LIVING WITHOUT OXYGEN

www.carleton.ca/~kbstorey
LITTORINA LITTOREA

- Marine gastropod (periwinkle)
- Found on Atlantic coast of Europe & N. America
- One of just a few intertidal species in the north
- Intertidal zone highly variable environment

Intertidal zone
TIDE POOLS

Anoxia ↔ Hyperoxia
High Temp ↔ Sub-Freeze
Acidification
Pollution, UV
Hypo ↔ Hyper Tonicity
Periodic Stress

METABOLIC DEPRESSION
METABOLISM IN ANOXIA

- mRNA synthesis
- Translation
- Fuel use (incl. CHO)
- BioSyn. / Degrad.
- Cell cycle

ATP turnover to <5% of normal
mRNA synthesis

**L. littorea**
In vitro Protein Synthesis

Anoxic Time Course

Relative amount of $^3$H Incorporation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2500</td>
</tr>
<tr>
<td>0.5 h</td>
<td>2000</td>
</tr>
<tr>
<td>1 h</td>
<td>1500</td>
</tr>
<tr>
<td>48 h</td>
<td>1000</td>
</tr>
<tr>
<td>R1 h</td>
<td>750</td>
</tr>
<tr>
<td>R5 h</td>
<td>500</td>
</tr>
<tr>
<td>R12 h</td>
<td>250</td>
</tr>
</tbody>
</table>
Polysome Analysis

30% Linear Sucrose Gradient

A254

Normoxia

Anoxia

Recovery
Anoxia Recovery

Incorporation of $^3$H (cpm x 10$^3$/mg/h)

<table>
<thead>
<tr>
<th>Time</th>
<th>Protein Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>eIF-2α</td>
</tr>
<tr>
<td>0.5 h</td>
<td>eIF-2α</td>
</tr>
<tr>
<td>1 h</td>
<td>eIF-2α-P</td>
</tr>
<tr>
<td>12 h</td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td></td>
</tr>
<tr>
<td>R1 h</td>
<td></td>
</tr>
<tr>
<td>R12 h</td>
<td></td>
</tr>
</tbody>
</table>

Protein Synthesis

Con 24h 1R
1. Metabolic rate reduction

2. Control by protein kinases (SAPKs, 2\textsuperscript{nd} messenger PKs)

3. Most GENES OFF!

4. Selective gene activation
PRINCIPLES OF METABOLIC ARREST

1. Metabolic rate reduction

2. Control by protein kinases (SAPKs, 2nd messenger PKs)

3. Most Genes OFF

4. Selective gene activation
Nucleus
GENES
ON/OFF
[Trans.F]

mRNAs

[i + e Factors]

PROTEINS

PATHWAYS

CHO

ATP

AA

PROT

SAPK

P

KINASES (2nd)

KINASES

SMW

ATP

ADP

FAT

GENES

MITO

ETC

Ca^{2+}

Na

K
Metabolic Rate Depression
CHANGES

- Few ‘SAP’ kinases activated
- * Thousands of processes OFF
- Gene ‘inactivation’ (RNA)
- Few Genes activated (1-2%)
PROTEIN KINASES

- 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 via interconversion of active vs subactive forms of protein substrates
- p38, ERK (1/2), JNK, AMPK, AKT (mTOR)
Pathway Control in MR Depression

Phospho / de-Phospho

- Glycolysis (GP, GS, PFK, PK)
- Fat synthesis (ATP-CL, ACC)
- CHO fuel use (PDH)
- Translation (eIF2α, eEF2)
- Ion pumps (NaK-ATPase, Ca-ATPase)

PATHWAY CONTROL IN MR DEPRESSION

Phospho / de-Phospho

NOVEL DISCOVERIES

• CHO: Hexokinase, G6PDH, Aldolase
• Key DH’s: GlutDH, IsocitDH, LDH(!)
• Energy: AMPD, Arginine Kinase
• Signaling: AMPK, GSK-3
• AOE: SOD, catalase, GST, GPox
NOVEL P/deP ENZYME CONTROL

GlutDH

ARG-K

G6PDH

LDH

Insights into the in vivo regulation of glutamate dehydrogenase from the foot muscle of an estivating land snail.

Regulation of tail muscle arginine kinase by reversible phosphorylation in an anoxia-tolerant crayfish.

Glucose-6-Phosphate Dehydrogenase Regulation in Anoxia Tolerance of the Freshwater Crayfish Orconectes virilis.

Regulation of liver lactate dehydrogenase by reversible phosphorylation in response to anoxia in a freshwater turtle.
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
Epigenetics:
- Stable changes in gene activity that do not involve changes in DNA sequence

Common mechanisms:
- DNA methylation
- Histone modification / histone variants e.g. acetylation, phosphorylation
- Regulatory non-coding RNAs
Evidence for a reduced transcriptional state during hibernation in ground squirrels

Pier Jr Morin*, Kenneth B. Storey

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Received 14 March 2006; accepted 4 August 2006
Available online 18 September 2006

Abstract

During mammalian hibernation, metabolic rate can be reduced to <5% of the euthermic rate as a result of suppression of multiple energy expensive metabolic processes. Gene transcription is one of these processes. This study examines mechanisms of transcriptional control that could contribute to lowering the rate of gene expression. Histone deacetylases (HDAC) have been linked to gene silencing and measured HDAC activity was detected in skeletal muscle of hibernating thirteen-lined ground squirrels, Spermophilus tridecemlineatus, but not in controls. Western blotting also showed that HDAC1 and HDAC4 protein levels were 1.21 and 1.34 times higher in muscle from torpid animals. Histone H3 was also evaluated by Western blotting. Total histone H3 and two forms of covalently modified histone H3 that are associated with active transcription (phospho-acetylated Lys 23) were significantly reduced by 38-39% in muscle during hibernation. Finally, RNA was measured using a PCR-based approach; activity in muscle from hibernating squirrels was only detected in one of the muscle samples, indicating that transcriptional activity was significantly reduced. These data support an overall decrease in transcriptional activity in skeletal muscle of hibernating animals accomplished by multiple molecular mechanisms.

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Transcription Suppression
MRD

- Phospho-Histone H3 (Ser10) levels reduced
  * Inhibits transcription
- Histone Deacetylase activity increased 80%
- Acetyl-Histone H3 (Lys23) levels reduced
  * Both inhibit transcription *
- HDAC 1 & 4 protein levels increased
- RNA Polymerase II activity decreased
MICRO RNA

- Size ~22 nucleotides
- Highly conserved across species
- Bind to 3’ UTR of mRNAs
  - Repression mechanism(s) seem to include:
    - Block translation of mRNA
    - Help bind mRNA into stress granules
    - Target mRNA for degradation
Micro RNAs in *Littorina littorea*

**FOOT MUSCLE:**
*Up-regulated by Freezing & Anoxia*

miR-1a-1* & miR-133a*
- myocyte proliferation & differentiation
- regulate *Mef2a* and *Gata4*, Tfs that promote muscle maintenance

miR-2a*
- anti-apoptotic action by targeting the pro-apoptotic protein, *Reaper*

---

Micro RNAs in *Littorina littorea*

**HEPATOPANCREAS:**
Up-regulated by Freezing & Anoxia

Major changes:
- miR-1a-1 up in freeze & anoxia (like in foot)
- miR-210 up in anoxia
- miR-29b up in freeze
MICRO RNA: Drosha & Dicer

imperfect complimentarity = translational repression

Ago-1

Dicer

Ago-2 (Slicer)

perfect complimentarity = RNA interference

DICER ENZYME IN L. littorea TISSUES

Dicer protein increased in both freezing & anoxia (immunoblots)

Elevated miRNA processing
ANOXIA INDUCED CHANGES

- Few ‘SAP’ kinases activated
- Gene ‘inactivation’ (mRNA)
- Few Genes activated
GENE CHIPS

Confirms by RT-PCR, Northern blots

Data Leads

Downstream genes

Tf

TRANSCRIPTION FACTOR PROFILING

ELISAs in plates

Confirm by EMSA

ROLE & CONTROL OF SYSTEM

Transgenics

Cell Assay

RNAi

Knock out

Epigenetics

FUNCTIONAL ASSAYS

Protein levels
- enzyme assay
- antibodies: protein
- functional analysis
  e.g. HIF → EPO →
Beyond gene chips: transcription factor profiling in freeze tolerance

Kenneth B. Storey
Institute of Biochemistry, Carleton University, Ottawa, Canada K1S 5B6; kenneth_storey@carleton.ca

Abstract
The Wood Frog, Rana sylvatica, is one of several terrestrially hibernating anurans that display natural freeze tolerance. The multifaceted biochemical responses to the cellular stresses imposed when ~65% of total body water is converted to extracellular ice have
GENE CHANGES IN Anoxic *L. littorea*

- Antioxidant Enzymes
- Shock proteins (GRP, HSP)
- Low oxygen Shock (HIF)
- Metallothionein & Ferritin
- Unknown: SARP, KVN, LRD
- Transcription factors

*Trans.factors ~1-2%*  

Oxidative stress: animal adaptations in nature

Abstract

As a consequence of aerobic life, all organisms must deal with the continuous generation of reactive oxygen species (O₂, H₂O₂, -OH) as byproducts of metabolism and defend itself against the harm that these can do to cellular macromolecules. Organisms protect themselves from such damage with both enzymatic and non-enzymatic antioxidant defenses. However, the reperfusion injuries noted after ischemic insult in mammalian organs and also to a host of reactive oxygen species produced when oxygenated blood is reintroduced demonstrate that the underlying defenses are not always adequate.

Key words:
- Reactive oxygen species
- Free radical damage
- Lipid peroxidation
- Antioxidant activity
- Peroxides tolerance
- Exudation
- Inflammation

Central theme in oxygen metabolism

Linked with cancer, aging, diabetes, hypoxia, etc.

Antioxidant enzymes as indicators of oxidative stress
REACTIVE OXIDATIVE SPECIES

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^- \] superoxide radical

\[ \text{O}_2^- + \text{H}_2\text{O} \rightarrow \text{HOO}' + \text{OH}^- \] hydroperoxyl radical

\[ \text{HOO}' + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2 \] hydrogen peroxide

\[ \text{H}_2\text{O}_2 + e^- \rightarrow \text{OH} + \text{OH}^- \] hydroxyl radical

\[ \text{Fe (III)} + \text{O}_2^- \rightarrow \text{Fe (II)} + \text{O}_2 \]

\[ \text{Fe (II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe (III)} + \text{OH} + \text{OH}^- \] Fenton reaction

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH} + \text{OH}^- \] Haber-Weiss reaction
ANTIOXIDANT DEFENSE

Iron storage:
- Ferritin (H & L chains)
- Transferrin receptor 2

Antioxidant enzymes
- SOD (1)
- GST (M5, A2)
- GPX (1, 4)
- Peroxiredoxin 1

Multiple subfamilies incl: Alpha, Kappa, Mu, Pi, Sigma, Theta

Some forms controlled by the antioxidant response element (ARE) via the Nrf2 transcription factor
**L. littorea FOOT MUSCLE:**
20 h anoxia & 4 h recovery at 10°C:
GST responses

Sigma class GSTs are prominent in marine molluscs.
L. littorea HEPATOPANCREAS
20 h anoxia & 4 h recovery at 10°C: GST responses

GST Isoforms in Littorina littorea Hepatopancreas

Relative levels

A1/2  A3  M3  P1  T1

Control  Anoxic  Recovery

a a a a
WHERE DO WE GO FROM HERE?

- Applications of MRD research
- Novel phosphorylations
- Atrophy, hypertrophy -- autophagy for survival
- Turning it all off -- microRNA
- Epigenetics & adaptation
- Life span extension
- Antioxidant Defense
- Cell cycle suppression
- Unity through evolution

NEW DIRECTIONS
ANOXIA SURVIVAL

<table>
<thead>
<tr>
<th>Constitutive factors</th>
<th>Metabolic downregulation</th>
<th>Basal maintenance</th>
<th>Recovery</th>
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</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>Hypoxia/energy failure sensing</td>
<td>ATP turnover rates minimal</td>
<td>Oxygen sensing</td>
</tr>
<tr>
<td>Opioid receptors</td>
<td>HIF-1α ↑, glycolysis ↑</td>
<td>Preserve network integrity</td>
<td>Ion channels ↑</td>
</tr>
<tr>
<td>GABA receptors</td>
<td>ATP ↓↑, adenosine ↑</td>
<td>Ion channel ↔</td>
<td>Electrical activity ↑</td>
</tr>
<tr>
<td>Hsp72, Hsc73</td>
<td>Ion channels ↓, electrical activity ↓</td>
<td>Electrical activity ↔</td>
<td>Protein synthesis ↑</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Glutamate, dopamine release ↓</td>
<td>Glutamate release ↔</td>
<td>ROS defenses ↑</td>
</tr>
<tr>
<td>PACAP 38</td>
<td>Protein synthesis ↓</td>
<td>Dopamine release ↔</td>
<td></td>
</tr>
<tr>
<td>Ascorbate</td>
<td>Hsp72 ↑, Hsc73 ↑</td>
<td>GABA release ↑</td>
<td></td>
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<tr>
<td>SOD</td>
<td>Suppression of ATP turnover rates</td>
<td>GABA receptors ↑</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Protein synthesis ↔</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hsp72 ↓, Hsc73 ↑</td>
<td></td>
</tr>
</tbody>
</table>

NEW DIRECTIONS - TBA

Big Science Edition:

1. GENOMES: Sequence all the genes to feel better!
   The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage.
   Shaffer HB,...et al Storey KB, Genome Biol. 2013 14(3): R28

2. Protein 2D: What about the Proteins
   OMICS – Proteomics
Novel phosphorylations

Regulation of skeletal muscle creatine kinase from a hibernating mammal

Khalil Abnous, Kenneth B. Storey *  
Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6  
Received 25 May 2007, and in revised form 9 July 2007  
Available online 22 August 2007

Abstract

Control over skeletal muscle energetics is critical in hibernation to sustain metabolic pathways and maintain thermogenesis during arousal. Creatine kinase (CK) has a key role in muscle function. In hibernating mammals, CK activity was ~20% lower during hibernation than in summer activity. Hibernation CK showed reduced affinity for ATP, which promoted endogenous protein kinase or phosphatase action, coupled with the release of ADP. Phosphorylation forms, showed that the CK in hibernating squirrels, Spermophilus richardsonii, was ~75% lower in hibernation than in summer activity. High and low phospho-CK was detected in hibernating animals. High and low phospho-CK showed different kinetic responses to kinase and phosphatase action.

Regulation of liver glutamate dehydrogenase by reversible phosphorylation in a hibernating mammal

Ryan A.V. Bell, Kenneth B. Storey *  
Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

ARTICLE INFO  
ABSTRACT

Glutamate dehydrogenase (GDH) is a key enzyme that links amino acid and carbohydrate metabolism in cells. Most important when organisms are confronted with extreme changes such as the presence and lack of food associated with winter. Many small mammals, such as voles, hibernate during the winter and in such conditions GDH is not active. Instead, the mammal cells actively synthesize glucose, which is then stored as glycogen. In cold, the animal will then use this energy in the spring. The enzyme GDH is a key enzyme in discussing the metabolic state. It can be activated by phosphorylation, which is a critical step in the metabolic cascade. In this study, we examined the activity of GDH in different hibernating animals and found that it was activated by phosphorylation. We also found that the enzyme was inhibited by ADP, which is consistent with the role of ADP in the metabolic cascade. Our results suggest that the molecular mechanism of phosphorylation and ADP inhibition is a key step in the metabolic cascade of small mammals.
Epigenetics in anoxia tolerance: a role for histone deacetylases.

Krivoruchko A, Storey KR

Institute of Biochemistry, Carleton University, Ottawa, ON, Canada. krivoruchko@gmail.com

Abstract

The importance of epigenetics has been established in many key biological processes; the epigenetic mechanism to animal survival of low oxygen conditions has never been examined. To determine if epigenetic mechanisms could be involved in natural anoxia tolerance, we have examined the anoxic response of transcripational silencers, histone deacetylases (HDACs), in tissues of a unique model system: the turtle Trachemys scripta elegans. Transcript and protein levels of all five HDACs rose 4-6 fold in skeletal muscle in response to 20 h of anoxia exposure. In addition, HDAC activity increased in response to 20 h of anoxia and levels of acetylated histone H3 (Lys 9 or Lys 23) decreased in liver displayed a milder response with HDAC1, -4, and -5 protein levels increasing by 1.5 fold. Acetylated histone H3 levels also decreased to 50-75% of control values. Only HDAC5 was unaffected in heart. Hdac5 transcript levels increased 2.1-2.3-fold and HDAC5 protein rose by 3.3-fold.
Unavoidable metabolic costs

Perspectives in Cell Cycle Regulation: Lessons from an Anoxic Vertebrate

Kyle K. Biggar and Kenneth B. Storey*

Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, K1S 5B6, Canada

Abstract: The ability of an animal, normally dependent on aerobic respiration, to suspend breathing and enter an anoxic state for long term survival is clearly a fascinating feat, and has been the focus of much recent research. Anoxia tolerant turtles are faced with periods of oxygen deprivation, numerous modifications as well as the implementation of translation and transcriptional control mechanisms. Though it is clear that anoxic survival relies on the suppression of ATP consuming processes, the mechanisms that allow anoxia tolerant vertebrates remain elusive. Several anoxia tolerant invertebrates and vertebrates have been shown to have lower rates of cell cycle arrest when presented with anoxic stress. Despite this, the cell cycle arrest can be beneficial to the organism. Understanding how vertebrates respond to anoxia can have important implications for the future of cellular proliferation and hypoxic tumor progression. This review of cell cycle control mechanisms in anoxic vertebrates and non-vertebrates should be useful for most investigators interested in cell cycle control, the activation of checkpoint kinases, and the development of new therapeutic strategies.
An Overview of Stress Response and Hypometabolic Strategies in *Caenorhabditis elegans*: Conserved and Contrasting Signals with the Mammalian System

Benjamin Lant and Kenneth B. Storey
Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada

Abstract

Stress response and hypometabolic strategies are conserved across species, and the nematode *C. elegans* is an excellent model organism for studying these processes. The dauer stage is a morphological and physiological state in which the nematode loses its motility and cell division activity. This period of development is triggered by environmental cues such as low oxygen and food availability. The suppression of cellular metabolism during the dauer stage is crucial for survival and development. The nematode employs a number of signaling pathways to control these processes, which are likely conserved in the mammalian system. This review discusses the conserved and contrasting signals in the nematode and mammalian systems, highlighting the differences and similarities in stress response and hypometabolism.
**Life span extension**


**Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.**

Krivoruchko A, Storey KB

Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, ON, CA.

**Abstract**

While mammals cannot survive oxygen deprivation for more than a few minutes without sustaining damage, some animals have mastered anaerobic life. Freshwater turtles belonging to the Trachemys species are champion facultative anaerobes of the vertebrate world, often surviving without oxygen for more than an hour. Their physiological and biochemical mechanisms that underlie anoxia tolerance in turtles include depression of mitochondrial activity, depression, post-translational modification of proteins, strong antioxidant defenses, activation of transcription factors, and enhanced expression of cytoprotective proteins. Turtles are also known to live longer and display characteristics of "negligible senescence". We propose that the robust stress-tolerance and long term anaerobiosis by turtles may also support the longevity of these animals. Many of the characteristics of natural anoxia tolerance, such as hypometabolism, antioxidant defense and the presence of transcription factors to play important roles in mammalian oxygen-recovery and survival. Understanding the mechanisms of anoxia tolerance in turtles could also aid in the understanding and treatment of disease processes that are associated with oxygen deprivation conditions. In the present review we discuss the recent advances in the molecular and physiological research in turtles and the potential links between this tolerance and longevity. 

---

**Diagram:**

- **STIMULATORY SIGNALS**
  - IkB Kinase
  - IkB
  - p50
  - p65

- **CYTOPLASM**
  - IkB
  - p50
  - p65

- **NUCLEUS**
  - Transcription
  - Immune response
  - Stress response
  - Antioxidant defenses
  - Cell growth and differentiation
  - Anti-apoptosis

- **Degradation by proteasome**

---

**Image:**

- A young animal and a turtle.
Littorina littorea, periwinkle
Distribution of Littorina littorea
Molecular Adaptation to Climate Change: Challenges for Amphibians & Reptiles

Freezing survival

Estivation

Anoxia tolerance

Temperature Adaptation in a Changing Climate

EDITED BY KENNETH B. STOREY AND KAREN TANINO
Invertebrate Anaerobiosis

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Funded by NSERC Canada
1. Metabolic rate depression
2. Kinase / phosphatase action
3. Reversible P-enzymes
4. Global suppression of transcription & translation
5. Selected genes up-regulated
GENE CHIPS

TRANSCRIPTION FACTOR PROFILING

Data Leads

Confirm by RT-PCR, Northern blots

ELISAs in plates

Confirm by EMSA

Tf

Downstream genes

ROLE & CONTROL OF SYSTEM

Transgenics

Cell Assay

RNAi

Knock out

Epigenetics

FUNCTIONAL ASSAYS

Protein levels
- enzyme assay
- antibodies: protein
- functional analysis e.g. HIF \( \rightarrow \) EPO \( \rightarrow \)
FREEZE TOLERANT ANIMALS

- TERRESTRIAL INSECTS
- INTERTIDAL MOLLUSCS & BARNACLES
- AMPHIBIANS & REPTILES:
  - FROGS (6 species)
  - HATCHLING PAINTED TURTLES
  - GARTER SNAKES
  - LIZARDS (some)
Thanks to:

C-W. Wu
S. Tessier
J.M. Storey

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Distribution of *Littorina littorea*
1. Metabolic rate depression
2. Overall suppression of transcription & translation
3. Alternative end products
4. Reversible phosphorylation of enzymes
5. Selected genes up-regulated
$\text{O}_2 + e^- \rightarrow \text{O}_2^{\cdot -}$  superoxide radical

$\text{O}_2^{\cdot -} + \text{H}_2\text{O} \rightarrow \text{HO'O}^+ + \text{OH}^-$  hydroperoxyl radical

$\text{HO'O}^+ + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2$  hydrogen peroxide

$\text{H}_2\text{O}_2 + e^- \rightarrow \cdot\text{OH} + \text{OH}^-$  hydroxyl radical

$\text{Fe(III)} + \text{O}_2^{\cdot -} \rightarrow \text{Fe(II)} + \text{O}_2$

$\text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \cdot\text{OH} + \text{OH}^-$  Fenton reaction

$\text{O}_2^{\cdot -} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{OH}^-$  Haber-Weiss reaction
ANTIOXIDANT ENZYMES

\[
\begin{align*}
O_2^- + H^+ & \rightarrow H_2O_2 \\
H_2O_2 & \rightarrow O_2 + H_2O \\
O_2^- + H_2O_2 & \rightarrow O_2 + OH^- + OH^- \\
& \text{superoxide dismutase} \\
& \text{catalase} \\
& \text{Fenton reaction} \\
& \text{Haber-Weiss reaction} \\
& \text{Fe salt catalyst} \\
& \text{Fe}^{2+} \\
& \text{Fe}^{3+} + OH^- + OH^- \rightarrow \text{peroxidation} \\
& \text{electrophile} \\
& \text{glutathione S-transferase} \\
& \text{glutathione reductase} \\
& \text{NADP}^+ \rightarrow \text{NADPH} \\
& \text{GSSG} \rightarrow \text{GSH} \\
& \text{selenium dependent glutathione peroxidase} \\
& GS-electrophile \\
\end{align*}
\]
Normoxia 48 Anoxia
Reversible suppression of protein synthesis in concert with polysome disaggregation during anoxia exposure in *Littorina littorea*

Kevin Larade and Kenneth B. Storey

*Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada*

Received 11 October 2001; accepted 3 December 2001

**Abstract**

Many marine invertebrates can live without oxygen for long periods of time, a capacity that is facilitated by the ability to suppress metabolic rate in anoxia to a value that is typically less than 10% of the normal aerobic rate. The present study demonstrates that a reduction in the rate of protein synthesis is one factor in the overall anoxia-induced metabolic suppression in the marine snail, *Littorina littorea*. The rate of [H]leucine incorporation into newly translated protein in hepatopancreas isolated from 48 h anoxic snails was determined to be 49% relative to normoxic controls. However, protein concentration in hepatopancreas did not change during anoxia, suggesting a coordinated suppression of net protein turnover. Analysis of hepatopancreas samples from snails exposed to 24–72 h anoxia showed a gradual disaggregation of polysomes into monosomes. A re-aggregation of monosomes into polysomes was observed after 3 h of aerobic recovery. Analysis of fractions from the ribosome profile using radiolabeled probe to detect α-tubulin transcripts confirmed a general decrease in protein translation during anoxia exposure (transcript association with polysomes decreased) with a reversal during aerobic recovery. Western blotting of hepatopancreas samples from normoxic, 24 h anoxic, and 1 h aerobic recovered snails demonstrated that eIF-2α is substantially phosphorylated during anoxia exposure and dephosphorylated during normoxia and aerobic recovery, suggesting a decrease in translation initiation during anoxia exposure. These results suggest that metabolic suppression during anoxia exposure in *L. littorea* involves a decrease in protein translation. (Mol Cell Biochem 232: 121–127, 2002)
Anoxia Recovery

Incorporation of $^3$H (cpm x $10^3$/mg/h)

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>0.5 h</th>
<th>1 h</th>
<th>12 h</th>
<th>48 h</th>
<th>R1 h</th>
<th>R12 h</th>
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Protein Synthesis

Con 24h 1R

eIF-2α
eIF-2α-P
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
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
GENES

Transcription
Control by transcriptional regulation

RNAs
Translation
Control by translational regulation

PROTEINS (ENZYMES)
Control by proteases
Degradation
Control by post-translational modification

INACTIVE ENZYME

Control at level of enzyme function
Glutathione S-transferases are a family with at least 9 subfamilies: e.g. Alpha, Kappa, Mu, Pi, Sigma, Theta

Function in transformation / detoxification of many compounds, including carcinogens, drugs, xenobiotics and products of oxidative stress.

Catalyze the reaction of reduced glutathione (GSH) with an acceptor molecule to form an S-substituted glutathione.

Gene expression of some forms are under control by the antioxidant response element (ARE) regulated by the Nrf2 transcription factor