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

TIDE POOLS

Anoxia ←→ Hyperoxia

High Temp ←→ Sub-Freeze

Acidification

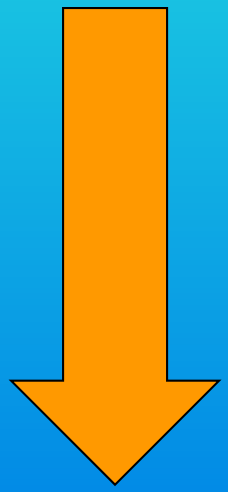
Pollution, UV

Hypo ←→ Hyper Tonicity

Periodic Stress

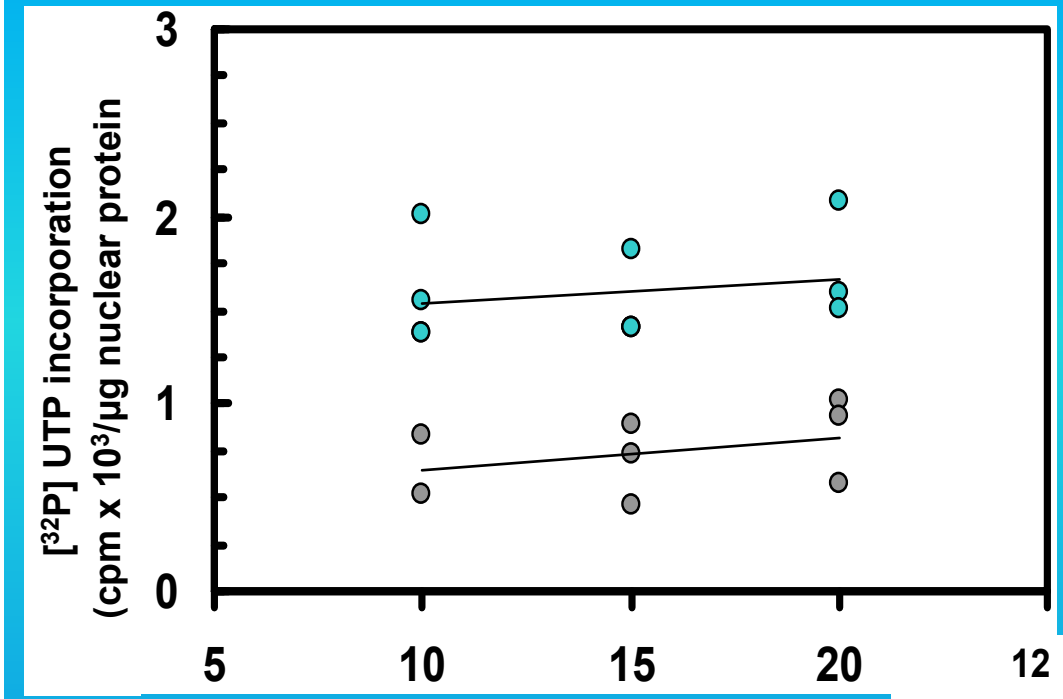
METABOLIC DEPRESSION

METABOLISM IN ANOXIA

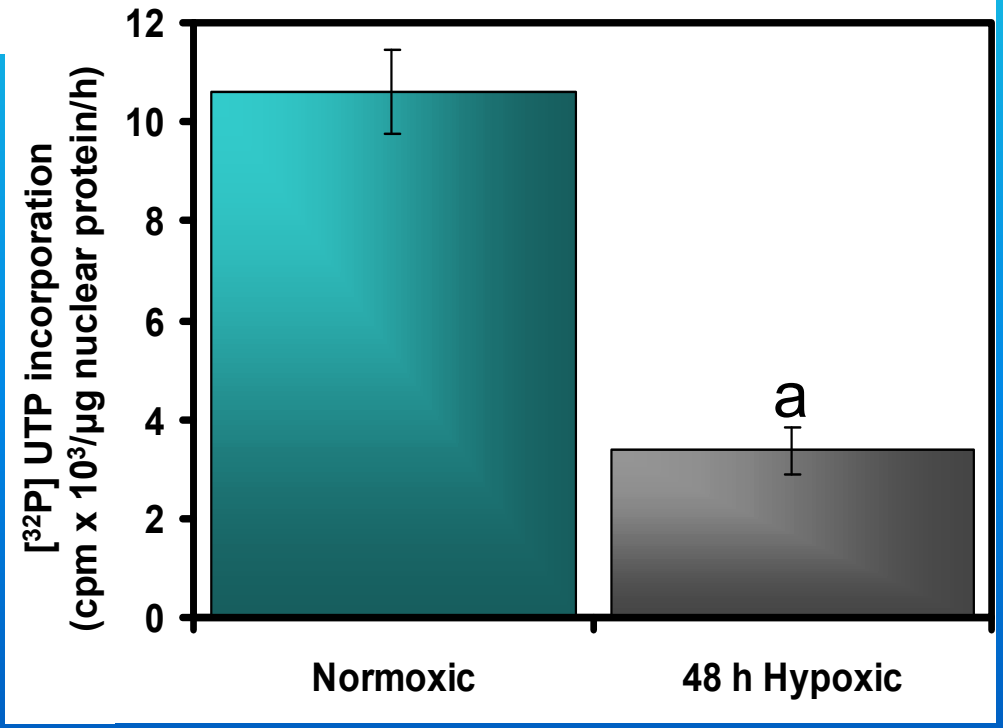


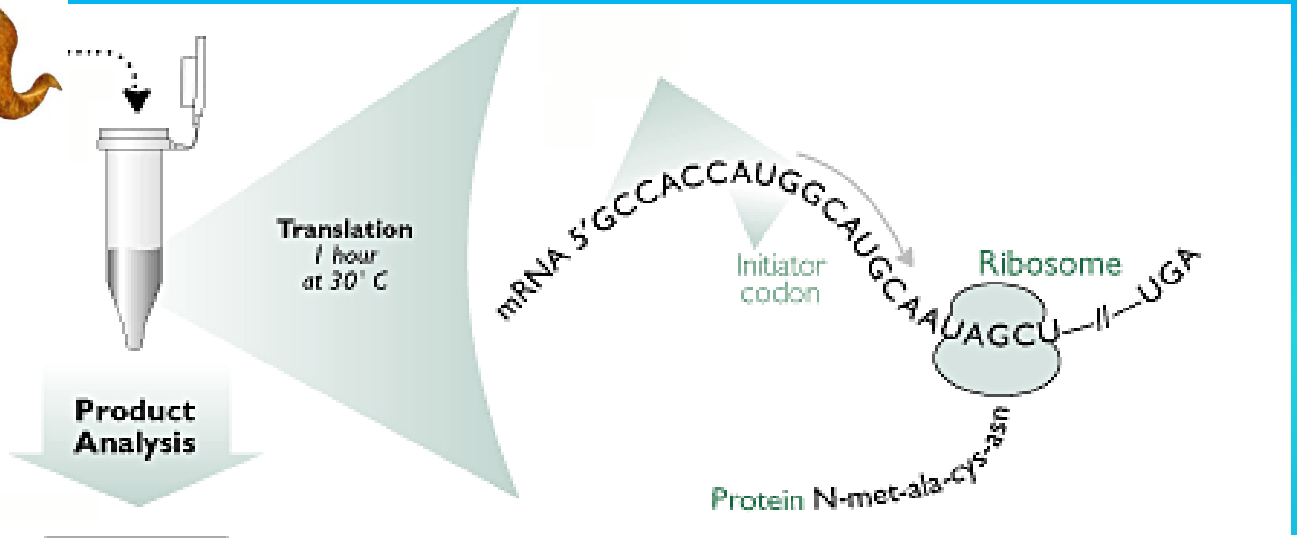
- mRNA synthesis
- Translation
- Fuel use (incl. CHO)
- BioSyn. / Degrad.
- Cell cycle

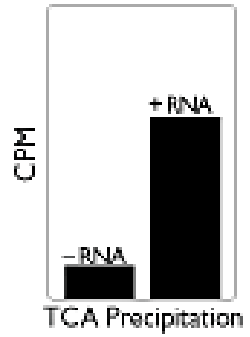
ATP turnover ↓ to <5% of normal



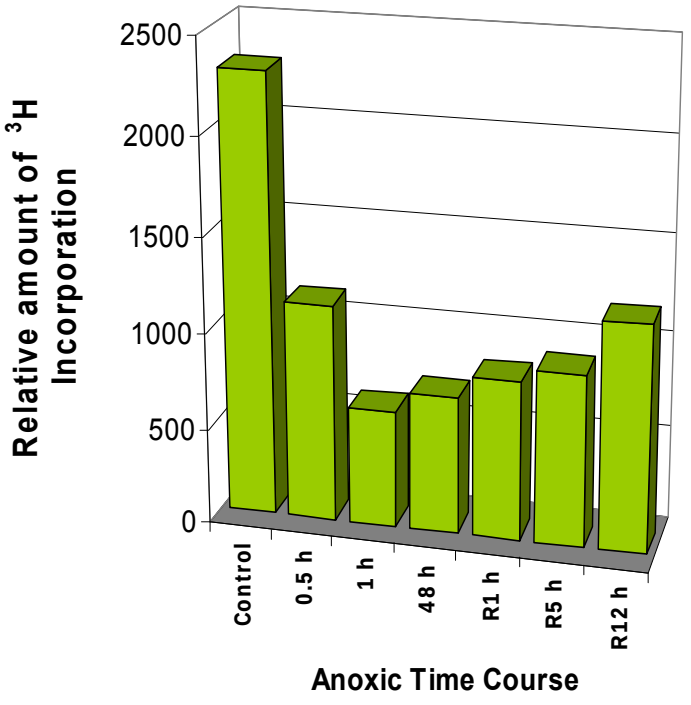
mRNA synthesis

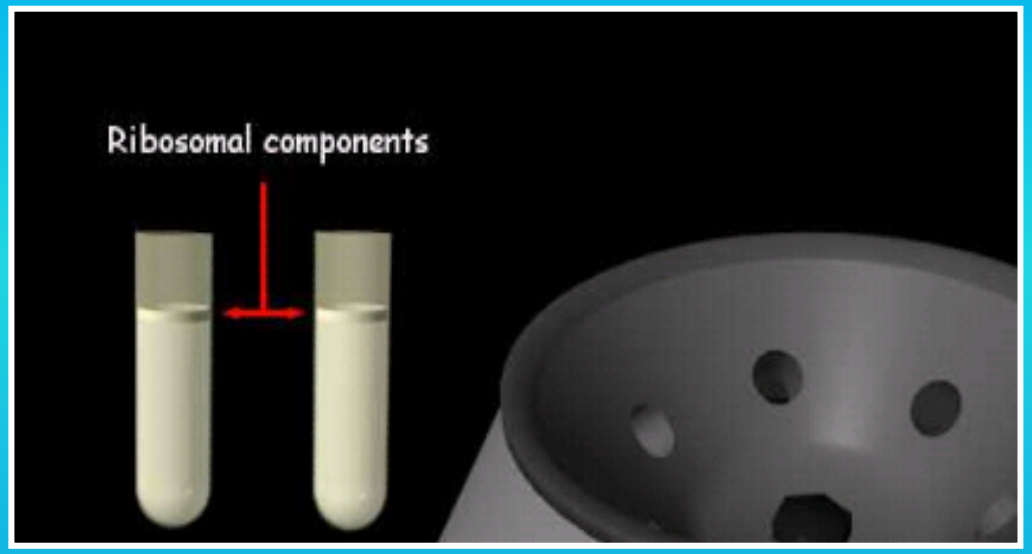


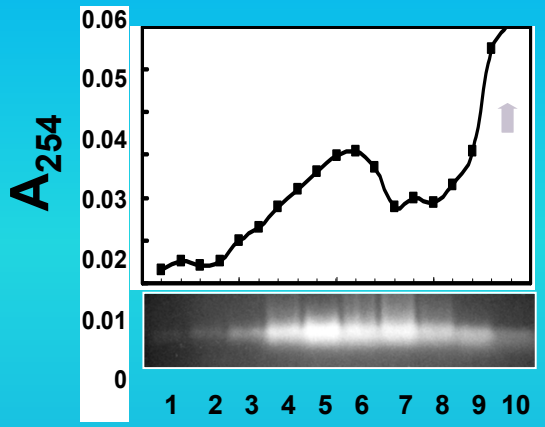


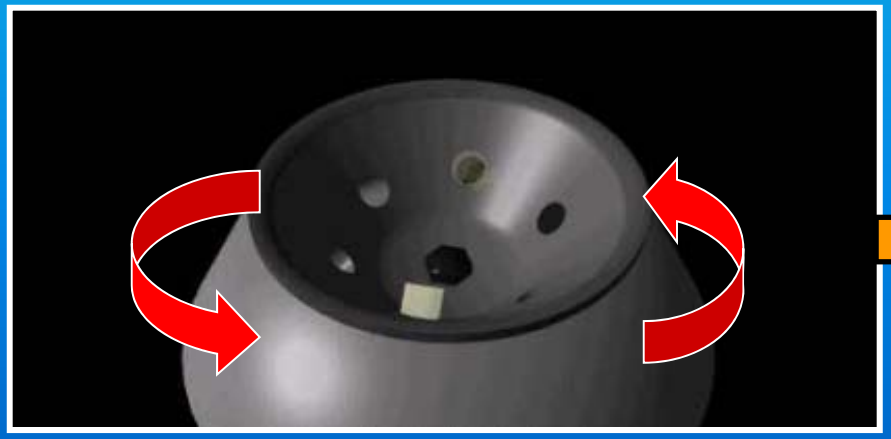


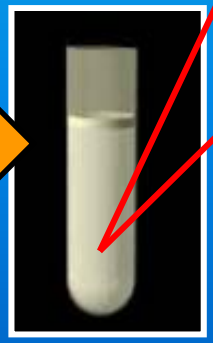
In vitro Protein Synthesis

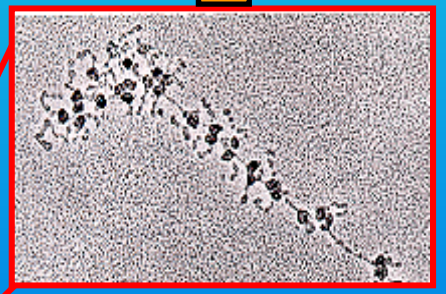




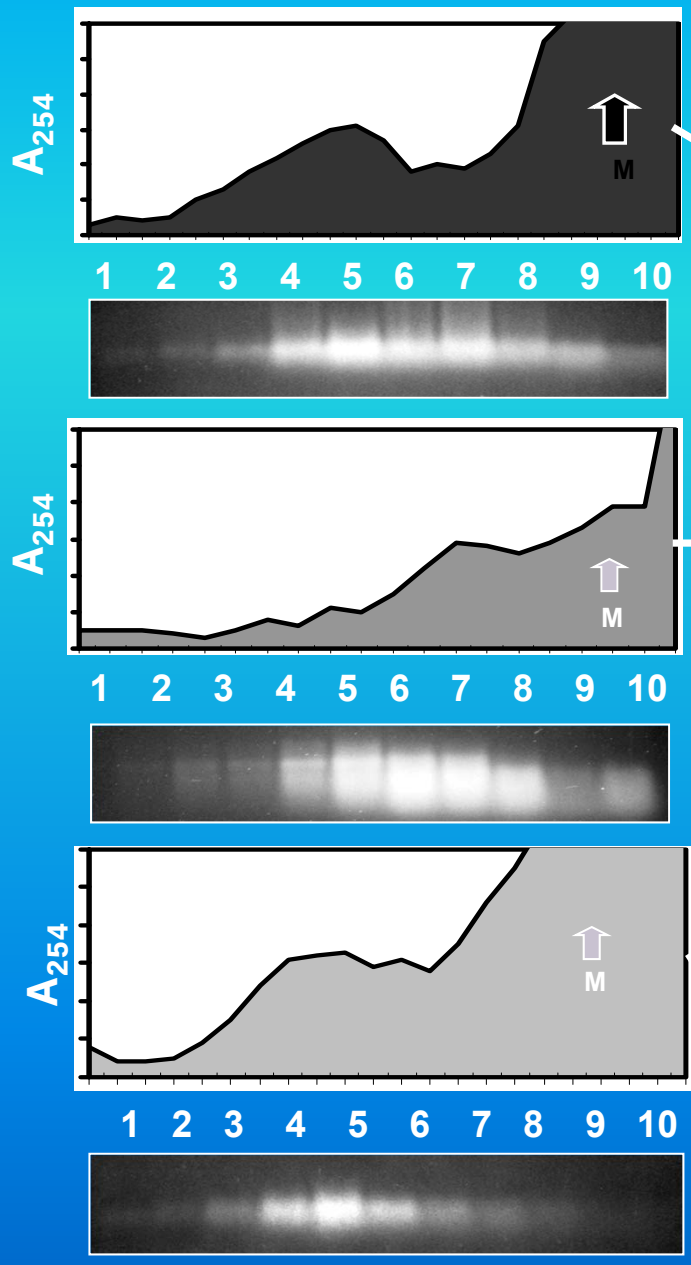


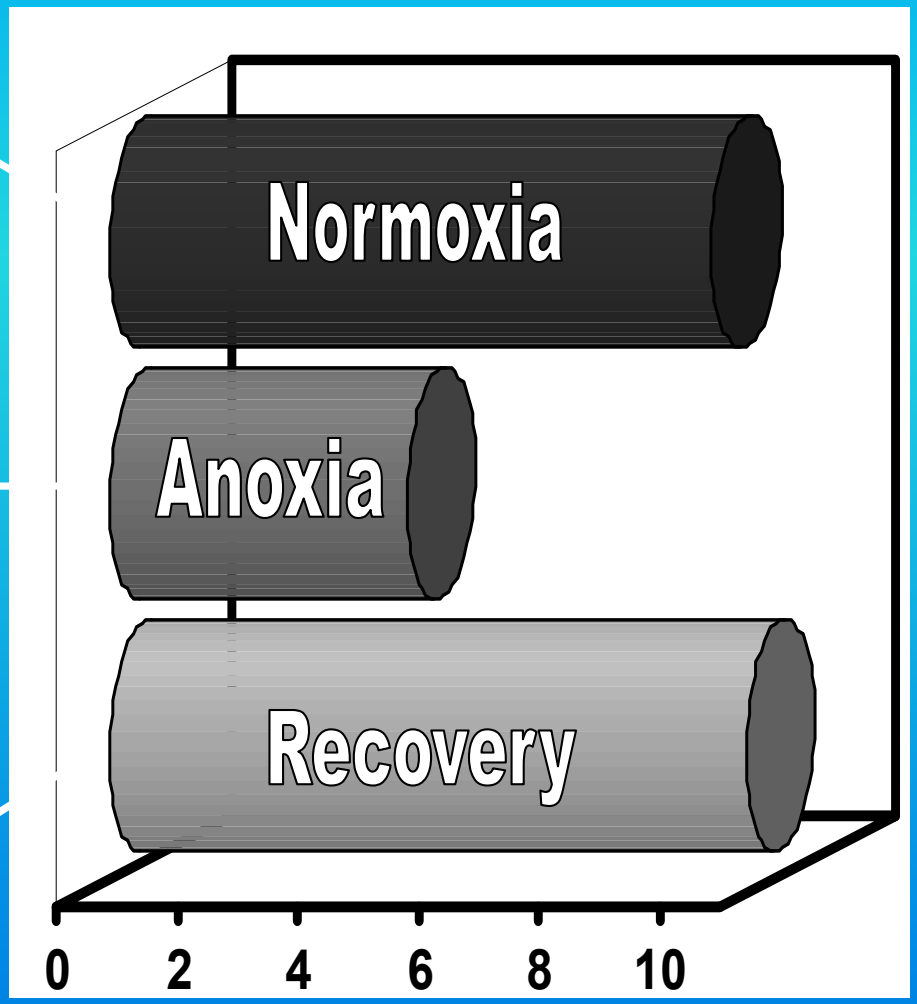




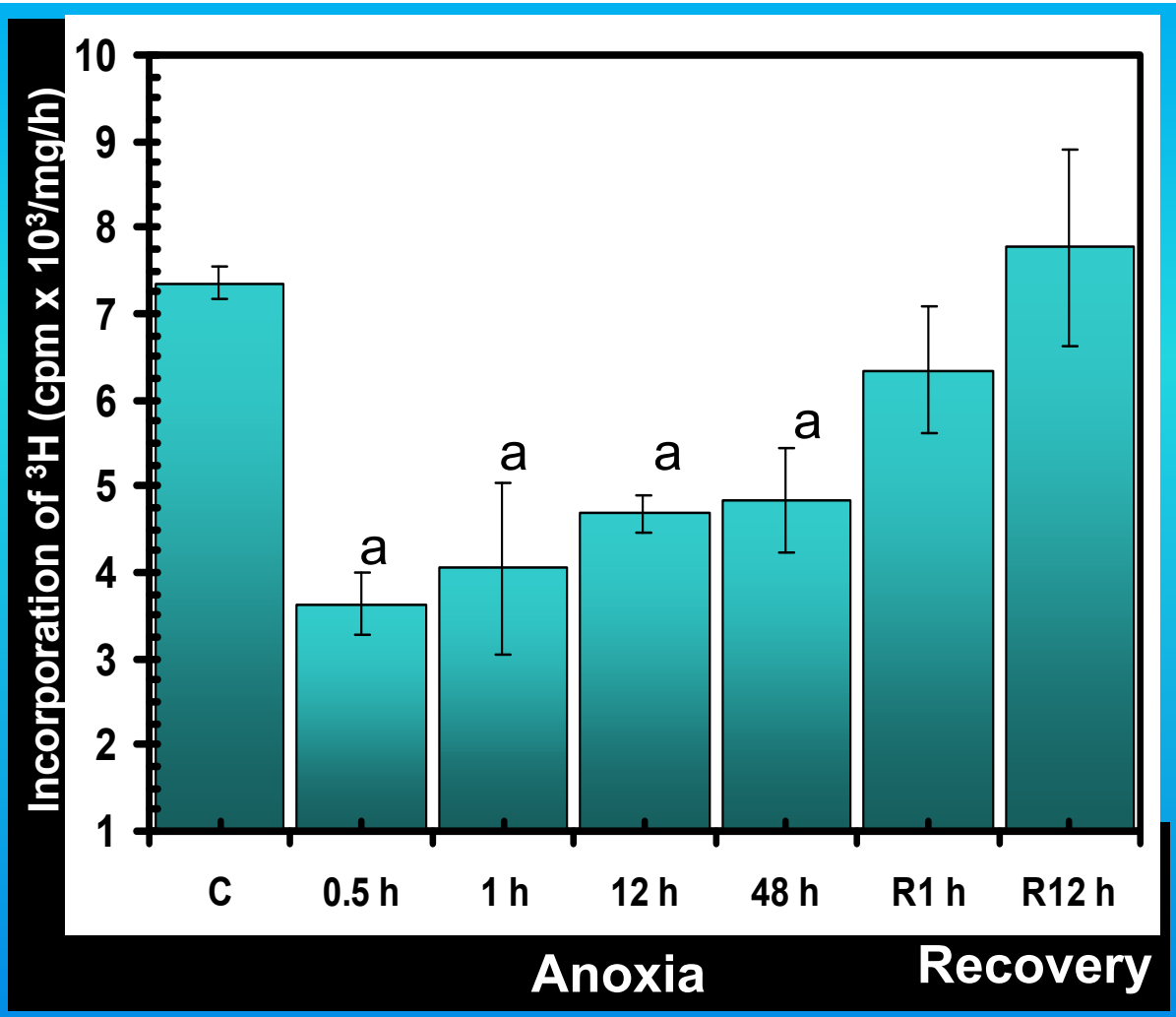


Polysome Analysis

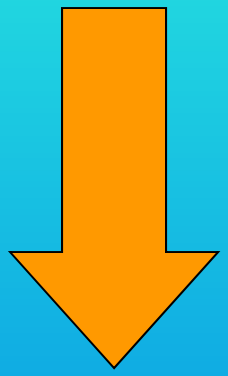


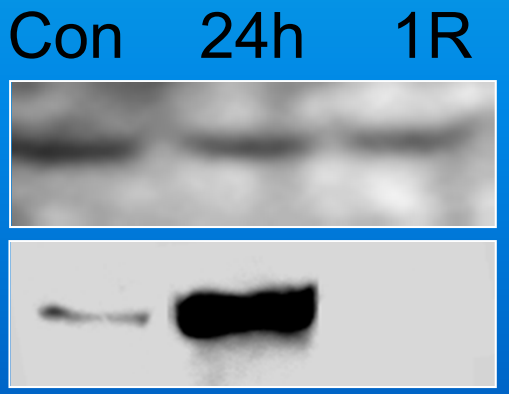


Polysome Analysis



Protein Synthesis



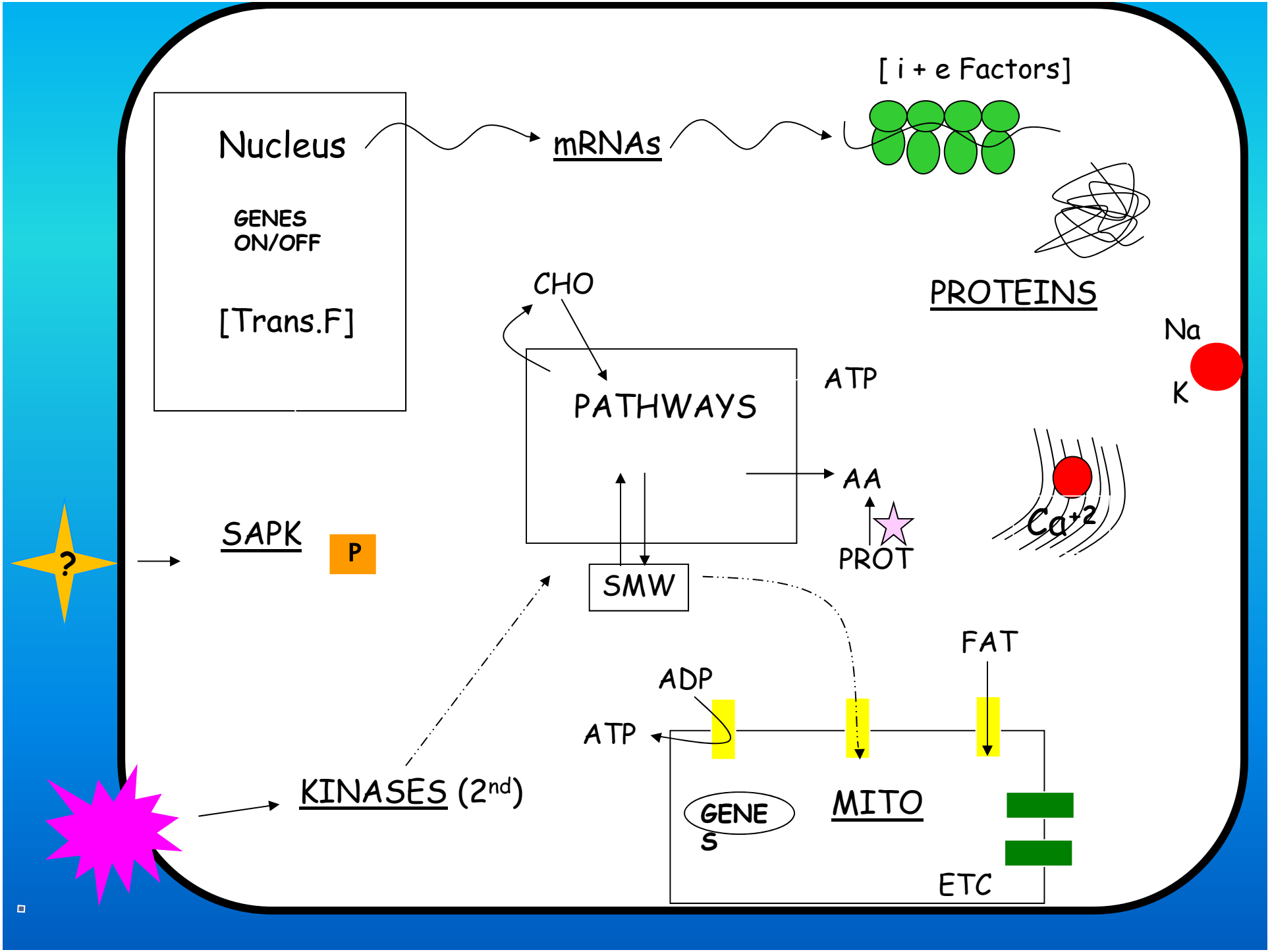


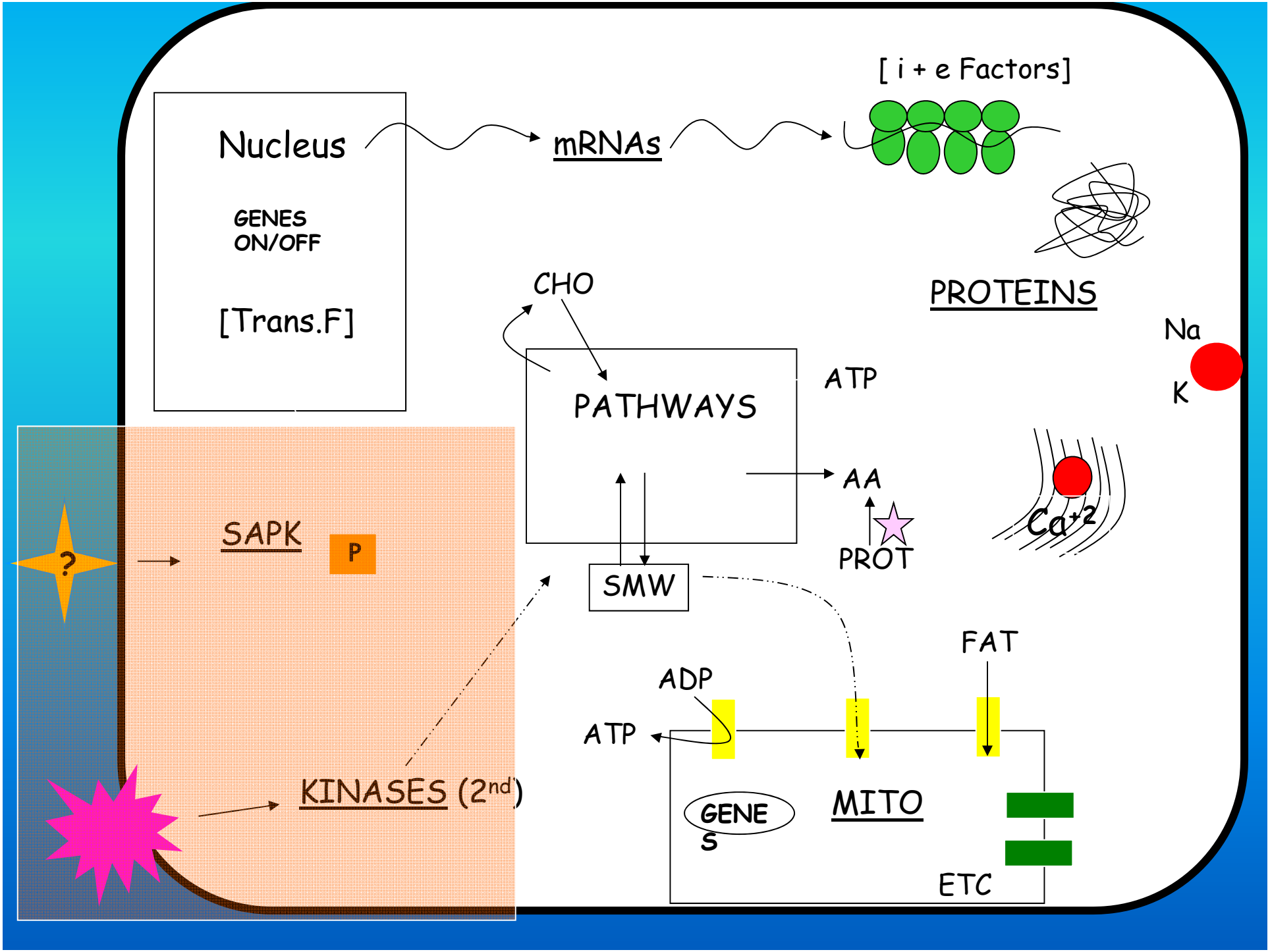
PRINCIPLES OF METABOLIC ARREST

1. Metabolic rate reduction
2. Control by protein kinases
   (SAPKs, 2nd messenger PKs)
3. Most GENES OFF !
4. Selective gene activation

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


[i + e Factors]
Nucleus
mRNAs
GENES
ON/OFF
[Trans.F]
PROTEINS
CHO
Na
K
ATP
PATHWAYS
AA
PROT
Ca+2
?
SAPK
P
SMW
FAT
ADP
ATP
KINASES (2nd)
GENE
S
MITO
ETC


[i + e Factors]
Nucleus
GENES
ON/OFF
[Trans.F]
mRNAs
PROTEINS
CHO
PATHWAYS
ATP
Na
K
AA
PROT
Ca+2
SMW
?
SAPK
P
KINASES (2nd)
FAT
ADP
ATP
GENE
S
MITO
ETC

GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

PROTEINS (ENZYMES)

No Modification

Control by proteases

Degradation

INACTIVE ENZYME

Covalent modification

Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation

Control at level of enzyme function

ACTIVE ENZYMES

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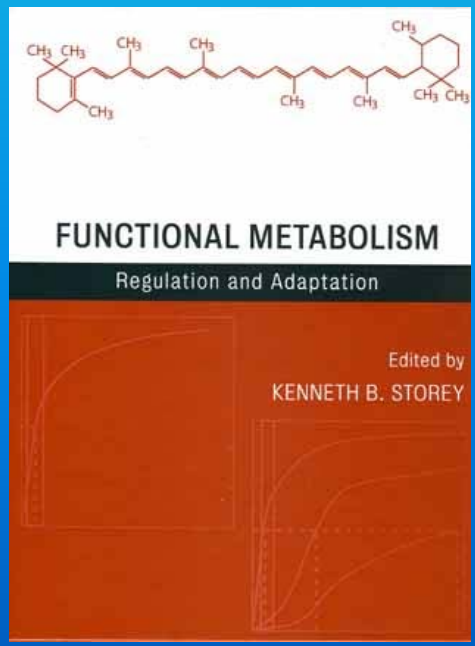
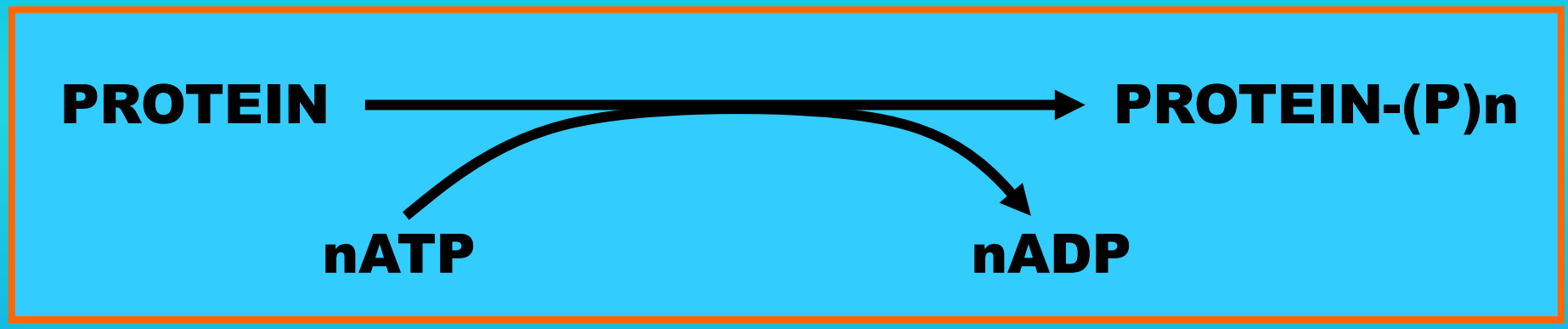


Metabolic Rate Depression CHANGES

- Few 'SAP' kinases activated
- * Thousands of processes OFF
- Gene 'inactivation' (⇩ RNA)
- Few Genes activated (1-2%)

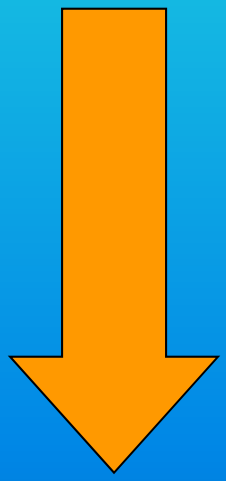
PROTEIN KINASES



- 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

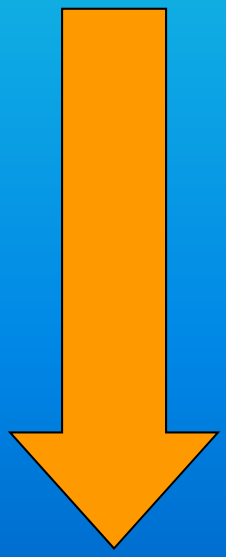


- **Glycolysis** **(GP, GS, PFK, PK)**
- **Fat synthesis** **(ATP-CL, ACC)**
- **CHO fuel use** **(PDH)**
- **Translation** **(eIF2α, eEF2)**
- **Ion pumps** **(NaK-ATPase, Ca-ATPase)**

Estivation: Storey & Storey 2012. J. Exp. Biol. 215:1425-33
Hibernation: Storey & Storey 2010. Adv Clin Chem 52:77-108
Hypometabolism: Storey & Storey 2007. J. Exp. Biol. 210, 1700-14

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

Enzyme Res. 2012;2012:317314. Epub 2012 Mar 26.

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

Bell RA, Dawson NJ, Storey KB.

Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6.

Abstract

Land snails, Otala lactea, survive in seasonally hot and dry environments by entering a state of aerobic torpor called estivation. During estivation, snails must prevent excessive dehydration and reorganize metabolic fuel use so as to endure prolonged periods without food. Glutamate dehydrogenase (GDH) was hypothesized to play a key role during estivation as it shuttles amino acid carbon skeletons into the Krebs cycle for energy production and is very
estivating conditions r
This kinetic difference
incubations that stimu
estivating land snail fo
during prolonged estiv

GlutDH

J Comp Physiol B. 2011 Oct;181(7):851-9. Epub 2011 Apr 26.

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

Dawson NJ, Storey KB.

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

Abstract

Freshwater crayfish, Orconectes virilis, can experience periodic exposures to hypoxia or anoxia due to low water flow (in summer) or ice cover (in winter) in their natural habitat. Hypoxia/anoxia disrupts energy metabolism and triggers mechanisms that to support ATP levels while often also suppressing ATP use. Arg
stores to buffer ATP levels
state and structural stabilit
significantly higher (by 59
was converted to a high p
exchange chromatography
the aerobic form, and (c) t
AK with protein kinase A c
be to suppress AK activity
catabolizing direction unde

ARG-K

Enzyme Res. 2011;2011:524906. Epub 2011 Oct 17.

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

Lant B, Storey KB.

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

Abstract

Glucose-6-phosphate dehydrogenase (G6PDH), the enzyme which catalyzes the rate determining step of the pentose phosphate pathway (PPP), controls the production of nucl
functions, including antioxidant
response to 20 h anoxic expos
0.008 mM to 0.012 ± 0.008 mM
mechanism involved is reversit
effects on anoxia on K(m) valu
whose proportions changed du
G6PDH kinetic properties. In a
Anoxia-induced phosphorylatio
NADPH supply for antioxidant

G6PDH

Comp Biochem Physiol B Biochem Mol Biol. 2012 Oct;163(2):221-8. Epub 2012 Jun 23.

Regulation of liver lactate dehydrogenase by reversible phosphorylation in response to anoxia in a freshwater turtle.

Xiong ZJ, Storey KB.

Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada.

Abstract

Lactate dehydrogenase (LDH) is the terminal enzyme of anaerobic glycolysis and key to hypoxia/anoxia survival by most animals. In this study, the effects of anoxic submergence (20 h at 7°C in nitrogen-bubbled water) were assessed on LDH from liver of an anoxia-tolerant freshwater turtle, the red-eared slider (Trachemys scripta elegans). Liver LDH from aerobic and anoxic turtles was purified to homogeneity in two steps. The kinetic properties and thermal stability of purified LDH were analyzed, revealing significant differences between the two enzyme forms in V(max), K(m) pyruvate, and I(50) pyruvate as well as melting temperature determined by differential scanning fluorimetry. The phosphorylation state of aerobic and anoxic forms of LDH was visualized by ProQ Diamond phosphoprotein staining, the results indicating that the anoxic form had a higher phosphorylation state. Incubation studies that promoted protein kinase versus protein phosphatase actions showed that changes in the phosphorylation state of aerobic and anoxic forms mimicked the anoxia-responsive changes in K(m) pyruvate and I(50) pyruvate. The high phosphate form of liver LDH that occurs in anoxic turtles appears to be a less active form. Turtle liver LDH was also subject to another form of posttranslational modification, protein acetylation, with a 70% higher content of acetylated lysine residues on anoxic versus aerobic LDH. This is the first study to show that LDH function in an anoxia-tolerant animal can be differentially modified between aerobic and anoxic states via the mechanism of posttranslational modification.

LDH

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

TURNING OFF GENES: Role of Epigenetics

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

Available online at www.sciencedirect.com
ScienceDirect
Cryobiology 53 (2006) 310–318
CRYOBIOLOGY
www.elsevier.com/locate/ycryo

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

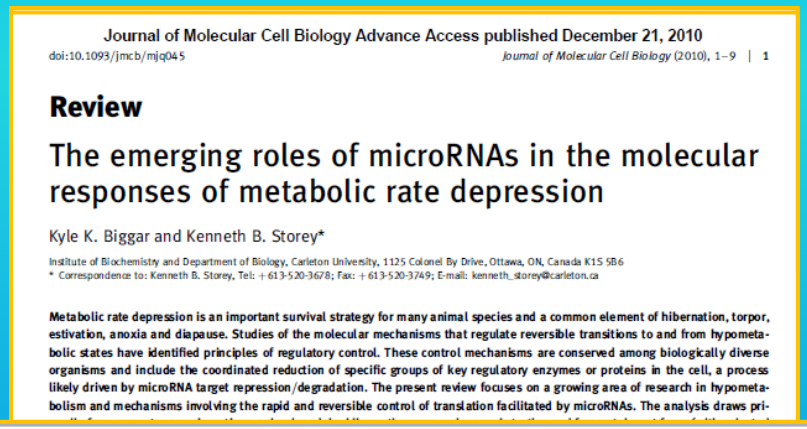
Abstract

During mammalian hibernation, metabolic rate can be reduced to <5% of the euthermic rate as a suppression of multiple energy expensive metabolic processes. Gene transcription is one of these examines mechanisms of transcriptional control that could contribute to lowering the rate of gen Histone deacetylases (HDAC) have been linked to gene silencing and measured HDAC activity skeletal muscle of hibernating thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, com controls. Western blotting also showed that HDAC1 and HDAC4 protein levels were 1.21-and 1. tively, in muscle from torpid animals. Histone H3 was also evaluated by Western blotting. Total hist but two forms of covalently modified histone H3 that are associated with active transcription (phos 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 value. These data support an overall decrease in transcriptional activity in skeletal muscle of hibe accomplished by multiple molecular mechanisms.

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



Journal of Molecular Cell Biology Advance Access published December 21, 2010

doi:10.1093/jmcb/mjq045 *Journal of Molecular Cell Biology* (2010), 1–9 | 1

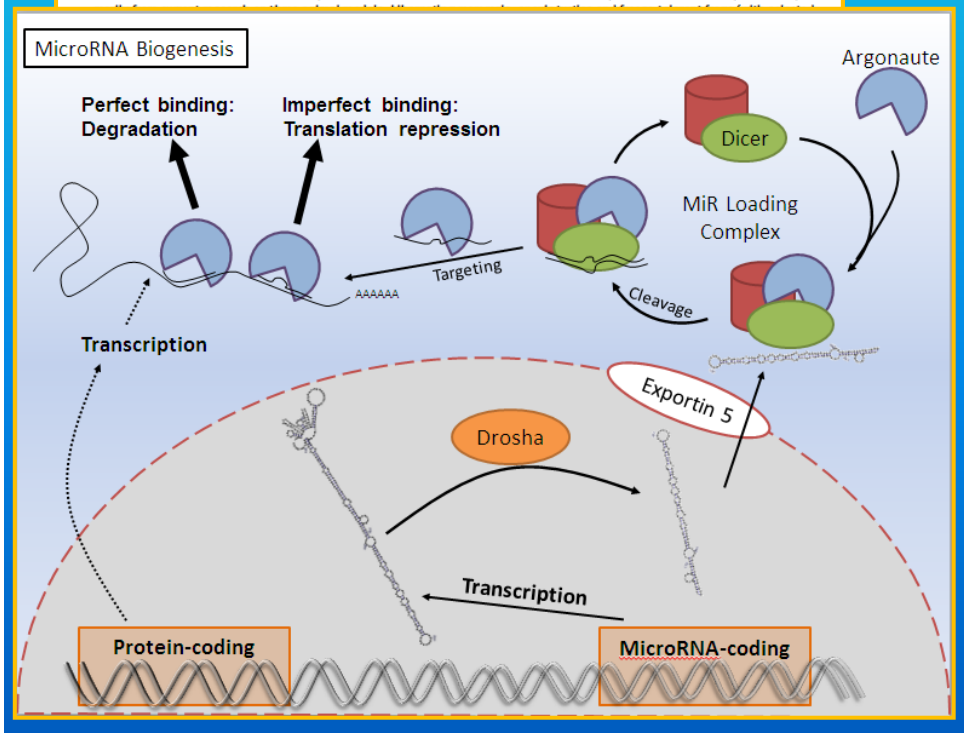
Review

The emerging roles of microRNAs in the molecular responses of metabolic rate depression

Kyle K. Biggar and Kenneth B. Storey*

Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
* Correspondence to: Kenneth B. Storey, Tel: +613-520-3678; Fax: +613-520-3749; E-mail: kenneth_storey@carleton.ca

Metabolic rate depression is an important survival strategy for many animal species and a common element of hibernation, torpor, estivation, anoxia and diapause. Studies of the molecular mechanisms that regulate reversible transitions to and from hypometabolic states have identified principles of regulatory control. These control mechanisms are conserved among biologically diverse organisms and include the coordinated reduction of specific groups of key regulatory enzymes or proteins in the cell, a process likely driven by microRNA target repression/degradation. The present review focuses on a growing area of research in hypometabolism and mechanisms involving the rapid and reversible control of translation facilitated by microRNAs. The analysis draws pri-

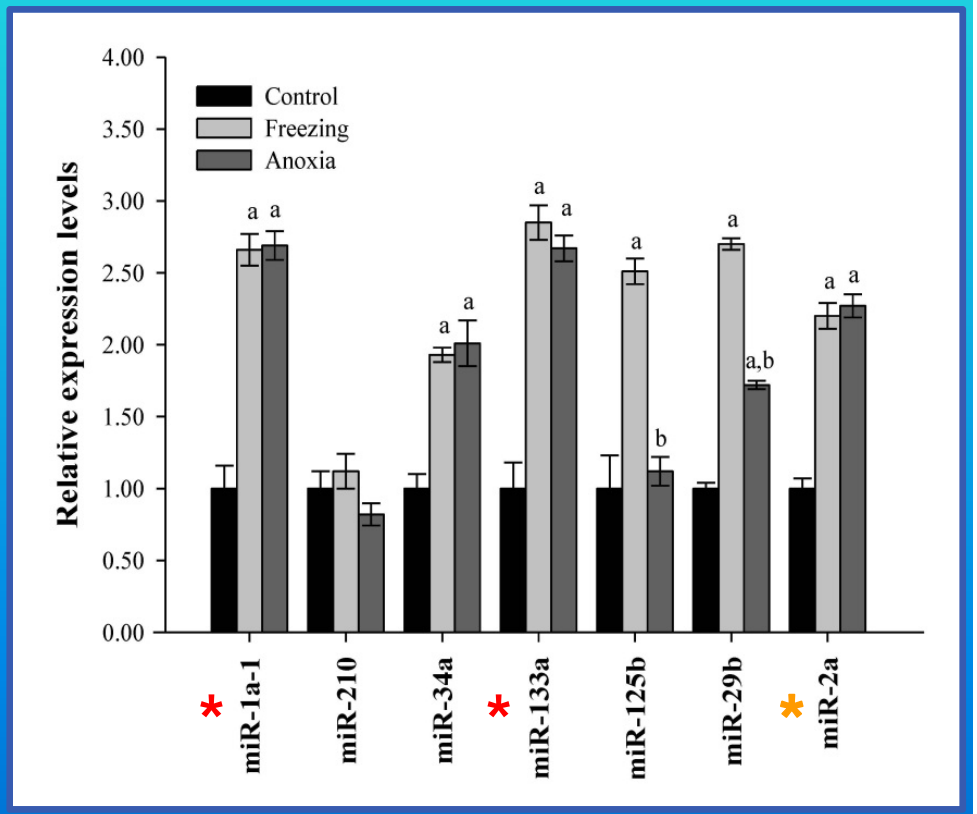


- Size ~22 nucleotides
- Highly conserved across species
- Bind to 3' UTR of mRNAs
- Repression mechanism(s) seem to include:
  - Block translation of mRNA
  - Help bind mRNA into stress granules
  - Target mRNA for degradation

Micro RNAs in *Littorina littorea* FOOT MUSCLE: Up-regulated by Freezing & Anoxia

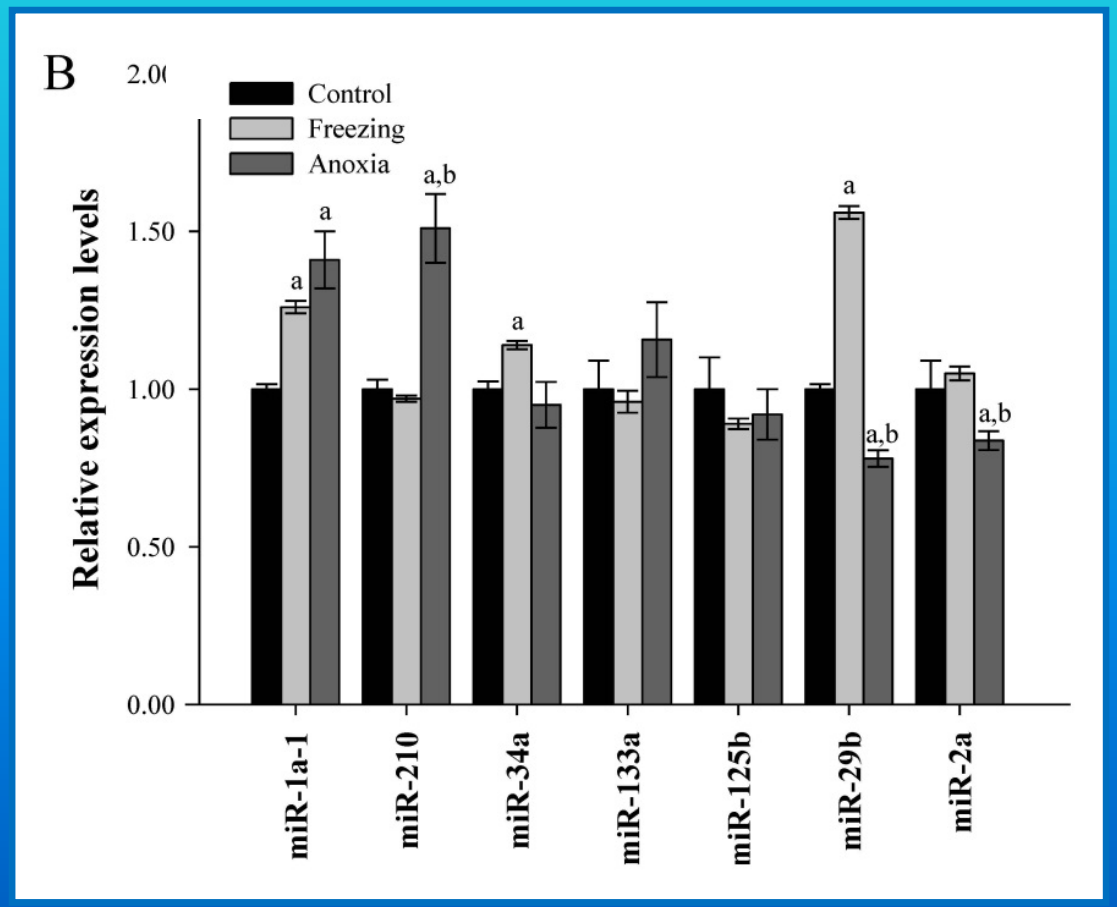
miR-1a-1* & miR-133a*
- myocyte proliferation & differentiation
-regulate *Mef2a* and *Gata4*, Tfs that promote muscle maintenance

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



Biggar, Kornfeld & Storey, 2011. Anal. Biochem. 416, 231-3.
Biggar, Kornfeld, Maistrovski & Storey, 2012. Genom. Proteom. Biotech. in press

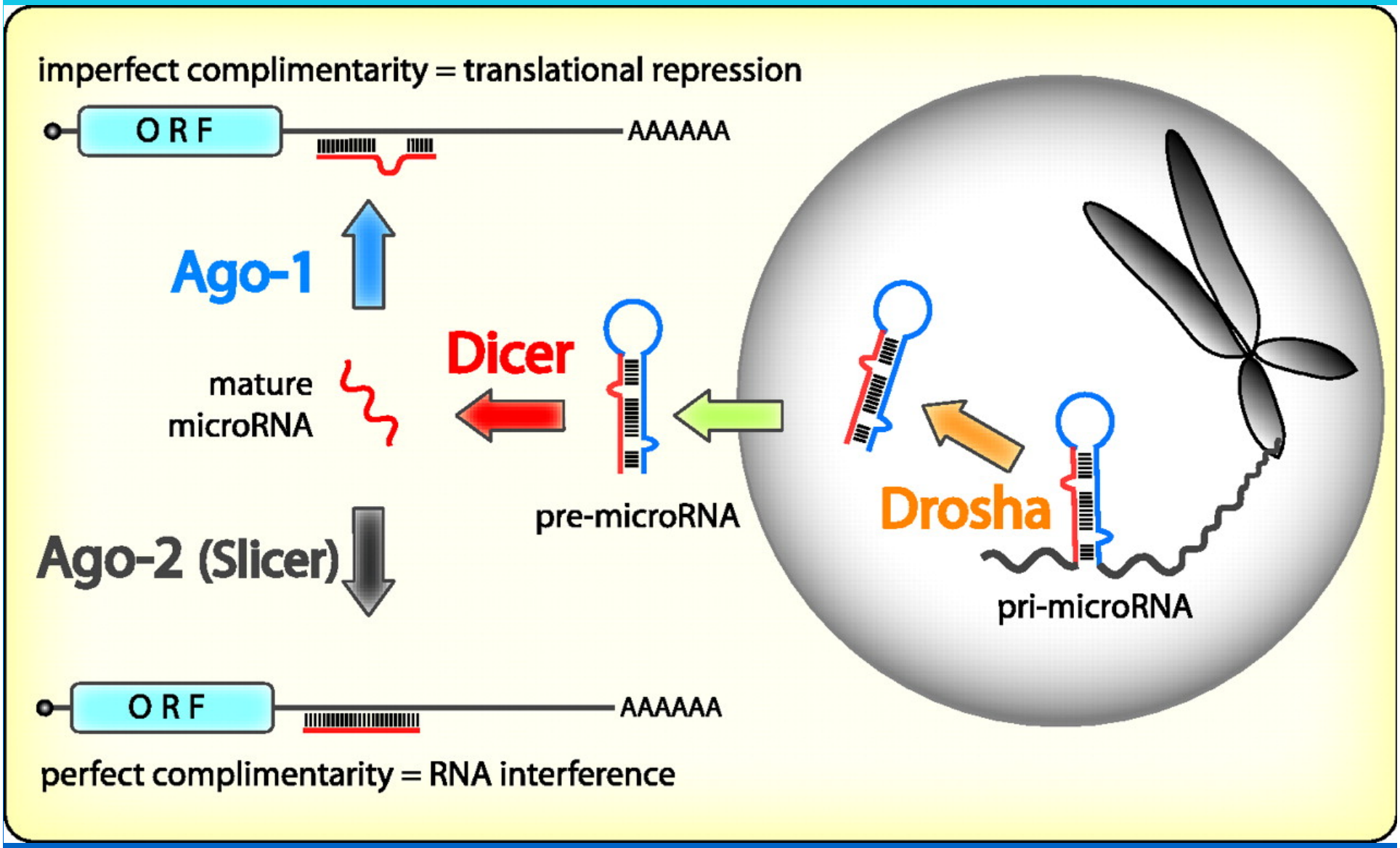
Micro RNAs in *Littorina littorea* HEPATOPANCREAS: Up-regulated by Freezing & Anoxia



Major changes:

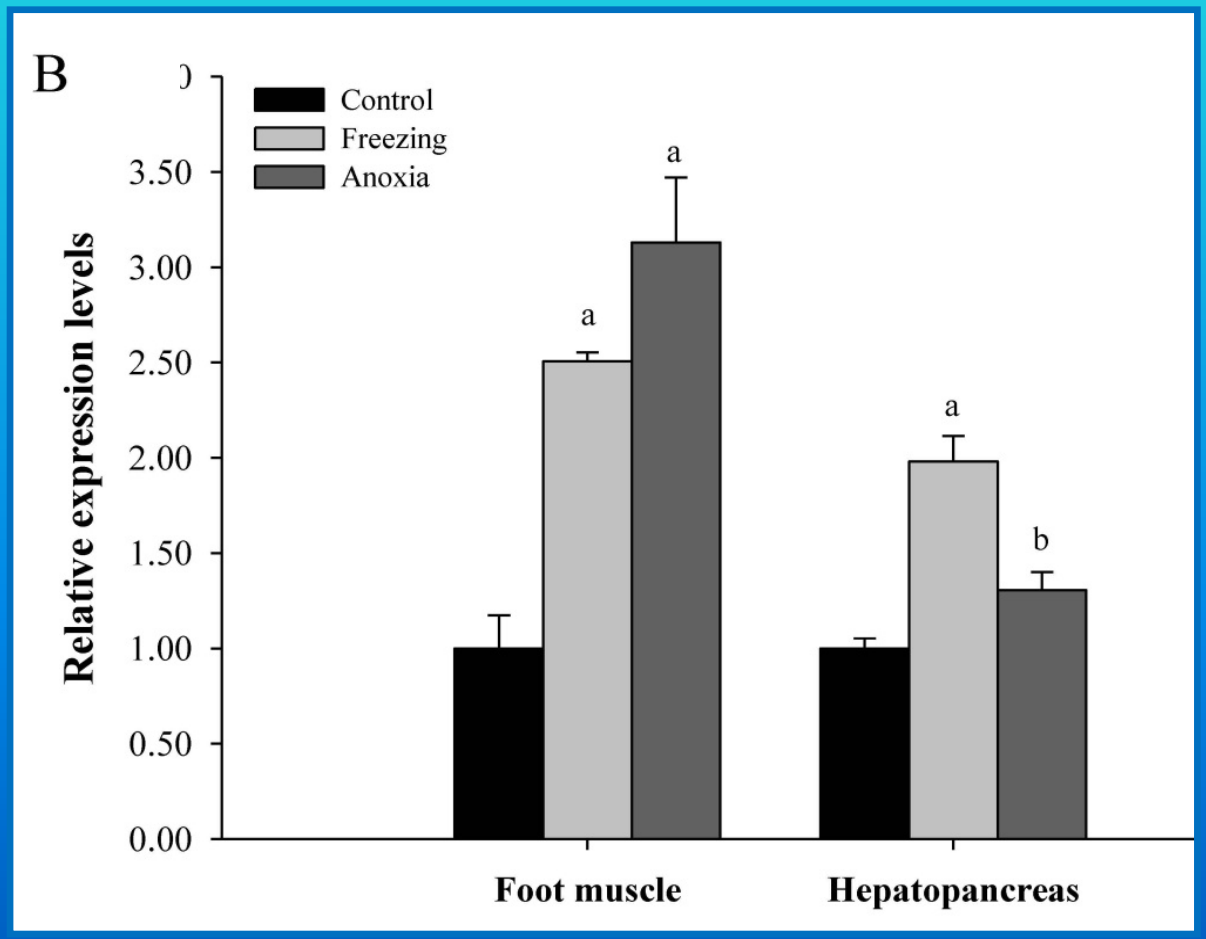
- miR-1a-1 up in freeze & anoxia (like in foot)
- miR-210 up in anoxia
- miR-29b up in freeze

MICRO RNA: Drosha & Dicer



Cuellar TL, McManus MT. J Endocrinol. 187(3):327-332, 2005.

DICER ENZYME IN *L. littorea* TISSUES

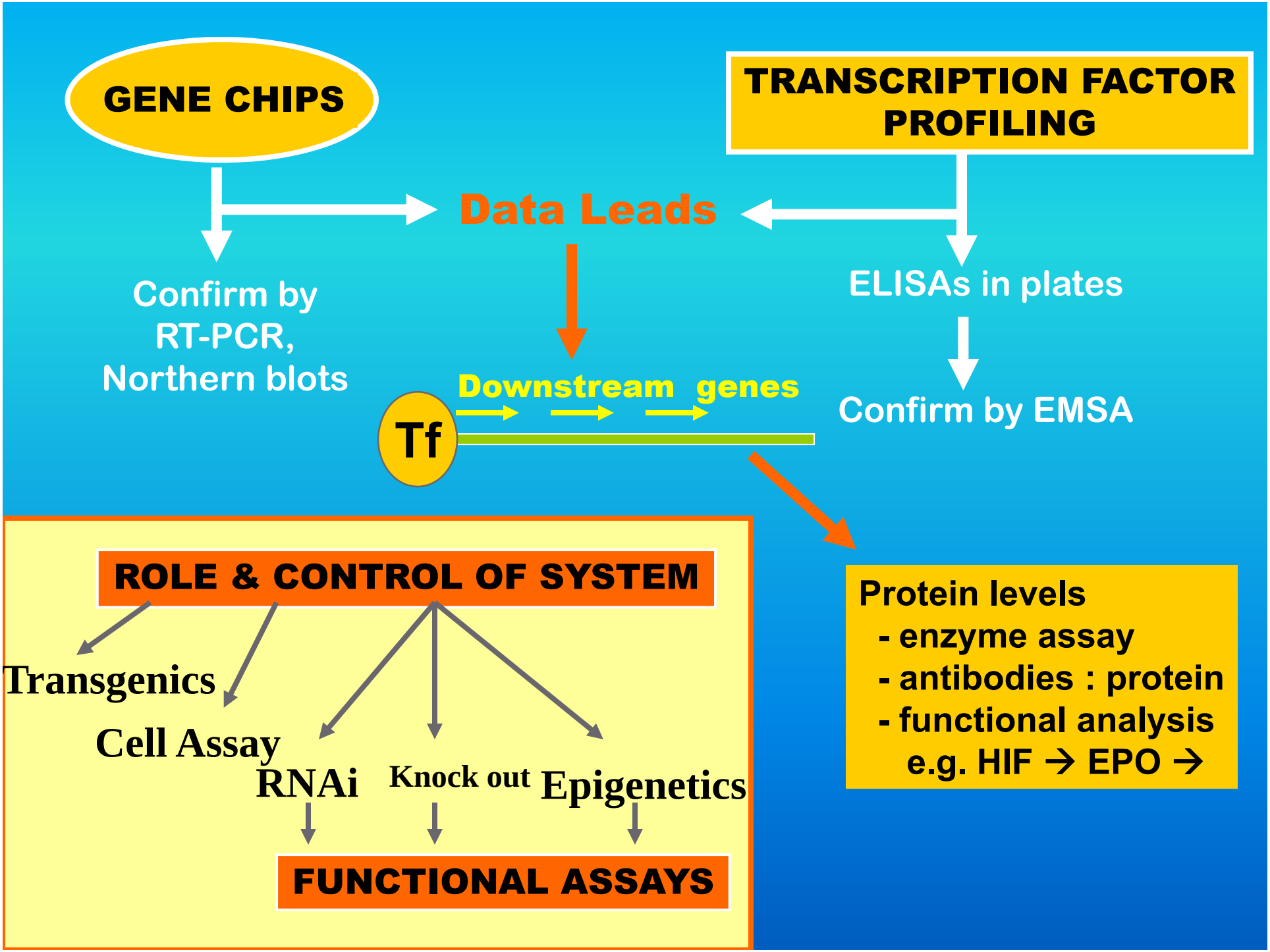


Dicer protein increased in both freezing & anoxia (immunoblots)

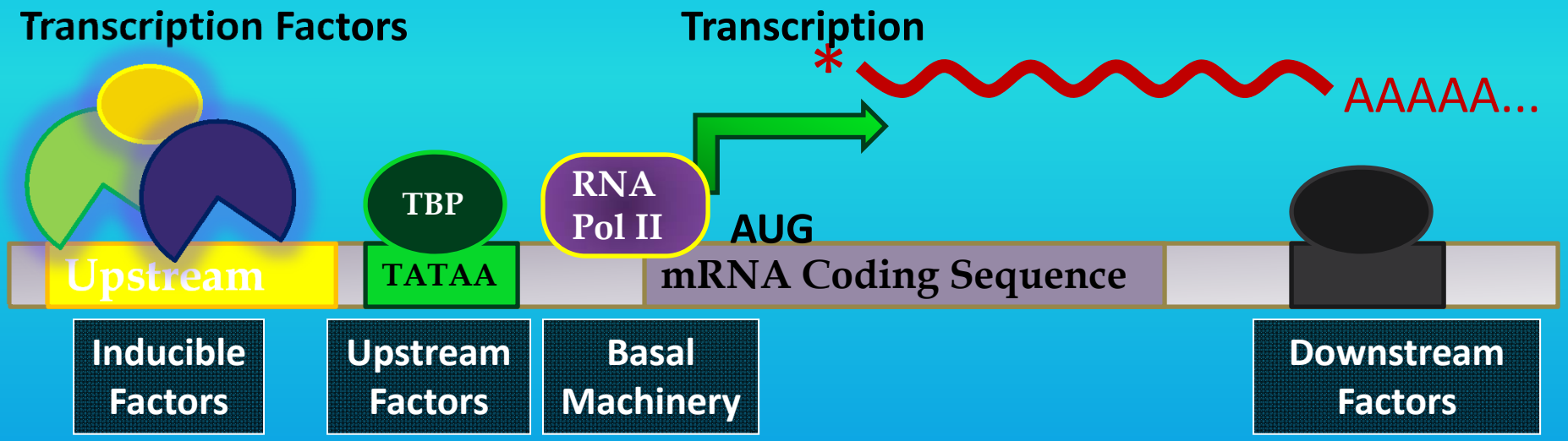
Elevated miRNA processing

ANOXIA INDUCED CHANGES

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


GENE CHIPS
TRANSCRIPTION FACTOR PROFILING
Data Leads
Confirm by RT-PCR, Northern blots
ELISAs in plates
Confirm by EMSA
Downstream genes
Tf
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 →

Regulation of Gene Transcription



10 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% ⬆

Larade & Storey. 2009. Curr. Genomics 10, 76-85

OXIDATIVE STRESS

Brazilian Journal of Medical and Biological Research (1996) 29: 1715-1733
ISSN 0100-879X

Oxidative stress: animal adaptations in nature

K.B. Storey

Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario, Canada K1S 5B6

Abstract

As a consequence of aerobic life, an organism must deal with the continuous generation of reactive oxygen species (O_2^-, H_2O_2, •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 nonenzymatic antioxidant defenses. However, the reperfusion injuries noted after ischemic insult in mammalian organs and ascribed to a burst of reactive oxygen species produced when oxygenated blood is reintroduced demonstrate that the antioxidant defenses of many organisms can be overwhelmed

Correspondence
K.B. Storey
Institute of Biochemistry and
Department of Biology
Carleton University
Ottawa, Ontario
Canada K1S 5B6
Fax: (613) 520-4389
E-mail: kbstorey@ccs.carleton.ca

Key words
- Reactive oxygen species
- Free radical damage
- Lipid peroxidation
- Anoxia tolerance
- Freeze tolerance
- Estivation
- Ischemia

ELSEVIER

Comparative Biochemistry and Physiology Part B 120 (1998) 437–448

CBP

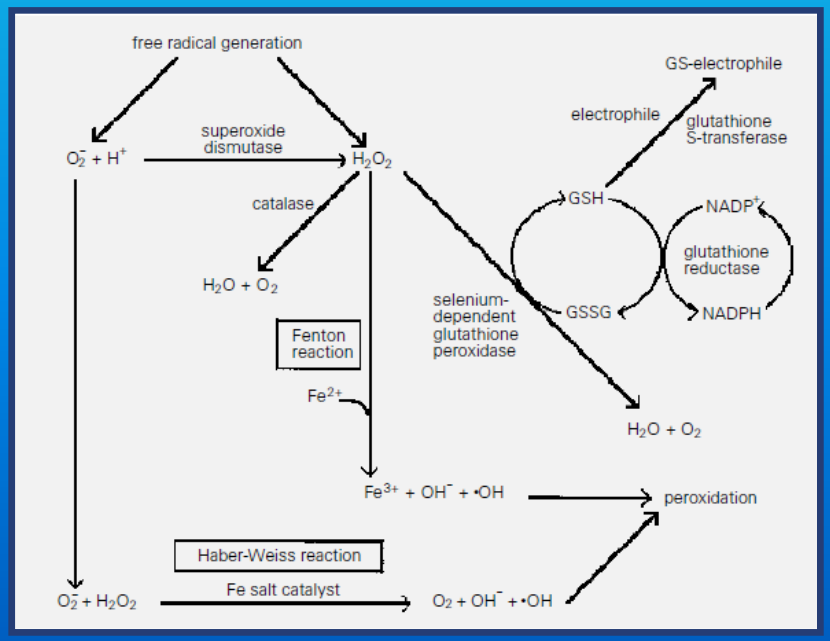
Review

Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails

Marcelo Hermes-Lima [a,*], Janet M. Storey [b], Kenneth B. Storey [b]

[a] *Departamento de Biologia Celular, Universidade de Brasilia, Brasilia 70910-900 DF, Brazil*
[b] *Department of Biology, Carleton University, Ottawa K1S 5B6, Canada*

Received 4 November 1997; received in revised form 16 May 1998; accepted 1 June 1998



- Central theme in oxygen metabolism
- Linked with cancer, aging, diabetes, hypoxia, etc.
- Antioxidant enzymes as indicators of oxidative stress

REACTIVE OXIDATIVE SPECIES

$O_2 + e^- \rightarrow O_2^{\cdot -}$ superoxide radical

$O_2^{\cdot -} + H_2O \rightarrow HOO^{\cdot} + OH^-$ hydroperoxyl radical

$HOO^{\cdot} + e^- + H^+ \rightarrow H_2O_2$ hydrogen peroxide

$H_2O_2 + e^- \rightarrow {}^{\cdot}OH + OH^-$ hydroxyl radical

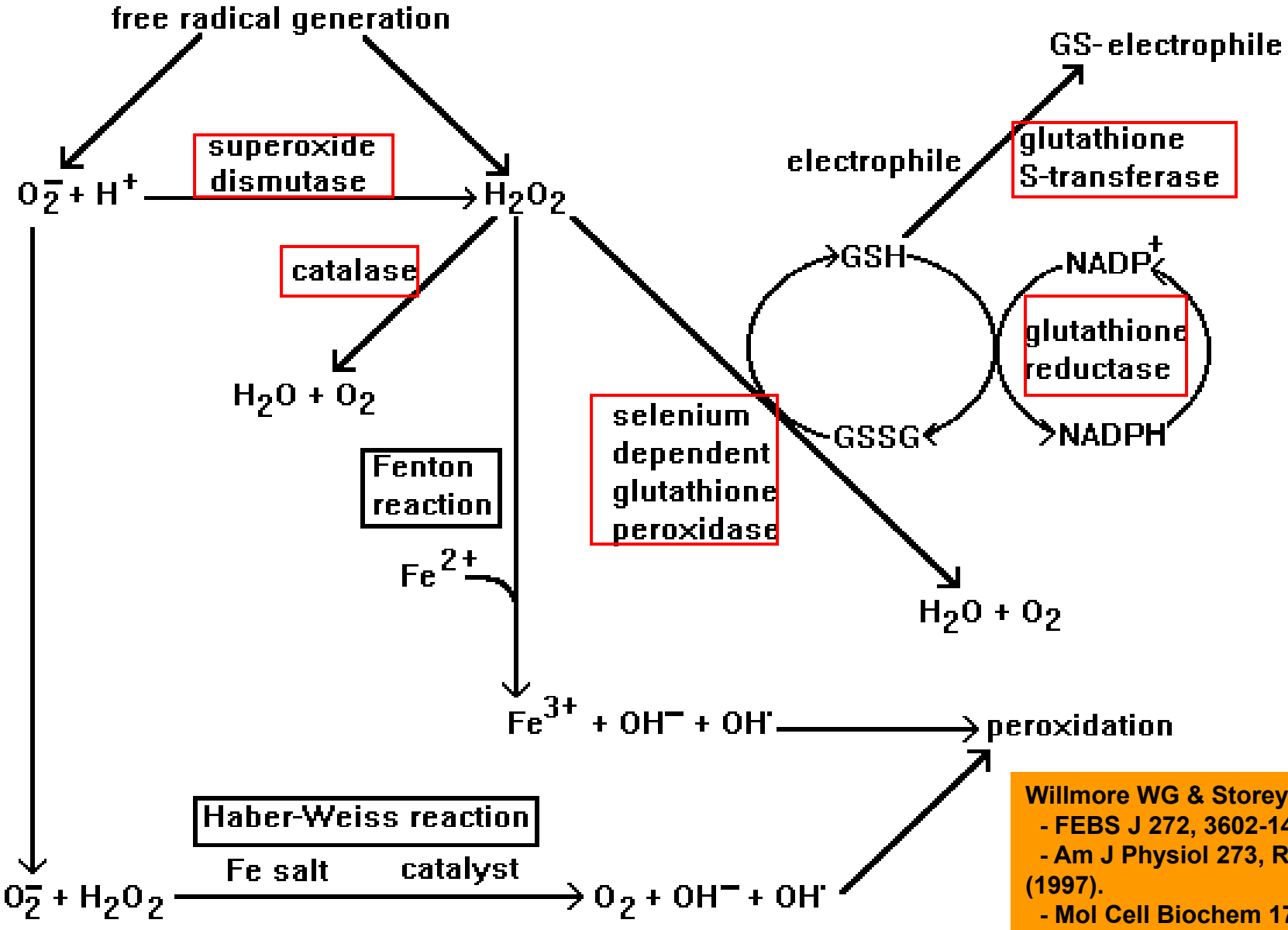
$Fe\,(III) + O_2^{\cdot -} \rightarrow Fe\,(II) + O_2$

$Fe\,(II) + H_2O_2 \rightarrow Fe\,(III) + {}^{\cdot}OH + OH^-$ Fenton reaction

---

$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + {}^{\cdot}OH + OH^-$ Haber-Weiss reaction

ANTIOXIDANT ENZYMES



Willmore WG & Storey KB
- FEBS J 272, 3602-14 (2005)
- Am J Physiol 273, R219-25 (1997).
- Mol Cell Biochem 170, 177-185 (1997)

ANTIOXIDANT DEFENSE

Iron storage:

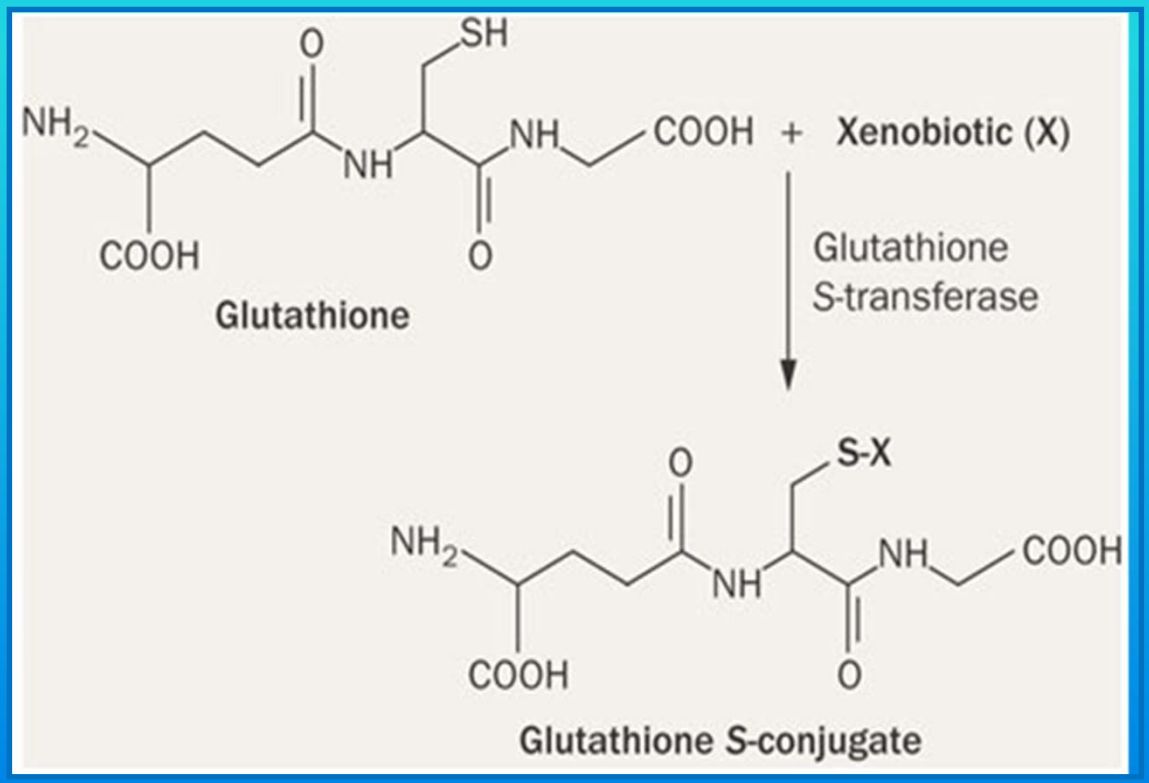
- **Ferritin (H & L chains)**
- **Transferrin receptor 2**

Antioxidant enzymes

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

Estivation: Storey & Storey 2012. J. Exp. Biol. 215:1425-33
Cold hardiness: Storey & Storey 2012. Can. J. Zool. 90: 456–475
Marine: Freire et al. 2011. *In:* Oxidative Stress in Aquatic Ecosystems. pp. 41-57

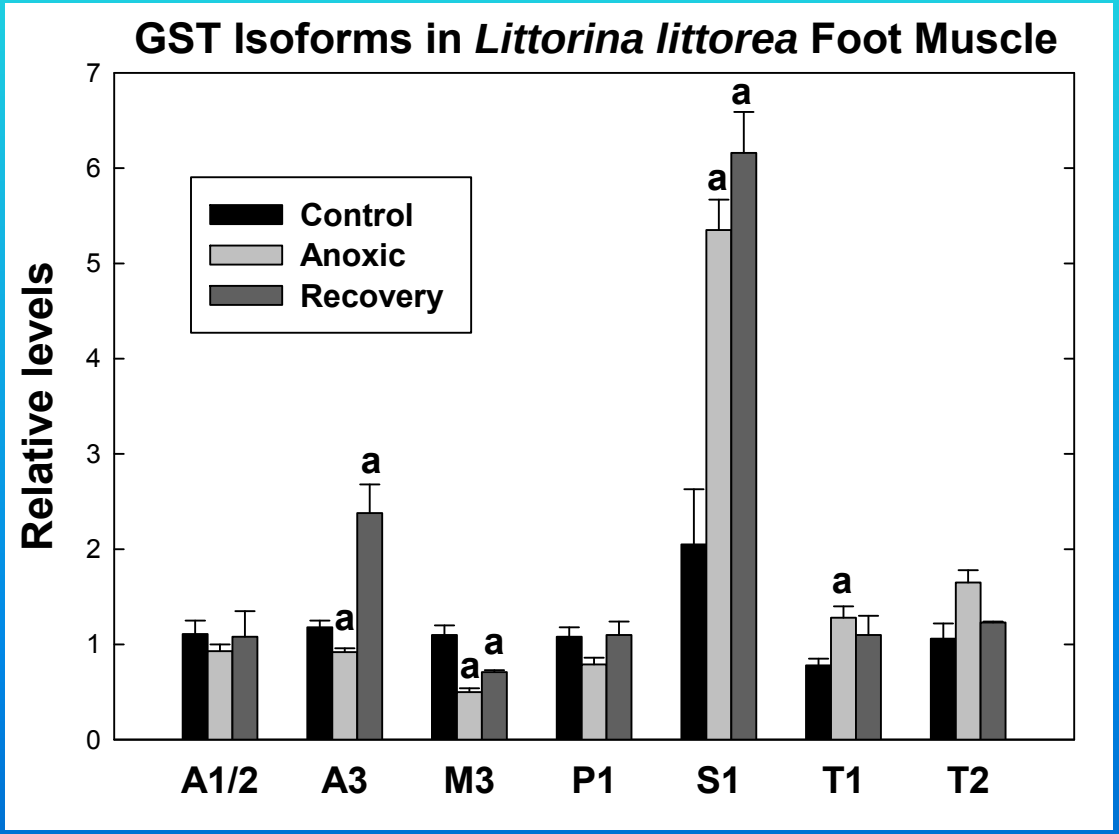
GLUTATHIONE *S* -TRANSFERASE



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

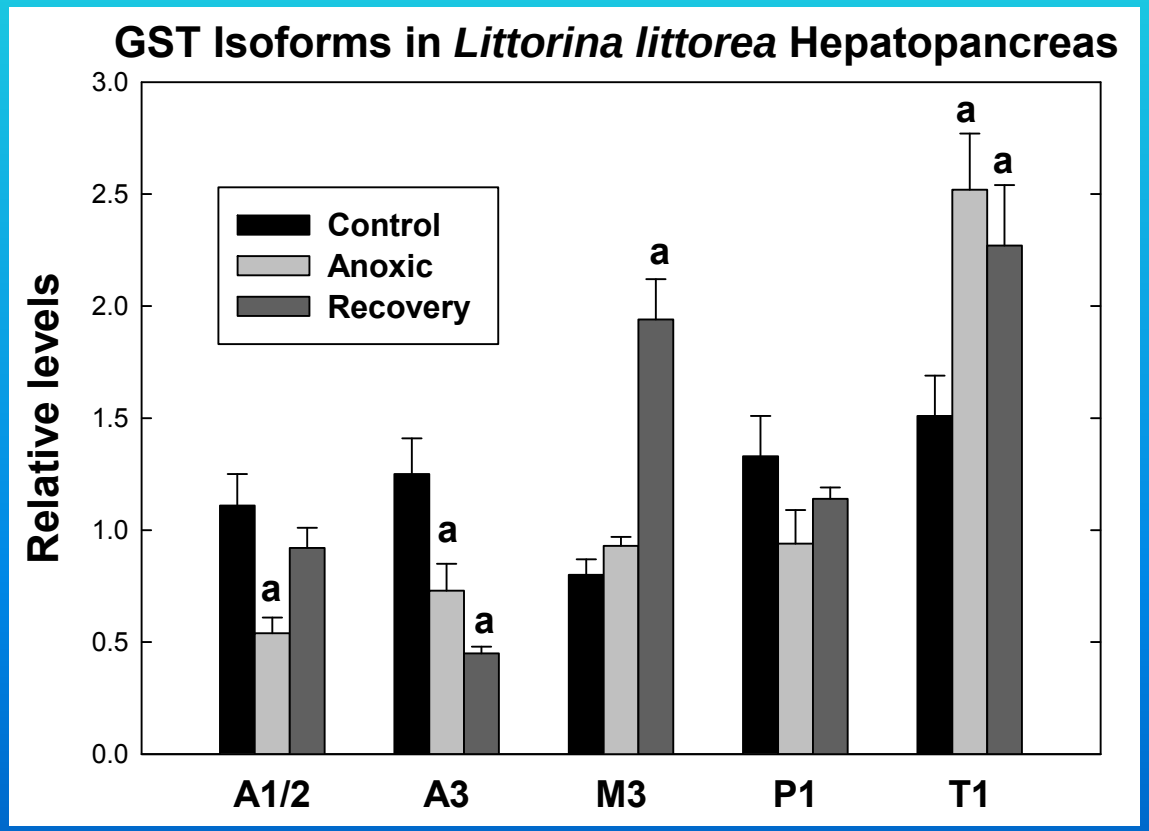
Some forms controlled by the antioxidant response element (ARE) via the Nrf2 transcription factor

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

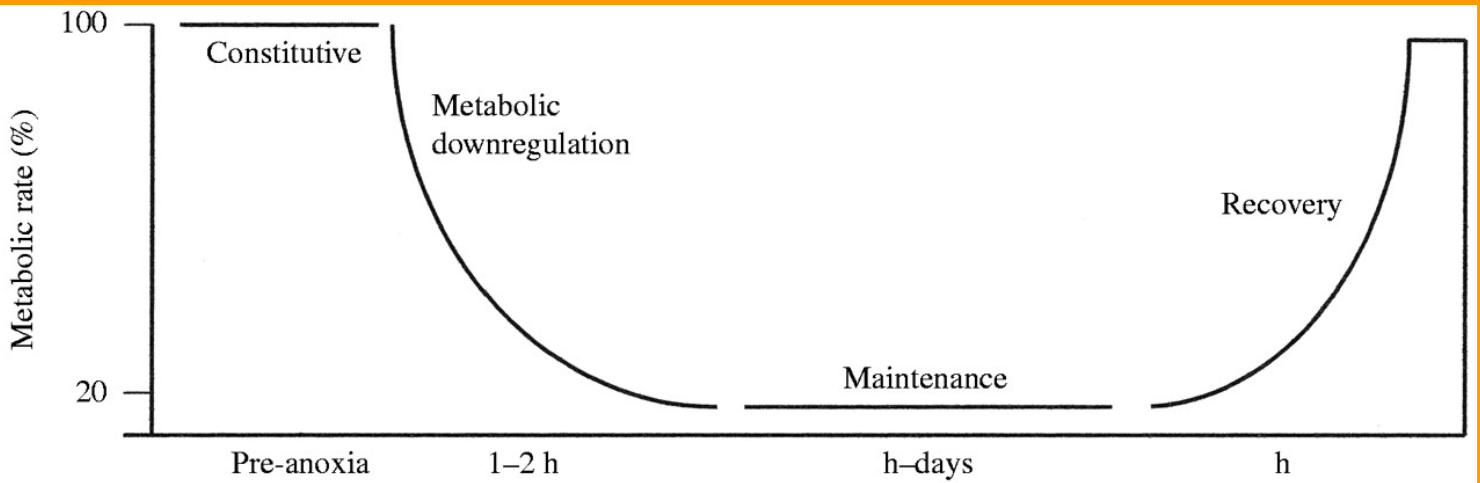


WHERE DO WE GO FROM HERE?

- 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



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

Lutz PL & Milton SL. 2004. J Exp Biol 207: 3141-3147

NEW DIRECTIONS - TBA

Big Science Edition:

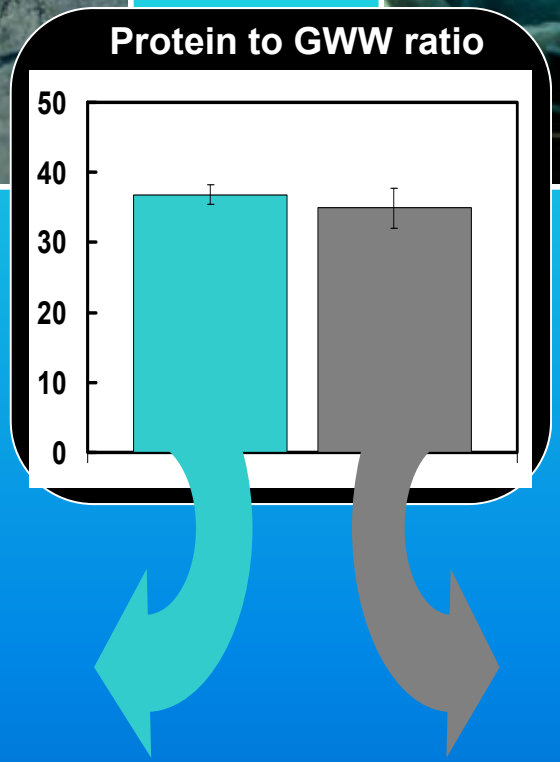
1. GENOMES: Sequence all the genes to feel better !

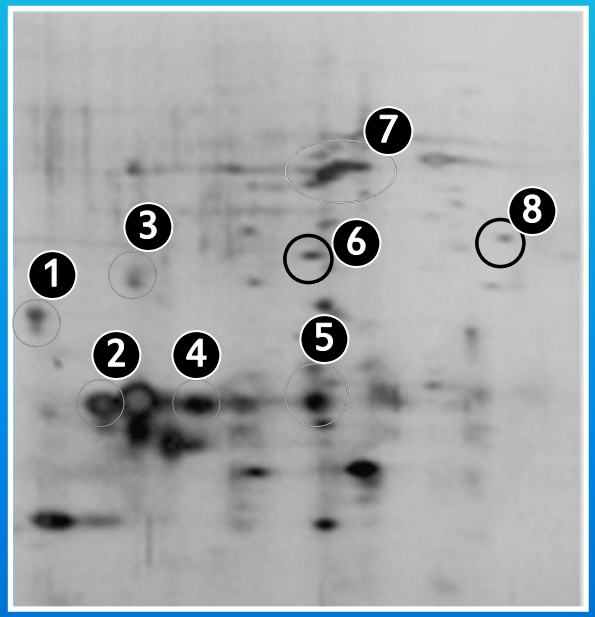
The western painted **turtle genome**, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage.

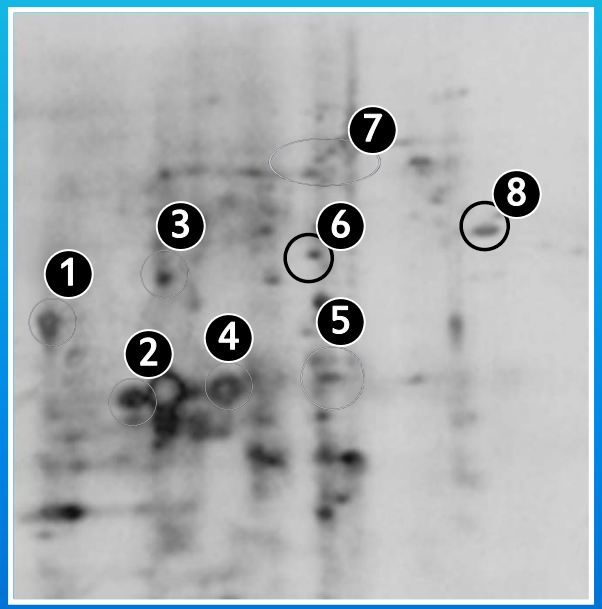
Shaffer HB,...et al Storey KB, Genome Biol. 2013 14(3): R28

2. Protein 2D: What about the Proteins

OMICS – Proteomics







Normoxia

48 Anoxia

Novel phosphorylations

Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 467 (2007) 10–19
ABB
www.elsevier.com/locate/yabbi

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 29 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 v
thermogenesis during arousal. Creatine kinase (CK)[1] has a key role in muscle
squirrels, *Spermophilus richardsonii*. CK activity was ~20% lower during hibe
mRNA was reduced by ~70%. Hibernator CK showed reduced affinity for AT
that promoted endogenous protein kinase or phosphatase action, coupled wi
phosphate forms, showed that soluble CK from euthermic squirrels was a mi
only phospho-CK was detected in hibernating animals. High and low phosph
substrates but did not differ in stability to urea denaturation. About 20–25%
bound CK showed different kinetic responses to kinase and phosphatase trea

Comparative Biochemistry and Physiology, Part B 157 (2010) 310–316
Contents lists available at ScienceDirect
Comparative Biochemistry and Physiology, Part B
journal homepage: www.elsevier.com/locate/cbpb

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

Ryan A.V. Bell, Kenneth B. Storey *

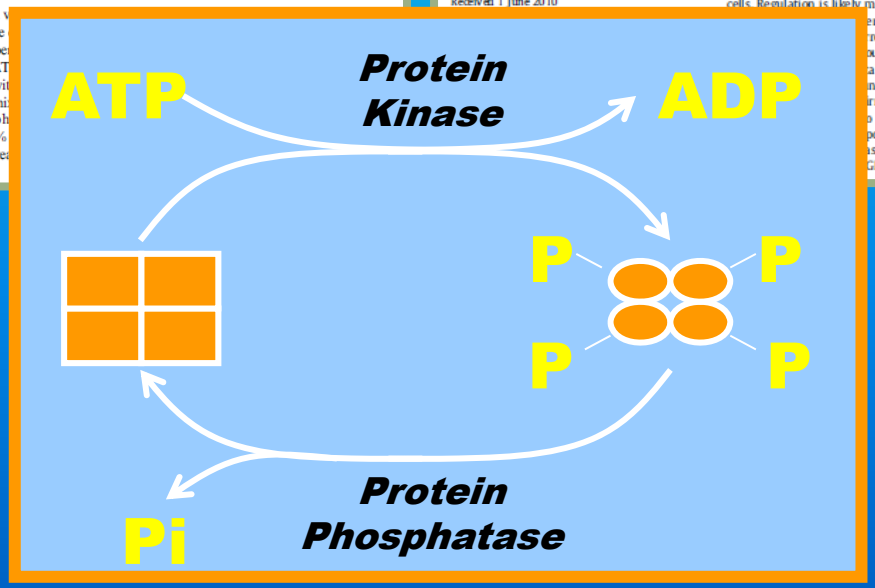
Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

ARTICLE INFO

Article history:
Received 1 June 2010

ABSTRACT

Glutamate dehydrogenase (GDH) is a key enzyme that links amino acid and carbohydrate metabolism in
cells. Regulation is likely most important when organisms are confronted with extreme stresses such as the
eratures and lack of food associated with winter. Many small mammals, such as
rels, *Spermophilus richardsonii*, cope with these conditions by hibernating. Animals
ound torpor where metabolic rate is greatly suppressed, body temperature drops to
abolic needs must be met from fixed internal body stores of fuels. To investigate
nder these conditions, kinetic properties of GDH were analyzed in liver from
irrels, revealing significant differences in V_{max}, K_m glutamate, K_a ADP and inhibition
o forms of GDH. These data suggested an activation of the glutamate-oxidizing
pometabolic state. Subsequent experiments suggested that the molecular basis of
s a change in the protein phosphorylation state of GDH between euthermia and
GDH appears to be dephosphorylated and activated when animals transition into



Epigenetics in Adaptation miRNA PLUS

Mol Cell Biochem. 2010 Sep;342(1-2):151-61. Epub 2010 May 1.

Epigenetics in anoxia tolerance: a role for histone deacetylases.

Krivoruchko A, Storey KB.

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

Abstract

The importance of epigenetics has been established in many key biological processes
mechanism to animal survival of low oxygen conditions has never been examined. To e
mechanisms could be involved in natural anoxia tolerance, we have examined the anox
transcriptional silencers, histone deacetylases (HDACs), in tissues of a unique model
turtle Trachemys scripta elegans. Transcript and protein levels of all five HDACs rose b
in skeletal muscle in response to 20 h of anoxia exposure. In addition, HDAC activity in
response to 20 h of anoxia and levels of acetylated histone H3 (Lys 9 or Lys 23) decrea
liver displayed a milder response with HDAC1, -4, and -5 protein levels increasing by 1
acetylated histone H3 levels also decreased to 50-75% of control values. Only HDAC5
heart; Hdac5 transcript levels increased 2.1-2.3-fold and HDAC5 protein rose by 3.3-fol

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Available online at www.sciencedirect.com

ScienceDirect

Cryobiology 53 (2006) 310–318

CRYOBIOLOGY

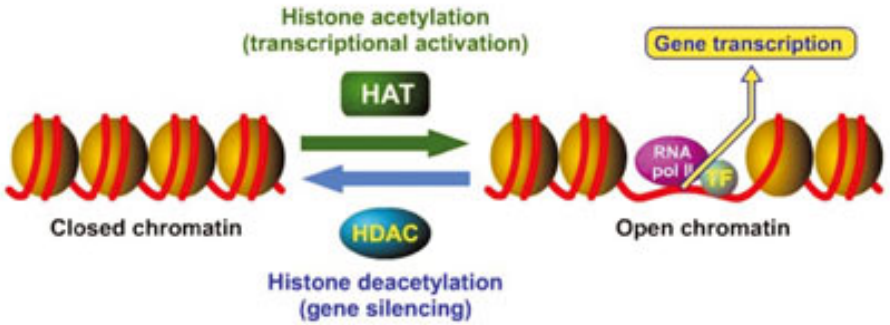
www.elsevier.com/locate/ycryo

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 Colo

Received 14 March 2006; accepted 4 August 2
Available online 18 September 2006



Unavoidable metabolic costs

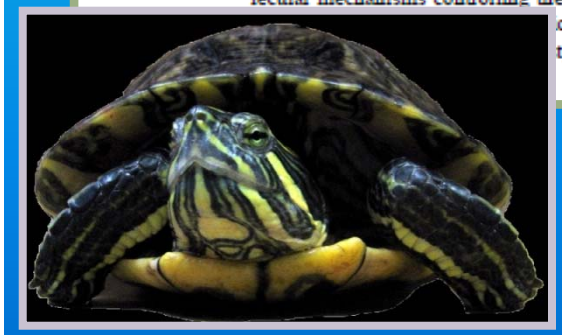
Current Genomics, 2009, 10, 573-584 573

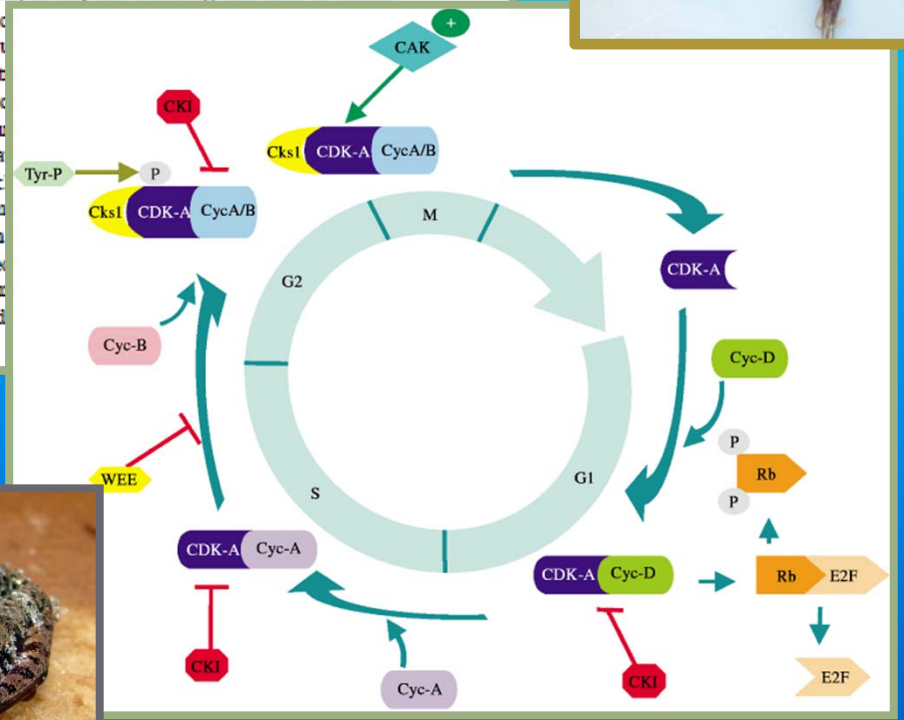
Perspectives in Cell Cycle Regulation: Lessons from an Anoxic Vertebrate

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 foc
anoxia tolerant turtles are faced with periods of oxygen deprivation, numerou
take place in order to facilitate vital reductions in ATP consumption. Such st
modifications as well as the implementation of translation and transcription co
though it is clear that anoxic survival relies on the suppression of ATP consu
anoxia tolerant vertebrates remain elusive. Several anoxia tolerant invertebra
cell cycle arrest when presented with anoxic stress. Despite this, the cell cyc
tolerant turtles. Understanding how vertebrates respond to anoxia can have im
cellular proliferation and hypoxic tumor progression are inescapably linked in
lecular mechanisms controlling these processes have profound clinical conse
c vertebrates and more specifically, the cor
t, the activation of checkpoint kinases, and





Unity through Evolution

Int. J. Biol. Sci. 2010, 6 9

International Journal of Biological Sciences
2010; 6(1):9-50

Review

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 ✉

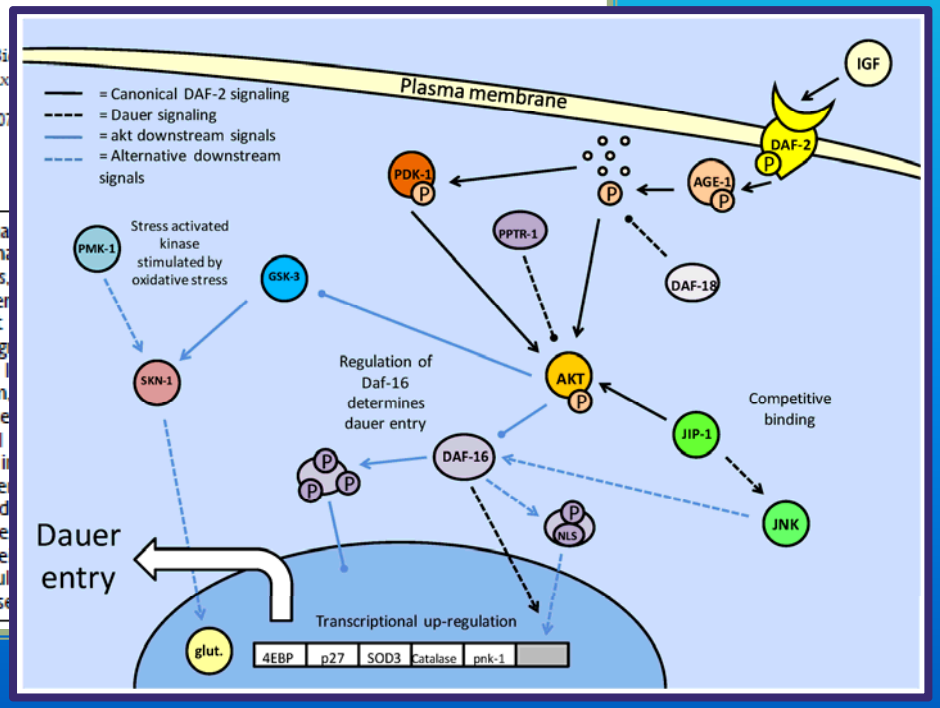
Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada

✉ Correspondence to: Kenneth B. Storey, Institute of Bi
Ottawa, Ont. K1S 5B6, Canada. Tel. +1 613 520 3678, Fax

Received: 2009.09.11; Accepted: 2009.11.25; Published: 2010.01.0

Abstract

Studies of the molecular mechanisms tha
physiological) have long been used to ma
model organism, *Caenorhabditis elegans*,
'dauer' stage. This period of developmer
in metabolic rate, triggered by ambient
ents. *C. elegans* employs a number of sig
unfavourable conditions and survive for l
The suppression of cellular metabolism
survival of nematodes through the daue
nisms that are fundamental to control
general, mammalian systems are highly i
temperatures and low oxygen), howeve
signal transduction pathways of nematod
protein targets in the stress response
maintained, and often differ only in the
outlines a framework of critical molecul
as therapeutic targets for addressing dise



WWCeD

Life span extension

Oxid Med Cell Longev. 2010 May-Jun;3(3):186-98.

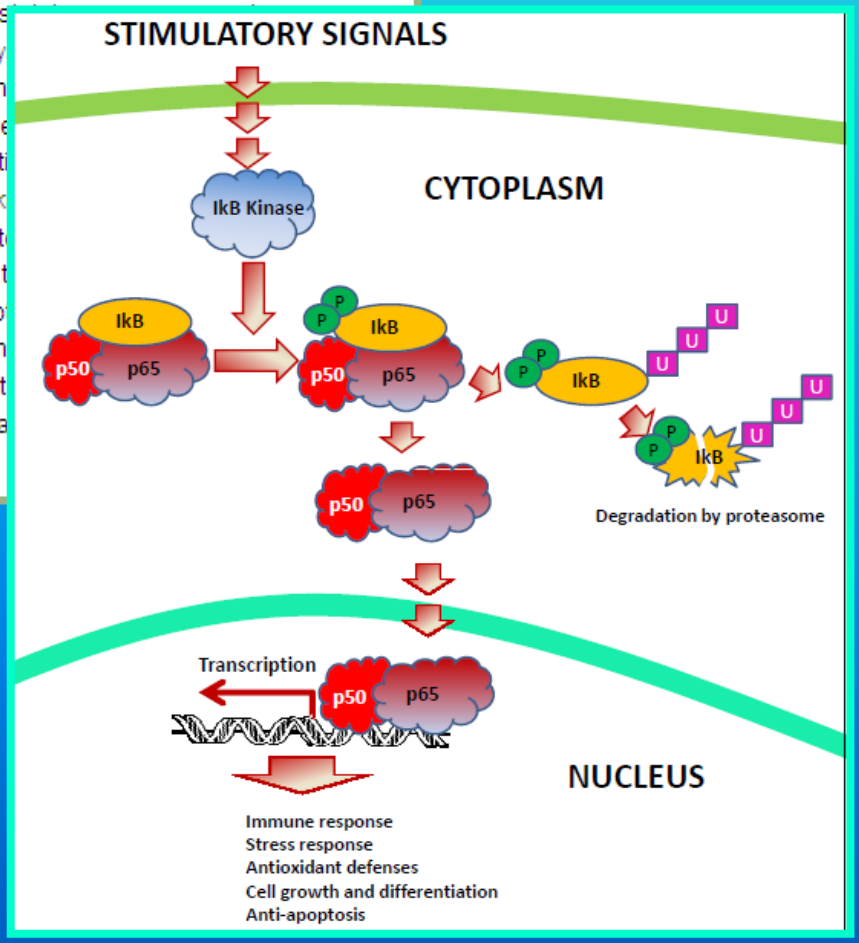
Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.

Krivoruchko A, Storey KB.

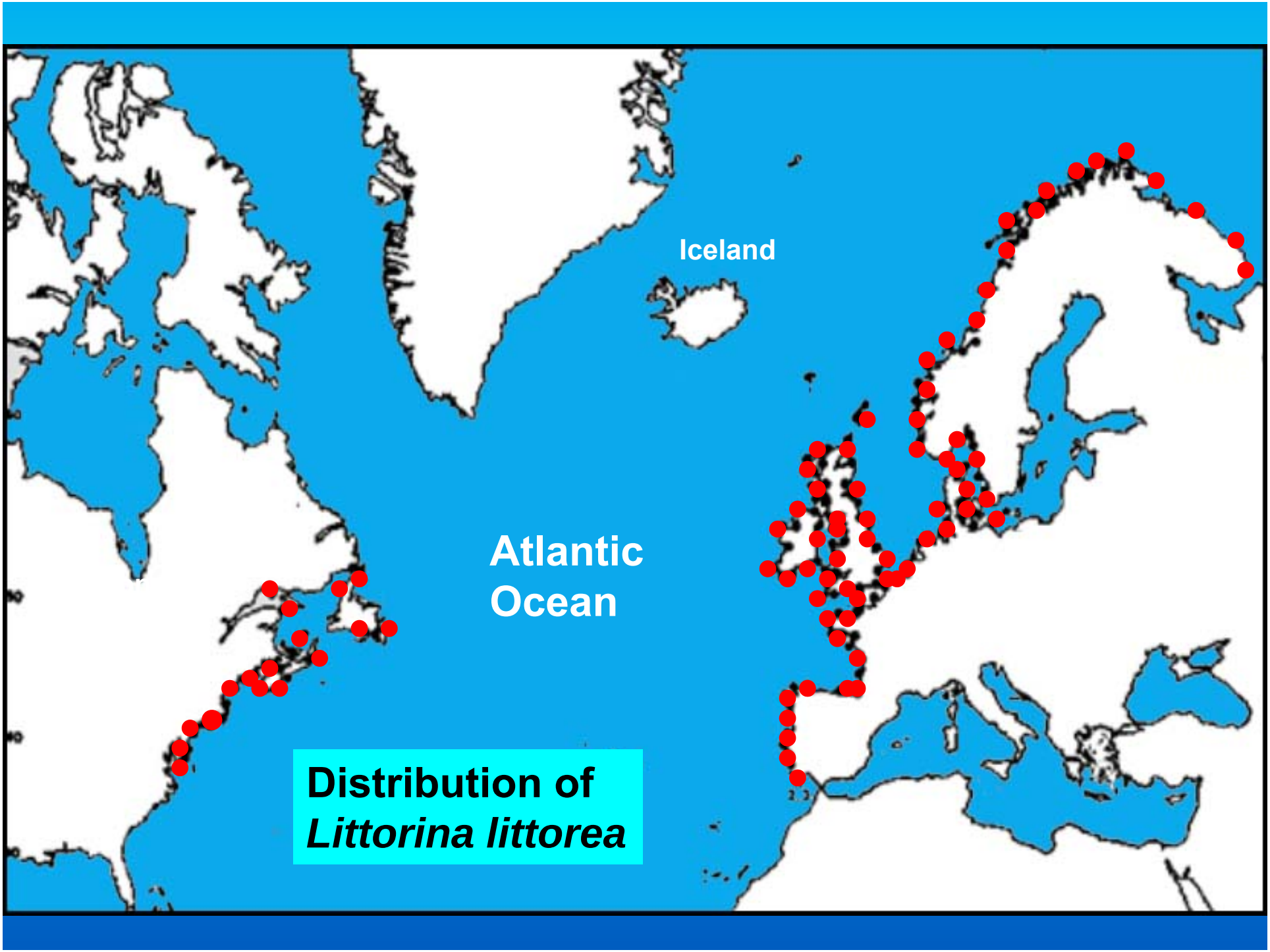
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, ON, CA.

Abstract

While mammals cannot survive oxygen deprivation for more than a few minutes without sus
some animals have mastered anaerobic life. Freshwater turtles belonging to the Trachemy
champion facultative anaerobes of the vertebrate world, often surviving without oxygen for m
physiological and biochemical mechanisms that underlie anoxia tolerance in turtles include
depression, post-translational modification of proteins, strong antioxidant defenses, activati
transcription factors, and enhanced expression of cytoprotective proteins. Turtles are also k
and display characteristics of "negligible senescence". We propose that the robust stress-t
long term anaerobiosis by turtles may also support the longevity of these animals. Many of t
natural anoxia tolerance, such as hypometaboli e pro
to play important roles in mammalian oxygen-re andin
oxygen could aid in the understanding and treat s that
In the present review we discuss the recent adv ecula
turtles and the potential links between this toler



***Littorina littorea*, periwinkle**


Iceland
Atlantic
Ocean
Distribution of
Littorina littorea

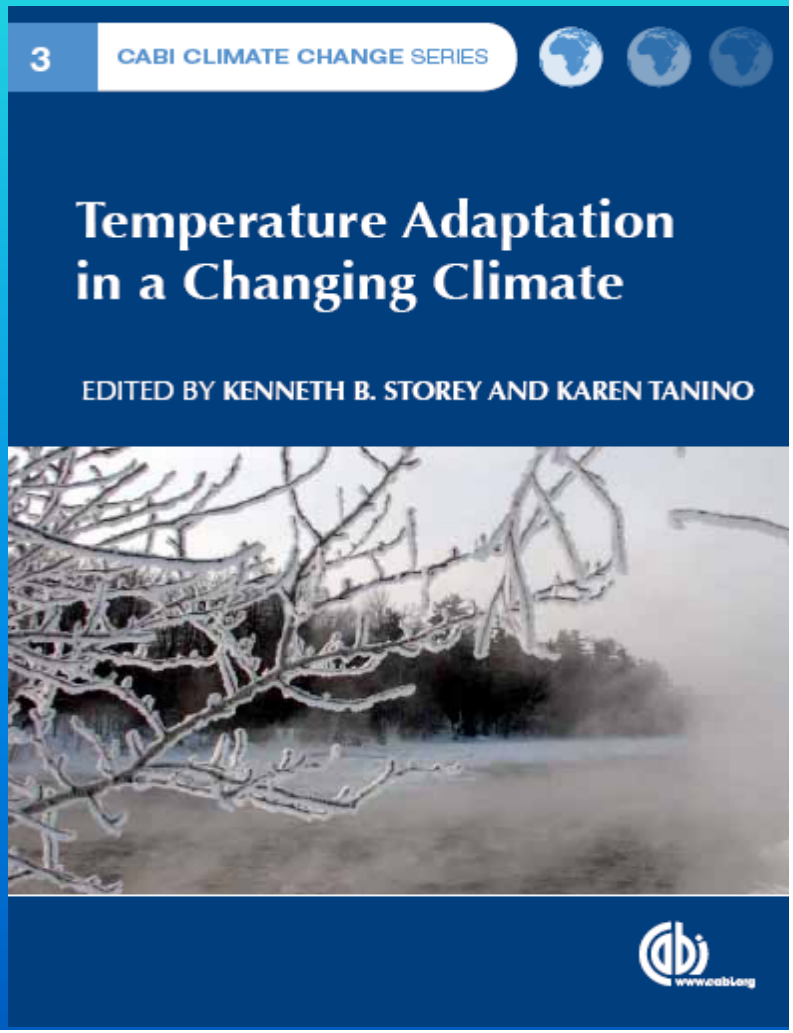
Molecular Adaptation to Climate Change:

Challenges for Amphibians & Reptiles

Freezing survival

Estivation

Anoxia tolerance



Invertebrate Anaerobiosis

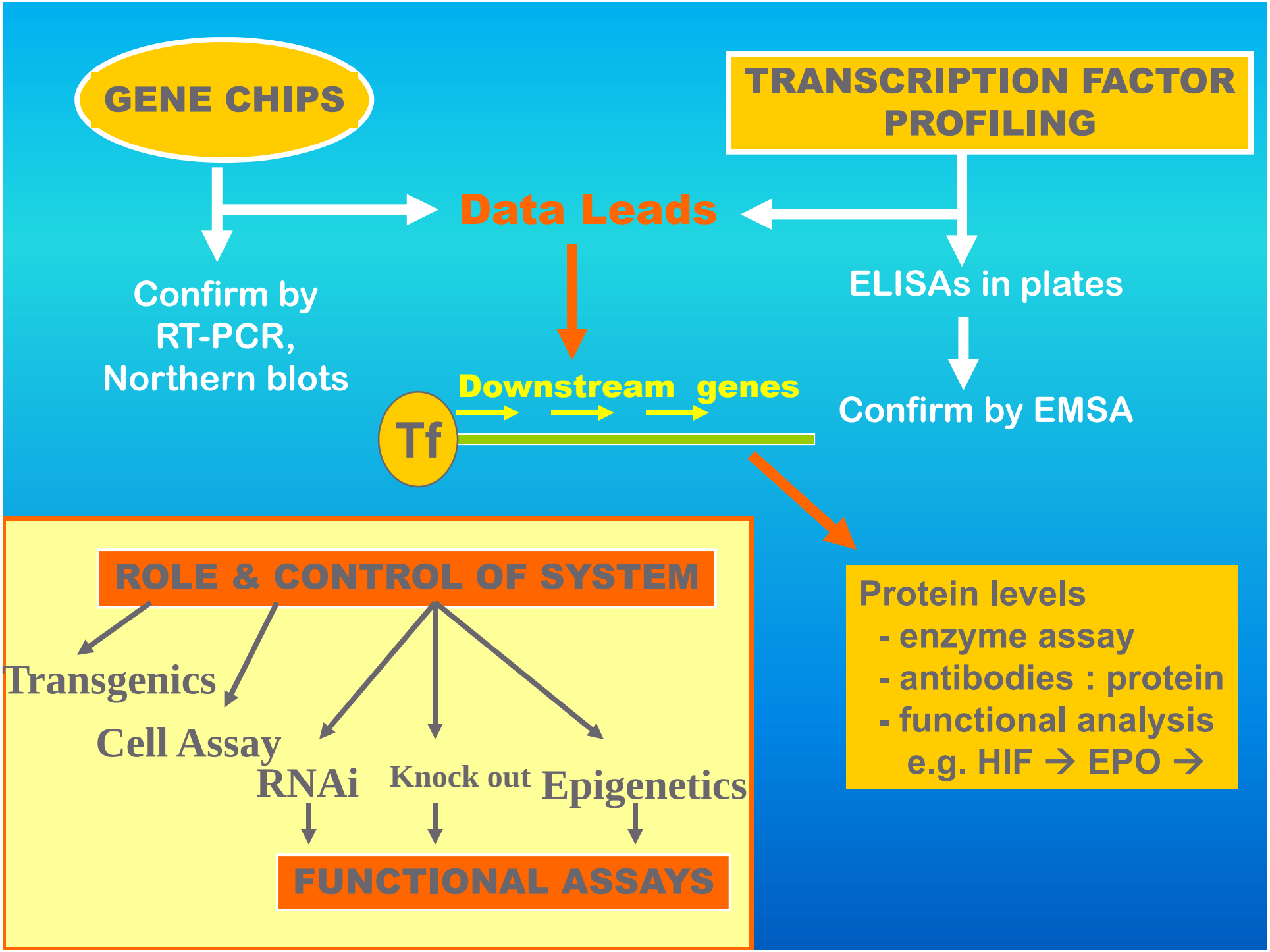
- J. STOREY
- S. BROOKS
- W. WILLMORE
- J. DUNCAN
- M. HERMES-LIMA
- S. GREENWAY
- T. ENGLISH
- E. RUSSELL
- T. PANNUNZIO
- J. MACDONALD
- K. LARADE
- R. WHITWAM
- S. KORYCAN
- B. MICHAELIDIS
- R. BELL
- J. LAMA
- B. LANT
- O. ANOZIE
- K. BIGGAR
- N. DAWSON

www.carleton.ca/~kbstorey

Funded by
NSERC Canada

PRINCIPLES OF HYPOXIA SURVIVAL

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
Downstream genes
Tf
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 →

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

Funded by NSERC Canada

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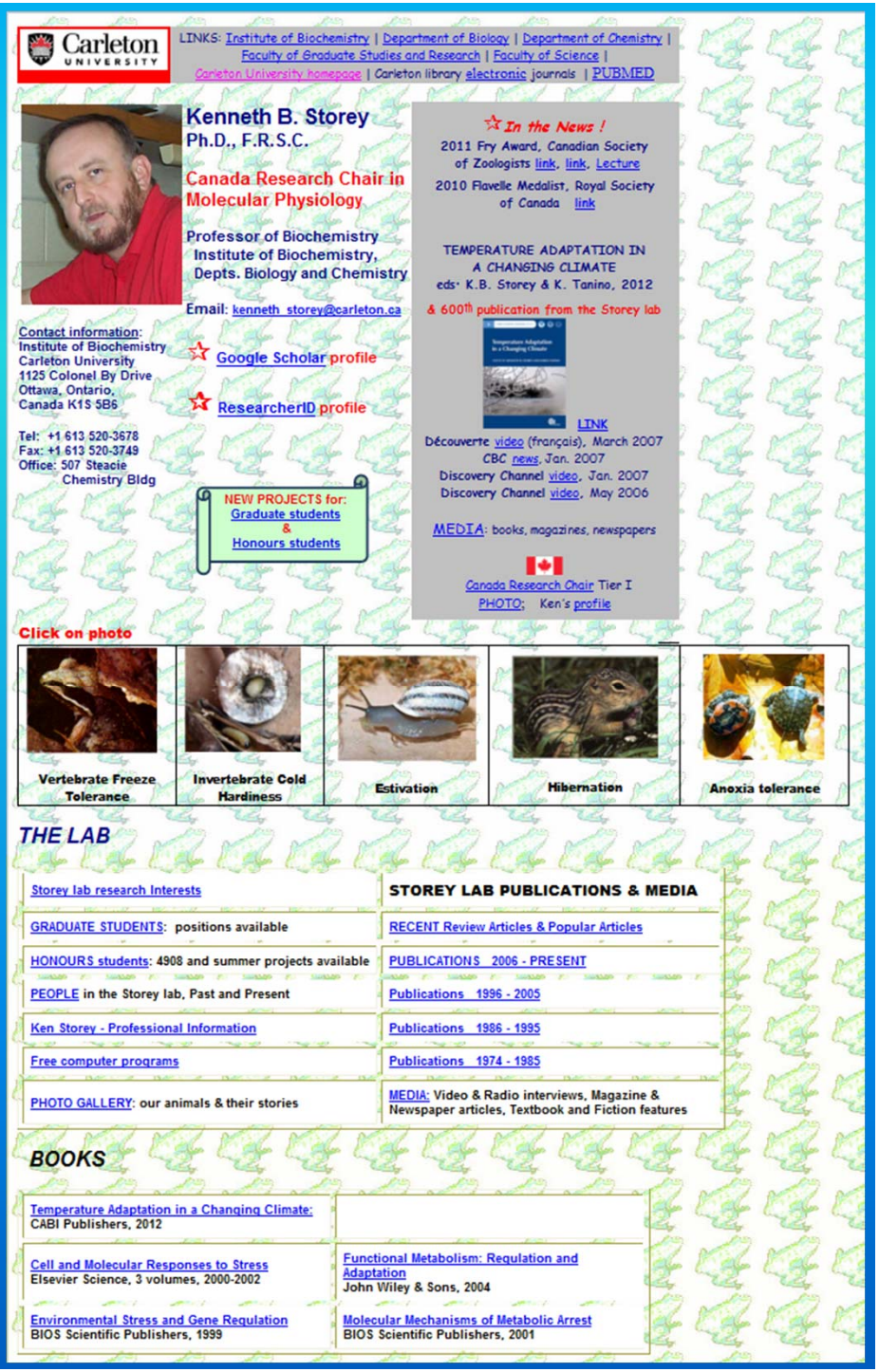
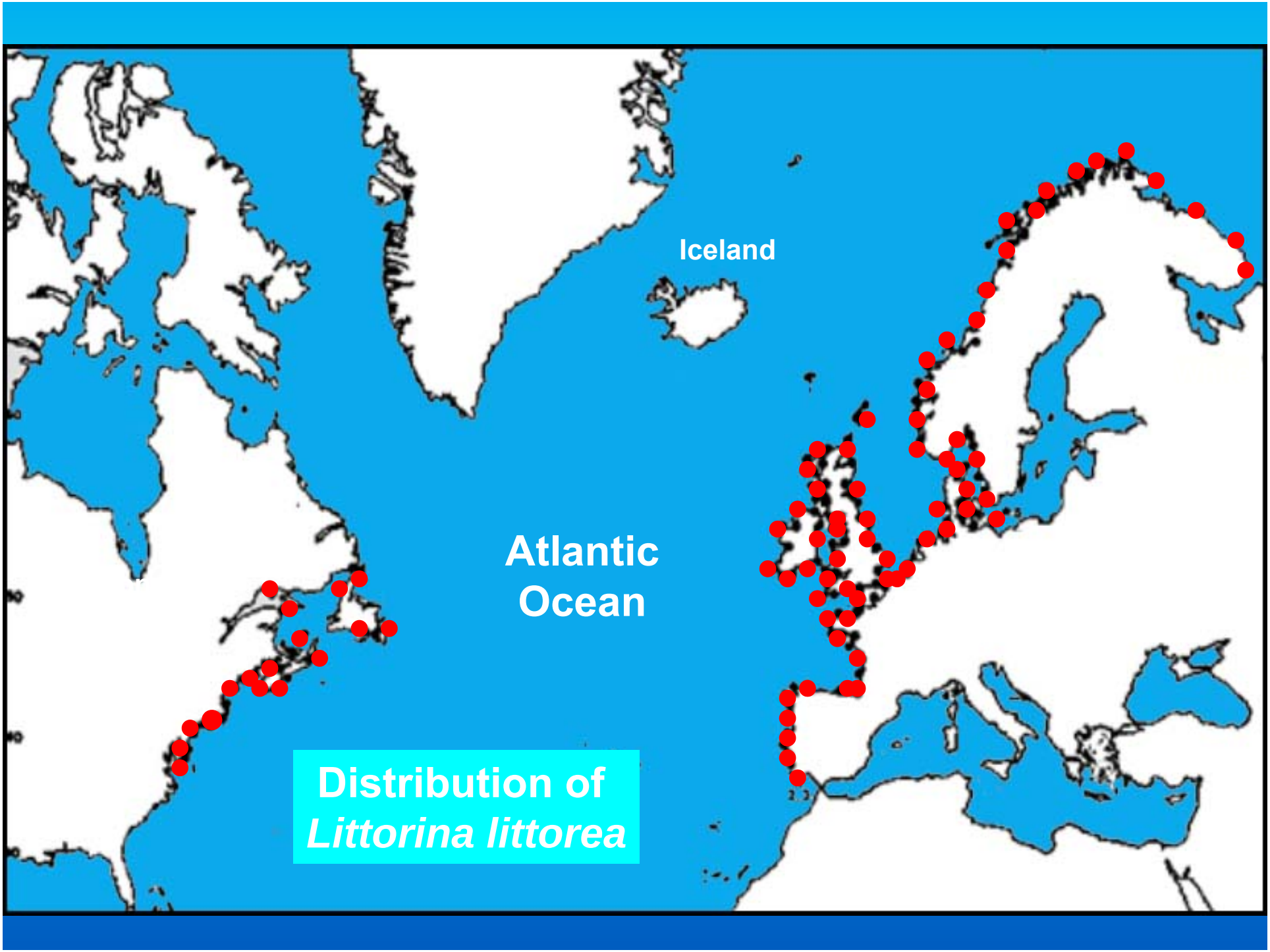



Iceland
Atlantic
Ocean
Distribution of
Littorina littorea

PRINCIPLES OF HYPOXIA SURVIVAL

1. Metabolic rate depression
2. Overall suppression of transcription & translation
3. Alternative end products
4. Reversible phosphorylation of enzymes
5. Selected genes up-regulated

$O_2 + e^- \rightarrow O_2^{\cdot -}$ superoxide radical

$O_2^{\cdot -} + H_2O \rightarrow HOO^{\cdot} + OH^-$ hydroperoxyl radical

$HOO^{\cdot} + e^- + H^+ \rightarrow H_2O_2$ hydrogen peroxide

$H_2O_2 + e^- \rightarrow {}^{\cdot}OH + OH^-$ hydroxyl radical

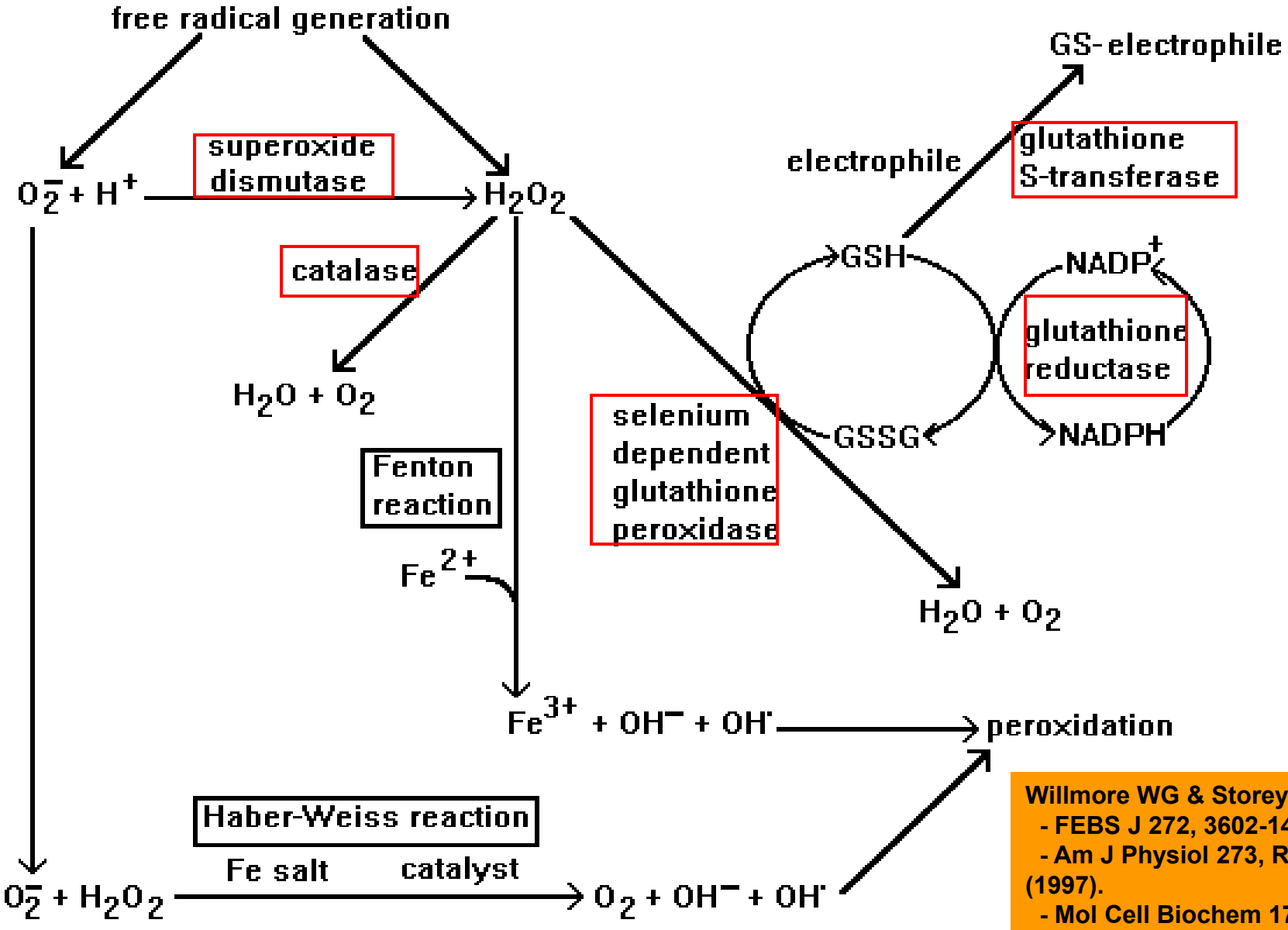
$Fe\,(III) + O_2^{\cdot -} \rightarrow Fe\,(II) + O_2$

$Fe\,(II) + H_2O_2 \rightarrow Fe\,(III) + {}^{\cdot}OH + OH^-$ Fenton reaction

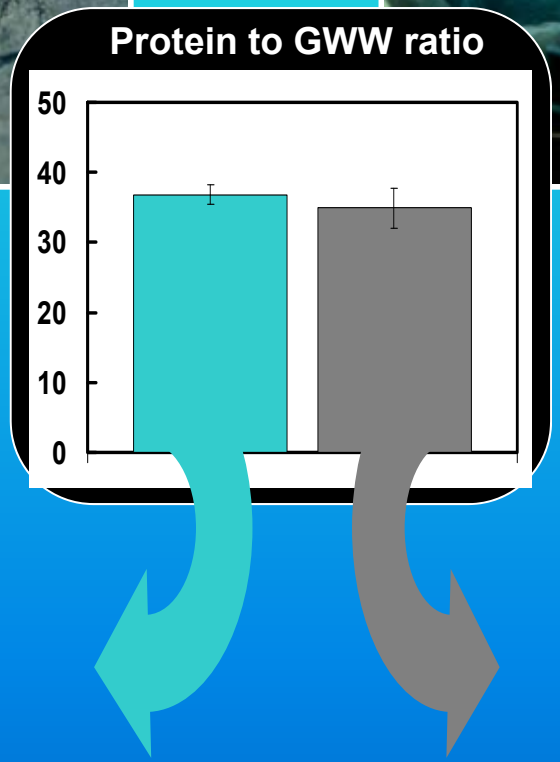
---

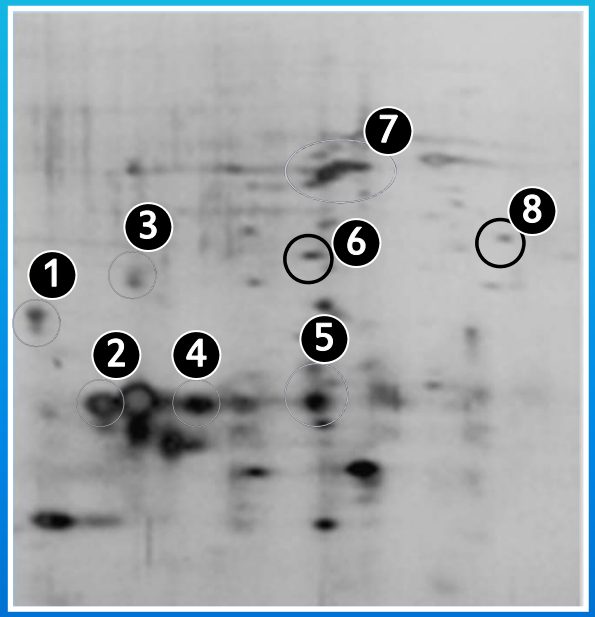
$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + {}^{\cdot}OH + OH^-$ Haber-Weiss reaction

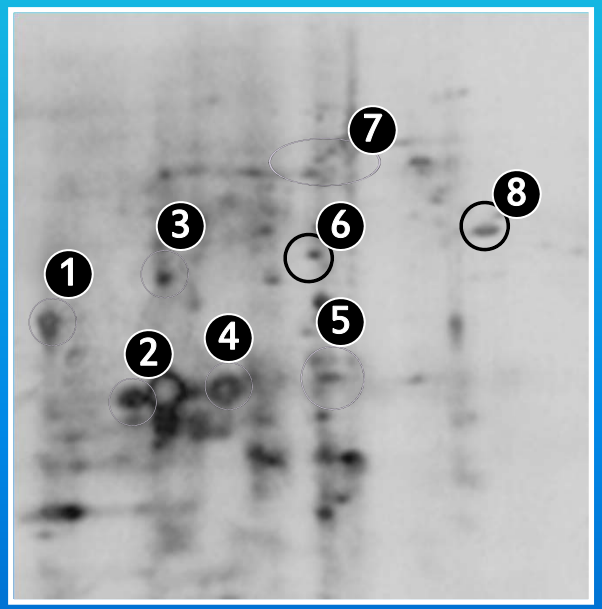
ANTIOXIDANT ENZYMES



Willmore WG & Storey KB
- FEBS J 272, 3602-14 (2005)
- Am J Physiol 273, R219-25 (1997).
- Mol Cell Biochem 170, 177-185 (1997)







Normoxia

48 Anoxia

Molecular and Cellular Biochemistry **232**: 121–127, 2002.

Reversible suppression of protein synthesis in concert with polysome disaggregation during anoxia exposure in *Littorina littorea*

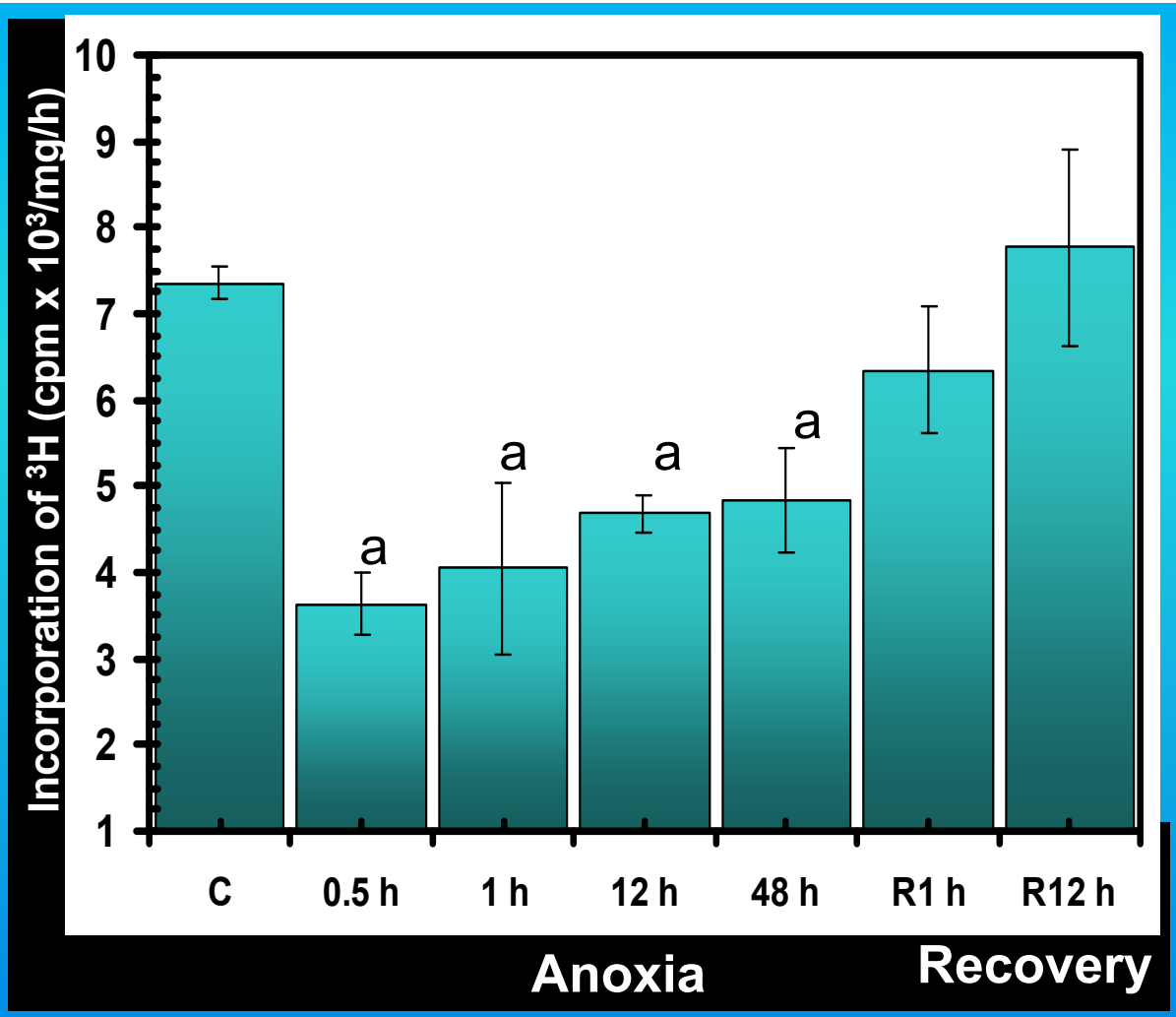
Kevin Larade and Kenneth B. Storey

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

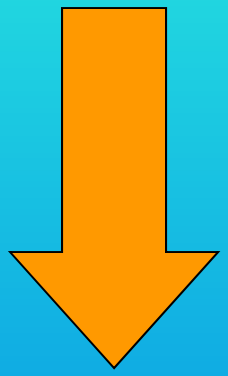
Received 11 October 2001; accepted 3 December 2001

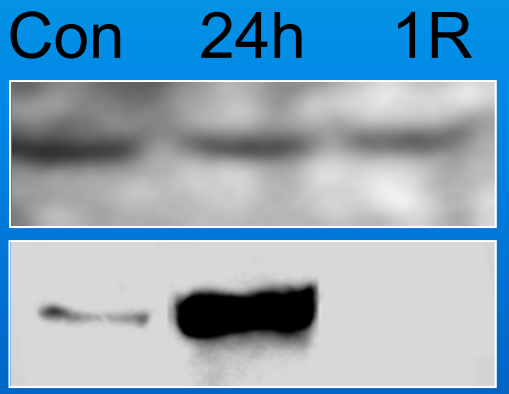
Abstract

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 [^{3}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)



Protein Synthesis





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

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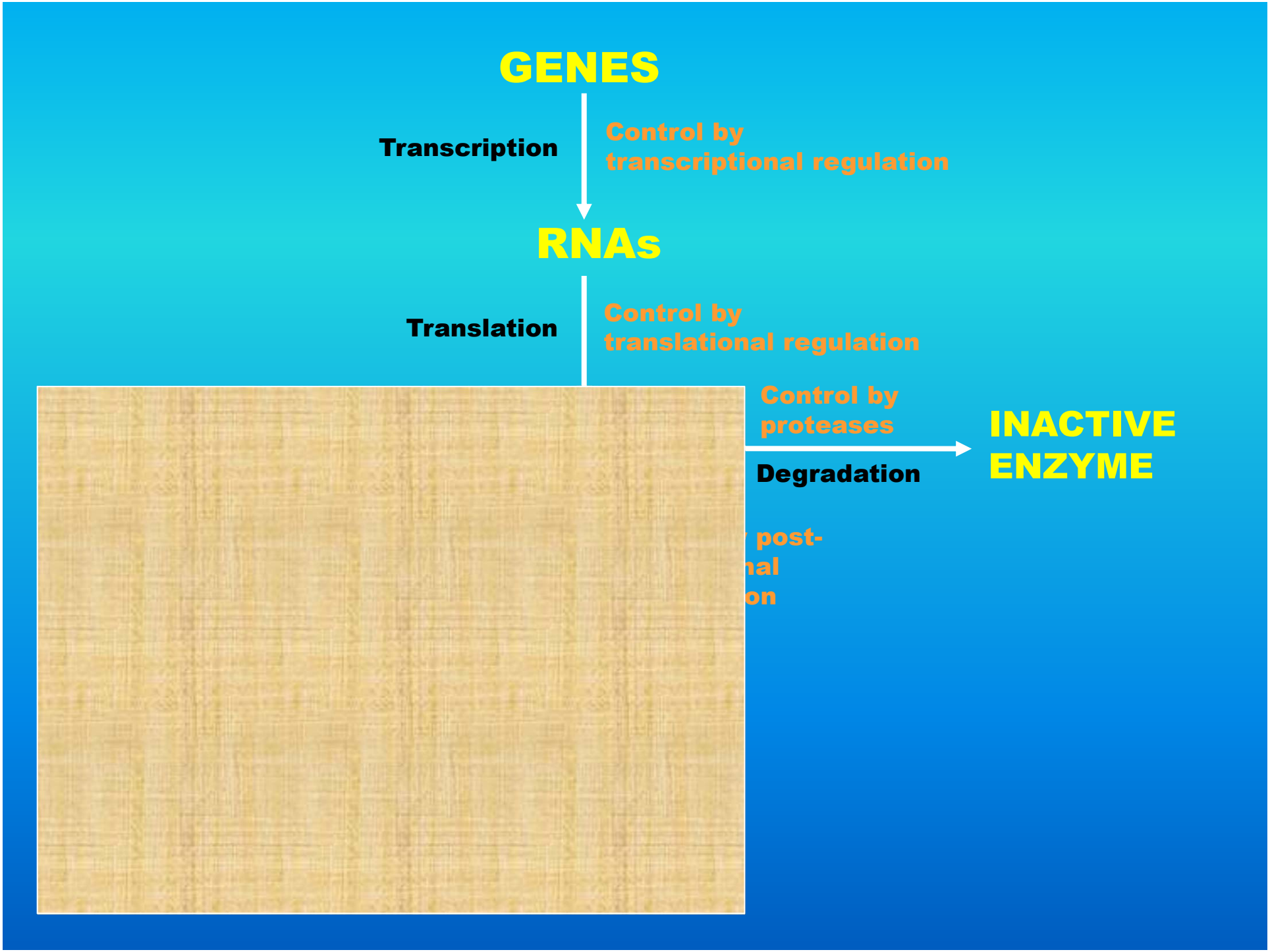
GENES
Transcription
Control by
transcriptional regulation
RNAs
Translation
Control by
translational regulation
Control by
proteases
INACTIVE
ENZYME
Degradation
post-
nal
on

Glutathione *S*-transferase

- 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