

LIFE IN THE COLD - An Ice Stor-e-y



www.carleton.ca/~kbstorey

FREEZE TOLERANT ANIMALS

- Insects (many)
- Intertidal molluscs & barnacles
- Amphibians & Reptiles
 - Frogs (6 species)
 - Hatchling turtles
 - Garter snakes
 - Lizards (some)

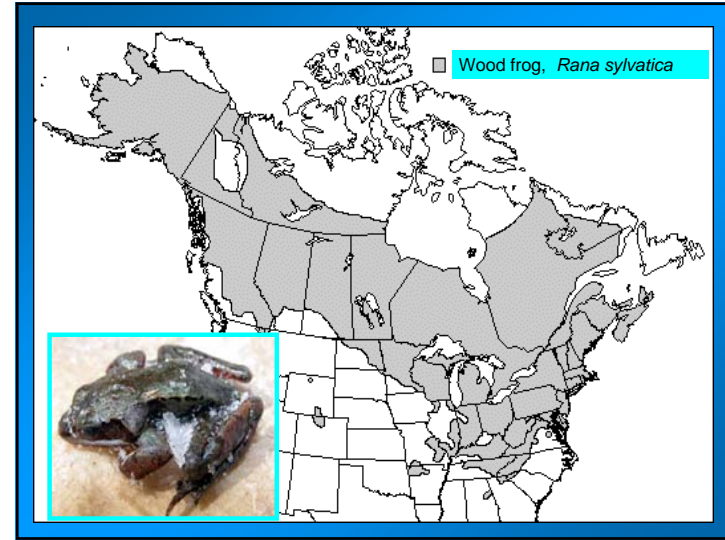


VERTEBRATE FREEZE TOLERANCE



WOOD FROG
Rana sylvatica





SURVIVING FREEZING

- Extracellular freezing only
- 65-70% of body water frozen
- Acclimation required
- Protectants
 - high glucose, glycerol, sorbitol
 - ice nucleators

UNFROZEN CELL

EXTRACELLULAR FREEZING BEGINS

no protection

with protection

Higher osmolality

water

Ice

Lower

INP

Glycerol

FREEZING WITHOUT PROTECTION

FREEZING WITH PROTECTION

WOOD FROG CRYOPROTECTANTS

- Blood glucose rises from ~5 mM to 200-400 mM
- Glucose triggered by ice formation
- Made from liver glycogen (180 mg/g)
- Liver is ~12% of body mass
- Glucose distribution via Blood:
 - Liver >
 - Core organs >
 - Periphery

Glucose, mM

Days frozen

Days thawed

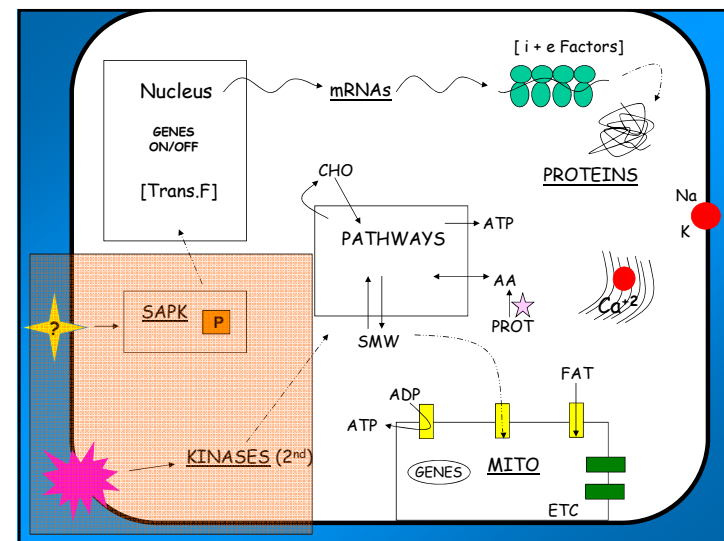
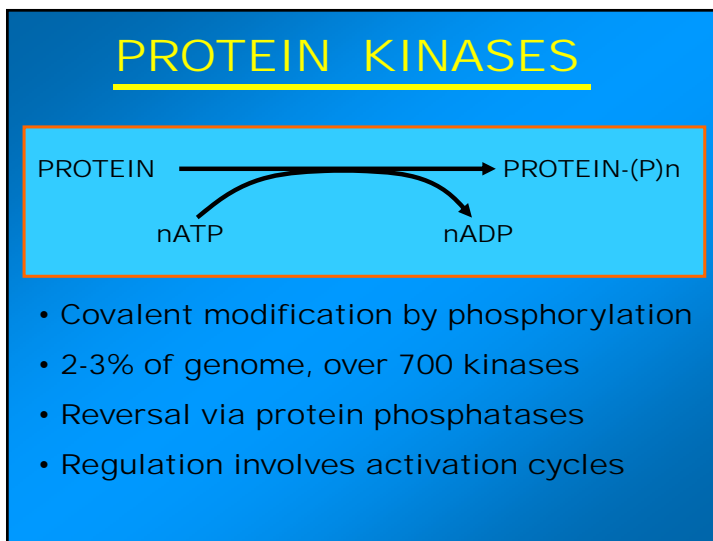
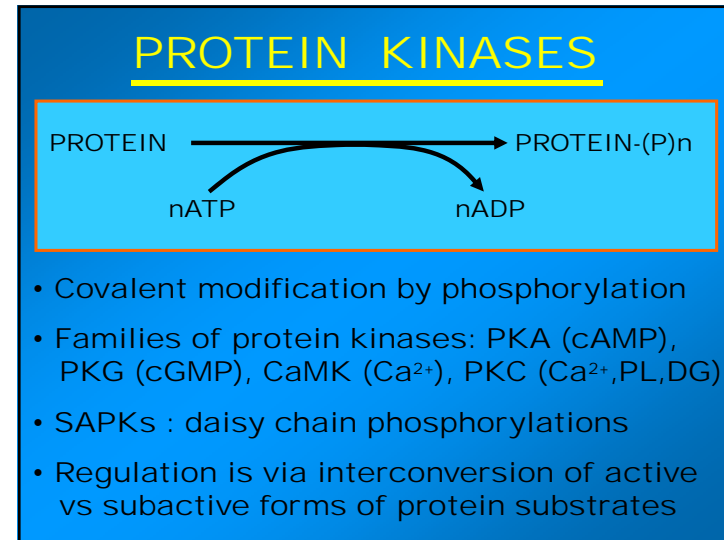
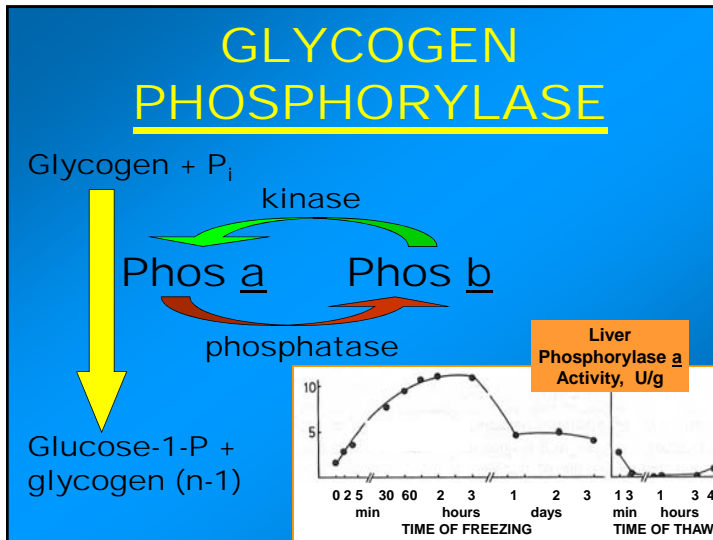
Blood

Liver

Heart

Kidney

Muscle



FREEZE INDUCED CHANGES

- Protein Synthesis slows to 1%
 - Pumps & channels closed
 - Energy Production slows to 5%
 - Energy Utilization slows to 2%
 - Few 'SAP' kinases activated
- Results: P38, AMPK, JUNK, ERK, PKA, PKC, PERK, Fuel Pathways
- Protein Phosphatases (1, 2A, 2C)

CELL PROCESSES



- DNA/RNA synthesis
- Protein synthesis
- Fuel metabolism
- Ion pumping
- Work done

ATP turnover ↓ to <5% of normal

FREEZE 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

FREEZE INDUCED GENE CHANGES

- Most Genes 'inactivated'
- mRNA decreases
- Epigenetic changes !
- Few Genes activated
 - transcription factors
 - shock proteins
 - antioxidants



FREEZE INDUCED GENE CHANGES

- Most Genes 'inactivated'
- mRNA decreases
- Epigenetic changes !
- Few Genes activated
 - transcription factors
 - shock proteins
 - antioxidants



TURNING OFF GENES: Role of Epigenetics

Epigenetics:

- **Stable changes in gene activity that do not involve changes in DNA sequence**

Common mechanisms:

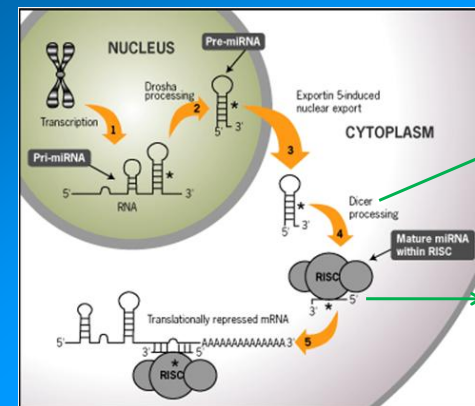
- **DNA methylation**
- **Histone modification / histone variants**
- **Regulatory non-coding RNAs**

microRNA (miRNA)

- **Small RNAs ~22 nucleotides in length**
- **Highly conserved across species**
- **Bind to 3' UTR of mRNAs**
- **All repression mechanism(s) yet to be defined, but include:**
 - **Block translation of mRNA**
 - **Help bind mRNA into stress granules**
 - **Target mRNA for degradation**



miRNA processing pathway



Steps of interest
in our lab:

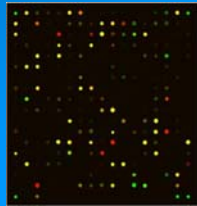
Dicer Protein
Expression

miRNA
Expression

Source: www.ambion.com

Storey Lab approach to miRNA

1) miRNA array comparing samples from control vs frozen/hibernating



2) Lead Identification

3) Q-PCR Validation

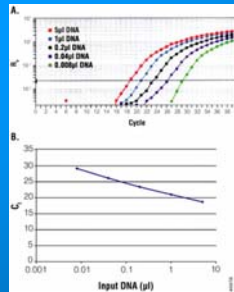


Image: www.esiqon.com

Image: www.promega.com

Are miRNAs differentially regulated in our animal models?

- **Yes!** Selected miRNAs were up-regulated in hibernating 13-lined ground squirrels
(Morin, Dubuc & Storey, 2008, Biochim Biophys Acta 1779:628-633)

• How about frogs?

New data for muscle of frozen wood frogs:

miRNA	Fold change	Process in higher mammals
Mir-1	5.0	Myogenesis
Mir-133a	5.4	Myogenesis
Mir-206	4.6	Myogenesis
Let-7	4.0	Cell cycle
Mir-26	3.4	Hypoxia
Mir-451	7.6	Erythropoiesis

FREEZE 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

GENE ACTIVATION (TRANSCRIPTION)

- **Global rate of mRNA synthesis depressed. Method: nuclear run-on**
- **Are selected genes up-regulated ?**
- **TO ASSESS GENE UP-REGULATION:**
What new mRNAs are created
- cDNA library, Gene Chip
Result: only **1 %** of Genes are **UP**

FREEZE INDUCED GENE CHANGES

- Most Genes 'inactivated'
- mRNA decreases
- Epigenetic changes !
- Few Genes activated
 - transcription factors
 - shock proteins
 - antioxidant enzymes

Carleton University | Institute of Biochemistry | Department of Biology | Department of Chemistry | Faculty of Graduate Studies and Research | Carleton University Homepage | Carleton Library | Carleton Library Journals | Carleton Library | Safety - MGS/Chimie | Storey Lab Chemical List

Kenneth B. Storey
Ph.D., F.R.S.C.
Canada Research Chair in Molecular Physiology

Professor of Biochemistry
Institute of Biochemistry,
and Departments of
"Biology and Chemistry"

Email: kenneth_storey@carleton.ca

Contact Information
Institute of Biochemistry
College of Natural Sciences
1125 Colonel By Drive
Ottawa, Ontario, Canada
K1S 5S6
Tel: +1 413 520-2628
Fax: +1 413 520-2569
Office: 508 Steacie
Chemistry Bldg

ISI Highly Cited Researcher
[Click to view record](#)

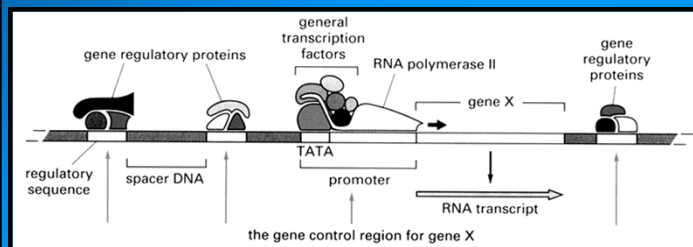
New book
FUNCTIONAL METABOLISM
REGULATION & ADAPTATION
edited by K.B. Storey. Wiley-Liss

In the News!
Diabetes *Diary*, NY Times May 2005
Suspended animation, US News & World Report, May 2005
DISCOVER *magazine*, Feb. 2005
Carleton U. *magazine*, Winter 2005
Washington Post *magazine*, Dec. 2004

THE LAB

Research interests	Professional Information
Positions available	NEW Reviews & Popular Articles
Lab personnel: Past and Present	RECENT PUBLICATIONS - 1996 - Present
Etc. computer programs	Publications - 1996 - 1995
Array II - info on running cDNA arrays	Publications - 1974 - 1995
PHOTO GALLERY - see animals & their studies	Book, magazine, newspaper articles & Etc.

Transcription Factors UP : GENES Respond



Epigenetics = OFF) :

- microRNA
- phospho-RNA Polymerase
- Histones modified
- HDAC / HAT changes



TRANSCRIPTION FACTORS

- ATF (Glucose Regulated Proteins)
- HIF (O₂), HSF (Hsp)
- NFkB (IκB-P), Nrf-2 (GST), NRF-1
- PPAR, PGC, RXR, chREBP, CREB-P
- STAT, SMAD, p53-P, HNF, AP (1,2)
- Methods: EMSA, PCR



TRANSCRIPTION FACTORS

- ATF (Glucose Regulated Proteins)
- HIF (O₂), HSF (Hsp)
- NFκB (IκB-P), **Nrf-2 (GST)**, NRF-1
- PPAR, PGC, RXR, chREBP, CREB-P
- STAT, SMAD, p53-P, HNF, AP (1,2)

• **Role :** ↓ MR, Diabetes, Organ Preservation

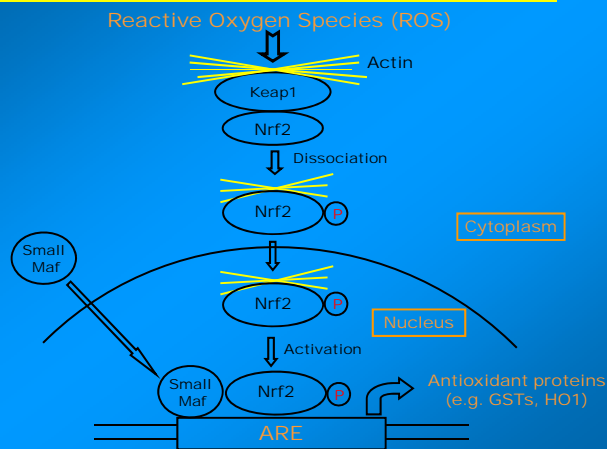


FREEZE-INDUCED GENES: WOOD FROGS

cDNA Library / Gene Chip

- Transcription Factors
NRF-2
- Antioxidant enzymes

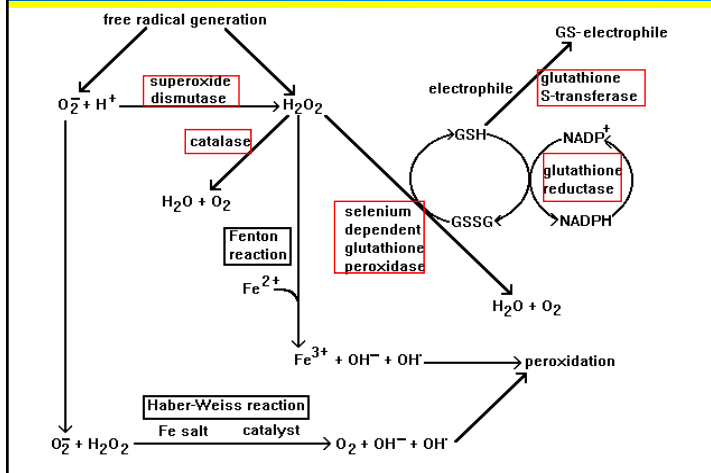
Nrf2 / ARE pathway



NRF-2

- Increased Nrf-2 protein & P-protein
- Increased Nrf-2 in the Nucleus
- Increased levels of co-Tf: MafG
- **Downstream gene activation:**
- mRNA Protein Synthesis.....
- GST, HO-1, HO-2, Peroxiredoxin

ANTIOXIDANT ENZYMES



Conclusions: Freezing

Activation of the Nrf2 pathway:

→ Tissue-specific activation, along with downstream gene protein products

→ Increased GST protein and activity



Result:

→ Detoxification of ROS, intracellular signaling control

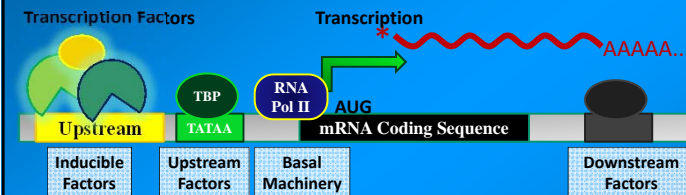
TRANSCRIPTION FACTORS

- ATF (Glucose Regulated Proteins)
- HIF (O₂), HSF (Hsp)
- NFκB (IκB-P), Nrf-2 (GST), NRF-1
- PPAR, PGC, RXR, chREBP, CREB-P
- STAT, SMAD, p53-P, HNF, AP (1,2)

• **Role :** ↓ MR, Diabetes, Organ Preservation



Regulation of Gene Transcription

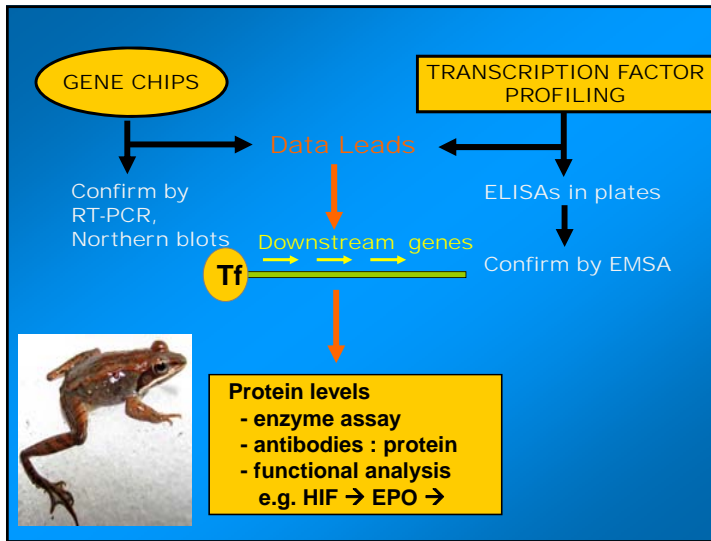


10 Beyond gene chips: transcription factor profiling in freeze tolerance

In: Hypometabolism in Animals: Hibernation, Torpor and Cryobiology (Lovegrove, B.G., and McKechnie, A.E., eds.) University of KwaZulu-Natal, Pietermaritzburg, pp. 101-108.
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



Where do we go from here?

- Novel proteins
- Novel phosphorylations
- Turning it all off -- microRNA
- Epigenetics & adaptation
- Life span extension
- Antioxidant defense
- Cell cycle suppression
- Climate Change & Winter



NEW DIRECTIONS

Freeze Tolerance & Medicine: Unique Animal Stress Model

Available online at www.sciencedirect.com

ELSEVIER

CRYOBIOLOGY

Cryobiology 49 (2004) 136–141

Strategies for exploration of freeze responsive gene expression: advances in vertebrate freeze tolerance

Kenneth B. Storey*

Organ Cryopreservation

Cryobiology

Journal homepage: www.elsevier.com/locate/cryo

Brief Communication

Glycation of wood frog (*Rana sylvatica*) hemoglobin and blood proteins: In vivo and in vitro studies*

Justin A. MacDonald^a, Thorsten Degenhardt^b, John W. Baynes^c, Kenneth B. Storey^{a*}

^a Department of Biochemistry and Molecular Biology, University of Guelph, 1386 Agricultural Drive, Guelph, ON, Canada N1G 2W1
^b Department of Family Science and Public Health Research Group, University of Guelph, Ontario, Canada, G1G 2R6, Guelph, Ontario
^c Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6

ABSTRACT

The effects of in vivo freezing and glucose crystallization on protein glycation were investigated in the wood frog, *Rana sylvatica*. Our studies revealed no difference in the fractional content of blood plasma analyzed from control, 21 h frozen and 18 h thawed wood frogs. Glycated hemoglobin (Glyc) decreased slightly with 48 h freezing exposure and was below control levels after 7 d recovery, while glycosylated serum albumin was unchanged by 48 h freezing but did increase after 7 d recovery. In vitro exposure to production in wood frog was similar to that of the 48 h that wood frog hemoglobin was glycosylated during freezing and recovery when in the glucose is highly saturated in vivo compared to in vitro. © 2004 Elsevier Inc. All rights reserved.

Diabetes model: resistance to damage by high glucose

NOVEL PROTEINS: FR10, FR47, Li16

Upregulation of a novel gene by freezing exposure in the freeze-tolerant wood frog (*Rana sylvatica*)

Qunta Cai^a, Kenneth B. Storey^{a*}

Abstract

A novel gene responsive to freezing exposure (FR10) was identified by screening a cDNA library constructed from liver of the freeze-tolerant wood frog (*Rana sylvatica*) for novel genes. The cDNA sequence and contained a single open reading frame of 306 bp, encoding a protein of 102 amino acids. Northern blot analysis revealed that FR10 was upregulated in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h. Northern blot analysis revealed that FR10 was upregulated in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h. Northern blot analysis revealed that FR10 was upregulated in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h.

Freeze-induced expression of a novel gene, *fr47*, in the liver of the freeze-tolerant wood frog, *Rana sylvatica*

J. Doyne McNally, Christopher M. Sturgeon, Kenneth B. Storey*

Abstract

The ability to tolerate the freezing of body fluids is well developed in an adaptation for winter survival in the freeze-tolerant wood frog (*Rana sylvatica*). The mechanisms supporting natural freeze tolerance have been shown to include antifreeze glycoprotein (AFGP) synthesis and protein synthesis in the liver of freeze-tolerant animals. A novel gene, *fr47*, was identified in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h. Northern blot analysis revealed that *fr47* was upregulated in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h, and in the liver of wood frogs exposed to freezing at -20 °C for 24 h and 48 h.

Identification and characterization of a novel freeze-inducible gene, *li16*, in the wood frog *Rana sylvatica*

J. Doyne McNally, Shao-Jia Wu, Christopher M. Sturgeon, and Kenneth B. Storey

Abstract

The wood frog (*Rana sylvatica*) survives for weeks during winter hibernation with up to 65% body water frozen in ice. Natural freeze tolerance includes both seasonal and freeze-induced molecular adaptations that control ice formation, deal with long-term ischemia, regulate cell volume changes, and protect neuromusculars. This report identifies and characterizes a novel freeze-inducible gene, *li16*, that codes for a protein of 116 amino acids. Northern blot analysis showed that *li16* transcript levels rose quickly during freezing to reach levels 3.7-fold higher than control values after 24 h, immunoblotting showed a parallel 2.4-fold rise in 116 protein. Regulatory influence on gene expression was assessed. Nuclear runoff assays confirmed that freezing initiated an increase in the rate of *li16* transcription, and analysis of signal transduction pathways via in vitro incubation of liver slices implicated a cAMP-mediated pathway in *li16* expression. Gene and protein expression in liver was also strongly stimulated by anoxia exposure, whereas the gene was less responsive to dehydration stress. The strong response of *li16* to both freezing and anoxia, and the rapid down-regulation of the gene when anoxia was discontinued, suggest that the *li16* protein may play a role in ischemia resistance during freezing.

Novel Phosphorylations

Regulation of hexokinase by reversible phosphorylation in skeletal muscle of a freeze-tolerant frog
 Christopher A. Denz¹, Kenneth B. Storey^{1*}
¹ Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6

Article Info
 Article history: Received 1 August 2009; Accepted 1 October 2009; Available online 15 October 2009

Creatine kinase regulation by reversible phosphorylation in frog muscle
 Christopher A. Denz¹, Kenneth B. Storey^{1*}
¹ Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Canada K1S 5B6

Article Info
 Article history: Received 1 October 2009; Accepted 15 October 2009; Available online 15 October 2009

Turning It All Off

Review
The emerging roles of microRNAs in the molecular responses of metabolic rate depression
 Kyle K. Biggar and Kenneth B. Storey^{a*}
^a Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6

Article Info
 Article history: Received 1 July 2009; Accepted 21 August 2009; Available online 9 September 2009

MicroRNA regulation below zero: Differential expression of miRNA-21 and miRNA-16 during freezing in wood frogs
 Kyle K. Biggar, Adrian Dubuc, Kenneth Storey^{a*}
^a Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6

Article Info
 Article history: Received 1 July 2009; Accepted 21 August 2009; Available online 9 September 2009

Epigenetics in Adaptation

Epigenetics in anoxia tolerance: a role for histone deacetylases.
 Invoruchio & Storey KB
Institute of Biochemistry, Carleton University, Ottawa, ON, Canada. invoruchio@gmail.com

Abstract
 The importance of epigenetics has been established in many key biological mechanisms to animal survival in low oxygen conditions has never been fully appreciated. In natural anoxia tolerance, we have examined transcriptional silencers, histone deacetylases (HDACs), in tissues of a turtle *Trachemys scripta elegans*. Transcript and protein levels of all five in skeletal muscle in response to 20 h of anoxia exposure. In addition, HDACs respond to 20 h of anoxia and levels of acetylated histone H3 (Lys 9) of liver displayed a milder response with HDAC1, -4, and -5 protein levels of acetylated histone H3 levels also decreased to 50-75% of control values heart. HDAC5 transcript levels increased 2.1-2.3 fold and HDAC5 protein

Evidence for a reduced transcriptional state during hibernation in ground squirrels
 Pier Jr Marin¹, Kenneth B. Storey^{1*}
¹ Institute of Biochemistry, Carleton University, Ottawa, ON, Canada. Available online 11 September 2009

Life Span Extension

Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.
 Invoruchio & Storey KB
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, ON, Canada.

Abstract
 While mammals cannot survive oxygen deprivation for more than a few minutes without sustaining severe organ damage, some animals have mastered anaerobic life. Freshwater turtles belonging to the champion facultative anaerobes of the vertebrate world, often surviving without physiological and biochemical mechanisms that underlie anoxia tolerance in depression, post-translational modification of proteins, strong antioxidant defense transcription factors, and enhanced expression of cytoprotective proteins. Turtles display characteristics of "negligible senescence". We propose that the long term anaerobiosis by turtles may also support the longevity of these animals. Natural anoxia tolerance, such as that of turtles, may play important roles in mammalian longevity. In the present review we discuss turtles and the potential links between anoxia tolerance and longevity.

STIMULATORY SIGNALS
 HSP, p53, p21, p27, p29, p30, p35, p39, p42, p44, p46, p51, p53, p57, p63, p65, p70, p75, p80, p85, p90, p95, p105, p107, p115, p130, p135, p140, p145, p150, p155, p160, p165, p170, p175, p180, p185, p190, p195, p200, p205, p210, p215, p220, p225, p230, p235, p240, p245, p250, p255, p260, p265, p270, p275, p280, p285, p290, p295, p300, p305, p310, p315, p320, p325, p330, p335, p340, p345, p350, p355, p360, p365, p370, p375, p380, p385, p390, p395, p400, p405, p410, p415, p420, p425, p430, p435, p440, p445, p450, p455, p460, p465, p470, p475, p480, p485, p490, p495, p500, p505, p510, p515, p520, p525, p530, p535, p540, p545, p550, p555, p560, p565, p570, p575, p580, p585, p590, p595, p600, p605, p610, p615, p620, p625, p630, p635, p640, p645, p650, p655, p660, p665, p670, p675, p680, p685, p690, p695, p700, p705, p710, p715, p720, p725, p730, p735, p740, p745, p750, p755, p760, p765, p770, p775, p780, p785, p790, p795, p800, p805, p810, p815, p820, p825, p830, p835, p840, p845, p850, p855, p860, p865, p870, p875, p880, p885, p890, p895, p900, p905, p910, p915, p920, p925, p930, p935, p940, p945, p950, p955, p960, p965, p970, p975, p980, p985, p990, p995, p1000.

Regulation by proteasome

Transcription
 Involvement in stress response, differentiation, cell growth and differentiation, cell apoptosis.

Unavoidable Metabolic Costs

RESEARCH ARTICLE
Regulation of Cell Cycle Components During Exposure to Anoxia or Dehydration Stress in the Wood Frog, *Rana sylvatica*
 RABIH ROUBAYEL, KYLE K. BIGGAR, AND KENNETH B. STOREY*
 Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario, Canada

ABSTRACT
 The wood frog (*Rana sylvatica*) exhibits a wet tolerance. The degree of stress tolerance depends on the proliferation of cells under hypometabolic states that helps to preserve. We hypothesized that the mechanisms involved in the establishment of the hypometabolic state required the proliferation of cells were evaluated using immunofluorescence comparing controls with animals subjected to gas atmosphere or dehydration to 40% of total body weight. Type A, B, D, and E decreased significantly under both stresses. Type C and F were significantly reduced in both muscle; however, an increase in the relative amount of Type B was observed in liver under both stresses. Type A and C were significantly reduced in both muscle and liver under both stresses. Type D and E were significantly reduced in both muscle and liver under both stresses. Type F was significantly reduced in both muscle and liver under both stresses. This study demonstrates that the regulation of cell cycle components is an integral part of the metabolic response to anoxia and dehydration stress (but not in muscle). This study is published in *Journal of Experimental Zoology*, 315:487-495.

Unity through Evolution

Int. J. Biol. Sci. 2010, 6, 9
International Journal of Biological Sciences
 © Copying International Publisher. All rights reserved.

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

WWCeD

ABSTRACT
 Studies of the molecular mechanisms that regulate the physiological responses to stress in the model organism, *Caenorhabditis elegans*, have long been used to understand the evolution of stress response and metabolic rate, triggered by ambient conditions. *C. elegans* employs a number of signaling pathways to regulate its metabolic rate under stress. The suppression of cellular metabolism during survival of nematodes through the dauer stage is a conserved mechanism that is fundamental to control of metabolism. In general, mammalian systems are highly sensitive to stress and low oxygen, however, signal transduction pathways of nematode protein targets in the stress response are conserved, and often differ only in the details. This review outlines a framework of critical molecular targets for addressing the

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

GENE CHIPS → Data Leads → **TRANSCRIPTION FACTOR PROFILING**

Confirm by RT-PCR, Northern blots → Downstream genes → **Tf** → Confirm by EMSA

ELISAs in plates → Protein levels - enzyme assay - antibodies : protein - functional analysis e.g. HIF → EPO →

ROLE & CONTROL OF SYSTEM

Transgenics → Cell Assay → RNAi → Knock out → Epigenetics → **FUNCTIONAL ASSAYS**

FREEZE TOLERANCE

- J. STOREY
- D. McNALLY
- J. MacDONALD
- T. CHURCHILL
- S. GREENWAY
- C. HOLDEN
- S. WU
- J. NILES
- J. DU
- A. DeCROOS

- K. BIGGAR
- O. AGUILAR
- R. ROUFAYEL
- C. BROOKS
- L. ZHENHONG
- Q. CAI
- F. SCHUELER
- S. BROOKS
- B. RUBINSKY
- R. BROOKS

Funded by NSERC Canada

www.carleton.ca/~kbstorey