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

HIBERNATION

13-LINED GROUND SQUIRREL

Ictidomys tridecemlineatus

HIBERNATION

Little Brown Bat
Myotis lucifugus

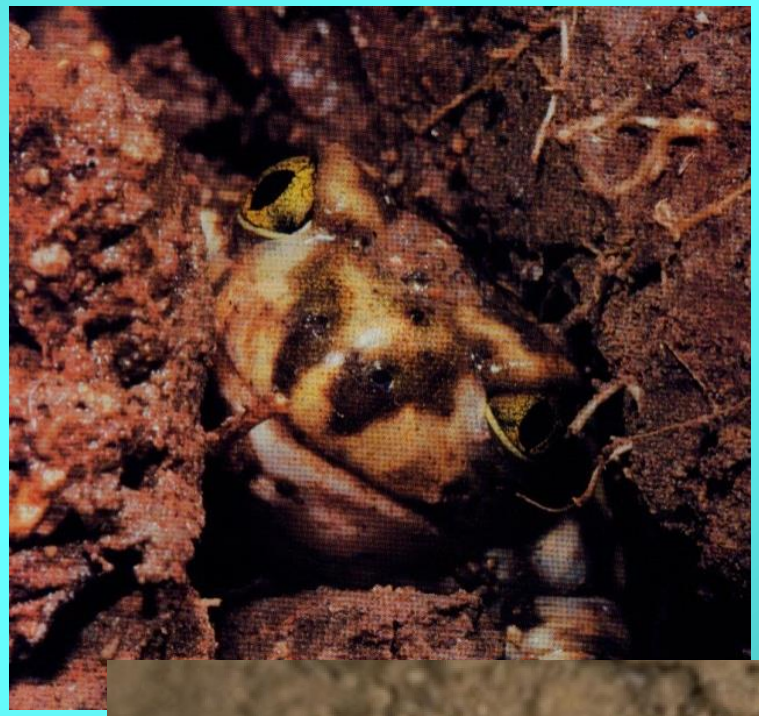
DAILY TORPOR

Grey mouse lemur,
Microcebus murinus

ESTIVATION

Milk snail
Otala lactea

ESTIVATION



Spadefoot toad
Scaphiopus holbrookii

ANOXIA

Painted turtle
Chrysemys picta

Periwinke
Littorina littorea

Red-eared turtle
Pseudemys scripta

FREEZING

Wood frog
Rana sylvatica

MAMMALS ON ICE:

Metabolic Rate Reduction

www.carleton.ca/~kbstorey

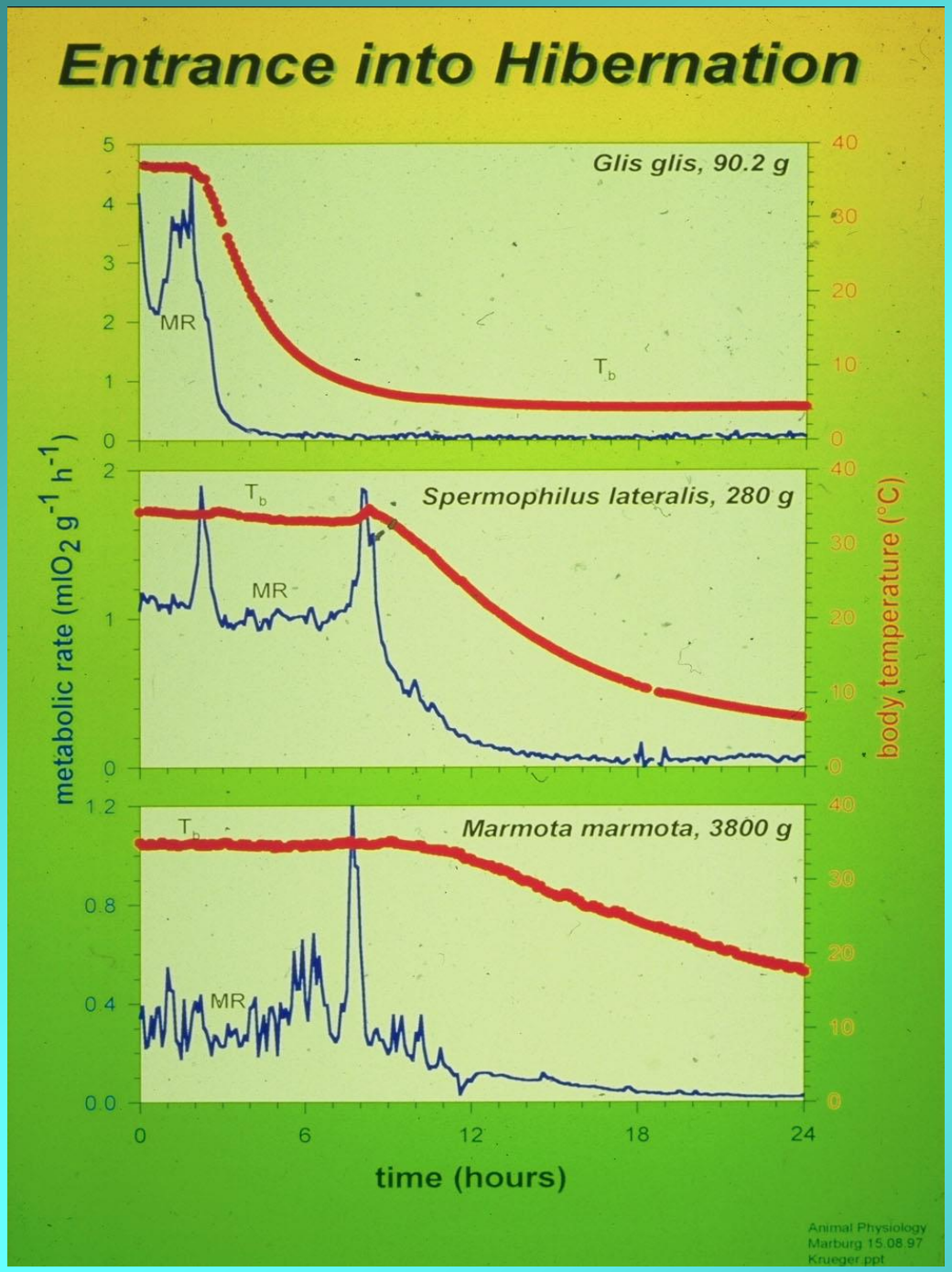
Model Hibernators

***Urocitellus richardsonii*, Richardson's ground squirrel**

***Ictidomys 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



- **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, 2nd messenger PKs)
3. Most Genes OFF
4. Selective gene activation

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

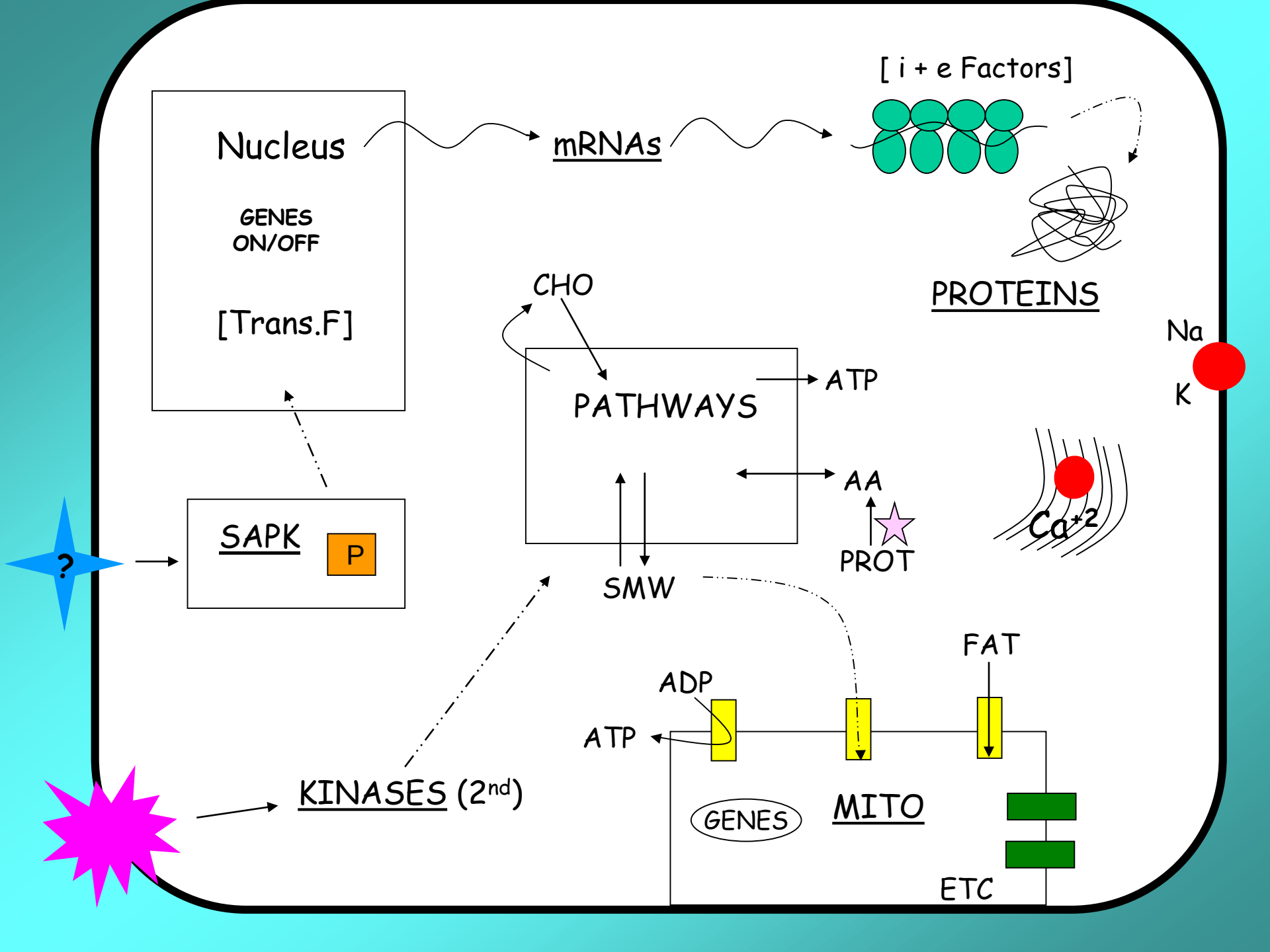
Freezing

Diapause

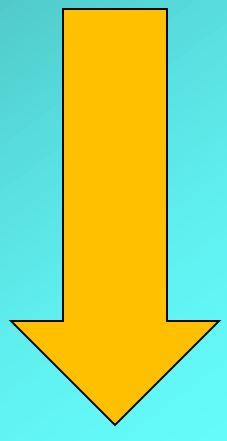
PRINCIPLES OF HIBERNATION

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

Same as with ALL MRD

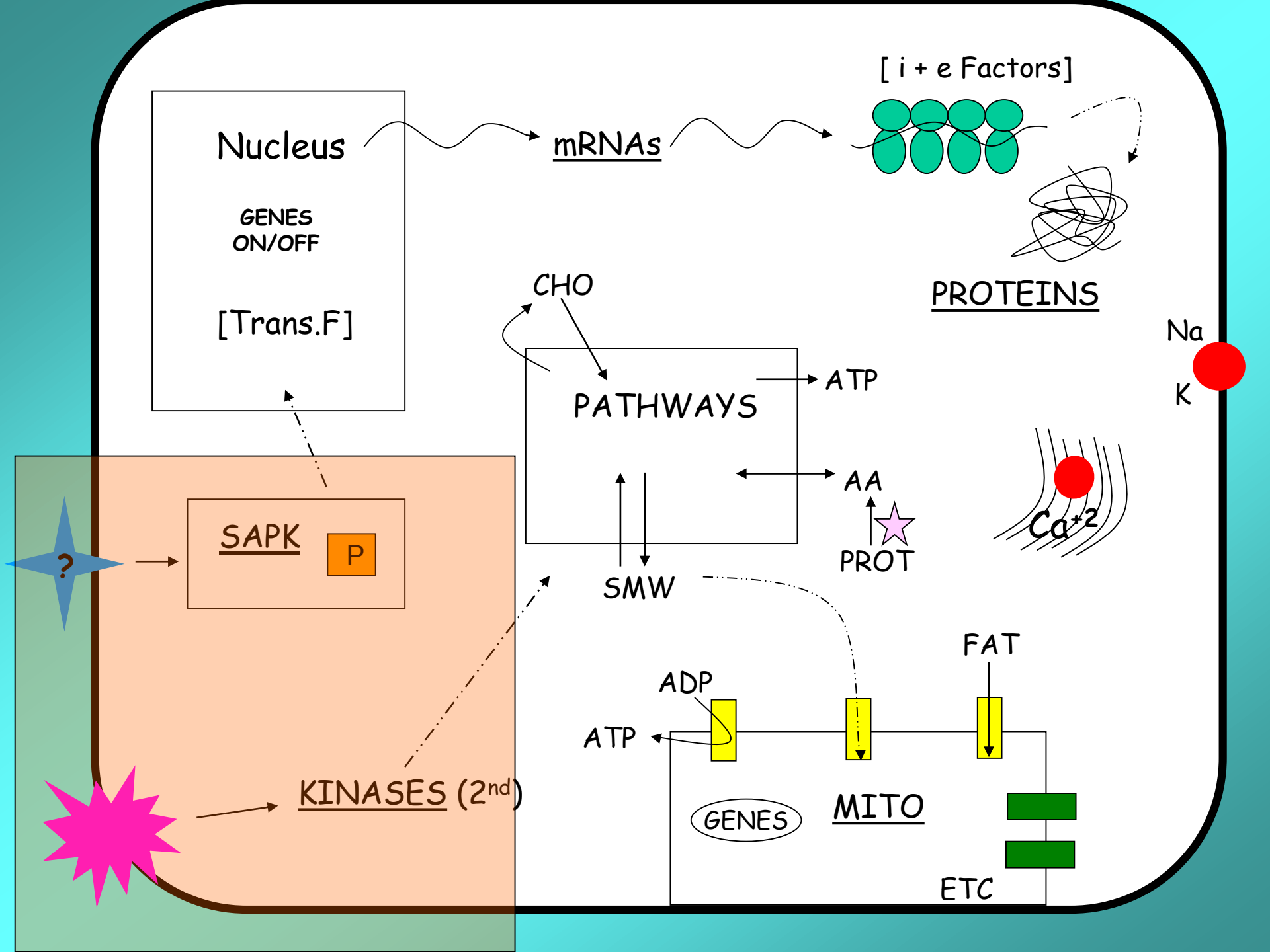

[i + e Factors]
Nucleus
GENES
ON/OFF
[Trans.F]
mRNAs
PROTEINS
CHO
PATHWAYS
ATP
AA
PROT
SMW
Na
K
Ca+2
?
SAPK
P
KINASES (2nd)
ADP
ATP
FAT
GENES
MITO
ETC

METABOLISM IN HIBERNATION



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

ATP turnover  to <5% of normal


[i + e Factors]
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Na
K
Ca+2
SAPK
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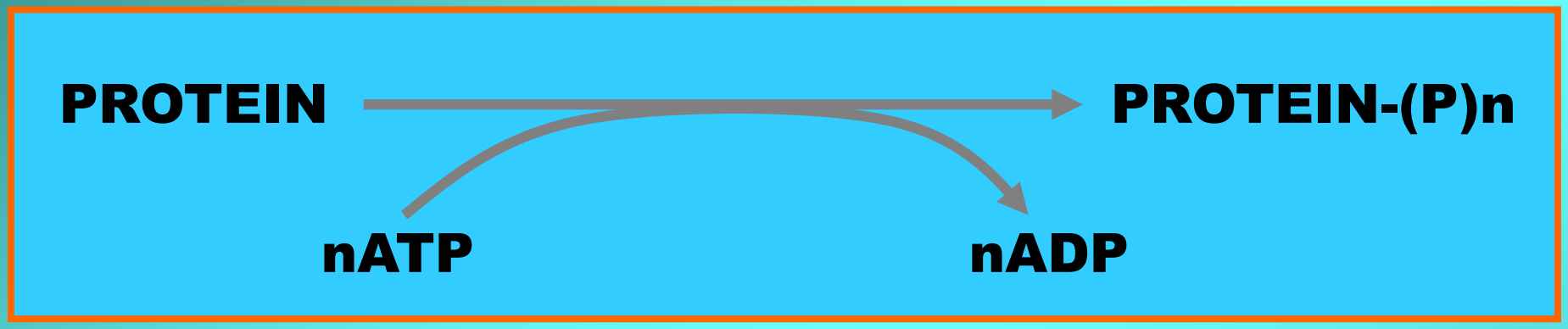
?
KINASES (2nd)
ADP
ATP
FAT
GENES
MITO
ETC

Metabolic Rate Depression CHANGES

- Few ‘SAP’ kinases activated
- * thousands of processes OFF

- 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)

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

No Modification

PROTEINS (ENZYMES)

Control by proteases

Degradation

INACTIVE ENZYME

Covalent modification

Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation

Control at level of enzyme function

ACTIVE ENZYMES

TURNING OFF GENES: Role of Epigenetics

Epigenetics:

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

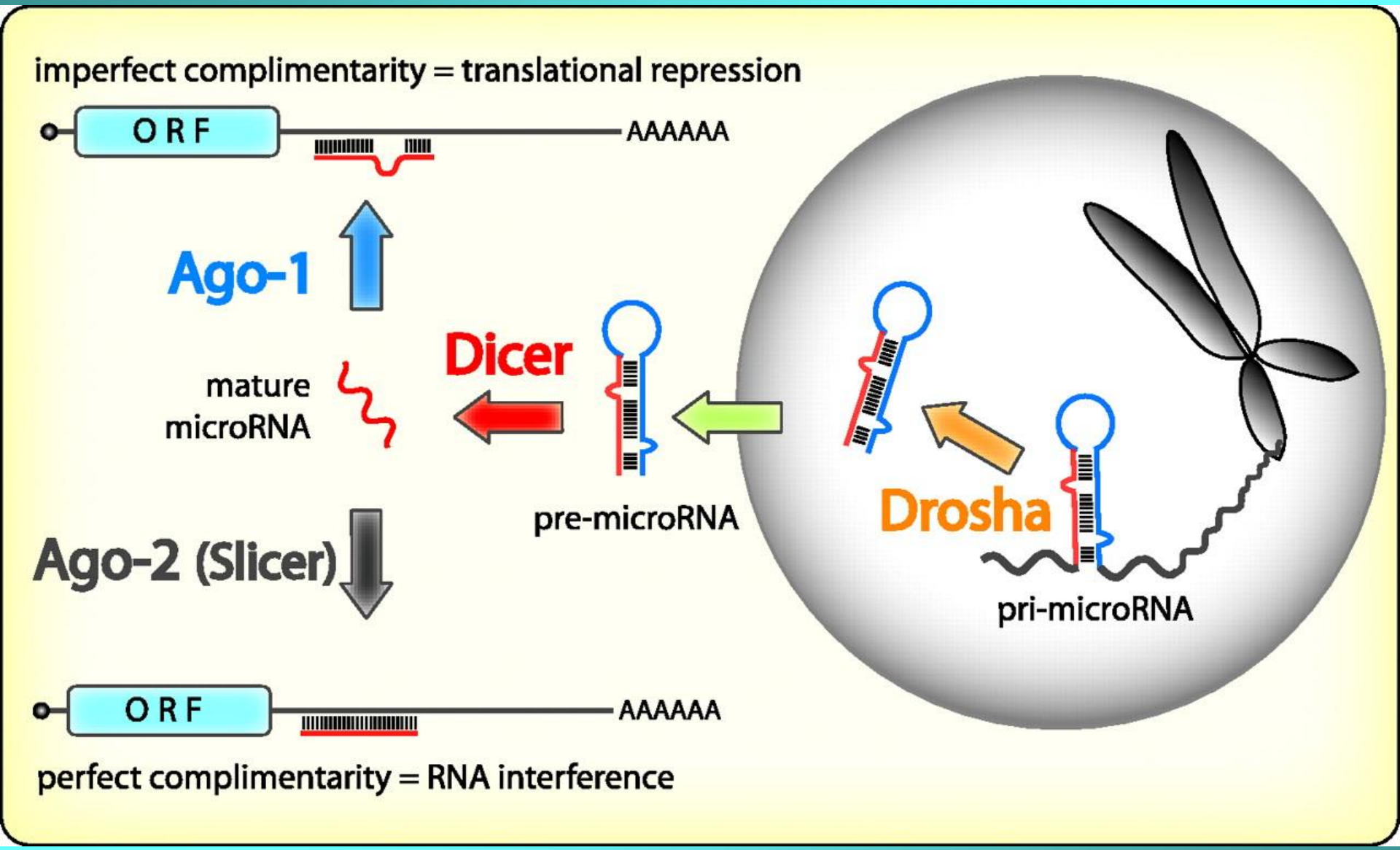
Common mechanisms:

- 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



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

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*

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Metabolic r
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bolic states
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marily from
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decrease pr
cell cycle ar
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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

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Article history:
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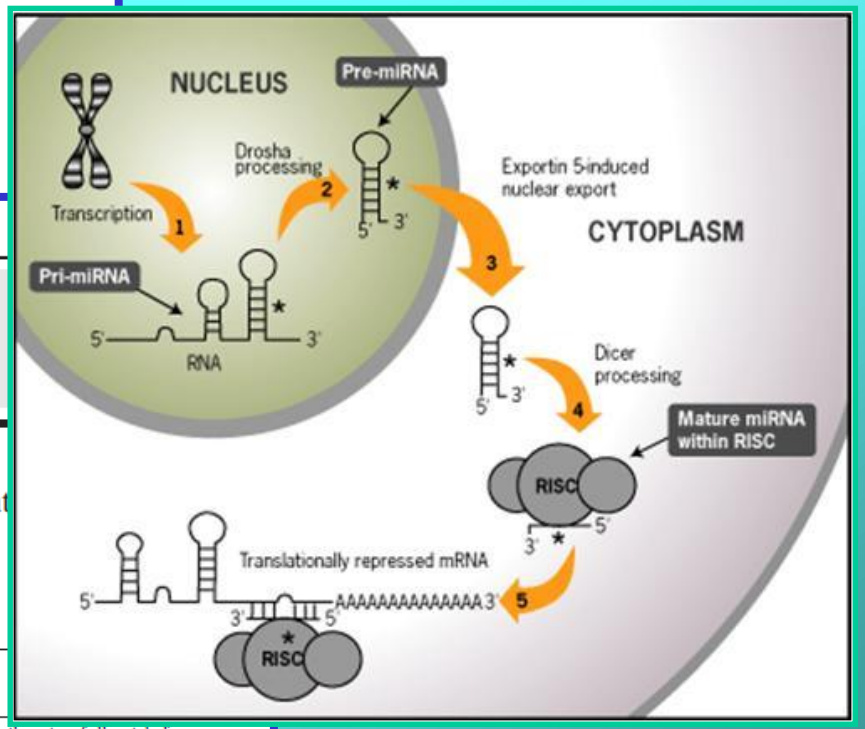
Keywords:
MicroRNA
Hibernation
Spermophilus tridecemlineatus
Dicer
Reversible control of translation

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



METABOLIC RATE DEPRESSION

Hibernation

Estivation

Anoxia

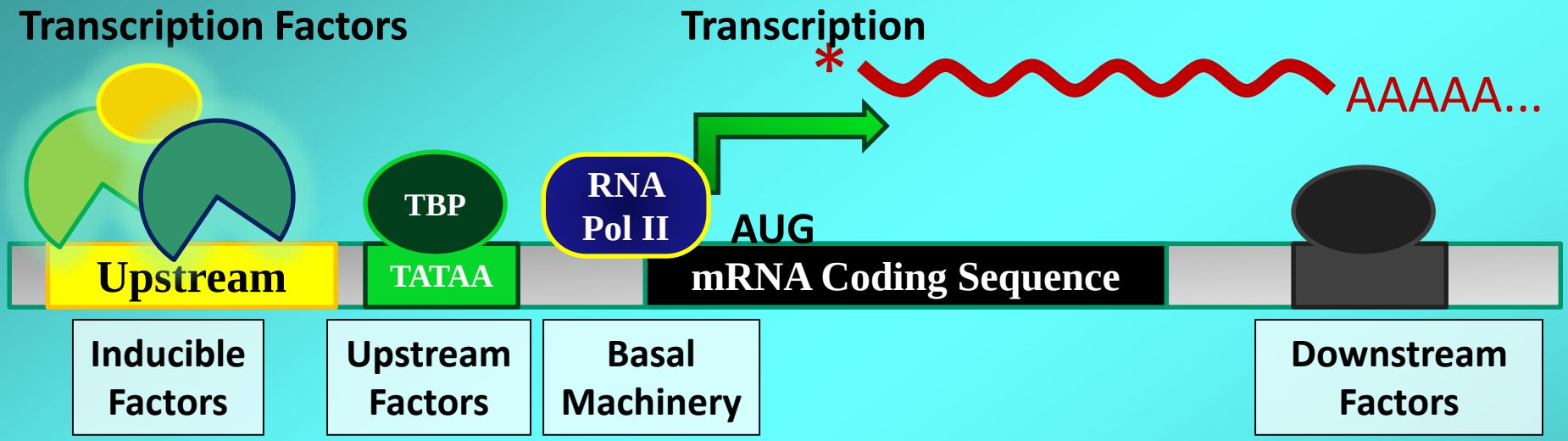
Freezing

Diapause

MRD CHANGES

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

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 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

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

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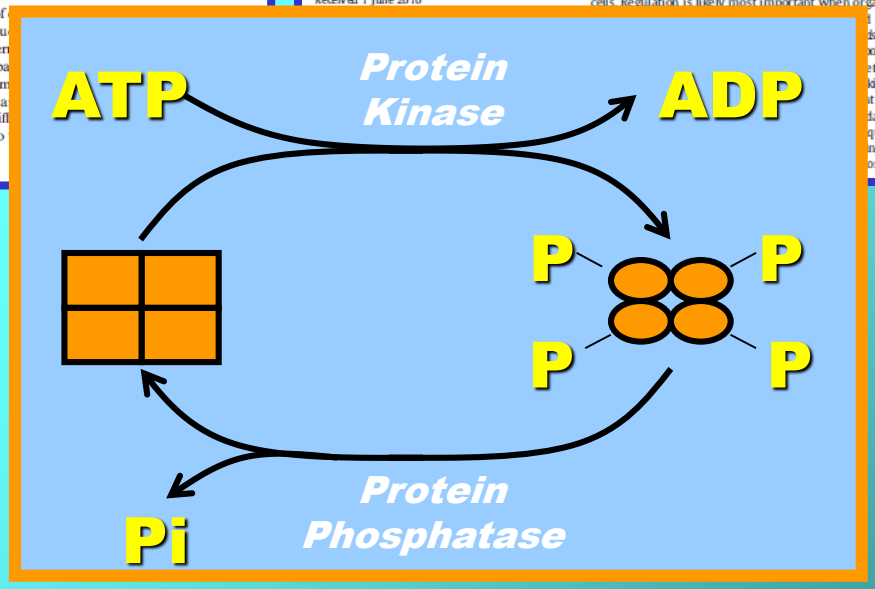
ARTICLE INFO

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 sphorylated and activated when animals transition into



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

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Available online at www.sciencedirect.com

ScienceDirect

Cryobiology 53 (2006) 310–318

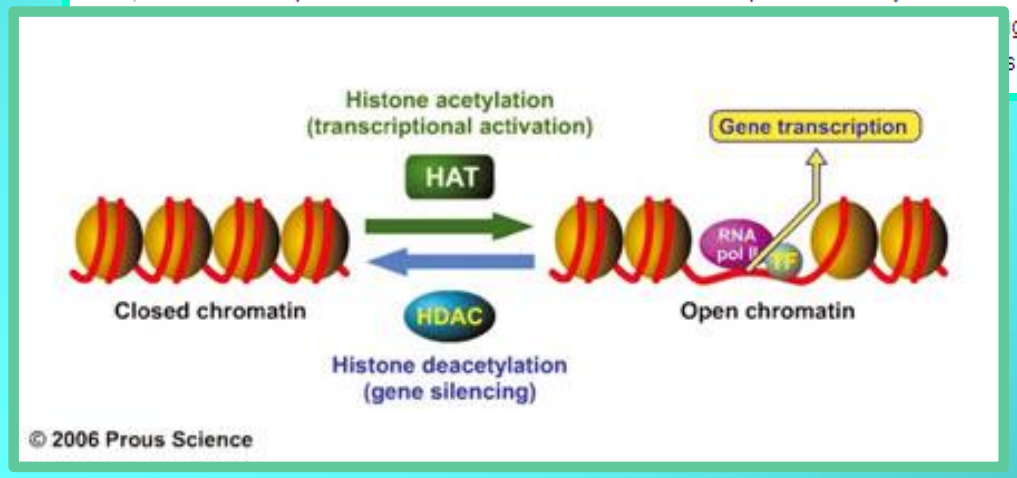
CRYOBIOLOGY

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



Life span extension

Oxid Med Cell Longev. 2010 May-Jun;3(3):186-98.

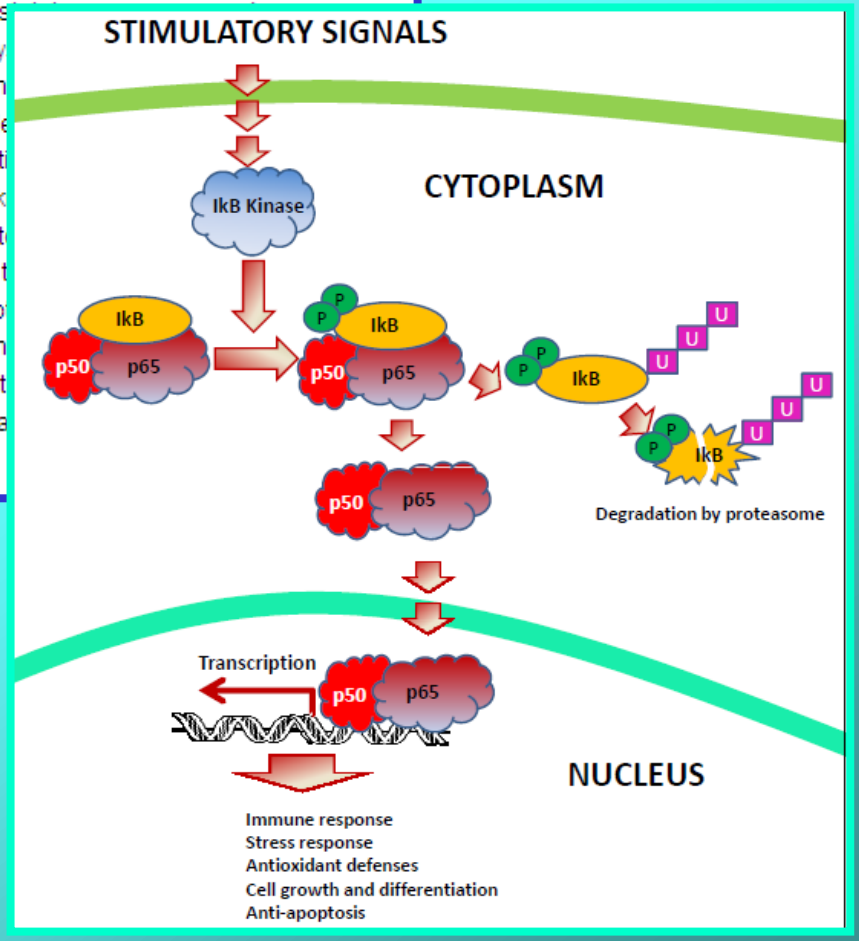
Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.

Krivoruchko A, Storey KB.

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

Abstract

While mammals cannot survive oxygen deprivation for more than a few minutes without sus
some animals have mastered anaerobic life. Freshwater turtles belonging to the Trachemy
champion facultative anaerobes of the vertebrate world, often surviving without oxygen for m
physiological and biochemical mechanisms that underlie anoxia tolerance in turtles include
depression, post-translational modification of proteins, strong antioxidant defenses, activati
transcription factors, and enhanced expression of cytoprotective proteins. Turtles are also k
and display characteristics of "negligible senescence". We propose that the robust stress-t
long term anaerobiosis by turtles may also support the longevity of these animals. Many of t
natural anoxia tolerance, such as hypometaboli e pro
to play important roles in mammalian oxygen-re andin
oxygen could aid in the understanding and treat s that
In the present review we discuss the recent adv ecula
turtles and the potential links between this toler



Atrophy – Hypertrophy

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

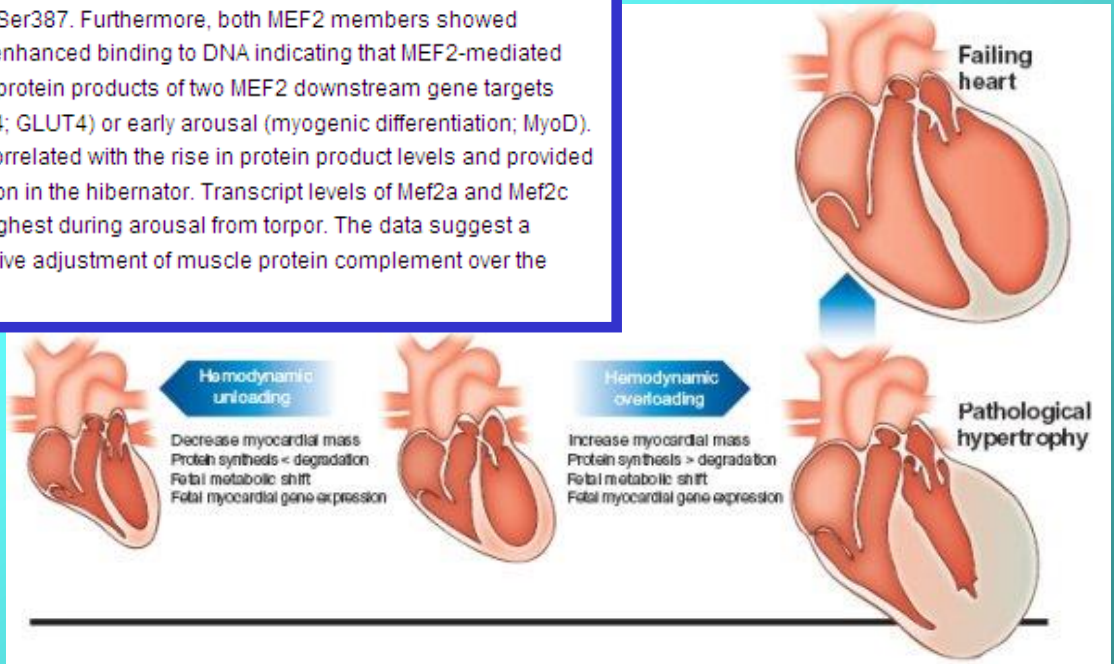
Expression of myocyte enhancer factor-2 and downstream genes in ground squirrel skeletal muscle during hibernation.

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 erns 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



Unavoidable metabolic costs

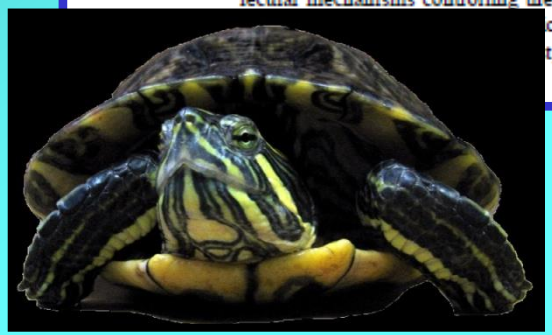
Current Genomics, 2009, *10*, 573-584

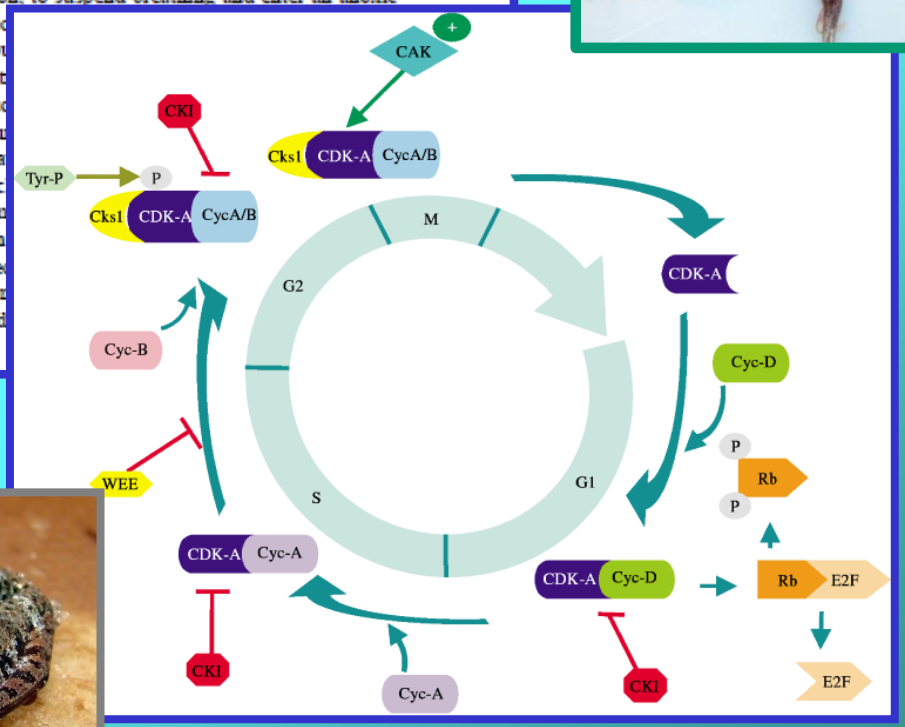
Perspectives in Cell Cycle Regulation: Lessons from an Anoxic Vertebrate

Kyle K. Biggar and Kenneth B. Storey*

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

Abstract: The ability of an animal, normally dependent on aerobic respiration, to suspend breathing and enter an anoxic state for long term survival is clearly a fascinating feat, and has been the foc
anoxia tolerant turtles are faced with periods of oxygen deprivation, numerou
take place in order to facilitate vital reductions in ATP consumption. Such st
modifications as well as the implementation of translation and transcription co
though it is clear that anoxic survival relies on the suppression of ATP consu
anoxia tolerant vertebrates remain elusive. Several anoxia tolerant invertebra
cell cycle arrest when presented with anoxic stress. Despite this, the cell cyc
tolerant turtles. Understanding how vertebrates respond to anoxia can have im
cellular proliferation and hypoxic tumor progression are inescapably linked in
lecular mechanisms controlling these processes have profound clinical conse
c vertebrates and more specifically, the cor
t, the activation of checkpoint kinases, and





Unity through Evolution

International Journal of Biological Sciences
2010; 6(1):9-50

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 ✉

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Ottawa, Ont. K1S 5B6, Canada. Tel. +1 613 520 3678, Fax

Received: 2009.09.11; Accepted: 2009.11.25; Published: 2010.01.0

Abstract

Studies of the molecular mechanisms tha
physiological) have long been used to ma
model organism, *Caenorhabditis elegans*,
'dauer' stage. This period of developmer
in metabolic rate, triggered by ambient
ents. *C. elegans* employs a number of sig
unfavourable conditions and survive for l
The suppression of cellular metabolism
survival of nematodes through the daue
nisms that are fundamental to control
general, mammalian systems are highly i
temperatures and low oxygen), howeve
signal transduction pathways of nematod
protein targets in the stress response
maintained, and often differ only in the
outlines a framework of critical molecul
as therapeutic targets for addressing dis

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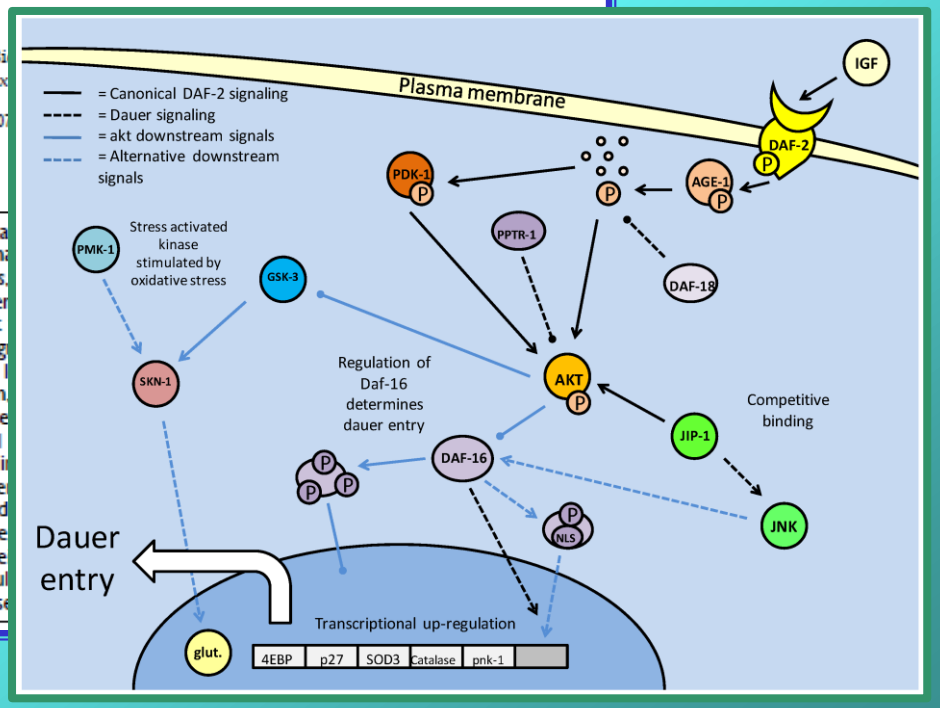
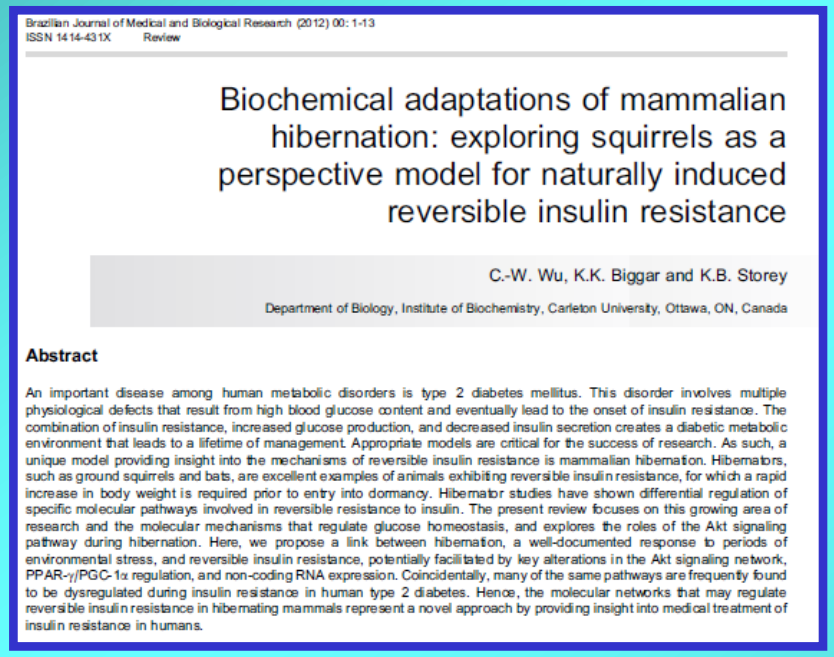


Hibernators as a model for metabolic disease?

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.

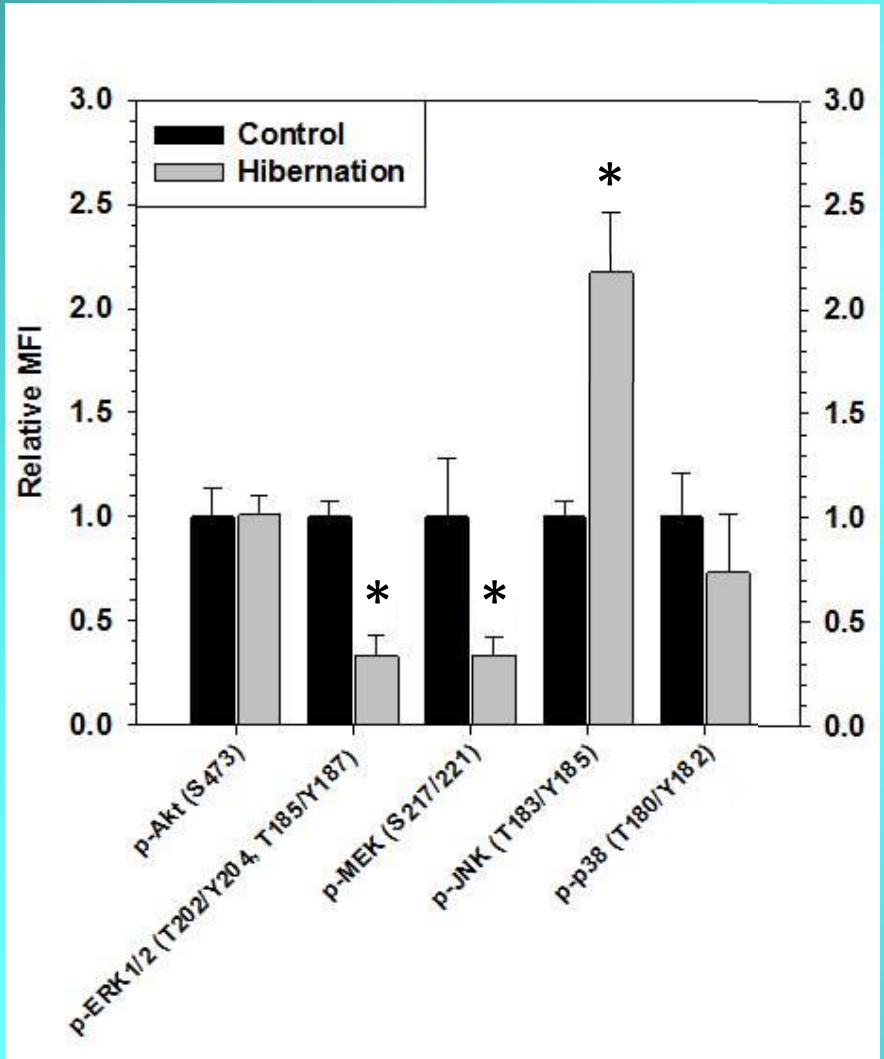
- **Hibernators show reversible insulin resistance**
- **Rapid weight gain, hyperinsulinemia during entry into hibernation, reversed in deep torpor**
- **Modifications to similar pathways: Glucose transport, Akt activity, PPAR-γ signaling**

PRIMATE HIBERNATION !!

Gray Mouse Lemur

Madagascar - western dry forests

MRD in Primate hibernation [Stress Kinases]

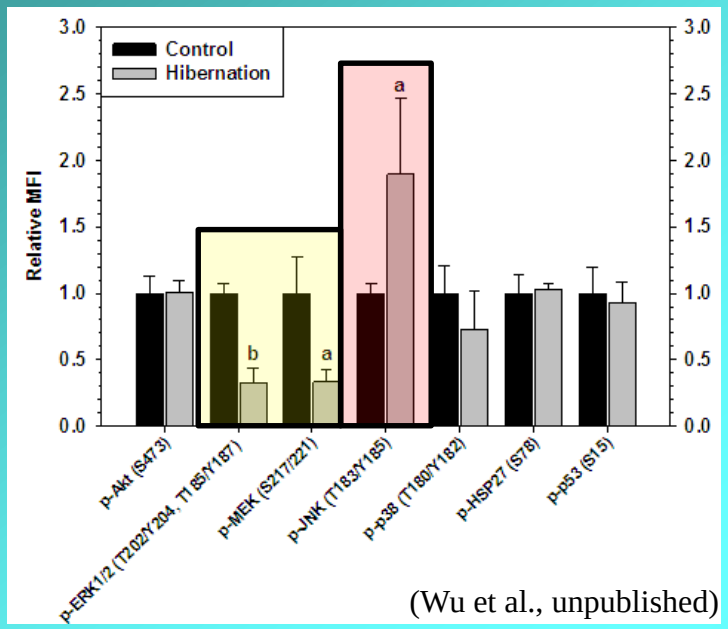


Biggar KK, Wu CW, Tessier SN, Zhang J & Storey KB

Gray Mouse Lemur

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

Primate Hibernation



(Wu et al., unpublished)

Gray mouse lemur

- 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

Primates !!

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

Metabolic rate depression: the biochemistry of mammalian hibernation.

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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.

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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
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.

Metabolic Rate Depression

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GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

No Modification

PROTEINS (ENZYMES)

Control by proteases

Degradation

INACTIVE ENZYME

Covalent modification

Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation

Control at level of enzyme function

ACTIVE ENZYMES