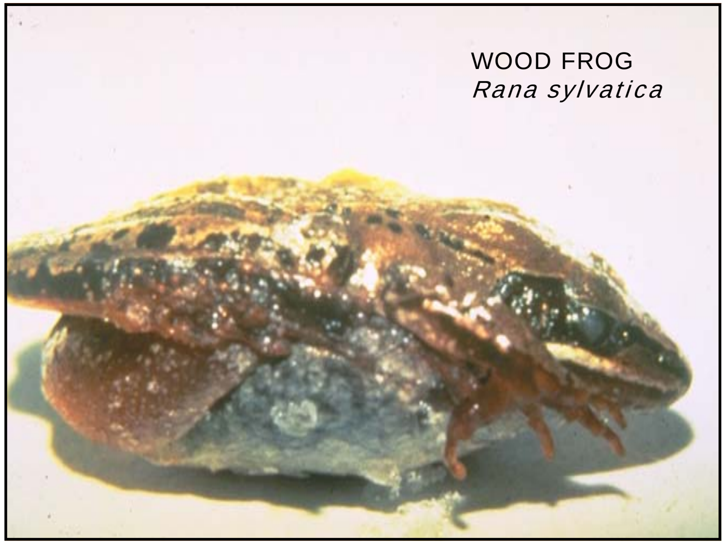
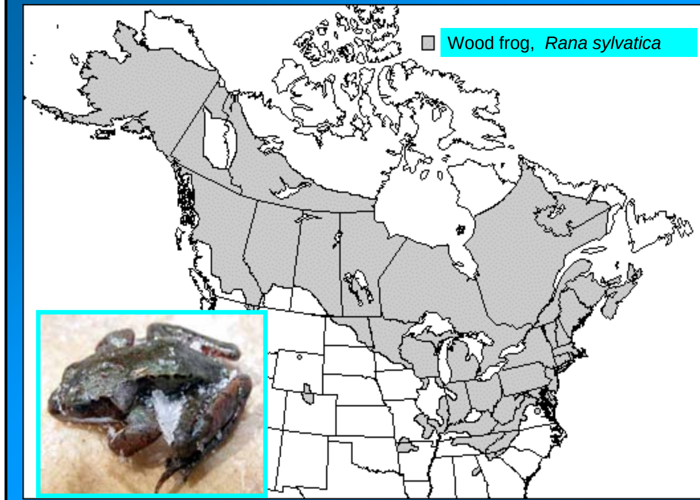

LIFE IN THE COLD - An Ice Stor-e-y
www.carleton.ca/~kbstorey


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


VERTEBRATE FREEZE TOLERANCE

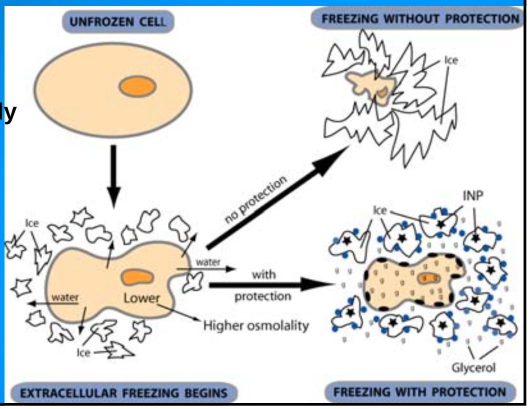

WOOD FROG
Rana sylvatica





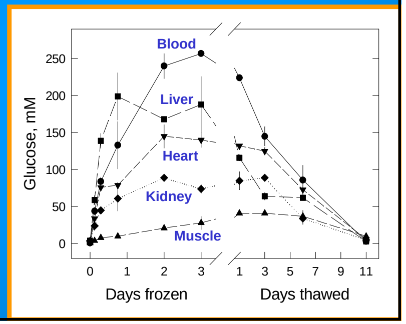
SURVIVING FREEZING

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



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



GLYCOGEN PHOSPHORYLASE

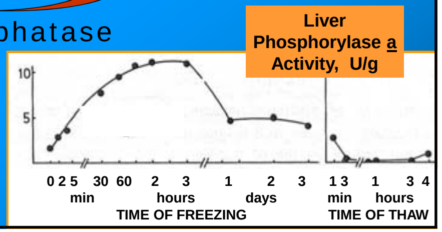
Glycogen + P_i

kinase

Phos a ⇄ Phos b

phosphatase

Glucose-1-P + glycogen (n-1)



PROTEIN KINASES

PROTEIN → PROTEIN-(P)n

nATP → nADP

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

PROTEIN KINASES

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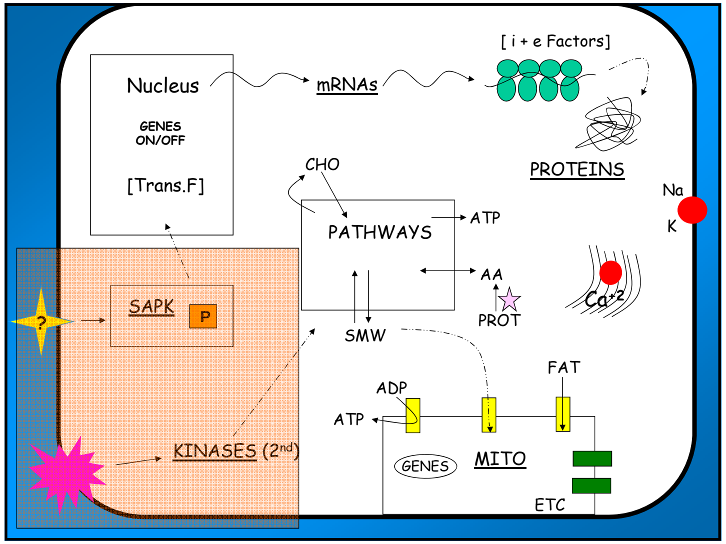
- Covalent modification by phosphorylation
- 2-3% of genome, over 700 kinases
- Reversal via protein phosphatases
- Regulation involves activation cycles



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

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- 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

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TURNING OFF GENES: Role of Epigenetics

Epigenetics:

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

Common mechanisms:

- DNA methylation

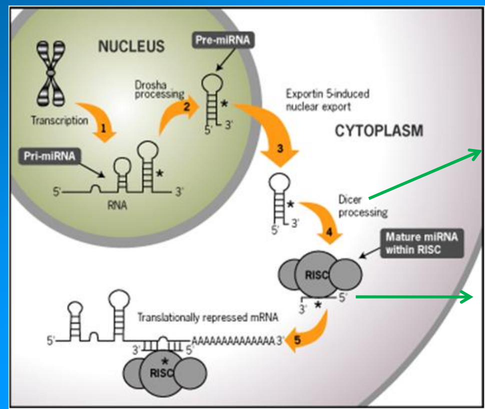
- Histone modification / histone variants

- 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:

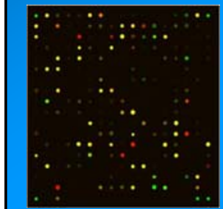
Dicer Protein Expression

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

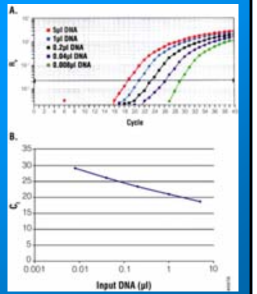


Image: www.exiqon.com

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

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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

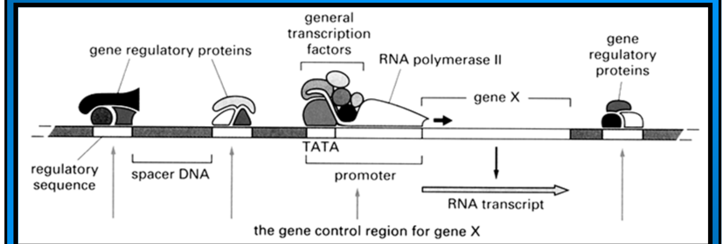
Result: only 1 % of Genes are UP

FREEZE INDUCED GENE CHANGES

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

cDNA Arrays
- Methods
- Materials
- Sources
- Publications

Transcription Factors UP : GENES Respond



Epigenetics = OFF) :
- **microRNA**
- **phospho-RNA Polymerase**
- **Histones modified**
- **HDAC / HAT changes**

TRANSCRIPTION FACTORS

- **ATF (Glucose Regulated Proteins)**
- **HIF (O_2), HSF (Hsp)**
- **NFkB (IkB-P), Nrf-2 (GST), NRF-1**
- **PPAR, PGC, RXR, chREBP, CREB-P**
- **STAT, SMAD, p53-P, HNF, AP (1,2)**

- **Methods: EMSA, PCR**

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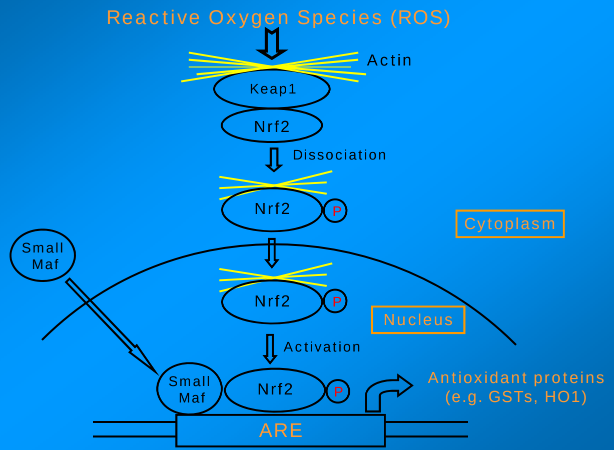
- **Role : ↓ MR, Diabetes, Organ Preservation**

FREEZE-INDUCED GENES: WOOD FROGS

cDNA Library / Gene Chip

- Transcription Factors
  NRF -2
- Antioxidant enzymes

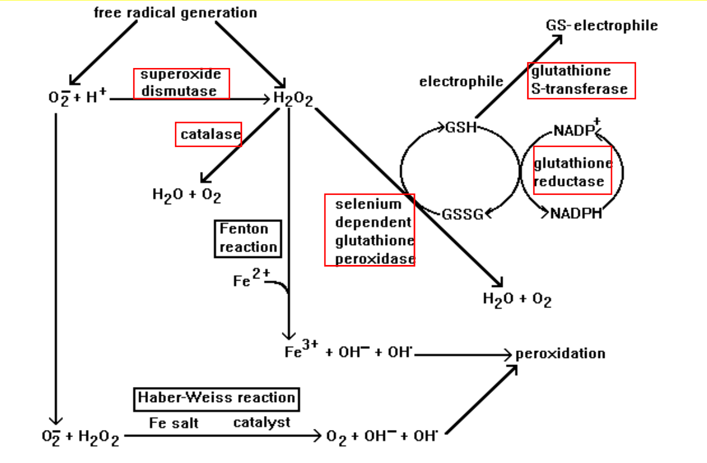
Nrf2 / ARE pathway



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**

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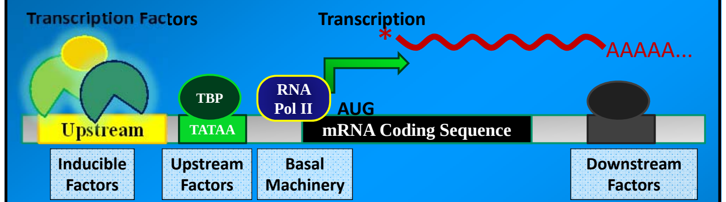
Regulation of Gene Transcription



10 **Beyond gene chips: transcription factor profiling in freeze tolerance**

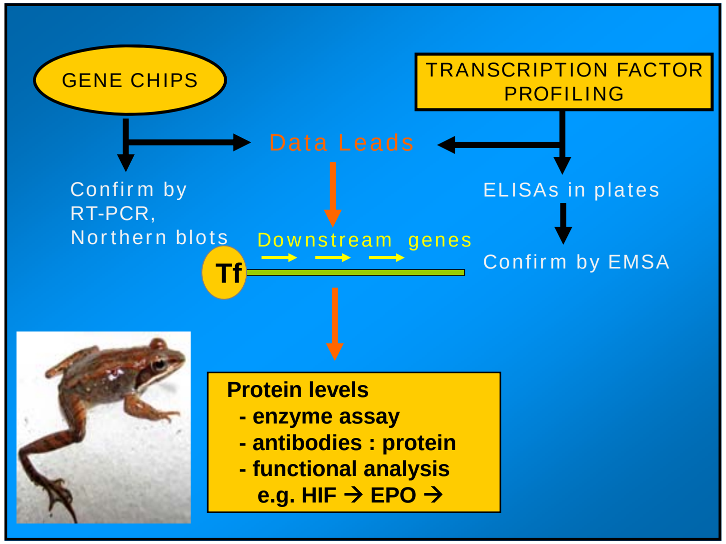
In: Hypometabolism in Animals: Hibernation, Torpor and Cryobiology (Lovegrove, B.G., and McKechnie, A.E., eds.) University of KwaZulu-Natal, Pietermaritzburg, pp. 101-108.

KENNETH B. STOREY

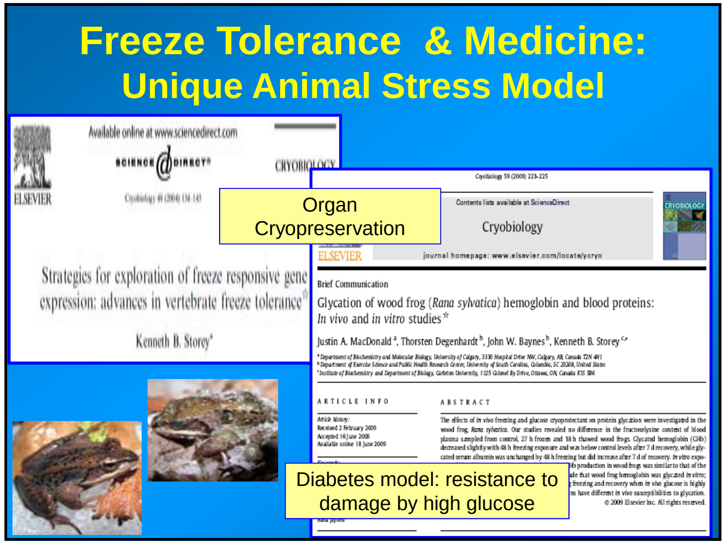
Institute of Biochemistry, Carleton University, Ottawa, Canada K1S 5B6; kenneth_storey@carleton.ca

Abstract

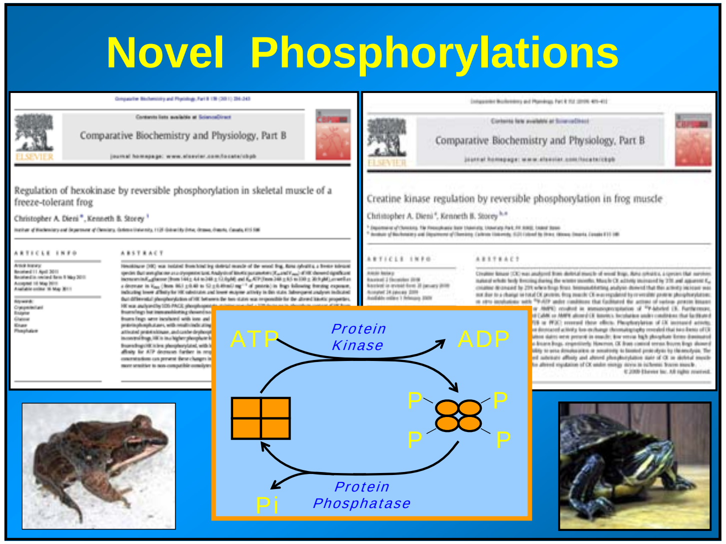
The Wood Frog, *Rana sylvatica*, is one of several terrestrially hibernating anurans that display natural freeze tolerance. The multifaceted biochemical responses to the cellular

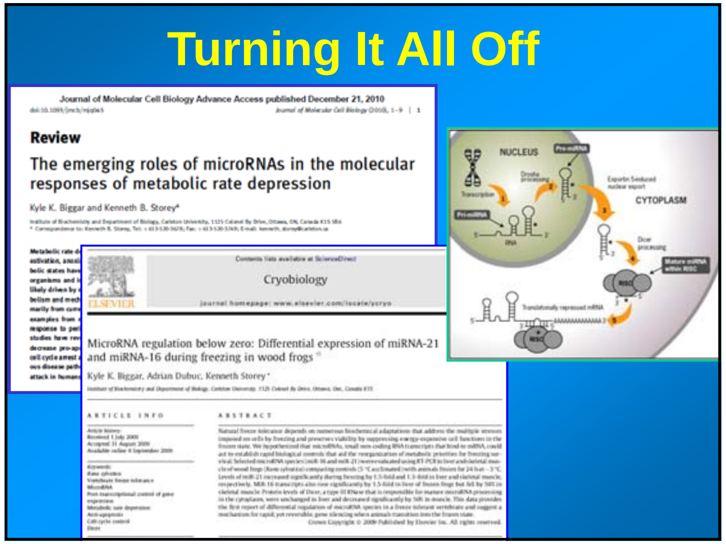

GENE CHIPS
TRANSCRIPTION FACTOR PROFILING
Data Leads
Confirm by RT-PCR, Northern blots
ELISAs in plates
Downstream genes
Tf
Confirm by EMSA
Protein levels
- enzyme assay
- antibodies : protein
- functional analysis
e.g. HIF → EPO →

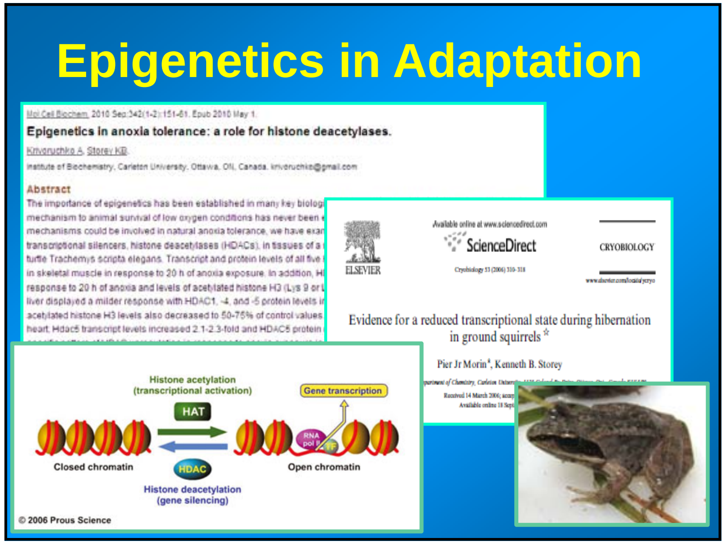

Where do we go from here?
• Novel proteins
• Novel phosphorylations
• Turning it all off -- microRNA
• Epigenetics & adaptation
• Life span extension
• Antioxidant defense
• Cell cycle suppression
• Climate Change & Winter
NEW DIRECTIONS


Freeze Tolerance & Medicine:
Unique Animal Stress Model
Organ Cryopreservation
Strategies for exploration of freeze responsive gene expression: advances in vertebrate freeze tolerance
Kenneth B. Storey
Cryobiology
Glycation of wood frog (Rana sylvatica) hemoglobin and blood proteins: In vivo and in vitro studies
Justin A. MacDonald, Thorsten Degenhardt, John W. Baynes, Kenneth B. Storey
Diabetes model: resistance to damage by high glucose


NOVEL PROTEINS:
FR10, FR47, Li16
GENE
Upregulation of a novel gene by freezing exposure in the freeze-tolerant wood frog (Rana sylvatica)
BBA
Freeze-induced expression of a novel gene, fr47, in the liver of the freeze-tolerant wood frog, Rana sylvatica
Identification and characterization of a novel freezing-inducible gene, li16, in the wood frog Rana sylvatica


Novel Phosphorylations
Comparative Biochemistry and Physiology, Part B
Regulation of hexokinase by reversible phosphorylation in skeletal muscle of a freeze-tolerant frog
Christopher A. Dieni, Kenneth B. Storey
Comparative Biochemistry and Physiology, Part B
Creatine kinase regulation by reversible phosphorylation in frog muscle
Christopher A. Dieni, Kenneth B. Storey
ATP
Protein Kinase
ADP
P
P
P
P
Pi
Protein Phosphatase


Turning It All Off
Journal of Molecular Cell Biology Advance Access published December 21, 2010
Review
The emerging roles of microRNAs in the molecular responses of metabolic rate depression
Kyle K. Biggar and Kenneth B. Storey
Cryobiology
MicroRNA regulation below zero: Differential expression of miRNA-21 and miRNA-16 during freezing in wood frogs
Kyle K. Biggar, Adrian Dubuc, Kenneth Storey
NUCLEUS
CYTOPLASM


Epigenetics in Adaptation
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
ScienceDirect
CRYOBIOLOGY
Evidence for a reduced transcriptional state during hibernation in ground squirrels
Pier Jr Morin, Kenneth B. Storey
Histone acetylation (transcriptional activation)
HAT
Gene transcription
Closed chromatin
Open chromatin
HDAC
Histone deacetylation (gene silencing)
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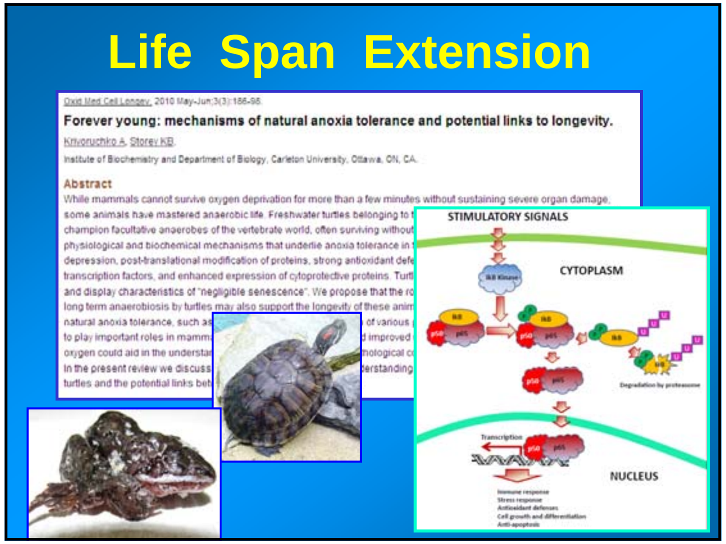
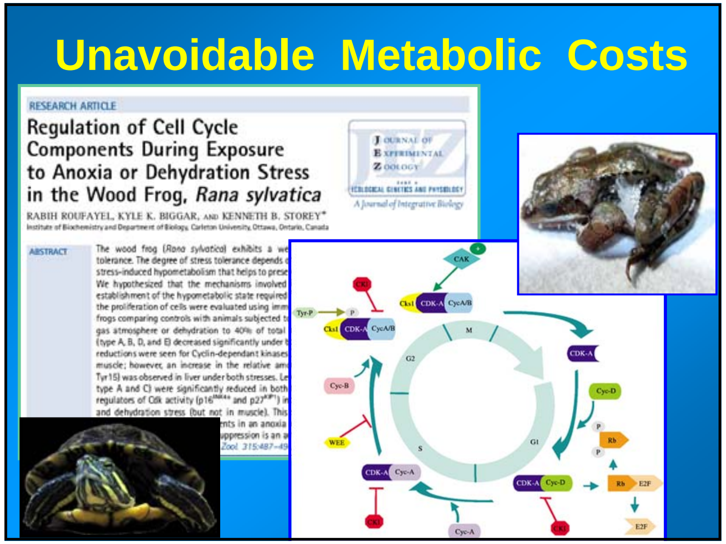
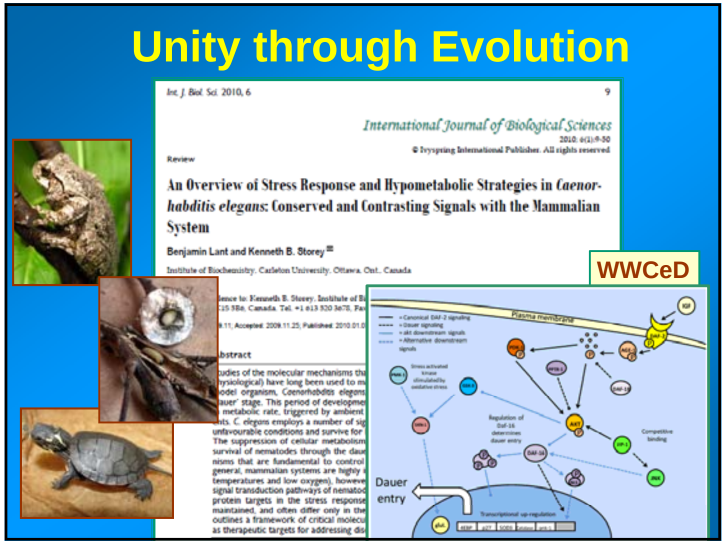
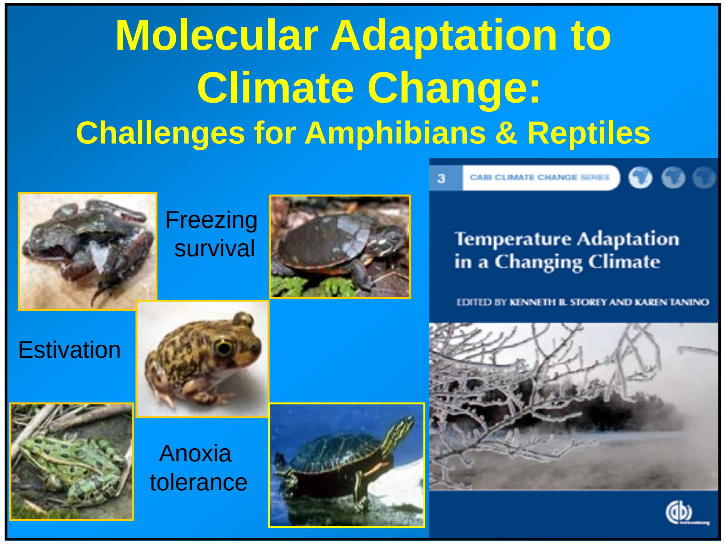
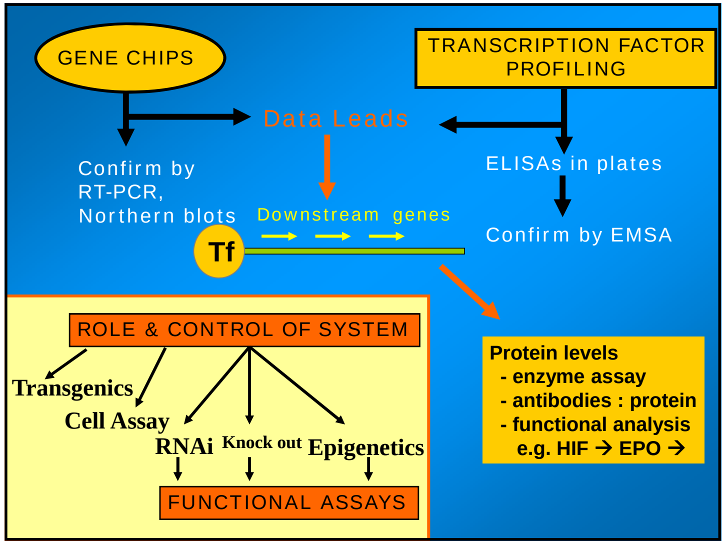

Life Span Extension
Oxid Med Cell Longev. 2010 May-Jun;3(3):186-98.
Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.
Krivoruchko A, Storey KB.
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, ON, CA.
Abstract
STIMULATORY SIGNALS
CYTOPLASM
NUCLEUS


Unavoidable Metabolic Costs
RESEARCH ARTICLE
Regulation of Cell Cycle Components During Exposure to Anoxia or Dehydration Stress in the Wood Frog, Rana sylvatica
RABIH ROUFAYEL, KYLE K. BIGGAR, AND KENNETH B. STOREY
ABSTRACT
CAK
CDK-A
CycA/B
CDK-A
Cyc-D
Rb
E2F
Cyc-A
Cyc-B
WEE
G1
G2
S
M


Unity through Evolution
Int. J. Biol. Sci. 2010, 6
International Journal of Biological Sciences
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
WWCeD
Plasma membrane
Dauer entry


Molecular Adaptation to Climate Change:
Challenges for Amphibians & Reptiles
Freezing survival
Estivation
Anoxia tolerance
CABI CLIMATE CHANGE SERIES
Temperature Adaptation in a Changing Climate
EDITED BY KENNETH B. STOREY AND KAREN TANINO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FUNCTIONAL ASSAYS
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- enzyme assay
- antibodies : protein
- functional analysis
e.g. HIF → EPO →


FREEZE TOLERANCE
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• D. McNALLY
• J. MacDONALD
• T. CHURCHILL
• S. GREENWAY
• C. HOLDEN
• S. WU
• J. NILES
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