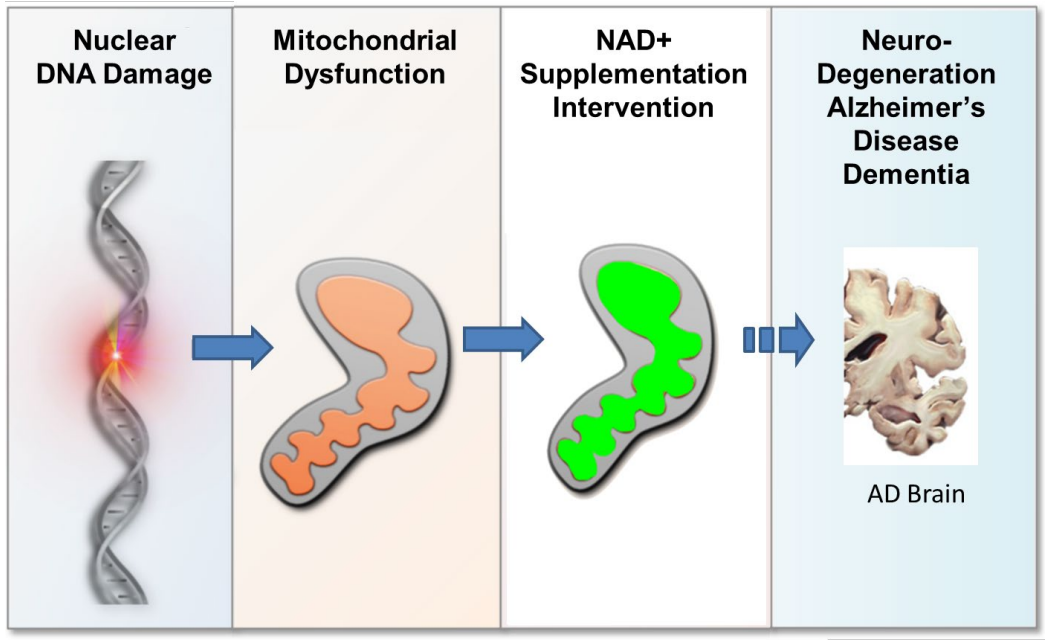
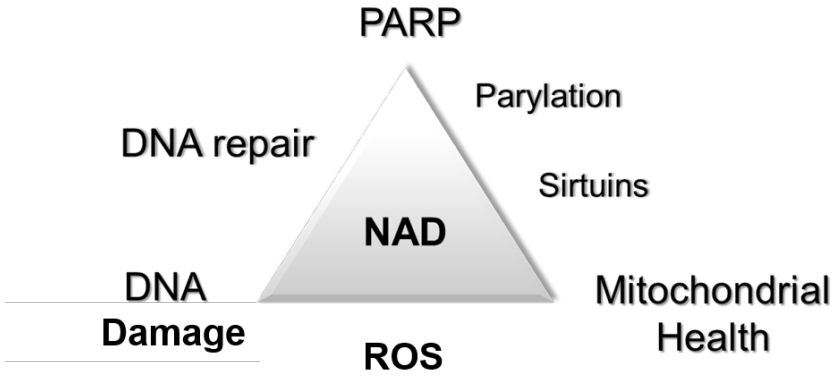
DNA damage signaling to mitochondria in neurodegeneration and aging

Vilhelm A. Bohr, M.D., Ph.D.
DNA Repair Section, National Institute on Aging, NIH
National Institute on Aging, Baltimore, MD





1978-82 MD Copenhagen
Meningitis clin study
DNA repair, PARP
Residencies, Neurology

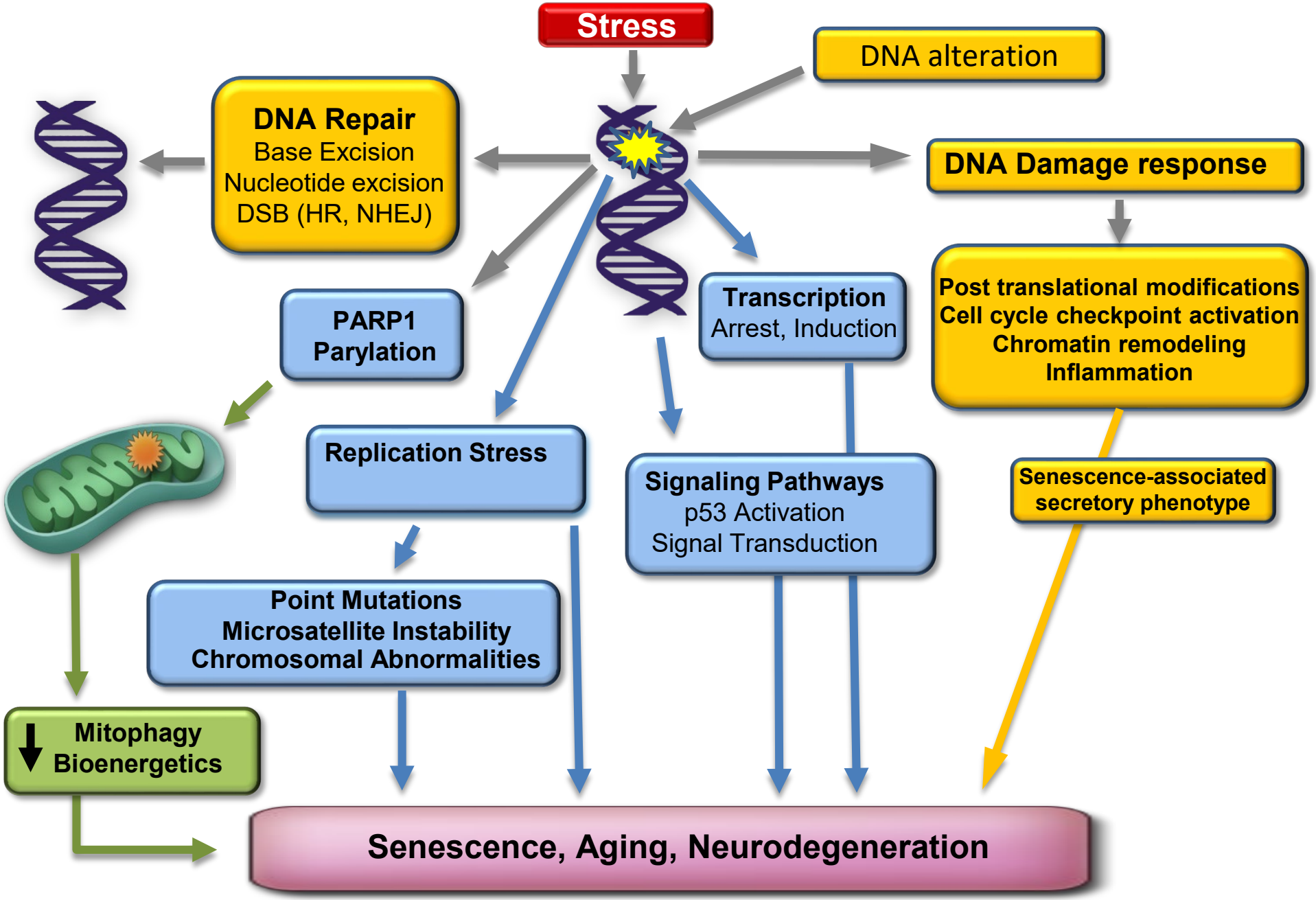
1982-86 Stanford
Preferential DNA repair
Transcription coupled DNA repair

1986-92 NCI
Transcription coupled DNA repair
Repair of different lesions
Mitochondrial DNA repair

1992-NIA
Transcription coupled DNA repair
Premature aging diseases
RecQs, CS, WS
Mitochondrial DNA repair
Neurodegeneration

Moving to more Translation, NAD supplementation, Intervention

DNA Damage Responses

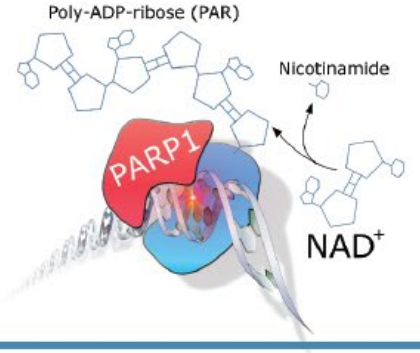


17 PARPS

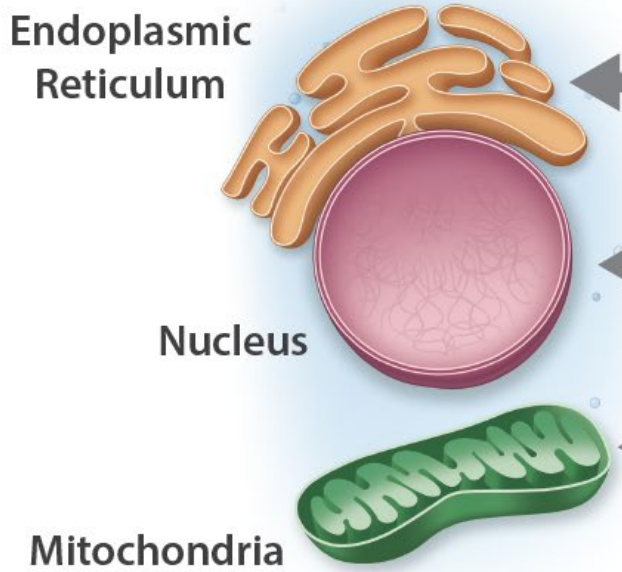
PARP1

DNA Damage

Parylation



Sub-compartments



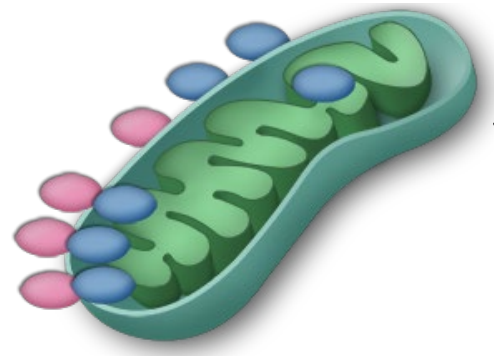
Nuclear-Mitochondrial Signaling

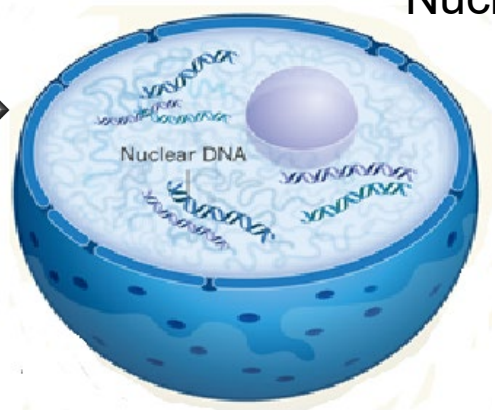
Mitochondria

Nucleus

ROS, Metabolites, UPR

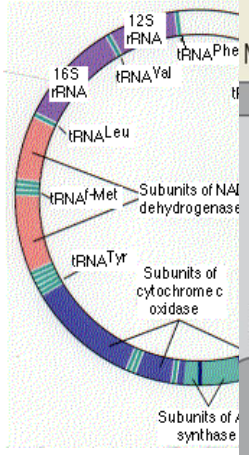
P53, SIRTs, PGC1α

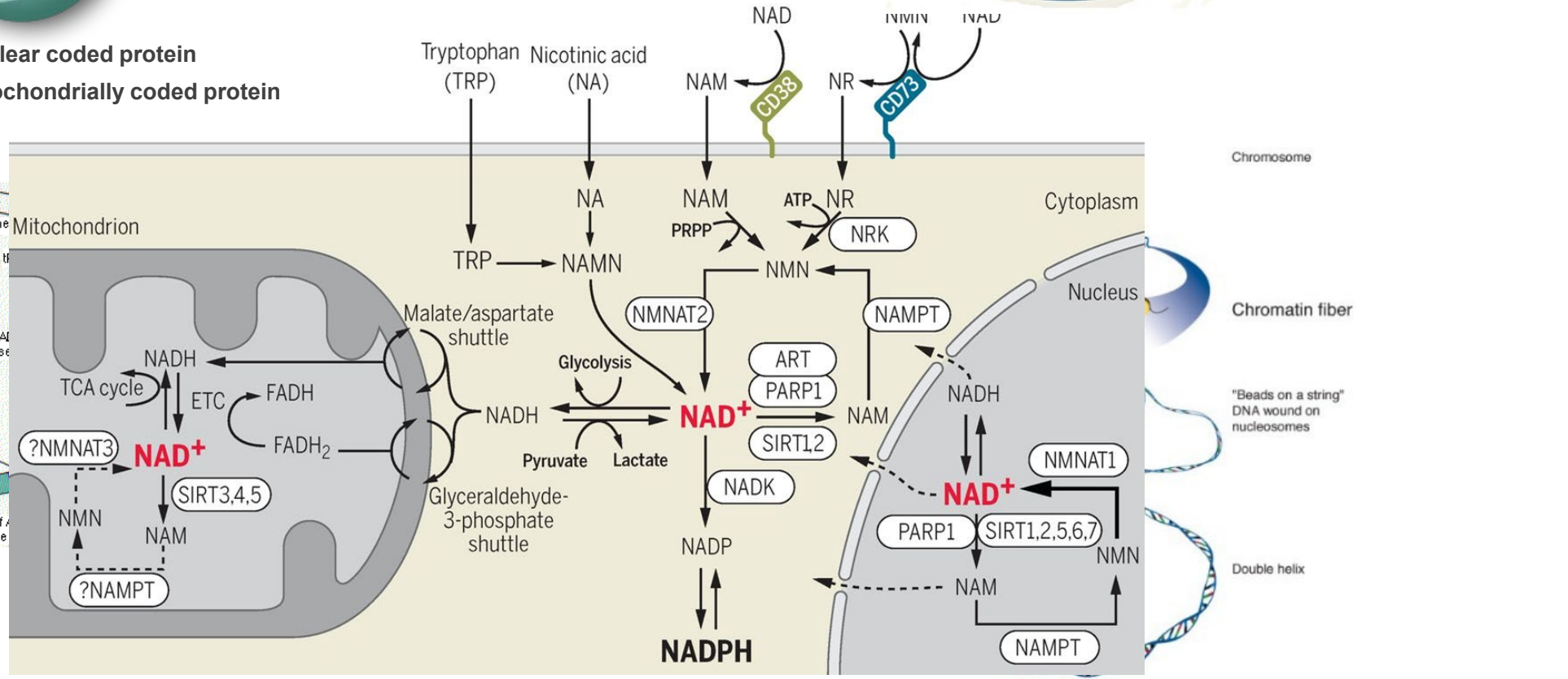




Nuclear coded protein

Mitochondrially coded protein

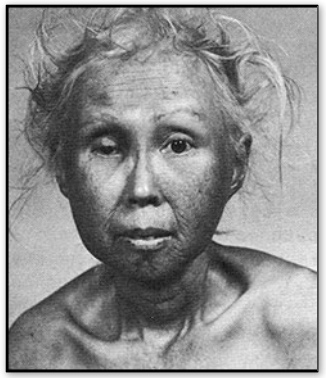




Segmental Progerias: Premature Aging Disorders

Premature Aging

14 Years



48 Years

Werner Syndrome
Cockayne Syndrome
Xeroderma Pigmentosum
Bloom Syndrome
Rothmund Thomson Syndrome
Hutchinson Gilford Progeria
Ataxia Telangiectasia

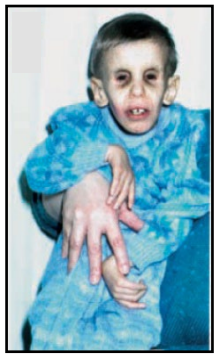
GOOD model systems for the study of aging

Genetic defects can be complemented in biochemical and cellular studies

Elite Aging

Jeanne Calment
122.5 Yrs

Cockayne Syndrome

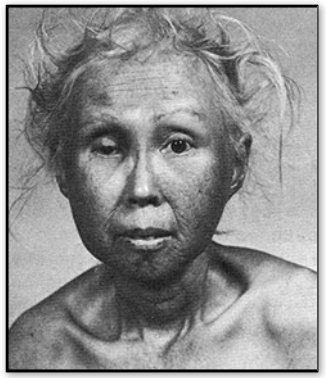


All these conditions are defective in DNA repair

Segmental Progerias: Premature Aging Disorders

Premature Aging

14 Years

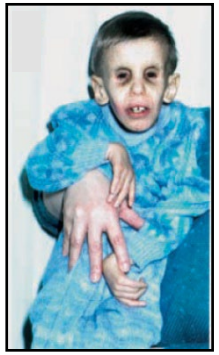


48 Years

Severe Neurodegeneration

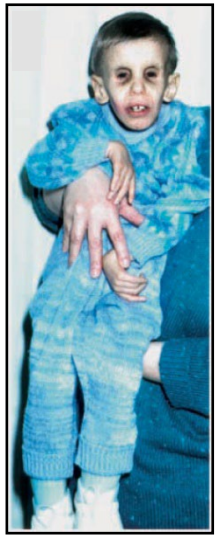
Cockayne Syndrome
Xeroderma Pigmentosum grp A
Ataxia Telangiectasia

Cockayne Syndrome

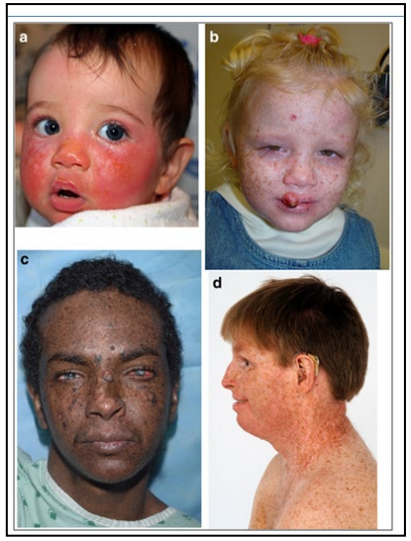


Nuclear DNA Damage leads to Mitochondrial Dysfunction

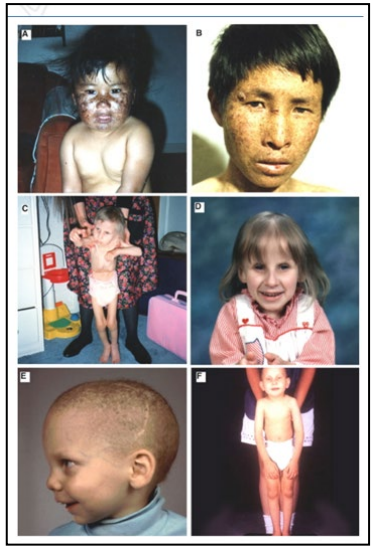
Cockayne Syndrome



Xeroderma Pigmentosum, Group A



Ataxia Telangiectasia



Diseases with DNA repair defects

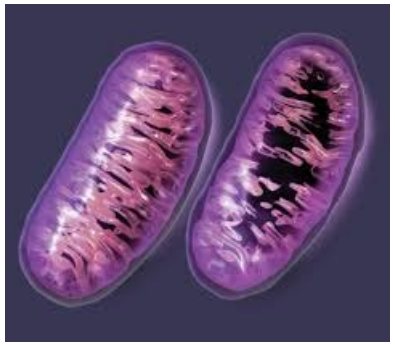
premature aging

severe neurodegeneration

Studies across Species

Scheibye-Knudsen et al. *J. Exp Med*. 2012
Scheibye-Knudsen et al., *Aging,* 2013
Fang, Scheibye-Knudsen et al., *Cell 2014*
Scheibye-Knudsen et al, *Cell Metabolism 2014*
Scheibye-Knudsen, Fang et al, *Autophagy 2014*
Scheibye-Knudsen, Fang et al., *Trends Cell Biol 2015*
Fang et al, *Nature Reviews, MCB 2016*
Fang et al, *Cell Metabolism*, *2016*
Fang et al, *Trends in Molecular Medicine*, *2017*
Scheibye-Knudsen et al, *PNAS 2017*

Damaged Mitochondrion



www.mito.db

Welcome to the mitochondrial disease database

DNA repair deficiency leads to mitochondrial dysfunction

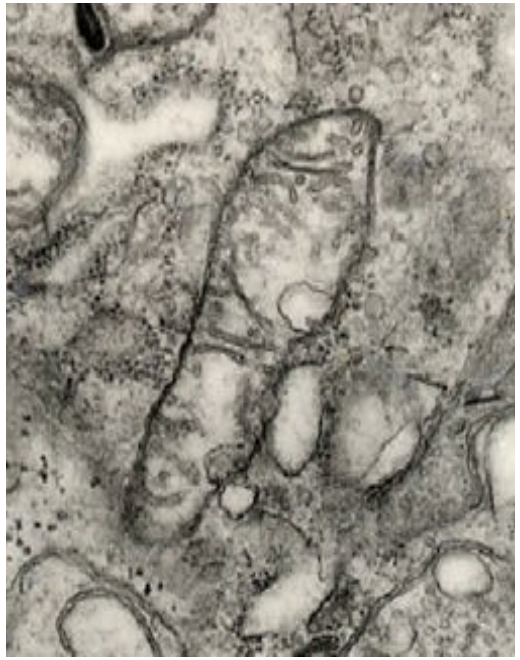
Premature aging diseases with deficient DNA repair:

Ataxia telangiectasia
Xeroderma pigmentosum Grp A
Cockayne syndrome

Human cells
Mouse models
Nematodes

Bioinformatics
Cell Biology
Biochemistry
Behavior

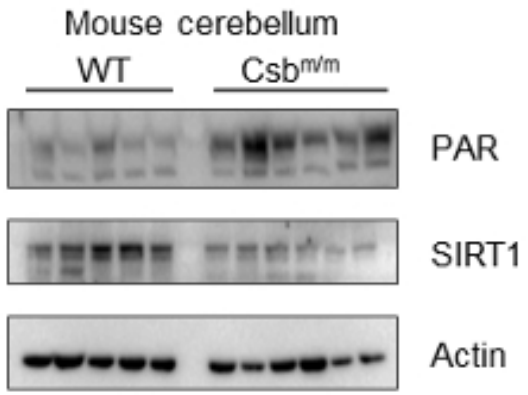
- INCREASED PARYLATION
- INCREASED MITOCHONDRIAL ROS
- INCREASED MITOCHONDRIAL MEMBRANE POTENTIAL
- DECREASED MITOPHAGY
- LOWER NAD^+
- DECREASED SIRTUIN ACTIVITIES
- MANY CLINICAL FEATURES SIMILAR
- TO MITOCHONDRIAL DISEASE



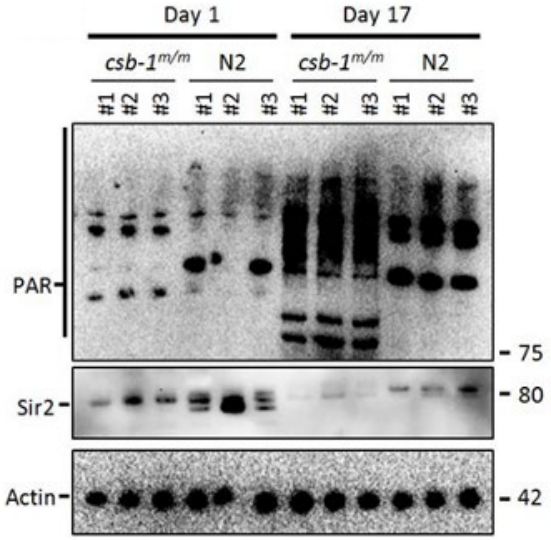
Werner syndrome
Aprataxia
Alzheimers Disease

PARylation across species in DNA Repair Disorders

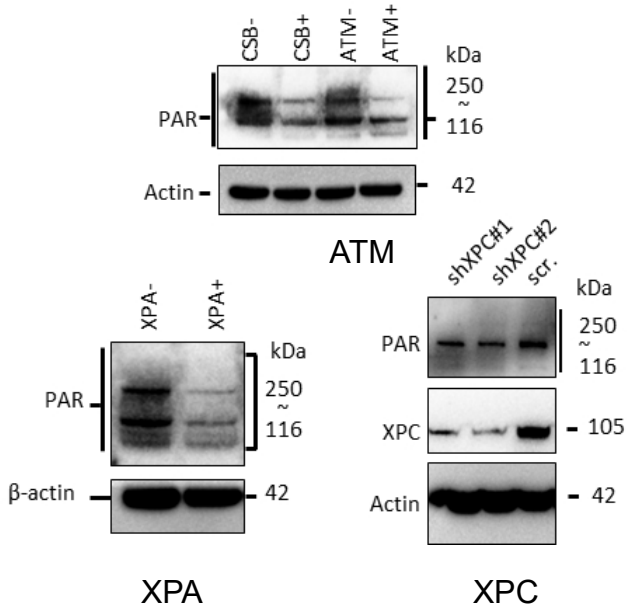
Mice



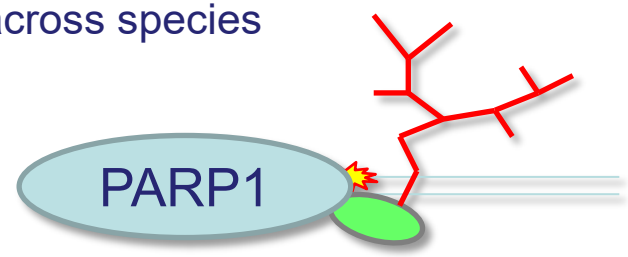
Worms



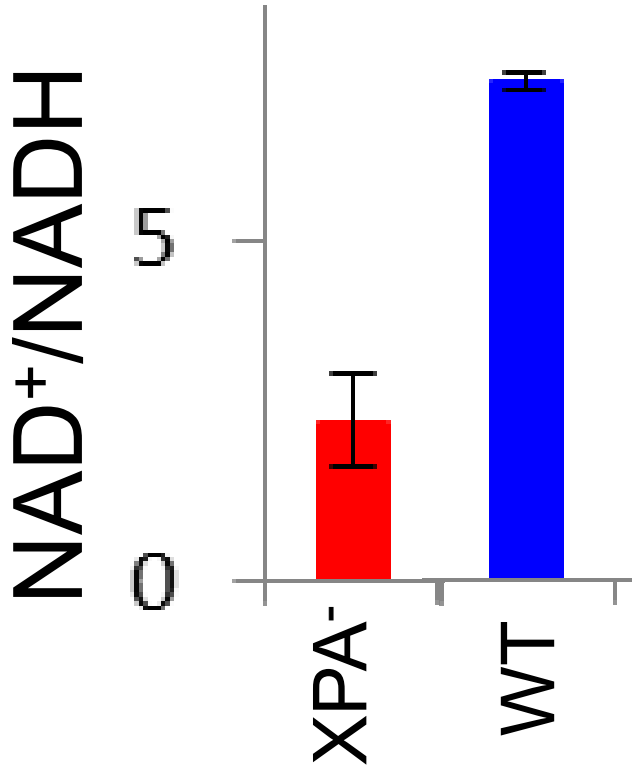
Human cells



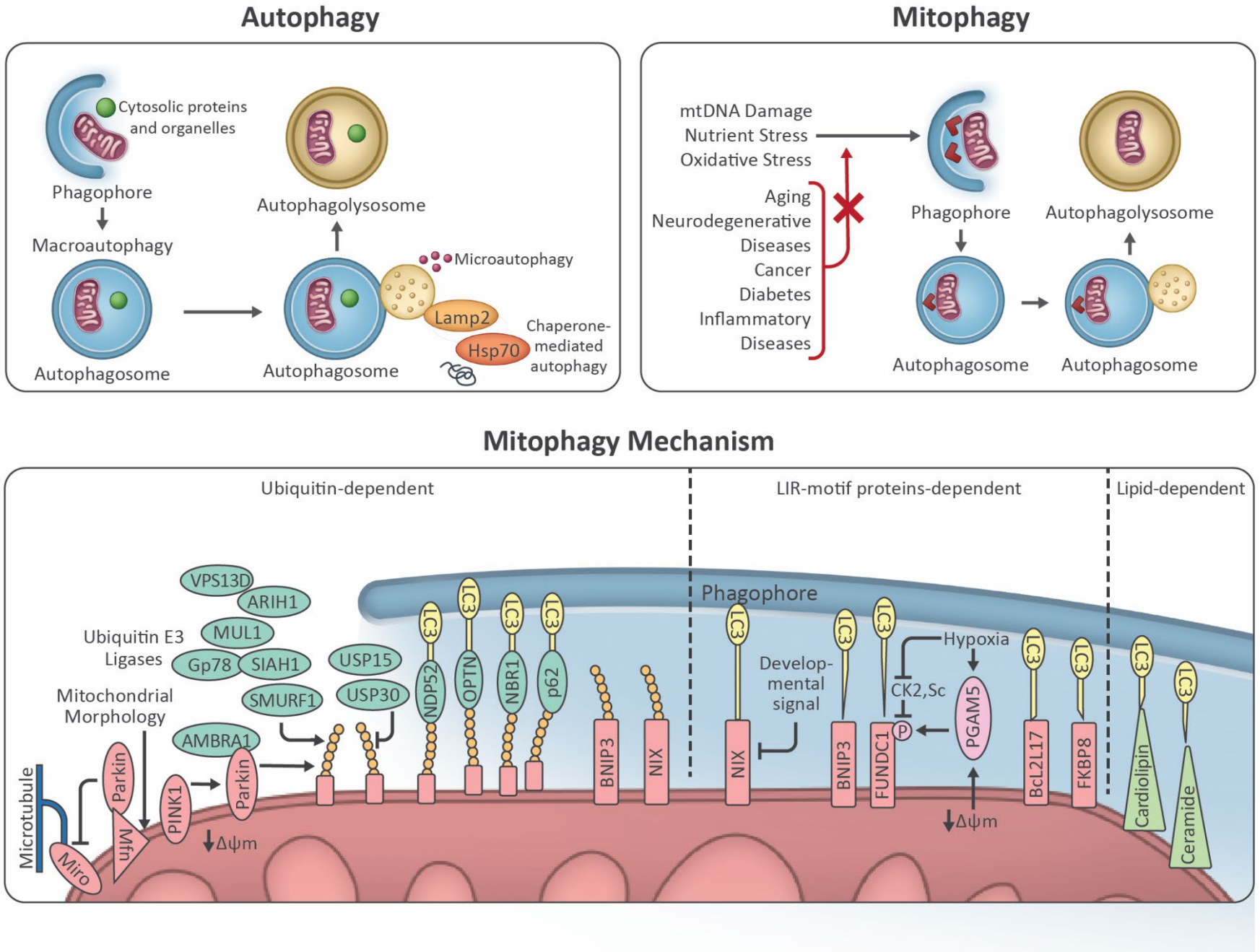
- Increased PARylation in DNA repair deficient disorders across species
- PARylation increases with age in the worm
- Lower Sirt1 expression across species



Decreased NAD^+/NADH in XPA^- Cells



Fang et al, Cell, 2014



Babbar et al, 2020

Linking Nuclear DNA Damage to Mitochondrial Dysfunction

Genome maintenance

DNA repair ↑

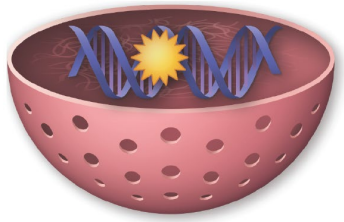
DNA repair defective premature aging disorders

Xeroderma pigmentosum grp A
Ataxia Telangiectasia
Cockayne syndrome

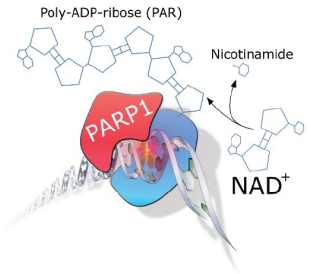
Alzheimer's disease

Aging

DNA damage ↑



Parylation

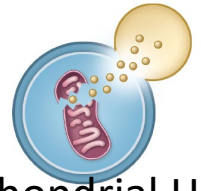


NAD ↓

Sirtuins ↓

Mitochondrial dysfunction

Mitophagy ↑



Mitochondrial Health ↑

Neurodegeneration

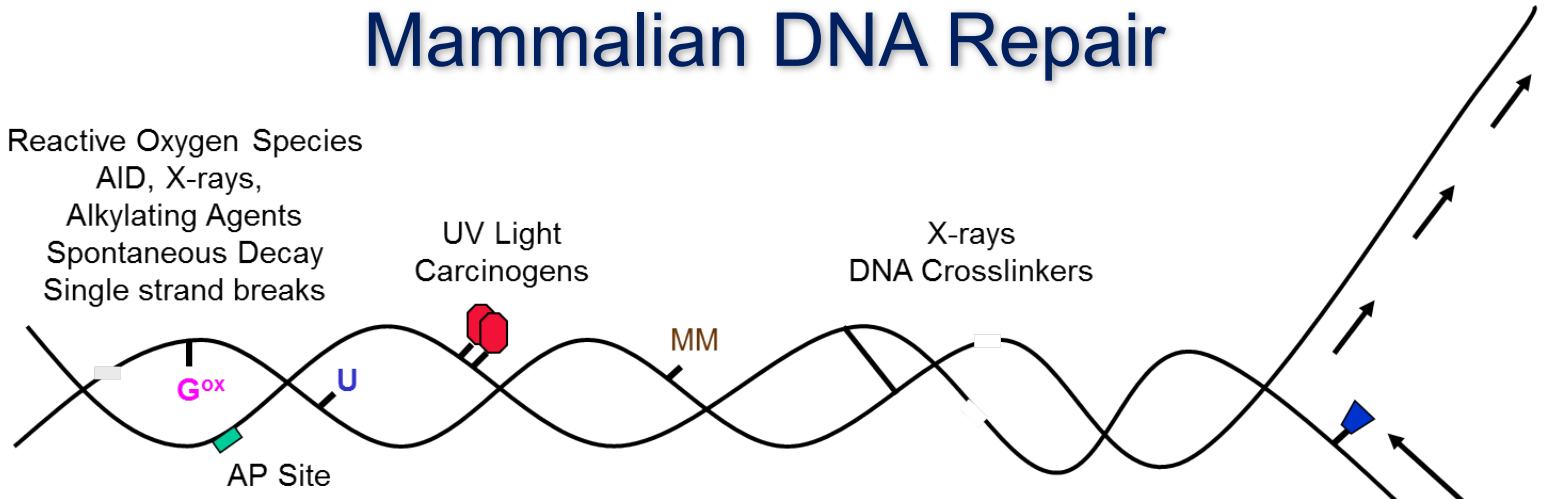
Interventions

NAD Supplementation

Nicotinamide Riboside (NR)

NMN

Mammalian DNA Repair



BASE EXCISION REPAIR

Short-patch | Long-patch | SSBR

NUCLEOTIDE EXCISION REPAIR

Gene-Specific | Global

TCR

MISMATCH REPAIR

RECOMBINATION REPAIR

Homologous | NHEJ | Alt-NHEJ

REPLICATION FORK ARREST

Various DNA Structures

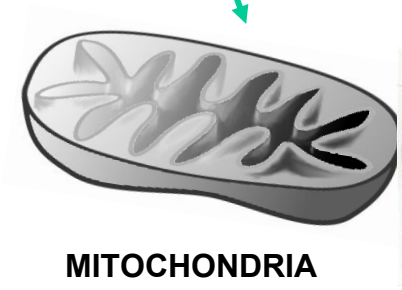
RECQ Helicases

CSB

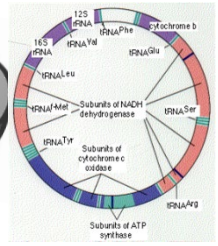
XPA

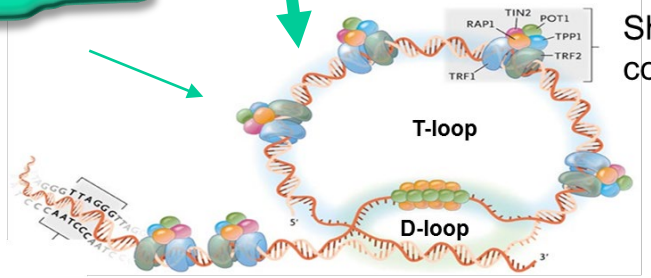
ATM

WRN



MITOCHONDRIA

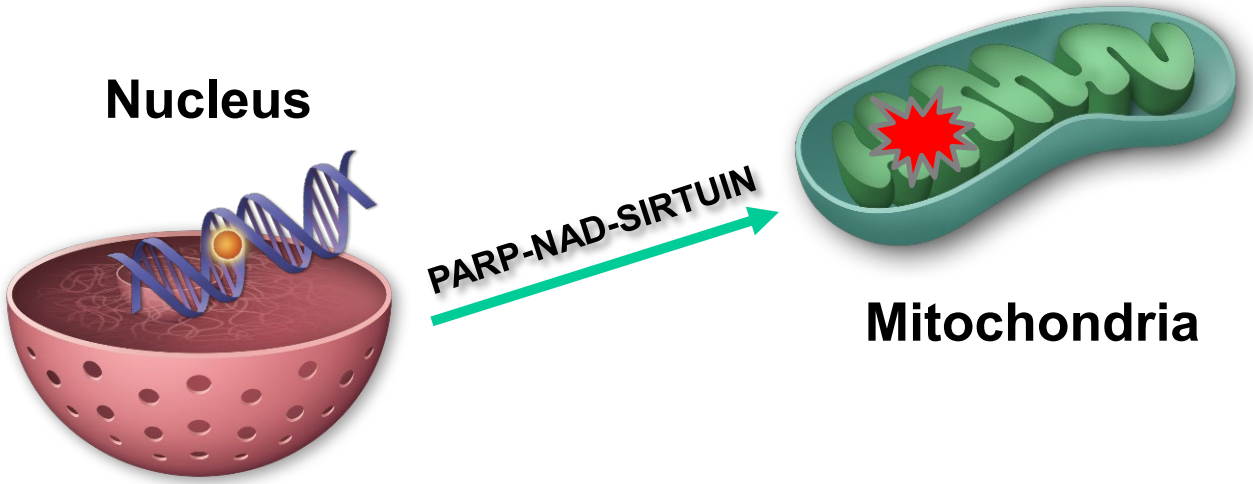




Shelterin complex

TELOMERES

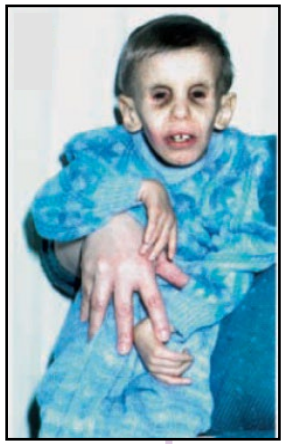
NUCLEAR DNA damage and mitochondrial dysfunction: EXTERNAL and INTERNAL signaling to mitochondria

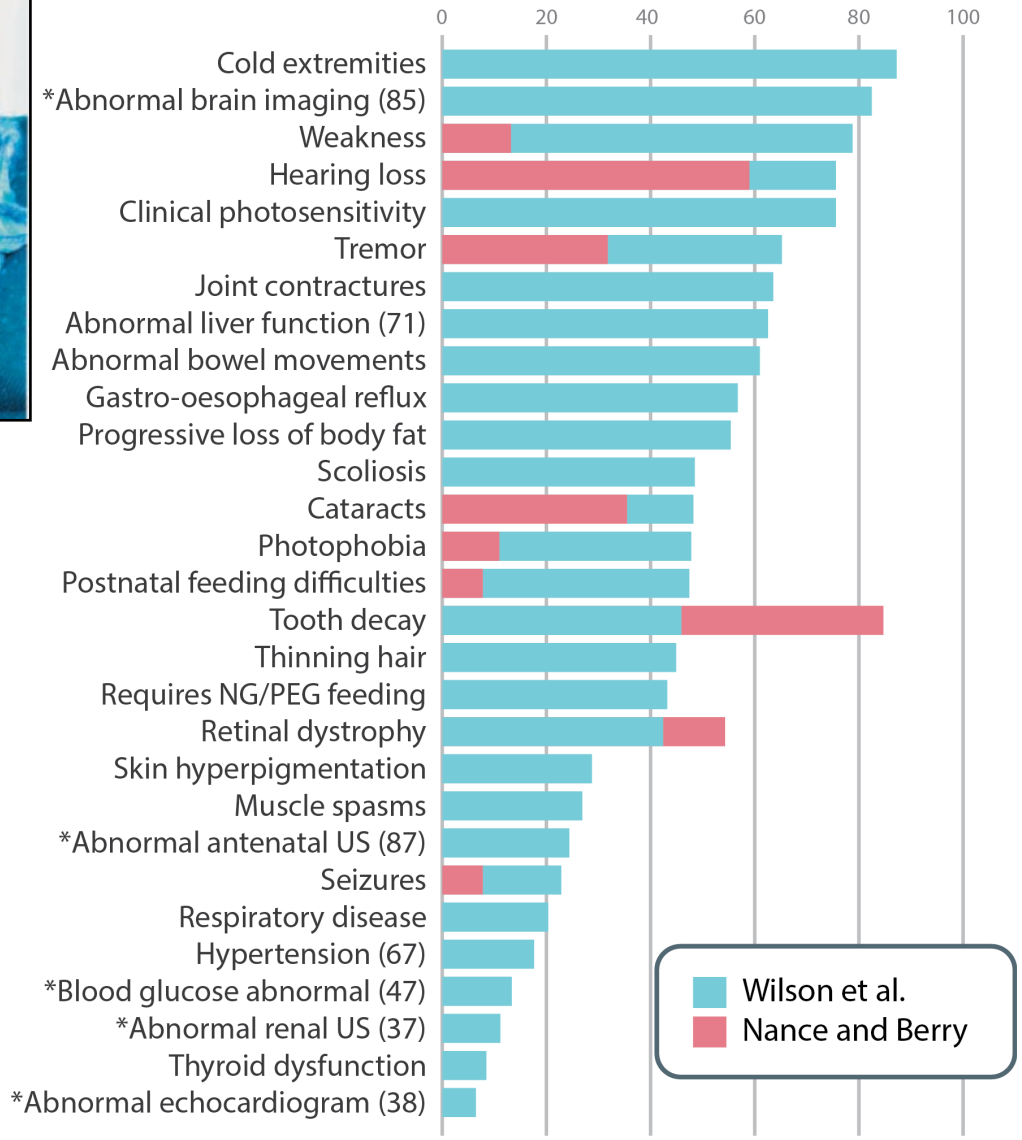


PRESENT IN THE NUCLEUS		PRESENT IN MITOCHONDRIA	
XPA	YES	XPA	NO
CSB/CSA	YES	CSB/CSA	YES
ATM	YES	ATM	??
DNA POLβ	YES	DNA POLβ	YES
WRN	YES	WRN	NO

Cockayne syndrome

- Accelerated aging disorder
- Rare autosomal recessive disease
- Clinical manifestations:
  - Neurodegeneration
    - Ataxia, sensorineural hearing loss, retinitis pigmentosum, seizures, muscle weakness, CNS lactic acidosis, cataracts, dental caries
    - Cerebellar atrophy and calcification
  - Cachectic dwarfism
  - Photosensitivity, but no cancer
- Mutation in CSB, 80%, and CSA, 20%
- CSA and CSB have similar phenotypes
  - Important to find convergences of CSA & CSB
  - Transcription coupled NER, transcription, rDNA, mitochondrial dysfunction





Scheibye-Knudsen et al., PNAS 2016,
Karikkineth et al 2016; Okur et al., 2019,
Lee, J-H. et al,. 2019

CSA and CSB are linked to multiple biological functions

DNA repair
TC-NER, BER

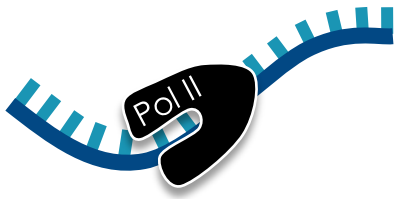
Mitochondrial abnormalities

Okur et al. *Aging Cell*, 2020

Scheibye-Knudsen et al. J Exp Med, 2012

rRNA synthesis

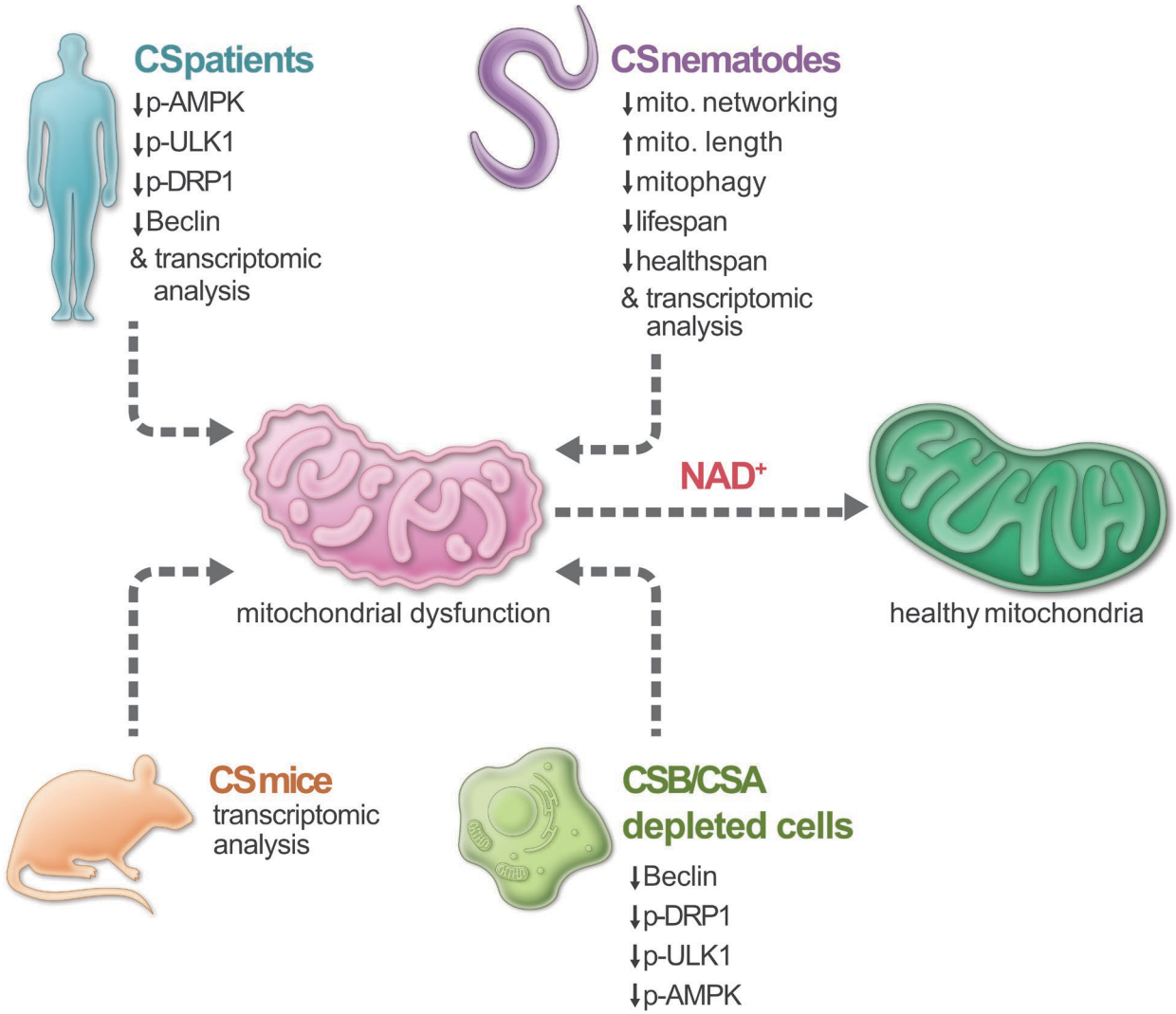
Okur *et al. Nucleic Acid Research*, 2020



Transcription
Epanchintsev et al. *Mol Cell*. 2017

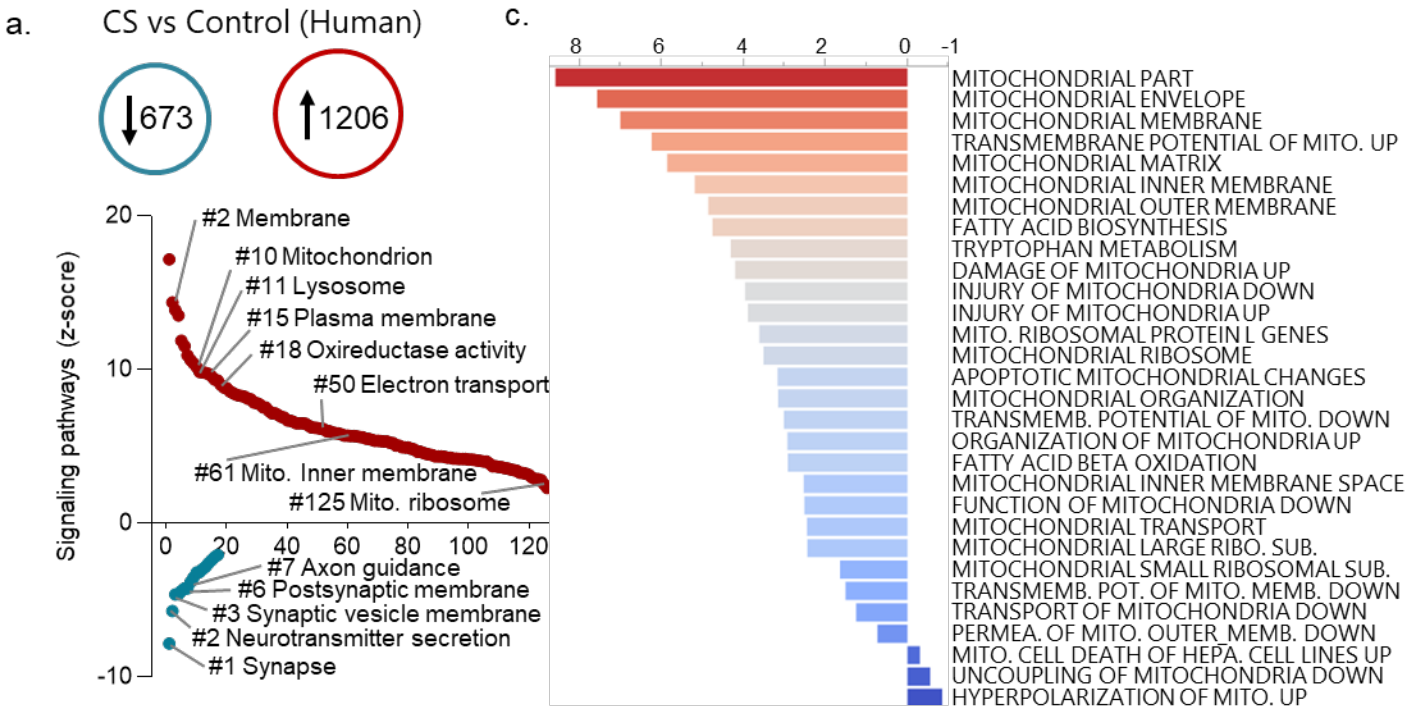
Chromatin Remodeling
Fousteri et al. *Mol Cell*. 2006

Cross species mitochondrial phenotype in Cockaynes syndrome Restoration with NR



Okur et al., Aging Cell 2020

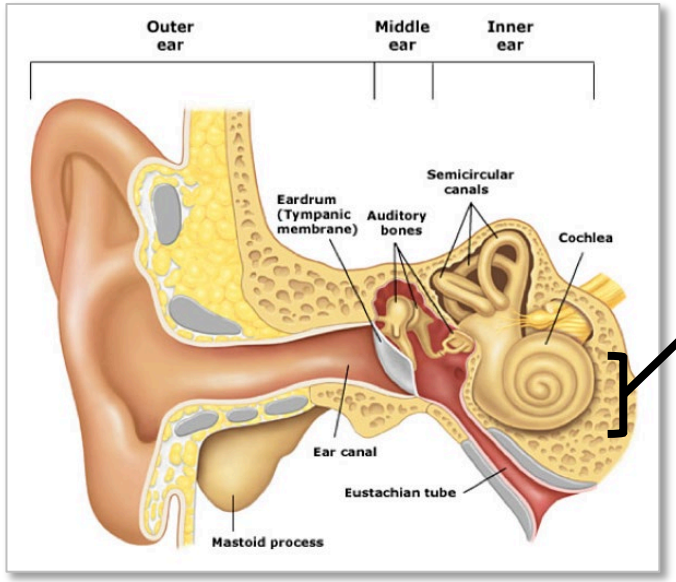
Transcriptomic analysis in CS patient postmortem brain tissue

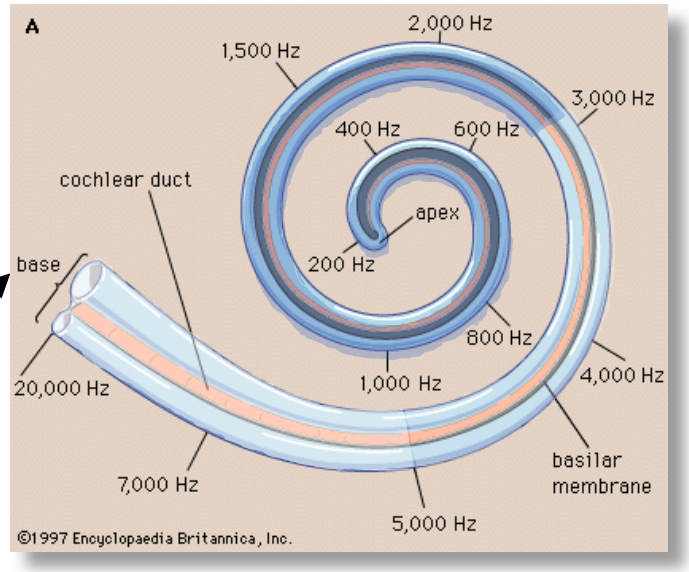


Okur et al, Aging Cell 2020

NAD⁺ Levels Are Reduced in Cochlea of CSA & CSB Mice

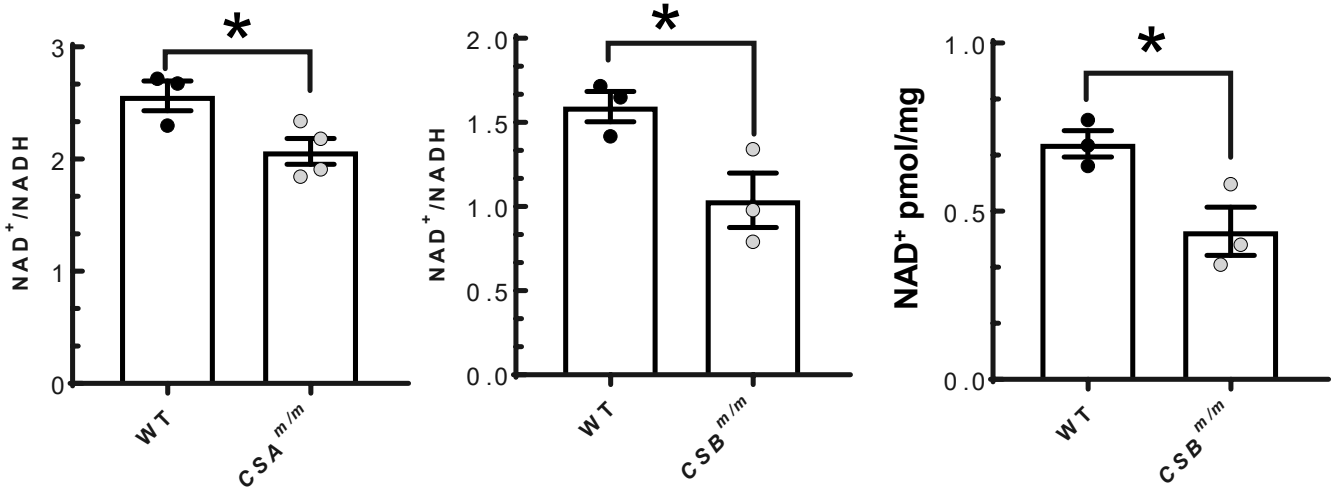
Ear Structure





Cochlea

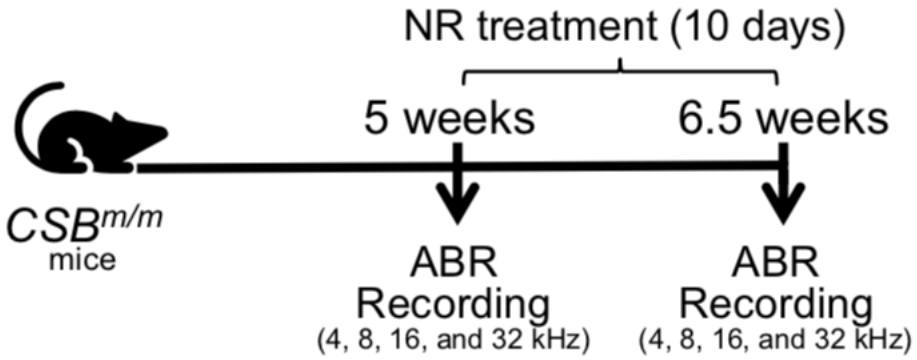
CS cochlea



Age: 6-7 months

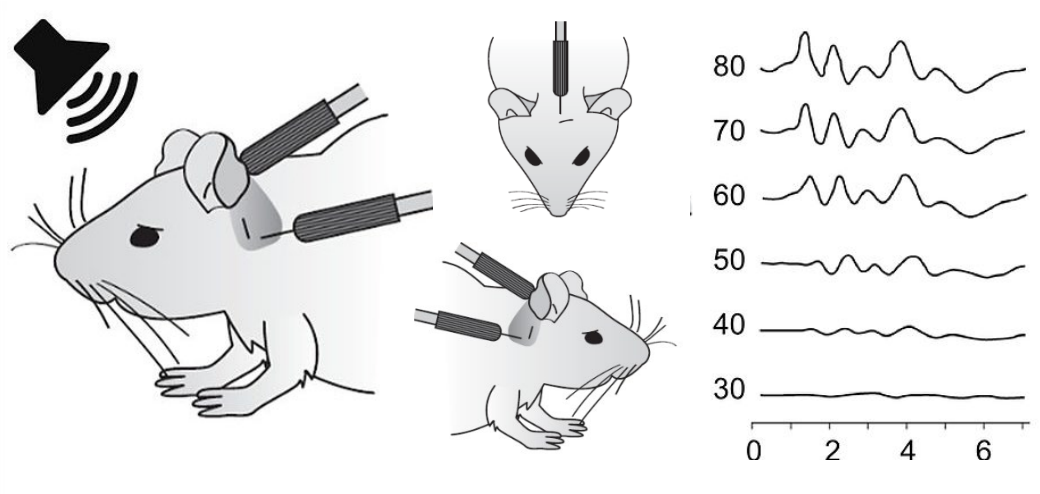
Okur, MN et al., NPJ Aging and Mech Dis., 2020

Short-term Treatment of CS Mice with Nicotinamide Riboside (NR)



CSB and CSA mice treated with 12 or 24 mM NR in drinking water

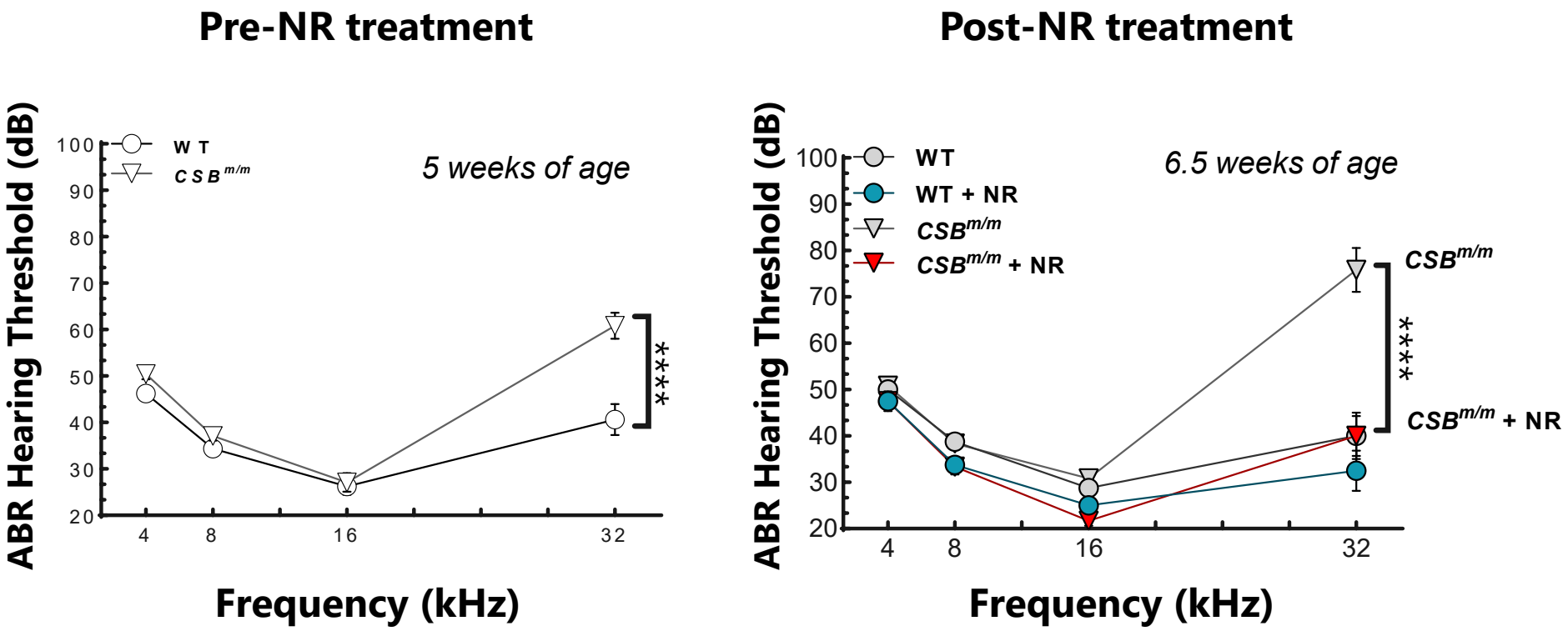
Auditory Brainstem Response (ABR) is used to measure hearing capacity in mice



Brain wave activity over time (ms)

ABR reveals **hearing thresholds**, which are the sound levels below which the ear is unable to detect any sound.

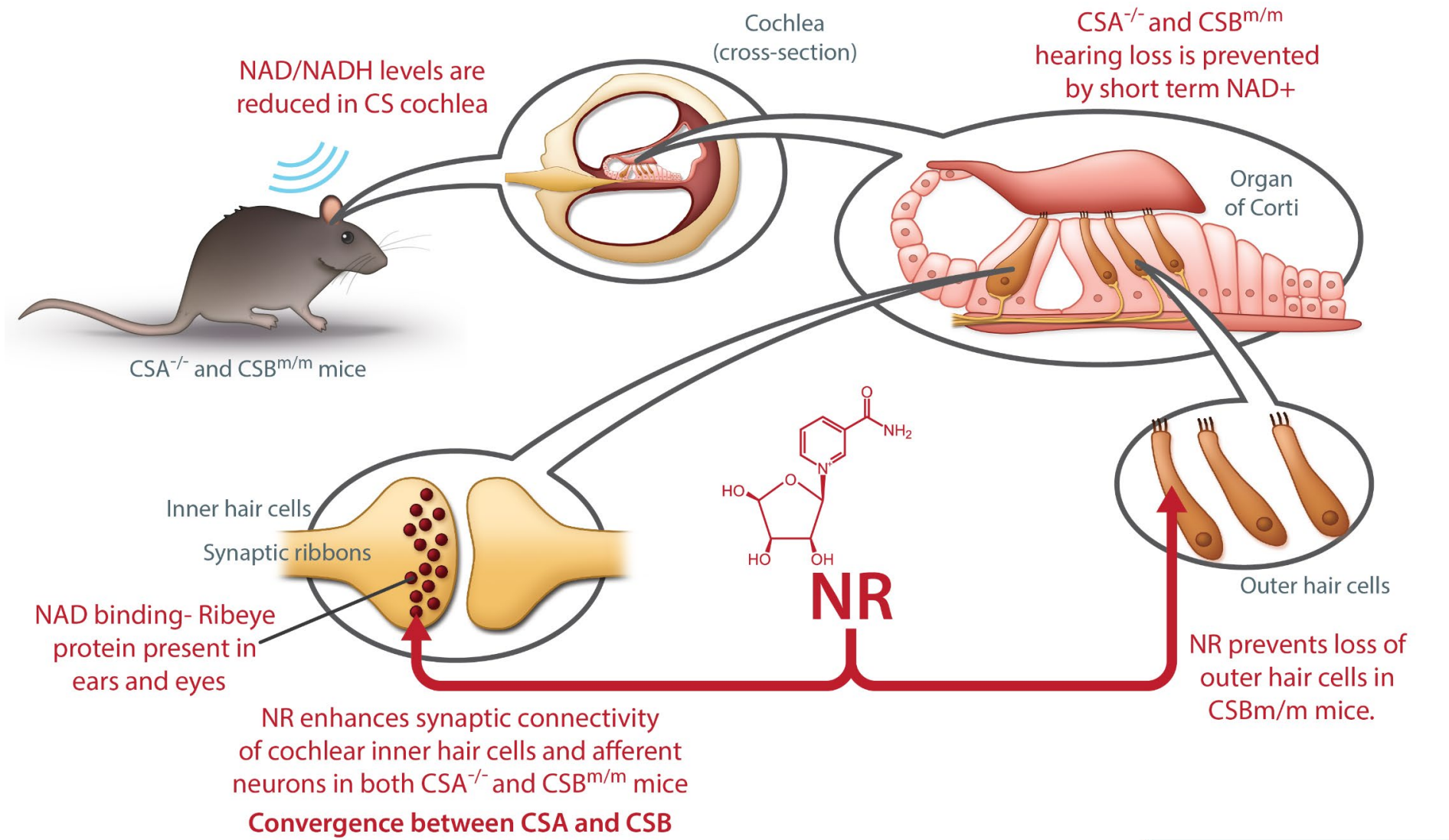
NR Intervention Reduces ABR Thresholds in CSB Mice



Mean ± S.E., p****<0.0001,
Two-way ANOVA with Tukey's post hoc test was used to determine significant difference

Okur, MN et al., NPJ Aging and Mech Dis., 2020

Conclusions



Okur, MN et al., NPJ Aging and Mech Dis., 2020

NR enhances synaptic connectivity in the cochlea of CS mice

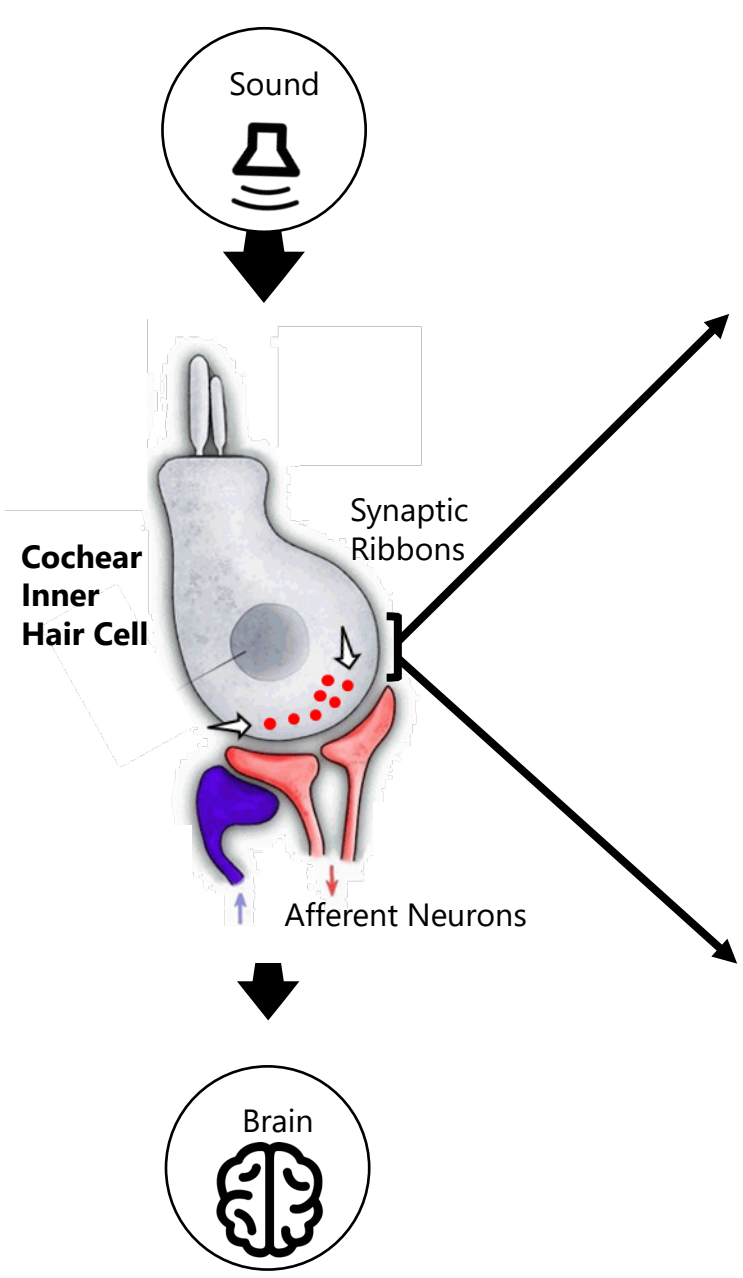
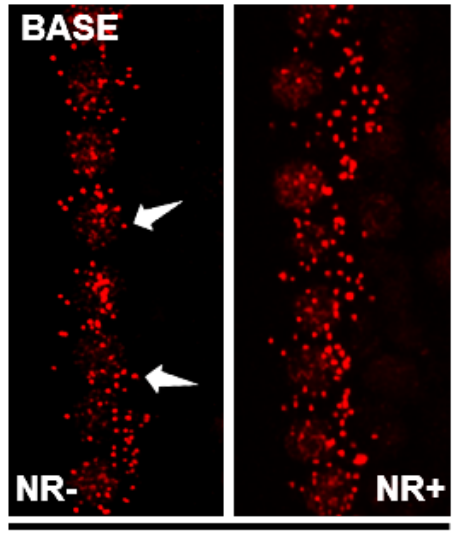
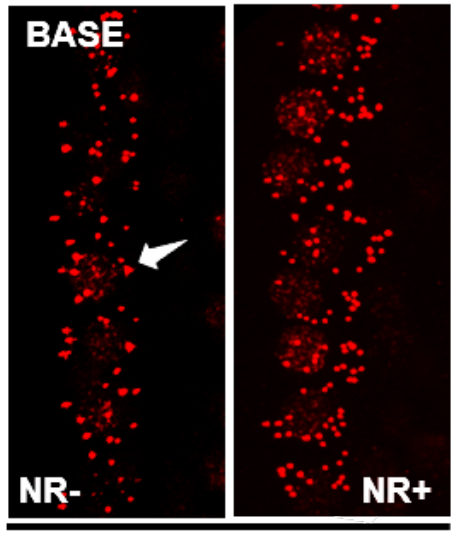


Image src: www.lifesci.sussex.ac.uk

Inner hair cels

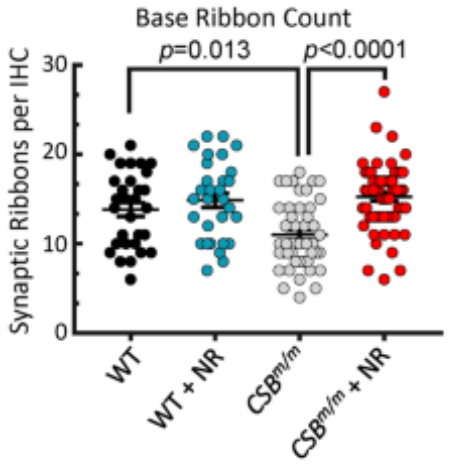


WT



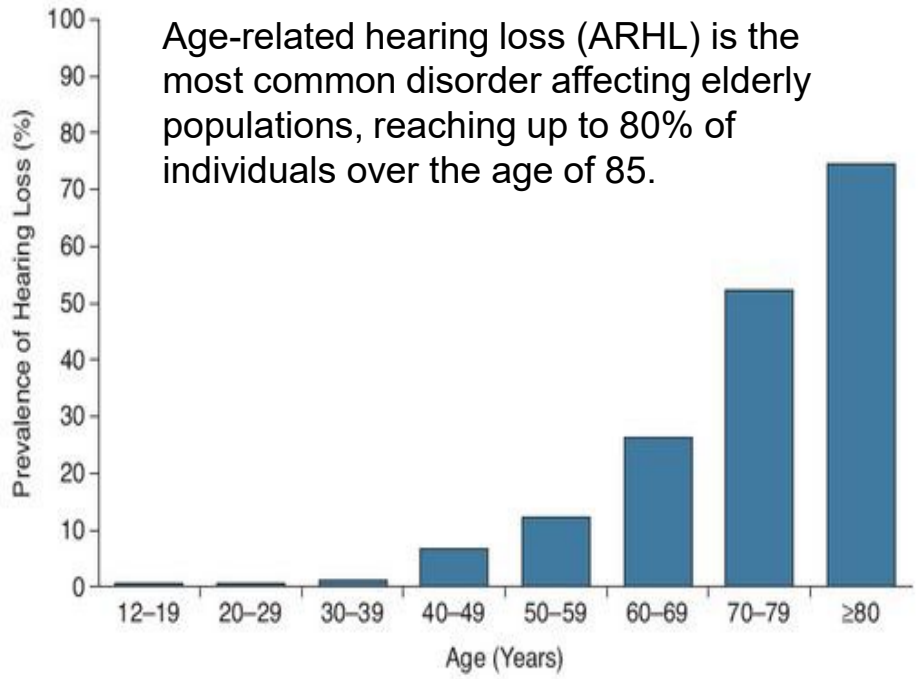
CSB

Anti-Ctbp2 : ribbon synapse marker



Age-related Hearing Loss

Age-related hearing loss (ARHL) is the most common disorder affecting elderly populations, reaching up to 80% of individuals over the age of 85.



Yamasoba et al., 2013 Hearing Research

Age related hearing loss
And
The hearing loss in Cockayne syndrome

Are both *Sensorineural hearing loss*

Clinical Intervention studies in Cockaynes and Werner syndromes

Werner syndrome

Chiba, Japan

Prospective, single-center, cross-over trial to verify safety and effectiveness of nicotinamide riboside for patients with Werner syndrome

M. Koshizaka Y. Maezewa, K. Yokote

Single-center, placebo-controlled, randomized, double-blind, cross-over studies

15-30 patients

Cockayne syndrome

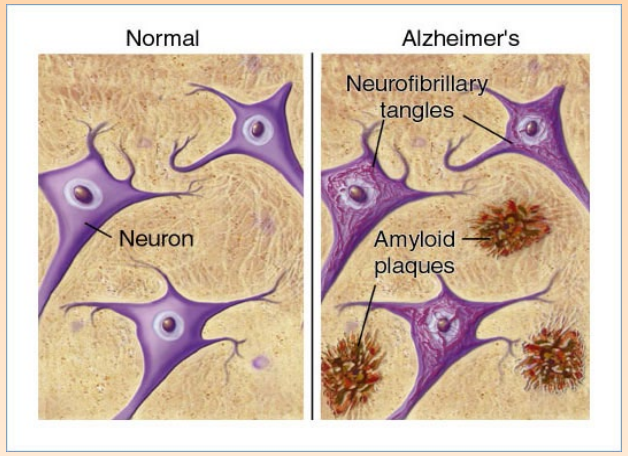
NIA

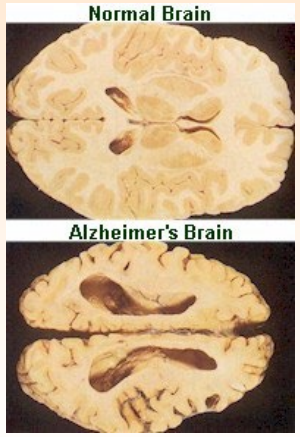
A. Karikkinith

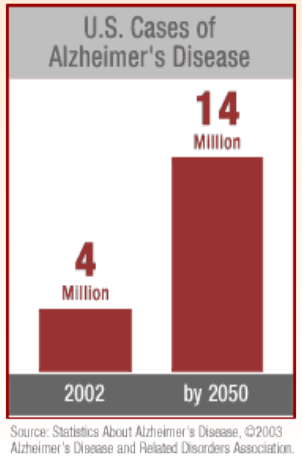
Chromadex

Alzheimer's Disease

- Tau pathology
- Beta cascade
- Vascular
- Transmission defects, glutamate
- DNA damage
- Mitochondrial dysfunction
- Senescence (all brain cell types)
- Protein misfolding







DNA Damage and Repair in Alzheimer Disease

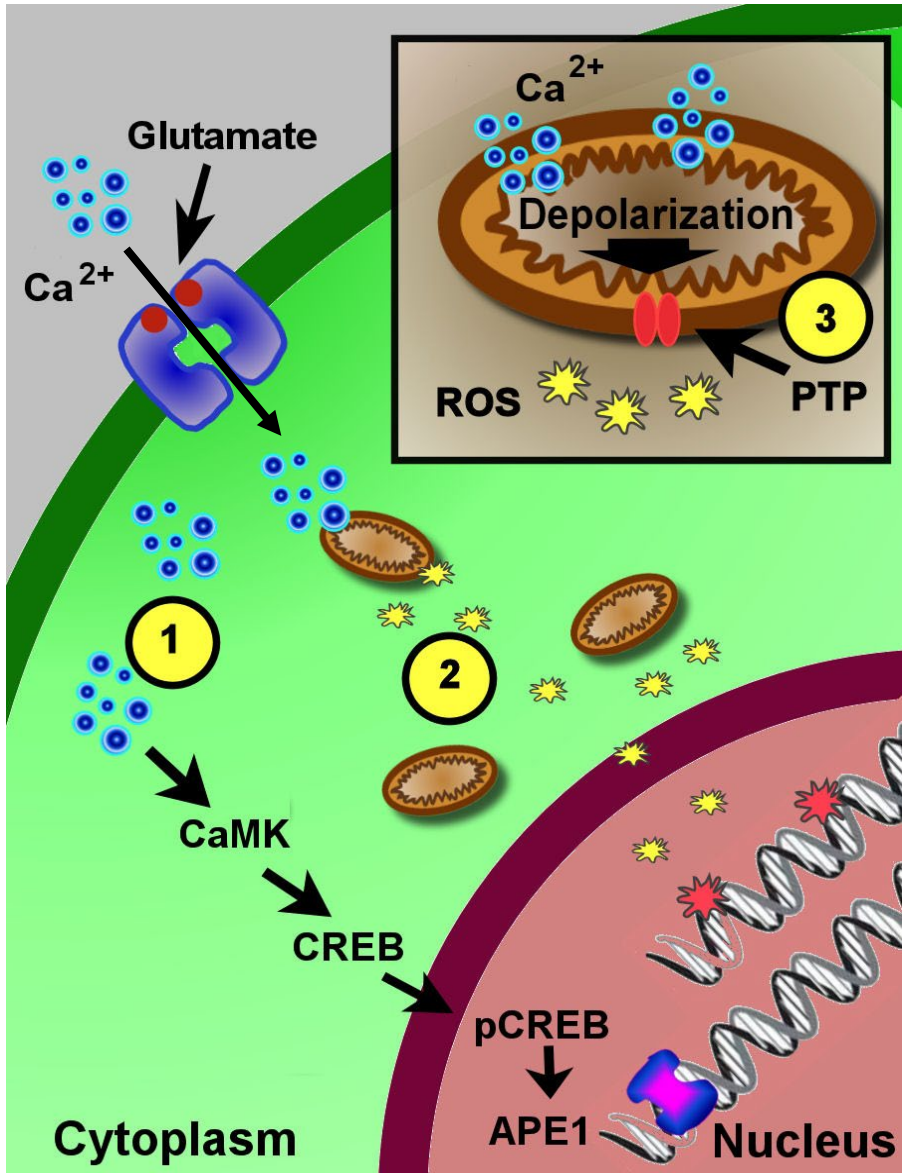
- Increased nuclear and mitochondrial DNA damage in postmortem brains from AD and Mild Cognitive Impairment (MCI) patients (Lowell and Markesbury)

  AD > MCI > Control

- Increased oxidative stress in AD

- Dysregulation of DNA repair enzymes

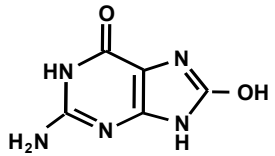
Physiological levels of glutamate stimulate a DNA repair pathway



Yang et al., 2010
Yang et al., 2011

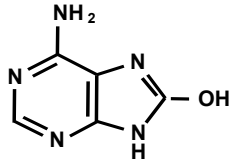
Repair of Endogenous DNA Damage by Base Excision Repair (BER)

8-oxoG

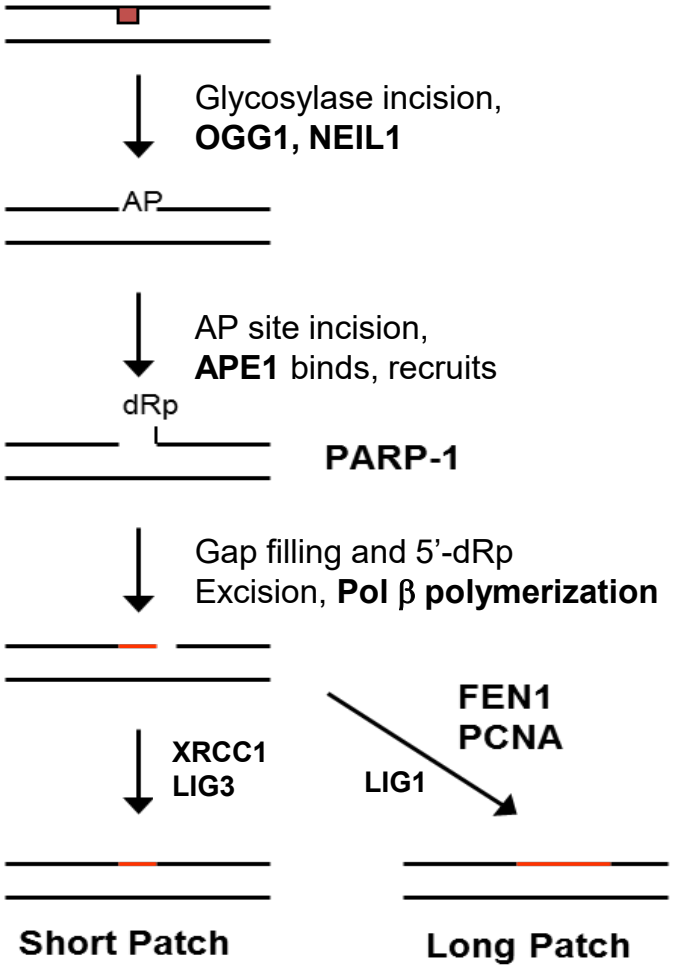


8-hydroxyguanine

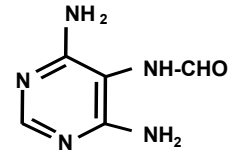
8-oxoA



8-hydroxyadenine

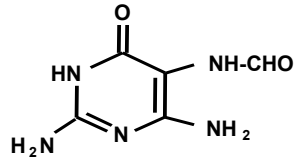


FapyA



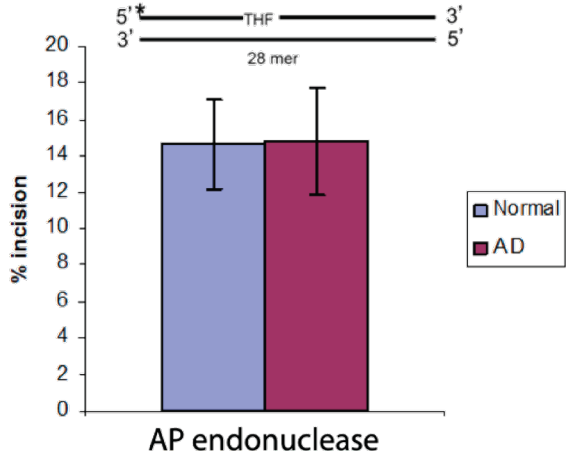
4,6-diamino -5-formamidopyrimidine

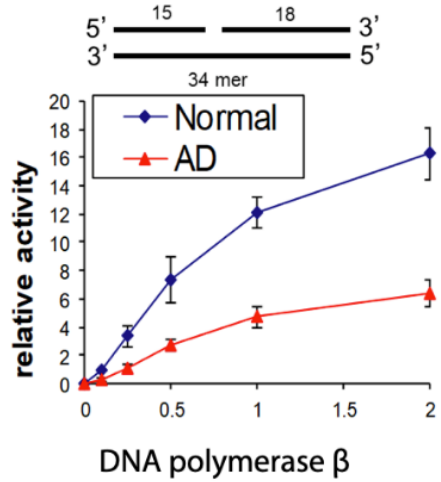
FapyG

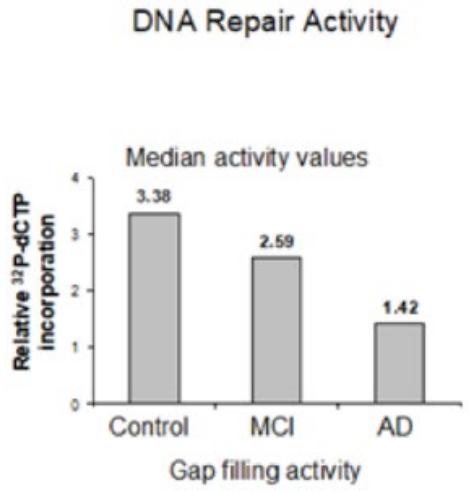


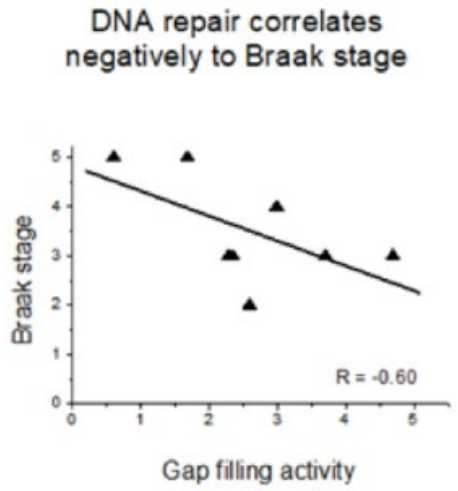
2,6-diamino -4-hydroxy -5-formamidopyrimidine

DNA repair declines in postmortem brain of AD and Mild Cognitive Impair patients relative to controls





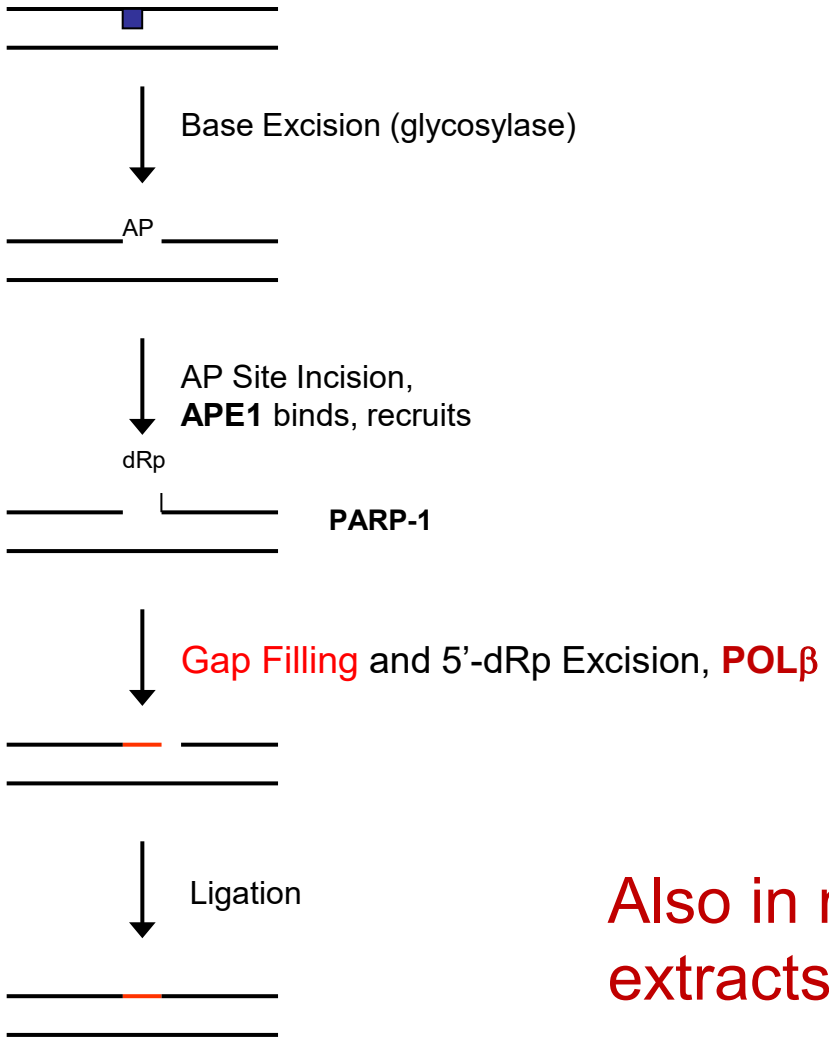




Weissman et al. 2007

Is BER a Disease Modifier for AD?

Polβ gap-filling step is deficient in AD postmortem brains



Wesimann NAR, 2007
Canugovi, Neurobiol Aging, 2014

Also in mitochondrial extracts from AD brains

Alzheimers Disease, DNA repair and Intervention

New Mouse
3xTg AD POLβ$^{-/+}$

Sykora et al. 2015
Masiak et al. 2017

Increased neuronal cell death
Decreased hippocampal volume
Deficient neurogenesis
Loss of synaptic plasticity
Memory loss

- Metabolic alteration
- Loss of olfaction
- Increased neuroinflammation
- Dysfunction of mitochondrial pathways and stress signaling

Alzheimers Disease, DNA repair and Intervention

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NAD Supplementation

Hou et al, 2018
Fang et al, 2019

Reduced neuroinflammation
Decreased p-tau
Improved LTP

- Improved memory and learning
- Improved DNA repair
- Increased mitophagy

Alzheimers Disease, DNA repair and Intervention

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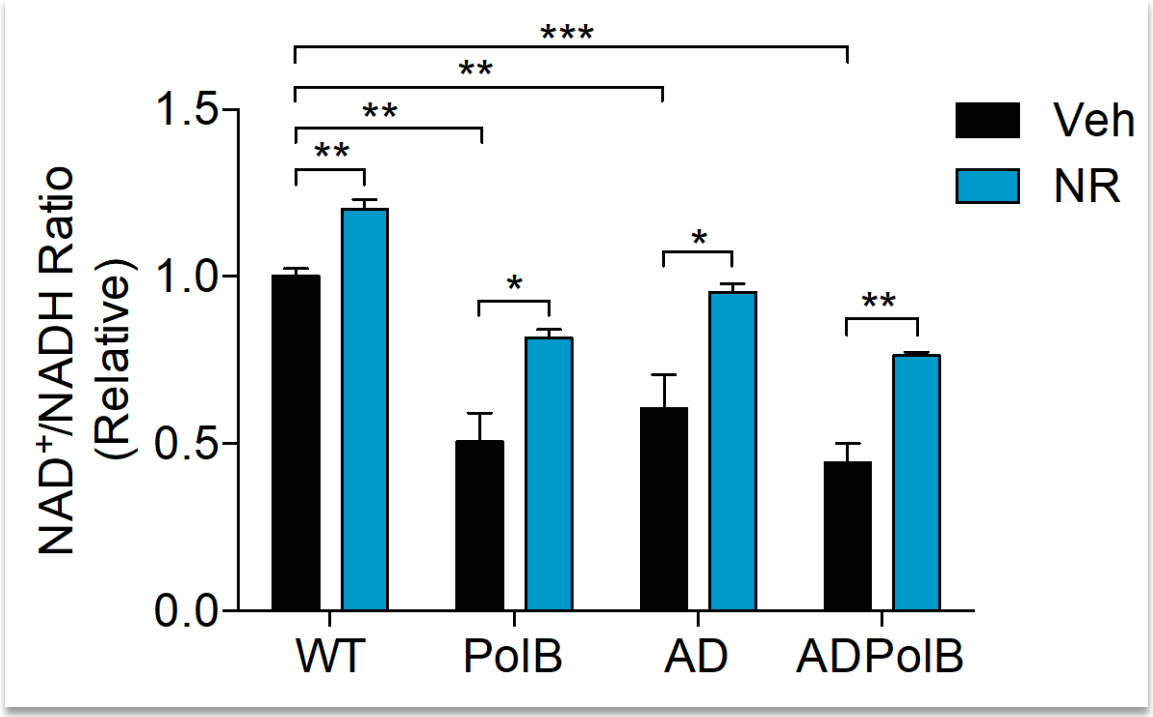
- Improved DNA repair
- Increased mitophagy

Precision Medicine

Demarest et al. 2020

Decreased complex 1 function in hippocampal mitochondria and metabolic shift in **females**

NAD^+/NADH ratio in brain is lower in AD mice and increases after Nicotinamde Riboside (NR) treatment



Study Design

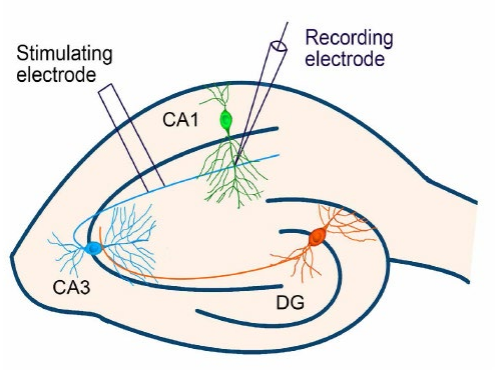
WT, PolB
AD, AD/PolB
(16-18 months)

NR (12 mM) treatment
for 3 months

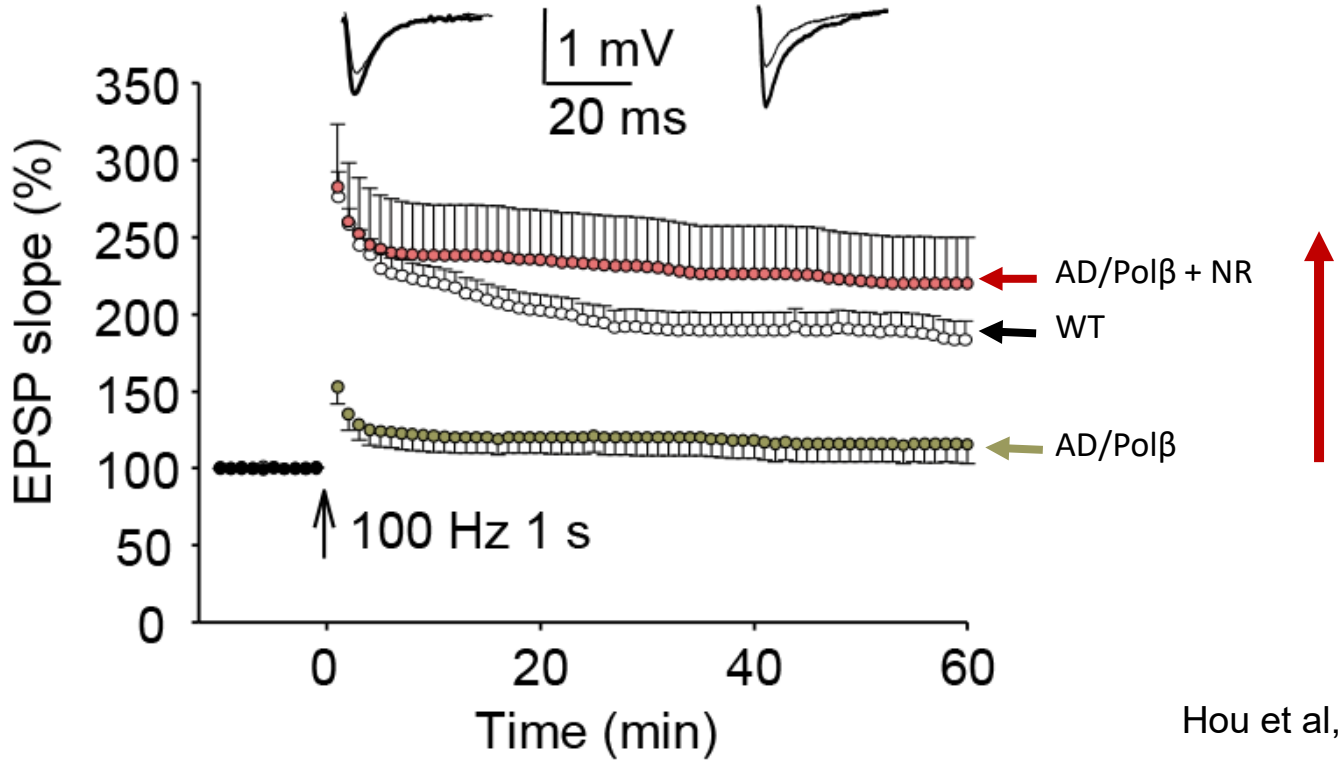
Behavior
LTP, IHC, WB etc.

Hou et al, PNAS 2018

NR Normalizes Synaptic Function, Memory in Long-Term Potentiation (LTP) Assay



LTP measured at the Schaffer collateral synapses



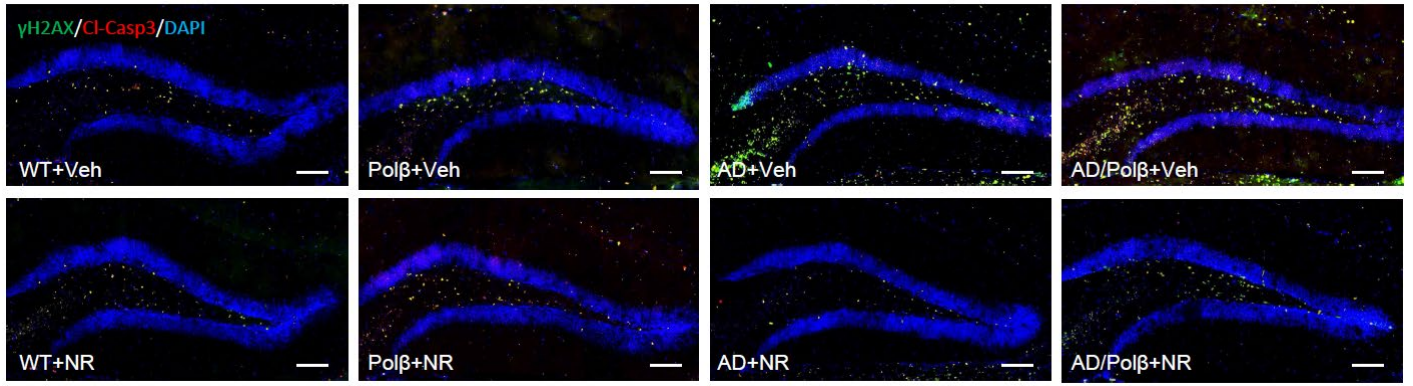
Hou et al, PNAS 2018

Intervention Study with NAD supplementation in the 3xTgAD-Polβ$^{+/-}$ mouse

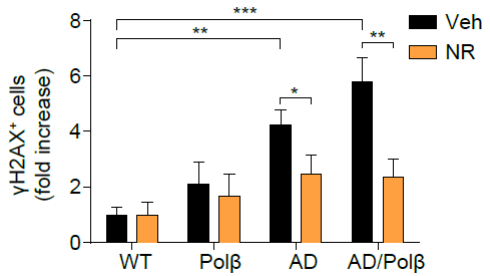
- NAD+/NADH is normalized
- NR improves learning & memory, and Long term potentiation
- NR increases neurogenesis and decreases neuroinflammation
- NR decreases tau phosphorylation
- DNA damage decreased after NR treatment

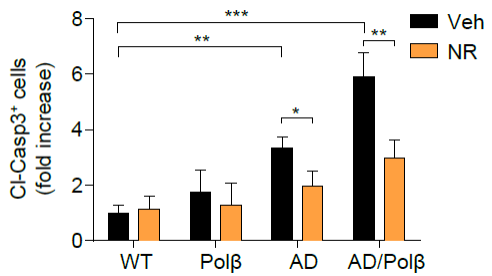
Hou et al, PNAS 2018

DNA damage is decreased after NR treatment in 3xTgAD and 3xTgAD/Polβ+/- mice

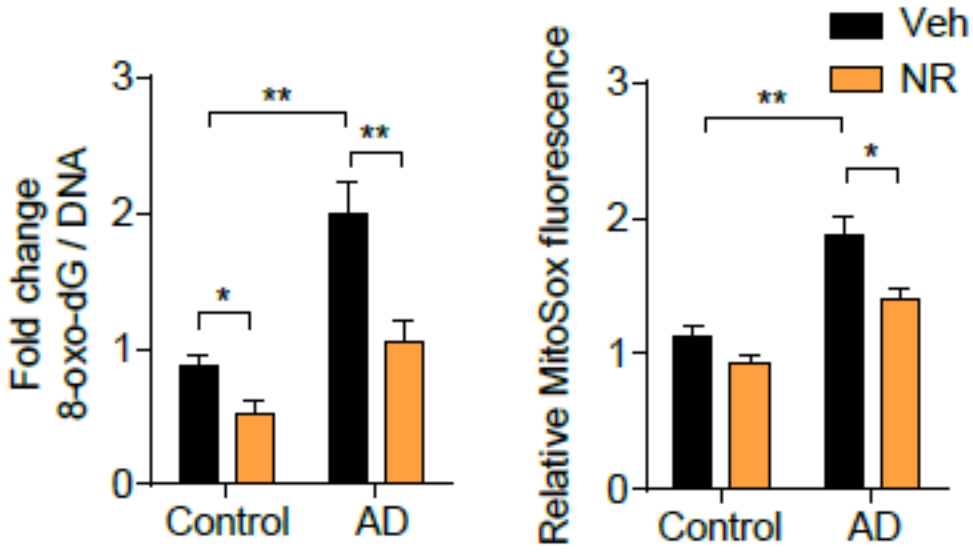


Scale bars, 100 μm.





Reduction of 8-oxoG and Mito ROS in human AD fibroblasts



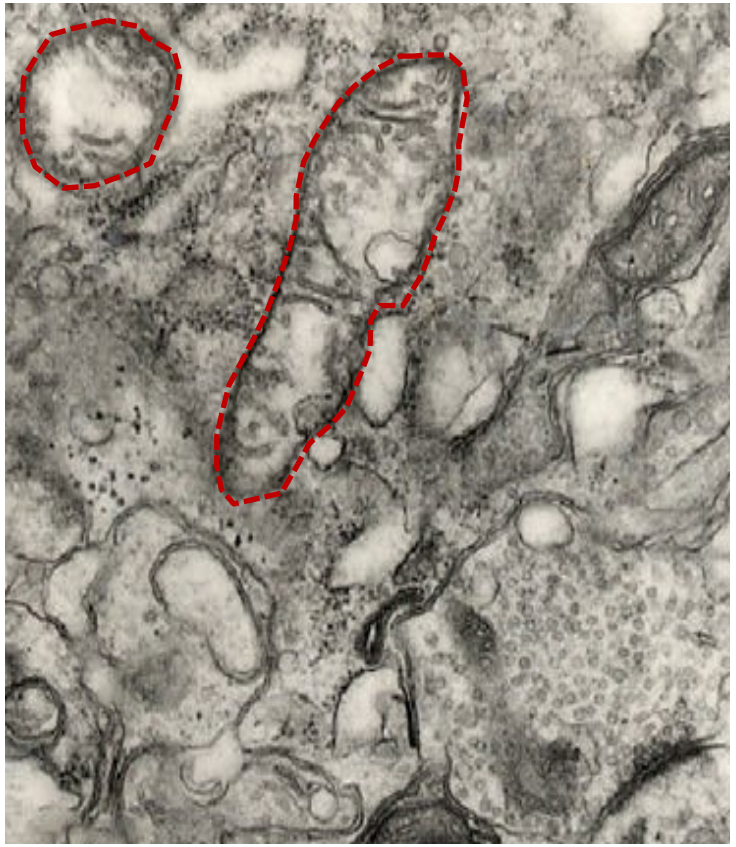
Abnormal mitochondria in human AD brain

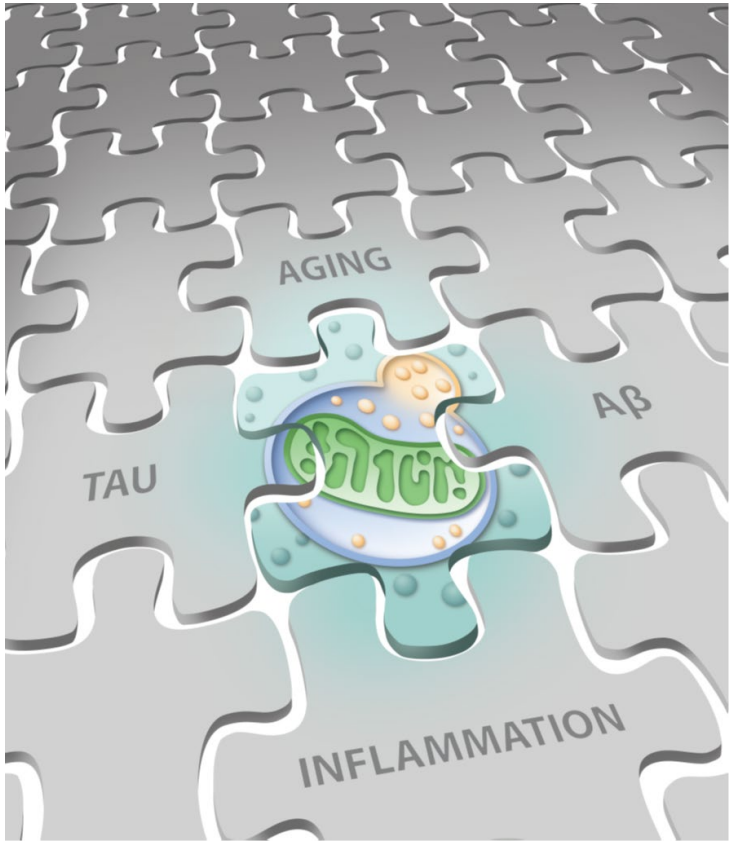
Mitochondria are critical for neuronal development, neuroplasticity, and survival

Disrupted mitochondrial health and neuronal metabolism are early features of AD

Mitochondrial dysfunction precedes Aβ and Tau pathologies. Aβ and Tau pathologies exacerbate mitochondrial dysfunction

Accumulation of damaged mitochondria are evident in AD human brain samples, possibly due to impaired mitophagy





Kerr et a., Gregi, Mattson, Bohr, Fang, Trends in Neurosciences, 2017
Mattson MP et al., Neuron, 2008
Mattson MP et al., Cell Metabolism, 2012

Baloyannis SJ, in book Neurodegenerative diseases

nature neuroscience

ARTICLES

https://doi.org/10.1038/s41593-018-0332-9

2019

Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease

Evandro F. Fang[1,2,12*], Yujun Hou[1,12], Konstantinos Palikaras[3,4,12], Bryan A. Adriaanse[5], Jesse S. Kerr[1], Beimeng Yang[1], Sofie Lautrup[1], Md Mahdi Hasan-Olive[2], Domenica Caponio[2], Xiuli Dan[1], Paula Rocktäschel[5], Deborah L. Croteau[1], Mansour Akbari[6], Nigel H. Greig[7], Tormod Fladby[8,9], Hilde Nilsen[2], M. Zameel Cader[5], Mark P. Mattson[10,11], Nektarios Tavernarakis[3,4] and Vilhelm A. Bohr[1,6*]

ALZ.ORG

nature REVIEWS DRUG DISCOVERY

RESEARCH HIGHLIGHT · 04 MARCH 2019

Turning up mitophagy in Alzheimer disease

Katie Kingwell

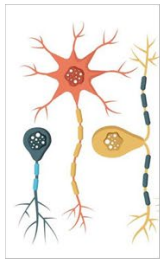
Accumulation of damaged mitochondria in neurons is a hallmark of neurodegenerative disorders including Alzheimer disease (AD). A new study in Nature Neuroscience reports impaired mitophagy — the process that removes damaged mitochondria — in patients and mouse models of AD, and identifies small-molecule mitophagy-inducing agents that improve AD signs and symptoms in preclinical models.

RELATED ARTICL

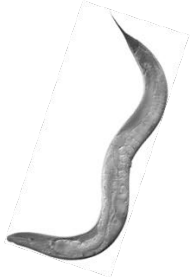
Mitochondria therapeutic ta common path

Cross-species studies in Alzheimers disease (AD)

AD postmortem brain



AD iPSCs



AD worms

AD mice

Mitophagy,

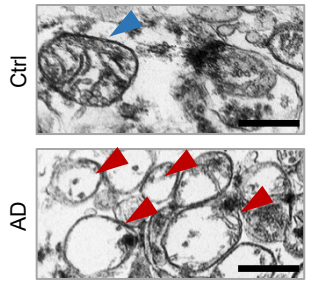
conserved mechanisms

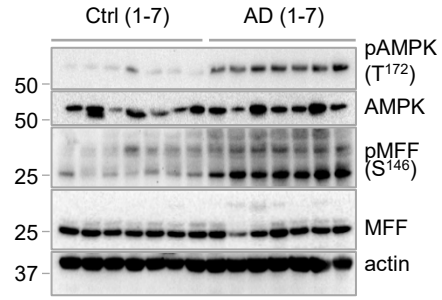
And intervention

Mitochondrial dysfunction and defective mitophagy in AD postmortem patient brain

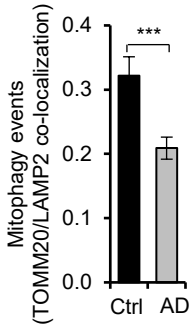
mitochondrial parameters from EM images in postmortem human hippocampal tissues from AD patients and age-matched healthy controls (n=7 individuals); representative set of EM

Mito. parameters	Human hippocampal tissues	
	Healthy ctrl. (n=203 mito.)	AD (n=300 mito.)
Mito. length (nm)	709±13	531±10***
Mito. diameter (nm)	567±8	409±7***
Area (μm²)/mitochondrion	0.346±0.010	0.194±0.006***
% Damaged mito.	21.7±1.7	71.9±2.8***

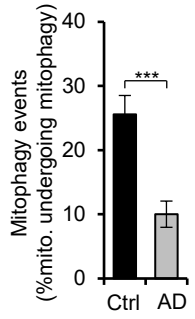




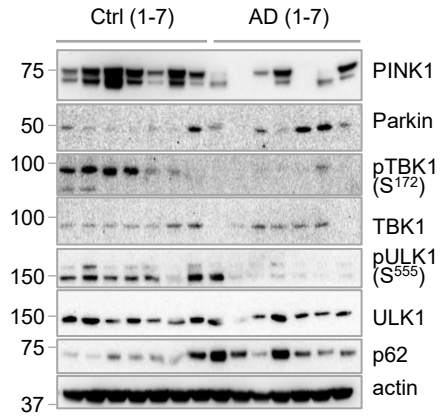
Proteins implicated in the AMPK pathway in postmortem human hippocampal tissues from AD patients and age-matched healthy controls



co-localization of the mitochondrial protein TOMM20 and the lysosomal protein LAMP2 protein using immunohistochemistry



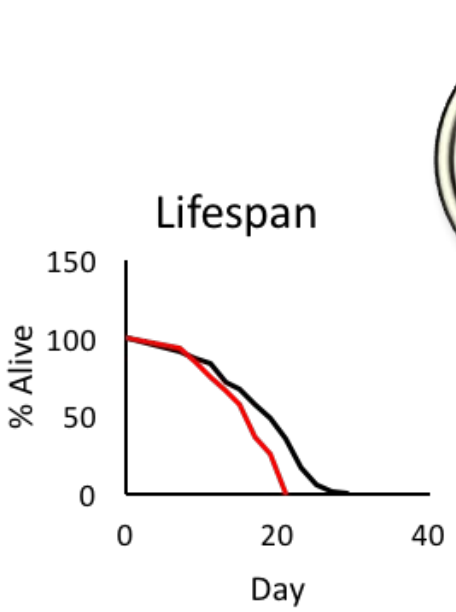
mitophagy-like events using EM images in postmortem human hippocampal tissues between AD patients and age-matched healthy controls

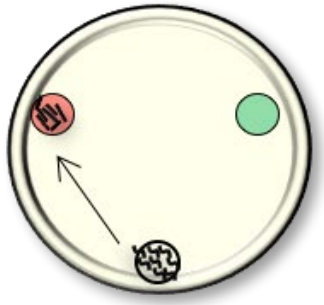


Changes in mitophagy proteins in postmortem human hippocampal tissues from AD patients and age-matched healthy controls (n=7 individuals)

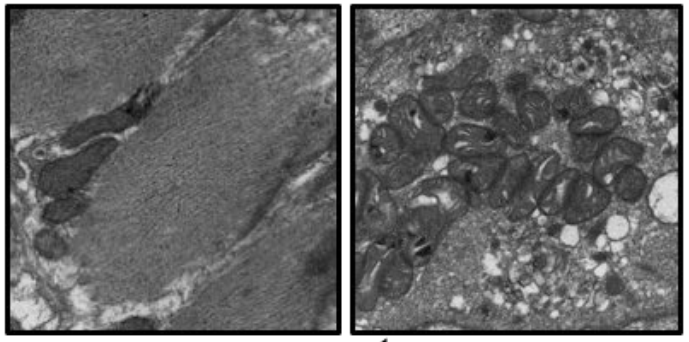
Fang et al, Nature Neuroscience, 2019

Nematode studies

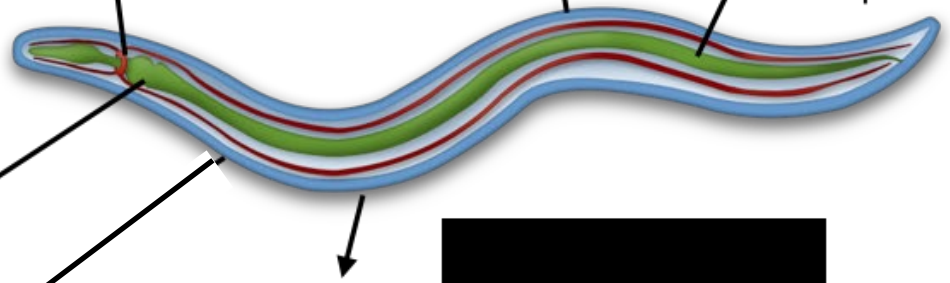




Chemotaxis/
Memory

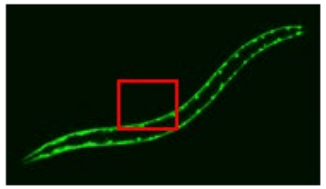


Mitochondrial
Morphology

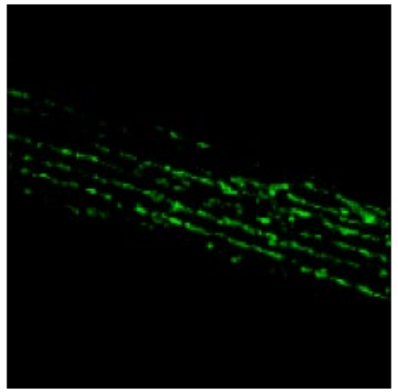


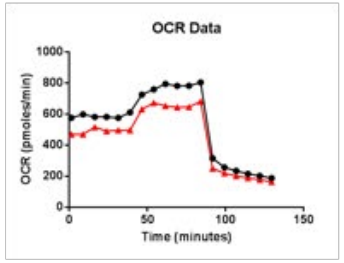
Healthspan

- Pharyngeal pumping
- Swimming



Mitochondrial
Networking





Seahorse
Analysis

Proteomics
Bioinformatics

Alzheimers worms

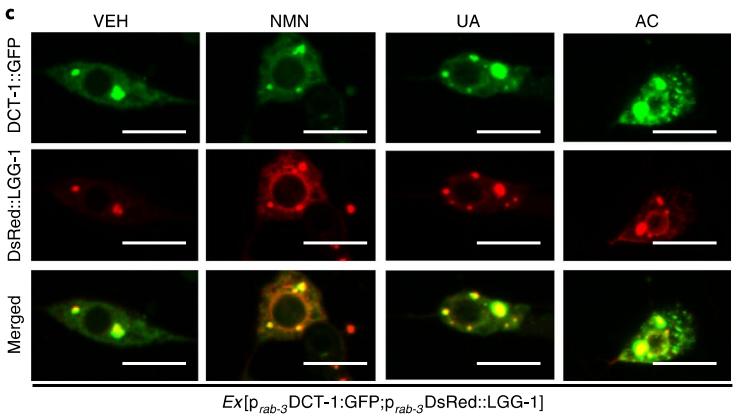
- Nematodes (c.elegans worms) are good models for AD (tau and abeta have memory loss
  - Screening for compounds that stimulate mitophagy

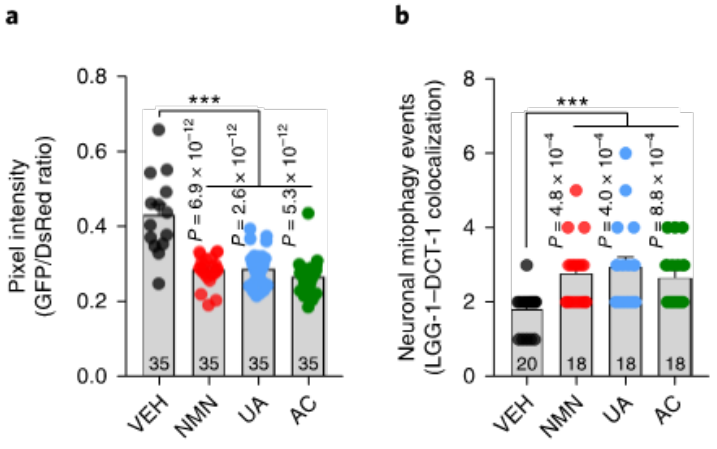
Quantification of Mitophagy in C. elegans

To quantify mitophagy dysfunction we use two distinct mitophagy biosensors:

I. **Colocalization of DsRed-LGG-1 and GFP-DCT-1**

II. **MtRosella's ratio of fluorescence intensity - GFP/DsRed**





We identified NMN, NR and Urolithin A as strong inducers

Fang, E., Hou, K. Palikaras, Tavernarakis, N. ……. Bohr VA
Nature Neuroscience 2019

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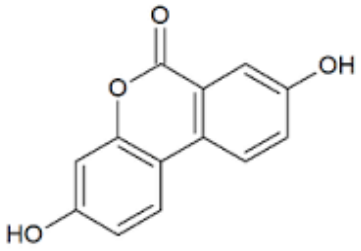
  - Screening for compounds that stimulate mitophagy

Urolithin A



Urolithin A is a metabolite compound resulting from the transformation of ellagitannins by the gut bacteria.

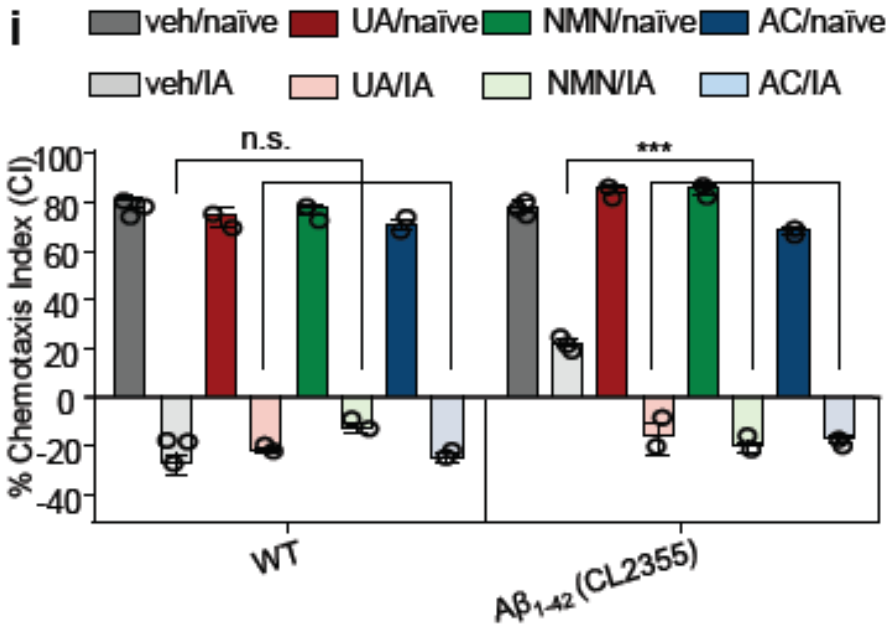
Ellagitannins are reported in dicotyledoneous angiospermes, and notably in species in the order Myrtales, such as the pomegranate.[4][5]

Fang, E., Hou, K. Palikaras, Tavernarakis, N. ……. Bohr VA
Nature Neuroscience 2019

Alzheimers worms

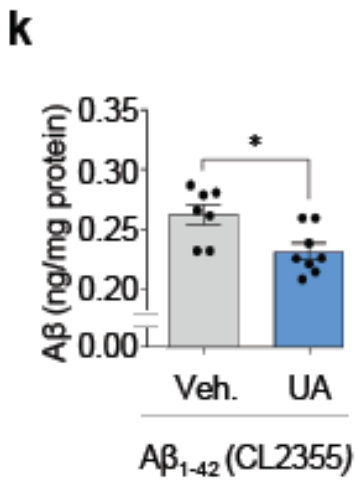
- Nematodes (c.elegans worms) are good models for AD (tau and abeta have memory loss

Mitophagy inducers, Urolithin A and Actinonin improve memory in Aβ worms in a pathway dependent manner



j

Aβ1-42 (CL2355)			
Mitophagy inducers	Mitophagy gene (memory dependent)		
	pink-1	*pdr-1*	*dct-1*
NMN	yes	yes	yes
UA	yes	yes	no
AC	yes	yes	no



Dct1-BNIP3
Pdr-1-PARKIN

Fang, E., Hou, K. Palikaras, Tavernarakis, N. ……. Bohr VA
Nature Neuroscience 2019

AD mice and IPSC cells

- Urolithin A (and NR and NMN) strongly improves learning and memory in AD mice
- Urolithin A improves mitochondrial function in human IPSC stem cells

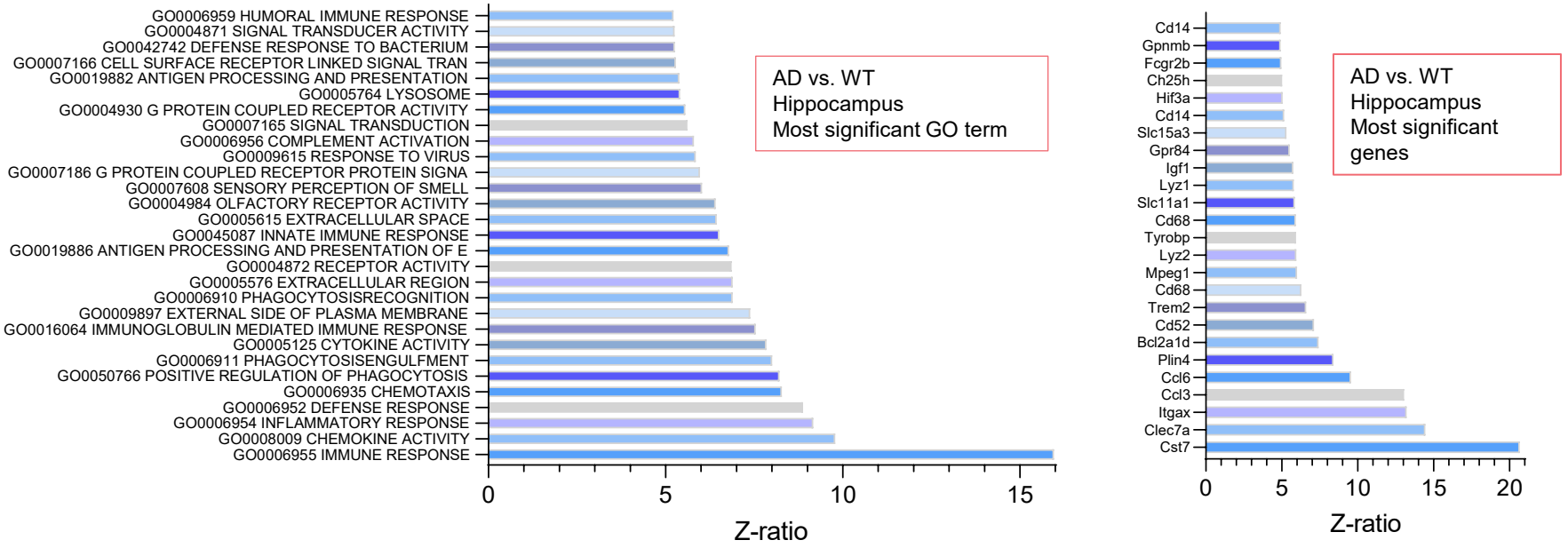
Mitophagy stimulation as future potential intervention in AD

Fang, E., Hou, K. Palikaras, Tavernarakis, N. ……. Bohr VA
Nature Neuroscience 2019

UA vs NR

- Mice, cells, worms, flies
- RNA seq, Nanostring, verification, Westerns, activity
- Bioinformatics
- Mitophagy and DNA repair pathway intersections

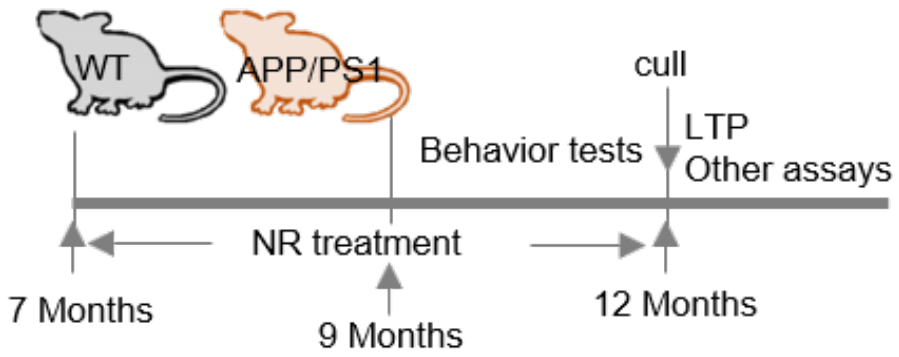
Microarray analysis of the hippocampus reveals abnormal neuroinflammation in APP/PS1 AD mice

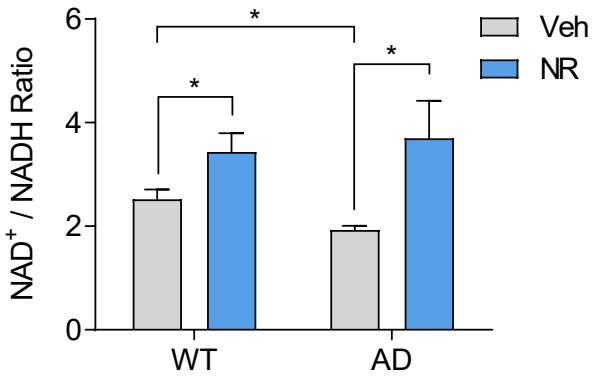


- The most changed pathways are all immune-related pathways, from the most: Immune response, chemokine activity, inflammatory response…
- The most changed genes are all microglia-related genes: Cst7, Clec7a, Itgax, Ccl3, Ccl6,…

Yujun Hou

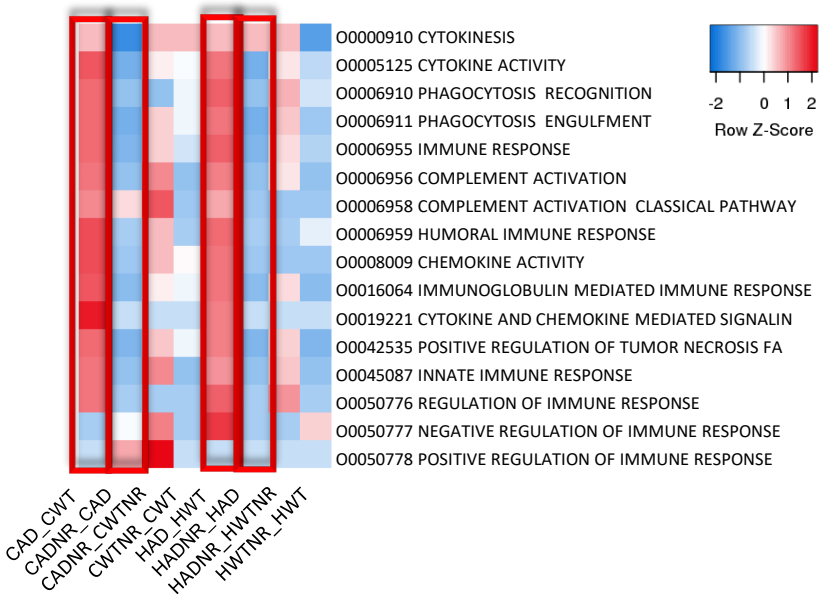
NR treatment increased NAD+/NADH in WT and APP/PS1 mouse brains

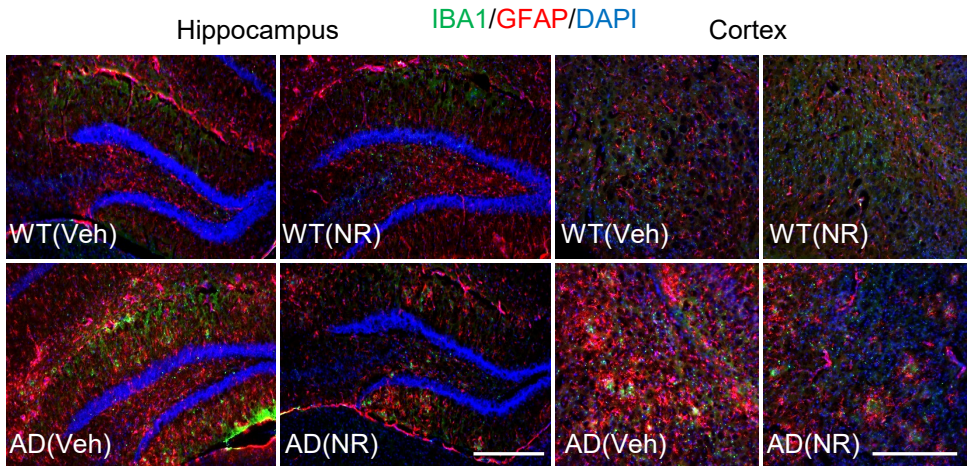


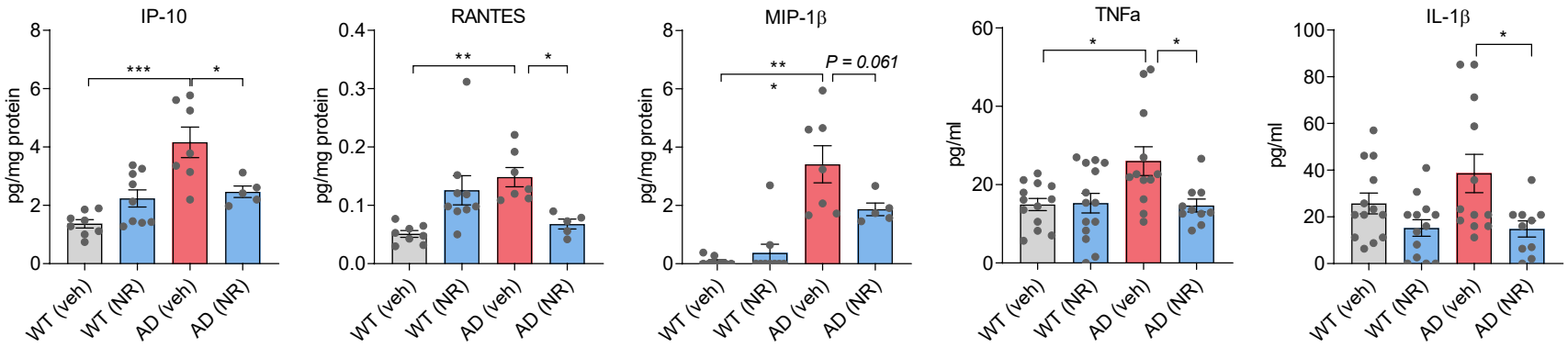


Hou et al, PNAS 2021

NR decreases abnormally activated astrocytes and microglia, and pro-inflammatory cytokines and chemokines

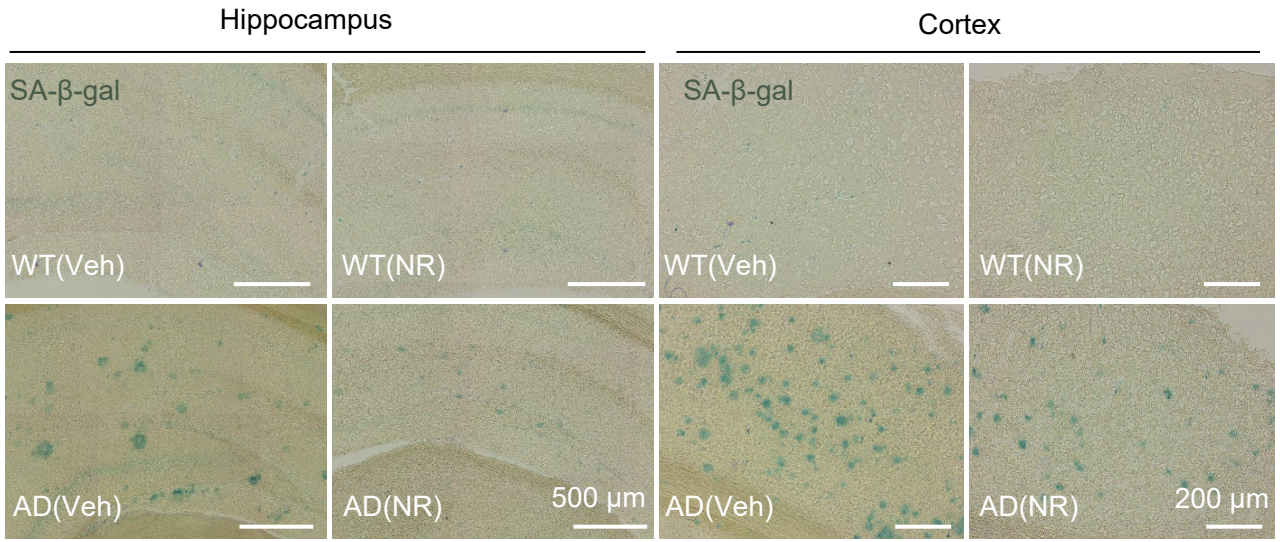


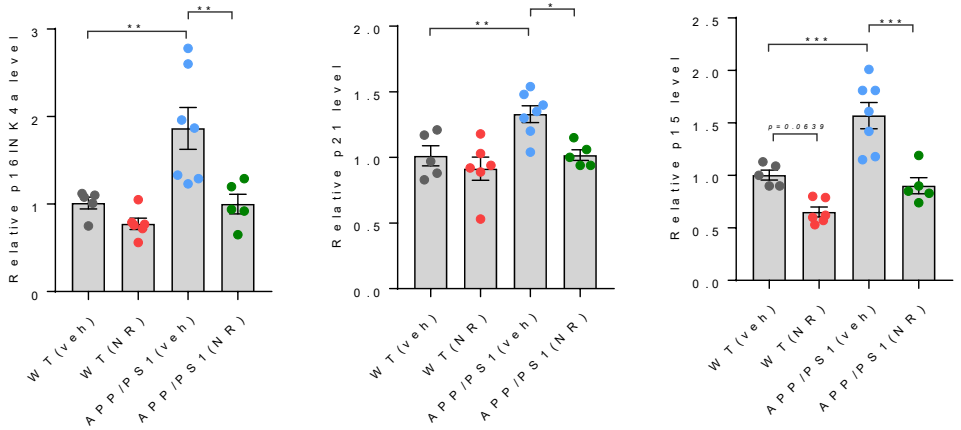




Hou et al, PNAS 2021

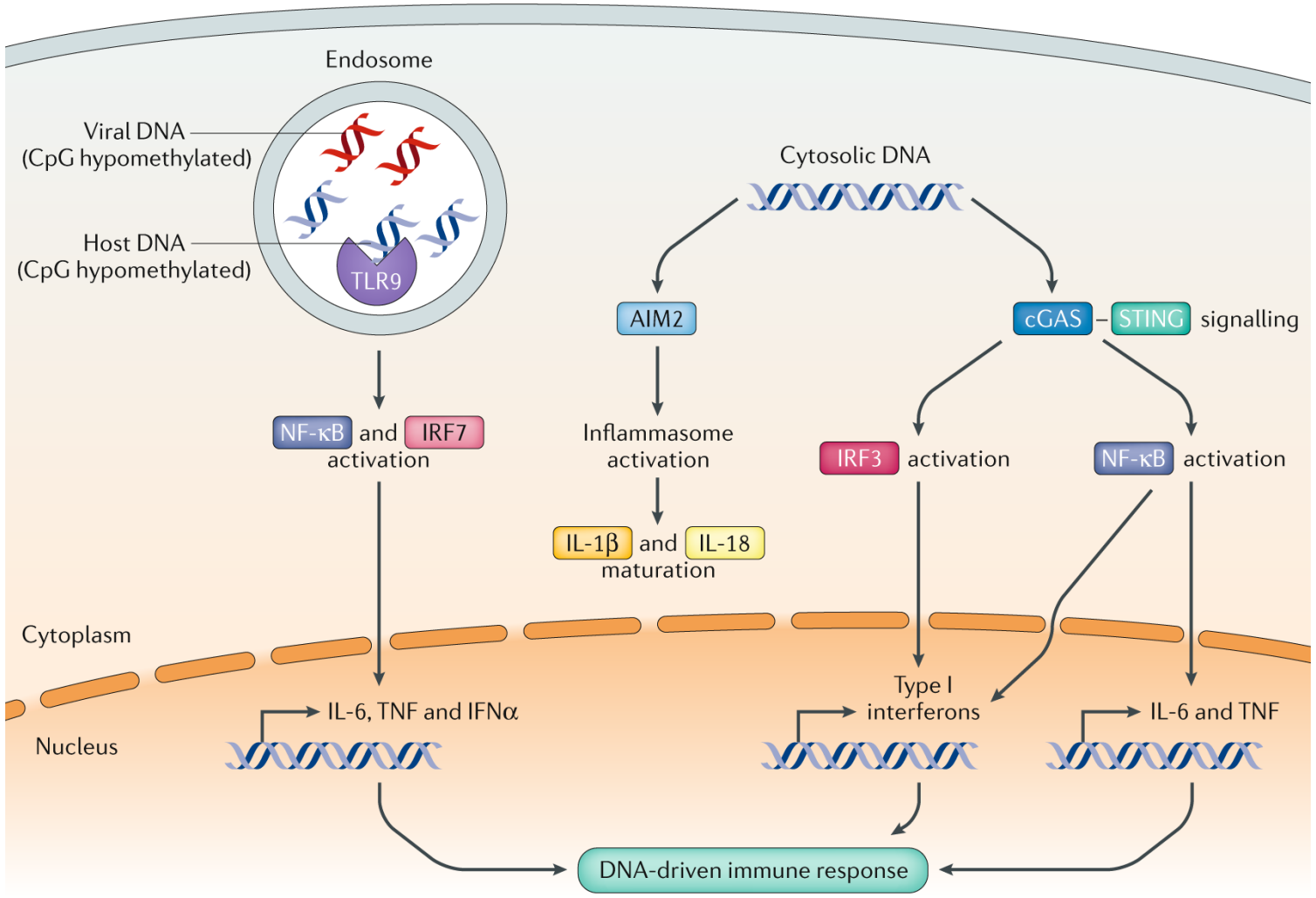
NR decreases cellular senescence in AD mice brains





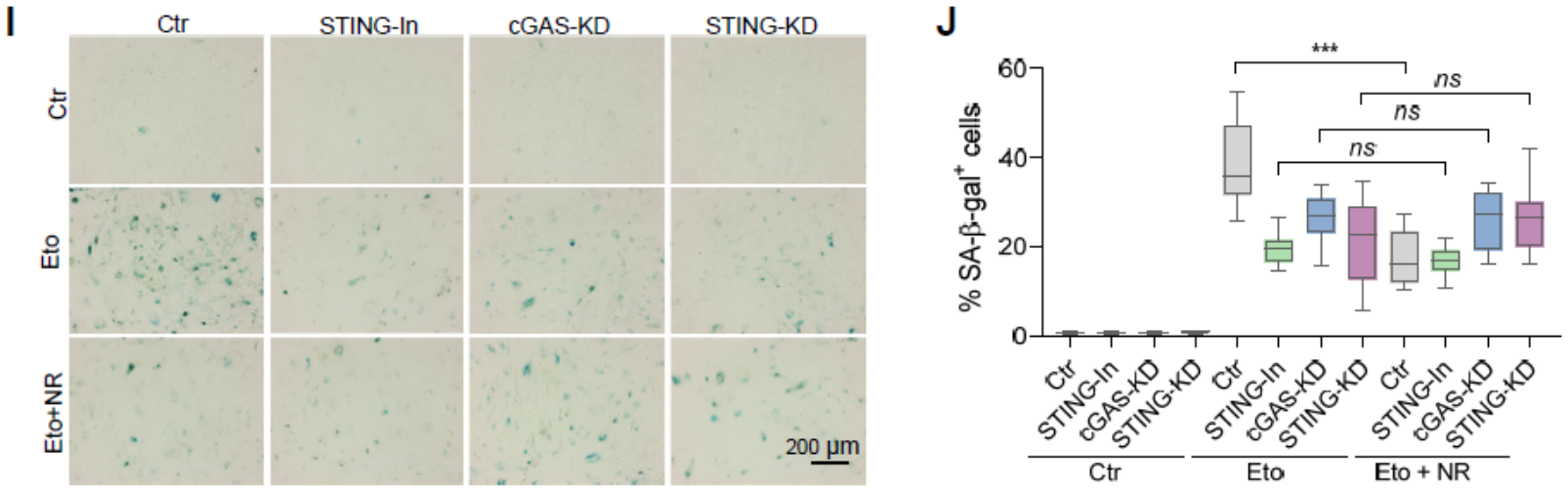
Hou et al, PNAS 2021

GAS STING signaling and cytoplasmic DNA



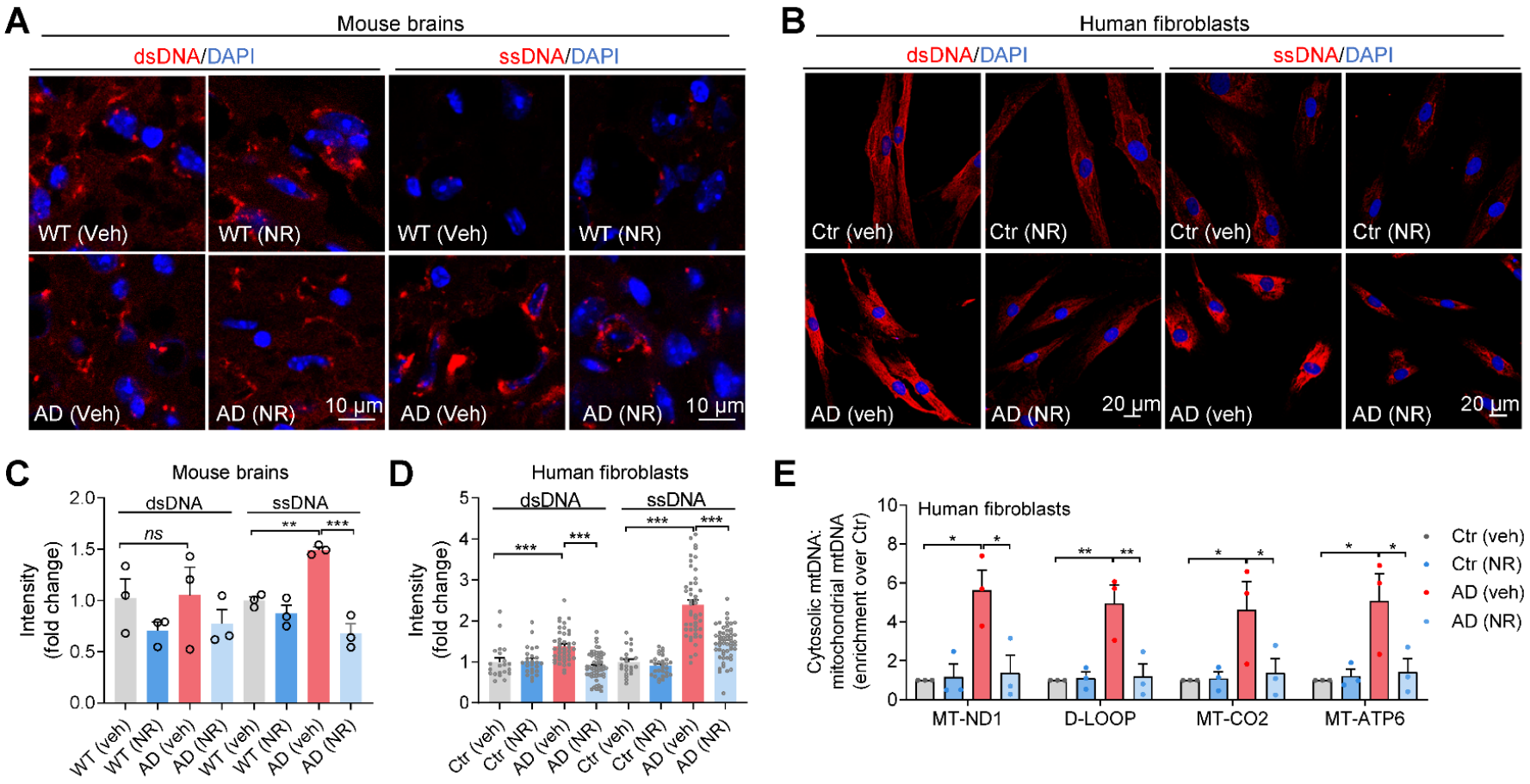
Motwani M et al, 2019
Paul, Snyder and Bohr, 2021

NR lowers cellular senescence in AD mouse brain through cGas-Sting



Hou et al, PNAS 2021

NR decreases cytosolic DNA in mouse brain and human fibroblasts



Hou et al, PNAS 2021

Conclusions

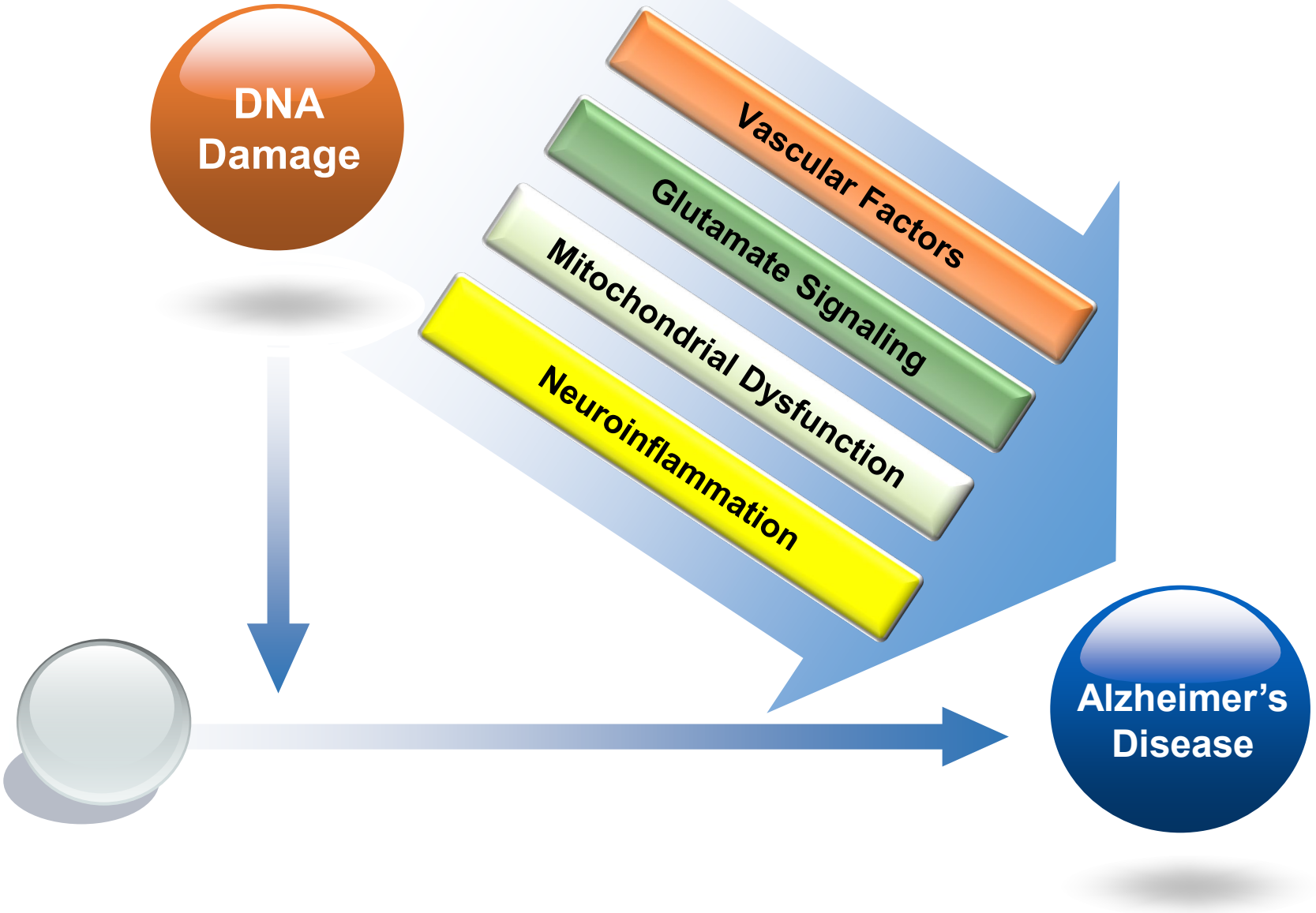
NR treatment attenuates neuroinflammation through downregulation of cGas Sting

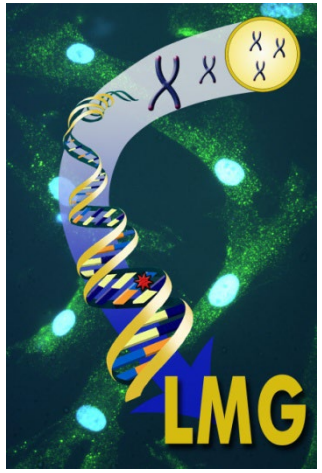
NR treatment reduces cytosolic DNAs in AD mouse brain and human AD fibroblasts

NR induces mitophagy in AD mouse brain

NR treatment normalizes cellular senescence in AD mouse brain

Hou et al, PNAS 2021


DNA Damage
Vascular Factors
Glutamate Signaling
Mitochondrial Dysfunction
Neuroinflammation
Alzheimer's Disease



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