MAMMALS ON ICE : *HIBERNATION*

www.carleton.ca/~kbstorey

Model Hibernators

Spermophilus richardsonii, Richardson's ground squirrel

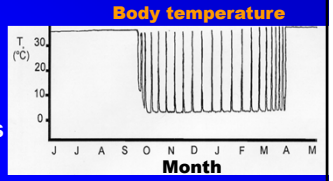
Spermophilus tridecemlineatus, 13-lined ground squirrel

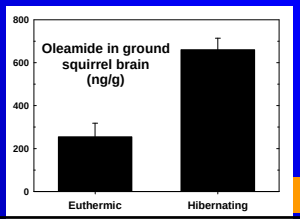
Myotis lucifugus, little brown bat

- Seasonal phenomenon
- Pre-hibernation hyperphagia
- Gain up to 40% of body mass
- Need polyunsaturated fats
- Find hibernaculum: dark, near 0°C

What happens?

- drop in body temperature
- reduced heart rate
- apnoic breathing
- some muscle atrophy
- periods of torpor lasting weeks
- non-REM sleep
- oleamide increases in brain

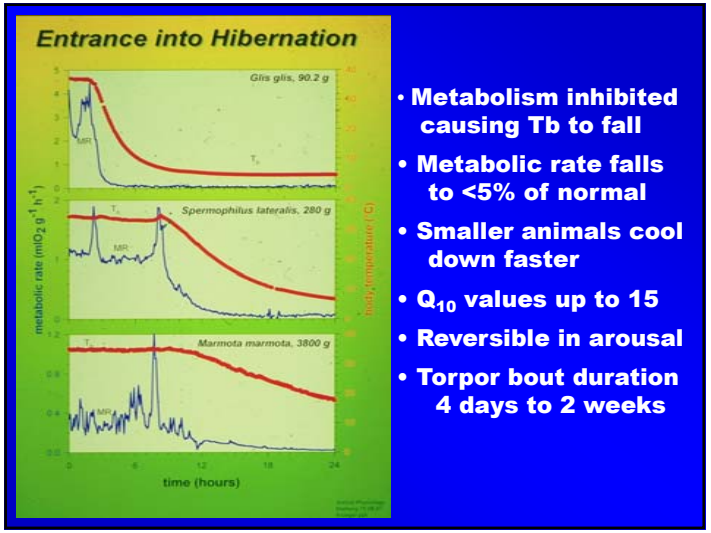




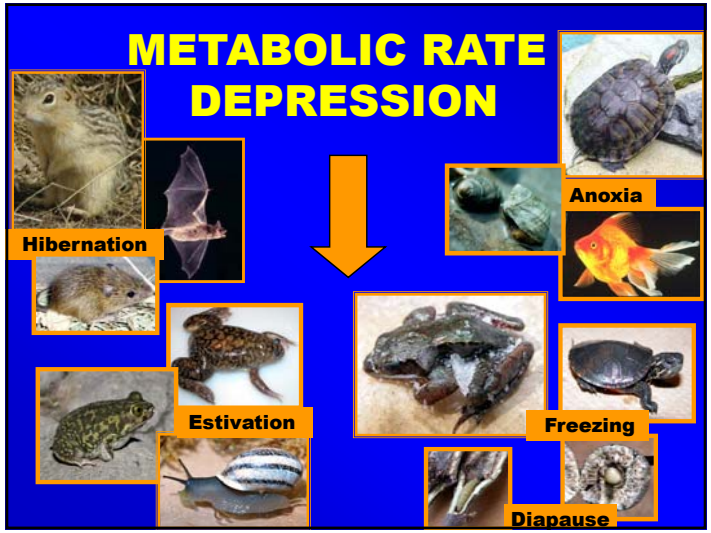
- suppression of carbohydrate oxidation
- RQ of 0.7 = lipid oxidation

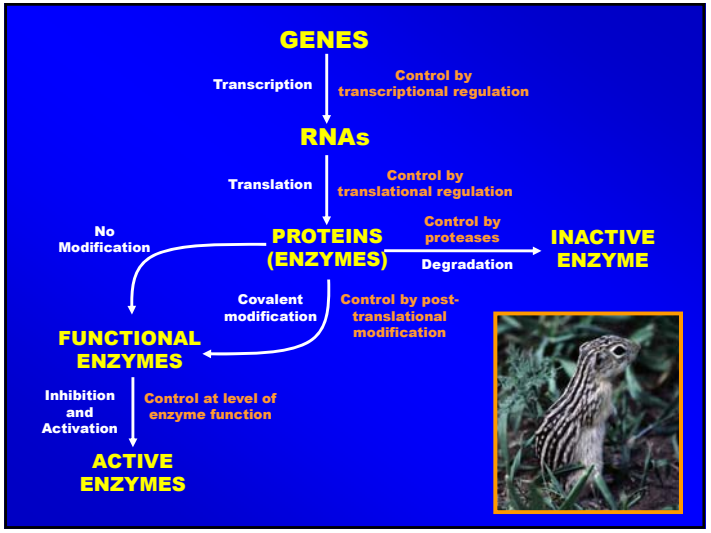
MR falls to fraction of normal

Stewart JM, Boudreau NM, Blakely JA & Storey KB. 2002. J. Thermal Biol. 27, 309-315.



- Metabolism inhibited causing Tb to fall
- Metabolic rate falls to <5% of normal
- Smaller animals cool down faster
- Q_{10} values up to 15
- Reversible in arousal
- Torpor bout duration 4 days to 2 weeks





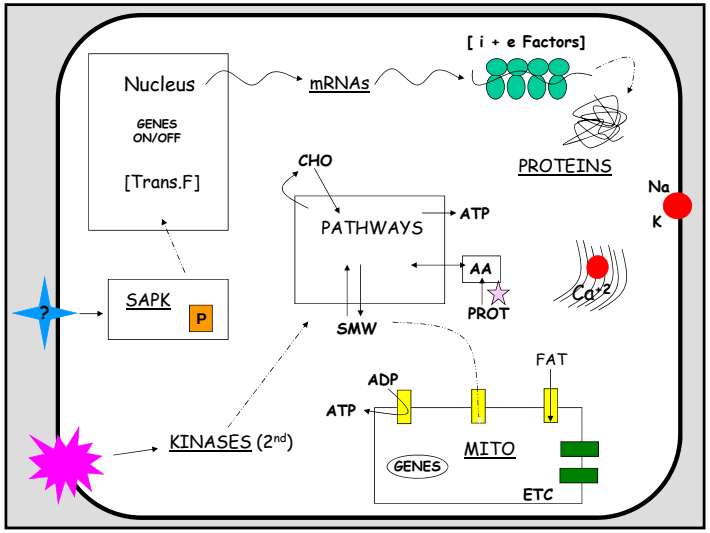
METABOLISM IN HIBERNATION

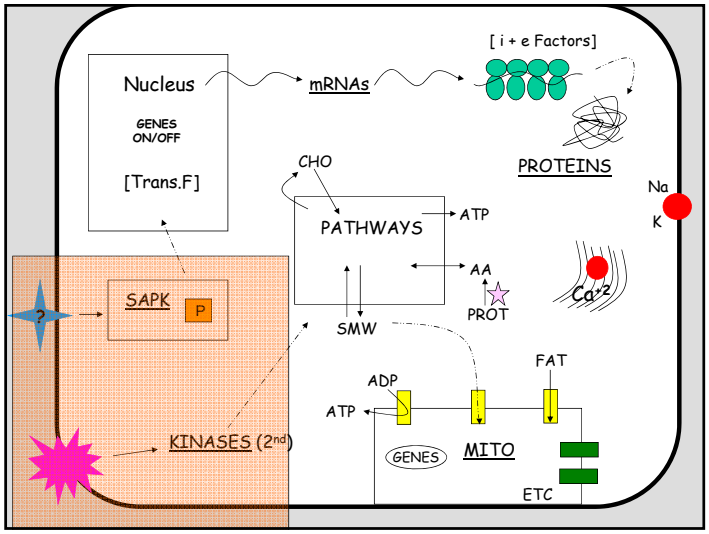
- mRNA synthesis
- Protein synthesis
- Ion Pumping
- Fuel use (esp. CHO)
- O_2 consumed

ATP turnover ↓ to <5% of normal

PRINCIPLES OF HIBERNATION

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



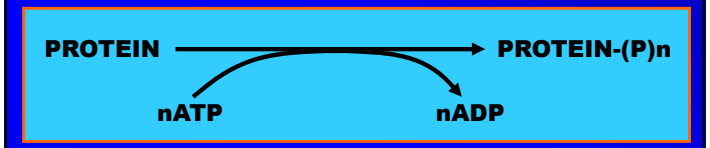


HIBERNATION INDUCED CHANGES

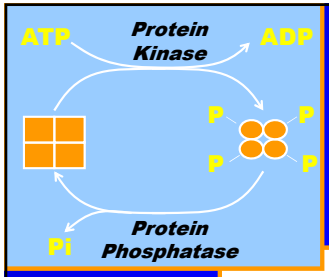
- Protein Synthesis slows to 1%
- Pumps & Channels closed
- Energy Production slows to 5%
- Energy Utilization slows to 2%
- Few 'SAP' kinases activated

- Gene 'inactivation' (↓ mRNA)
- Few Genes activated (1-2%)

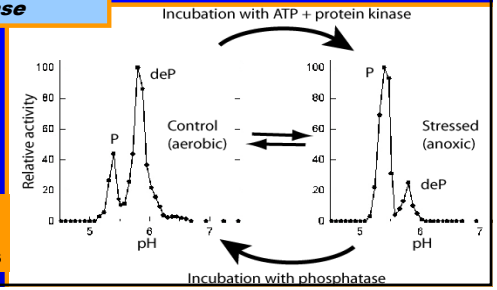
PROTEIN KINASES



- 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)



Reversible phosphorylation control of enzymes



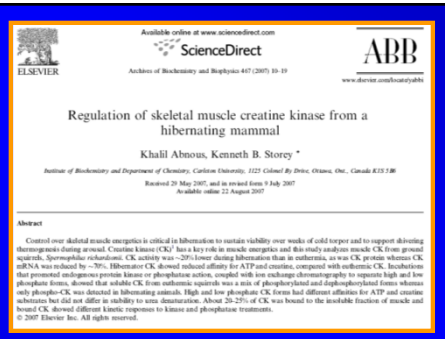
P & deP enzymes separate on ion exchange columns

PATHWAY CONTROL IN HIBERNATION

Phospho / de-Phospho

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

- *the usual suspects, TextBook*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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

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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 viability over weeks of cold torpor and to support shivering thermogenesis during arousal. Creatine kinase (CK_t) has a key role in muscle energetics and this study analyzes muscle CK from ground squirrels, *Spermophilus richardsonii*. CK activity was ~20% lower during hibernation than in euthermia, as was CK protein whereas CK mRNA was reduced by ~70%. Hibernator CK showed reduced affinity for ATP and creatine, compared with euthermic CK. Incubations that promoted endogenous protein kinase or phosphatase action, coupled with ion exchange chromatography to separate high and low phosphate forms, showed that soluble CK from euthermic squirrels was a mix of phosphorylated and dephosphorylated forms whereas only phospho-CK was detected in hibernating animals. High and low phosphate CK forms had different affinities for ATP and creatine substrates but did not differ in stability to urea denaturation. About 20–25% of CK was bound to the insoluble fraction of muscle and bound CK showed different kinetic responses to kinase and phosphatase treatments.

1. Novel PhosphoEnzymes: BioInformatics + Phospho-analyses
2. ^{32}P-ATP labeling studies
3. Purification / Properties
4. Structure / Function
5. Phospho-sites

Post-translational Modifications: The Next Generation

Novel Phosphorylation Control
CK, GDH, Hexokinase, G6PDH, LDH, NADP-IDH, α-GPDH, AMPD, GAPDH, FBPase, Antioxidant enzymes

PTM: Acetylation, Methylation, SUMOylation

HIBERNATION INDUCED CHANGES

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- **Pumps & channels closed**
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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 e.g. acetylation, phosphorylation**
- **Regulatory non-coding RNAs**


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CRYOBIOLOGY
ELSEVIER
Cryobiology 53 (2006) 310–318
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 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.

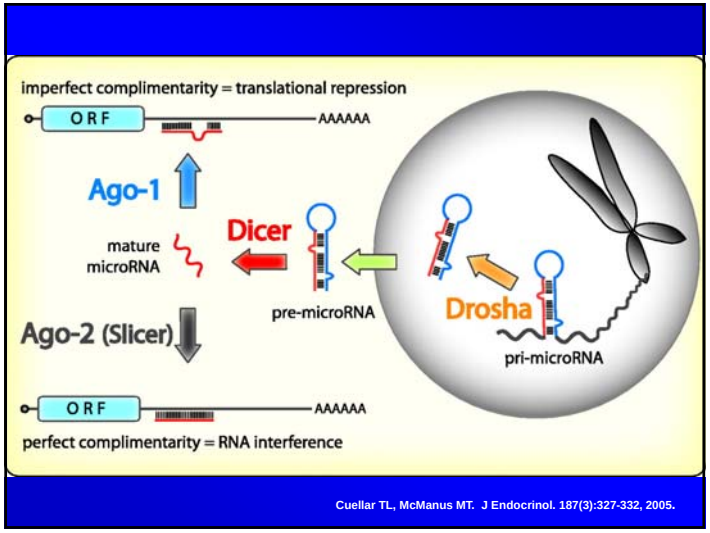
Transcription Suppression in Hibernator Muscle

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

Regulatory non-coding RNAs

microRNA

- Small RNAs ~22 nucleotides in length
- Highly conserved across species
- Bind to 3' UTR of mRNAs
- Could be 1000, affect 60 % of genes
- Disease involvement
- Act to :
  - Block translation of mRNA
  - Target mRNA for degradation



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

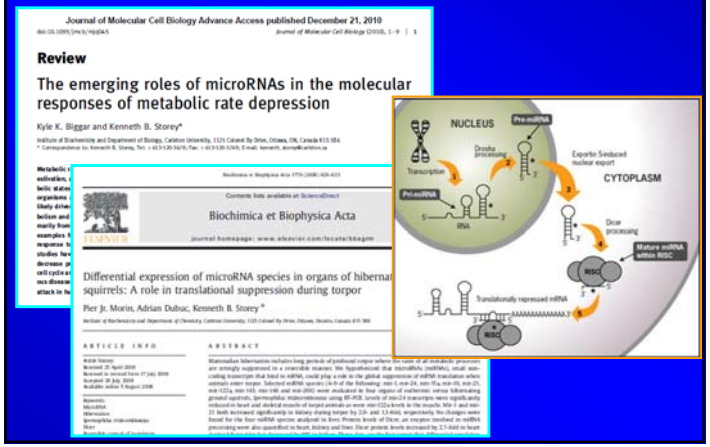
Are miRNAs differentially regulated in hibernators?

- Yes! Selected miRNAs were regulated in heart, muscle & kidney of hibernating 13-lined ground squirrels

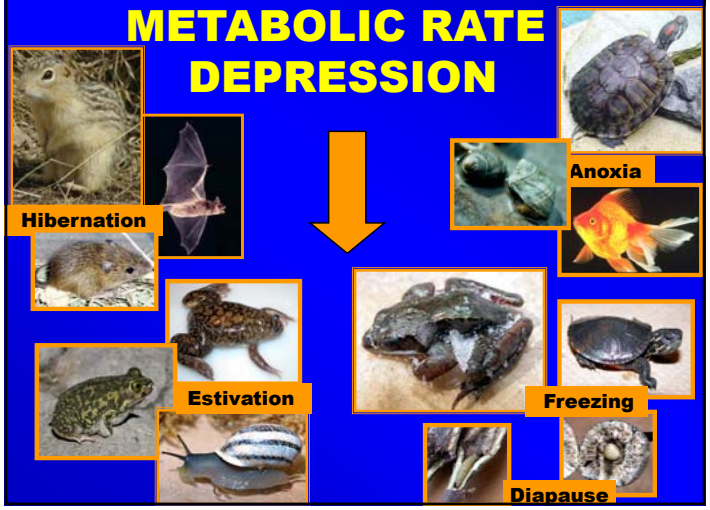
(Morin, Dubuc & Storey, 2008, Biochim Biophys Acta 1779:628-633)

miRNA	Fold change	Process in higher vertebrates
Mir-1	2.0	Myogenesis
Mir-133a	2.4	Myogenesis
Mir-206	2.6	Myogenesis
Let-7	2.0	Cell cycle
Mir-26	2.4	Hypoxia
Mir-451	2.6	Erythropoiesis

Turning it all off



METABOLIC RATE DEPRESSION



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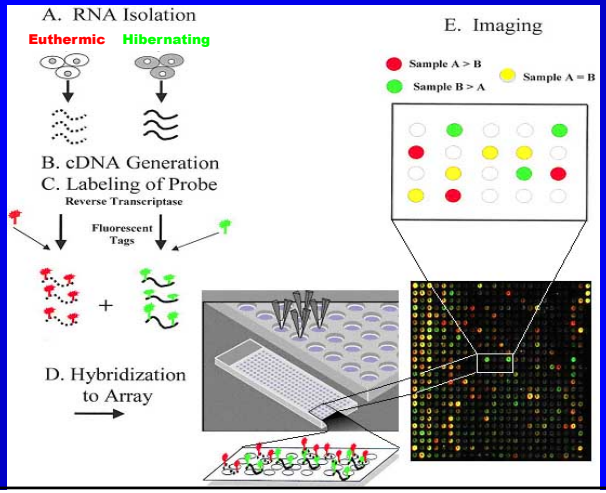
ROLE OF TRANSCRIPTION

- Global rate of mRNA synthesis depressed. Method: nuclear run-on
- Are selected genes up-regulated ?
- TO ASSESS GENE UPREGULATION:

What new mRNAs are created
- cDNA library, Gene Chip

Sequenced genome(s) as of 2011

cDNA ARRAY SCREENING

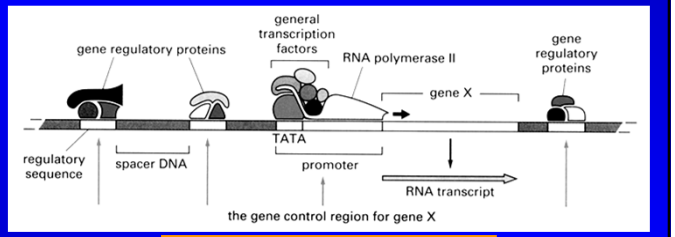


cDNA Arrays
- Methods
- Materials
- Sources
- Publications

GENE CHANGES IN HIBERNATION

- cDNA Library screen
  - Mitochondrial Genes
  - AOE
  - FABP, CPT, etc.
  - Shock proteins (GRP, HSP)
  - Transcription factors
- DNA Chip ~1-2% ⬆

CONTROL REGION OF A TYPICAL EUKARYOTIC GENE



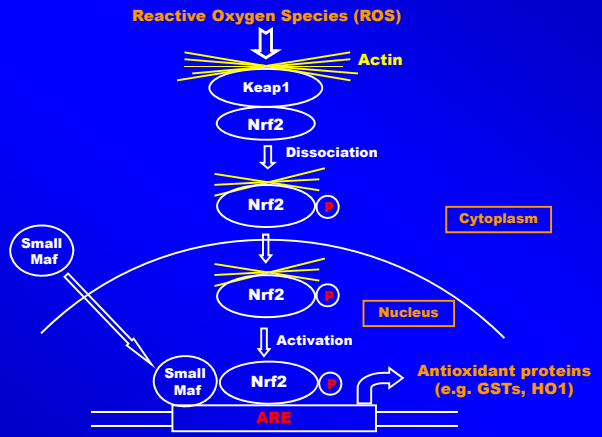
Epigenetics = OFF) :
- microRNA
- phospho-RNA Polymerase
- Histones modified
- HDAC / HAT changes

TRANSCRIPTION FACTORS

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

- Methods: EMSA, CHiP

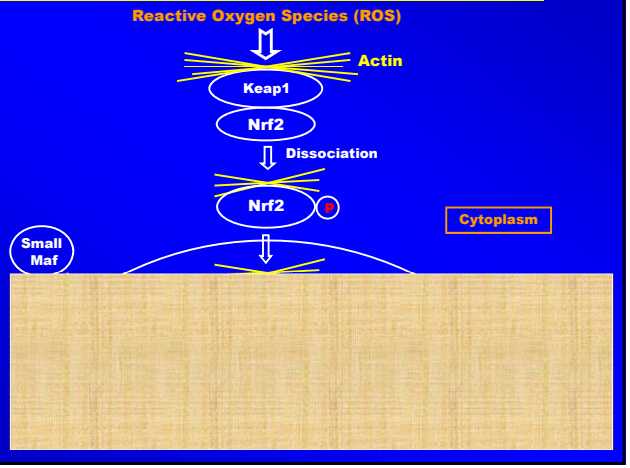
Nrf2/ARE pathway



Nrf-2

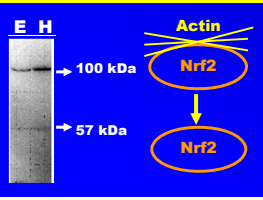
- Increased Nrf-2 protein & P
- Increased Nrf-2 in the Nucleus
- Increased levels of co-Tf: MafG
- Downstream gene activation:
- GST, HO-1, HO-2, Peroxiredoxin
- Thioredoxin, SOD (Cu/Zn & Mn)

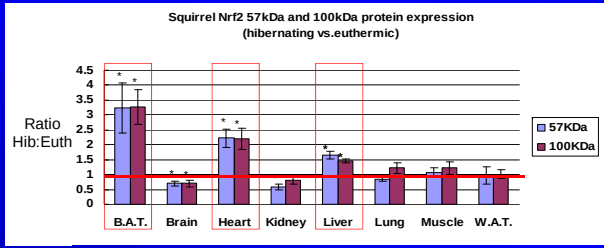
Nrf2/ARE pathway



Protein Regulation of Nrf2

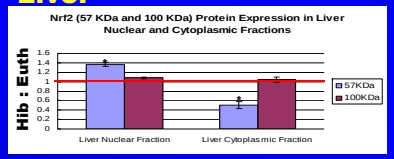
Euthermic vs Hibernating

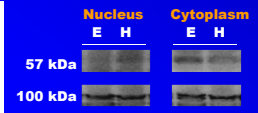




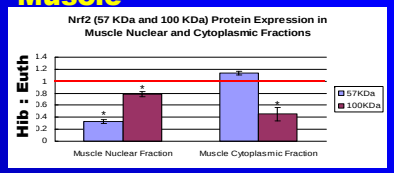
Nrf2 distribution between nuclear and cytoplasmic fractions

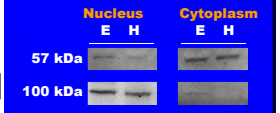
Liver



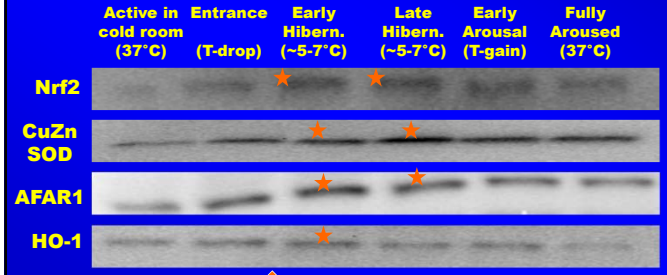


Muscle





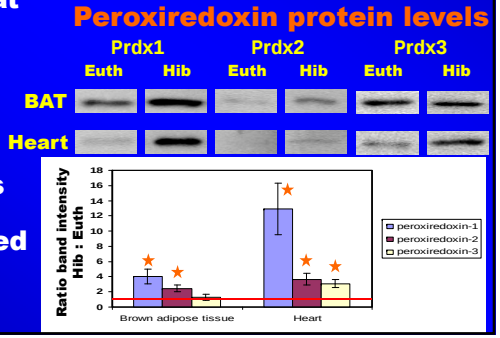
Nrf2 Timecourse in Heart



- Nrf2 protein ↑ in early and late hibernation
  → Up-regulation "cascade".

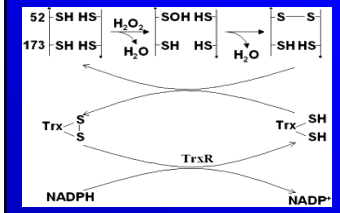
Peroxiredoxins

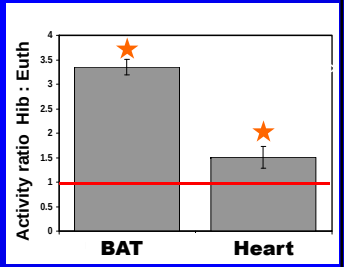
- Detoxify / reduce hydroperoxides
- Expressed at high levels
- ARE in promoter region of Prdx genes
- Nrf2 activated



Peroxiredoxin Activity

- Protein level correlates with increased activity
- Assays in BAT and heart with thioredoxin, thioredoxin reductase and NADPH:





Kim et al., 2005

Nrf Conclusion

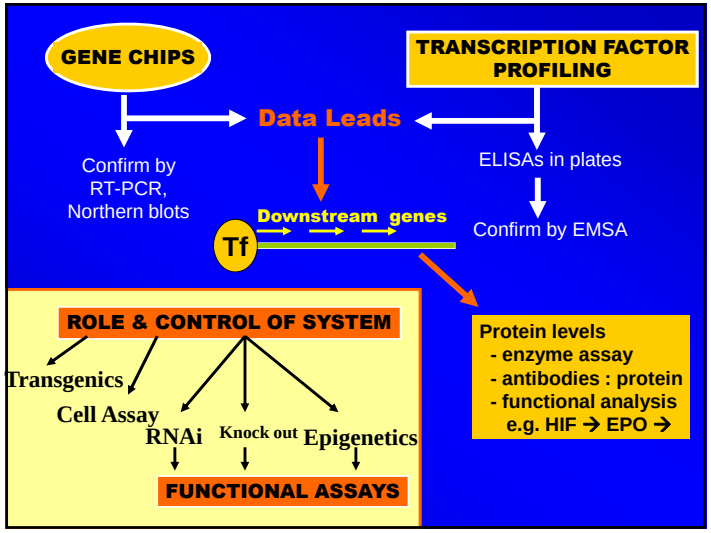
Activation of the Nrf2 pathway:

→ Activated in early-late torpor, along with downstream gene protein products

→ Increased PRDX, HO & TRX protein and activity

Result:

→ Detoxification of ROS, intracellular signaling control



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

PRIMATE HIBERNATION!!
GREY MOUSE LEMUR

Hibernation and medicine

Adv Clin Chem. 2010;52:77-108.

Metabolic rate depression: the biochemistry of mammalian hibernation.

Storey KB, Storey JM.

Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada. kenneth_storey@carleton.ca

Abstract

During winter hibernation, small mammals fall into long periods of deep cold torpor where metabolic rate is suppressed by > 90% and core body temperature can fall to near 0 degrees C. Studies with hibernators illustrate the molecular regulatory mechanisms that coordinate the suppression of metabolic functions during torpor, reprioritize energy use, and preserve/stabilize macromolecules to support long-term viability during cold torpor. This review explores mechanisms including post-translational modification of proteins, differential regulation of enzymes, global suppression of transcription and translation including a ro
transcription factors. The
relevant to issues in clini
and atrophy resistance.

Primates !!

Gerontology. 2010;56(2):220-30. Epub 2009 Jul 14.

Out cold: biochemical regulation of mammalian hibernation - a mini-review.

Storey KB

Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada. kenneth_storey@carleton.ca

Abstract

Hibernating mammals offer an intriguing example of natural torpor and illustrate the regulatory mechanisms that control
cell preservation strategies that support long-term viability in a hypometabolic state. These
roving the hypothermic preservation of human organs for transplant, and guidelines that
r as an intervention strategy in human medicine. Recent advances in hibernation research
contribute to metabolic depression by orchestrating the global suppression of ATP-
tion including multiple forms of post-translational modification of proteins/enzymes
(Sylation), mRNA storage mechanisms, and differential expression of microRNA species.
also contributed new advances in understanding the range of cell functions that are
ut some critical preservation strategies that aid long-term viability in a torpid state. These
erones and the implementation of the unfolded protein response, and the enhancement of
) to control the actions of extracellular proteases in clotting and inflammation responses.

Novel phosphorylations


ScienceDirect

ABB

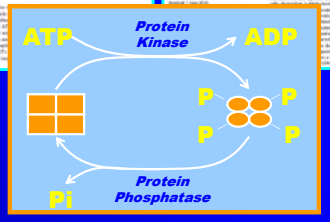
Regulation of skeletal muscle creatine kinase from a hibernating mammal

Khalil Abnous, Kenneth B. Storey *


Comparative Biochemistry and Physiology, Part B

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

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



Atrophy – Hypertrophy

Mol Cell Biochem. 2010 Nov;344(1-2):151-62. Epub 2010 Jul 9.

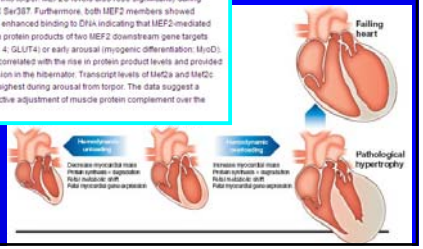
Expression of myocyte enhancer factor-2 and downstream genes in ground squirrel skeletal muscle during hibernation.

Tessier SN, Storey KB

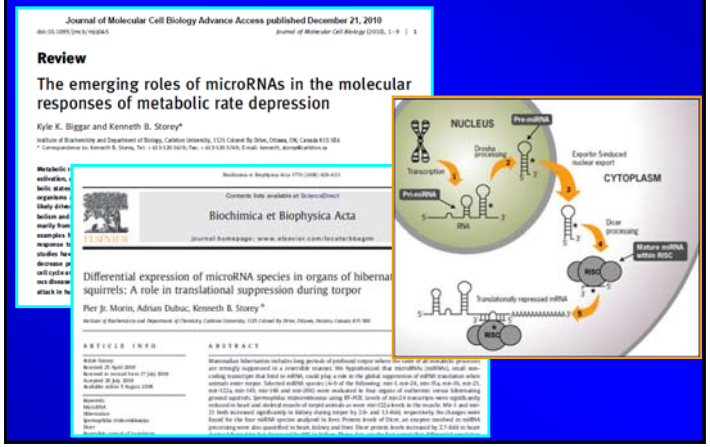
Institute of Biochemistry & Department of Biology, Carleton University, Ottawa, ON, Canada.

Abstract

Myocyte enhancer factor-2 (MEF2) transcription factors regulate the expression of a variety of genes encoding contractile proteins and other proteins associated with muscle performance. We proposed that changes in MEF2 levels and expression of selected downstream targets would aid the skeletal muscle of thirteen-lined ground squirrels (Spermophilus tridecemlineatus) in meeting metabolic challenges associated with winter hibernation, e.g., cycles of torpor-arousal, body temperature that can fall to near 0°C, long periods of inactivity that could lead to atrophy. MEF2A protein levels were significantly elevated when animals were in torpor (maximally 2.8-fold higher than in active squirrels) and the amount of phosphorylated active MEF2A Thr312 increased during entrance into torpor. MEF2C levels also rose significantly during entrance and torpor as did the amount of phosphorylated MEF2C Ser387. Furthermore, both MEF2 members showed elevated amounts in the nuclear fraction during torpor as well as enhanced binding to DNA indicating that MEF2-mediated gene expression was up-regulated in torpid animals. Indeed, the protein products of two MEF2 downstream gene targets
increased in muscle during torpor (glucose transporter isoforms 4; GLUT4) or early arousal (myogenic differentiation; MyoD).
MyoD mRNA transcript levels correlated with the rise in protein product levels and provided
MEF2-mediated gene expression in the hibernator. Transcript levels of Mef2a and Mef2c
ms with levels of both being highest during arousal from torpor. The data suggest a
gene transcription in the selective adjustment of muscle protein complement over the

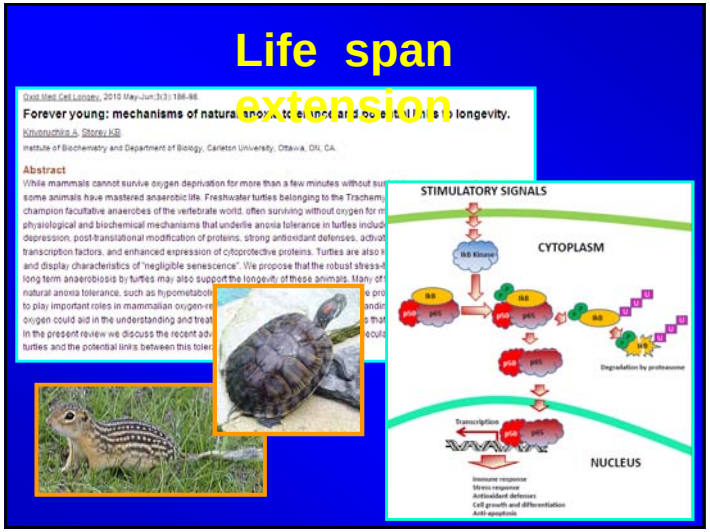


Turning it all off

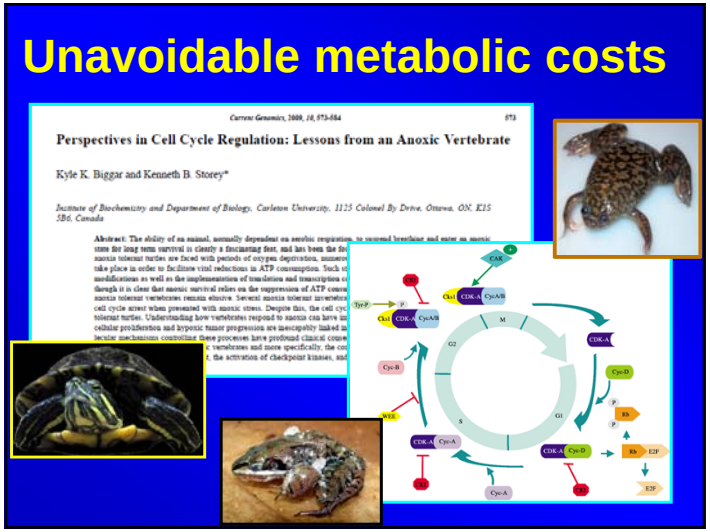


Epigenetics in Adaptation

Life span extension



Unavoidable metabolic costs



Unity through Evolution

WWCeD

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