3rd Annual
NAD+ Summit

TALES ABOUT NAD+

3rd Annual NAD Summit

January 27-28th
Catamaran Hotel
San Diego, California

Vince Giuliano, PhD
www.agingsciences.com

With **Major contribution** by Dr. James P Watson

For More Information on NAD+ Biology and Aging

Easy to Read Blogs on NAD+

Part I – NAD^+ de novo synthesis, NAD^+ salvage pathway, the 7 Sirtuins, CD38, the 17 PARPs, Circadian regulation of NAD^+ synthesis, Critical role of NAD^+ in the mitochondria and NAD+ deficiency causing pseudohypoxic state

Part II – Redox vs Cofactor roles of NAD^+, restoring cellular NAD^+ levels, Major functions of SIRT1 and SIRT6

Part III – 30 major factors that control SIRT1 gene expression, SIRT1 enzyme activity, and SIRT1-mediated aging

Part IV – NQO1 regulation of NAD^+/NADH ratio, NAD^+ Mand Warburg metabolism, NAD^+ and inflammation

Part V – Conflicting roles of extracellular NAMPT enzyme (eNAMPT) and why it causes systemic inflammation

Part VI – How Google is getting into NAD+ businesss, why Google's strategy will fail, why NR/NMN/NAD+ will not stop aging, (coming)

Mostly by Jim Watson

Vince Giuliano, PhD
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SUMMARY OF TODAY'S LECTURE

1. Fundamental "NAD+ Stories"

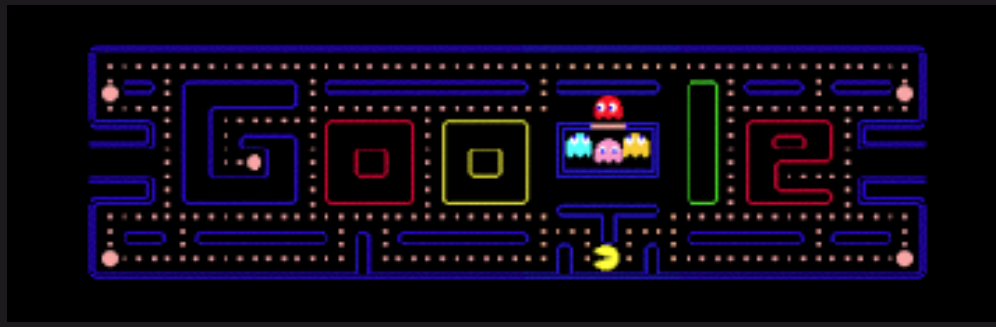
   Co-enzyme vs Cofactor functions of NAD+, declining NAD+, declining NAD+/NADH ratios, and declining cellular redox potentials with aging, NAD+ functions in each subcellular compartment, sources of NAD+ biosynthesis in the cell

2. NAD+ "Chamber of Horrors"

   Some of the main things that can go horribly wrong if you don't have enough NAD+ in your body or your or if your NAD+/NADH ratio goes screwy

3. NAD+ "Pac-men"

   Enzymes that can eat up your NAD+ while you are not looking – SIRTs, PARPs, CD38, MARTs, SARM-TIR




One dark, dark night, your
NAD/NADH ratio
dropped really low
NAD+
Stories

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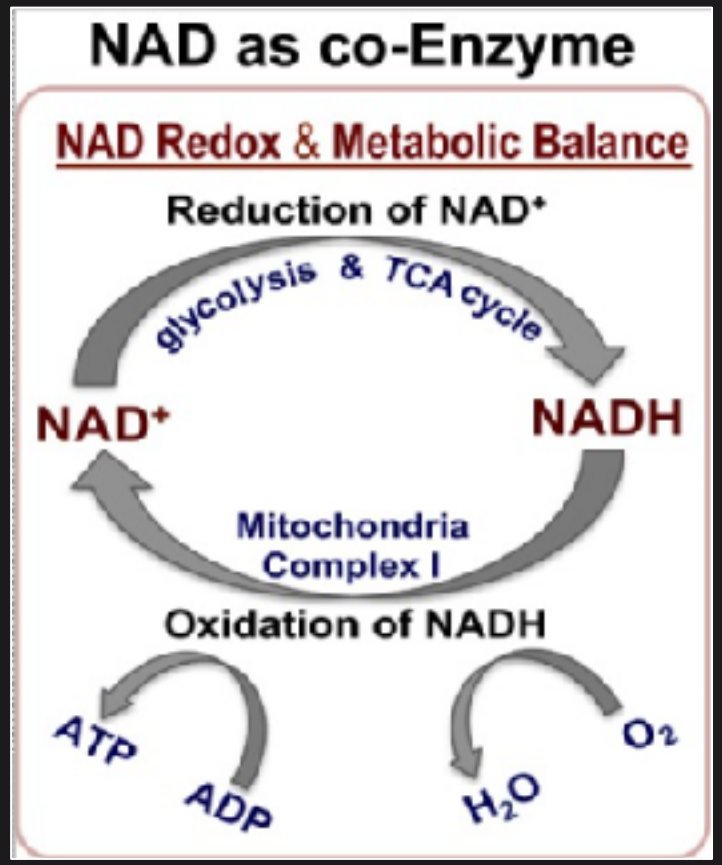
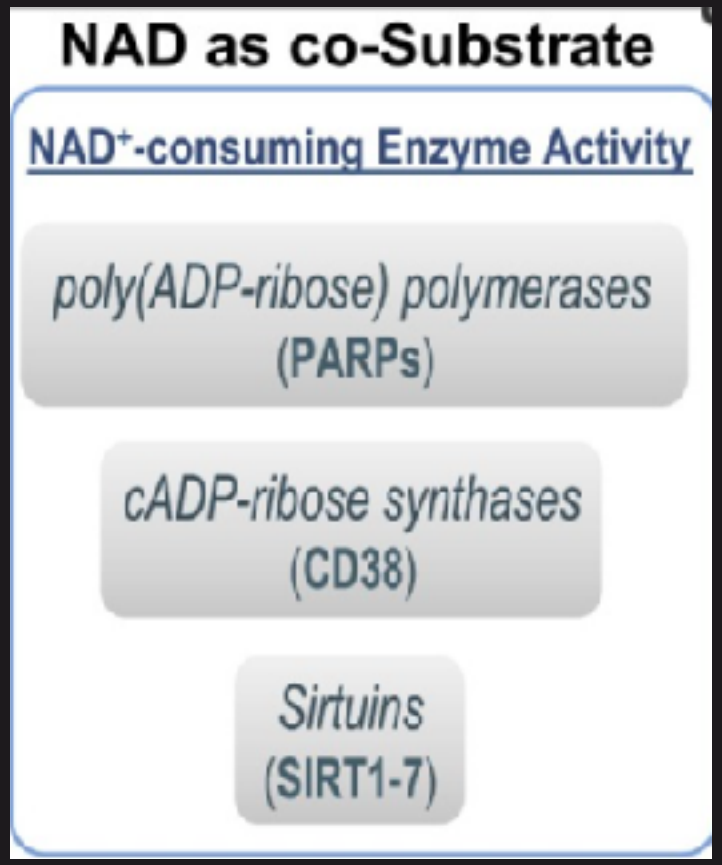
FUNDAMENTAL NAD+ STORY:

NAD+ CONSUMING VS NON-CONSUMING PROCESSES



NAD+ Is NOT Consumed



NAD+ Is Consumed

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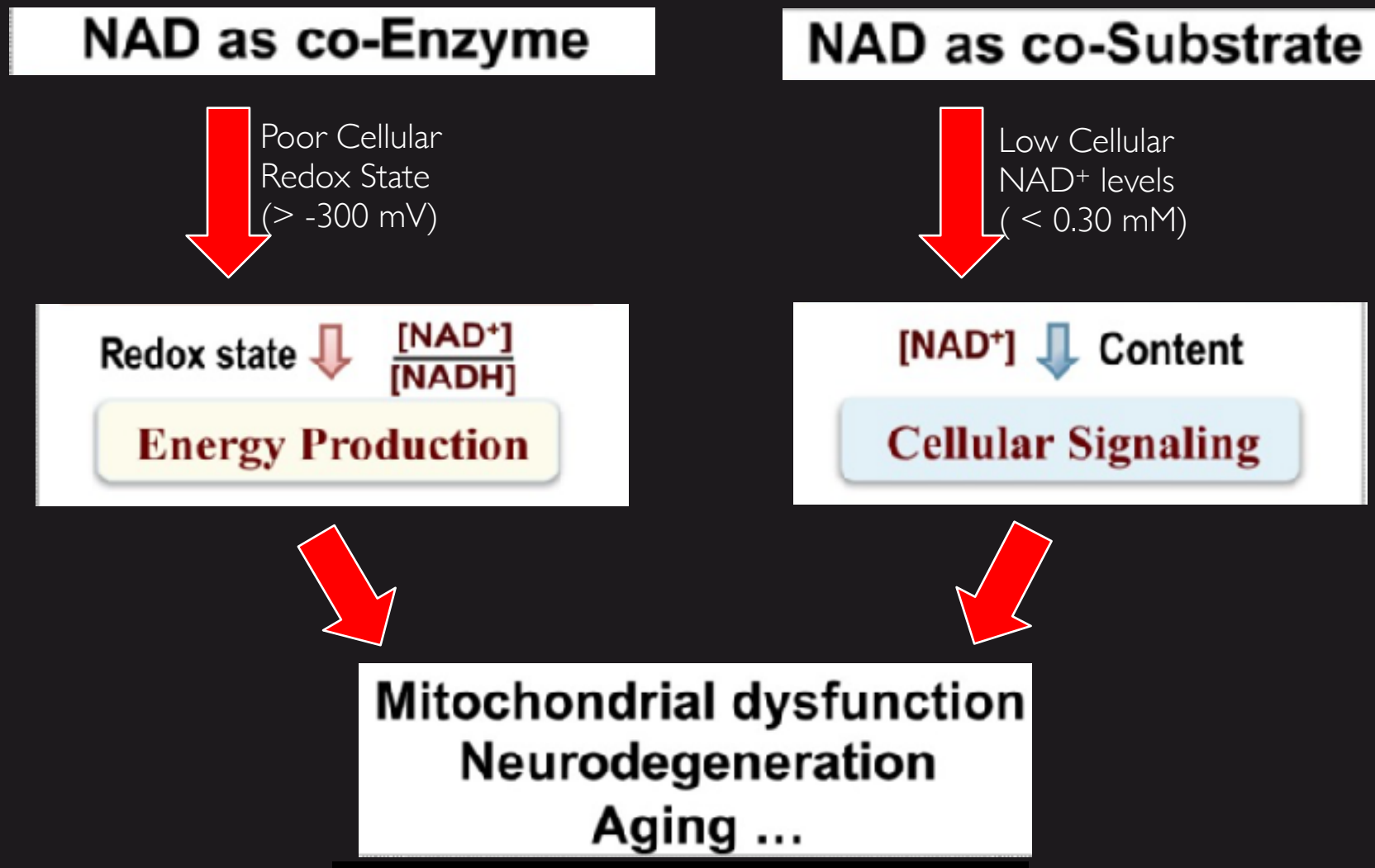
FUNDAMENTAL NAD+ AGING STORIES:

AGING IS MANIFESTED BY TWO NAD+-RELATED STORIES



* Zhu, et.al., *PNAS*, March, 2015, Vol 112, pp 2876- 2881

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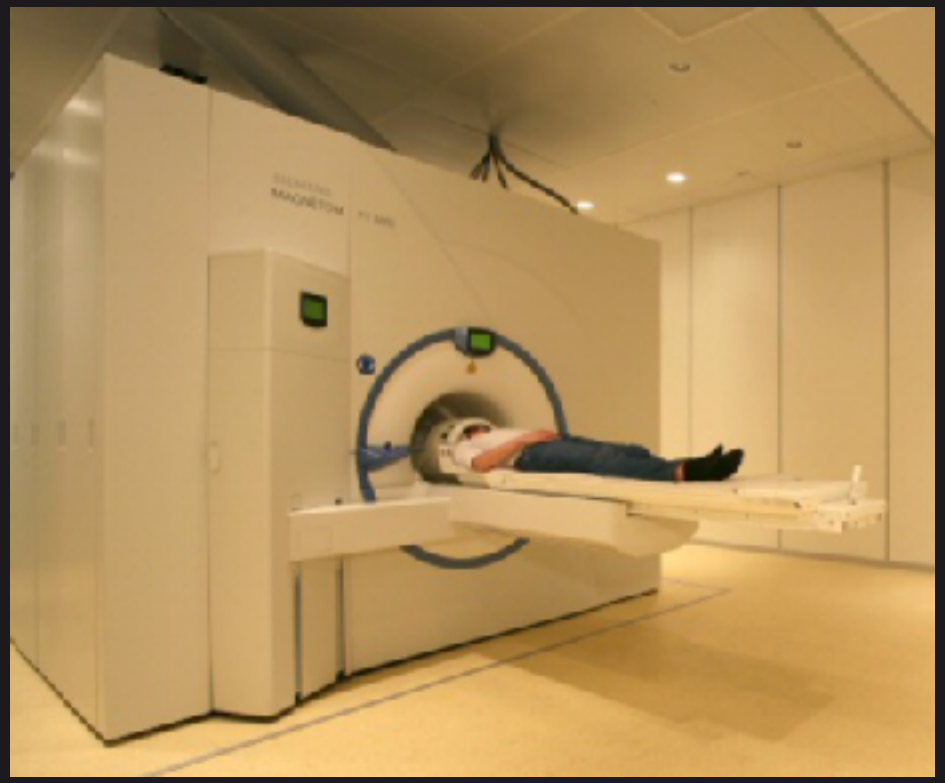
MEASURING NAD+ IN THE BRAIN

Measuring NAD+ in vivo in Live Humans



- Brain Ultrahigh-field (7 T) ^{31}P Magnetic Resonance Spectroscopy
- Measures brain ATP, NAD^+, and NADH simultaneously
- NAD^+/NADH ratio derivation:

$$RP = RP^{\emptyset} + \frac{RT}{zF}\ln\left(\frac{[NAD^+]}{[NADH]}\right) = RP^{\emptyset} + \frac{RT}{zF}\ln(RX),$$

* Zhu, et.al., *PNAS*, March, 2015, Vol 112, pp 2876- 2881

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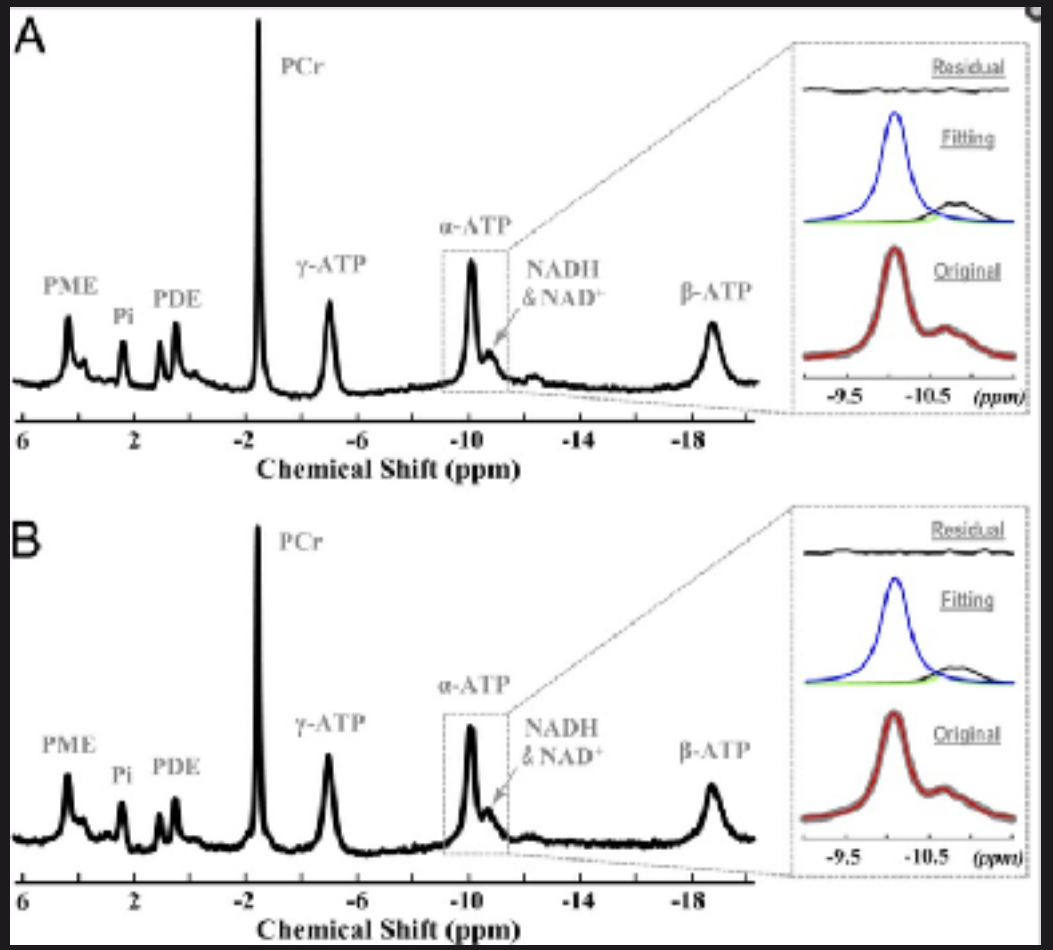
MEASURING NAD+ IN THE BRAIN

Ultrahigh-field (7 T) ^{31}P Magnetic Resonance Spectroscopy



18 year-old healthy male
ATP, NADH & NAD+ in occipital lobe

36 year-old healthy male
ATP, NADH & NAD+ in occipital lobe

* Zhu, et.al., *PNAS*, March, 2015, Vol 112, pp 2876- 2881

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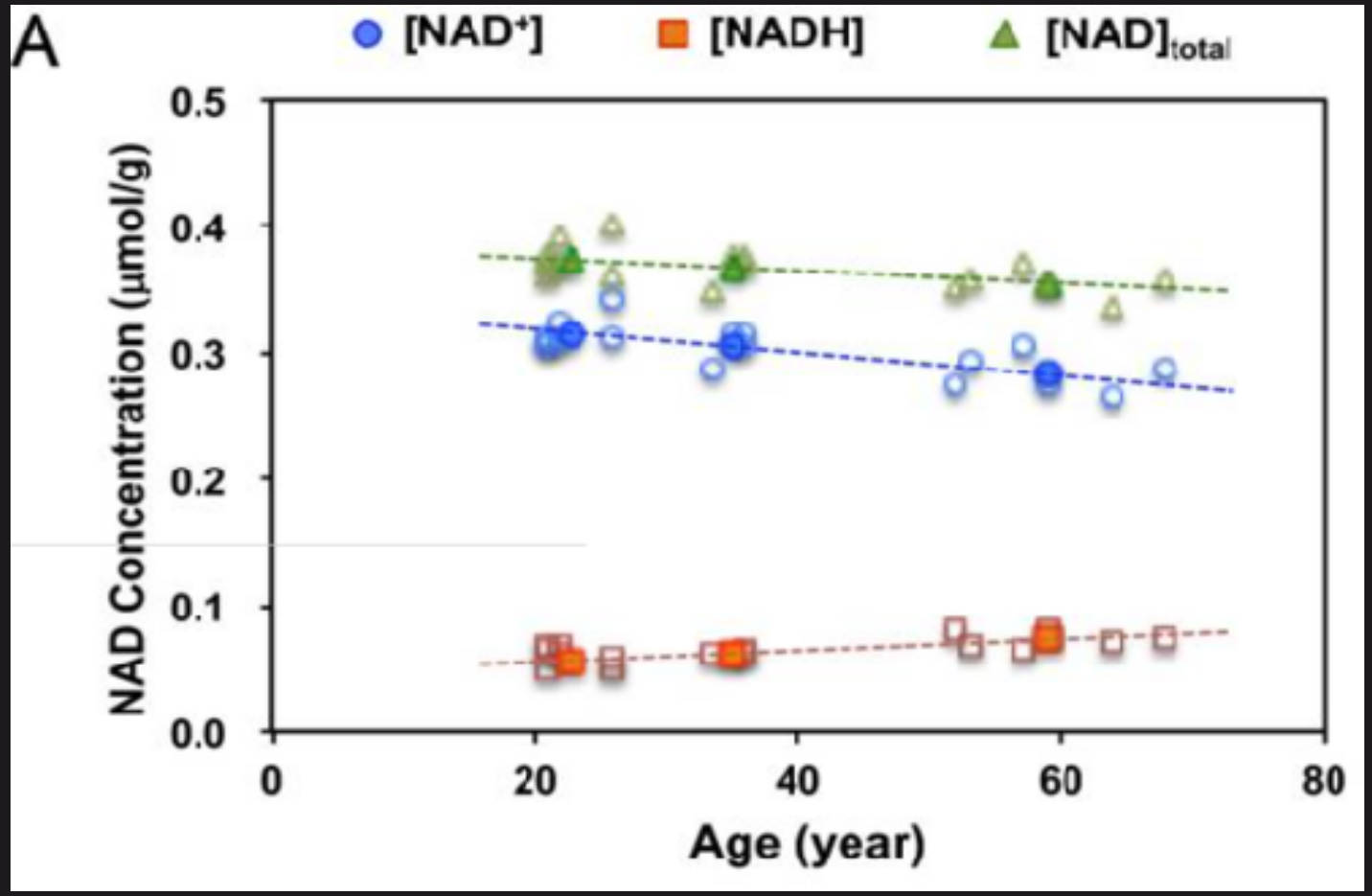
FUNDAMENTAL NAD+ STORIES

Intracellular NAD+ Levels Decline with Aging in the Brain



In Vivo Measurement of NAD+ in the Brains of 17 healthy patients, using 31P (7T) MRS Imaging *

* Zhu, et.al., *PNAS*, March, 2015, Vol 112, pp 2876- 2881

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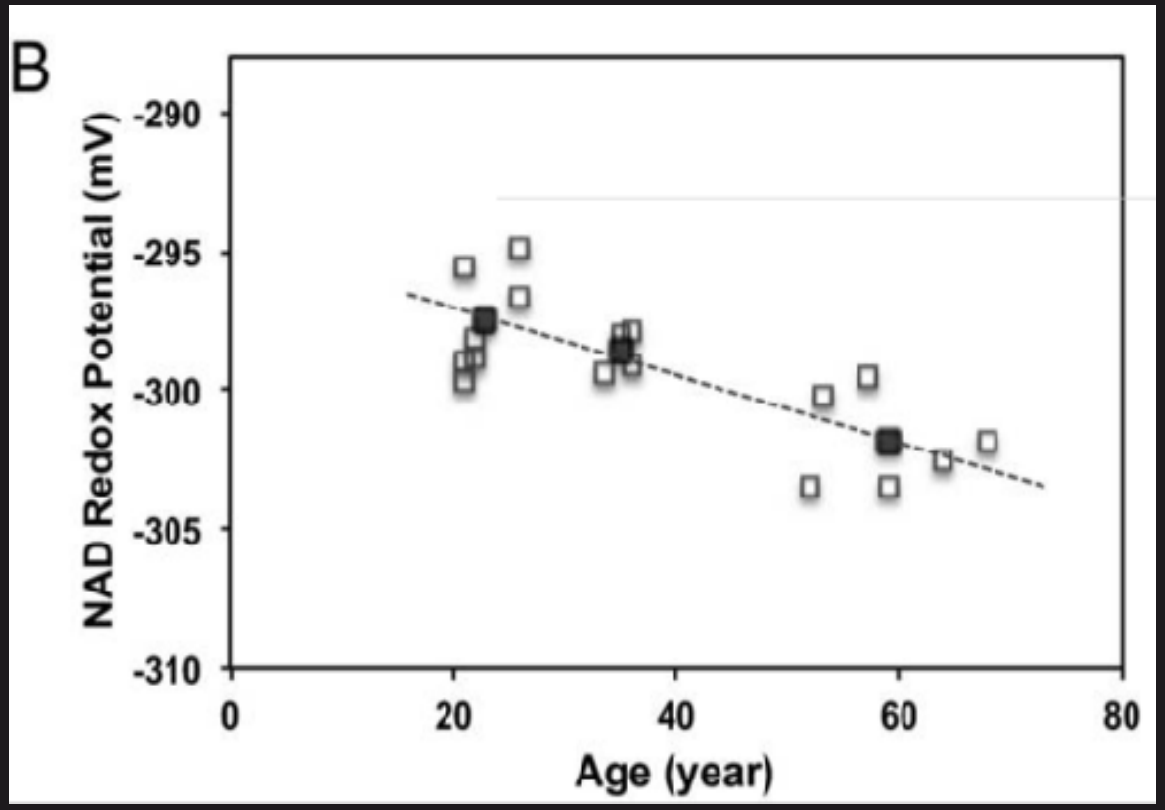
Redox Potential of Brain Cells Decline with Aging



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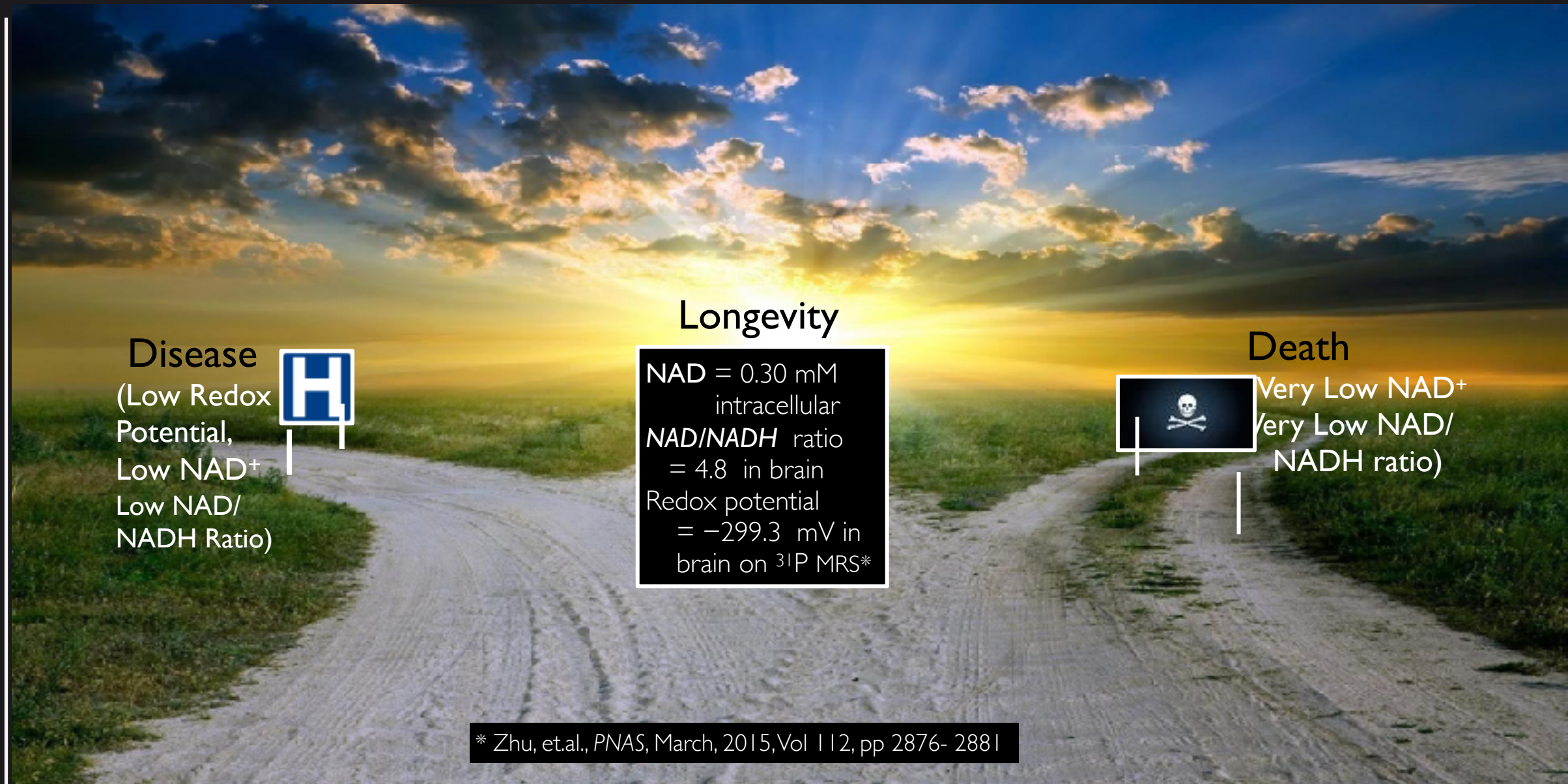
THE 3 OUTCOMES OF NAD+ LEVELS



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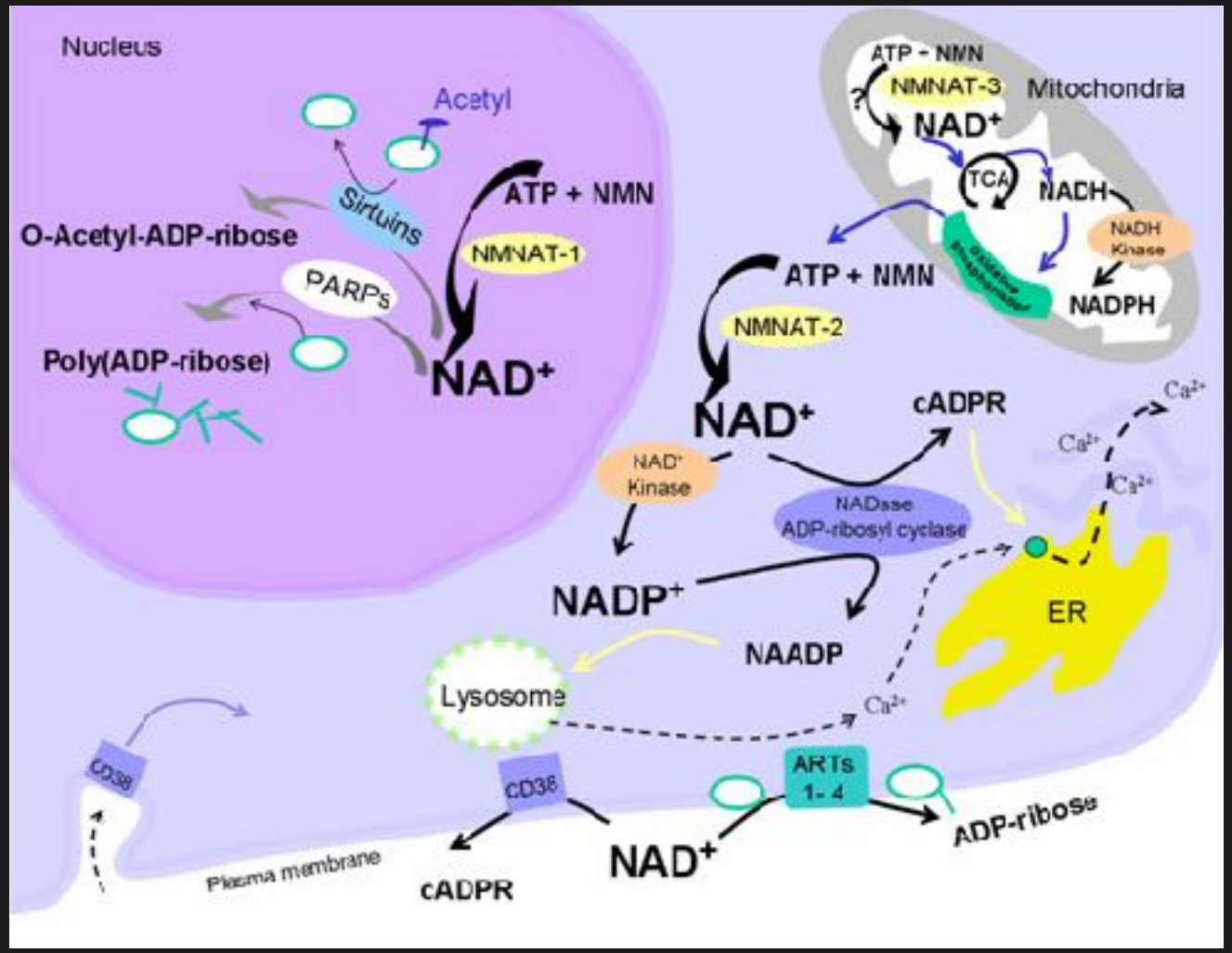
FUNDAMENTAL NAD+ STORIES

NAD+ and NADP+ Play a vital and unique role in each subcellular compartment of the cell



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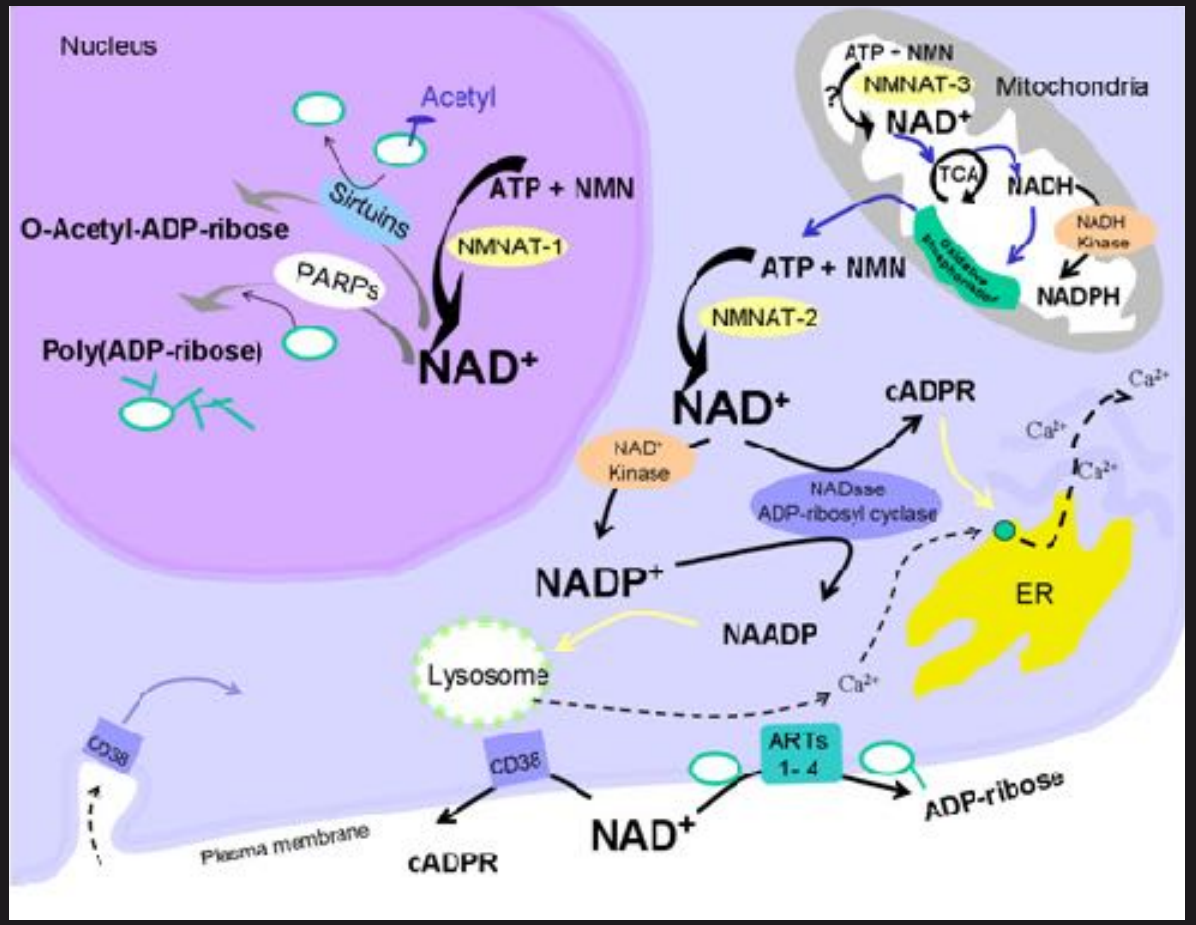
UNIQUE SUBCELLULAR ROLES OF NAD+ AND NADP+

Roles of NAD+ in Cell Nucleus

- **SIRT1** – Histone deacetylation, repetitive DNA silencing
- **SIRT6** – Histone ADP-ribosylation telomere maintenance
- **SIRT7** – Vital role in nucleolus
- **PARP1** – DNA repair
- **PARP2** – DNA repair
- **Tankyrases (aka PARP)** – Telomere maintenance
- **NMNAT-1** – converts NMN into to NAD+ in the nucleus



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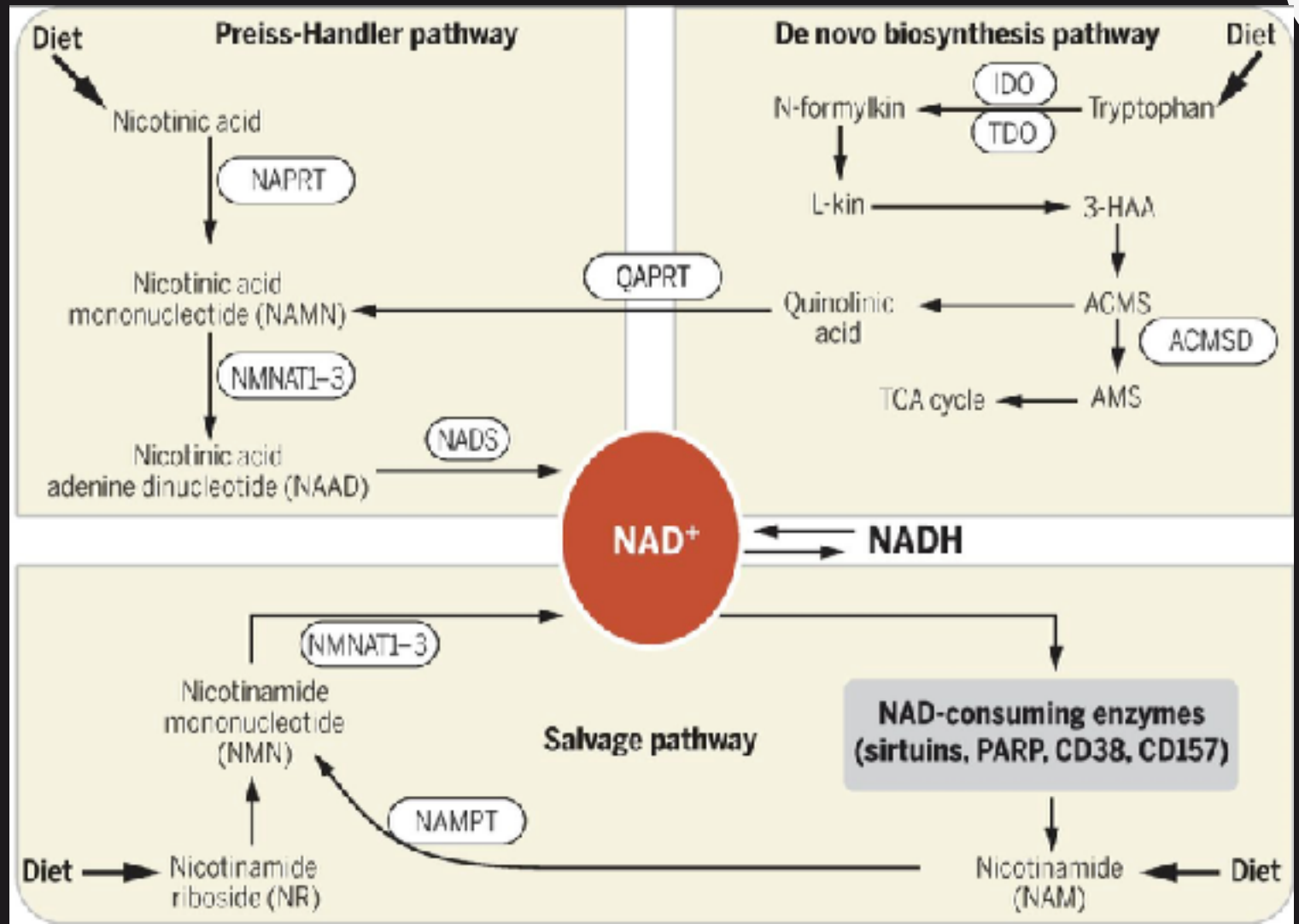
"Mommie, Where does NAD+ Come From"?

5 Ways to Get NAD+

- **Preiss-Handler Pathway** (NAD+ made from dietary nicotinic acid)
- **De novo Pathway** (NAD+ made from tryptophan via quinolinic a.)
- **Salvage Pathway** (NAD+ made from nicotinamide, byproduct of NAD+ consumption by SIRT, etc.)
- **Exogenous NAD+ precursors** (made from Nicotinamide Riboside or Nicontinamide Mononucletide)
- **Exogenous NAD+** (given IV, intra-nasally, or transdermally with iontophoresis)



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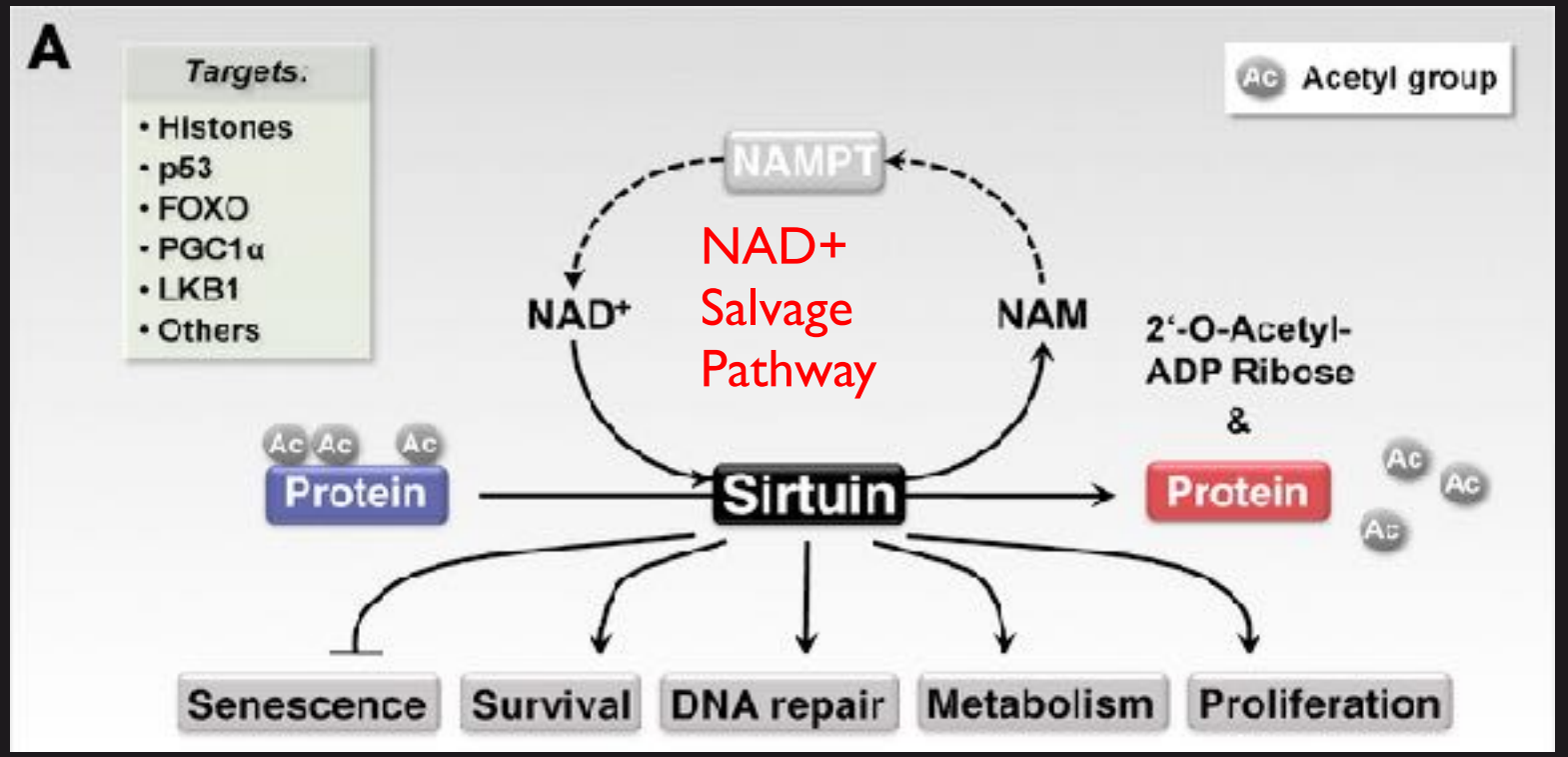
THE "7 SIRTUIN STORIES"

The "**7 Sirtuin Stories**" are the most well-studied roles of NAD+ as a cofactor* in the cell.

*(i.e. where NAD+ is consumed, vs being converted to NADH)



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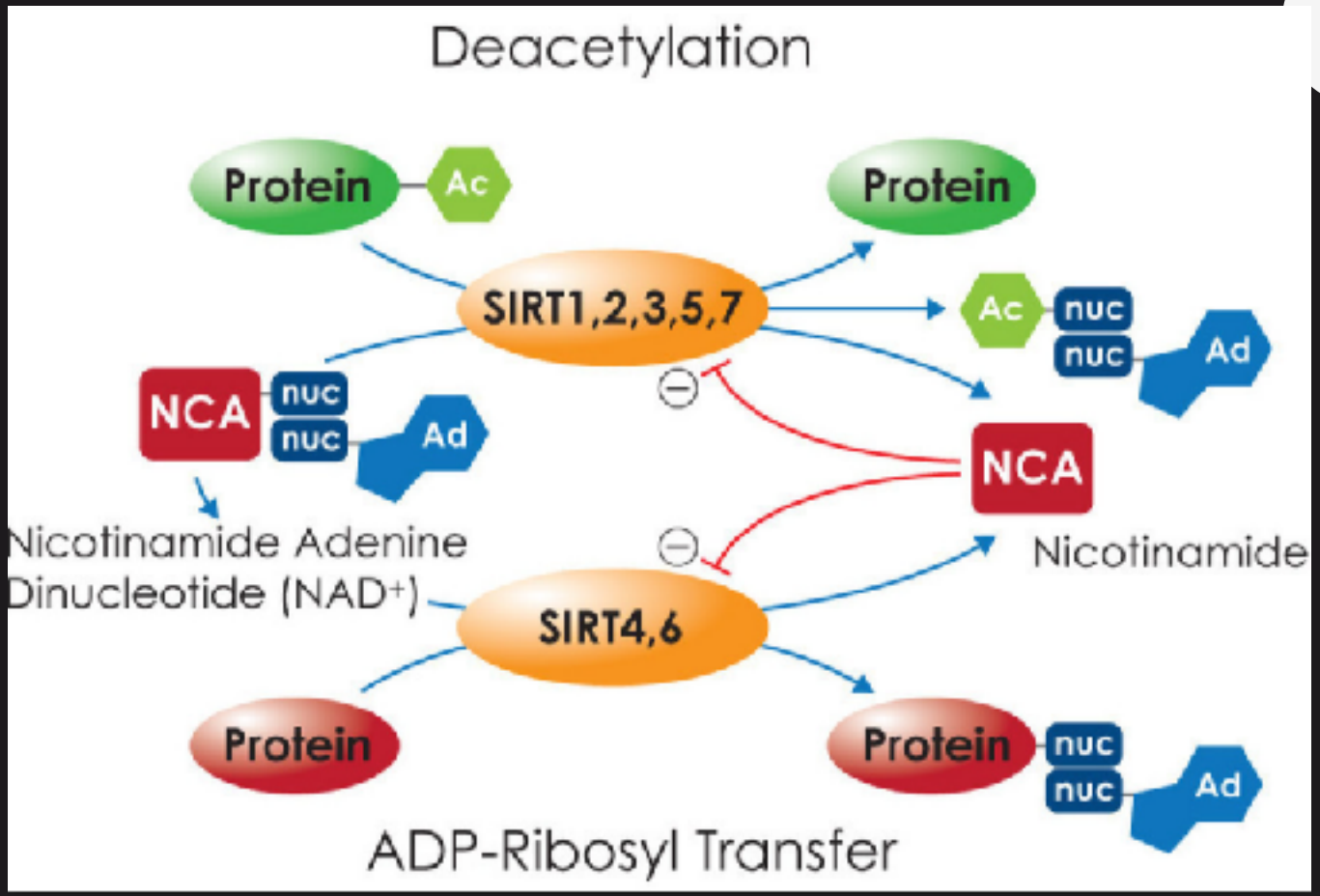
THE "SIRTUIN STORY"

- NAD+ is an essential substrate for the production of Sirtuins
- NAD+ drives the Sirtuin-dependent deacetylation of many key enzymes, including eNOS, NF-kB, PGC-1a, FoxOs, LKB1, and IRS1/IRS2.
- NAD+ drives the Sirtuin-dependent ADP-ribosylation of many proteins, including histones
- By NAD+-dependent deacetylation of these enzymes, Sirtuins thereby affect every major molecular pathway that involves aging.



This is why many researchers believe restoring NAD+ levels in the cell is so important for affecting the "cause" of aging, rather than the "effects" of aging.

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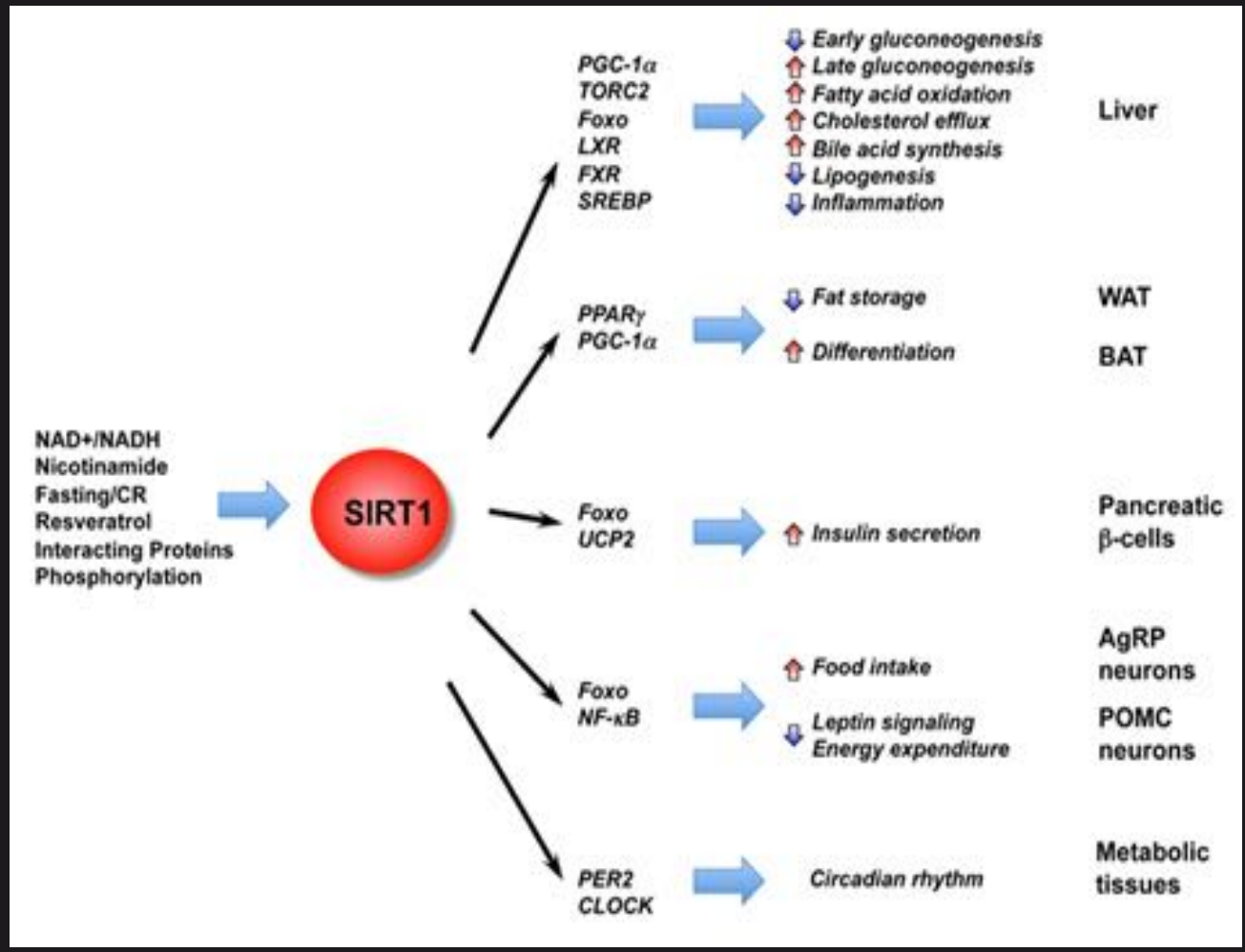
The "*SIRT1 Story*"

Key Points about SIRT1

- Only SIRT activated by Resveratrol (SIRT2-7 are not activated by resveratrol)
- Plays role in the nucleus and in the cytoplasm (histone deacetylation and transcription factor nuclear localization from cytoplasm)
- Key role in glucose metabolism and insulin secretion (TORC2, FoxO, UCP2,
- Key role in lipid trafficking (LXR, FXR, SREBP, PPAR, PGC-1)
- Key role in circaidian clock (PER2, CLOCK)
- Key role in reducing inflammation (FoxO, NF-kB)



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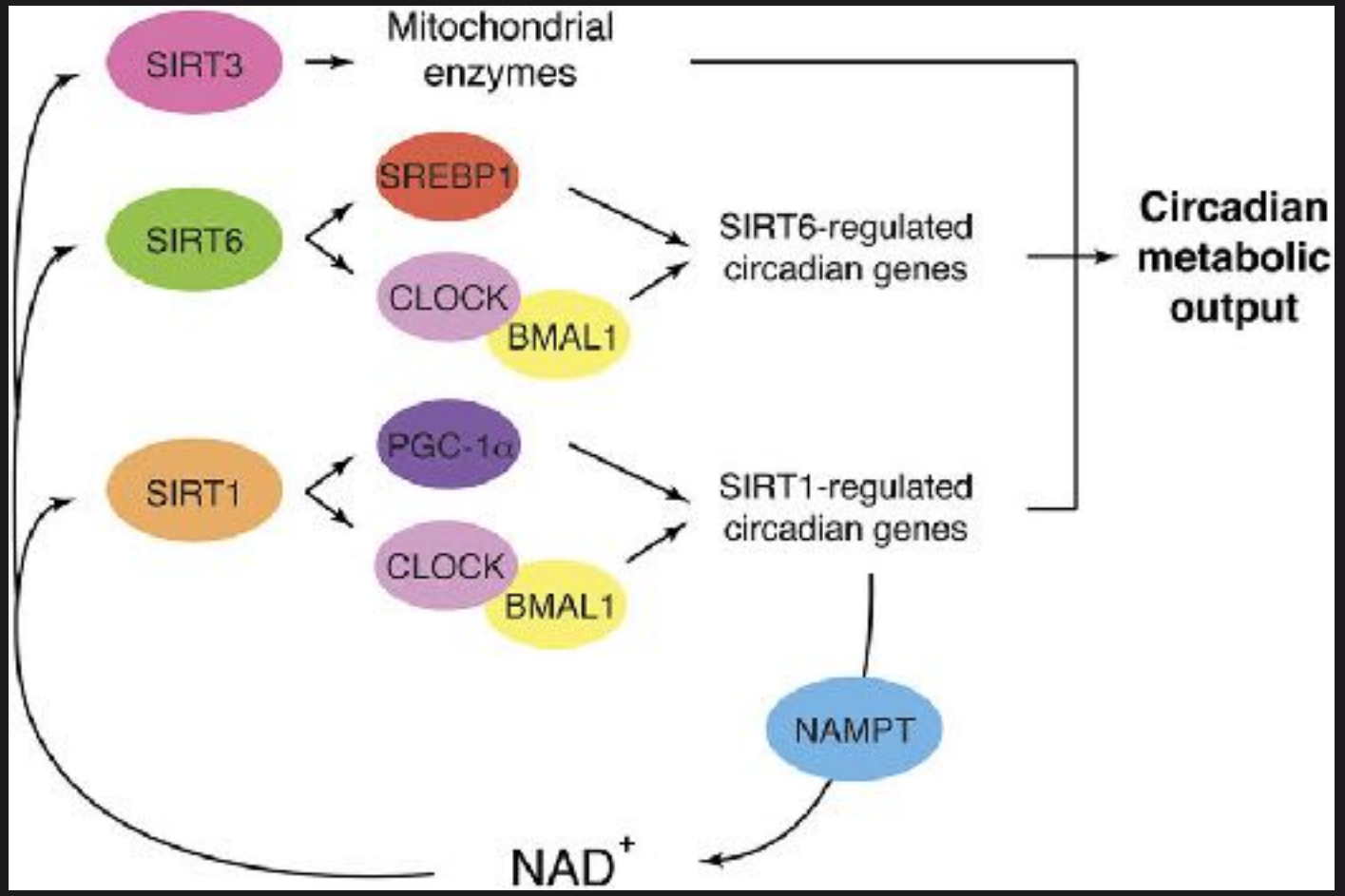
The "*SIR-cadian Clock Story*"

Sirtuins & Circadian Clocks

NAD+ and Sirtuins play vital roles in **circaidian clock** regulation of gene expression. Approximately 11% of the human genome Must be either "turned on" Or "turned off" by this SIRT1, SIRT3, and SIRT6 dependent molecular clock system, present in all 37 trillion human cells.



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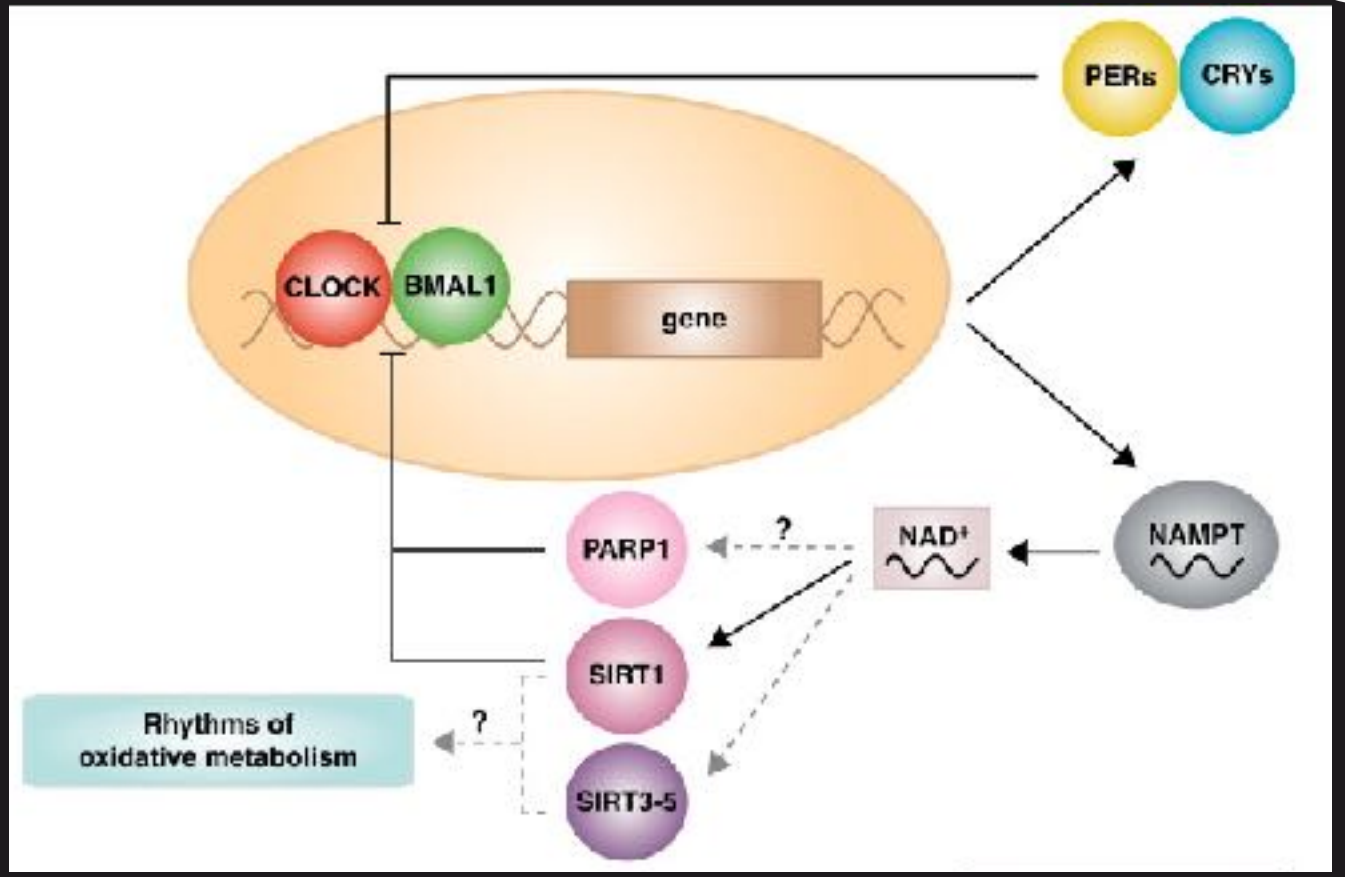
The "*Circadian Gene Expression of NAMPT Story*"

Circadian NAMPT Expression

- NAD+ salvage is regulated by the rate-limiting step in the salvage cycle, which is the enzyme NAMPT.
- NAMPT gene expression occurs in a circadian fashion – highest in the evening and at night.
- If your circadian rhythms and body clocks are off, NAD+ and Sirtuin enzyme levels may be too low, resulting in body circadian clocks not work well.



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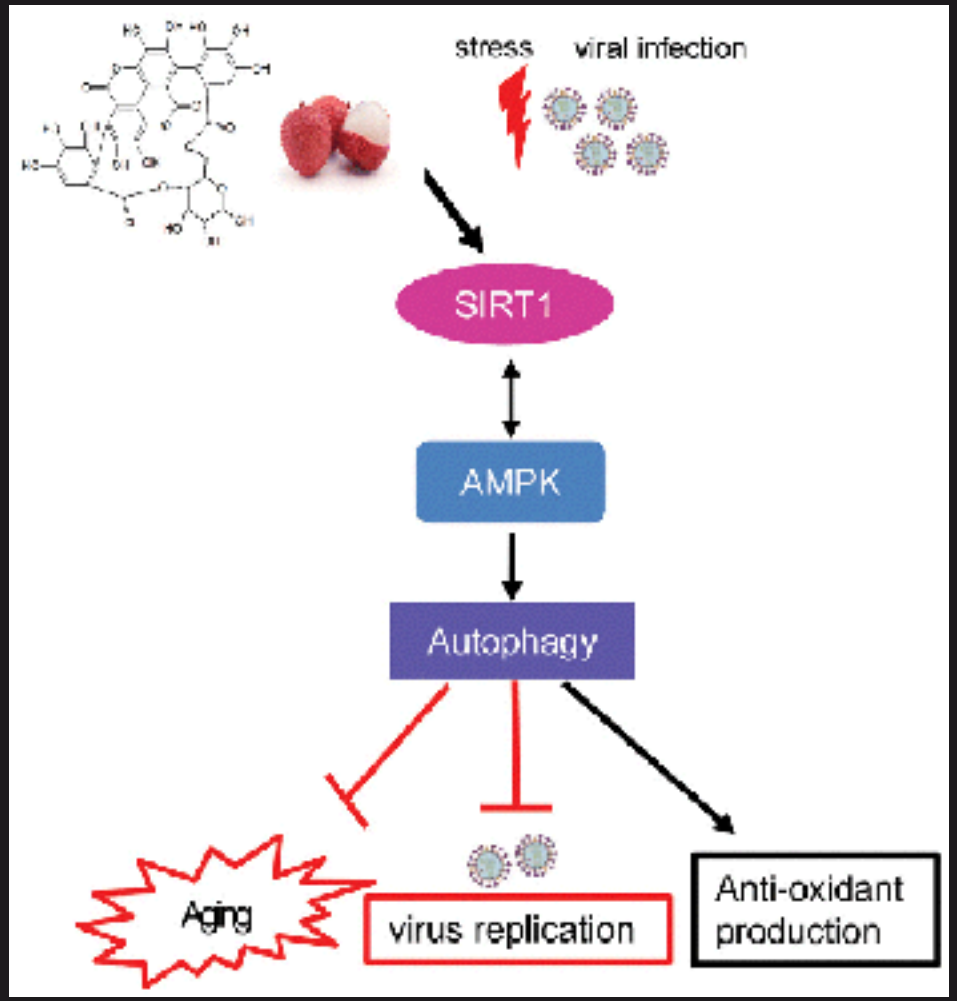
The "*Autophagy Story*"

NAD+ and SIRT1 play vital roles in " **cellular house cleaning**", akac autophagy. Autophagy is the only way cells can get rid of old damaged mitochondria, damaged cell membranes, and damaged, misfolded proteins. Also important in preventing virus infections, anti-oxidant protection and preventing aging.



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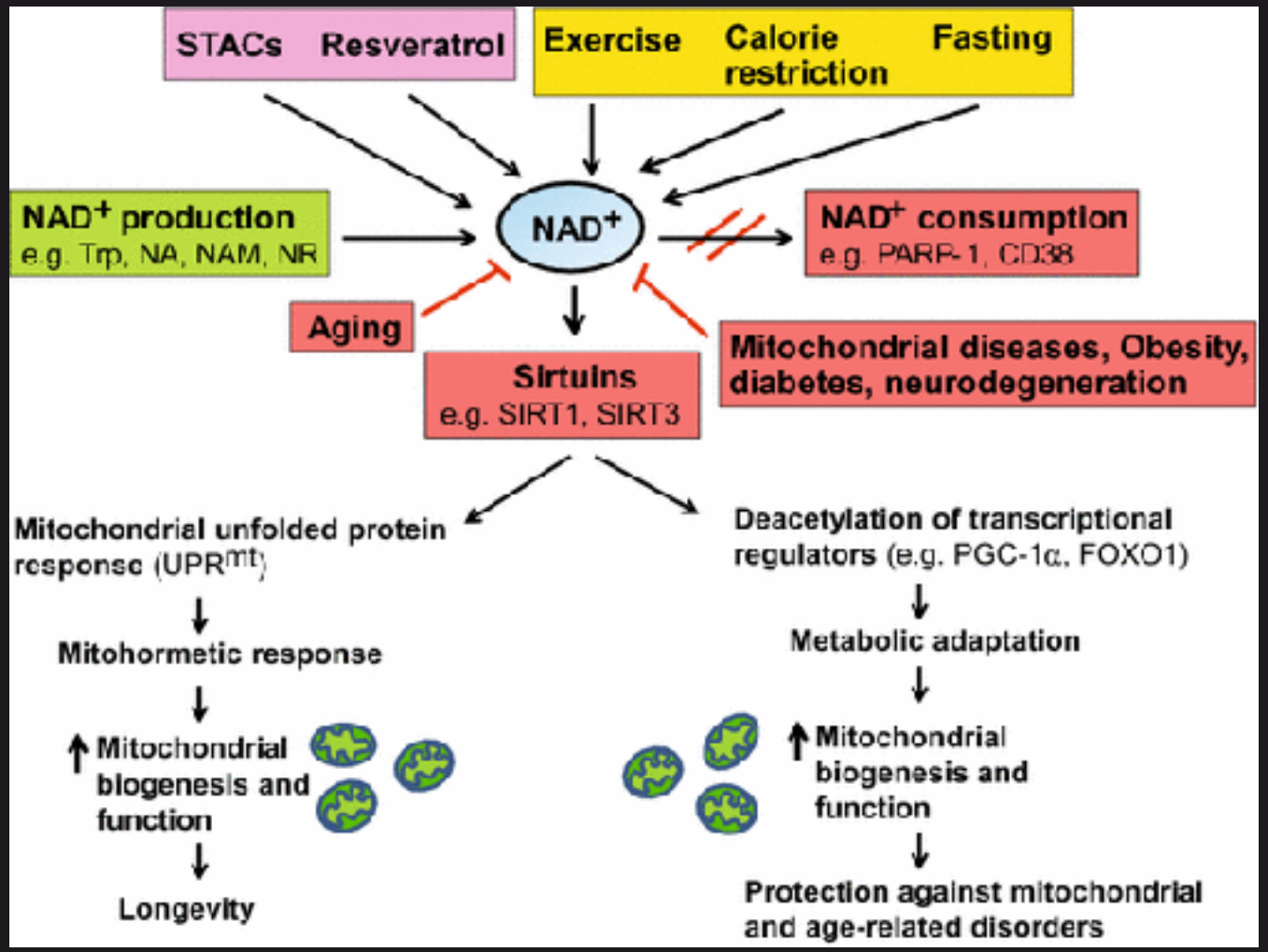
The "*Mitochondria-NAD+ Story*"

NAD+, Sirtuins and Mitochondrial function

NAD+ and Sirtuins are required for making new mitochondria (aka mitochondrial biogenesis) and mitochondrial function. SIRT1 and SIRT3 are key for Mitochondrial biogenesis. SIRT3, SIRT4, and SIRT5 are key for mitochondrial function. SIRT3-5 are all localized to inside of the mitochondria



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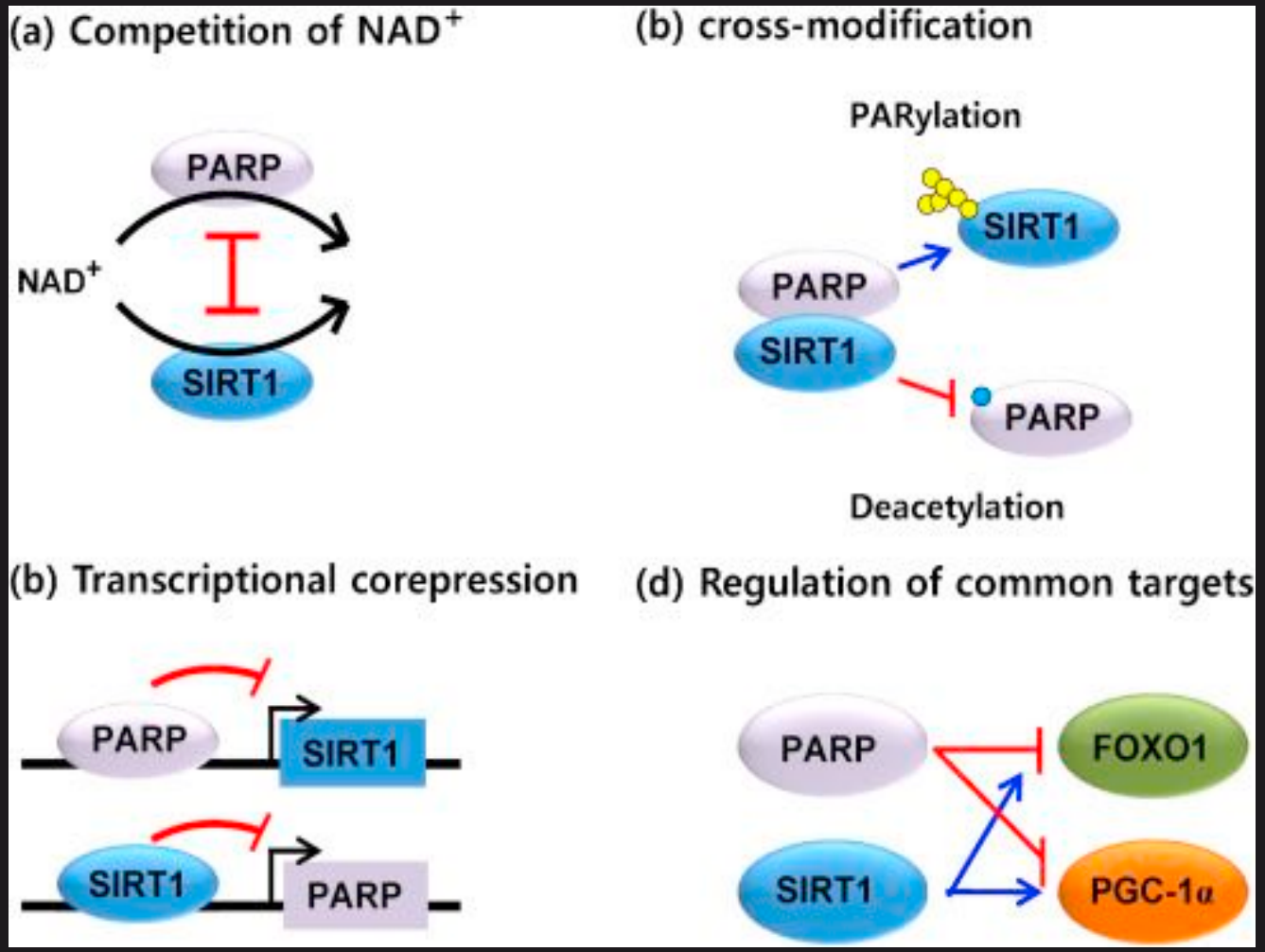
The "*PARP-SIRT Competion" for NAD+ Story*"

- PARPs and SIRTs both require plenty of NAD+ and a high NAD/NADH ratio
- Because of their mutal dependence on NAD+ to function, there is a natural competition between the Sirtuin enzymes and the PARP enzymes.
- PARP enzymes and SIRT1 enzyme have mutual negative feedback on each other. PARPs inhibit SIRT1 and SIRT1 inhibits PARP.
- PARPs and SIRT1 have opposite effects on FoxO1 and PCG-1a



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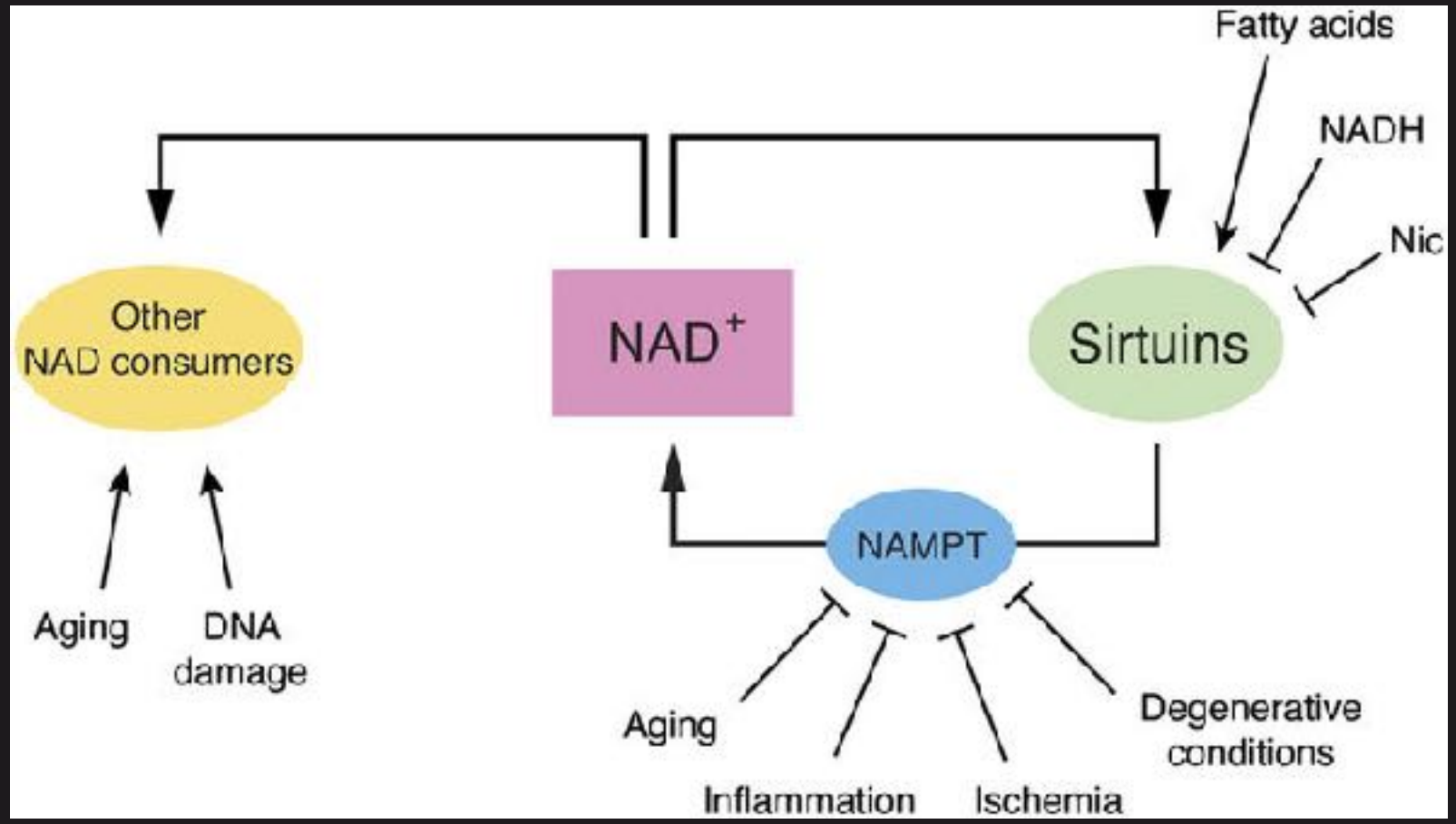
NAD+ AND AGING

- Aging and Age-related degenerative conditions contribute to both declining production of NAD+
- Aging, inflammation, ischemia, and other degenerative conditions inhibit the NAD+ salvage pathway from making more NAD+
- Fatty acids, NADH, and nicotinamide all inhibit Sirtuins



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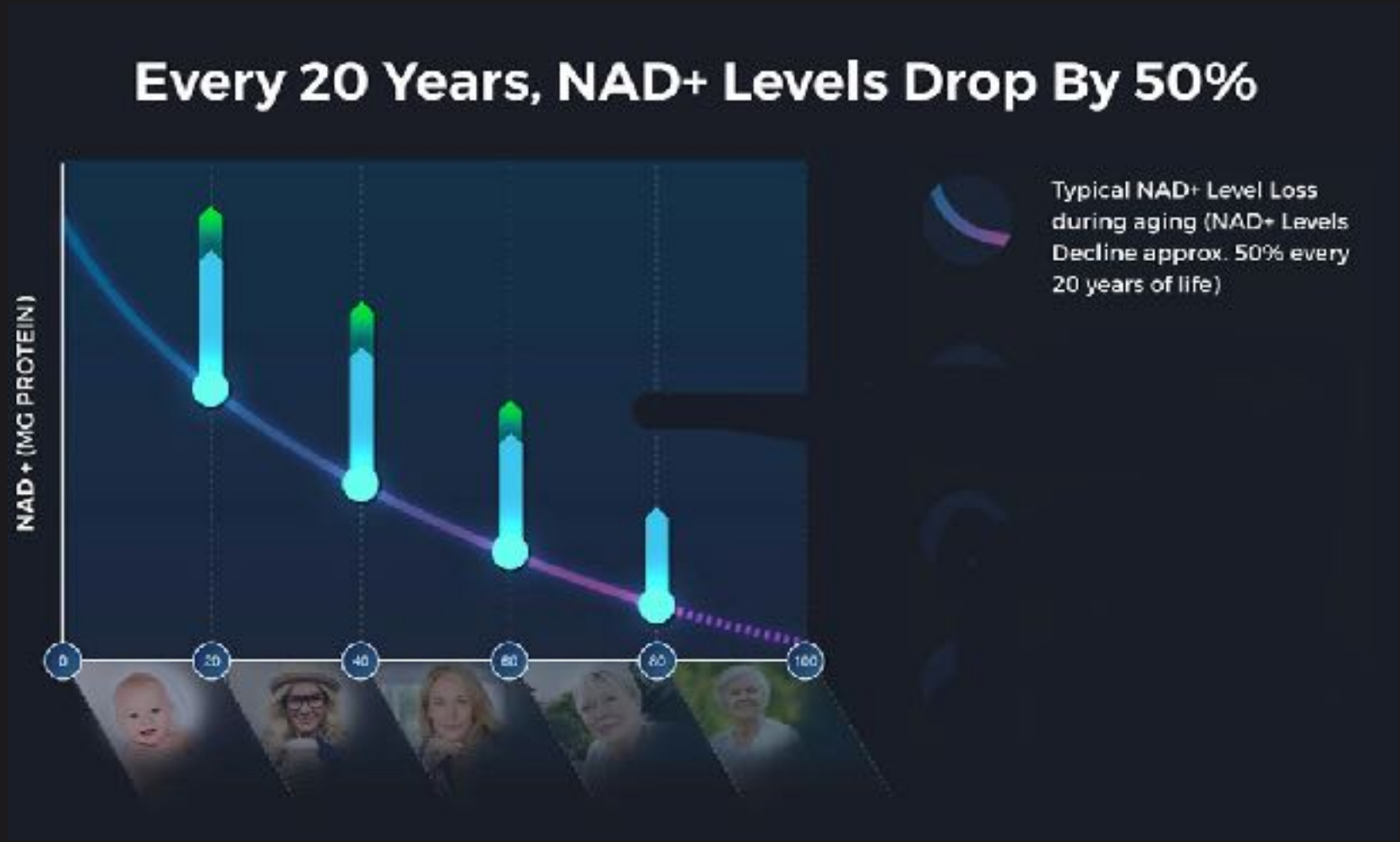
The "*Declining NAD+ Story*"



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The "*Declining NAD+ Story*"

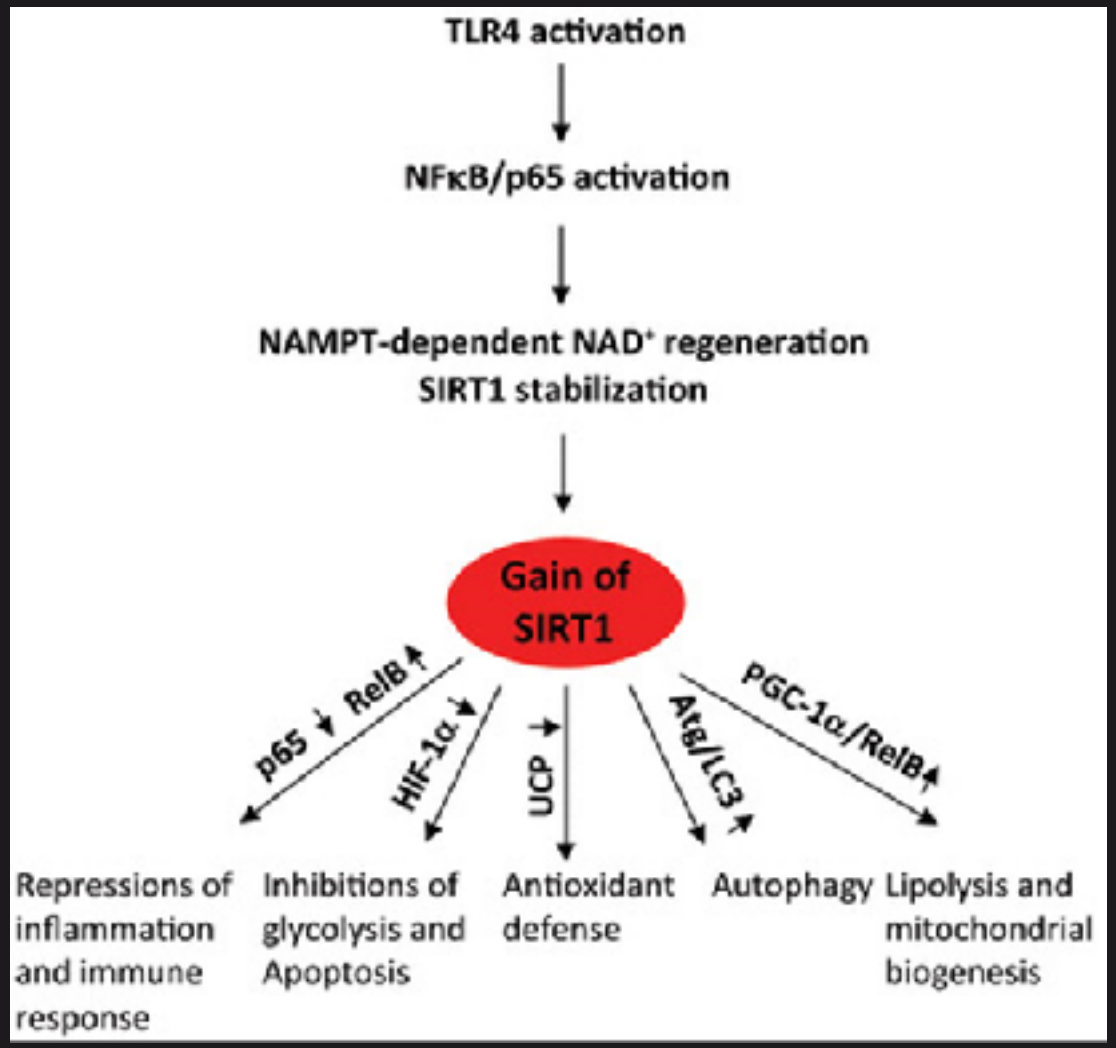
Nice Normal Tale:

When the nad salvage cycle is working well and there is plenty of sirt1, lots of good stuff happens.

Even when triggered By inflammation

Scary, Horrible Tale:

When NAD+ is scarce due to aging, sickness, competiton or pac-men factors, and sirt1 is scarce, expect the opposite consequence



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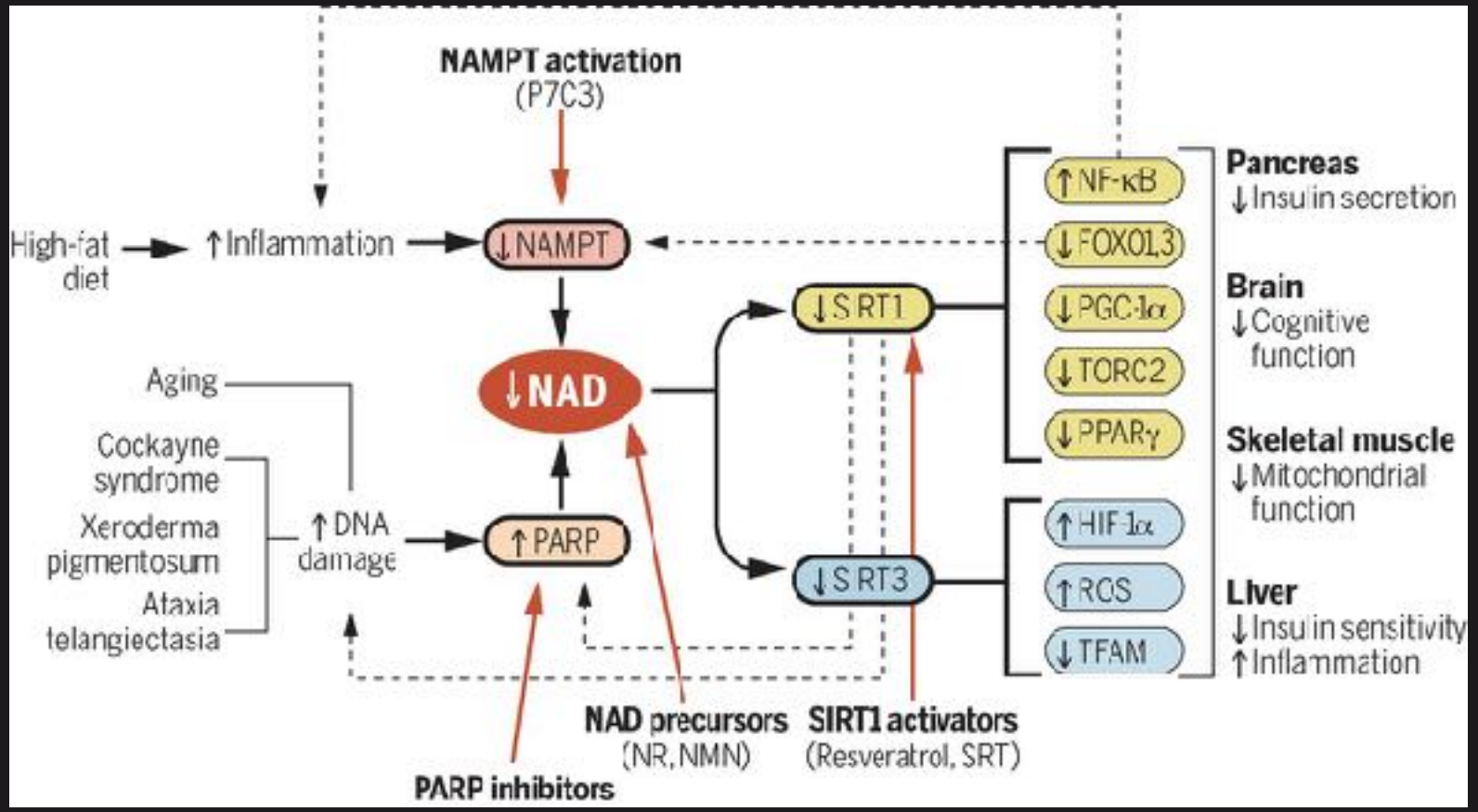
The "Declining NAD+ Story"

- With aging there is not enough NAD+ to satify the multiple needs for it. its usages in the body are triggered and rationed, with negative conseques across the body.
- Declining NAD+ Induces a Pseudohypoxic State Disrupting Nuclear-Mitochondrial Communication during Aging



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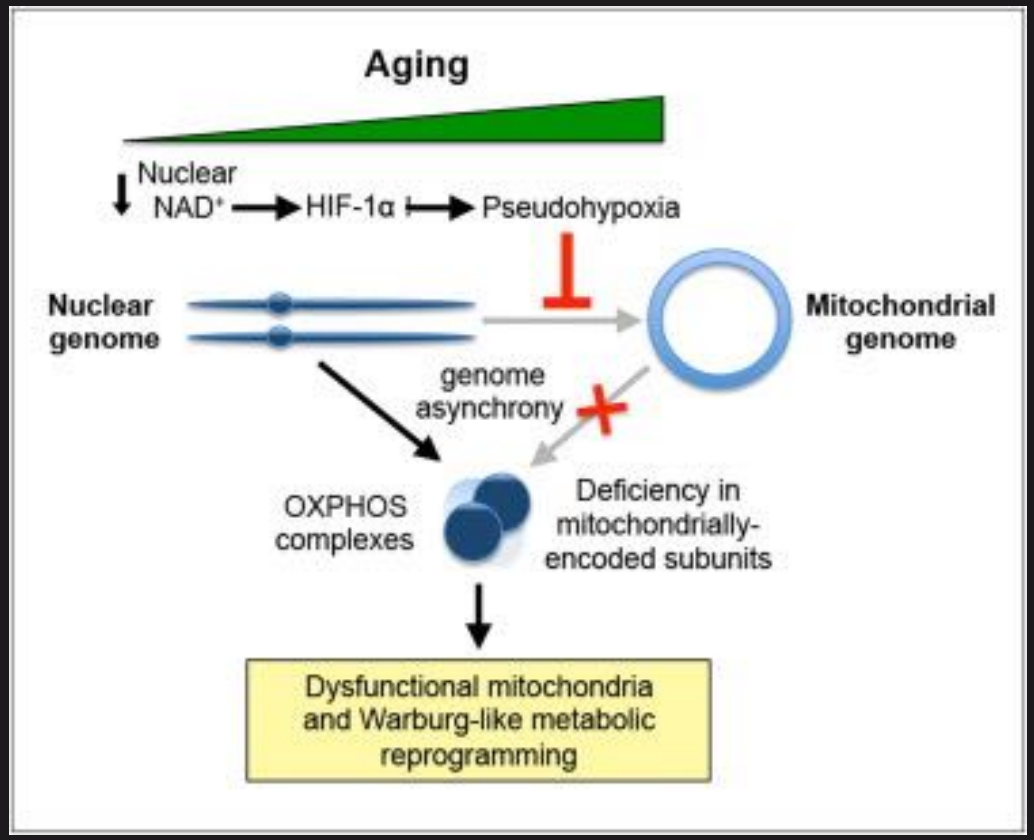
The "*Declining NAD+-Pseudohypoxia Story*"

Declining NAD+ in Aging & Mitochondrial Dysfuction

- A specific decline in mitochondrially encoded genes occurs w/muscle aging
- Nuclear NAD+ levels regulate mitochondrial homeostasis independently of PGC-1α/β
- Declining NAD+ during aging causes pseudohypoxia, which disrupts OXPHOS function
- Raising nuclear NAD+ in old mice with intraperitoneally administered NMN temporarily reversed pseudohypoxia and mitochondrial dysfunction



Ref: Gomes, et.al. Declining NAD+ Induces a Pseudohypoxic State Disrupting Nuclear-Mitochondrial Communication during Aging, Cell, December, 2013, Volume 155, pp 1624-1638.

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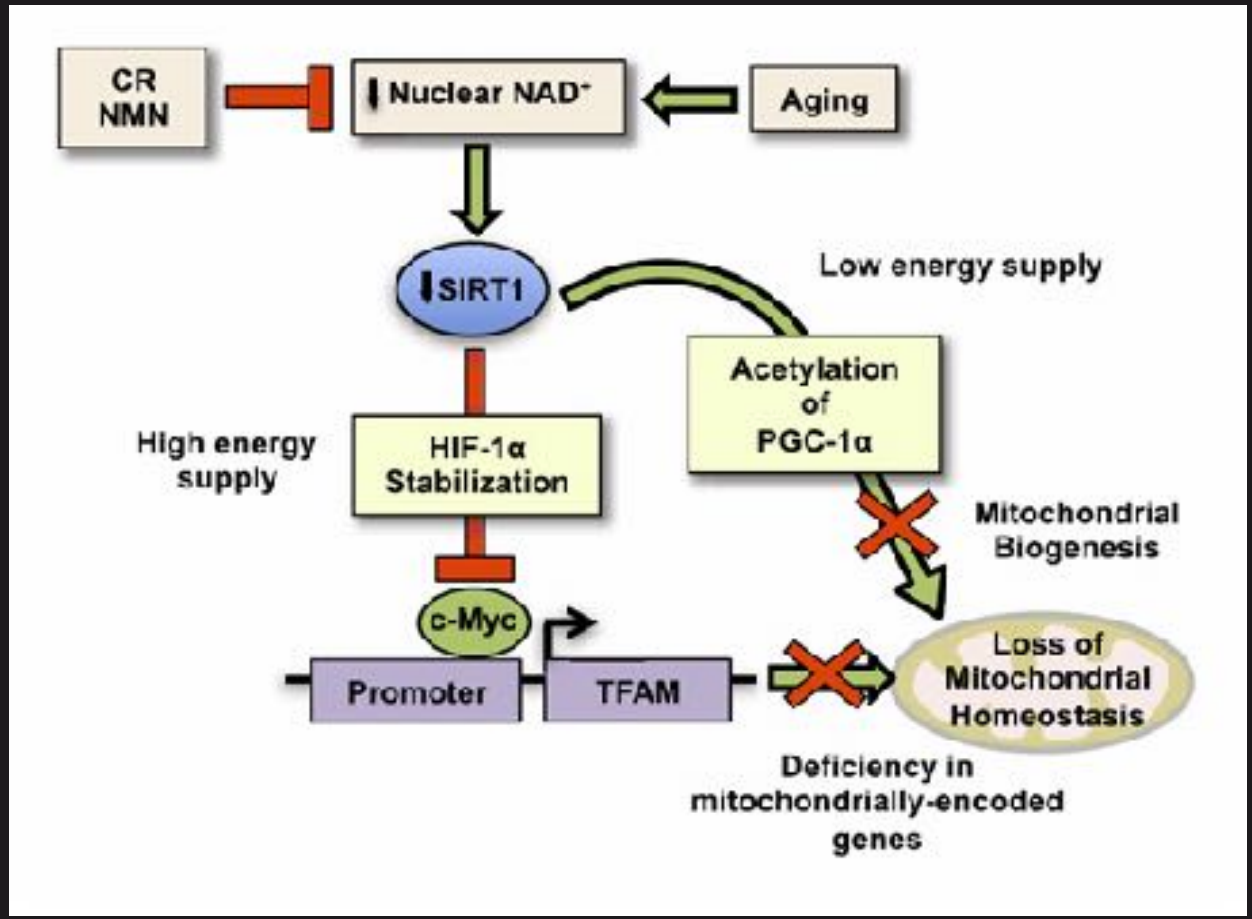
The "*Declining NAD+-Pseudohypoxia Story*"

How a Decline In Nuclear NAD+ Causes Mitochondrial Dysfuction

- Decline in nuclear NAD+ occurs with aging and overeating
- This produces a decline in SIRT1 activity, which has 2 consequences:
  1. HIF-1 stabilization
  2. Lack of deacetylation of PGC-1α
- This inhibits c-Myc activation of TFAM, which is the transcription factor for mitochondrial genes
- Loss of PGC-1α activation further stops mitochondrial biogenesis



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THE LOW NAD+ CHAMBER OF HORRORS

- **Inadequate Sirtuin function** – SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7
- **Inadequate PARP function** – PARP1, PARP2, PARP3, etc. Tankyrases
- **Inadequate DNA repair** – PARPs and SIRTs needed for DSB repair
- **Genomic Instability** => aneuploidy, gene mutations, polyploidy, etc.
- **Mitochondrial biogenesis fails** => no ATP
- **Mitochondrial dysfunction/death** => no ATP, no fat β-oxidation, ⇧ apoptosis
- **Excessive mitochondrial ROS** => aging
- **Warburg metabolism** => lactic acidosis
- **Compromised stress resistance**

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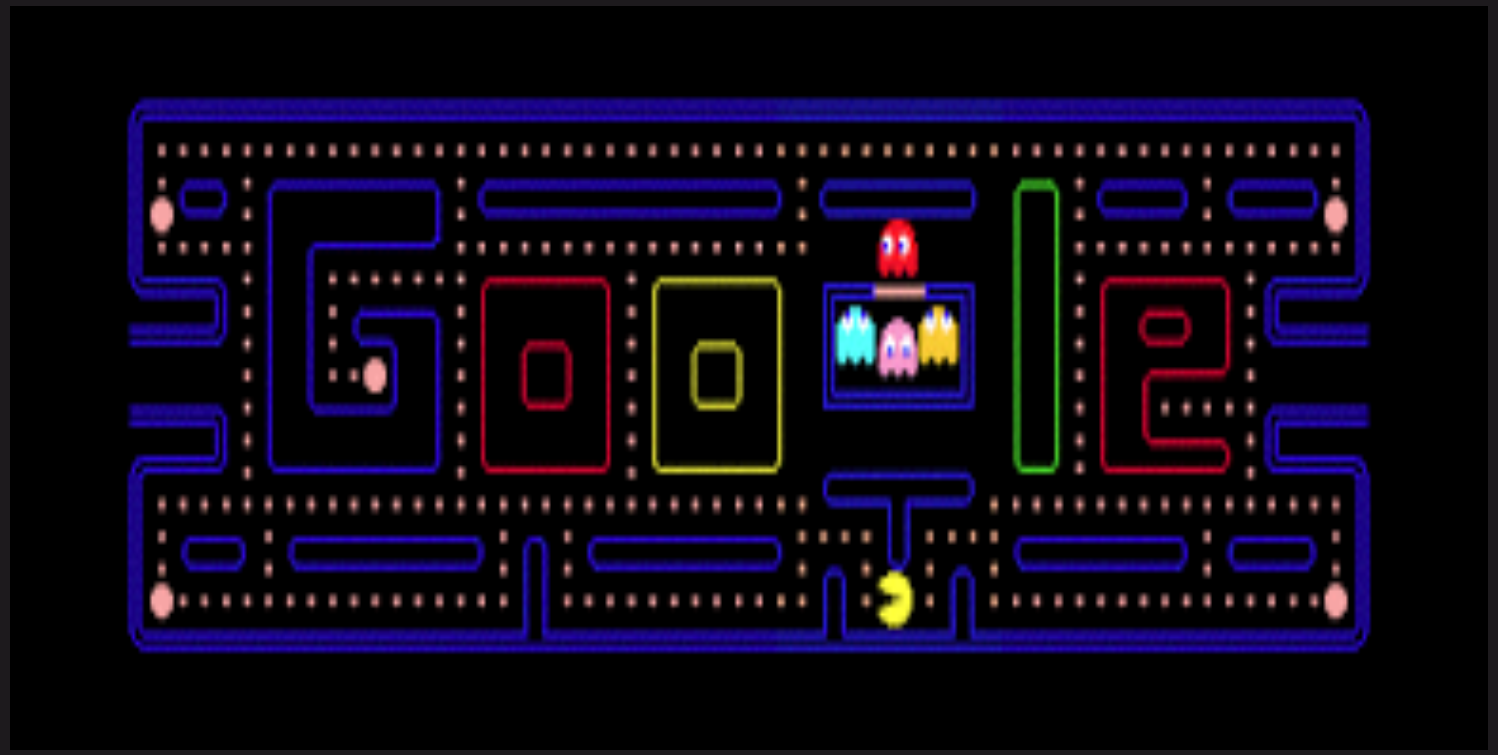
THE LOW NAD+ CHAMBER OF HORRORS

- **Cellular senescence** – increased SASP, chronic tissue inflammation, aging
- **Impaired autophagy** – no clearance of old mitochondria, misfolded proteins, etc.
- **Endoplasmic reticulum stress** => cell death
- **Failure of histone deacetylation** => epigenetic and circaidian gene regulation fails
- **Reduced antioxidant defenses** => ROS damage, lipid oxidation, protein carbonylation
- **Microtubule "railway" transport failure** => axon transport failure in the CNS
- **Inflammasome activation**
- **Impaired misfolded protein clearance**
- **Inactivated tumor suppressor proteins** – Ex: p53

PACMEN WHO EAT NAD+ STORIES

- PARP enzymes
- CD38 enzymes
- High fat diet
- NQO1 problem



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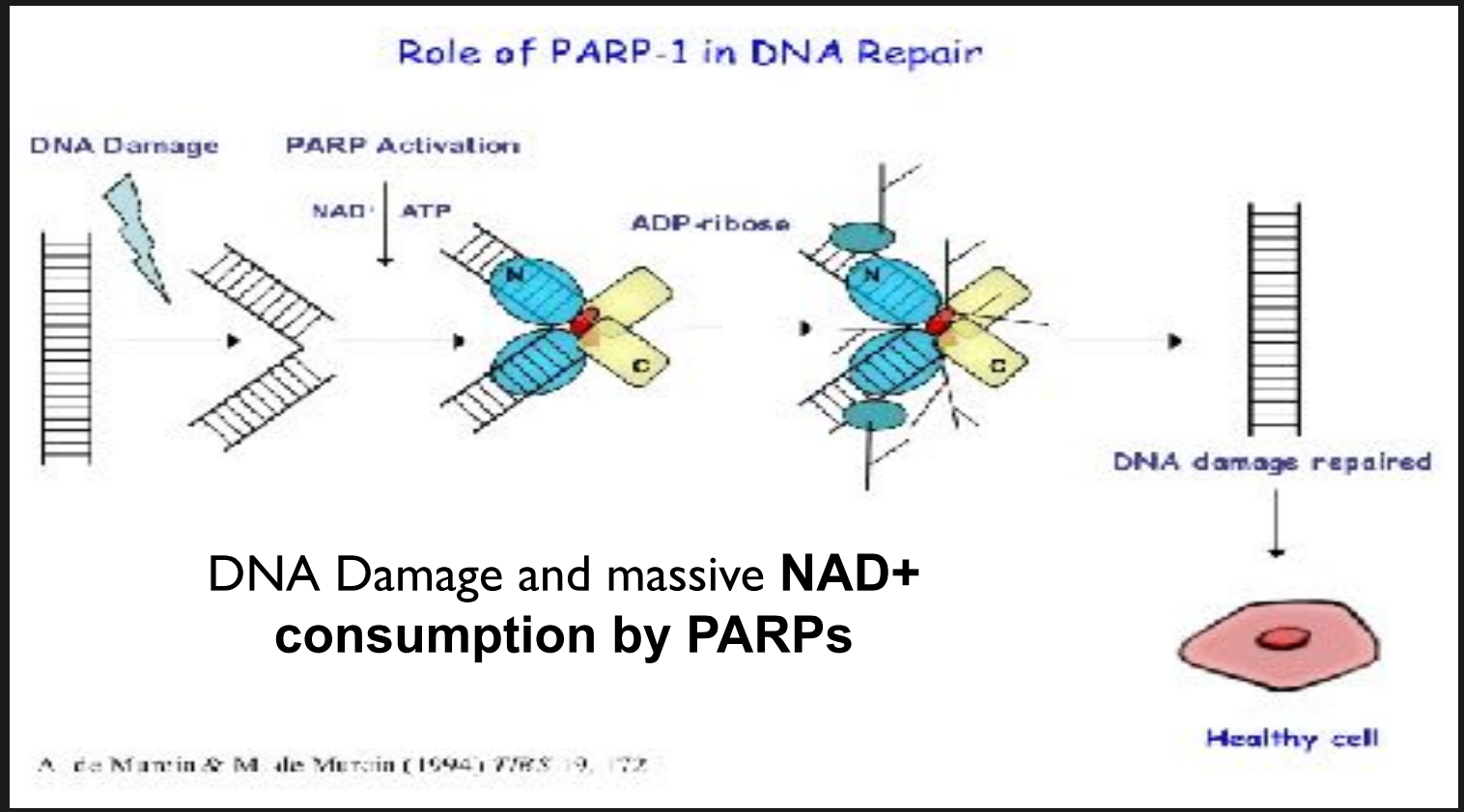
PACMEN WHO EAT NAD+ STORIES

The "*PARP-consuming NAD+ Story*"

- PARPs 1 and 2 are nuclear enzymes that are implicated in many cellular processes including DNA repair
- DNA damage is a relatively common event in the life of a cell and may lead to mutation, cancer, and cellular or organismic death.
- An important cell response to DNA damage is PARP activation, which consumes massive amounts of NAD+
- PARP hyperactivation occurs with crystal meth use



DNA Damage and massive **NAD+ consumption by PARPs**

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A NEW NAD+ STORY FROM 2017

How NAD+ binds in a pocket between DBC and PARP

The Nudix Homology Domain
A new function of NAD+

- DBC is an inhibitor of PARP1 , which prevents DNA repair
- With aging NAD+ concentrations decline, DBC1 is increasingly bound to PARP1, compromising PARP1 DNA repair and causes DNA damage to accumulate
- This process can be rapidly reversed by restoring the abundance of NAD+

A conserved NAD+ binding pocket that regulates protein-protein interactions during aging

Jun Li,[1] Michael S. Bonkowski,[1] Sébastien Moniot,[2] Dapeng Zhang,[3†] Basil P. Hubbard,[1†] Alvin J. Y. Ling,[1] Luis A. Rajman,[1] Bo Qin,[4] Zhenkun Lou,[4] Vera Gorbunova,[5] L. Aravind,[3] Clemens Steegborn,[2] David A. Sinclair[1,6‡]

DNA repair is essential for life, yet its efficiency declines with age for reasons that are unclear. Numerous proteins possess Nudix homology domains (NHDs) that have no known function. We show that NHDs are NAD+ (oxidized form of nicotinamide adenine dinucleotide) binding domains that regulate protein-protein interactions. The binding of NAD+ to the NHD domain of DBC1 (deleted in breast cancer 1) prevents it from inhibiting PARP1 [poly(adenosine diphosphate–ribose) polymerase], a critical DNA repair protein. As mice age and NAD+ concentrations decline, DBC1 is increasingly bound to PARP1, causing DNA damage to accumulate, a process rapidly reversed by restoring the abundance of NAD+. Thus, NAD+ directly regulates protein-protein interactions, the modulation of which may protect against cancer, radiation, and aging.

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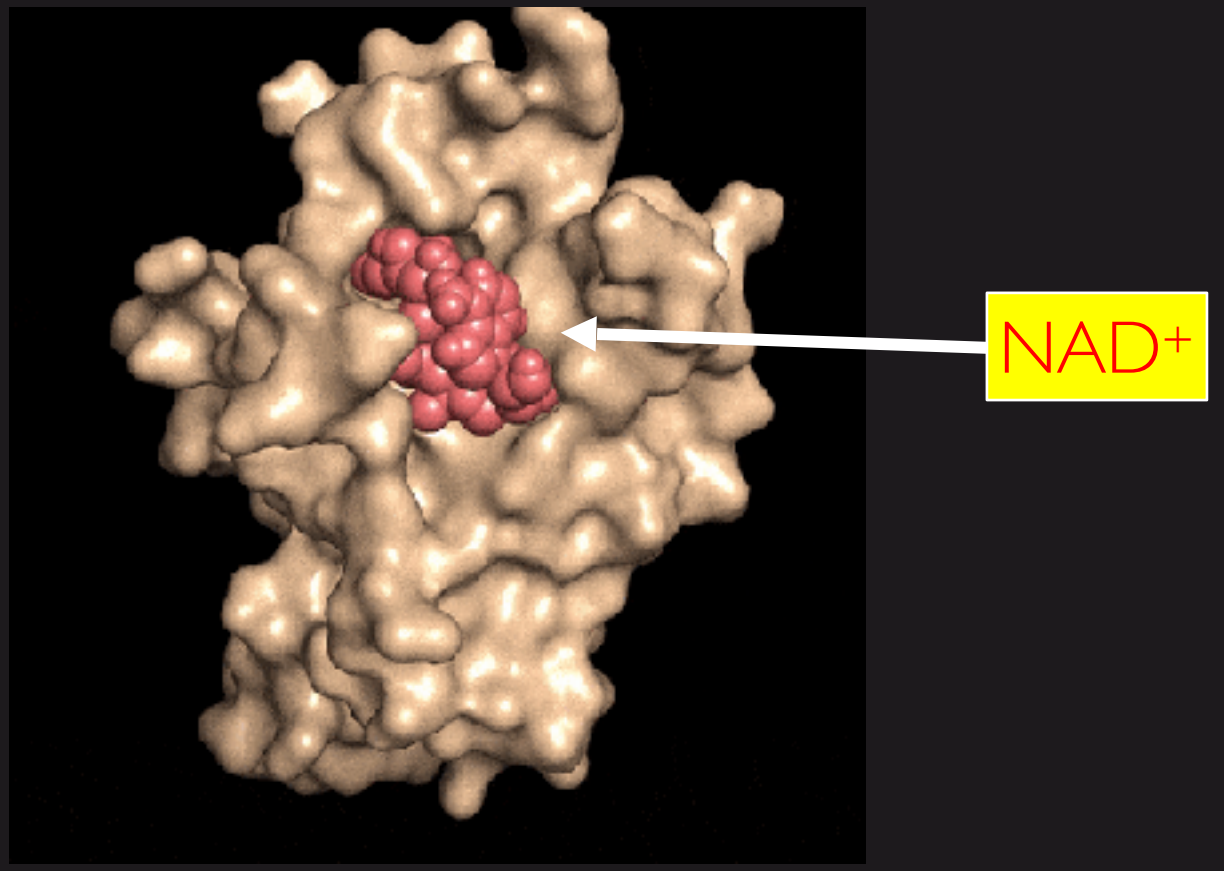
A NEW NAD+ STORY FROM 2017

How NAD+ binds in a pocket between DBC and PARP

The Nudix Homology Domain
A new function of NAD+

- The Nudix Homology Domain (NHD) is simply a binding pocket formed by two proteins
- Unless the two proteins are bound together, the "pocket" cannot be visualized or detected in the lab
- This is why this new role of NAD+ remained unknown for so long



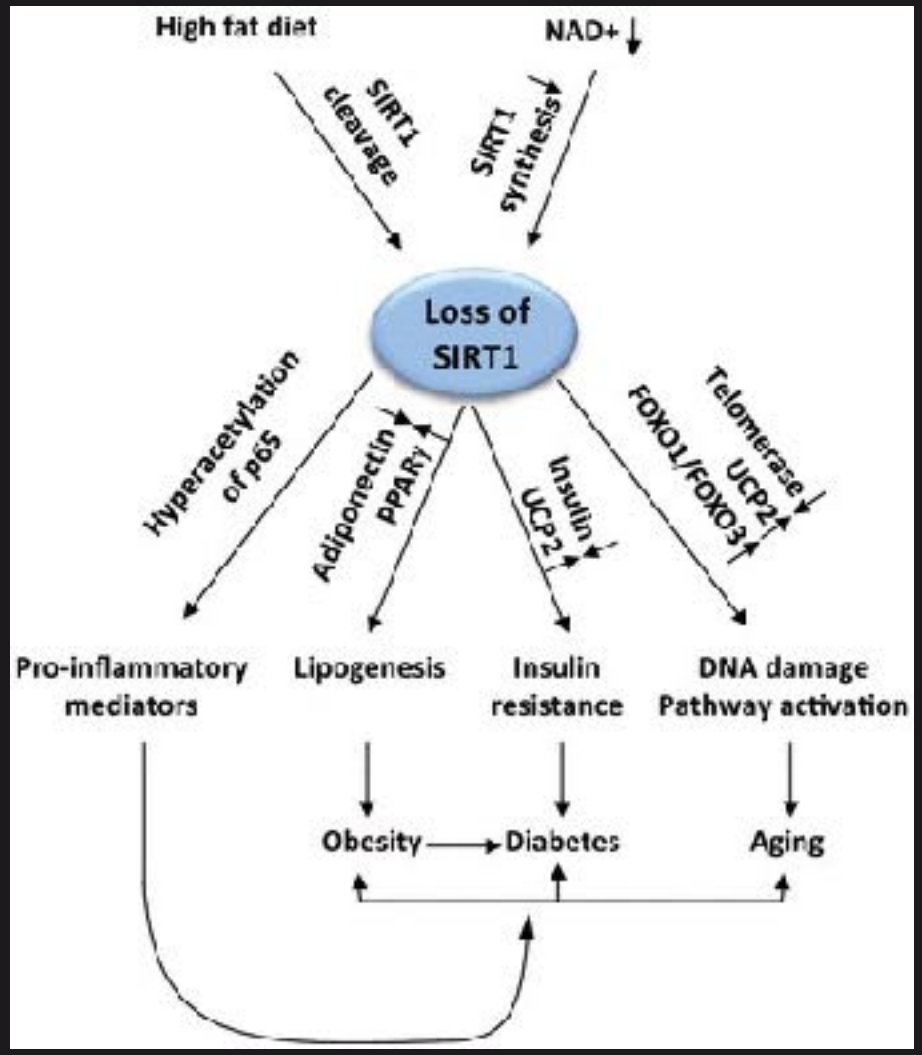
3rd Annual
NAD+ Summit

FUNDAMENTAL NAD+ STORIES

Fat: The "*Dietary NAD+ Destroyer*"

How To Destroy all your NAD+ at the Dinner Table!

- High fat diet triggers SIRT1 cleavage
  => destroying all of the SIRT1 function
- SIRT1 loss triggers 4 bad things:
  1. Inflammation (IL-6, TNF-α, IL-1β, etc.)
  2. Fat generation (via adiponectin, PPARγ)
  3. Insulin resistance (via UCP2)
- The pro-inflammatory cytokines have a "positive feedback" on the formation of obesity, diabetes, and aging



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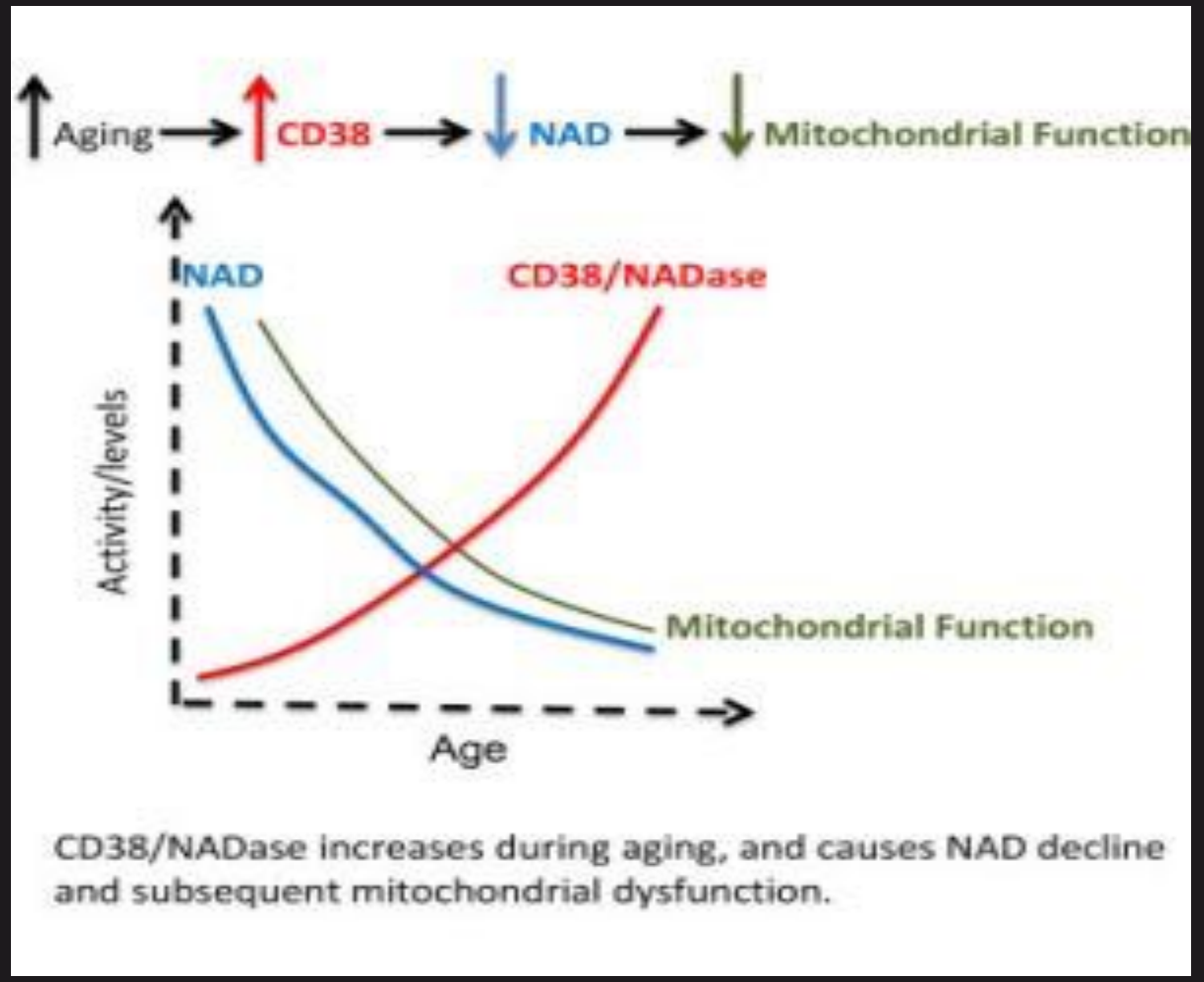
PACMEN WHO EAT NAD+

CD38: The "*Extracellular NADase Story*"

Why IV NAD+ can be DESTROYED before it even enter the Cell (Brain)

- CD38 is one of several "ectoenzymes" found outside the cell that are
- CD38 is probably the #1 Extracellular "NAD consumer" (PARPs are the #1 intracellular consumer, under normal conditions)
- CD38 consumes NAD+ and makes cyclic-ADP ribose (cADPR), but does this every inefficiently!
- CD38 gene expression increases with aging, exacerbating the problem



CD38/NADase increases during aging, and causes NAD decline and subsequent mitochondrial dysfunction.

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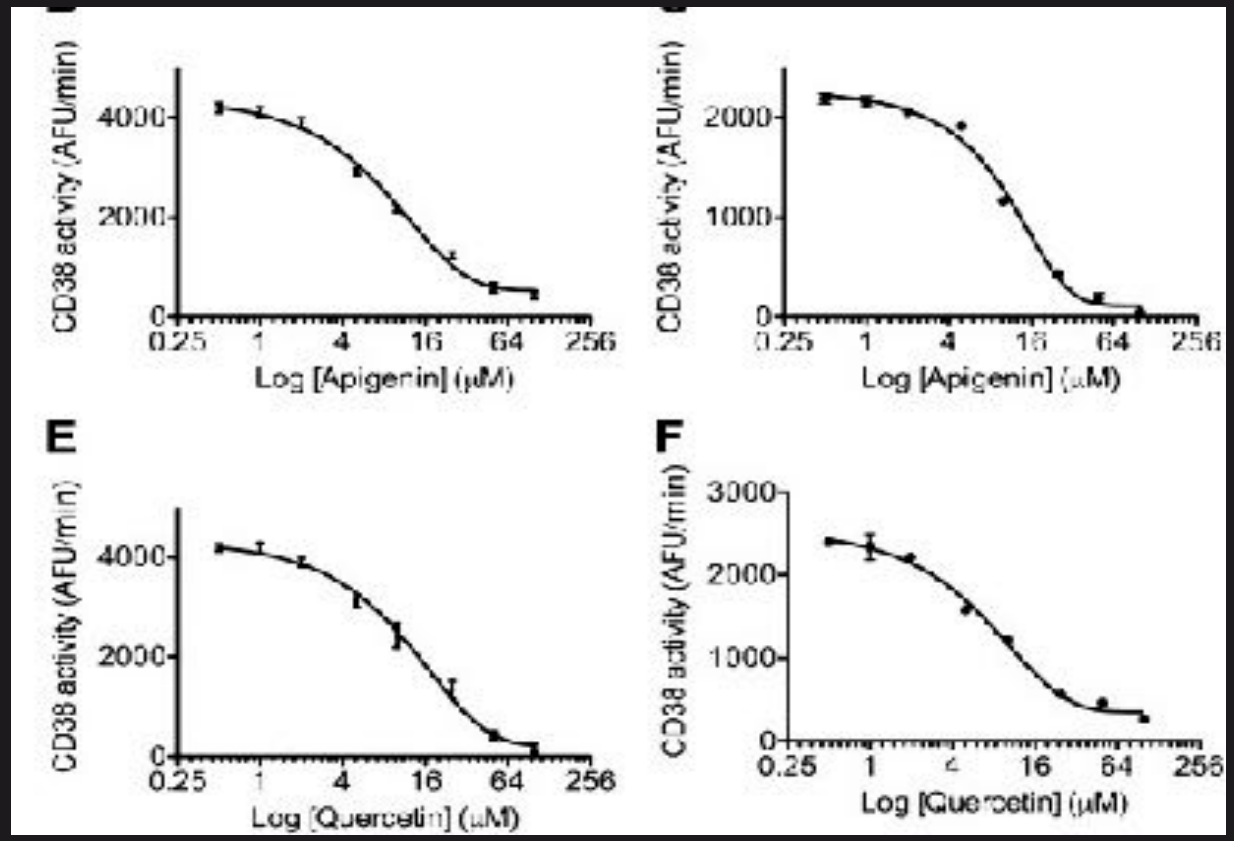
CD38: The "*Extracellular NADase Story*"

How to Blog NAD+ from being destroyed by CD38!

- CD38 can be blocked by natural compounds, founded in apple skins
- Apigenin, flavonoid found in apple skins. It is a powerful inhibitor of CD38.
- Treatment of cell cultures with apigenin increased NAD levels in the cells, reduced global acetylation of proteins, and reduced the acetylation of p53 and RelA-p65 subunits of NF-kB.
- Quercetin is also a powerful inhibitor of CD38 in-vitro as well.



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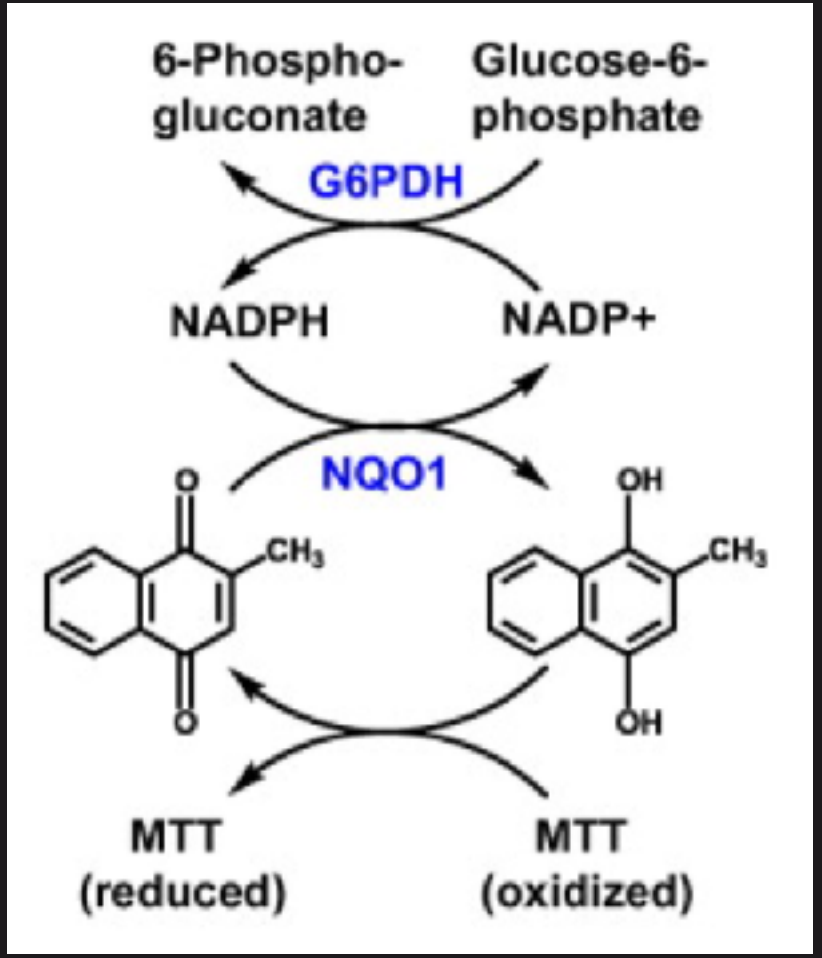
FUNDAMENTAL NAD+ STORIES

NQ01: The NAD+/NADH ratio destoyer"

- What is of prime importance for health and longevity may not be the actual concentration of NAD+ in cells or cell nuclei, but rather the NAD+/NADH ratio
- This ratio may not be affected by NAD+ precursor supplements or the rate of production of NAD+, and rather be driven by other matters such as expression of the NQ01 gene, a "longevity gene"
- NQ01 stands for a gene and protein NAD(P)H: quinone oxidoreductase 1 that is induced by Nrf2 but down-regulated by "epigenetic reader" proteins known as BET proteins.
- Expression of the NQ01 gene drops off with aging.



FUNDAMENTAL NAD+ STORIES

*NQ**01**:The NAD⁺/NADH ratio destoyer''*

- NQO1 regulates the ratio of NAD/NADH and the ratio of NADP/NADPH by oxidizing NADH to NAD+, the needed form.
- NQO1 is one of the few proteins that oxidizes NADH to NAD+
- Thus NQ01 regulates aging independently of NAD content.
- The longevity gene NQO1 regulates blood pressure by eNOS, ACE, and an LKB1/AMPK-mediated preservation in GTPCH-1
- B-Lapachone, a product from the Lapacho tree found in the Andes, solves the NAD/NADH ratio problem by triggering “futile cycling” of B-lapachone)

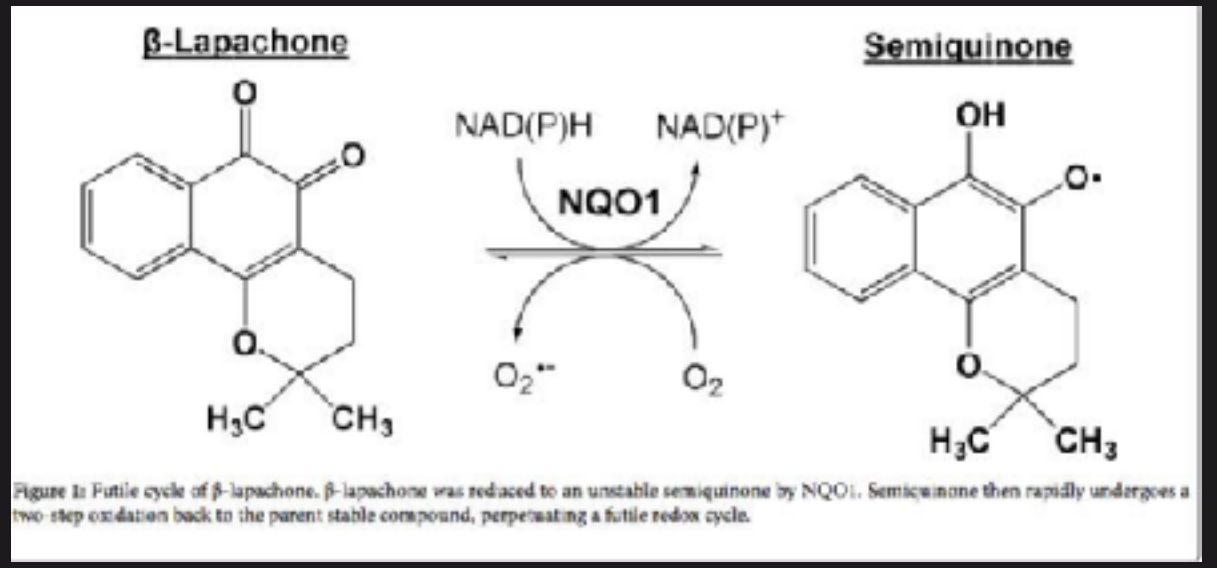


Figure 1: Futile cycle of β-lapachone. β-lapachone was reduced to an unstable semiquinone by NQO1. Semiquinone then rapidly undergoes a two-step oxidation back to the parent stable compound, perpetuating a futile redox cycle.

FUNDAMENTAL NAD+ STORIES

*Upregulating NQ**01** with a Natural Product*

- NQ01 is an “antioxidant response element” gene activated by Nrf2 and the many natural processes and phytosubstances that activate Nrf2
- E.g. quercetin increases Nrf2-mediated gene expression of NQO1.
- Beta-lapachone in particular increases NQO1 enzyme activity, a fact established by multiple studies
- Beta lapachone is found in the bark of the lapacho tree, is possibly in lapacho tea
- But probably not in a concentration sufficient for drinking lapacho tea to make a difference by upregulating the NQ01 gene

FUNDAMENTAL NAD+ STORIES

*Upregulating NQ**01** with a Natural Product*

- Research establishes beta lapachone in rats
  elevates the ratio of NAD/NADH in endothelial cells
  increases eNOS activity via an AMPK-dependent mechanism

- As a consequence, reduces blood pressure
  The elevation in the NAD/NADH ratio also results in a reduced cleavage and secretion of ACE into the bloodstream, thereby reducing Angiotensin II formation.

- As a result of the eNOS-mediated method and the ACE-reduction mediated molecular mechanism, resolved hypertension in rats.

- As a result of all this research, NQO1 activation has been recently proposed as a strategy for controlling hypertension

B-lapacho Tree

Conclusions and Practical Consequences

1. Horrible things can and will happen if you do not have enough NAD+or you're NAD+/NADH ratio is too low.

2. Most of the NAD+ you depend on is recirculated in the NAD Salvage cycle.

- 3. The extended version of the NAD Salvage cycle involves several other Cycles including ones connected with circadian rhythms, and with mitochondrial well being and mitochondrial biogenesis.
- 4. Important actors in The NAD Salvage cycle and in the biological activities of NAD are the sirtuins, sister enzymes that are responsible for many of the impacts of normal or too low NAD+..
- 5.Many things can go wrong in these Cycles which feed upon and affect each other, and these things can result in too low NAD+. There are many NAD+ Pac Men factors.

Conclusions and practical interventions

opportunities for this to happen,, the body may not be able to restore healthy NAD+ and NAD+/ NAD ratios without an extraordinary intervention. The NAD salvage cycle possibly requires rebooting. This may not be possible through normal interventions such as dietary ones.

7. I strongly conjecture that IV NAD infusions are now the best and possibly only way to provide such a rebooting intervention. Clinicians who are involved with NAD infusions at this conference have told me that they definitely have noticed a switching process that often takes place as a result of an infusion to a basically more healthy constitutional state.

Conclusions and practical interventions

of Pac Man factors there are of course many things that can be done, starting of course with respecting healthy sleep, dietary and lifestyle patterns. Dietary supplements that could be a significant help could include NR, resveratrol. Apigenin, and many other plant polyphenols. Again, health of the processes that produces NAD depends on health of numerous other body pathways.

9. I believe the control of chronic systemic inflammation is an additional key factor in maintaining health especially in aging. And there are some important things that can be done about that as well. That is another very important conversation.

For More Informationgeneral on NAD+ Biology and Aging

www.agingsciences.com

Again, thqnks to Jim Watson for his incredible contribution to making this presentation and to me in general,

AGING SCIENCES CORE TEAM:

JAMES P. WATSON, M.D.
MELODY WINNIG, M.I.S.
MICHAEL GIULIANO, M. S.
VINCE GIULIANO PHD

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