Redox Signaling, Oxidative Stress and Neurodegenerative Diseases

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Redox Signaling, Oxidative Stress and Neurodegenerative Diseases

• How oxidative stress happens in Neurodegeneration?
  – Alzheimer’s disease
  – Huntington’s disease
  – Amyotrophic Lateral Sclerosis
  – Parkinson’s disease

• Parkinson’s disease
  – Energy/redox metabolism
  – Gene-environment interactions
  – Signaling and Redox modulation
Neurodegenerative diseases

- Neurodegenerative diseases are defined as **hereditary and sporadic conditions** which are characterized by **progressive nervous system dysfunction**.

- These disorders are often associated with **atrophy of the affected central or peripheral structures of the nervous system** and include: Alzheimer's Disease and other dementias, Parkinson's Disease, Multiple Sclerosis, Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's Disease), Huntington's Disease, Prion Diseases, and others.

- There is no cure for these diseases.
Main mechanisms associated with neurodegeneration

- **Energy and Metabolism dysfunction**
  - Oxidative Phosphorylation, Glycolysis
  - Impaired ATP-dependent processes (Synaptic transmission, protein quality control mechanisms)
  - ATP is required to maintain antioxidant systems
  - Glucose metabolism regulates the PPP (NADPH)

- **Mitochondrial dysfunction**
  - Energy failure
  - Signaling (Calcium, pro-cell death molecules)
  - Increase steady state levels of reactive oxygen species (oxidative damage)

- **Dysfunction in Protein Quality Control Mechanisms.**
  - Protein Folding
  - Protein degradation
    - Proteasome
    - Autophagy
  - Impaired mitochondria turnover and accumulation of oxidized biomolecules
Oxidative stress is a common event in the pathogenesis of neurodegenerative diseases

- **Parkinson's disease (PD)**
  - Mitochondrial toxins, dopamine oxidation
  - Decreased antioxidant levels (GSH)
  - Lipid peroxidation, protein (carbonyls and nitrotyrosines) and nucleic acid oxidation

- **Alzheimer's disease (AD) and mild cognitive impairment.**
  - Increased lipid-peroxidation (CSF), protein (carbonyls and nitrotyrosines) and nucleic acid oxidation
  - Oxidative stress influences Aβ formation and Aβ has pro-oxidant effects

- **Huntington's disease (HD)** – Energy metabolism
Table II. Diverse molecular modifications observed in Alzheimer’s diseased brains.

<table>
<thead>
<tr>
<th>Modifications in Alzheimer’s disease</th>
<th>Observation</th>
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<tbody>
<tr>
<td>Oxidative protein modifications</td>
<td>Increases in protein carbonyls and other protein oxidation products (glutamic and aminoadipic semi-aldehydes), increased levels of methionine sulfoxide oxidized SOD, creatine kinase and glutamine synthetase UCHL1 is heavily oxidized</td>
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<tr>
<td>Nitrative protein modifications</td>
<td>Increased levels of nitrotyrosine neurofibrillary tangles Other nitrated proteins are β-actin, α-enolase and triosephosphate isomerase</td>
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<tr>
<td>Lipid peroxidation</td>
<td>Increased levels of HNE</td>
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<tr>
<td>Oxidative DNA damage</td>
<td>Increases in 8OHdG and other base oxidation products in mitochondria and nucleus</td>
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<tr>
<td>Mitochondrial dysfunction</td>
<td>Decreased activities of complex IV, alpha-ketoglutarate dehydrogenase, pyruvate dehydrogenase</td>
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<tr>
<td>Proteasome dysfunction</td>
<td>Decreased proteasomal activity, abnormal accumulation of proteins containing polyubiquitin</td>
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</table>

Table III. Diverse molecular modifications observed in Parkinson’s diseased brains.

<table>
<thead>
<tr>
<th>Modifications in Parkinson’s disease</th>
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<td>Oxidative protein modifications</td>
<td>Increased levels of protein carbonyls in substantia nigra, oxidized SOD1 Increased levels of oxidized dopamine (cysteinyl-DOPA and cysteinyl-dopamine)</td>
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<td>Nitrative protein modifications</td>
<td>Increased levels of nitrotyrosine in α-synuclein and Lewy bodies</td>
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<td>Lipid peroxidation</td>
<td>HNE-products in Lewy bodies and increased peroxides</td>
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<td>Oxidative DNA damage</td>
<td>Increased levels of DNA oxidation product 8OHdG in mitochondrial and total DNA</td>
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<td>Glutathione</td>
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<tr>
<td>Mitochondrial dysfunction</td>
<td>Decreased activities of complex I and alpha-ketoglutarate dehydrogenase</td>
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<tr>
<td>Proteasome dysfunction</td>
<td>Genetic defects in inherited Parkinson’s disease, decreased activities in sporadic Parkinson’s disease, decrease UCHL1 activity</td>
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<tr>
<td>Oxidized dopamine</td>
<td>Auto-oxidation of free non-vesicle dopamine to quinone</td>
</tr>
</tbody>
</table>
ROS and oxidative stress contribute to the aging process

**Exogenous Factors**
- Environmental conditions
- Lifestyle
- Stress conditions

**Endogenous sources of ROS**
- Modulation of signal transduction pathways
- Changes in gene expression
- Cellular responses: inflammation, survival, proliferation, death
- Systematic responses: aging, organ dysfunction, frailty, diseases

- Accumulation of molecular injury: DNA, protein, lipids
- Organelle damage (e.g., mitochondria, peroxisomes)

**Passage of Time**
Alzheimer’s disease

- The two core pathological hallmarks of Alzheimer's disease are amyloid plaques and neurofibrillary tangles.
- The amyloid cascade hypothesis suggests that deposition of amyloid β (Aβ) triggers neuronal dysfunction and death in the brain.
- Genetic causes of Alzheimer's disease include mutations of the genes encoding amyloid precursor protein (APP) and presenilin 1 (PSEN1) and PSEN2. PSEN1 and PSEN2 mutations affect concentrations of Aβ1–42 because the presenilin proteins form part of γ secretase, which cleaves APP to produce Aβ.
- Many treatable medical conditions are also associated with an increased risk of Alzheimer's disease, including stroke, diabetes (inflammation).
A) Amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation, neural plasticity and iron export.

Amyloidogenic processing is initiated by β-secretase beta-site amyloid precursor protein–cleaving enzyme 1 (BACE-1), releasing a shortened sAPPβ. The C99 fragment is a γ-secretase substrate, generating Aβ and AICD (amyloid precursor protein intracellular domain).

B) Soluble Aβ is prone to aggregation. Protofibrils (upper) and annular or pore-like profiles (lower) are intermediate aggregates.

Self-association of Aβ monomers into oligomers (right) is dependent on concentration (left immunoblot) and is promoted by oxidizing conditions (lane 2) and divalent metal conditions (lane 3, right IB).

NEJM Volume 362:329-344
**Tau hyperphosphorylation**

- **Tau proteins are proteins that stabilize microtubules.** Four repeat sequences (R1-R4) make up the microtubule-binding domain (MBD).

- **Normal phosphorylation of tau occurs on serine and threonine residues.**

- **When followed by proline (P),** these amino acids are phosphorylated by glycogen synthase kinase 3 (GSK-3β), cyclin-dependent kinase (cdk5), or mitogen-activated protein kinase (MAPK). **Nonproline-directed** kinases phosphorylating tau are Akt, Fyn, protein kinase A (PKA), calcium–calmodulin protein kinase 2 (CaMKII), and microtubule affinity-regulating kinase (MARK).

- **Excessive kinase, reduced phosphatase activities, or both cause hyperphosphorylated tau to detach and self-aggregate and to destabilize microtubules.**
Alzheimer’s disease and oxidative stress

- Amyloid β shows **peroxidative activity** on cell and organelle membrane lipids yields the mitochondrial toxins hydroxynonenal (HNE) and malondialdehyde.

- Cellular **Aβ directly attacks electron transport complex IV** (cytochrome c oxidase) and **key Krebs-cycle enzymes** (α-ketoglutarate and pyruvate dehydrogenase) and damages mitochondrial DNA (mtDNA), leading to fragmentation.

- **Lipid peroxidation products also promote tau phosphorylation** and aggregation, which in turn inhibit complex I.

- **Neuroinflammation and metal-induced ROS**

- Contradictory results have been found with respect to the effect of dietary intake of antioxidants, such as vitamin E in reducing the risk or the rate of progression of Alzheimer’s disease.
Alzheimer’s disease and RNS

- NMDAR hyperactivation triggers generation of NO and subsequent S-nitrosylation of neuronal proteins, contributing to synaptic damage and eventually neuronal death.
- Soluble oligomers of Aβ oligomers, can facilitate neuronal NO production in both NMDAR-dependent and -independent manners.
- S-Nitrosylation of the fission-inducing protein Drp1 (dynamin-related protein 1, forming SNO-Drp1) can contribute to synaptic damage and neuronal cell death by triggering excessive mitochondrial fission and bioenergetic impairment.

Huntington’s disease and oxidative stress

- Huntington disease (HD) is an autosomal dominant neurodegenerative disease.
- HD is caused by a CAG (glutamine) repeat expansion in the first exon of the HTT gene that encodes huntingtin (Htt).
- γ-Aminobutyric acid (GABAergic) medium spiny neurons of the striatum that contain enkephalin or substance P and project to the globus pallidus and substantia nigra are particularly vulnerable in HD.
- Defects in energy metabolism, particularly mitochondrial function, represent a common thread in studies of HD pathogenesis in humans and animal models.
- Impaired oxidative phosphorylation, oxidative stress, impaired mitochondrial calcium handling, abnormal mitochondria trafficking, deregulation of key factors of mitochondrial biogenesis, such as the transcriptional coactivator PPARγ coactivator-1α (PGC-1α), and decreased glycolisis.

Huntington’s disease and oxidative stress

- Mitochondrial toxins such as malonate and 3-nitropropionic acid (3-NP) inhibit succinate dehydrogenase (SDH), which is part of complex II of the electron transport chain.

- Neuroprotection: Coenzyme Q₁₀ is an electron acceptor from complex I and complex II that also has antioxidant activity. Creatine and triacetyluridine can buffer intracellular energy stores.

Amyotrophic lateral sclerosis

- Amyotrophic lateral sclerosis (ALS) is a paralytic disorder caused by motor neuron degeneration.
- The causes of most cases of ALS are as yet undefined. Excessive excitatory tone, protein misfolding, impaired energy production, abnormal calcium metabolism, altered axonal transport and activation of proteases and nucleases.
- Several factors are proposed to instigate these phenomena, including latent infections by viral and non-viral agents, toxins (for example, insecticides and pesticides) and autoimmune reactions.
- The protein products of these mutated genes are cytosolic Cu/Zn superoxide dismutase (SOD1), alsin, senataxin (SETX), synaptobrevin/VAMP (vesicle-associated membrane protein)-associated protein B (VAPB) and dynactin have been reported to cause ALS.
- About 20–25% of all familial ALS cases arise because of mutations in SOD1, the protein product of which accounts for 0.1–0.2% of the cellular proteins in the CNS.
- TDP-43 protein (known as TARDBP) accounts for approximately 2-6% of all familial ALS cases and is found aggregated in sporadic cases.
• In the aberrant redox chemistry model, mutant superoxide dismutase 1 (SOD1) is unstable and aberrant chemistry is mediated by promiscuous interaction with non-conventional substrates.
  – Hydrogen peroxide (H₂O₂) or peroxynitrite (ONOO⁻) can react with reduced SOD1 (SOD1-Cu⁺).
  – Molecular oxygen (O₂) can react aberrantly with Zn-deficient SOD1 to generate an excess of superoxide anion (O₂⁻).
  – The unstable protein can also release free copper and/or zinc, which might be toxic.

• In the protein toxicity model, conformationally altered mutant SOD1 forms toxic, proteinaceous deposits.
  – Aggregated SOD1 inhibits chaperone and/or proteasome activity, with subsequent misfolding and insufficient clearance of numerous proteins.
  – Alternatively, these aggregates could sequester, inactivate or enhance the toxicity of other proteins crucial for cellular processes.

Nat Rev Neurosci. 2006 Sep;7(9):710-23.
Multiple sclerosis and oxidative stress

- Multiple sclerosis is a **chronic inflammatory disease of the central nervous system**, associated with demyelination and **neurodegeneration**. The mechanisms of tissue injury are currently poorly understood, but recent data suggest that **mitochondrial injury** may play an important role in this process.

- Both in MS and in neurodegenerative diseases, inflammation initiates microglial activation.

- In MS, inflammation is immune-mediated but in neurodegenerative diseases the primary causes of inflammation remain speculative.

- **Activated microglia produce O$_2^-$ and •NO reactive species that combine to induce primary synthesis of peroxynitrite.**

- Peroxynitrite inhibits glutamate transporters and/or activates glutamate receptors causing glutamate overload.

- Excitotoxicity entails mitochondrial dysfunction, calcium overload.

**AUTO-TOXIC LOOP IN NEURODEGENERATION**

- Multiple sclerosis
  - Immune mediated inflammation
  - (microglial activation)
- Production of O$_2^-$ and •NO
- Peroxynitrite
  - Production of O$_2^-$
  - Ca$_2^+$ overload → •NO
  - Mitochondrial energy metabolism impairment
  - Glutamate overload
  - Glutamate transporters inhibition
  - and/or
  - Glutamate receptor activation
- ALS, Parkinson, Alzheimer
  - ??? mediated inflammation
  - (microglial activation)
The underlying cause of brain pathology in all prion disorders is PrP-scrapie (PrP(Sc)), a beta-sheet-rich conformation of a normal glycoprotein, the prion protein (PrP(C)).

In prion disorders, imbalance of brain-iron homeostasis is observed before end-stage disease and worsens with disease progression, implicating iron-induced oxidative stress in disease pathogenesis.


What causes Parkinson’s disease

- Parkinson’s disease (PD) is a **chronic progressive neurodegenerative disorder** that leads to shaking (tremors) and difficulty with walking, movement, and coordination. Parkinson's may lead to a deterioration of all brain functions, and an early death.

- Loss of **dopamine neurons** from the substantia nigra pars compacta leads to deficiency of dopamine in the caudate and putamen (“striatum”).

- Currently, there is no treatment to cure or stop PD progression.

- The exact cause of PD is unknown.
  - Familial (Hereditary) forms of PD (~10%) α-synuclein, Parkin, DJ-1, PINK1 and LRRK2 genes.
  - Sporadic (Idiopathic) PD  
    ~5% are linked to genetic alterations **Environmental** or occupational factors.
Products of PD-associated genes that affect mitochondrial function and oxidative stress

Rare inherited mutations in genes encoding electron transport chain components have been associated with parkinsonism.

- **Parkin** is partially localized to the outer mitochondrial membrane,
- **LRRK2** associates, at least in part, with the outer mitochondrial membrane
- **HTRA2** is a mitochondrial serine protease, the release of which might be involved in apoptotic cell death.
- **PINK1** is a mitochondrial serine–threonine kinase that affords protection against oxidative stress and acts with Parkin to regulate the balance of mitochondrial fission and fusion.
- **DJ-1** is relocated to mitochondria under conditions of oxidative stress and is thought to be neuroprotective under such conditions.
- The **α-synuclein** protein has an amino-terminal mitochondrial targeting sequence and, when overexpressed or under conditions of acidification, is at least partially associated with the inner mitochondrial membrane, where it might cause direct damage.

DJ-1 in Parkinson’s disease

Chemical Exposure → Oxidative Stress Insult

Age

Loss of function mutation in DJ-1

SH

Increased sensitivity to oxidative stress

Accelerated death of DA neurons in SNpc

Parkinson’s Disease

DJ-1

Over-oxidation of key cysteine & increase in acidic isoforms

Decreased protective activity
Experimental models of PD

- A single cause for PD is unlikely to emerge. Experimental models address different hallmarks of PD, depending on the question asked…
  - Mitochondrial dysfunction
  - Dopamine metabolism
  - Oxidative Stress
  - Genetic mutations
  - Impairment in protein quality control mechanisms

- There is no experimental PD model that recapitulates all aspects of PD.
- Genetic models induce poor DA loss.
- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used extensively as a model of PD.
- While epidemiological evidence strongly suggests a link between environmental exposures (pesticides, heavy metals), environmental exposures, by themselves, are not the cause of PD.
What makes a dopaminergic neuron the target?

- Dopamine and Iron (pro-oxidant)
- Dopaminergic neurons consume a significant amount of energy due to:
  - Pacemaking activity
  - Vesicle transport
  - Action potential and membrane potential maintenance through the unmyelinated axon

The axonal arbors of single nigrostriatal dopaminergic neurons in rat brain. The image shows axon fibers in the neostriatum. Red and blue lines indicate the axon fibers located in the striosome and matrix compartments of the neostriatum. A single dopamine neuron can influence up to 5% of all neurons in the neostraitum or ~75,000 neurons.

Oxidative stress in substantia nigra

- Using transgenic mice that expressed a redox-sensitive variant of green fluorescent protein targeted to the mitochondrial matrix, it was demonstrated that normal autonomous pacemaking created an oxidant stress that was specific to vulnerable SNc dopaminergic neurons but not in neurons in the ventral tegmental area.

- The oxidant stress engaged induced transient, mild mitochondrial depolarization or uncoupling.

- Knocking out DJ-1 (PARK7), which is a gene associated with an early-onset form of Parkinson's disease, down-regulated the expression of two uncoupling proteins (UCP4 (SLC25A27) and UCP5 (SLC25A14)), compromised Ca2+-induced uncoupling and increased oxidation of matrix proteins specifically in SNc dopaminergic neurons.

*Nature.* 2010 Dec 2;468(7324):696-700
Redox regulation of Parkinson’s Disease

- Redox cycling
- Thiols
  - GSH
  - Cysteine modifications
  - Trx/Prx/Srx
- Oxidative Stress
- Hydrogen sulfide
- Redox regulated transcription factors
- Mitochondria
- Inflammation
Why have antioxidant clinical trials failed?

- Antioxidant supplementation has for the most part failed to achieve meaningful effects in PD.
  - Lack of specificity
  - Administration of antioxidants might not have been given early enough
  - Not all cases arise from the same causes
  - Wrong experimental models
  - Oxidative stress is not the main/single cause in neurodegeneration
Environmental agents and Parkinson’s disease

- Environmental agents linked to increased incidence/risk to develop Parkinson’s disease
  - **Pesticides**
  - **Heavy Metals**

- The U.S. is the country as a whole with the world’s highest prevalence of PD.

- For residents of highly agricultural areas, pesticides are environmental risk factors of major public health concern for populations directly working with them or indirectly exposed by residential proximity to crop fields.


- Not a single environmental toxicant can be the cause of sporadic PD.
Mitochondrial Dysfunction:
Redox Homeostasis vs Bioenergetics

Redox homeostasis

Bioenergetics

Figure 12-8
Molecular Cell Biology, Sixth Edition
© 2008 by W. Freeman and Company
Alterations in Glucose Metabolism and the Pentose Phosphate Pathway (PPP)

A

B

C

Intracellular

Extracellular

The PPP is Hijacked to Mediate PQ Toxicity

A

Paraquat 0 0.1 0.2 0.5 1 [mM]

G6PD

GAPDH

Cell Death (Fold Increase)

Control Rotenone MPP+ PQ 6-OHDA

Cell viability (PI uptake)

B

Control PQ 0.5 PQ 0.75

% of max

- 6-AN

+ 6-AN

Paraquat impairs NADPH-dependent antioxidant systems

- Cell permeable GSH protects against paraquat
- Overexpression of GR and catalase (cytosol or mitochondria) does not prevent paraquat toxicity
Paraquat toxicity *in vivo*

**A**
- **PBS**
- **MPTP**

**B**
- **Control**
- **PQ**

**C**
- **TH**
- **PSSG**
- **Merge**

**Figure**
- "Control + PQ" vs "PBS + PQ"

**Graph**
- TH + neurons counts
- PBS vs MPTP

**Antioxid Redox Signal** 2012; 17(12):1676-93.
Paraquat toxicity *in vivo*

- **Midbrain**
  - PC3: 14.9%
  - PC2: 17.5%
  - PC1: 31.1%
  - P: 1.36e-04

- **Striatum**
  - PC3: 13.3%
  - PC2: 22.0%
  - PC1: 29.7%
  - P: 9.39e-04

- **Cortex**
  - PC3: 12.6%
  - PC2: 17.2%
  - PC1: 42.3%
  - P: 0.02
Gene-Environment Interactions in PD

The Multi-hit Hypothesis

SNCA mutations & SNPs (A53T, A30P, E46K)
- Parkin Mutations
- Loss-of-function mutations in DJ-1, Parkin
- Abnormal signal transduction
  - Aberrant kinase activity
    - Altered substrate specificity
    - Inappropriate protein phosphorylation

Protein aggregation
- Natively unfolded α-synuclein
- Protofibrils
- Insoluble aggregates

UPS dysfunction
- Proteasomal inhibition
- Decreased protein degradation

Mitochondrial dysfunction
- Impaired mitochondrial integrity
- Reduced ATP production
- Increased free radicals

Reduced antioxidant response

SNPs in HLA & inflammatory cytokines

Neuroinflammation
- (microglial recruitment, proliferation & activation)
  - Inflammatory cytokines
  - Chemokines
  - Reactive free radicals
  - Eicosanoids
  - Excitatory amino acids
  - Proteases

Oxidative stress

Brain trauma
- CNS infection

Environmental toxicants
- (pesticides & heavy metals & other environmental toxins)

Progress in Neurobiology
**Gene-Environment Interactions in Parkinson’s Disease**

- Parkinson’s disease is a multifactorial disease: **ageing, genetics and environment.**

- The formation of intracellular aggregates (Lewy bodies) of which a major component is the protein **α-synuclein**, is a pathological hallmark.

- **Mitochondrial dysfunction and energy failure** induced by environmental toxicants can lead to α-synuclein misfolding and aggregation by an impairment in protein quality control mechanisms.
\(\alpha\)-synuclein Potentiates PQ Toxicity and Metabolic Dysfunction

A

\[
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\text{MOI} & \text{Empty} & 15 & \text{-} & \text{-} & \text{-} \\
\alpha\text{-synuclein} & \text{-} & 2.5 & 5 & 10 & 15 \\
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B

Cell survival (%)

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C

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\begin{array}{ccc}
\alpha\text{-syn} & PQ & \alpha\text{-syn} + PQ \\
\end{array}
\]

Anandhan A et al., J. Biol Chem [submitted]
α-synuclein Potentiates PQ Toxicity and Metabolic Dysfunction

A

Relative NMR intensity to control (100)

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Glucose 6-P</th>
<th>Fructose 1,6-bisP</th>
<th>Ribose 5-P</th>
<th>NADH/NADPH</th>
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<tr>
<td>Control</td>
<td>α-syn</td>
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Cell survival (%)

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<tr>
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<th>Alpha syn</th>
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SGLT

Glucose

Phlorizin

STF-31

Glucose

Anandhan A et al., J. Biol Chem [submitted]
Ascorbic Acid and Inhibition of the PPP Protect Against $\alpha$-synuclein + PQ

**A**

<table>
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**B**

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*Energy Failure & Oxidative Stress*

Glucose $\rightarrow$ PPP $\rightarrow$ NADPH

H$_2$O$_2$

Down-Regulation of AMPK

$\alpha$-synuclein

Autophagy

Anandhan A et al., J. Biol Chem [submitted]
Understanding α-synuclein (Gene) – Environment Interactions

• α-synuclein: More questions than answers

• Interactions between genes and environmental exposures in dopaminergic cells should be:
  - **Different:** Not all genes might be expected to interact similarly with all environmental exposures
  - **Specific:** A gene alteration could be expected to interact similarly with other environmental exposures in order to mediate toxicity
Paraquat and Manganese Interact with α-synuclein

Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-κB)

A

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<th>MnCl₂ (mM)</th>
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<td>A53T</td>
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<td>-</td>
</tr>
</tbody>
</table>

Nitric Oxide (•NO) regulates PQ/α-synuclein Toxicity: iNOS and -ONOO (peroxynitrite)

A

B

C

Inhibition of p38 Protects Against PQ/α-synuclein Toxicity

A

Control
PQ
SB203580
SB203580+PQ

Cell survival (%)

Empty Alpha syn A53T

B

PQ (µM)

Empty + + - - - - + + - - - - - -
α-Syn - - + + - - - - - - - - - - -
A53T - - - - + + + + + + + + + +
SB203580 - + - + - + - + - + - - -
p-NF-κB 1 1 1.2 2.5 2.8 6.2 1 0.8 2.9 2.2 4.2 3.4
NF-κB β-Actin

α-synuclein (duplications and mutations)
Paraoquat
Manganese

Dopaminergic cells

NF-κB in Neurodegeneration: Inflammation and Neuronal Survival

- Inducible NF-κB is primarily associated with inflammatory processes and ROS formation.
- Constitutive neuronal NF-κB activation is required for neuronal function (anti-apoptotic).
Redox regulation of NF-κB: Where should we start?

Direct redox regulation
- Nitrosylation
- Carbonylation
- Nitration
- Alkylation
- Sulfhydration
- Trx/TrxR, Grx

Redox-activated signaling
- Nrf2, Foxo3a

NF-κB signaling

- Oxidative DNA-damage
  - ATM, CK2
- Stress-activated signaling
  - MAPKs, Akt
- UPS
  - Changes in the levels of NF-κB dimers

Energy failure
- AMPK, mTOR

ROS

ROS

ROS
Impairment of NF-κB translocation/signaling

- **MG132** (µM) - 0, 0.05, 0.1, 0.2, 0.4
- **NF-κB**
- **p-NF-κB**
- **p-p38**
- **p38**

<table>
<thead>
<tr>
<th>Cell number</th>
<th>Control</th>
<th>NF-κB GFP reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP-fluorescence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **MnCl₂ [mM]** - 0, 0.25
- **GFP (NF-κB expression)**

- **Paraquat [µM]** - 0, 50
- **GFP (NF-κB expression)**

- **MG132 [µM]** - 0, 0.1, 0.2, 0.4
- **GFP (NF-κB expression)**

- **Control**
- **NF-κB GFP reporter**
- **α-Syn**
- **A53T**

* Sign indicates statistical significance.
Summary

• Three main mechanisms linked to neurodegeneration.
  – Energy Failure
  – Mitochondrial dysfunction
  – Dysfunction in Protein Quality Control Mechanisms.

• Three major risk factors involved in neurodegeneration: Age, genes and environment.

• Alterations in redox homeostasis in neurodegeneration are not isolated events.

• Both oxidative damage and signaling are involved in neurodegeneration

• THINK OUTSIDE THE BOX!!!!
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