**TURTLE HYPOXIA**

- Metabolism reduced to 10% of normoxia
- Anoxic survival for months at 7°C
- Glycogen catabolized; up to 200 mM lactate accumulated
- Shell dissolves to buffer acid load and lactate stored in shell

Herbert & Jackson (1985) Physiol Zool 58:655

**METABOLISM IN ANOXIA**

- mRNA synthesis
- Protein synthesis
- Ion Pumping
- Fuel use
- O₂ consumed

ATP turnover to <5% of normal

**PRINCIPLES OF ANOXIA SURVIVAL**

1. Metabolic rate reduction
2. Control by protein kinases (SAPKs, 2nd messenger PKs)
3. Selective gene activation
**AONXIA INDUCED CHANGES**

- Protein Synthesis slows to 1%
- Pumps & Channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few ‘SAP’ kinases activated
- Gene ‘inactivation’ ( mRNA )
- Few Genes activated (1-2%)

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**Nucleus GENES ON/OFF**

- mRNAs
- [i + e Factors]

**PROTEINS**

- Ca\(^{+2}\)
- KINASES (2nd)
- SMW
- CHO
- AA
- ATP
- PROT
- FAT
- MITO
- ETC

**PROTEIN KINASES**

- PROTEIN \(\rightarrow\) PROTEIN\(-(P)n\)
- nATP \(\rightarrow\) nADP

- Covalent modification by phosphorylation
- Families of protein kinases: PKA (cAMP), PKG (cGMP), CaM (Ca\(^{+2}\)), PKC (Ca\(^{+2}\), PL,DG)
- SAPKs : daisy chain phosphorylations
- Regulation is via interconversion of active vs subactive forms of protein substrates

**Reversible phosphorylation control of enzymes**

- Incubation with ATP + protein kinase
- P & deP enzymes separate on ion exchange columns
PROTEIN PHOSPHORYLATION & GLYCOLYSIS
- Protein kinase A, PKA
- Protein kinase C (Brain)
- Protein phosphatase 1, 2A, 2C


METABOLIC RATE DEPRESSION
- Hibernation
- Anoxia
- Estivation
- Freezing
- Diapause

ANOXIA INDUCED CHANGES
- Protein Synthesis slows to 1%
- Pumps & channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few ‘SAP’ kinases activated
- Gene ‘inactivation’ ( mRNA )
- Few Genes activated (1-2%)

ROLE OF TRANSCRIPTION
- Global rate of mRNA synthesis depressed. Method: nuclear run-on
- Are selected genes up-regulated?
- TO ASSESS GENE UPREGULATION:
  - cDNA library
  - Gene Chip
cDNA Arrays
- Methods
- Materials
- Sources
- Publications

Gene Changes in Turtle Anoxia
- cDNA Library & Chip
  (~2% putative up-regulated)
  - Transcription Factors
  - Mitochondrial Genes
  - Protease inhibitors
  - Shock proteins (Hsps)
  - Antioxidant enzymes
  - Ferritin H & L

Antioxidant Defense
- Iron storage:
  - Ferritin (H & L chains)
  - Transferrin receptor 2
- Antioxidant enzymes
  - SOD (1)
  - GST (M5, A2)
  - GPX (1, 4)
  - Peroxiredoxin 1

The Good And The Bad Of Oxygen
The Good
1) Fuels normal aerobic metabolism
2) More than 200 enzymes use O2
3) Eliminates toxins (xenobiotics) via cytochrome P450
4) Produce O2 via photosynthesis

The Bad
1) Reactive oxygen species (ROS) damage macromolecules, deplete GSH, vitamins
2) ROS produced by normal aerobic metabolism & must be destroyed
3) Heavy metals catalyze formation of particularly dangerous ROS
4) Associated with disease & ageing

In: R.C. Reech et al., eds. Hypoxia and Exercise, Springer, NY
Reactive Oxygen Species: The Bad Guys

- O₂ → O₂⁻ → H₂O₂ → OH⁻

Superoxide - forms when O₂ acquires a single electron - relatively short-lived

Hydrogen Peroxide - formed from superoxide - not a radical, is long-lived - passes readily through membranes

Hydroxyl Radical - formed from H₂O₂ (with Fe²⁺ or Cu⁺) - HIGHLY REACTIVE - very short-lived

GENE CHANGES IN TURTLE ANOXIA

- cDNA Library & Chip (~2% putative up-regulated)
  - Transcription Factors
    - Mitochondrial Genes
    - Protease inhibitors
    - Shock proteins (Hsps)
    - Antioxidant enzymes
    - Ferritin H & L

CONTROL REGION OF A TYPICAL EUKARYOTIC GENE

We Study:
- Transcriptional regulation
  - Changes in mRNA levels
- Translational regulation
  - Changes in protein levels
- Post-translational regulation
  - Changes in post-translational modifications
  - Changes in subcellular distribution
**Transcription Factors**
- Master regulators of gene expression
- Respond to intra- or extracellular signals
- Bind to promoter regions of specific genes
- Mediate DNA transcription to mRNA

**Nuclear Factor kappa B (NF-κB)**
- Dimeric transcription factor, composed of subunits including p65, p50, p52, c-Rel and Rel B
- Activated by: Stress, Cytokines, Free radicals, UV
- Functions: Immune response, Development, Cell growth, Apoptosis, Stress response

**NF-κB**
- CONTROL: NF-κB dimer is sub-active in cytoplasm, bound to IκB
- STRESS: IκB phosphorylated & degraded
- Free NF-κB dimer: moves to nucleus, binds to DNA, transcription of downstream genes

**IκB Phosphorylation**
- IκB is (P) in liver & brain at 5h of anoxia
- Elevated (P) of IκB frees NF-κB dimer to move into the nucleus

**P-IκB protein levels in turtle tissues**
- Time course for IκB phosphorylation in liver
NF-κB p65 and p50 are upregulated during 5 h of anoxia.

NF-κB pathway is activated in turtle liver and brain in anoxia.

Ferritin heavy chain:
- Sequesters iron
- Can hold up to 4,500 atoms of iron
- 24 subunits: light (19 kDa) and heavy (21 kDa)
- Limits iron-catalyzed ROS production via the Fenton reaction
**Ferritin and Heme Oxygenase -1**

Help minimize free iron levels in cells

- **Ferritin:** Binds iron; Heavy & Light chains

- **Heme oxygenase -1:**
  - Degrades heme, a source of redox active iron
  - Free iron then stored into ferritin

Iron can be a source of oxidative stress:

- Catalyzes production of Hydroxyl radicals via Fenton reaction:

\[
\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{HO}^{-} + \text{HO}^{-} + \text{Fe}^{3+}
\]

Hydroxyl radical is very reactive and responsible for most oxidative stress-mediated damage.

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**Hypoxia / Ischemia**

- **Sensitive Animals** (most mammals)
  - Energy deficit (high ATP demand)
  - Disruption of ions and depolarization
  - Release of excitotoxic GLU,
  - Excess intracellular Ca^{2+}
  - Oxidative Stress (+ reperfusion)
  - Cell Death

- **Tolerant Animals** (e.g. turtles, carp)
  - Decrease ATP demand (Metabolic Arrest)
  - Adenosine as a Retaliatory Molecule
### ANOXIA SURVIVAL IN TURTLE BRAIN

**Table of Metabolic-Degradation Processes:**
- Hypoglycemia (Glucose-Insulin signal)
- ATP turnover rates (Mitochondrial activity)
- Glutamate depolarization
- Glycolysis
- Protein synthesis
- Metabolic-Degradation
- ATP turnover rates (Mitochondrial function)
- Glutamate depolarization
- Glycolysis
- Protein synthesis

**Metabolic-Degradation Processes:**
- Hypoglycemia (Glucose-Insulin signal)
- ATP turnover rates (Mitochondrial activity)
- Glutamate depolarization
- Glycolysis
- Protein synthesis

**Anoxia Survival in Turtle Brain**

BRAIN GENES
Up-regulated in turtle anoxia (DNA array)

- GABA transporter
- GABA receptor

Adult T. s. elegans

BRAIN GENES
Up-regulated in turtle anoxia (DNA array)

- Adenosine receptor
- 5’Nucleotidase

Adult T. s. elegans

BRAIN GENES
Up-regulated in turtle anoxia (DNA array)

- GABA transporter
- GABA receptor
- Adenosine receptor
- Serotonin receptor

BRAIN GENES
Up-regulated in turtle anoxia (DNA array)

- GABA transporter
- GABA receptor
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- Serotonin receptor

**cDNA ARRAY SCREENING**

A. RNA Isolation
B. cDNA Generation
C. Labeling of Probe
D. Hybridization to Array

**ANTIOXIDANT ENZYMES**

- Free radical generation
- Superoxide dismutase
- Catalase
- Glutathione peroxidase
- Selenium-dependent glutathione peroxidase
- Glutathione reductase
- GS-electrophile
- NADPH
- NADP+ oxidized
- Peroxidation

**Major effects of anoxia on cellular energetic turnover**

**TRANSPORTERS / RECEPTORS**