 143 normal unrelated individuals over the age of 60 years.
- Shorter telomeres in blood DNA had poorer survival
  - 3.18-fold higher mortality rate from heart disease (95% CI 1.36-7.45, p=0.0079)
  - 8.54-fold higher mortality rate from infectious disease (1.52-47.9, p=0.015)
- Telomere shortening in human beings contributes to mortality in many age-related diseases.

- Cawthon RM et al. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*. 2003 Feb 1;361(9355):393-5.

Telomere Length, Mortality, Disease

- N= 472, 432
- Reduced LTL was associated with increased
  - Overall mortality (HR, 1.08; 95% CI, 1.07-1.09)
  - Cardiovascular (HR, 1.09; 95% CI, 1.06-1.12)
  - Respiratory (HR, 1.40; 95% CI, 1.34-1.45)
  - Digestive (HR, 1.26; 95% CI, 1.19-1.33)
  - Musculoskeletal (HR, 1.51; 95% CI, 1.35-1.92)
  - COVID-19 (HR, 1.15; 95% CI, 1.07-1.23) mortality
- Schneider, C et al. Association of Telomere Length With Risk of Disease and Mortality. *JAMA Intern Med.* 2022 Mar 1;182(3):291-300.

The Impact of Telomere Shortening

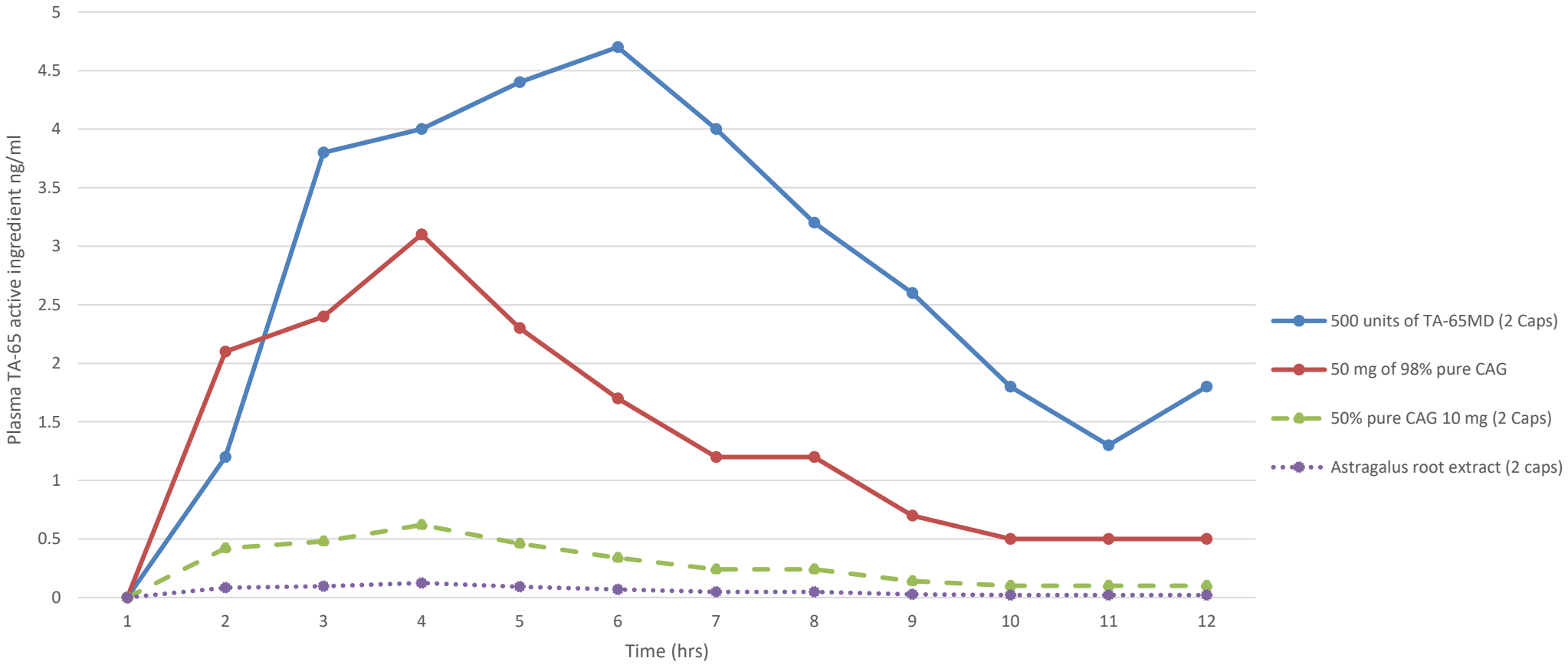
- "Short telomeres are associated with increased risks for human bladder, head, neck, lung, and renal cell cancers”
- Fraternal twins with the shortest telomeres had a three times greater risk of death than their co-twins with the longest telomere measurements

- Wu X et al. Telomere Dysfunction: A Potential Cancer Predisposition Factor (2003) *J National Cancer Inst.* 2003 Aug 20;95(16)1211-18.
- Johansson, S et al. Telomere length predicts survival independent of genetic influences (2007) *Aging Cell*, 2007.

Astragalus

- Chinese medicine for more than 2,000 years for healing and diabetes
- Primary chemical constituent is cycloastragenol (CAG) (TAT2)
- Used as a tonic to improve:
  - Lungs
  - Adrenal glands
  - Gastrointestinal tract
  - Metabolism
  - Healing
  - Fatigue

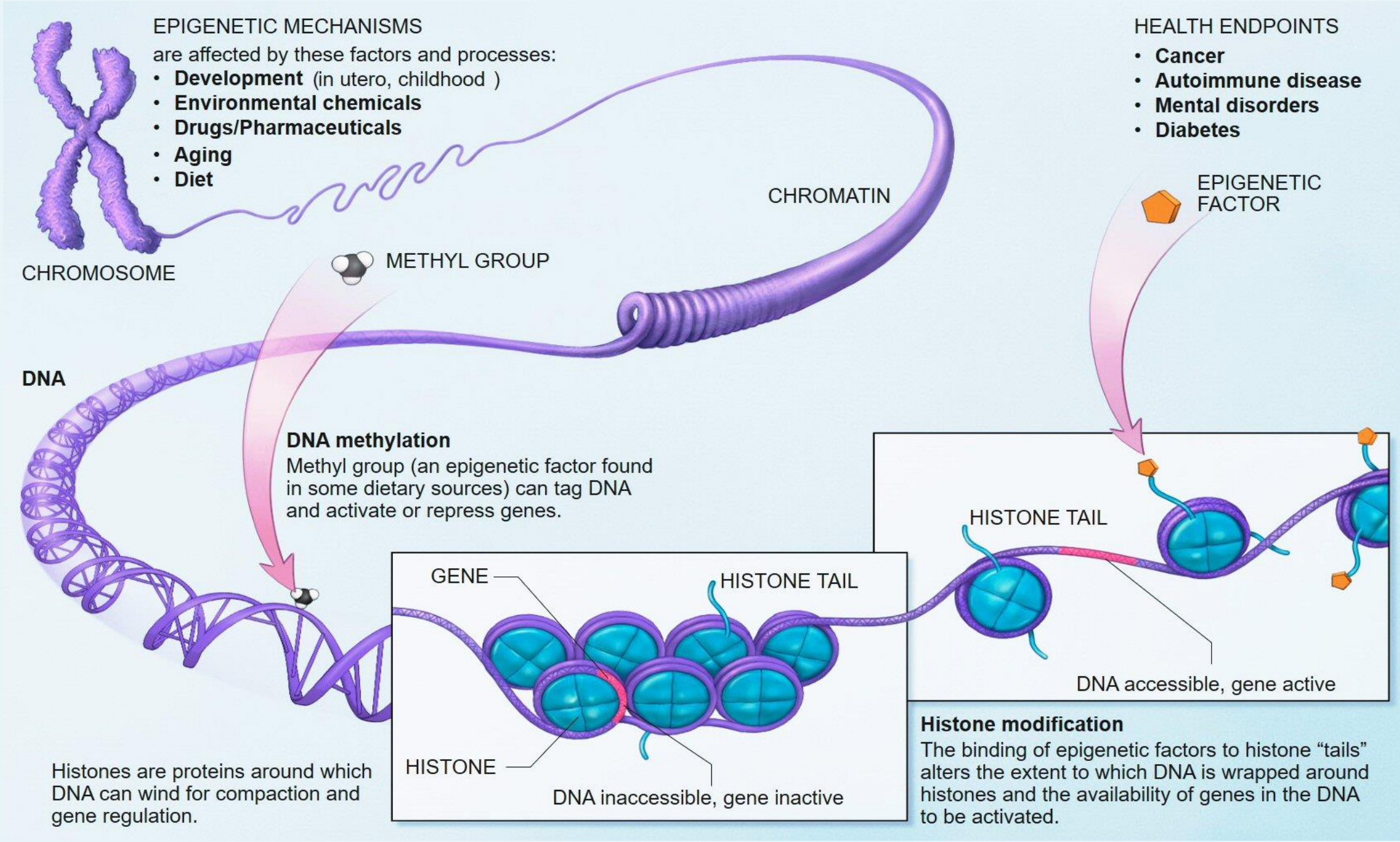
Bioavailability of TA-65



TA-65MD provides more than five times the bioavailability as compared to 98% pure CAG.

Epigenetics

- The study of changes in gene expression that do not involve changes to the underlying DNA sequence
- Change in phenotype without a change in genotype — which affects how cells read the genes.
- Epigenetic clock – biomarker of aging
- Measures DNA methylation, histones and other biomarkers



https://upload.wikimedia.org/wikipedia/commons/d/dd/Epigenetic_mechanisms.jpg

TERT and Epigenetics

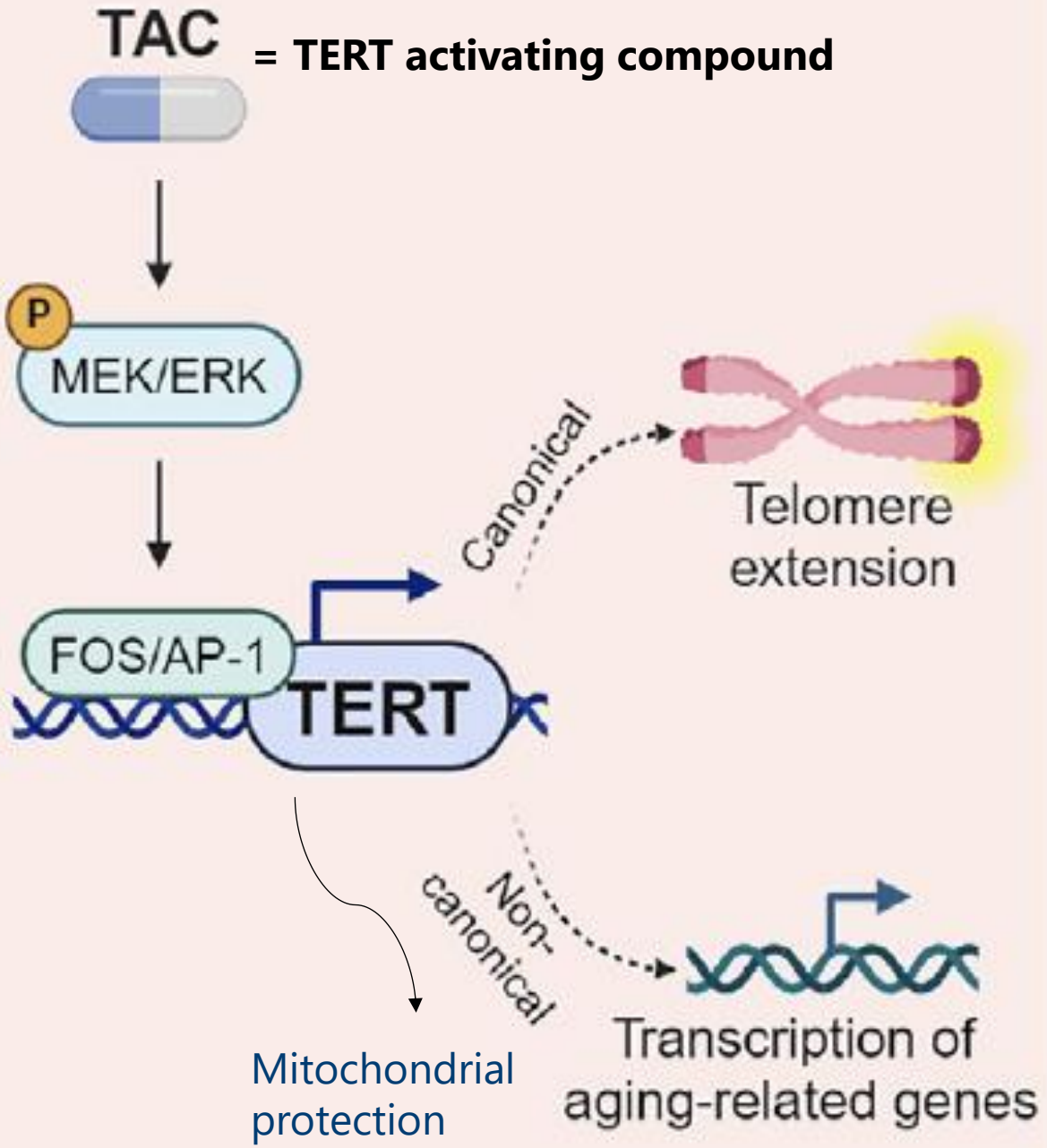
- TAC =TERT Activating Compound
- Used in Shim study to activate telomerase (as does TA-65)
- TERT is linked to all hallmarks of aging
- TERT gene is epigenetically repressed with aging
- TAC restores TERT levels to promote telomere maintenance and reprogram gene expression
- TAC in aged mice reduces senescence

- Shim et al., TERT activation targets DNA methylation and multiple aging hallmarks, *Cell* (2024)

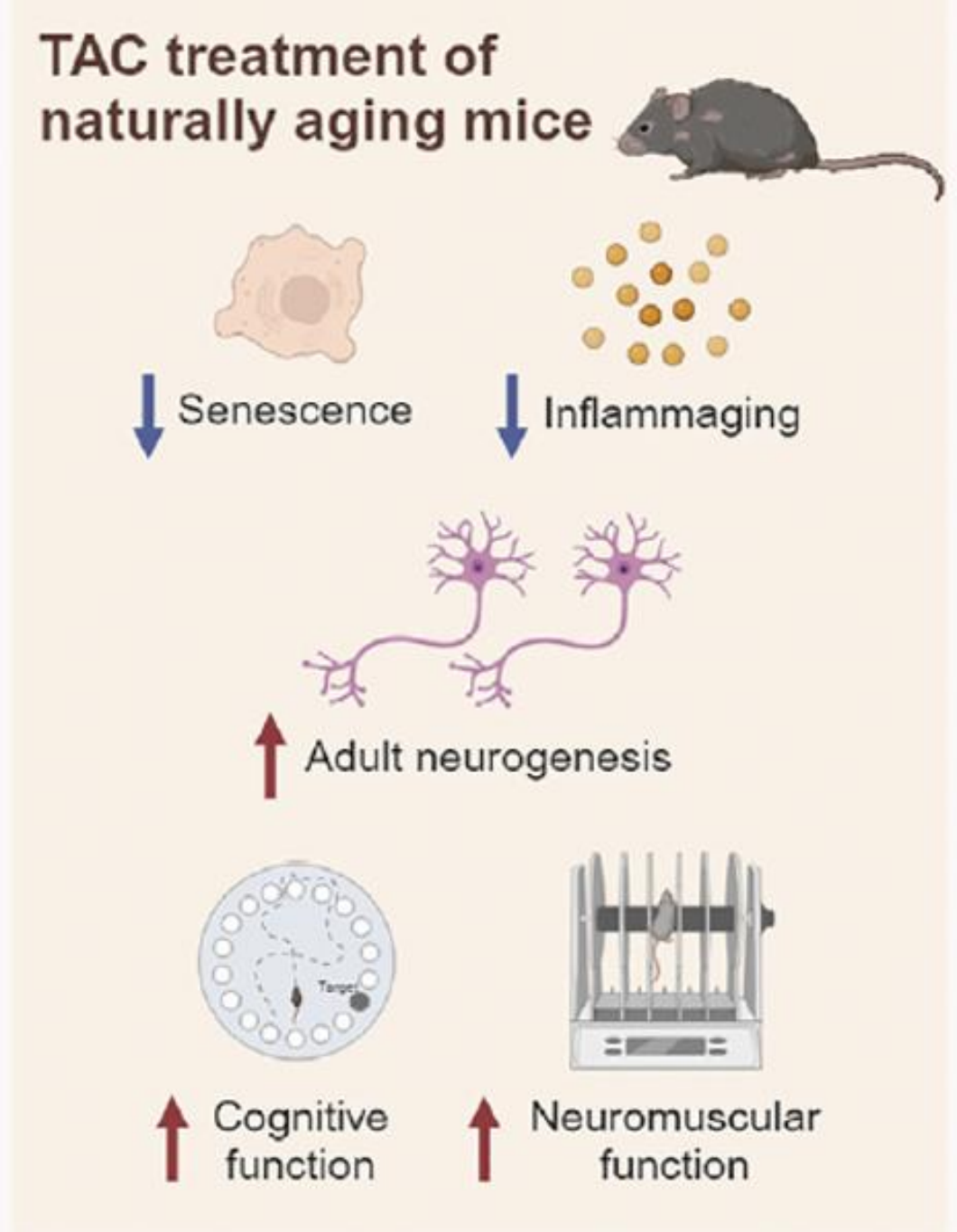
TAC and Aging

- TAC administration in very aged mice alleviates multiple aging hallmarks such as **cellular senescence** and **systemic inflammation**
- Promotes new neuron formation with improved cognitive ability
- Enhances neuromuscular function
- Well tolerated with no evidence of toxicity
- Brain
  - Increases neurotrophic factors
  - Stimulates adult neurogenesis
  - Preserves cognitive function
  - Without evident toxicity including cancer risk
- TERT activation is a strategy to mitigate multiple aging hallmarks and associated pathologies

- Shim et al, TERT activation targets DNA methylation and multiple aging hallmarks, *Cell* 2024



Shim et al., TERT activation targets DNA methylation and multiple aging hallmarks, *Cell* (2024)



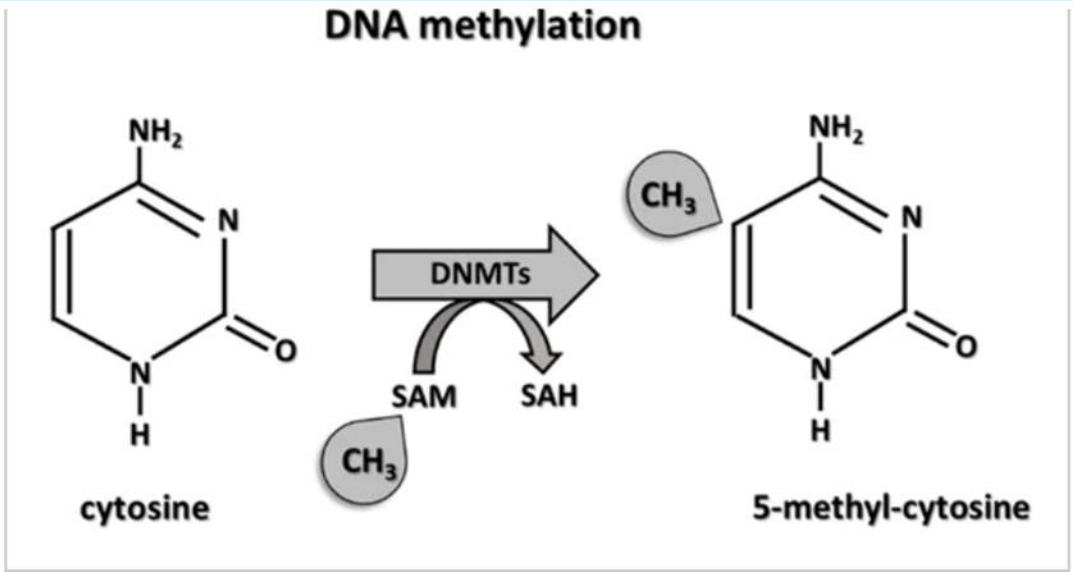
Shim et al., TERT activation targets DNA methylation and multiple aging hallmarks, *Cell* (2024)

What are DNMTs = DNA Methyl transferases?

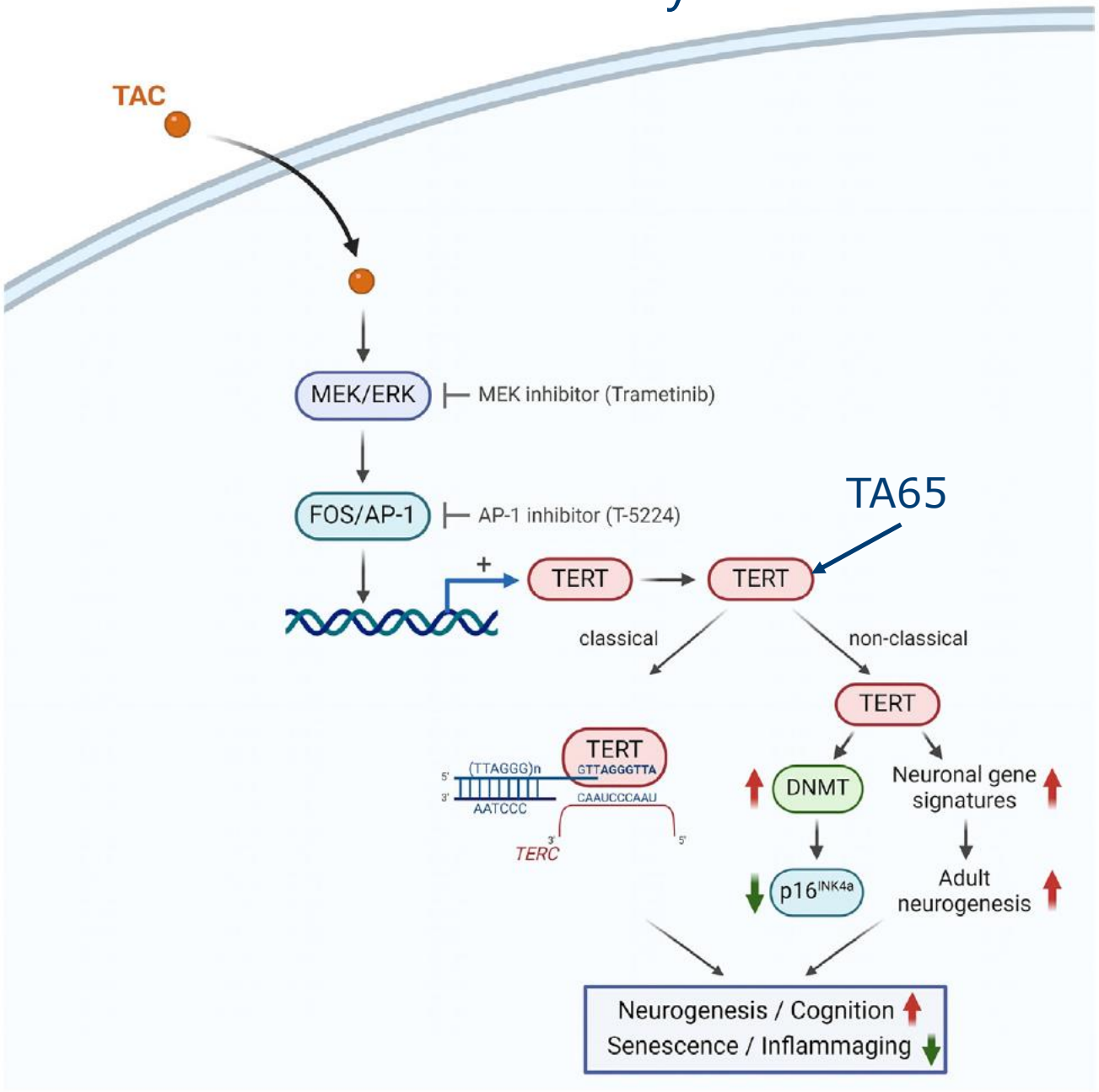
- DNMTs are the enzymes that catalyze the transfer of a methyl group from SAM to the 5-carbon position of cytosine residues
- Several types of DNMTs have been discovered, including DNMT1, DNMT3A, and DNMT3B, each with specific roles in establishing and maintaining DNA methylation patterns.
- DNA methylation plays a critical role in brain function, including neurogenesis, neuronal differentiation, synaptogenesis, learning, and memory

SAM = S-adenosylmethionine
SAH = S-adenosylhomocysteine

- Ciechomska M et al. DNA Methylation as a Future Therapeutic and Diagnostic Target in Rheumatoid Arthritis. *Cells*. 2019 Aug
- Xie J et al. Dynamic Regulation of DNA Methylation and Brain Functions. *Biology* (Basel). 2023 Jan



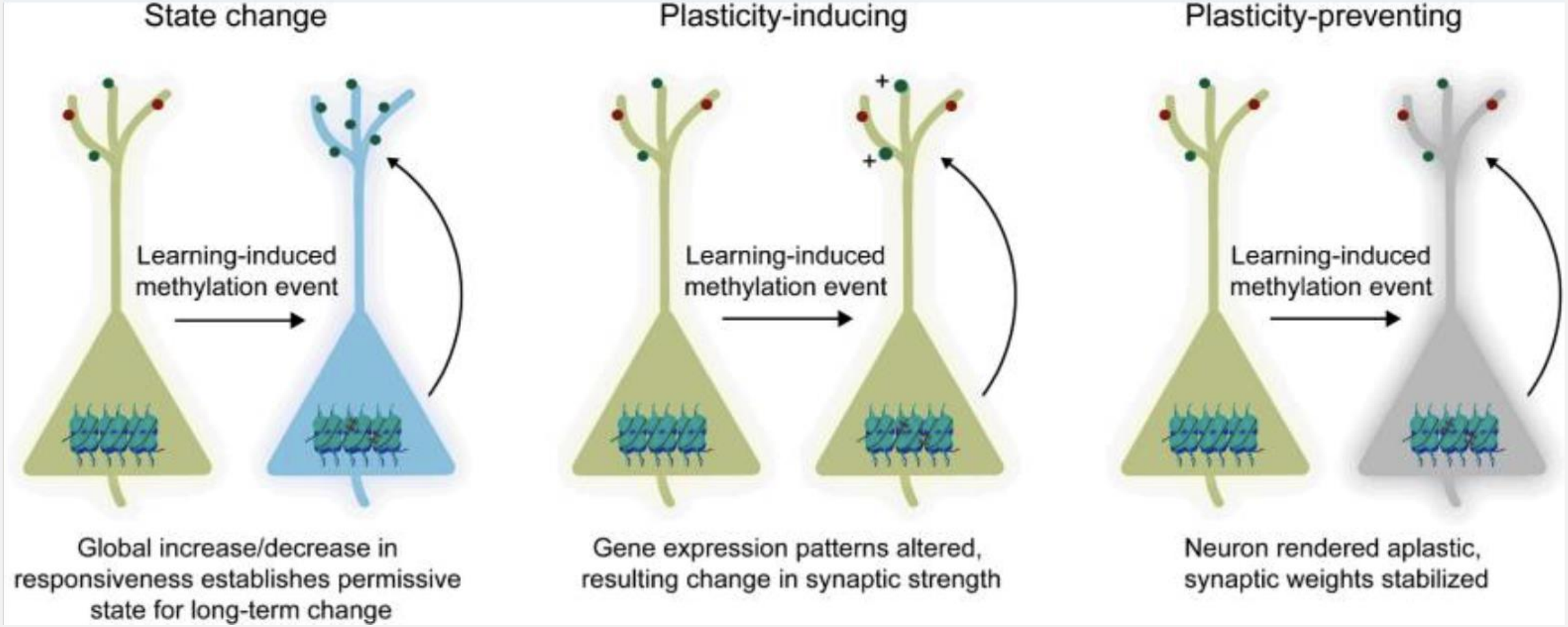
DNMT = DNA Methyl transferase



- TERT induction reduces tissue senescence by **DNMT3B-mediated promoter hypermethylation**
- Reduces inflammation
- Enhances adult neurogenesis and cognitive function by promoting hippocampal transcriptomic signatures.

- Shim et al., TERT activation targets DNA methylation and multiple aging hallmarks, *Cell* (2024)

Methylation - Responsible for memory



Day et al. DNA methylation and memory formation. *Nat Neurosci*. 2010 Nov

When telomeres become critically short
Loss of genome protection 3 bad options:

- Senescence
- Senescence associated secretory phenotype = SASP = Inflammation
- Genomic Instability: Mutation that can lead to malignancy


SASP

Telomerase protects DNA

- Adds TTAGGG to prevent replicative senescence and prevent Senescence Associated Secretory Phenotype (SASP)
- SASP induces chronic inflammation
  - Telomerase prevents inflammation
- What about post-mitotic cells?
- What shape is mitochondrial DNA?
- Telomerase has essential role in mtDNA and in non-replicating cells

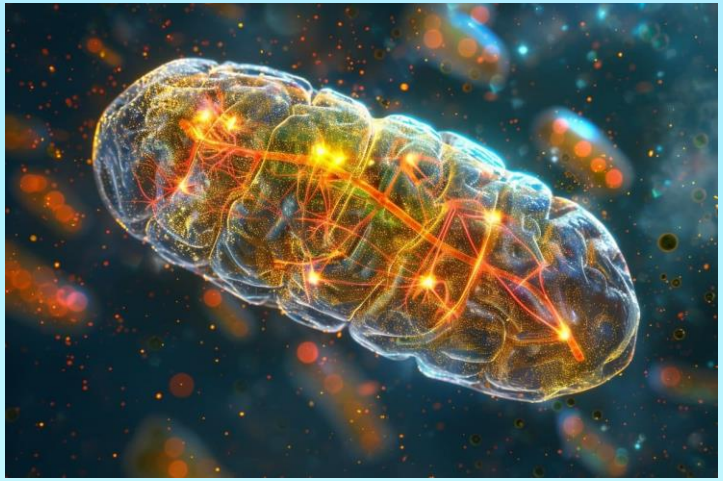
- Sikora E et al. Cellular senescence in ageing, age-related disease and longevity. *Curr Vasc Pharmacol*. 2014;12(5):698-706.

Mitochondrial DNA

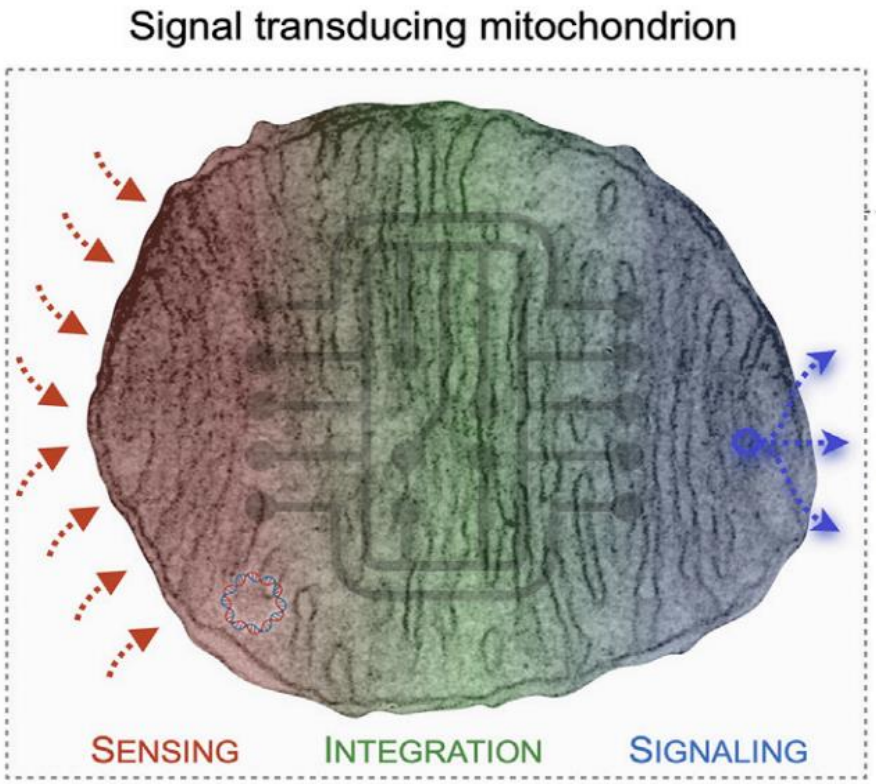
"You are only as good as your mitochondria"

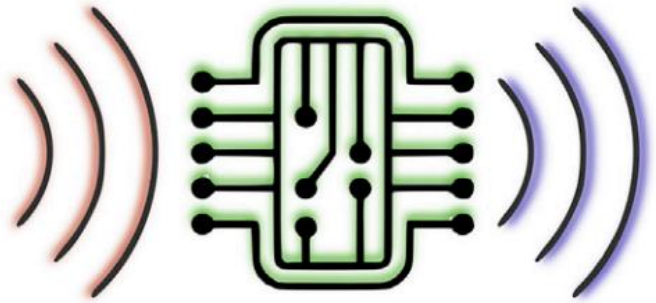
Mitochondria

- 10–20% of TERT resides within the mitochondria under normal physiological conditions
- Increases to ~80% in the presence of high ROS.



- Sharma, N. K. et al (2012). Human telomerase acts as a hTR-independent reverse transcriptase in mitochondria. *Nucleic Acids Research*
- Ahmed S. et al (2008). Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. *Journal of Cell Science*, 2008





"Mitochondria are the processor of the cell"

Mitochondria not only are the "batteries" of the cell producing ATP

Mitochondria also are the information processors of the cell – cellular communication

Picard, Martin et al.
Mitochondrial signal transduction *Cell Metabolism,* November 2022

“Intelligent Antioxidant”

- Free radical damage is one of the fundamental causes of the degeneration of aging
- In anti-aging medicine we attempt to control ROS with anti-oxidants, coenzyme Q 10, vitamin C, stimulating glutathione etc.
- Non-canonical actions
- Telomerase connection:
  - Shuttling of TERT from nucleus to mitochondria as needed.
  - If mitochondria are protected against free radicals there is more TERT to protect nucleus.
  - Increased oxidative stress shortens telomeres
  - Prevents vicious cycle

hTERT is “Dual Targeted”

- Nucleus - prevents telomere erosion leading to senescence and genomic instability
- Also protects terminally differentiated post mitotic cells
- Mitochondria – has a different function since mitochondrial DNA does not contain telomeric structures.
- Non telomeric activities hTERT in the nucleus:
  - Cell cycle regulation
  - Modulation of cellular signaling and gene expression,
  - Augmentation of proliferative lifespan as well as DNA damage responses
- Mitochondrial hTERT
  - Reduces reactive oxygen species, DNA damage and apoptosis

- Ale-Agha N et al. Cellular functions of the dual-targeted catalytic subunit of telomerase, telomerase reverse transcriptase--potential role in senescence and aging. *Exp Gerontol.* 2014 Aug;56:189-93.

Non-canonical roles of TERT

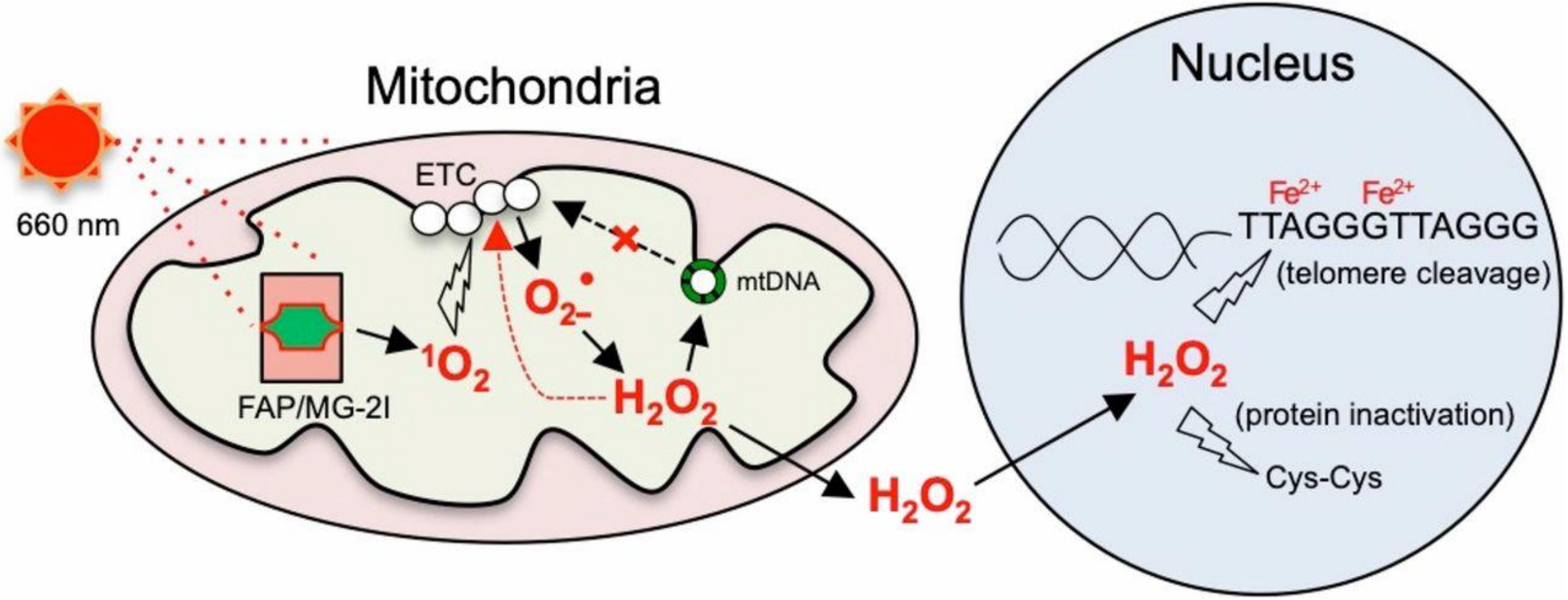
- Non-canonical activities of TERT show
  - Cellular protective effects
  - Nuclear TERT protects against cell death following **double-stranded DNA damage**, **independent** of its role in telomere length maintenance.
- Emerging non-canonical roles of TERT
  - Regulation of non-telomeric DNA damage responses
  - Promotion of cell growth and proliferation
  - Acceleration of cell cycle kinetics
  - Control of mitochondrial integrity following oxidative stress

- Thompson, C et al. Non-canonical Functions of Telomerase Reverse Transcriptase: Emerging Roles and Biological Relevance. *Curr Top Med Chem*. 2020;20(6):498-507.

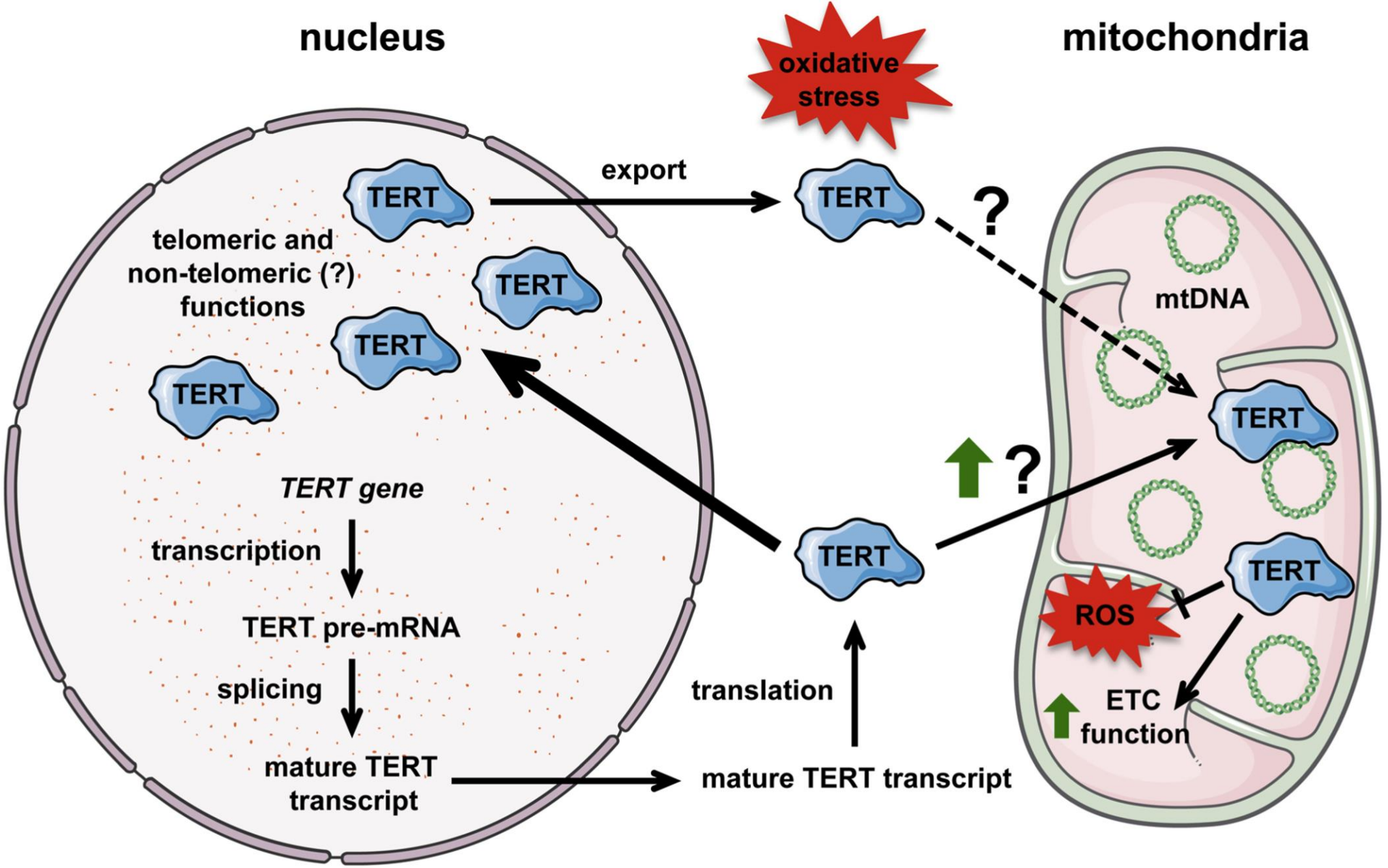
Telomerase Protects Nuclear DNA from Mitochondrial ROS

- Singlet oxygen induces oxidative damage to mitochondrial electron transport chain (ETC)
- Initiates a secondary wave of superoxide and hydrogen peroxide generation
- Hydrogen peroxide generated by mitochondria damages mtDNA, which amplifies the damage to the Electron Transport Chain
- No overall nuclear DNA damage with adequate telomeres and telomerase

- Wei Qian et al. *PNAS* 2019;116:37



Wei Qian et al. *PNAS* 2019;116:37



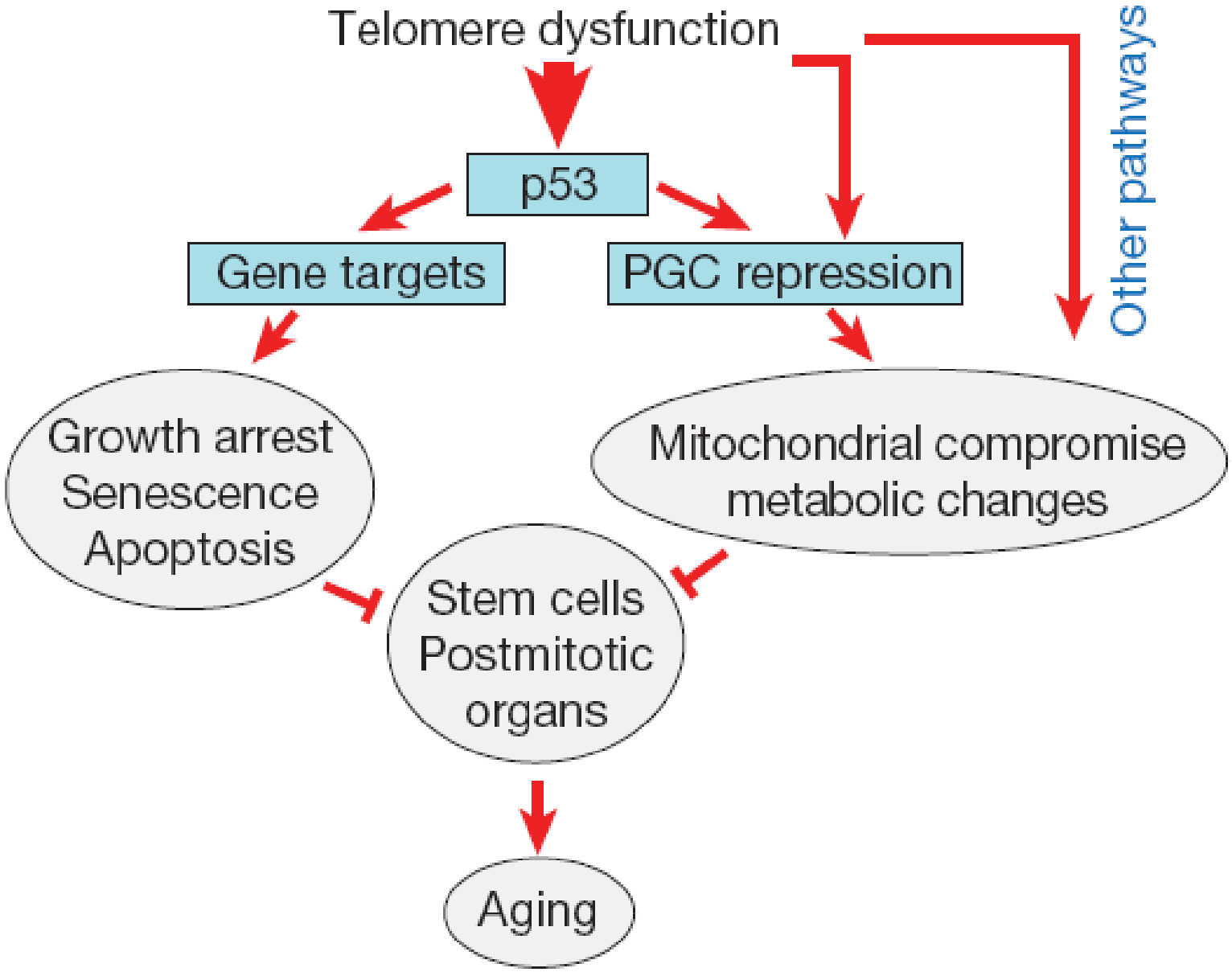
Rosen J et al. Non-canonical functions of Telomerase Reverse Transcriptase – Impact on redox homeostasis. *Redox Biology* July 2020,

- Mitochondrial TERT--
  - Reduces ROS production
  - Protects mtDNA against damage
  - Inhibits apoptosis
  - Enhances respiratory chain activity particularly complex 1
- Most important for mitochondrial protection in rapid metabolism post mitotic tissues like the heart

- Ale-Agha N et al. Cellular functions of the dual-targeted catalytic subunit of telomerase, telomerase reverse transcriptase--potential role in senescence and aging. *Exp Gerontol.* 2014 Aug;56:189-93.

Telomere Dysfunction, **PGC-1α** and Mitochondrial Compromise

- Telomere dysfunction activates p53-mediated cellular growth arrest, senescence and apoptosis
- Telomere dysfunction drives progressive atrophy and functional decline in high-turnover tissues.
- Telomere dysfunction leads to Peroxisome Proliferator-activated receptor gamma, coactivator 1 alpha **(PGC-1α**) **repression** leads to senescence through…
- **Impaired mitochondrial biogenesis and function**, decreased gluconeogenesis, cardiomyopathy, and increased reactive oxygen species
- Ergün Sahin et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature.* 2011 February 17; 470(7334): 359–365.

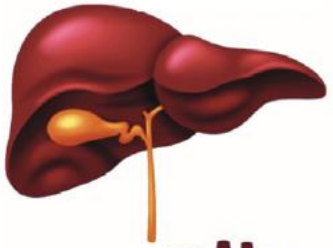


Ergün Sahin et al. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature.* 2011 February 17; 470(7334): 359–365.

PGC-1 alpha

Adipose tissue

Adaptative thermogenesis
Fatty acid oxidation
Differentiation to brown adipose tissue

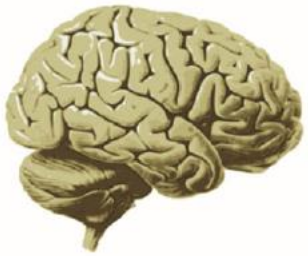


Liver

β-Oxidation of fatty acids
Gluconeogenesis
Regulation of TCA cycle

Heart

Fatty acid oxidation
Glucose metabolism
ATP production
Contractile function



Cerebrum

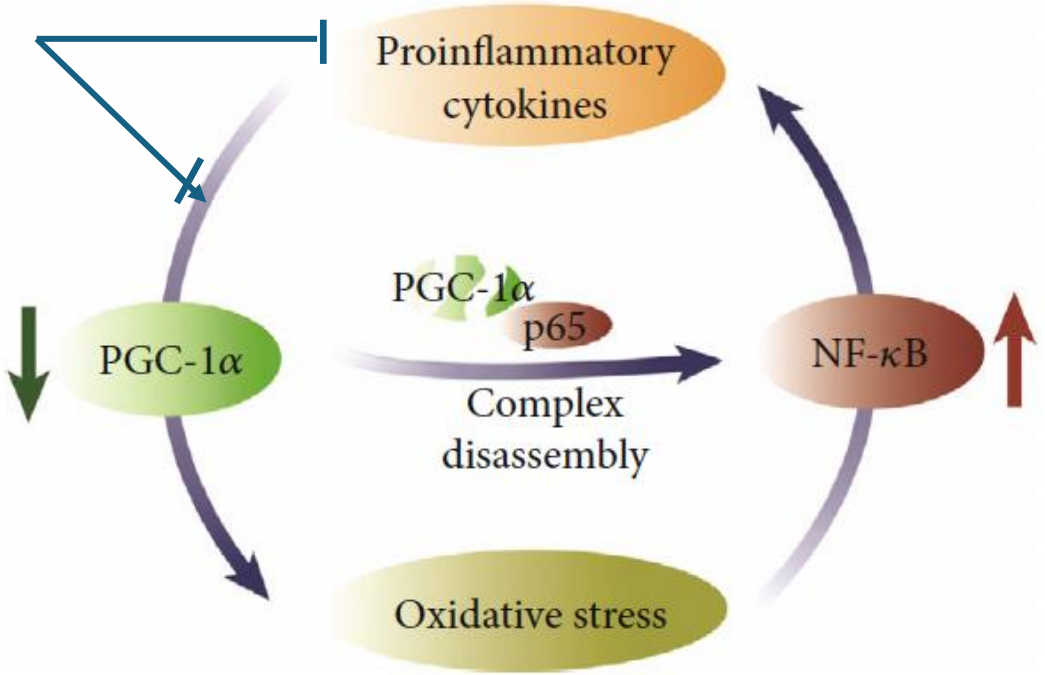
Neuronal activity
ATP production
Regulation of sodium pumps in astrocytes
Regulation of neurofilaments proteins

Skeletal muscle

ATP production
Energetic metabolism
Endurance exercise adaptation

Rius-Pérez S et al. PGC-1α, Inflammation, and Oxidative Stress: An Integrative View in Metabolism. Oxid Med Cell Longev. 2020 Mar 2020 Mar

Telomerase
TERT



Published Peer reviewed TA-65 papers

- Salvador L, et al. A Natural Product Telomerase Activator **Lengthens Telomeres in Humans:** A Randomized, Double Blind, and Placebo Controlled Study. *Rejuvenation Res*. 2016 Mar 30.

- Dow CT, Harley CB Evaluation of an oral telomerase activator for early **age-related macular degeneration** - a pilot study.. *Clin Ophthalmol*. 2016 Jan 28;10:243-9.

- Reichert S, Bize P, Arrivé M, Zahn S, Massemin S, Criscuolo Experimental increase in telomere length leads to **faster feather regeneration**. F. *Exp Gerontol*. 2014 Apr;52:36-8.

- Harley CB, Liu W, Flom PL, Raffaele JM. A natural product telomerase activator as part of a health maintenance program: **metabolic and cardiovascular** response. *Rejuvenation Res.* 2013 Oct;16(5):386-95.

- Molgora, B et al. Functional assessment of pharmacological telomerase activators in human T cells. *Cells*. 2013 Jan 14;2(1):57-66.

- Bernardes de Jesus B, Schneeberger K, Vera E, Tejera A, Harley CB, Blasco MA. The telomerase activator TA-65 **elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence.** *Aging Cell.* 2011 Aug;10(4):604-21.
- **Harley CB**, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, Raffaele JM. A natural product telomerase activator as part of a health maintenance program. *Rejuvenation Res.* 2011 Feb;14(1):45-56.
- Fernandez M et al. TA-65, A Telomerase Activator **improves Cardiovascular Markers** in Patients with Metabolic Syndrome. *Current Pharmaceutical Design*, 2018, 24, 1-7
- Kokubun T et al Telomerase Plays a Pivotal Role in **Collateral Growth Under Ischemia** by Suppressing Age-Induced Oxidative Stress, Expression of p53, and Pro-Apoptotic Proteins Collateral Growth Related to Telomerase.. *Int Heart J* 2019; 60: 736-745
- Eyolfon E et al. Sexually Dimorphic Behavioral and Genetic Outcomes Associated With Administration of TA65 (A Telomerase Activator) Following Repetitive **Traumatic Brain Injury**: A Pilot Study**.** *Front Neurol* . 2020 Feb 18;11:98.
- Wan, T et al. Increased telomerase improves motor function and alpha-synuclein pathology in a transgenic mouse model of **Parkinson's disease** associated with enhanced autophagy. *Prog Neurobiol.* 2021 Apr; 199: 101953.
- Maier, Rebecca et al. Telomerase Activation to Reverse Immunosenescence in Elderly Patients With **Acute Coronary Syndrome**: Protocol for a Randomized Pilot Trial**.** *JMIR Res Protoc*. 2020 Sep 23;9(9):e19456

Gunasekaran Singaravelu , Calvin B Harley , Joseph M Raffaele , Pratheesh Sudhakaran , Anitha Suram. Double-Blind, Placebo-Controlled, Randomized Clinical Trial Demonstrates **Telomerase Activator TA-65 Decreases Immunosenescent CD8+CD28- T** Cells in Humans. *OBM Geriatrics* 2021, volume 5, issue 2

Alshinnawy A et al. Astragalus *membranaceus* and *Punica granatum* alleviate infertility and kidney dysfunction induced by aging in male. Turk J Biol. (2020) 44: 166-175

TA-65 restored youthful levels of sperm function and kidney function in old rats

Ameera Saeed Alshinnawy. **Telomerase activator-65** and pomegranate peel **improved the health status of the liver in aged rats**; multi-targets involved. Iran J Basic Med Sci . 2021 Jun

Gabriele Saretzki· **Telomerase in Brain**: The New Kid on the Block and Its Role in Neurodegenerative Diseases *Biomedicines.* 2021 May.

Niloofar Ale-Agha. Mitochondrial Telomerase Reverse Transcriptase **Protects From Myocardial Ischemia/Reperfusion** Injury by Improving Complex I Composition and Function. 2021 Dec 2021 10.1161/*CirculationAHA*.

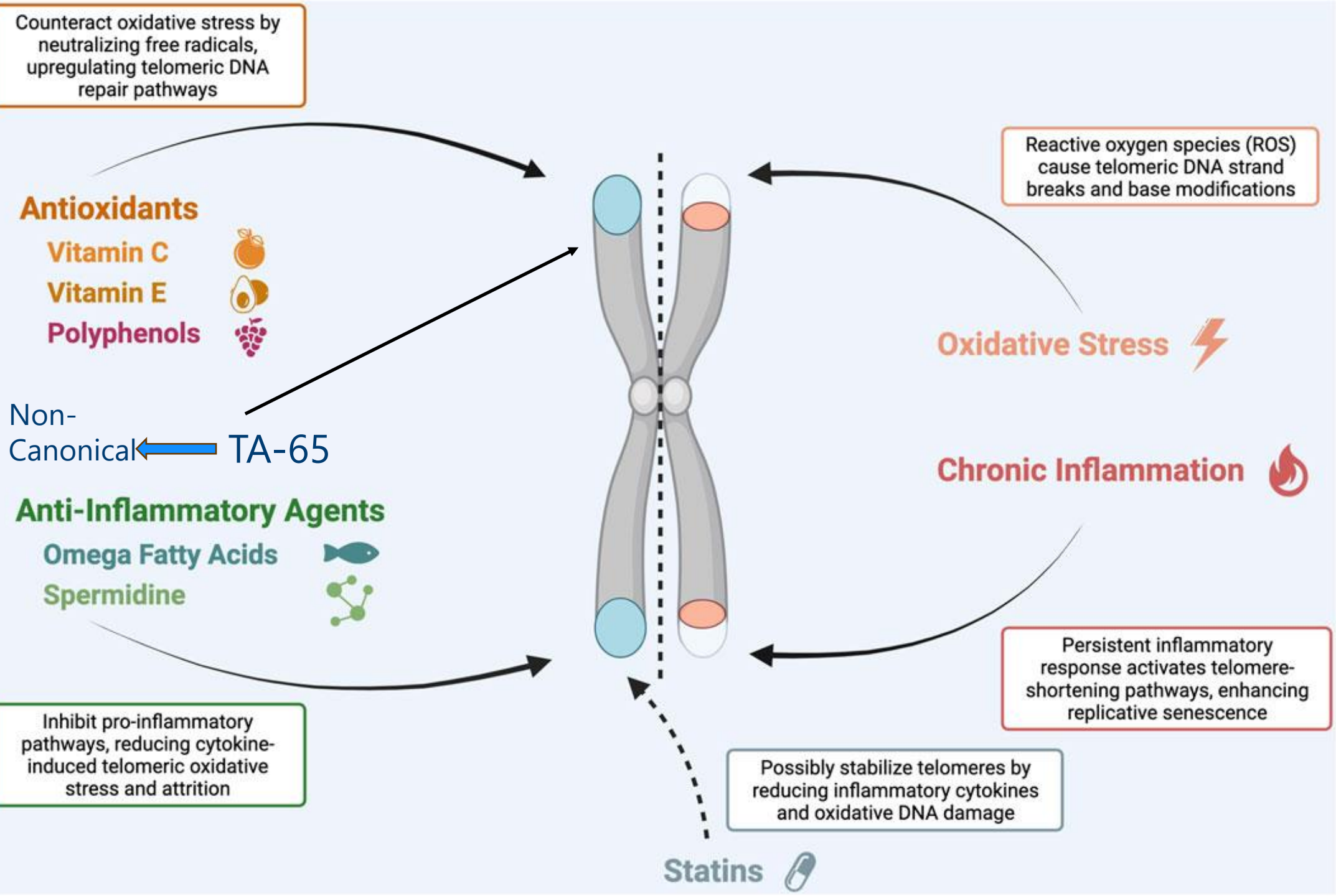
De Sousa Lages, A et al. Therapeutics That Can Potentially Replicate or **Augment the Anti-Aging Effects of Physical Exercise.** *Int. J. Mol. Sci.* 2022

Bawamia B. et al. Activation of telomerase by TA-65 enhances immunity and reduces inflammation post myocardial infarction. Geroscience . 2023 Apr.

Tiendrébéogo, A.J. *et al.* The telomerase activator **TA-65 protects from cigarette smoke-induced small airway remodeling in mice through extra-telomeric effects**. *Sci Rep* (2023)

2024 Peer Reviewed Articles

- Schellnegger M et al. Unlocking **longevity**: the role of telomeres and its targeting interventions. *Front Aging*. 2024
- Li S et al. Upregulation of mitochondrial telomerase reverse transcriptase **mediates the preventive effect of physical exercise** on pathological cardiac hypertrophy via improving mitochondrial function and inhibiting oxidative stress. *Biochim Biophys Acta Mol Basis Dis*. 2024 Jan
- Shim et al., TERT activation targets DNA **methylation** and multiple aging hallmarks, *Cell* (2024)



Schellnegger M et al. Unlocking longevity: the role of telomeres and its targeting interventions *Front. Aging*, 24 January 2024 Sec. Healthy Longevity Volume 5 - 2024

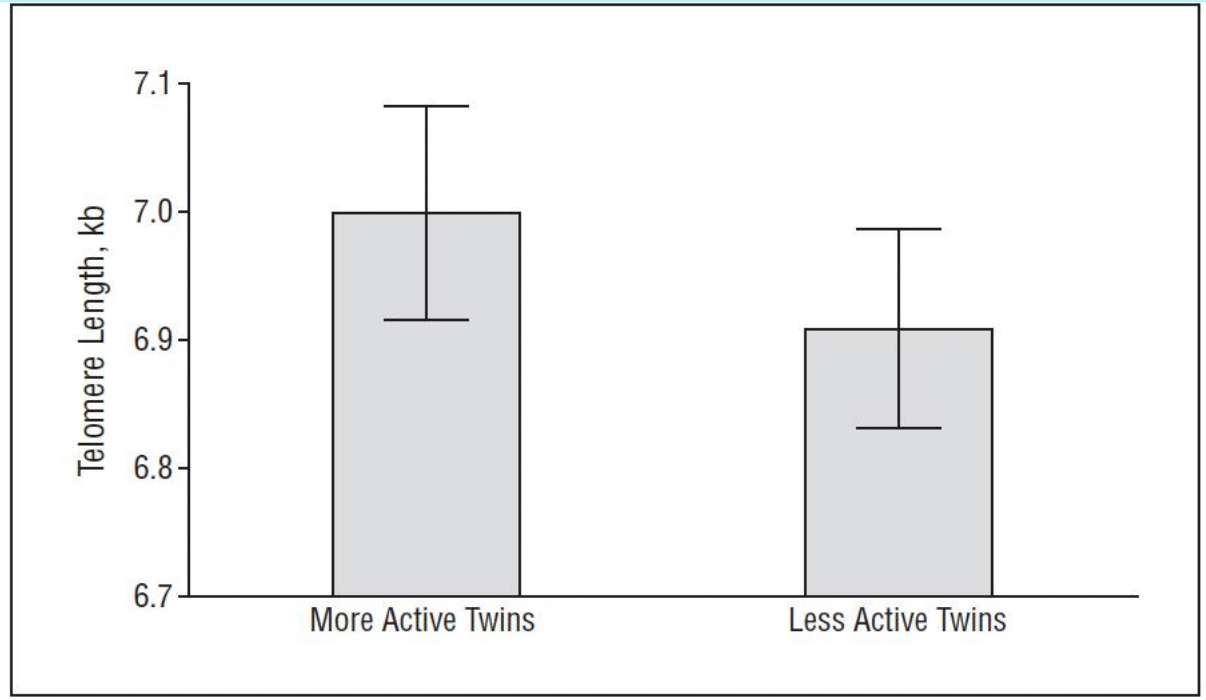
What Can Be Done To Keep Telomeres Long?

- Nutraceuticals
  - Kiecolt-Glaser JK et al. **Omega-3** fatty acids, oxidative stress, and leukocyte telomere length: A randomized controlled trial. *Brain Behav Immun.* 2013 Feb;28:16-24.
  - Lui M et al. **Resveratrol** protects against age-associated infertility in mice. *Hum Reprod*. 2013 Mar;28(3):707-17
  - Liu JJLiu et al. Plasma **Vitamin D** biomarkers and leukocyte telomere length. *Am J Epidemiol*. 2013 Jun 15;177(12) : 1411-7.
  - Zijian Xia et al. Telomerase: A Target for Therapeutic Effects of **Curcumin** and a Curcumin Derivative in Ab1-42 Insult In Vitro. *PLOS ONE* 1 July 2014.Volume 9. Issue 7
  - Richards JB et al. **Homocysteine** levels and leukocyte telomere length**.** *Atherosclerosis*. 2008 Oct.

- Garcia-Calzon S. et al. **Dietary total antioxidant** capacity is associated with leukocyte telomere length in a children and adolescent population. *Clinical Nutrition* 34 (2015) 694-699
- Bandi Hari Krishna et al. Association of Leukocyte Telomere Length with Oxidative Stress in **Yoga** Practitioners. *Journal of Clinical and Diagnostic Research.* 2015 Mar, Vol-9(3): CC01-CC03
- Werner C et al. Effects of physical **exercise** on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J Am Coll Cardiol*. 2008 Aug 5;52(6):470-82.
- Bijnens E et al. Lower placental telomere length may be attributed to **maternal residential traffic exposure**; a twin study. *Environ Int.*2015 Jun;79:1-7
- Laia Hernandez et al. **Aging and radiation**: bad companions. *Aging Cell* (2015) 14, pp153–161
- Marta Crous-Bou et al. **Mediterranean diet** and telomere length in Nurses' Health Study: population-based cohort study. *BMJ* 2014; 349
- Jacobs TL, Epel ES, Lin J, Blackburn E, Wolkowitz OM, Bridwell DA, Zanesco AP, Aichele SR, Sahdra BK, MacLean KA, King BG, Shaver PR, Rosenberg EL, Ferrer E, Wallace BA, Saron CD Intensive **meditation** training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology*. 2011 Jun;36(5):664-81

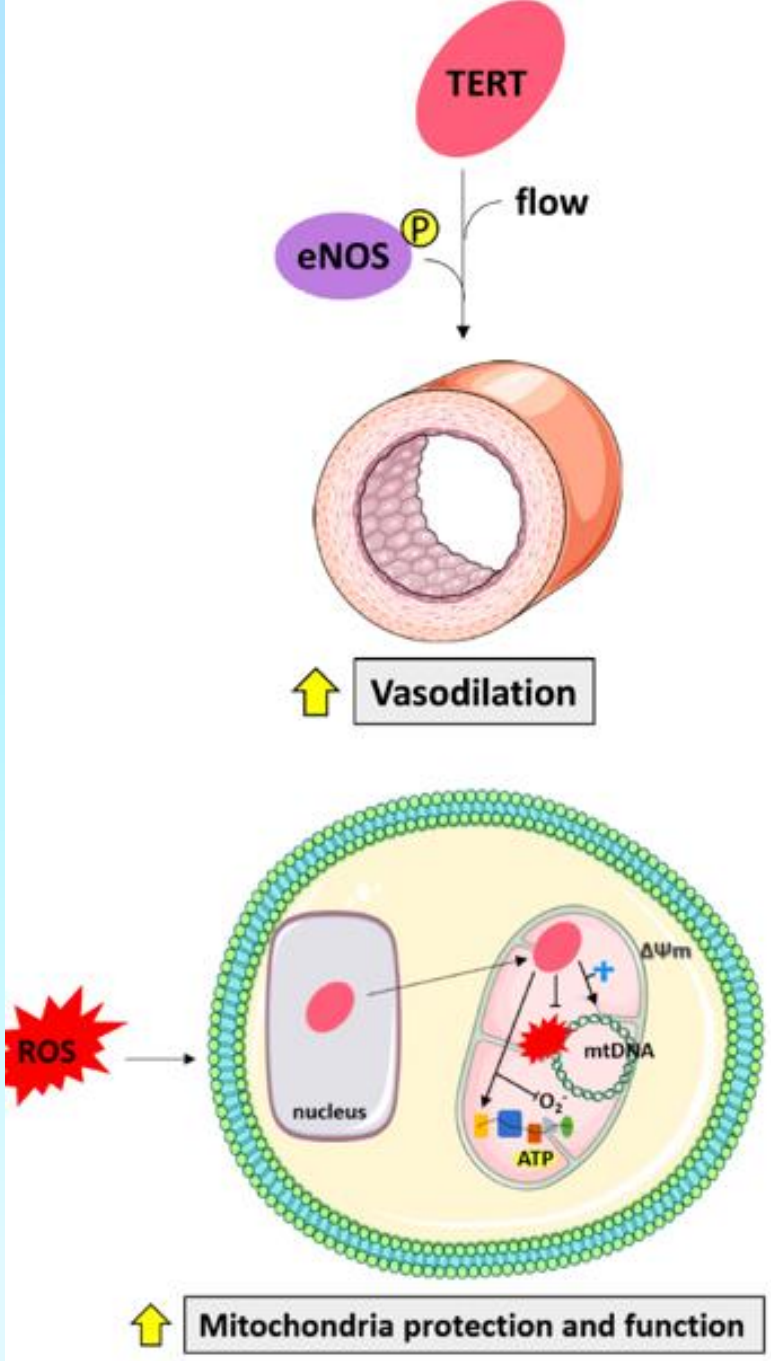
Exercise and TERT

- Two way street
- Exercise increases telomerase and TERT
- TERT increases exercise capacity



Cherkas L et al. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med*. 2008 Jan

TERT Increase Nitric Oxide (NO)

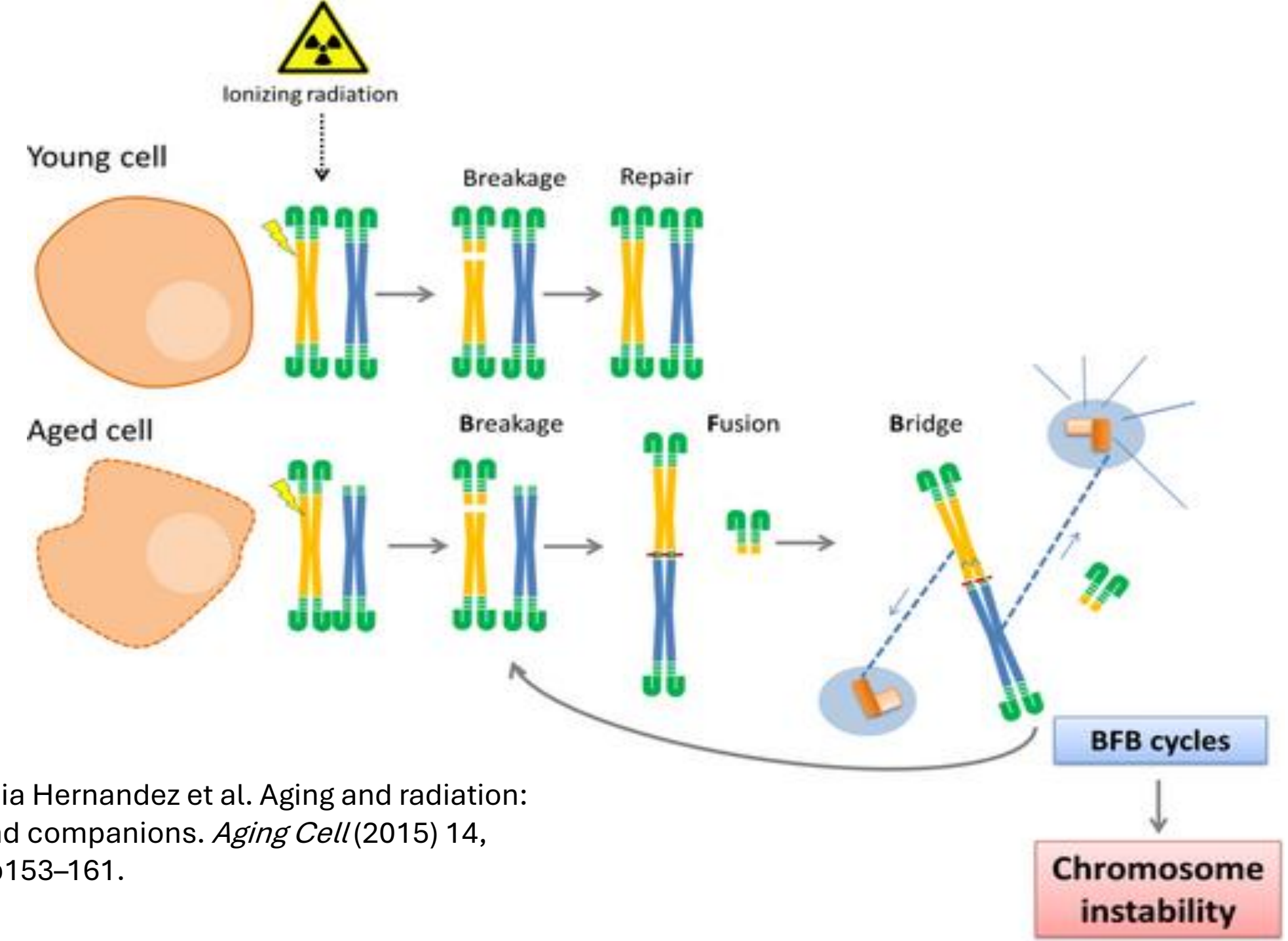


Denham J. Canonical and extra-telomeric functions of telomerase: Implications for healthy ageing conferred by endurance training. *Aging Cell*. 2023 Jun

Radiation and Aging – Bad Companions

- Age-dependent telomere attrition contributes to human carcinogenesis
- Ionizing radiation induces new DNA double-strand breaks (DSBs)
- Dysfunctional repair leads to end-to-end fusions and DSB-end fusions between different chromosomes
- Breakage–fusion–bridge cycle (BFB cycles) lead to rise in chromosome instability
- Initiates or promote a carcinogenic process.

- Laia Hernandez et al. Aging and radiation: bad companions. *Aging Cell* (2015) 14, pp153–161.



Laia Hernandez et al. Aging and radiation: bad companions. *Aging Cell* (2015) 14, pp153–161.

Effect of TA-65 on Telomere Length in Humans

A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A Randomized, Double Blind, and Placebo Controlled Study. Salvador L, et al. *Rejuvenation Res.* 2016 Mar 30.

- Randomized, double-blind, placebo-controlled study of 97 men and women (50-84 years old)- all CMV+
- Shows statistically significant lengthening of telomeres ***in humans*** *(3, 6, 9, and 12 months)(p<.005)*

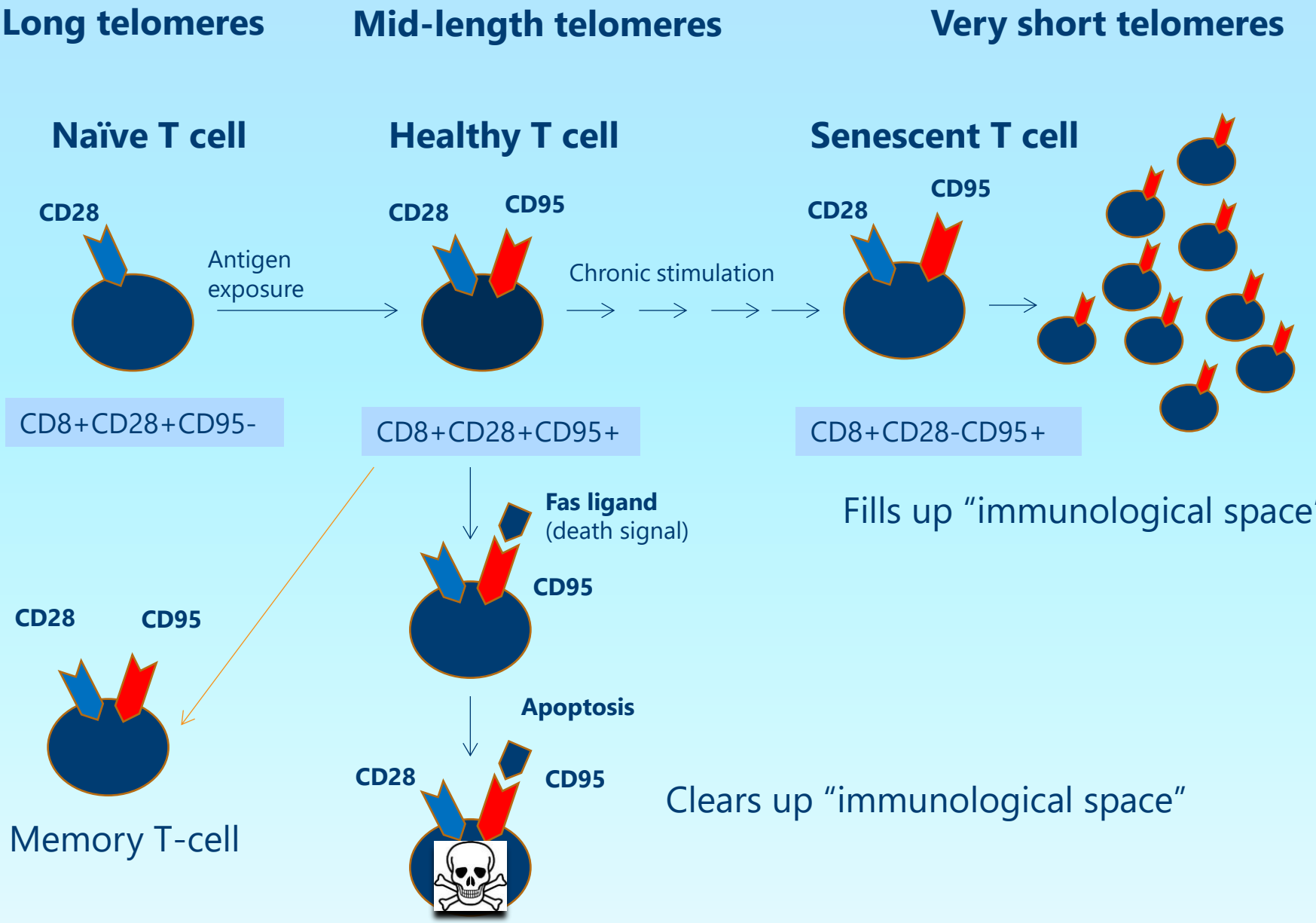
TA-65 ® Group
Increase in median telomere length

Time (months)	Increase in length (base pairs)
3 months	+384 (± 195) bp *
6 months	+158 (± 164) bp
9 months	+526 (± 167) bp *
12 months	+533 (± 183) bp *

Placebo Group
Decrease in median telomere length

Time (months)	Decrease in length (base pairs)
3 months	-24 (± 106) bp
6 months	none
9 months	-170 (± 106) bp *
12 months	-288 (± 101) bp *

* Statistically significant



Courtesy - Joseph Raffaele MD

CD8+CD28- T cells

- Functional CD8+ cytotoxic T lymphocytes drive the adaptive immune response to cancer
- Downregulation of CD28 is a hallmark of senescent T cells, needed for activation of T cells
- Increased CD8+CD28- senescent populations present in solid and hematogenous tumors
- T cell senescence can be induced by several factors including aging, telomere damage, tumor-associated stress
- Senescent CD8+CD28– T cells display **immunosuppressive functions in cancer**
- Associated with **atherosclerosis and inflammation**

Huff W. et al. The Evolving Role of CD8+CD28- Immunosenescent T Cells in Cancer Immunology Int. *J. Mol. Sci*. 2019, 20, 281

TA-65 Decreases Immunosenescent CD8+CD28- T Cells in Humans
Primary Endpoint: Effect on Immuno-senescence

- N=500
- TA65 100 mg, 250 mg, 500 mg daily and 250 mg twice daily compared to placebo

Findings after 9 months:

- Senescent T cells decreased
- Naive T cells increased
- All p values for data. <.001
- Results independent of CMV status

Gunasekaran Singaravelu , Calvin B Harley , Joseph M Raffaele , Pratheesh Sudhakaran , Anitha Suram. Double-Blind, Placebo-Controlled, Randomized Clinical Trial Demonstrates Telomerase Activator TA-65 Decreases Immunosenescent CD8+CD28- T Cells in Humans. *OBM Geriatrics* 2021, volume 5, issue 2

TA-65 decreases senescent T cells (CD8+CD28-) in humans

Group	No. of Subjects	Baseline (mean ± SE) cells/μL	End of Study (mean ± SE) cells/μL	Change in mean*	% of change in mean	p value †
Placebo	72	123 ± 16	127 ± 22	+4	+3%	0.624
TA-65 (100 Units)	86	191 ± 17	167 ± 15	-24	-13%	<0.001
TA-65 (250 Units)	94	161 ± 15	138 ± 12	-24	-14%	<0.001
TA-65 (500 Units)	92	148 ± 15	129 ± 12	-18	-13%	<0.001
TA-65 (250 Units) b.i.d.	80	134 ± 18	117 ± 15	-17	-13%	<0.001

† *p* values <0.05 are indicated in red; p values are estimated by Student's *t*-test *Change in mean = End of study – baseline; SE = Standard error of mean

TA-65 decreases senescent T cells (CD8+CD28-) in humans

Group	No. of Subjects	Baseline (mean ± SE) cells/μL	End of Study (mean ± SE) cells/μL	Change in mean*	% of change in mean	p value †
Placebo	72	123 ± 16	127 ± 22	+4	+3%	0.624
TA-65 (all groups)	352	153 ± 7	136 ± 7	-17	-13%	<0.001

"No product related toxicity or serious adverse events (SAEs) were observed for this study."

† *p* values <0.05 are indicated in red; p values are estimated by Student's *t*-test *Change in mean = End of study – baseline; SE = Standard error of mean

Will TA-65 increase cancer risk?

- Short telomeres increase most cancer risks
- TA-65 transiently induces telomerase
- TA-65 compensates for telomere loss but does not overexpress telomerase
- Most cancers have permanently induced telomerase
- Some cancers do not express telomerase
- Preclinical studies with TA-65 show no increases cancer risks
- No reports of human cancers associated with TA-65
- Over 20,000 active users of TA-65
- GRAS status

Telomerase and Cancer

- Telomerase is not an oncogene
- No studies suggest that telomerase induction causes cancer
- Germ cell line has been expressing telomerase for eons
- Cancer cells express telomerase
- Since cancer cells already express telomerase, telomerase activation is not a concern
- Cancer cells require multiple other properties not effected by telomerase
  - Loss of contact inhibition
  - Failure of apoptotic pathways
    - Loss of p53 pathway function
    - Loss of Retinoblastoma pathway (pRb)

TA 65: Telomeres, Health Span, Cancer

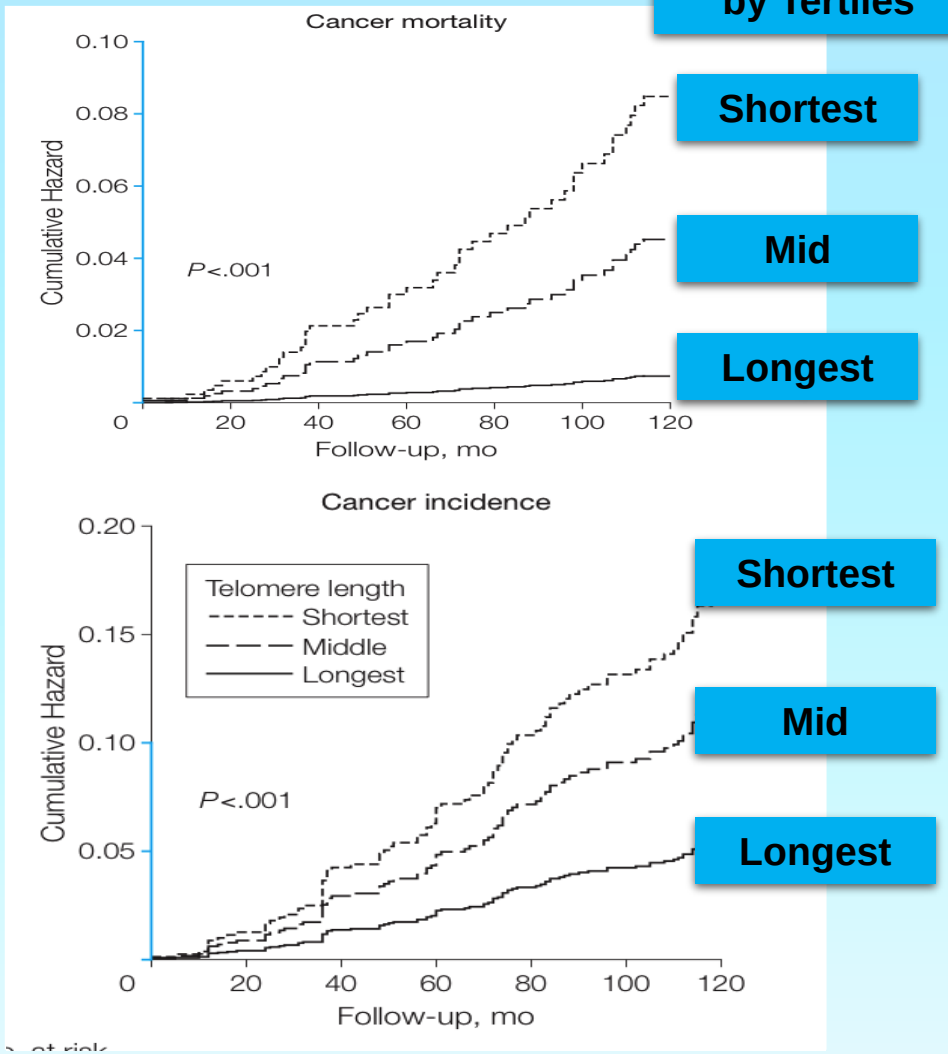
- Mice treated with TA 65 x 4 months
- Telomerase Activator stimulates telomerase activity and elongates short telomeres
- Significant decrease of very short telomeres (telomeres < 2, 3 and 4 Kb)
- Improved
  - Glucose tolerance
  - Epidermal thickness
  - Good hair day
  - Bone density
  - Higher hemoglobin
  - No increased cancer incidence

Bernardes de Jesus et al. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. *Aging Cell.* 2011,10(4):604-2

Short Telomeres Predict Cancer Incidence & Death

Cancer

- 800 men, women, 45 to 84y, tracked 10 yrs
- 92 cases of incidence, 44 of mortality
- Shortest v. longest tertile hazard ratio
  - **3.1** cancer incidence
  - **11.1** cancer mortality



Willeit P et al. Telomere length and risk of incident cancer and cancer mortality. *JAMA*. 7,69-75 (2010)

Shorter telomeres predict cancer and mortality

- Duggan C et al. Change in peripheral blood leukocyte telomere length and mortality in breast cancer survivors. *J Natl Cancer Inst*. 2014 Apr
  - **Shorter telomeres**
    - **Increased Breast cancer mortality**
    - **Increased all cause mortality**
- Mucciardi, G et al. Telomere instability in papillary **bladder** urothelial carcinomas: Comparison with grading and risk of recurrence. *Indian J Urol*. 2014 Jul
- Thorvaldsdottir B et al. Telomere length is predictive of **breast cancer** risk in **BRCA2** mutation carriers. *Cancer Epidemiol Biomarkers Prev.*2017 Feb
- Mendelsohn AR, Larrick JW. Ectopic expression of telomerase safely increases health span and life span. *Rejuvenation Res*. 2012 Aug
  - Transient induction of TERT by an astragalus-derived compound increases health span **without an increase in cancer** incidence
- Wang Y et al. Astragaloside in **cancer chemoprevention** and therapy. *Chin Med J April* 2023

Cycloastrogenol **Transiently** Activates Telomerase

Results:

- TAT2 (Cycloastrogenol) can transiently activate telomerase
- Slows telomere loss
- Increases replicative capacity
- Enhances immune function in CD8+ T lymphocytes
- Fauce SR, Jamieson BD, Chin AC, Mitsuyasu RT, Parish ST, Ng HL, Kitchen CM, Yang OO, Harley CB, Effros RB. Telomerase-based pharmacologic enhancement of antiviral function of human CD8+ T lymphocytes. *J Immunol*. 2008

Neuro

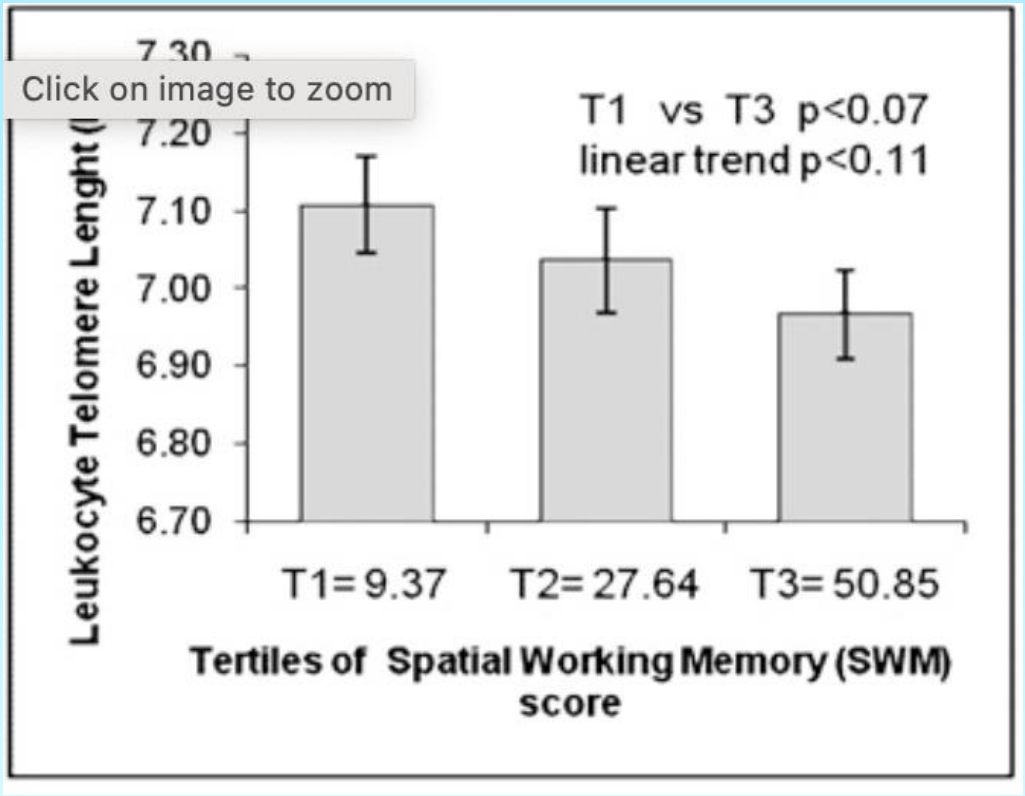
- Alexander Karabatsiakis et al. Telomere shortening in leukocyte subpopulations in **depression**. *BMC Psychiatry* 2014

- Smith JA et al. Oxidative stress, DNA damage, and the telomeric complex as therapeutic targets in acute **neurodegeneration.** *Neurochem Int*. 2013 Apr;62(5):764-75
  - Preventing DNA damage promotes neuronal survival and enhances **neurological recovery.**
  - Telomerase is a novel therapeutic target in the treatment of **neurodegeneration**

Neuro

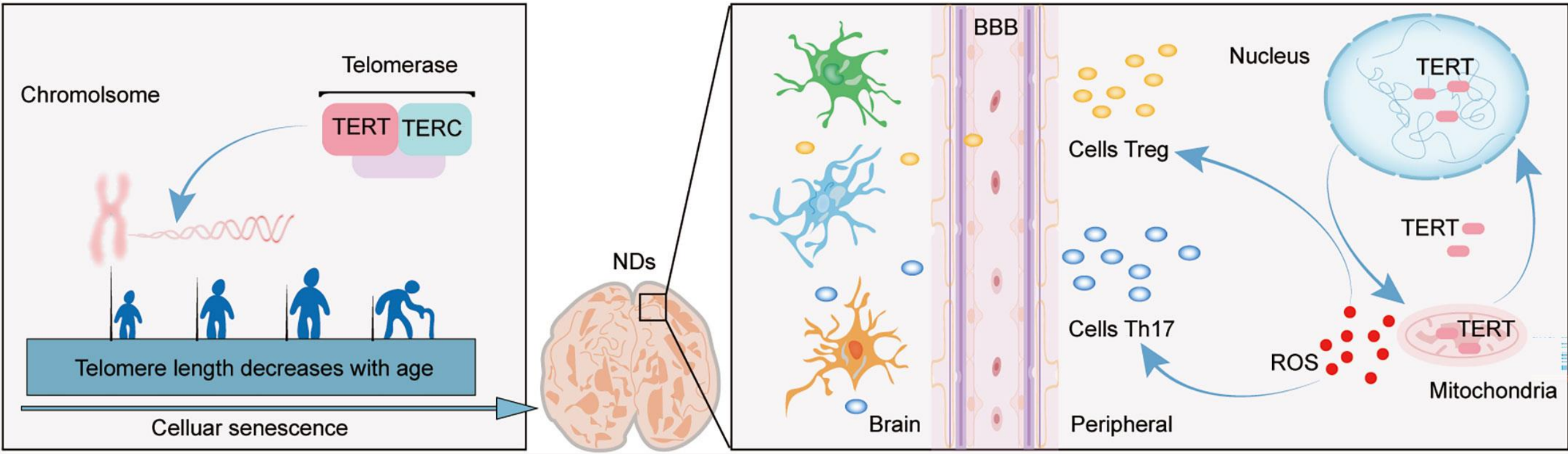
- Eyolfon E et al. Sexually Dimorphic Behavioral and Genetic Outcomes Associated With Administration of TA65 (A Telomerase Activator) Following Repetitive **Traumatic Brain Injury**: A Pilot Study**.** *Front Neurol.* 2020 Feb
- Zhou QG et al. Hippocampal TERT Regulates Spatial **Memory** Formation through Modulation of Neural Development. *Stem Cell Reports*. 2017 Jul 25
- Wan, T et al. Increased telomerase improves motor function and alpha-synuclein pathology in a transgenic mouse model of **Parkinson's** disease associated with enhanced autophagy. *Prog Neurobiol.* 2021 Apr
  - Only one activator, **TA-65**, resulted in a decrease of reactive oxygen species from brain mitochondria
- Saretzki, G.· Telomerase in Brain: The New Kid on the Block and Its Role in **Neurodegenerative** Diseases *Biomedicines.* 2021 May.
  - TERT in brain - benefit for the amelioration of brain aging and neurodegenerative diseases such as **AD** and PD.
  - TA-65 discussed as therapy

Leucocyte telomere length correlates with cognitive function



- Valdes A et al. Leukocyte telomere length is associated with cognitive performance in healthy women. *Neurobiol Aging*. 2010 Jun

Relationship between TERT, Aging and Neurodegenerative Diseases



- **Cycloastrogenol** stimulates TERT to regulate brain inflammation
- Treg CD4+ suppress cytokines and inflammation
- Th17 CD4+ pro and anti-inflammatory actions, anti-inflammatory in Brain
- TERT lower in Alzheimer's, Parkinson's, and ALS
- Xin Yu et al. Telomerase reverse transcriptase and neurodegenerative diseases *Front Immunol* . 2023 Mar

Cardio

- Zimnitskaya OV et al. . Leukocyte Telomere Length as a Molecular **Biomarker** of Coronary Heart Disease. Genes (Basel). 2022 Jul
  Leukocyte telomere length (LTL) is associated with the development of widespread cardiovascular diseases such as CHD and essential hypertension

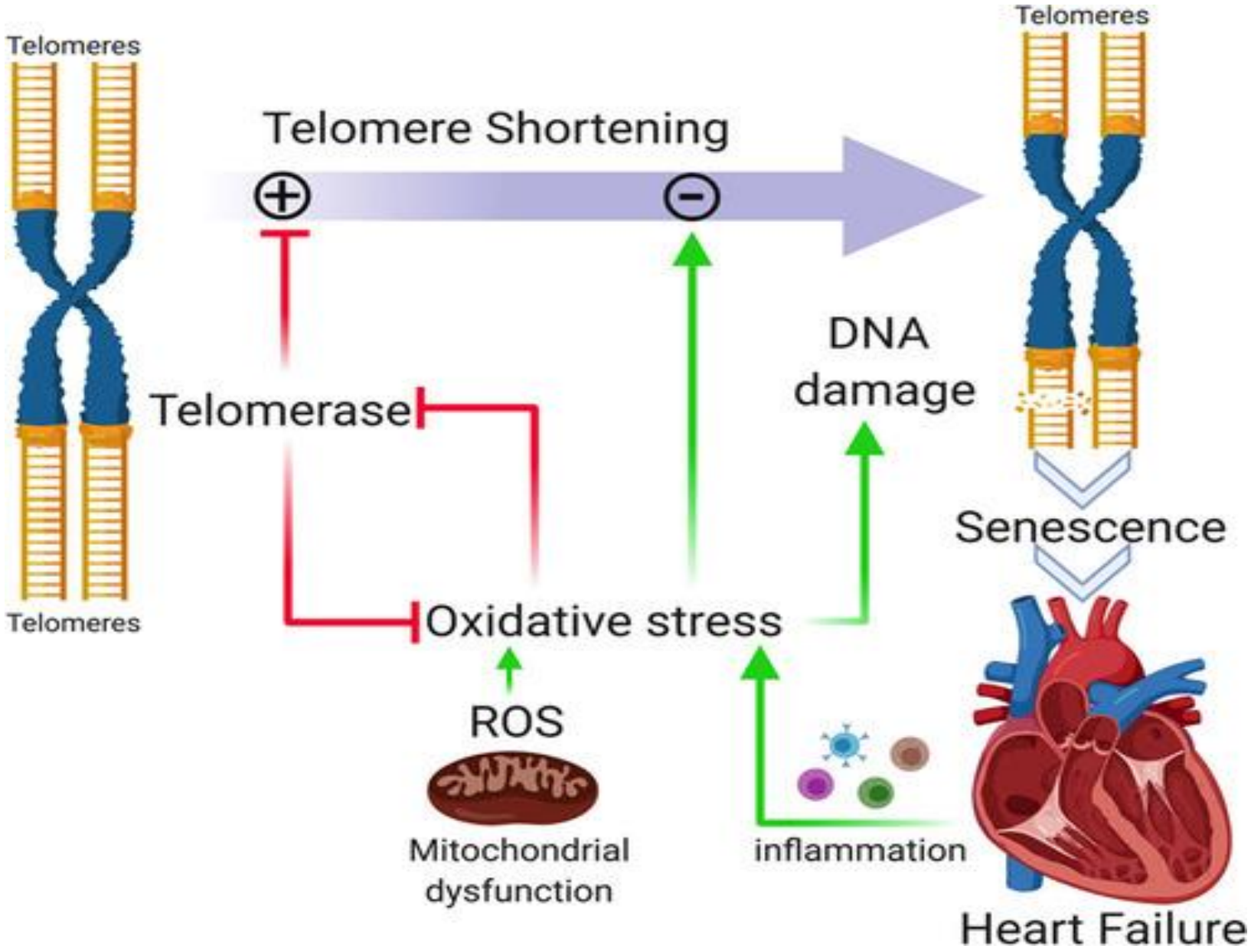
- Hoffmann. J. Telomerase as a **Therapeutic Target** in Cardiovascular Disease, *Arteriosclerosis, Thrombosis, and Vascular Biology.* Volume: 41, Issue: 3, 2021
  - The atheroprotective role of mitochondrial TERT - antioxidative function and vascular cell regeneration.
  - The noncanonical functions of TERT - beneficial in the treatment of cardiovascular aging and disease.

- Ale-Agha, N. Mitochondrial Telomerase Reverse Transcriptase **Protects From Myocardial Ischemia/Reperfusion Injury** by Improving Complex I Composition and Function. Dec 2021 *Circulation AHA*.
  - An increase in mitochondrial TERT levels, induced by treatment with TA-65, could be beneficial in ischemia/reperfusion injury.

Telomeres and CVD

- Critically short telomeres lead to cellular senescence and apoptosis
- Contributes to the development of atherosclerosis and predispose to **plaque instability**
- LTL reflects the burden of oxidative stress and inflammation
- Effective biomarker for risk stratification for atherosclerosis and CVDs.
- Improving telomere length is a target for treating CVD

- Yeh JK et al. Telomeres and Telomerase in Cardiovascular Diseases. *Genes (*Basel). 2016 Sep

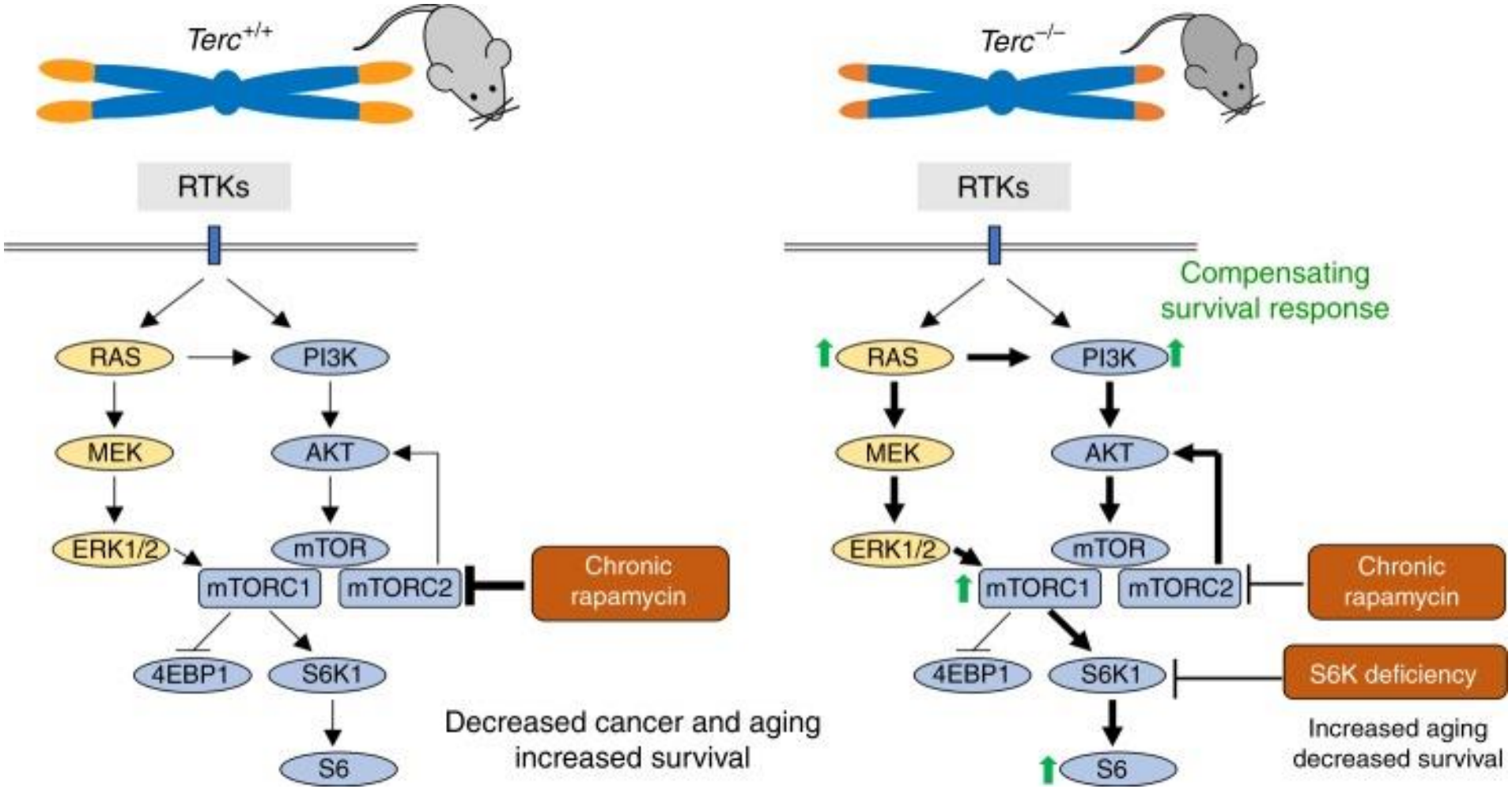


Jedrzej Hoffmann. Telomerase as a Therapeutic Target in Cardiovascular Disease, *Arteriosclerosis, Thrombosis, and Vascular Biology.* Volume: 41, Issue: 3, 2021

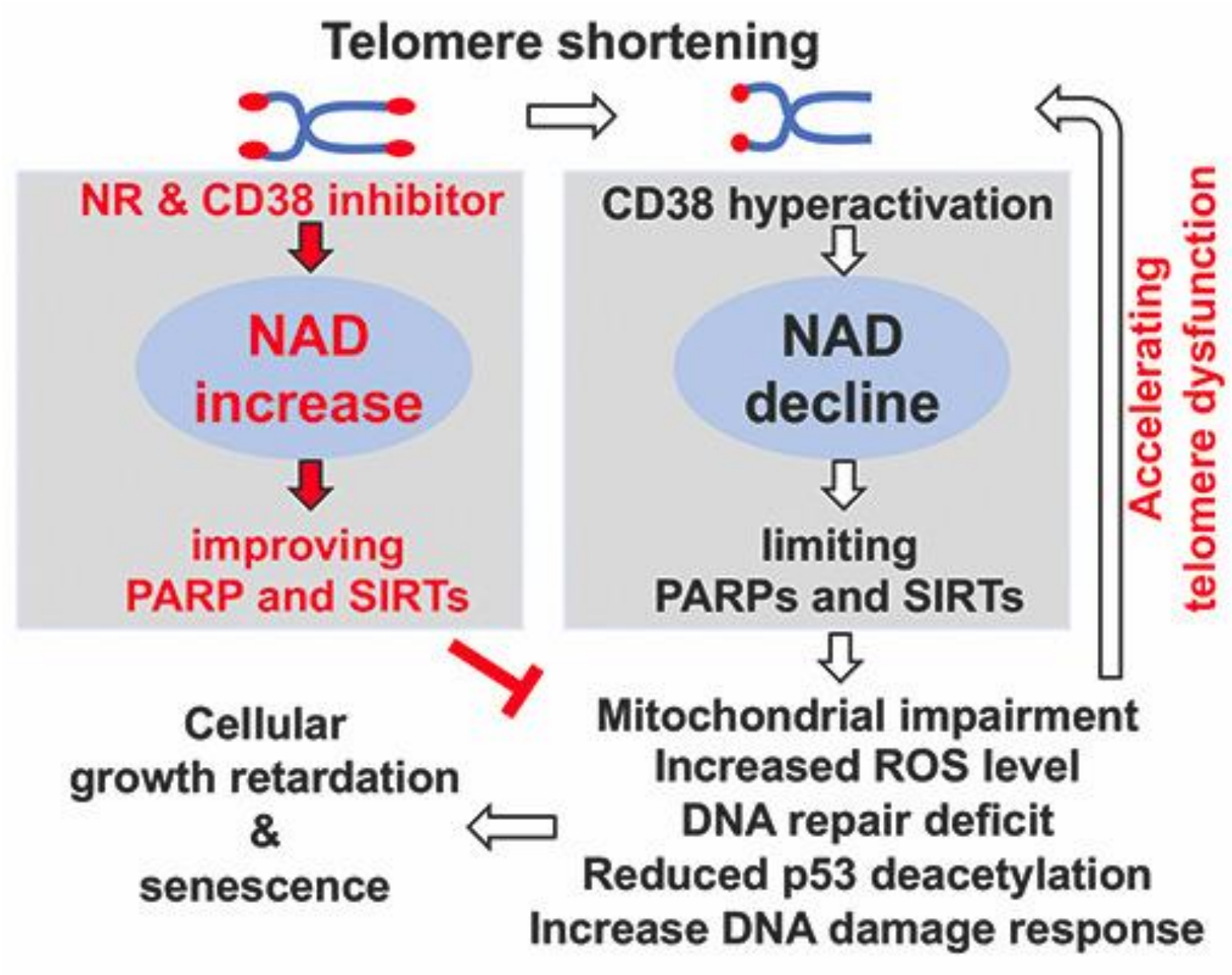
Telomeres, TA-65 – Covid-19

- Wang et al. Shorter leukocyte telomere length is associated with **adverse COVID-19 outcomes**: A cohort in UK Biobank. *EBioMedicine* 70 (2021)
- Aviv A. Telomeres and COVID-19. *FASEB J.* 2020 Jun;34(6):7247-7252.
  - Short telomere length identifies patients more likely to **die** from the SARS-CoV-2 infection, regardless of age
  - **TA-65 is a potential intervention in COVID-19**
- Mongelli, A et al. Evidence for Biological Age Acceleration and Telomere Shortening in COVID-19 Survivors. *Int. J. Mol. Sci*. 2021 June, 22
  - **Long Covid**

Fix your telomeres before taking rapamycin



Ferrara-Romeo I et al. The mTOR pathway is necessary for survival of mice with short telomeres. *Nat Commun* . 2020 Mar

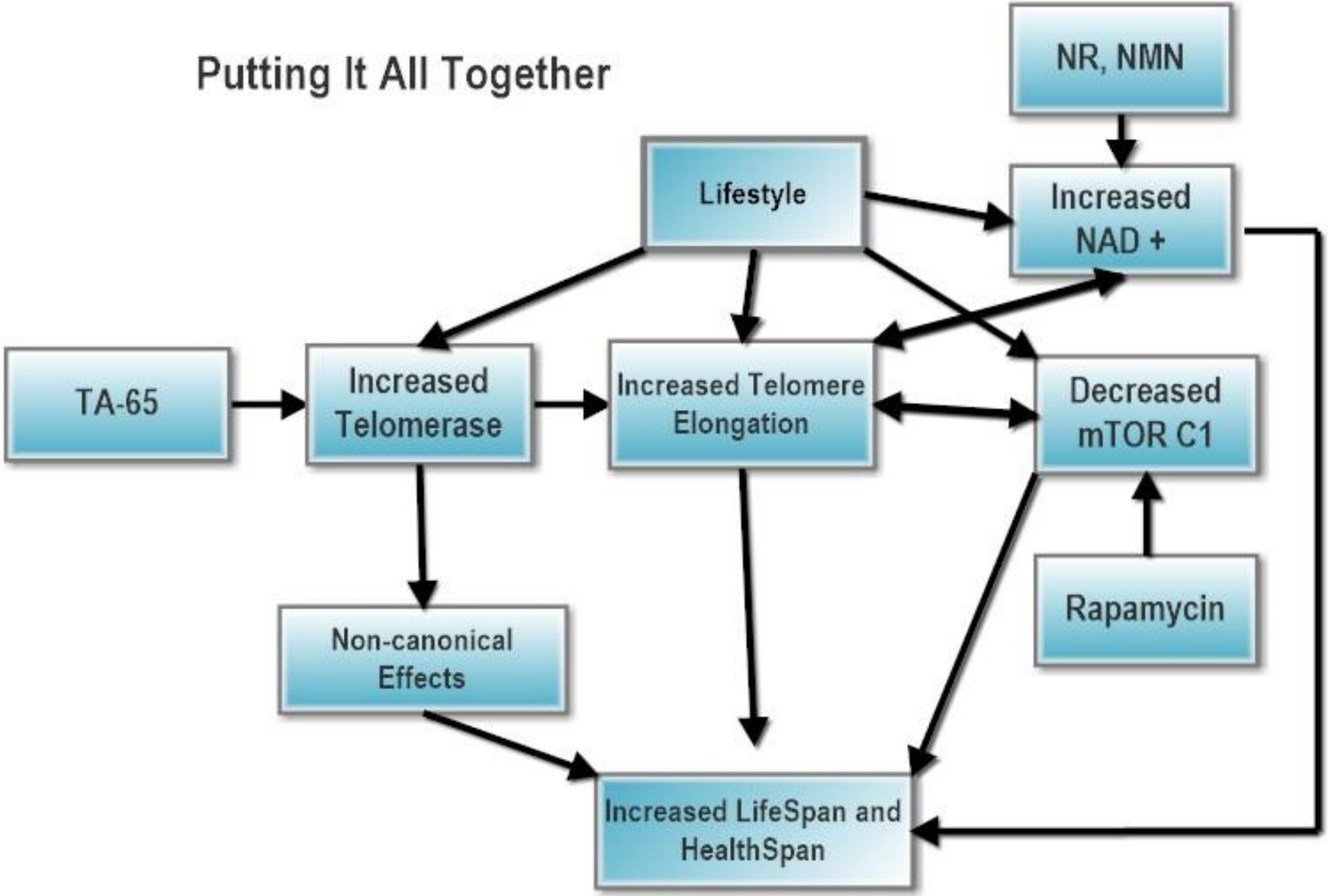


Chongkui Sun Kun et al. **Re-equilibration of imbalanced NAD metabolism ameliorates the impact of telomere dysfunction.** *The EMBO Journal*. Art 16, Sep 2020

It all fits together

- Everything we are trying to do for optimal health and quality of life has a telomere/telomerase connection
- Avoiding and controlling oxidative stress
- Nutrition
- Time restricted eating
- Meditation
- Exercise
- Controlling Mental Stress, environmental toxicity, radiation exposure
- Optimizing hormones
- Optimizing Nitric Oxide (NO)
- NAD+ increase, mTORC1 inhibition
- Activating Telomerase

Putting It All Together



Safe Over More Than 20 Years Of Studies

- Early safety studies done in the 1990's at California biotech company, Geron, before selling their telomerase activation technology and patents to TA Sciences.
- Safety studies began at T.A. Sciences in 2002
- In use by humans since 2007
- More than 20,000 people currently taking TA65®MD
- No reports of any significant adverse events

October 13, 2014
TA-65 "Generally Recognized As Safe"

BURDOCK GROUP
Consultants

Be It Known to All Interested Parties, That
Telomerase Activation Sciences, Inc.
Have Provided Substantive Data Leading to a Determination of
"Generally Recognized as Safe" (GRAS) Status of
TA-65®

According to the rights and privileges conferred in §201(s) (et seq.) of the Federal Food, Drug and Cosmetic Act, Experts, by virtue of their training and experience, have determined that the above mentioned substance, when produced in accordance with current Good Manufacturing Practice (cGMP), is safe under the intended conditions of use and is "Generally Recognized as Safe" (GRAS) by scientific procedures when used as a food ingredient in specific food categories at specified levels and for the purpose(s) indicated in the GRAS dossier, as approved by the GRAS Expert Panel.

Edward L. Carmines, Ph.D. - Expert Panelist — Date 10/6/14

Nancy J. Szabo, Ph.D. – Burdock Group Scientific Monographer — Date 03OCT2014

I. Glenn Sipes, Ph.D., Fellow ATS and AAAS - Expert Panelist — Date 10/7/14

George A. Burdock, Ph.D., DABT, FACN – President, Burdock Group — Date

John A. Thomas, Ph.D., D.A.T.S., F.A.C.T. - Expert Panelist — Date 10/8/14

GRAS

STATE OF FLORIDA
COUNTY OF ORANGE
Affirmed and subscribed before me this 13th day of October, 2014, by George A. Burdock, Ph.D., personally known to me.

Notary Public State of Florida
Gina Strollo Radcliff
My Commission EE026886
Expires 11/26/2014

Anecdotal reports

- Skin
- Presbyopia- Vision improvement
- Energy
- Increased memory and attention
- Improved peripheral neuropathy
- Aerobic capacity
- Immune function
    - Less viral and bacterial infections

- “I notice it will interfere with my sleep if I take it at night so I take one in the morning and one mid afternoon.. In conclusion I would say it gives me more energy.”
- “I stopped my amlodipine and my blood pressure is the same and is OK”
- “My fasting glucose has decreased from 110-120 to 90-105”
- “5 mg of Cialis works now where only 20 mg worked before with only change being addition of TA-65”
- “I can remember faces and names better”
- ”Finally something works for my ADD”

“ I no longer need a nap in the afternoon”

“I am breathing easier when I come up from being held underwater by a large wave”

“Friends ask me if I had a recent esthetic procedure, and I haven’t”

“I’m glad a half tablet works as well as a whole one”

“My memory is better; I don’t struggle to find missing word or name”

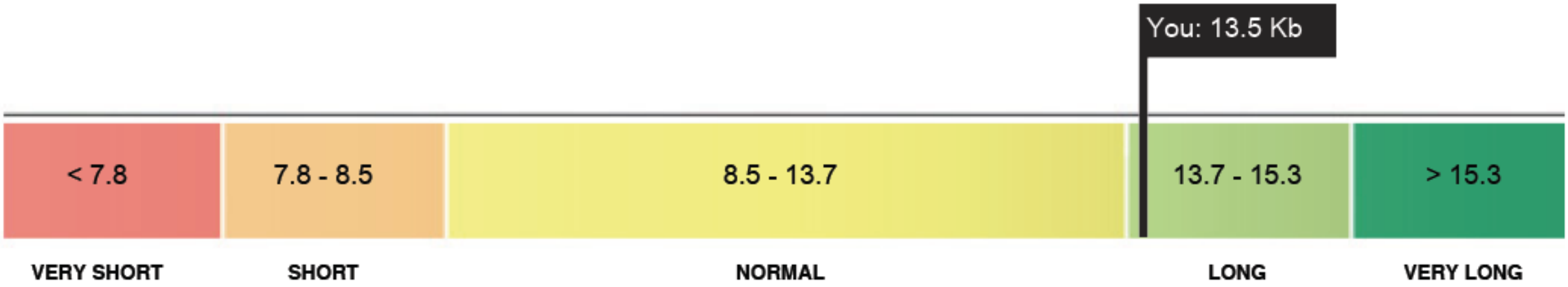
”My aerobic capacity has improved with no other changes other than TA-65”

“I no longer need by reading glasses”

1. Your telomere length

Median Telomere Length: 13.5 Kb

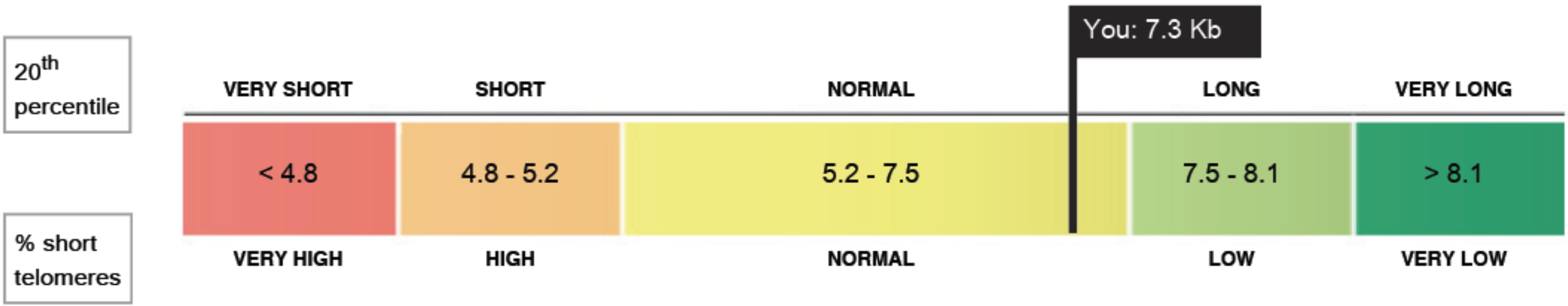
Your median telomere length is estimated to be normal compared to Life Length´s database population.



Median Telomere Length (MTL) = 13.5 ± 0.1 Kb

20th percentile: 7.3 Kb

Your 20th percentile is estimated to be normal compared to Life Length's database population. This implies that your percentage of short telomeres is normal compared to the same database.



Case Study with Life Length Testing
TA 65 with anti aging management

Male retired scientist H/o renal CA	2012	2013	2016
Chrono Age	71	72	75
Bio Age	83	76	75
Median Telomere / Kb	6.3	7.0	9.7

Transient Telomerase Activation with TA-65

- Lengthens telomeres
- “Generally recognized as safe”
- Improves % critically short telomeres
- Protects Mitochondrial DNA as well as nuclear DNA
- Repair of tissue damage
- Improved Quality of Life
- Esthetic benefits –skin
- Improves immune markers, Restores CD8+28+ T cells from CD8+CD28-
- Improves metabolic biomarkers
- Improves AMD
- Improves function of non-mitotic tissues such as neurons and cardiac myocytes
- May lower cancer risks and protect against radiation
- **Synergistic with other anti-aging treatments such as Lifestyle, Intermittent fasting, Testosterone, Estrogen, Melatonin and Growth Hormone**

Clinical Pearl for Optimized Telomeres

- Don’t stay awake all night while exposed to pesticides when under high stress with hormone deficiencies and radiation while getting no exercise and eating white bread…and take TA-65

Why use TA-65?

- In preventive/regenerative medicine we want to optimize health and fitness for our patients and ourselves
- We want to use multiple modalities: Lifestyle, nutraceuticals, hormone optimization.
- Telomere optimization through TA-65 is a powerful addition of another approach to health and fitness.
- We have adequate data that TA-65 works to increase telomerase and lengthen telomeres and that it is safe.
- We have convincing data that TA-65 improves immune function in T cells
- Telomerase also protects us against inflammation, atherosclerosis, protects mitochondria and is necessary for optimal brain function
- Telomerase optimizes epigenetics

- We could wait 20 years for a prospective double-blind controlled outcome study but….
- We could use the information we have now to prevent the constant erosion of our telomeres, our DNA and our lives.