The Living Dead: Metabolic Arrest and the Control of Biological Time
HIBERNATION

13-LINED GROUND SQUIRREL
Ictidomys tridecemlineatus
Hibernation

Little Brown Bat
Myotis lucifugus
DAILY TORPOR

Grey mouse lemur, *Microcebus murinus*
ESTIVATION

Milk snail
*Otala lactea*
ESTIVATION

Spadefoot toad
Scaphiopus holbrookii
ANOXIA

Painted turtle
*Chrysemys picta*

Red-eared turtle
*Pseudemys scripta*

Periwinkle
*Littorina littorea*
FREEZING

Wood frog
*Rana sylvatica*
MAMMALS ON ICE:
Metabolic Rate Reduction

www.carleton.ca/~kbstorey
Model Hibernators

*Mylotis lucifugus*, little brown bat

*Ictidomys tridecemlineatus*, 13-lined ground squirrel

*Urocitellus richardsonii*, Richardson’s 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
• 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
PRINCIPLES OF HIBERNATION

1. Metabolic rate reduction
2. Control by protein kinases (SAPKs, 2\textsuperscript{nd} messenger PKs)
3. Most Genes OFF
4. Selective gene activation
METABOLIC RATE DEPRESSION

Hibernation

Estivation

Freezing

Diapause

Anoxia
PRINCIPLES OF HIBERNATION

1. Metabolic rate reduction

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

3. Most Genes OFF

4. Selective gene activation

Same as with ALL MRD
Nucleus

GENES ON/OFF

[Trans.F]

mRNAs

PROTEINS

Ca^{2+}

KINASES (2nd)

SAPK

PATHWAYS

CHO

ATP

AA

PROT

SMW

KETC

P

FAT

GENES

MITO

ETC

ATP

ADP
METABOLISM IN HIBERNATION

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

ATP turnover to <5% of normal
Nucleus

GENES
ON/OFF

[Trans.F]

mRNAs

PROTEINS

Ca^2+

KINASES (2nd)

SAPK

PATHWAYS

CHO

ATP

AA

PROT

SMW

FAT

MITO

GENES

ETC

[ i + e Factors]

Na

K
Metabolic Rate Depression CHANGES

- Few ‘SAP’ kinases activated
- * thousands of processes OFF
- Gene ‘inactivation’ ( mRNA )
- Few Genes activated (1-2%)
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)
MRD CHANGES

Few ‘SAP’ kinases activated

- Gene ‘inactivation’ (mRNA)
- Few Genes activated (1-2%)
GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

PROTEINS (ENZYMES)

Control by proteases

Degradation

FUNCTIONAL ENZYMES

Control at level of enzyme function

Inhibition and Activation

Covalent modification

ACTIVE ENZYMES

Degradation

INACTIVE ENZYME

No Modification

Degradation

Covalent modification

Degradation
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
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
imperfect complementarity = translational repression

Ago-1

mature microRNA

Dicer

pre-microRNA

Ago-2 (Slicer)

perfect complementarity = RNA interference

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, a physiological state in which energy expenditure and growth are significantly decreased, likely drives hibernation and torpor. Many examples of hibernation in animals and the effects of cold on the body, as demonstrated in a study with hibernating ground squirrels, show that a decrease in oxygen consumption is accompanied by a decrease in cell cycle activity and the overall survival of the animal. The differential expression of microRNA species in the organs of hibernating ground squirrels: A role in translational suppression during torpor

Fier Jr. Morin, Adrian Dubuc, Kenneth B. Storey*

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

Article Info

Article history:
Received 25 April 2008
Received in revised form 17 July 2008
Accepted 28 July 2008
Available online 5 August 2008

Keywords:
MicroRNA
Hibernation
Spermophilus tridecemlineatus
Dicer

Abstract

Mammalian hibernation includes long periods of profound torpor where the rates of all metabolic processes are strongly suppressed in a reversible manner. We hypothesized that microRNAs (miRNAs), small non-coding transcripts that bind to mRNA, could play a role in the global suppression of mRNA translation when animals enter torpor. Selected miRNA species (4–9 of the following: mir-1, mir-24, mir-15a, mir-19a, mir-21, mir-122, mir-145, mir-146 and mir-206) were evaluated in four organs of euthermic versus hibernating ground squirrels, Spermophilus tridecemlineatus using RT-PCR. Levels of mir-24 transcripts were significantly reduced in heart and skeletal muscle of torpid animals as were mir-122a levels in the muscle. Mir-1 and mir-21 both increased significantly in kidney during torpor by 2.0- and 1.3-fold, respectively. No changes were found for the four miRNA species analyzed in liver. Protein levels of Dicer, an enzyme involved in miRNA processing were also quantified in heart, kidney and liver. Dicer protein levels increased by 2.7-fold in heart during torpor, by 1.9 fold in kidney and did not change in liver. These results suggest that miRNAs are involved in the global suppression of mRNA translation during hibernation.
METABOLIC RATE DEPRESSION

Hibernation

Estivation

Freezing

Diapause

Anoxia
MRD CHANGES

- Few ‘SAP’ kinases activated
- Gene ‘inactivation’ ( mRNA )
- Few Genes activated (1-2%)
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 HIBERNATION

• Some Activated:
  - Mitochondrial Genes
  - AOE
  - FABP, CPT, etc.
  - Shock proteins (GRP, HSP)
  - Transcription factors

• DNA Chip ~1-2%
Where do we go from here?

Nature’s Tools for MRD

- Novel Enzyme Controls
- Atrophy, Autophagy
- Turning it all off -- microRNA
- Epigenetics & adaptation
- Life span extension
- Antioxidant Defense
- Cell cycle suppression
- Unity through evolution

NEW DIRECTIONS
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 viability over weeks of environmental hypothermia during annual hibernation. Creatine kinase (CK) has a key role in muscle energetics and this study investigated the regulation of CK in hibernating species such as the northern fur seal (Callorhinus ursinus). CK activity was ~20% lower during hibernation than in euthermic animals, and mRNA was reduced by ~30%. Hibernator CK showed reduced affinity for ATP and creatine, compared with non-hibernating species, and CK activity was coupled with exchange phosphates, such that CK from a euthermic species is a mix of phosphorolysed CK and phospho-CK was detected in hibernating animals. High and low phosphorus CK forms had different substrate affinities, but did not differ in the activity of ATP or phosphate treatment. About 20% of CK was bound to ADP and bound CK showed different kinetic responses to kinases and phosphatase treatments.

© 2007 Elsevier Inc. All rights reserved.
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 but the mechanism to animal survival of low oxygen conditions has never been examined. To establish mechanisms could be involved in natural anoxia tolerance, we have examined the anoxia-responsive transcriptional silencers, histone deacetylases (HDACs), in tissues of a unique model for an anoxia-tolerant species, the turtle Trachemys scripta elegans. Transcript and protein levels of all five HDACs rose by 1.3- to 2-fold in skeletal muscle in response to 20 h of anoxia exposure. In addition, HDAC activity in the muscle, liver, and heart displayed a milder response to 20 h of anoxia and levels of acetylated histone H3 (Lys 9 or Lys 23) decreased to 50-75% of control values. Only HDAC5 responded similarly in the liver, Hdac5 transcript levels increased 2.1-2.3-fold and HDAC5 protein increased 3.3-fold. Overall, HDACs are key players in the regulation of gene expression during anoxia exposure.

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

Krivoruchko A, Storey KB.

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

Abstract

While mammals cannot survive oxygen deprivation for more than a few minutes without sustaining damage, some animals have mastered anaerobic life. Freshwater turtles belonging to the Trachemys species, champion facultative anaerobes of the vertebrate world, often surviving without oxygen for more than 24 hours, provide important physiological and biochemical mechanisms that underlie anoxia tolerance in turtles includes antioxidant defenses, activation of antioxidant transcription factors, and enhanced expression of cytoprotective proteins. Turtles are also key models to test hypotheses and display characteristics of "negligible senescence". We propose that the robust stress-tolerance and long term anaerobiosis by turtles may also support the longevity of these animals. Many of the physiological and molecular mechanisms that contribute to natural anoxia tolerance, such as hypometabolism, antioxidant response, and enhanced expression of cytoprotective proteins, could aid in the understanding and treatment of human diseases that are associated with aging. In the present review we discuss the recent advances in our understanding of the biochemistry and molecular biology underlying natural anoxia tolerance in turtles and the potential links between this tolerance and longevity.
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 evidence of MEF2-mediated gene expression in the hibernator. Transcript levels of Mef2a and Mef2c genes with levels of both being highest during arousal from torpor. The data suggest a selective gene transcription in the selective adjustment of muscle protein complement over the
Unavoidable metabolic costs
Unity through Evolution

An Overview of Stress Response and Hypometabolic Strategies in *Caenorhabditis elegans*: Conserved and Contrasting Signals with the Mammalian System

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

Abstract

Studies of the molecular mechanisms that control metabolism (physiological) have long been used to model organism, *Caenorhabditis elegans*, “dauer” stage. This period of development is driven by unfavorable conditions and survival for *C. elegans* employs a number of signals sensitive to unfavorable conditions and survive for life. The suppression of cellular metabolism and survival of nematodes through the dauer mechanisms are fundamental to control general, mammalian systems are highly diverse (temperatures and low oxygen), however, signal transduction pathways of nematode protein targets in the stress response are maintained, and often differ only in the overall. This outlines a framework of critical molecules and pathways as therapeutic targets for addressing disease.
Hibernators as a model for metabolic disease?

- Hibernators show reversible insulin resistance
- Rapid weight gain, hyperinsulinemia during entry into hibernation, reversed in deep torpor
- Modifications to similar pathways: Glucose transport, Akt activity, PPAR-γ signaling

Type 2 Diabetes mellitus
PRIMATE HIBERNATION!!
Gray Mouse Lemur

Madagascar - western dry forests
MRD in Primate hibernation
[Stress Kinases ]

- Warm Hibernator
- Daily and Seasonal Torpor
- Uncharacterized model
- First molecular studies

Biggar KK, Wu CW, Tessier SN, Zhang J & Storey KB
Primate Hibernation

- Novel model of hibernation
  - Short term torpor in mild climate (Tb falls to ~20°C)
- Reduction of ERK signaling
- Activation of JNK stress response
Hibernation and medicine

Metabolic rate depression: the biochemistry of mammalian hibernation.

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 posttranslational modification of proteins, differential regulation of enzymes, global suppression of transcription and translation including a role for microRNA and atrophy resistance.

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

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 may prove useful in the hypothermic preservation of human organs for transplant, and guidelines that could be used as an intervention strategy in human medicine. Recent advances in hibernation research have contributed to metabolic depression by orchestrating the global suppression of ATP-consuming processes including multiple forms of post-translational modification of proteins/enzymes (O-linked N-acetylglucosamine), mRNA storage mechanisms, and differential expression of microRNA species. These studies have also contributed new advances in understanding the range of cell functions that are put to some critical preservation strategies that aid long-term viability in a torpid state. These include the implementation of the unfolded protein response, and the enhancement of strategies to control the actions of extracellular proteases in clotting and inflammation responses.
GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

PROTEINS (ENZYMES)

Control by proteases

Degradation

FUNCTIONAL ENZYMES

No Modification

Covalent modification

Control by post-translational modification

ACTIVE ENZYMES

Inhibition and Activation

Control at level of enzyme function

INACTIVE ENZYME