Controlling Biological Time: Nature has the BluePrint

Kenneth B. Storey, Carleton University, Ottawa
www.carleton.ca/~kbstorey

Nature's BioPreservation [Organs-OFF]		
TEMP	ANIMAL	OUTCOME
High	Lemur	√
	Marsupial	√
Low	Squirrel	√
	Bat	√
_____0°C_____		
Frozen	Frog	√
	Turtle	√

Nature's [NEW !] Mechanisms
Posttranslational modifications
Epigenetics
MicroRNA
Gene suppression
Selective gene activation

In Vitro Preservation Technology		
TEMP	FLOW	OUTCOME
High	+	√
	-	X
Low	+	√ (?)
	-	√
_____0°C_____		
Frozen	-	Y & N
Vitrified	-	√ (?)

In Vitro Preservation Technology

TEMP	FLOW	OUTCOME
High	+	√
	-	X
Low	+	√ (?)
	-	√
	0°C	
Frozen	-	Yes & No
Vitrified	-	√ (?)

Nature's BioPreservation
[Organs-OFF]

TEMP	ANIMAL	OUTCOME
High	Lemur	√
	Marsupial	√
Low	Squirrel	√
	Bat	√
	0°C	
Frozen	Frog	√
	Turtle	√

NATURE'S [NEW !] MECHANISMS

Posttranslational modifications

Epigenetics

MicroRNA

Gene suppression

Selective gene activation

Nature's BioPreservation [Organs-OFF]		
TEMP	ANIMAL	OUTCOME
High	Lemur	√
	Marsupial	√
Low	Squirrel	√
	Bat	√
_____0°C_____		
Frozen	Frog	√
	Turtle	√

Nature's [NEW !] Mechanisms
Posttranslational modifications
Epigenetics
MicroRNA
Gene suppression
Selective gene activation

In Vitro Preservation Technology		
TEMP	FLOW	OUTCOME
High	+	√
	-	X
Low	+	√ (?)
	-	√
_____0°C_____		
Frozen	-	Y & N
Vitrified	-	√ (?)

Nature's Way

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

Freezing

Diapause

Nature's Tools for Reversible Organ Preservation

* Novel Enzyme Controls
* Epigenetics & adaptation
* Turning it all off -- microRNA

Novel Enzyme Controls

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 viability over weeks of thermogenesis during arousal. Creatine kinase (CK)[1] has a key role in muscle energetics and this stu squirrels, *Spermophilus richardsonii*. CK activity was ~20% lower during hibernation than in euther mRNA was reduced by ~70%. Hibernator CK showed reduced affinity for ATP and creatine, compa that promoted endogenous protein kinase or phosphatase action, coupled with ion exchange chrom phosphate forms, showed that soluble CK from euthermic squirrels was a mix of phosphorylated a only phospho-CK was detected in hibernating animals. High and low phosphate CK forms had dif substrates but did not differ in stability to urea denaturation. About 20–25% of CK was bound to bound CK showed different kinetic responses to kinase and phosphatase treatments.

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 *

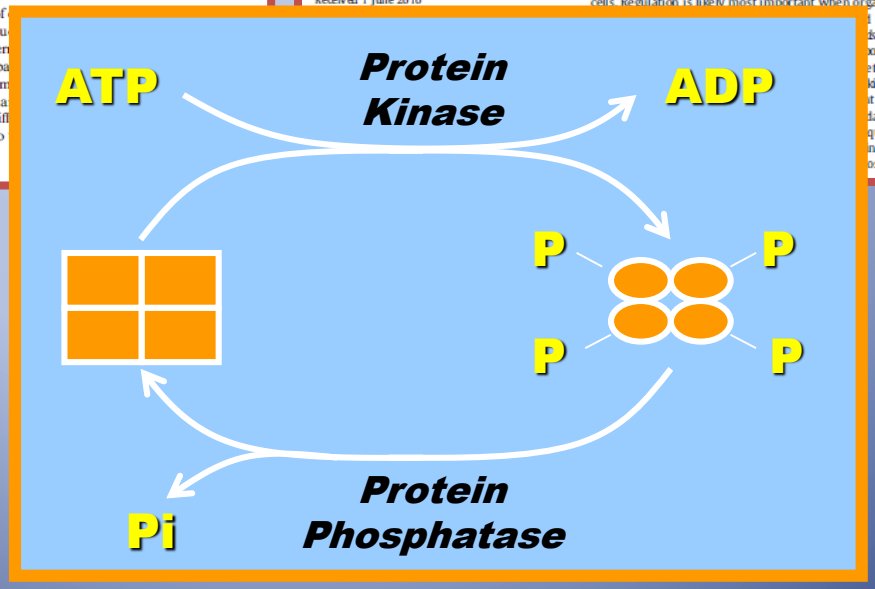
Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

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 associated with winter. Many small mammals, such as *sonii*, cope with these conditions by hibernating. Animals olic rate is greatly suppressed, body temperature drops to t from fixed internal body stores of fuels. To investigate kinetic properties of GDH were analyzed in liver from t differences in V_{max}, K_m glutamate, K_a ADP and inhibition data suggested an activation of the glutamate-oxidizing quent experiments suggested that the molecular basis of n phosphorylation state of GDH between euthermia and osphorylated and activated when animals transition into



Phospho / de-Phospho PATHWAY CONTROLS

- Affects all pathways
- Regulation in minutes
- Reversed by protein phosphatases
- Metabolic cost = less than 1 % total energy
- A dozen new enzymes unknown to textbooks are NOVEL !

- 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*

Posttranslational Modification: The Next Generation

Novel Phosphorylation Control of

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

PTMs: Acetylation, Methylation, SUMOylation, etc.

TURNING OFF GENES: Role of Epigenetics

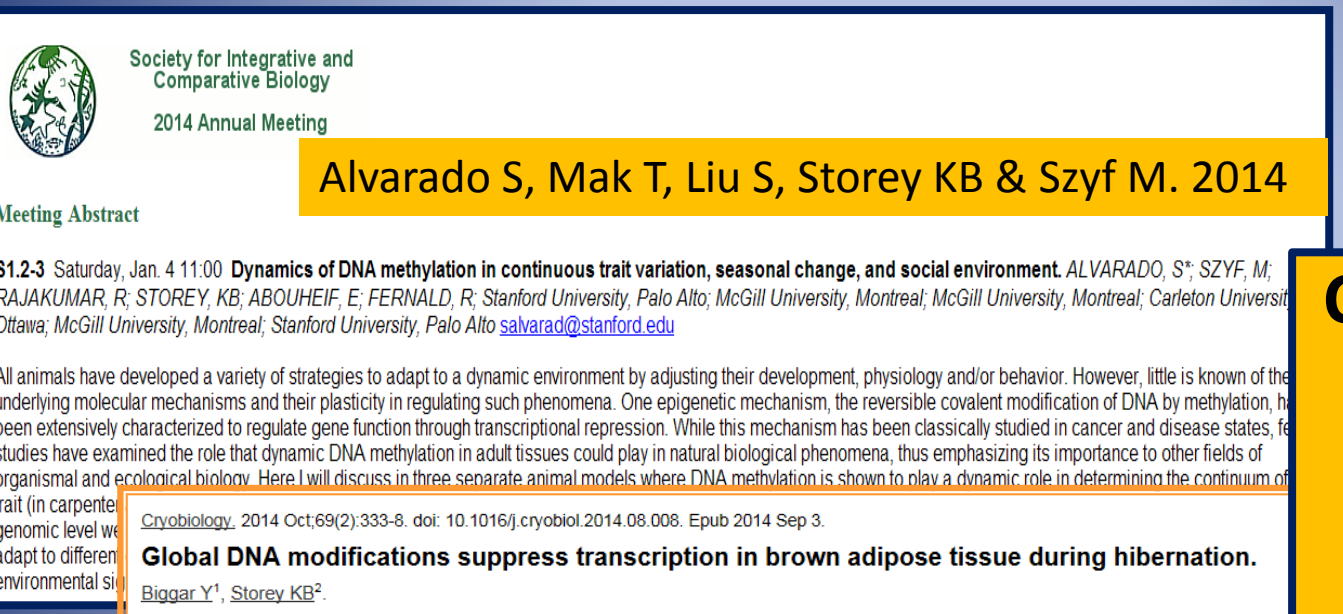
Epigenetics:

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

Common mechanisms:

- DNA methylation
- Histone modification / histone variants
  e.g. acetylation, methylation, phosphorylation
- Regulatory non-coding RNAs (microRNA)
- "Hiding messages"

DNA Methylation & Mammalian Hibernation



Society for Integrative and Comparative Biology

2014 Annual Meeting

Alvarado S, Mak T, Liu S, Storey KB & Szyf M. 2014

Meeting Abstract

S1.2-3 Saturday, Jan. 4 11:00 **Dynamics of DNA methylation in continuous trait variation, seasonal change, and social environment.** *ALVARADO, S*; SZYF, M; RAJAKUMAR, R; STOREY, KB; ABOUHEIF, E; FERNALD, R; Stanford University, Palo Alto; McGill University, Montreal; McGill University, Montreal; Carleton Universit Ottawa; McGill University, Montreal; Stanford University, Palo Alto* salvarad@stanford.edu

All animals have developed a variety of strategies to adapt to a dynamic environment by adjusting their development, physiology and/or behavior. However, little is known of the underlying molecular mechanisms and their plasticity in regulating such phenomena. One epigenetic mechanism, the reversible covalent modification of DNA by methylation, h been extensively characterized to regulate gene function through transcriptional repression. While this mechanism has been classically studied in cancer and disease states, fe studies have examined the role that dynamic DNA methylation in adult tissues could play in natural biological phenomena, thus emphasizing its importance to other fields of organismal and ecological biology. Here I will discuss in three separate animal models where DNA methylation is shown to play a dynamic role in determining the continuum of trait (in carpente genomic level we adapt to differen environmental si

Cryobiology. 2014 Oct;69(2):333-8. doi: 10.1016/j.cryobiol.2014.08.008. Epub 2014 Sep 3.

Global DNA modifications suppress transcription in brown adipose tissue during hibernation.

Biggar Y[1], Storey KB[2].

Author information

Abstract

Hibernation is crucial to winter survival for many small mammals and is characterized by prolonged periods of torpor during whic controls are applied to suppress energy-expensive cellular processes. We hypothesized that one strategy of energy conservatio in gene transcription imparted by reversible modifications to DNA and to proteins involved in chromatin packing. Transcriptional regulation during hibernation was examined over euthermic control groups and five stages of the torpor/arousal cycle in brown adipose tissue of thirteen-lined ground squirrels (Ictidomys tridecemlineatus). Brown adipose is crucial to hibernation success because it is responsible for the non-shivering thermogenesis that rewarms animals during arousal. A direct modification of DNA during torpor was revealed by a 1.7-fold increase in global DNA methylation during long term torpor as compared with euthermic controls. Acetylation of histone H3 (on Lys23) was reduced by about 50% when squirrels entered torpor, which would result in increased chromatin packing (and transcriptional repression). This was accompanied by strong increases in histone deacetylase protein levels during torpor; e.g. HDAC1 and HDAC4 levels rose by 1.5- and 6-fold, respectively. Protein levels of two co-repressors of transcription, MBD1 and HP1, also increased by 1.9- and 1.5-fold, respectively, in long-term torpor and remained high during early arousal. MBD1, HP1 and HDACs all returned to near control values during interbout indicating a reversal of their inhibitory actions. Overall, the data presents strong evidence for a global suppression of transcription during torpor via the action of epigenetic regulatory mechanisms in brown adipose tissue of hibernating thirteen-lined ground squirrels.

Changes in DNA methylation & DNMTs restrict gene transcription during torpor

Epigenetics in Adaptation Histone Code [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 but the
mechanism to animal survival of low oxygen conditions has never been examined. To establis
mechanisms could be involved in natural anoxia tolerance, we have examined the anoxia-resp
transcriptional silencers, histone deacetylases (HDACs), in tissues of a unique model for anox
turtle Trachemys scripta elegans. Transcript and protein levels of all five HDACs rose by 1.3-4.
in skeletal muscle in response to 20 h of anoxia exposure. In addition, HDAC activity in the mus
response to 20 h of anoxia and levels of acetylated histone H3 (Lys 9 or Lys 23) decreased to 4
liver displayed a milder response with HDAC1, -4, and -5 protein levels increasing by 1.6-2.1-fo
acetylated histone H3 levels also decreased to 50-75% of control values. Only HDAC5 respond
heart; Hdac5 transcript levels increased 2.1-2.3-fold and HDAC5 protein rose by 3.3-fold. Overa
ges
sary in this hypometabolic state.

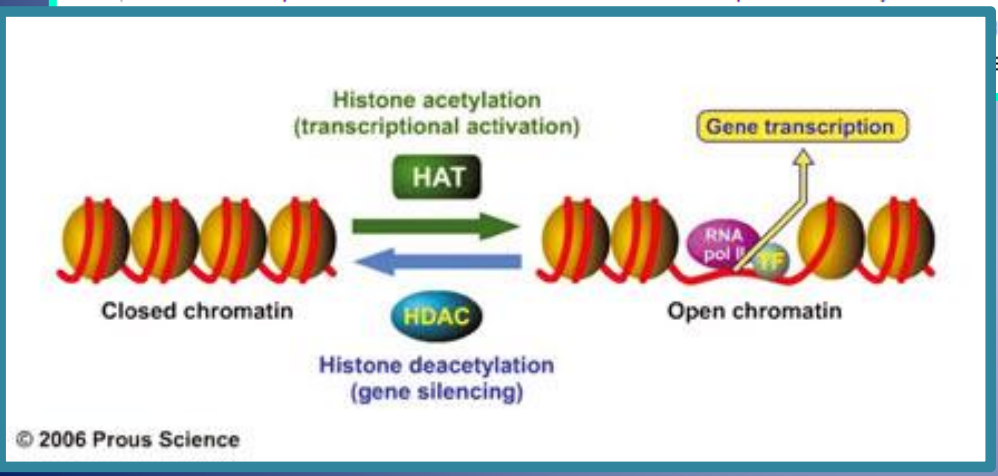
Available online at www.sciencedirect.com
ScienceDirect
CRYOBIOLOGY
ELSEVIER
Cryobiology 53 (2006) 310–318
www.elsevier.com/locate/ycryo

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



Turning it all off

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 r
estivation, a
bolic states
organisms
likely drive
bolism and
marily from
examples f
response to
studies hav
decrease pr
cell cycle ar
ous disease
attack in hu

Biochimica et Biophysica Acta 1779 (2008) 628–633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Differential expression of microRNA species in organs of hibernat squirrels: A role in translational suppression during torpor

Pier 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

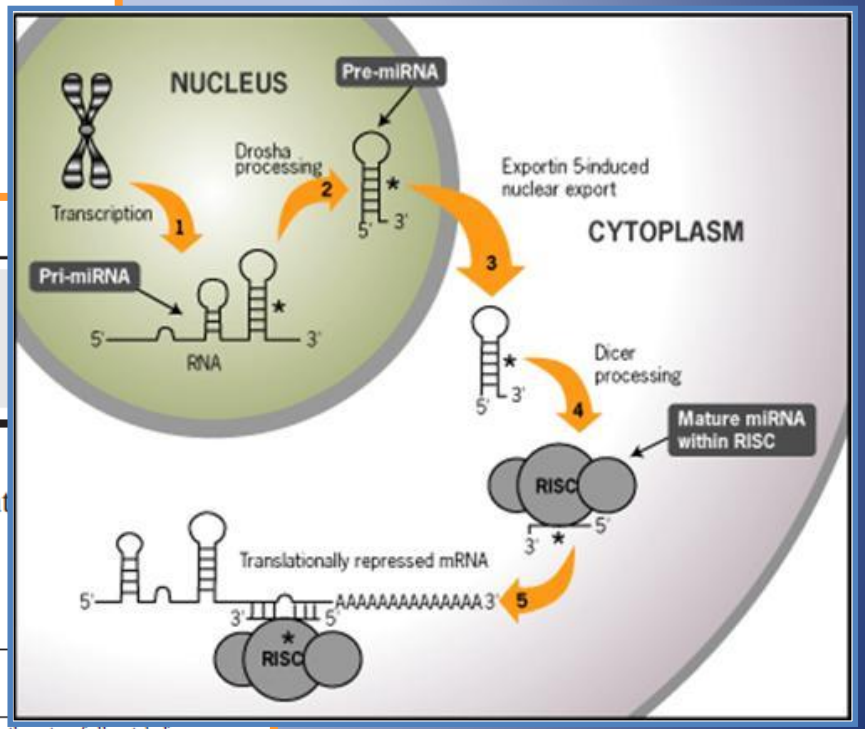
ARTICLE INFO

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
Reversible control of translation

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-16, mir-21, mir-122a, mir-143, 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 hibernation but decreased by 60% in kidney. These data are the first report that differential regulation



Nature's Way

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

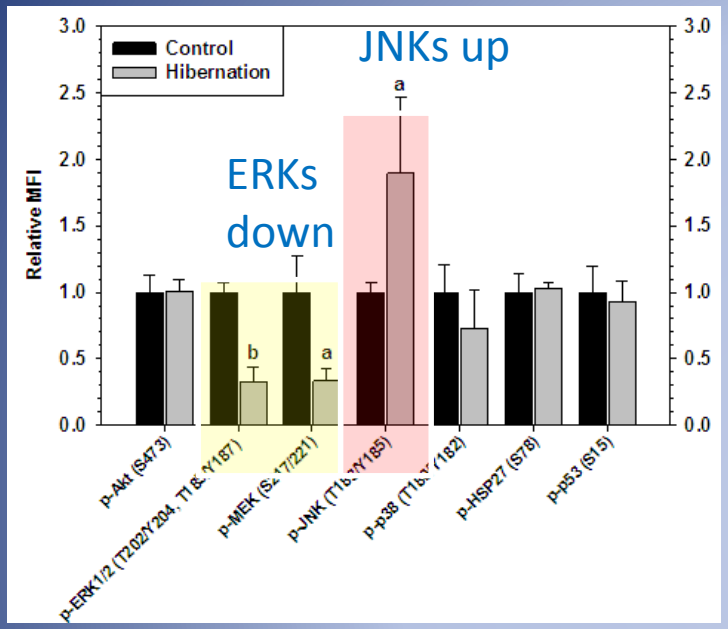
Freezing

Diapause

PRIMATE HIBERNATION !!
Gray Mouse Lemur

Madagascar
- western dry
forests

Primate Hibernation



Gray mouse lemur

Novel model of hibernation
- **both Daily and Seasonal Torpor**
- **torpor in mild climate -- body temperature may fall to just 20-30°C**

First molecular studies -- reveal common mechanisms to humans:
- **changes in stress-activated kinase signaling**
- **changes in insulin and AMPK signaling pathways**
- **changes in gene expression & microRNA**
- **suppression of protein synthesis**
- **antioxidants and chaperone protectants**

Storey lab: 6 paper series in Genom. Proteom. Bioinform. 2015, in press

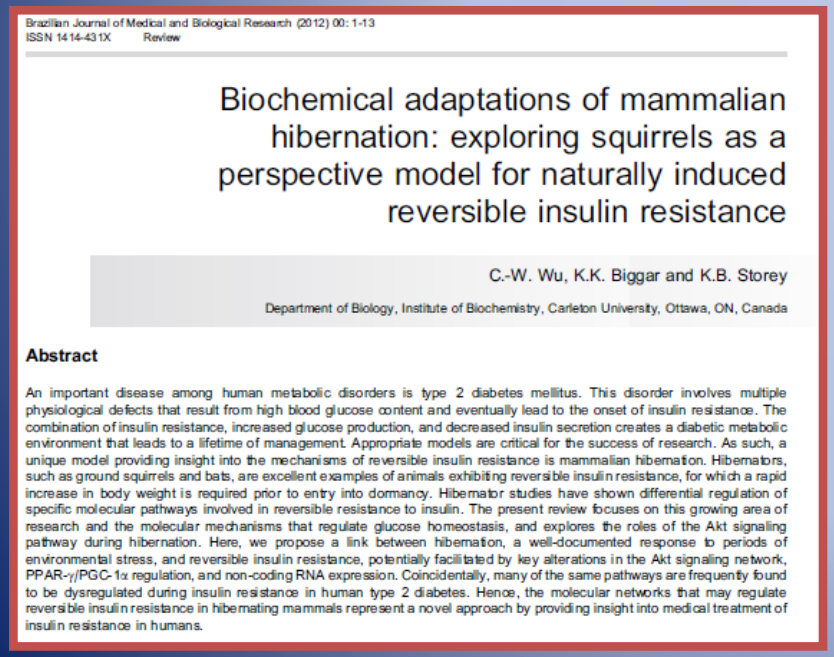
Metabolic Rate Depression

- J. STOREY
- S. EDDY
- D. HITTEL
- J. MacDONALD
- A. FAHLMAN
- P. MORIN
- C. HOLDEN
- H. MEHRANI
- J. NI
- M. HAPSATOU
- K. Abnous
- A. Krivoruchko
- R. Bell
- S. TESSIER
- C-W. WU
- K. Biggar
- J. Zhang
- Y. Maistrovski Biggar
- S. BROOKS
- C. FRANK
- J. HALLENBECK
- D. THOMAS
- A. RUBTSOV
- J. STEWART

www.carleton.ca/~kbstorey

Hibernators as a model for metabolic disease?

Type 2 Diabetes mellitus



Brazilian Journal of Medical and Biological Research (2012) 00: 1-13
ISSN 1414-431X Review

Biochemical adaptations of mammalian hibernation: exploring squirrels as a perspective model for naturally induced reversible insulin resistance

C.-W. Wu, K.K. Biggar and K.B. Storey

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

Abstract

An important disease among human metabolic disorders is type 2 diabetes mellitus. This disorder involves multiple physiological defects that result from high blood glucose content and eventually lead to the onset of insulin resistance. The combination of insulin resistance, increased glucose production, and decreased insulin secretion creates a diabetic metabolic environment that leads to a lifetime of management. Appropriate models are critical for the success of research. As such, a unique model providing insight into the mechanisms of reversible insulin resistance is mammalian hibernation. Hibernators, such as ground squirrels and bats, are excellent examples of animals exhibiting reversible insulin resistance, for which a rapid increase in body weight is required prior to entry into dormancy. Hibernator studies have shown differential regulation of specific molecular pathways involved in reversible resistance to insulin. The present review focuses on this growing area of research and the molecular mechanisms that regulate glucose homeostasis, and explores the roles of the Akt signaling pathway during hibernation. Here, we propose a link between hibernation, a well-documented response to periods of environmental stress, and reversible insulin resistance, potentially facilitated by key alterations in the Akt signaling network, PPAR-γ/PGC-1α regulation, and non-coding RNA expression. Coincidentally, many of the same pathways are frequently found to be dysregulated during insulin resistance in human type 2 diabetes. Hence, the molecular networks that may regulate reversible insulin resistance in hibernating mammals represent a novel approach by providing insight into medical treatment of insulin resistance in humans.

- 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

Hibernation and medicine

Primates !!

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 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 ro
transcription factors. The
relevant to issues in clini
and atrophy resistance.

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
e cell preservation strategies that support long-term viability in a hypometabolic state. These
proving 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-
ation including multiple forms of post-translational modification of proteins/enzymes
lOylation), 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
perones and the implementation of the unfolded protein response, and the enhancement of
s) to control the actions of extracellular proteases in clotting and inflammation responses.

Cryobiology 69 (2014) 333–338

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Global DNA modifications suppress transcription in brown adipose tissue during hibernation ☆

Yulia Biggar, Kenneth B. Storey *

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

ARTICLE INFO

Article history:
Received 6 January 2014
Accepted 20 August 2014
Available online 3 September 2014

Keywords:
Ground squirrel hibernation
Ictidomys tridecemlineatus
Transcriptional repression
DNA methylation
Histone acetylation
HP1
MBD1

ABSTRACT

Hibernation is crucial to winter survival for many small mammals and is characterized by prolonged periods of torpor during which strong global controls are applied to suppress energy-expensive cellular processes. We hypothesized that one strategy of energy conservation is a global reduction in gene transcription imparted by reversible modifications to DNA and to proteins involved in chromatin packing. Transcriptional regulation during hibernation was examined over euthermic control groups and five stages of the torpor/arousal cycle in brown adipose tissue of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). Brown adipose is crucial to hibernation success because it is responsible for the non-shivering thermogenesis that rewarms animals during arousal. A direct modification of DNA during torpor was revealed by a 1.7-fold increase in global DNA methylation during long term torpor as compared with euthermic controls. Acetylation of histone H3 (on Lys23) was reduced by about 50% when squirrels entered torpor, which would result in increased chromatin packing (and transcriptional repression). This was accompanied by strong increases in histone deacetylase protein levels during torpor; e.g. HDAC1 and HDAC4 levels rose by 1.5- and 6-fold, respectively. Protein levels of two co-repressors of transcription, MBD1 and HP1, also increased by 1.9- and 1.5-fold, respectively, in long-term torpor and remained high during early arousal. MBD1, HP1 and HDACs all returned to near control values during interbout indicating a reversal of their inhibitory actions. Overall, the data presents strong evidence for a global suppression of transcription during torpor via the action of epigenetic regulatory mechanisms in brown adipose tissue of hibernating thirteen-lined ground squirrels.

Cryobiology. 2014 Oct;69(2):333-8. doi: 10.1016/j.cryobiol.2014.08.008. Epub 201

Global DNA modifications suppress transcription in

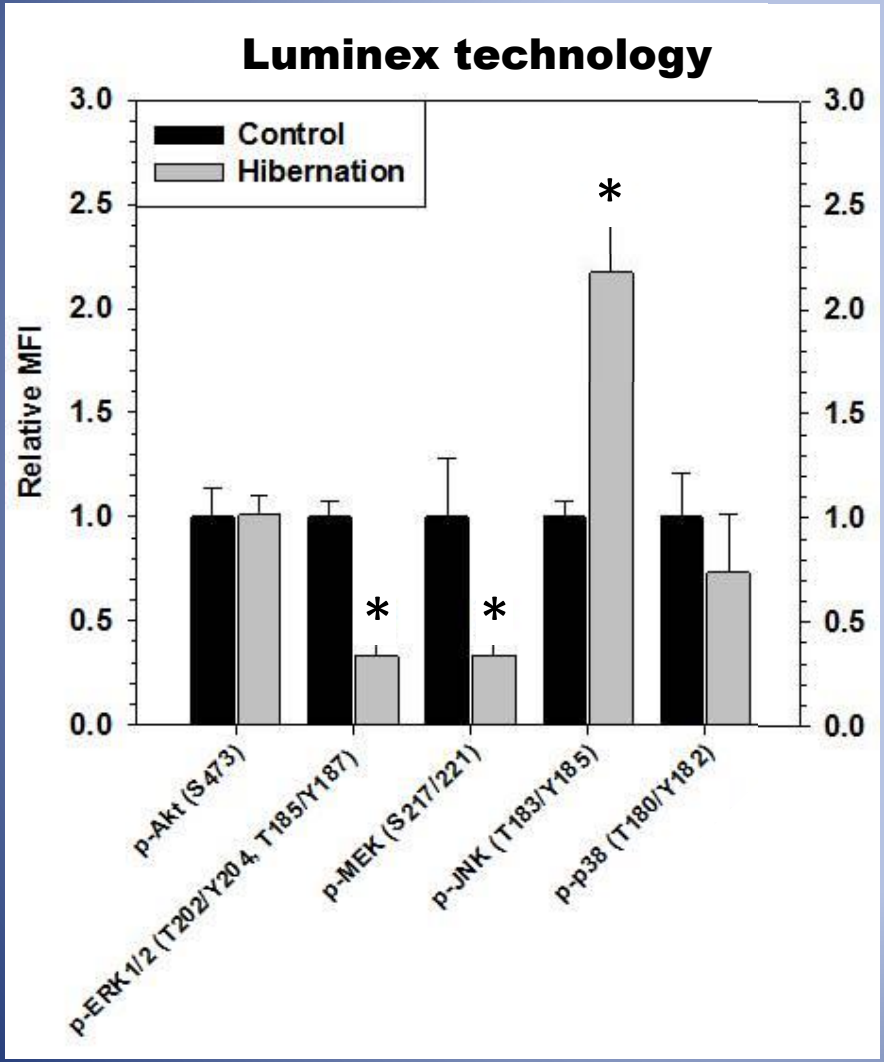
Biggar Y[1], Storey KB[2].

Author information

Abstract

Hibernation is crucial to winter survival for many small mammals and is characterized by prolonged periods of torpor during which strong global controls are applied to suppress energy-expensive cellular processes. We hypothesized that one strategy of energy conservation is a global reduction in gene transcription imparted by reversible modifications to DNA and to proteins involved in chromatin packing. Transcriptional regulation during hibernation was examined over euthermic control groups and five stages of the torpor/arousal cycle in brown adipose tissue of thirteen-lined ground squirrels (Ictidomys tridecemlineatus). Brown adipose is crucial to hibernation success because it is responsible for the non-shivering thermogenesis that rewarms animals during arousal. A direct modification of DNA during torpor was revealed by a 1.7-fold increase in global DNA methylation during long term torpor as compared with euthermic controls. Acetylation of histone H3 (on Lys23) was reduced by about 50% when squirrels entered torpor, which would result in increased chromatin packing (and transcriptional repression). This was accompanied by strong increases in histone deacetylase protein levels during torpor; e.g. HDAC1 and HDAC4 levels rose by 1.5- and 6-fold, respectively. Protein levels of two co-repressors of transcription, MBD1 and HP1, also increased by 1.9- and 1.5-fold, respectively, in long-term torpor and remained high during early arousal. MBD1, HP1 and HDACs all returned to near control values during interbout indicating a reversal of their inhibitory actions. Overall, the data presents strong evidence for a global suppression of transcription during torpor via the action of epigenetic regulatory mechanisms in brown adipose tissue of hibernating thirteen-lined ground squirrels.

MRD in Primate hibernation [Stress Kinases]



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

Gray Mouse Lemur

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