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

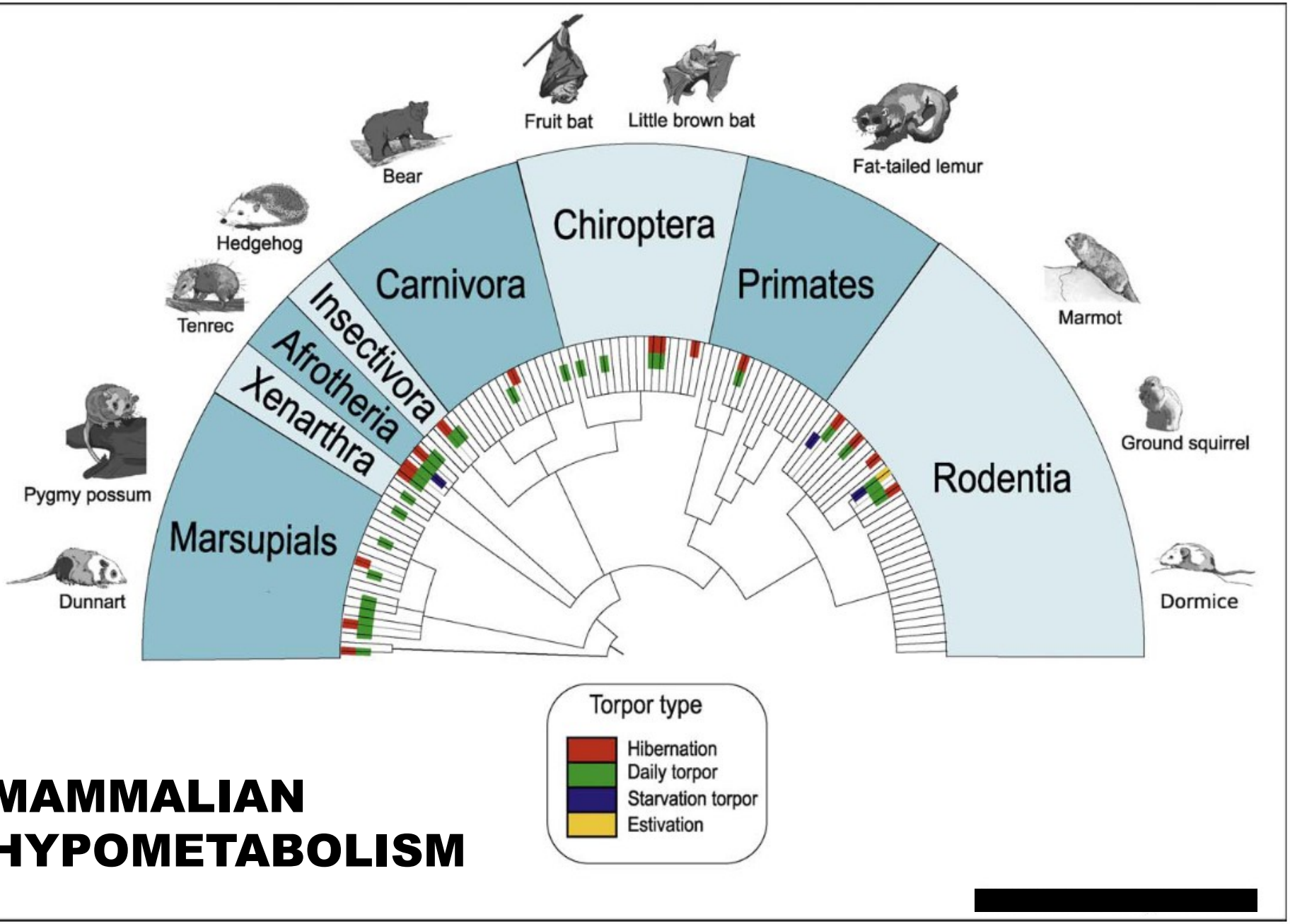
Hibernation

Estivation

Anoxia

Freezing

Diapause



MAMMALIAN HYPOMETABOLISM

Melvin RG & Andrews MT, Trends Endocrinol. Metab. 20: 490-8, 2009

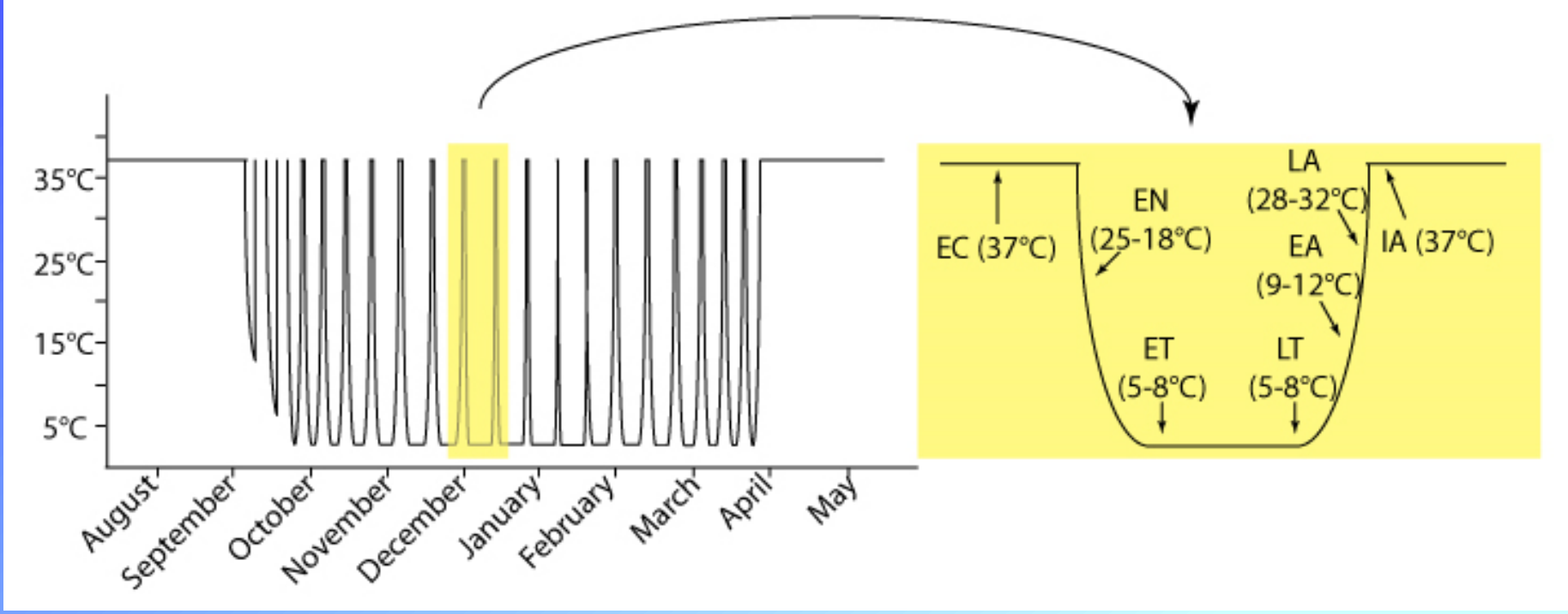
Model Hibernators

Spermophilus richardsonii, Richardson's ground squirrel

Spermophilus tridecemlineatus, 13-lined ground squirrel

Myotis lucifugus, little brown bat

TORPOR-AROUSAL IN HIBERNATORS



Animal studies by Dr. JM Hallenbeck and Dr. DC McMullen, NIH

Figure adapted from Nelson *et al.* 2009

COLD HIBERNATION

Lessons from mammalian hibernators: molecular insights into striated muscle plasticity and remodeling.
Tessier SN, **Storey KB**.
Biomol Concepts. 2016, 7(2):69-92. PMID: 26982616

Life in the cold: links between mammalian hibernation and longevity.
Wu CW, **Storey KB**.
Biomol Concepts. 2016, 7(1):41-52. PMID: 26820181

Insight into post-transcriptional gene regulation: stress-responsive microRNAs and their role in environmental stress survival of tolerant animals.
Biggar KK, **Storey KB**.
J Exp Biol. 2015, 218(Pt 9):1281-9. PMID: 25954040

Regulation of hypometabolism: insights into epigenetic controls.
Storey KB.
J Exp Biol. 2015, 218(Pt 1):150-9. PMID: 25568462

To be or not to be: the regulation of mRNA fate as a survival strategy during mammalian hibernation.
Tessier SN, **Storey KB**.
Cell Stress Chaper. 2014, 19(6):763-76. PMID: 24789358

Biochemical adaptations of mammalian hibernation: exploring squirrels as a perspective model for naturally induced reversible insulin resistance.
Wu CW, Biggar KK, **Storey KB**.
Braz J Med Biol Res. 2013, 46(1):1-13. PMID: 23314346

Biochemical adaptations of mammalian hibernation: exploring squirrels as a perspective model for naturally induced reversible insulin resistance.
Wu CW, Biggar KK, **Storey KB**.
Braz J Med Biol Res. 2013, 46(1):1-13. PMID: 23314346

The emerging roles of microRNAs in the molecular responses of metabolic rate depression.
Biggar KK, **Storey KB**.
J Mol Cell Biol. 2011, 3(3):167-75. PMID: 21177365

Out cold: biochemical regulation of mammalian hibernation - a mini-review.
Storey KB.
Gerontology. 2010, 56(2):220-30. PMID: 19602865

Metabolic rate depression: the biochemistry of mammalian hibernation.
Storey KB, Storey JM.
Adv Clin Chem. 2010, 52:77-108. PMID: 21275340

MONITO del MONTE

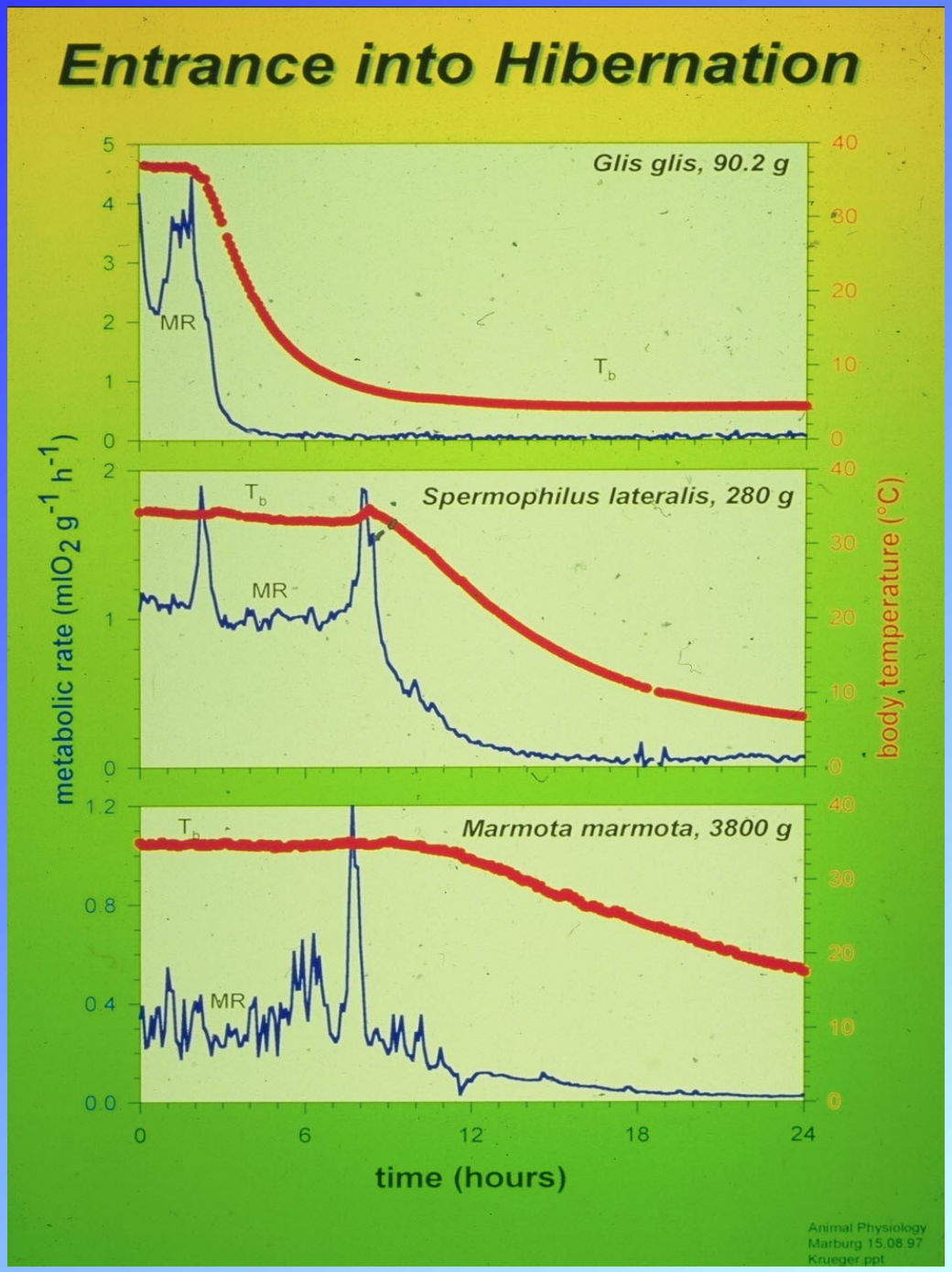
Dromiciops gliroides

South American marsupial

TORPOR

Gray mouse lemur,
Microcebus murinus

BEARS !



- 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

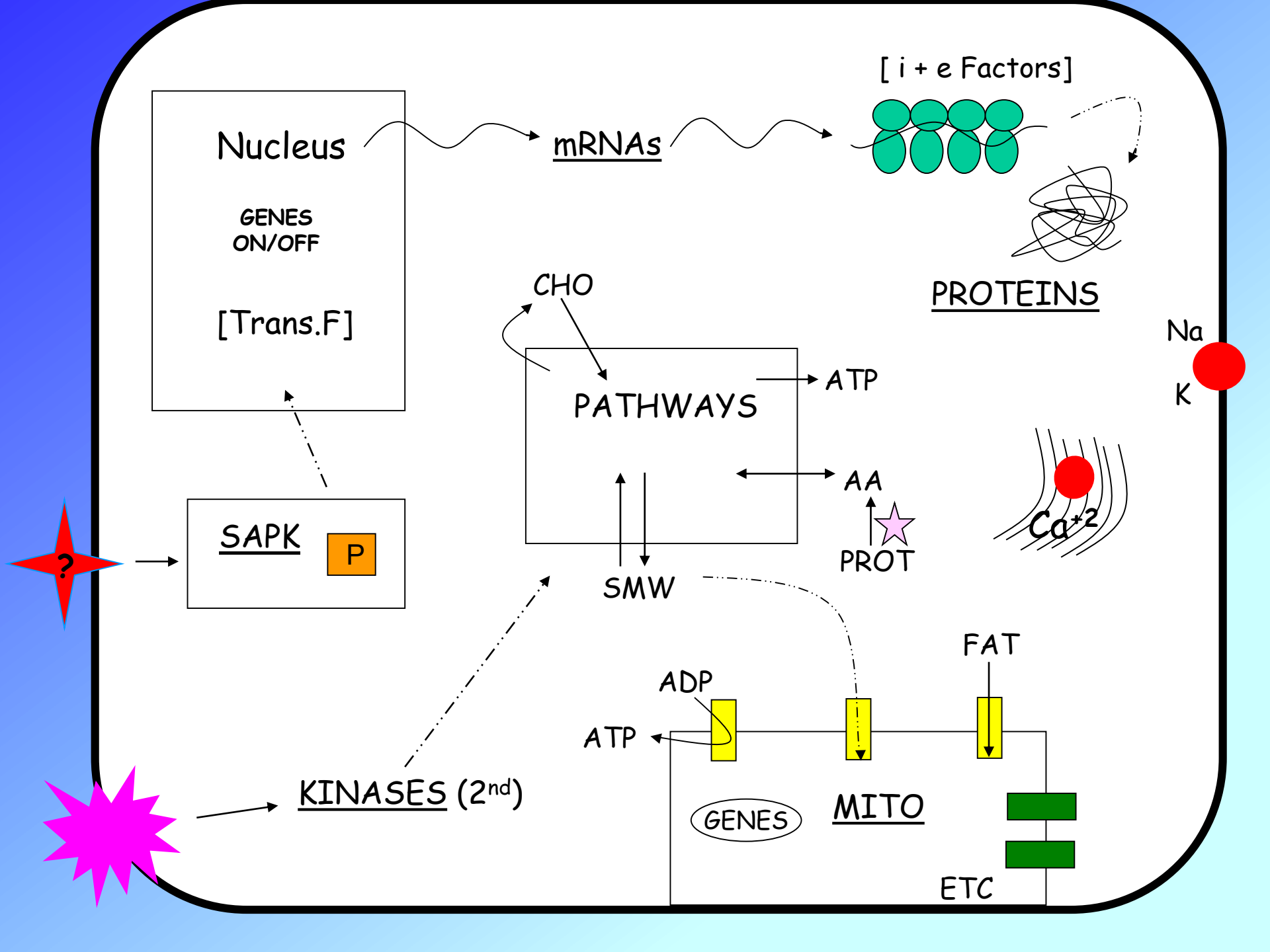
1. Metabolic rate reduction
2. Cold or Warm temperature
3. Most Genes & Processes OFF
4. miRNA Control of Pathways
5. Epigenetics as Central Controller

Same for ALL MRD

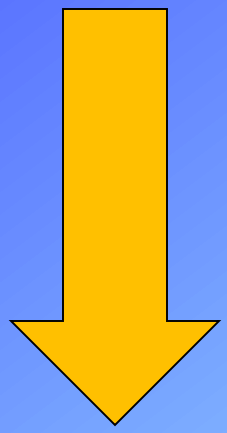
PRINCIPLES OF HIBERNATION

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

Same for ALL MRD

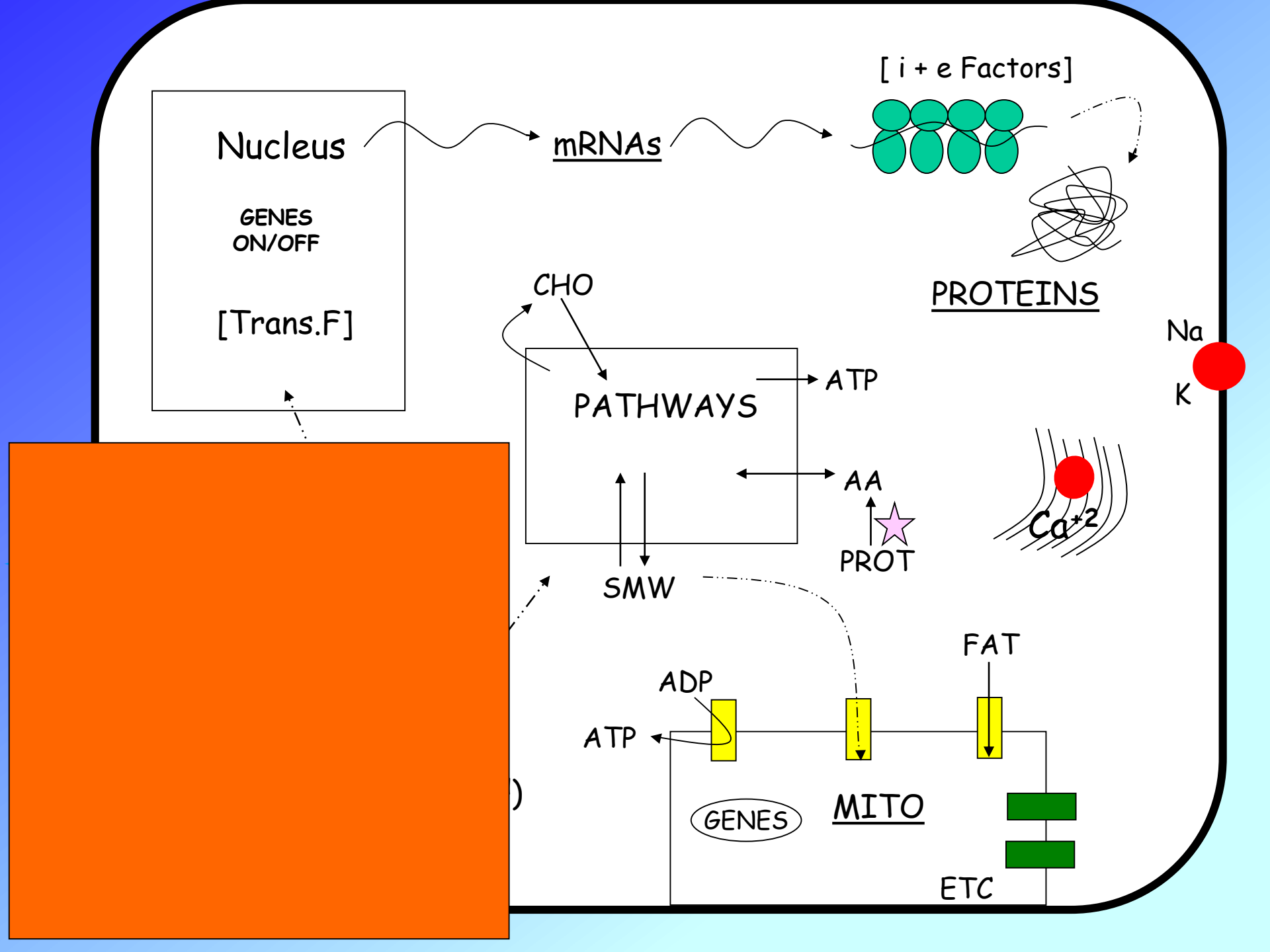

[i + e Factors]
Nucleus
GENES
ON/OFF
[Trans.F]
mRNAs
PROTEINS
CHO
PATHWAYS
ATP
AA
PROT
SMW
Na
K
Ca+2
SAPK
P
?
KINASES (2nd)
ADP
ATP
FAT
GENES
MITO
ETC

METABOLISM IN HIBERNATION



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

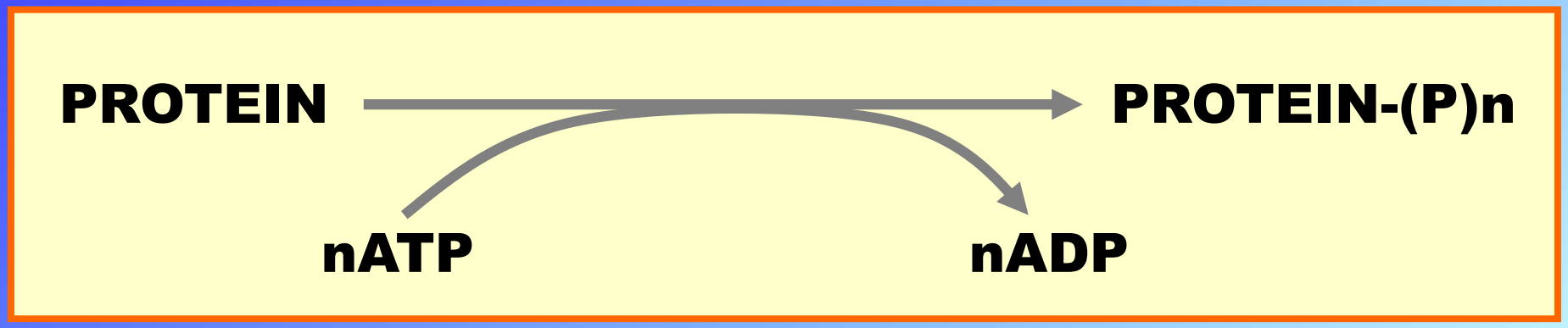
ATP turnover ↓ to <5% of normal


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

Metabolic Rate Depression CHANGES

- ***Thousands of processes OFF***
- **Gene ‘inactivation’ (⇩ mRNA)**
- **Few Genes activated (1-2%)**

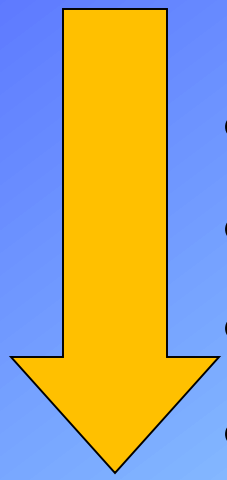
PROTEIN KINASES



- 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)

PATHWAY CONTROL IN HIBERNATION

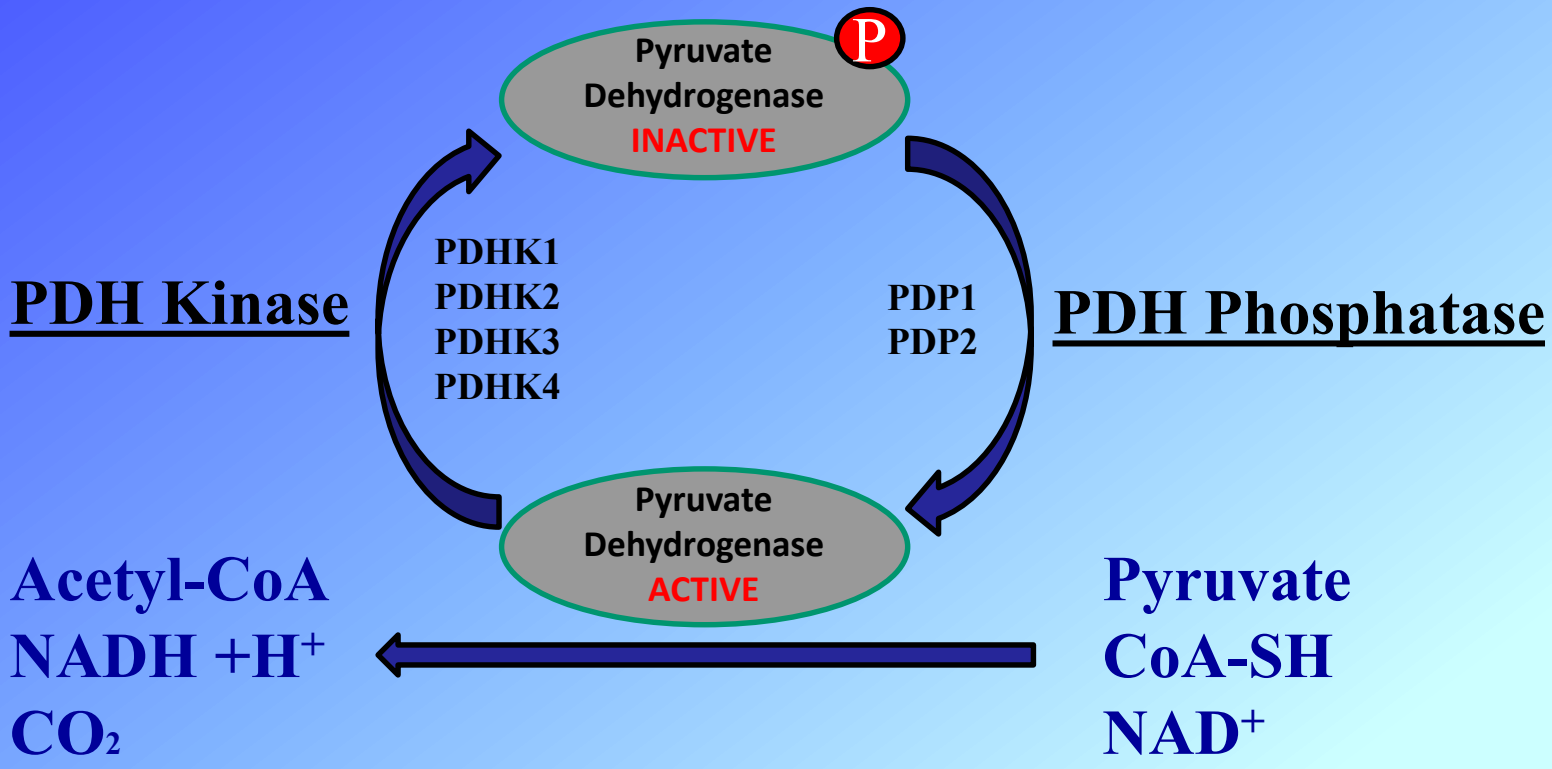
Phospho / de-Phospho

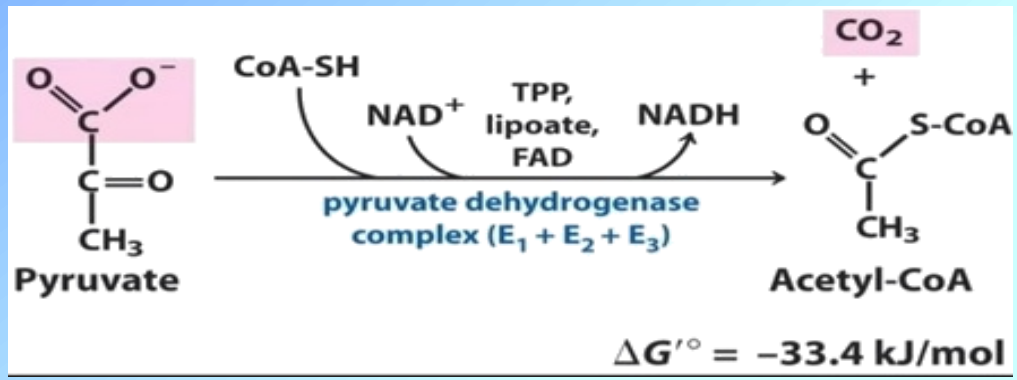


- **Glycolysis** **(GP, GS, PFK, PK)**
- **Fat synthesis** **(ATP-CL, ACC)**
- **CHO fuel use** **(PDH)**
- **Translation** **(eIF2α, eEF2)**
- **Ion pumps** **(NaK, Ca-ATPase)**

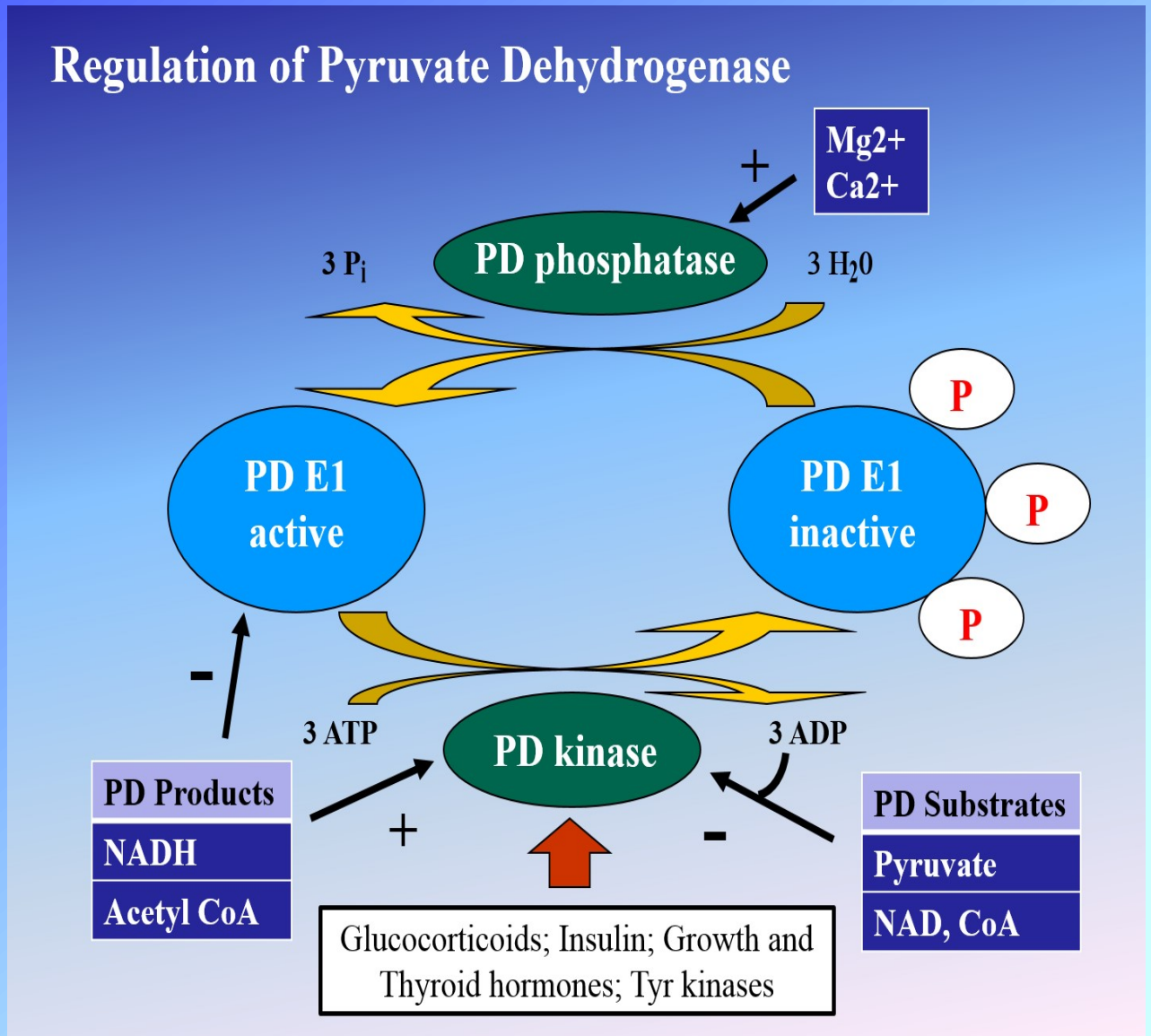
- ***the usual suspects, TextBook***

Pyruvate Dehydrogenase Regulation





Phosphorylation of one or more Ser sites → INACTIVATES pSer232, pSer293, pSer300



Metabolic Rate Depression CHANGES

Few ‘SAP’ kinases activated

- **Few Genes activated (1-2%)**

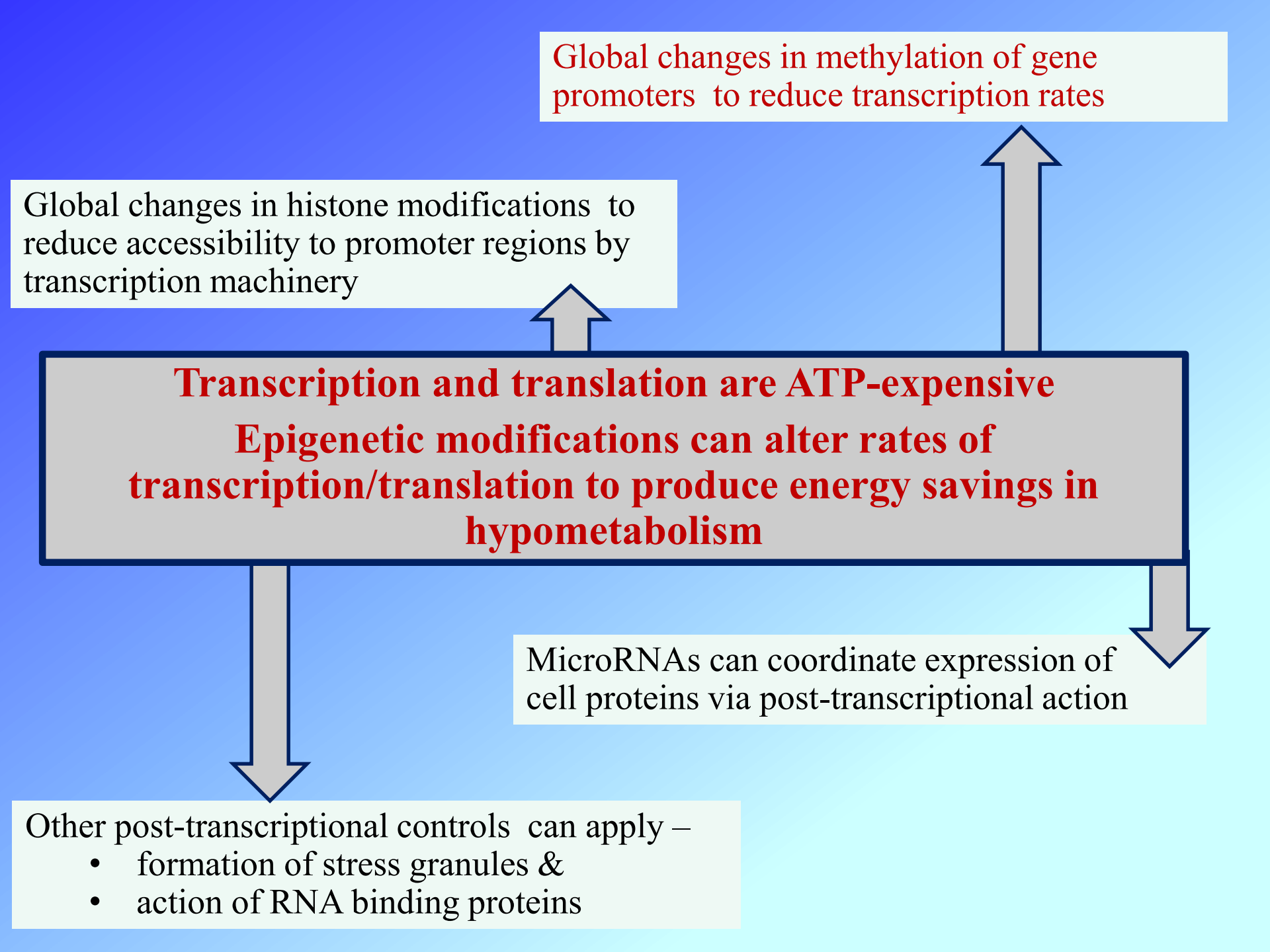
TURNING OFF GENES: Role of Epigenetics

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**


Global changes in methylation of gene promoters to reduce transcription rates
Global changes in histone modifications to reduce accessibility to promoter regions by transcription machinery
Transcription and translation are ATP-expensive
Epigenetic modifications can alter rates of transcription/translation to produce energy savings in hypometabolism
MicroRNAs can coordinate expression of cell proteins via post-transcriptional action
Other post-transcriptional controls can apply –
• formation of stress granules &
• action of RNA binding proteins

TURNING OFF GENES: Role of Epigenetics

Epigenetics:

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Common mechanisms:

- **DNA methylation**
- **Histone modification / histone variants**
  e.g. acetylation, phosphorylation
- **Regulatory non-coding RNAs [miRNA]**

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DNA Methylation & Mammalian Hibernation

J Exp Biol. 2015 Apr 23. pii: jeb.116046. [Epub ahead of print]

Dynamic changes in global and gene specific DNA methylation during hibernation in adult thirteen-lined ground squirrels, Ictidomys tridecemlineatus.

Alvarado S[1], Mak T[2], Liu S[2], Storey KB[3], Szyf M[4].

Author information

Abstract

Hibernating mammals conserve energy in the winter by undergoing prolonged bouts of torpor, interspersed with brief arousals back to euthermia. These bouts are accompanied with a suite of reversible physiological and biochemical changes; however, much remains to be discovered about the molecular mechanisms involved. Given the seasonal nature of hibernation, it stands to reason that underlying plastic epigenetic mechanisms should exist. One such form of epigenomic regulation involves the reversible modification of cytosine bases in DNA by methylation. DNA methylation is well-known to be a mechanism that confers upon DNA its cellular identity during differentiation in response to innate developmental cues. However, it has recently been hypothesized that DNA methylation also acts as a mechanism for adapting genome function to changing external environmental and experiential signals over different time scales, including during adulthood. Here, we tested the hypothesis that DNA methylation is altered during hibernation in adult wild animals. This study evaluated global changes in DNA methylation in response to hibernation in the liver and skeletal muscle of thirteen-lined ground squirrels along with changes in expression of DNA methyltransferases (DNMT1/3B) and methyl binding domain proteins (MBDs). A reduction in global DNA methylation occurred in muscle during torpor phases whereas significant changes in DNMTs and MBDs were seen in both tissues. We also report dynamic changes in DNA methylation in the promoter of the myocyte enhancer factor 2C (mef2c) gene, a candidate regulator of metabolism in skeletal muscle. Taken together, these data show that genomic DNA methylation is dynamic across torpor-arousal bouts during winter hibernation, consistent with a role for this regulatory mechanism in contributing to the hibernation phenotype.

Changes in DNA methylation & DNMTs restrict gene transcription during torpor

Alvarado, S., Mak, T., Liu, S., Storey, K.B., and Szyf, M. 2015. J. Exp. Biol. 218: 1787-1795

Histone Deacetylases & Mammalian Hibernation

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 ☆

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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 a result of coordinated suppression of multiple energy expensive metabolic processes. Gene transcription is one of these and the present study examines mechanisms of transcriptional control that could contribute to lowering the rate of gene expression in torpor. Histone deacetylases (HDAC) have been linked to gene silencing and measured HDAC activity was 1.82-fold higher in skeletal muscle of hibernating thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, compared with euthermic controls. Western blotting also showed that HDAC1 and HDAC4 protein levels were 1.21-and 1.48-fold higher, respectively, in muscle from torpid animals. Histone H3 was also evaluated by Western blotting. Total histone H3 was unchanged but two forms of covalently modified histone H3 that are associated with active transcription (phosphorylated Ser 10 and acetylated Lys 23) were significantly reduced by 38–39% in muscle during hibernation. Finally, RNA polymerase II activity was measured using a PCR-based approach; activity in muscle from hibernating squirrels was only 57% of the euthermic value. These data support an overall decrease in transcriptional activity in skeletal muscle of hibernating animals that is accomplished by multiple molecular mechanisms.

Histone deacetylases allow histones to wrap around DNA more tightly during torpor

Global changes in methylation of gene promoters to reduce transcription rates

Global changes in histone modifications to reduce accessibility to promoter regions by transcription machinery

Transcription and translation are ATP-expensive.

Epigenetic modifications can alter rates of transcription/translation to produce energy savings in hypometabolism

MicroRNAs can coordinate expression of cell proteins via post-transcriptional action

Other post-transcriptional controls can apply

- **formation of stress granules &**
- **action of RNA binding proteins**

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

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* Correspondence to: Kenneth B. Storey, Tel: +613-520-3678; Fax: +613-520-3749; E-mail: kenneth_storey@carleton.ca

Metabolic r… estivation, … bolic states … organisms … likely drive… bolism and … marily from … examples f… response to … studies hav… decrease p… 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 hibernating ground squirrels: A role in translational suppression during torpor

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

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MicroRNA
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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

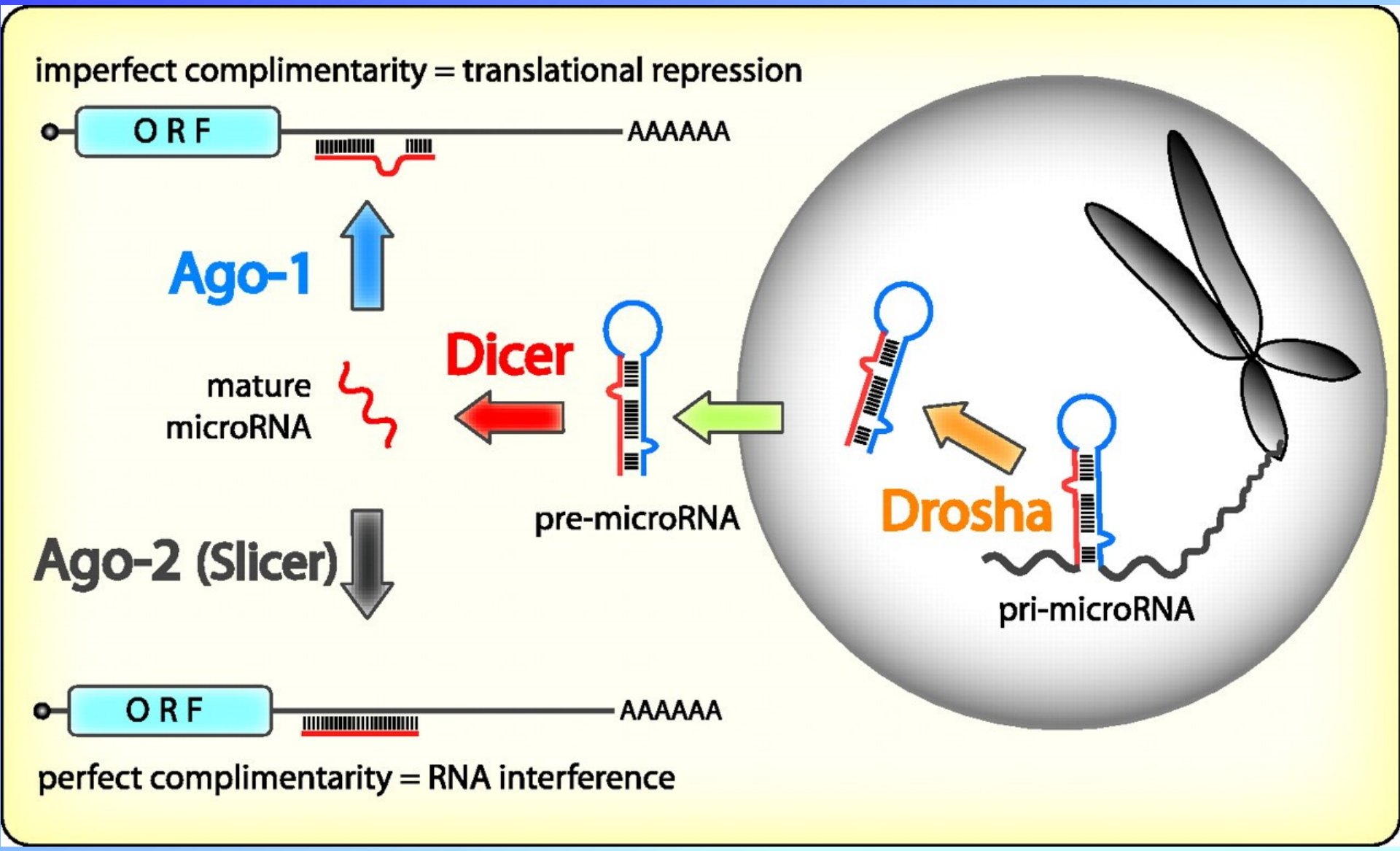
miRNAs & Dicer enzyme show organ-specific changes in mammalian hibernation

Regulatory non-coding RNAs

microRNA

- Small RNAs ~22 nucleotides in length
- Highly conserved across species
- Reach out to ALL cell processes
- Could be 1000, affect 85 % of genes
- Disease involvement
- Act to :
  - Block translation of mRNA
  - Target mRNA for degradation

MICRO RNA: Drosha & Dicer



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

MicroRNA & Hibernation

Physiol Genomics 48: 388–396, 2016.
First published April 15, 2016; doi:10.1152/physiolgenomics.00005.2016.

Analysis of microRNA expression during the torpor-arousal cycle of a mammalian hibernator, the 13-lined ground squirrel

Cheng-Wei Wu, Kyle K. Biggar,* Bryan E. Luu,* Kama E. Szereszewski, and Kenneth B. Storey
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, Ontario, Canada

Submitted 6 January 2016; accepted in final form 4 April 2016

Wu CW, Biggar KK, Luu BE, Szereszewski KE, Storey KB. Analysis of microRNA expression during the torpor-arousal cycle of a mammalian hibernator, the 13-lined ground squirrel. *Physiol Genomics* 48: 388–396, 2016. First published April 15, 2016; doi:10.1152/physiolgenomics.00005.2016.—Hibernation is a highly regulated stress response that is utilized by some mammals to survive harsh winter conditions and involves a complex metabolic reprogramming at the cellular level to maintain tissue protections at low temperature. In this study, we profiled the expression of 117 conserved microRNAs in the heart, muscle, and liver of the 13-lined ground squirrel (*Ictidomys tridecemlineatus*) across four stages of the torpor-arousal cycle (euthermia, early torpor, late torpor, and interbout arousal) by real-time PCR. We found significant differential regulation of numerous microRNAs that were both tissue specific and torpor stage specific. Among the most significant regulated microRNAs was *miR-208b*, a positive regulator of muscle development that was found to be upregulated by fivefold in the heart during late torpor (13-fold during arousal), while decreased by 3.7-fold in the skeletal muscle, implicating a potential regulatory role in the development of cardiac hypertrophy and skeletal muscle atrophy in the ground squirrels during torpor. In addition, the insulin resistance marker *miR-181a* was upregulated by 5.7-fold in the liver during early torpor, which supports previous suggestions of hyperinsulinemia in hibernators during the early stages of the hibernation cycle. Although microRNA expression profiles were largely unique between the three tissues, GO annotation analysis revealed that the putative targets of upregulated microRNAs tend to enrich toward suppression of progrowth-related processes in all three tissues. These findings implicate microRNAs in the regulation of both tissue-specific processes and general suppression of cell growth during hibernation.

tional level, with reversible protein phosphorylation shown to play an integral role in the regulation of key glycolytic enzymes, histone modifications, RNA polymerase II activity, and protein translation initiation (17, 30, 39).

Recent discoveries of microRNAs (miRNAs) have introduced a new dimension of cellular regulation that is highly conserved among species ranging from nematodes, fruit flies, to human (3). MiRNAs are small noncoding RNA transcripts that are ~22 nucleotides in length and are known to exert posttranscriptional control by binding to target mRNAs near the 3′-untranslated region (UTR) to promote translational silencing through either sequestration or degradation. Transcripts targeted by miRNAs have been shown to localize to cytoplasmic foci, which can serve as sites for mRNA storage or degradation leading to translational repression (23). We have recently shown evidence for the formation during hibernation of stress-induced granules that comprised RNA-binding proteins, and these could serve as potential mRNA storage foci that would complement the regulatory roles of miRNAs during torpor (40). A single miRNA can regulate hundreds of genes, and a single gene can be targeted by multiple miRNAs, creating a complex network that is thought to regulate up to 60% of all protein-coding genes in human (21). We have previously reported the regulatory roles of miRNAs during hibernation and have begun to show miRNA regulation as part of a global response to other environmental stressors that include estivation, anoxia, and freezing, with select miRNAs

- **Skeletal muscle atrophy**
- **Cardiac hypertrophy**
- **Insulin resistance**
- **Suppression of cell growth**

Species specific microRNA detection

Analytical Biochemistry 462 (2014) 32–34

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Notes & Tips

High-throughput amplification of mature microRNAs in uncharacterized animal models using polyadenylated RNA and stem–loop reverse transcription polymerase chain reaction

Kyle K. Biggar [1], Cheng-Wei Wu [1], Kenneth B. Storey *

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

ARTICLE INFO

Article history:
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Keywords:
Hibernation
RT–PCR
Polyadenylation
Ictidomys tridecemlineatus
MicroRNA amplification

ABSTRACT

This study makes a significant advancement on a microRNA amplification technique previously used for expression analysis and sequencing in animal models without annotated mature microRNA sequences. As research progresses into the post-genomic era of microRNA prediction and analysis, the need for a rapid and cost-effective method for microRNA amplification is critical to facilitate wide-scale analysis of micr-oRNA expression. To facilitate this requirement, we have reoptimized the design of amplification primers

Advanced method for miRNA expression analysis in species not genome-sequenced
-- key to comparative models

Nucleic Acids Research

Nucleic Acids Res. 2015 Nov 16; 43(20): e138. PMCID: PMC4787757
Published online 2015 Jul 10. doi: 10.1093/nar/gkv698

A framework for improving microRNA prediction in non-human genomes

Robert J. Peace,[1] Kyle K. Biggar,[2,3] Kenneth B. Storey,[2] and James R. Green[1,*]

ABSTRACT

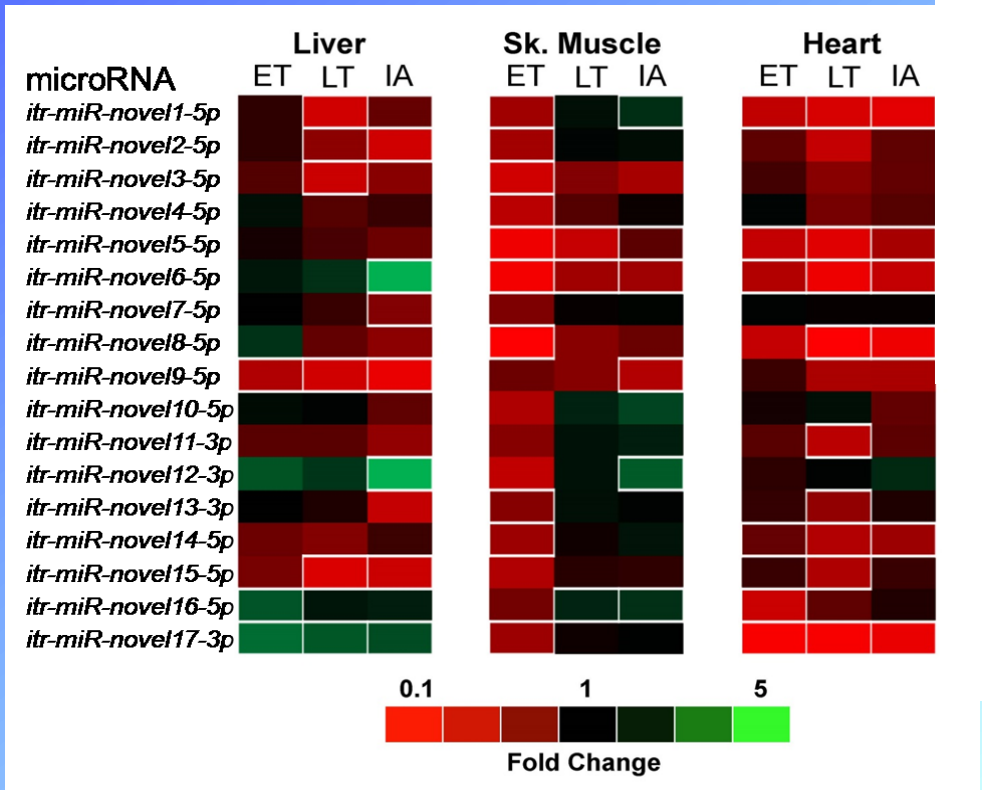
Go to:

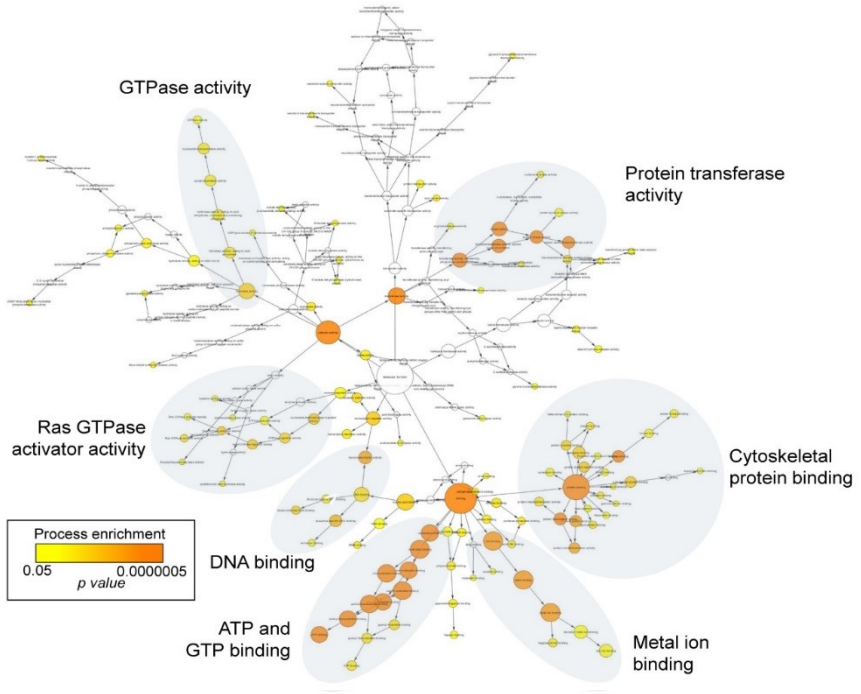
The prediction of novel pre-microRNA (miRNA) from genomic sequence has received considerable attention recently. However, the majority of studies have focused on the human genome. Previous studies have demonstrated that sensitivity (correctly detecting true miRNA) is sustained when human-trained methods are applied to other species, however they have failed to report the dramatic drop in specificity (the ability to correctly reject non-miRNA sequences) in non-human genomes. Considering the ratio of tru miRNA sequences to pseudo-miRNA sequences is on the order of 1:1000, such low specificity prevents the application of most existing tools to non-human genomes, as the number of false positives overwhelms

SMIRP – species specific miRNA prediction of NOVEL miRNAs in diverse species

Novel miRNA: Verification and Quantification

Novel microRNAs in 13-lined ground squirrels
(*Ictidomys tridecemlineatus*)





FEBS Letters
FEBS PRESS science publishing by scientists

Torpor-responsive expression of novel microRNA regulating metabolism and other cellular pathways in the thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*

Bryan E. Luu*, Kyle K. Biggar*, Cheng-Wei Wu and Kenneth B. Storey

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

Other Animals: Hibernating Marsupial

Dromiciops gliroides
Monito del Monte

Do different hibernators utilize the same strategies?

- **Studied highly conserved microRNAs in liver and skeletal muscle**

MARSUPIAL TORPOR

SCIENTIFIC REPORTS

SCIENTIFIC REPORTS | 6:24627 | DOI: 10.1038/srep24627

OPEN

The hibernating South American marsupial, *Dromiciops gliroides*, displays torpor-sensitive microRNA expression patterns

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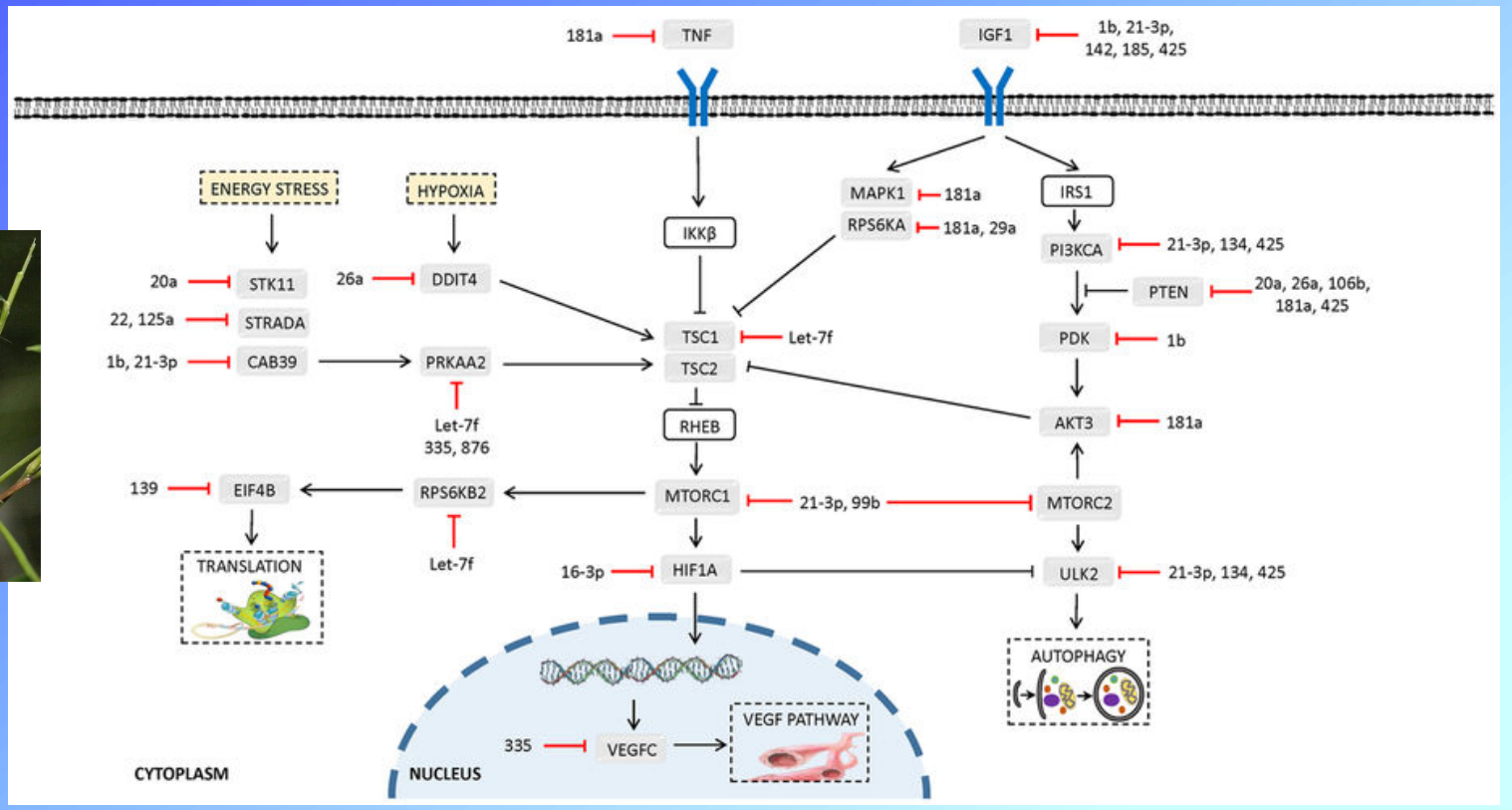
Hanane Hadj-Moussa[1,*], Jason A. Moggridge[1,*], Bryan E. Luu[1], Julian F. Quintero-Galvis[2], Juan Diego Gaitán-Espitia[3], Roberto F. Nespolo[2] & Kenneth B. Storey[1]

When faced with adverse environmental conditions, the marsupial *Dromiciops gliroides* uses either daily or seasonal torpor to support survival and is the only known hibernating mammal in South America. As the sole living representative of the ancient Order Microbiotheria, this species can provide crucial information about the evolutionary origins and biochemical mechanisms of hibernation. Hibernation is a complex energy-saving strategy that involves changes in gene expression that are elicited in part by microRNAs. To better elucidate the role of microRNAs in orchestrating hypometabolism, a modified stem-loop technique and quantitative PCR were used to characterize the relative expression levels of 85 microRNAs in liver and skeletal muscle of control and torpid *D. gliroides*. Thirty-nine microRNAs were differentially regulated during torpor; of these, 35 were downregulated in liver and 11 were differentially expressed in skeletal muscle. Bioinformatic analysis predicted that the downregulated liver microRNAs were associated with activation of MAPK, PI3K-Akt and mTOR pathways, suggesting their importance in facilitating marsupial torpor. In skeletal muscle, hibernation-responsive microRNAs were predicted to regulate focal adhesion, ErbB, and mTOR pathways, indicating a promotion of muscle maintenance mechanisms. These tissue-specific responses suggest that microRNAs regulate key molecular pathways that facilitate hibernation, thermoregulation, and prevention of muscle disuse atrophy.

- Activation of mTOR
- Activation of MAPKs
- Tissue-specific responses:
  - Hibernation
  - Thermal regulation
  - Disuse atrophy

Monito del Monte
from Chile

Hibernating Marsupial

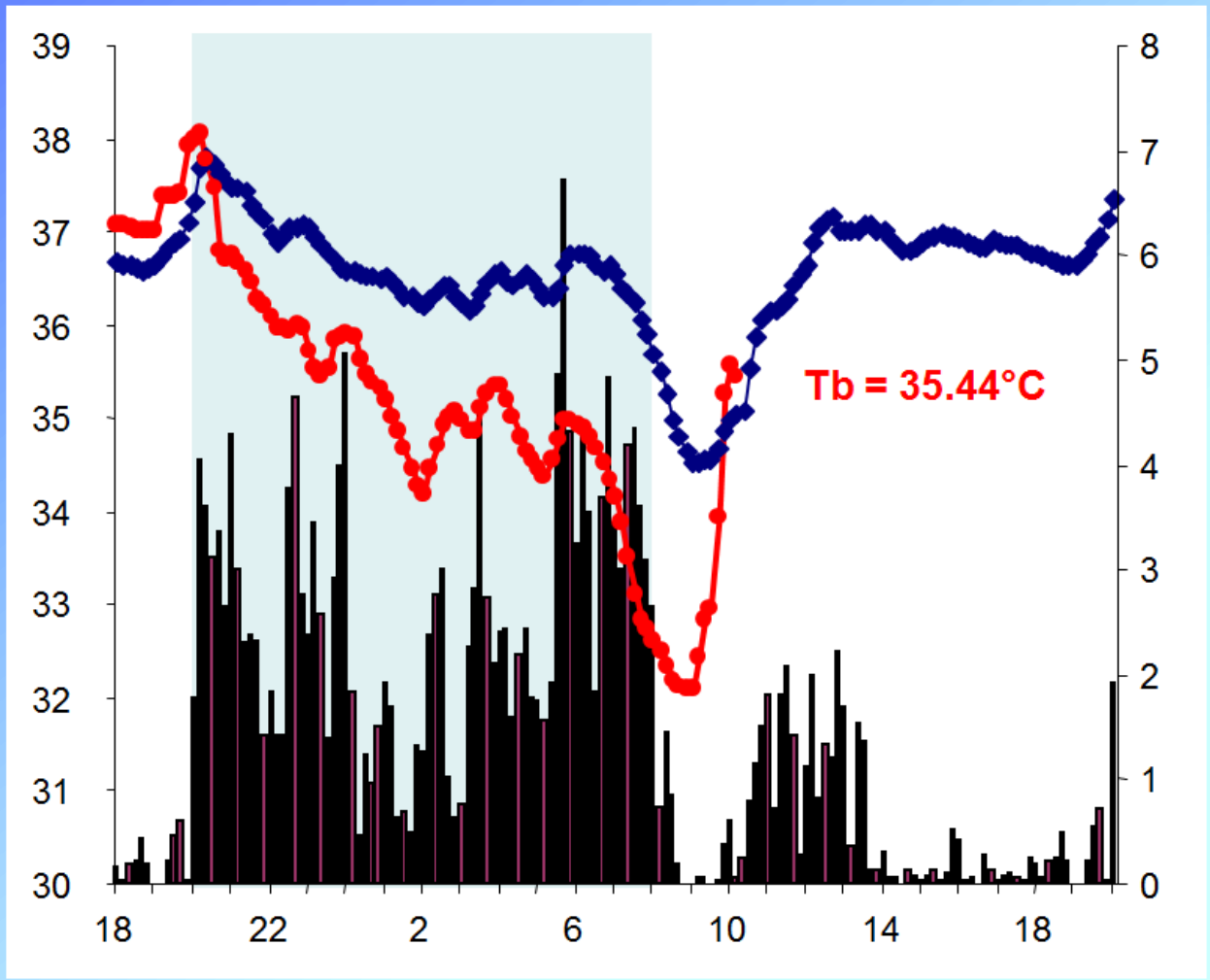


- MicroRNAs in marsupial and placental hibernators behave similarly
- Target energy-expensive processes while activating pro-survival responses

LEMUR model

- Primates, native to Madagascar
- Use daily torpor while sleeping
- Hibernate long term to deal with chronic food shortages in the dry season
- The most closely related species to man that exhibit natural hypometabolism
- Enter torpor at high ambient temperatures (T_b ~28-32°C) i.e. not confounded by the additional biochemical adaptations needed for low temperature function

PRIMATE TORPOR: GRAY MOUSE LEMUR



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PREFACE

The Gray Mouse Lemur: A Model for Studies of Primate Metabolic Rate Depression

Kenneth B. Storey *,[a]

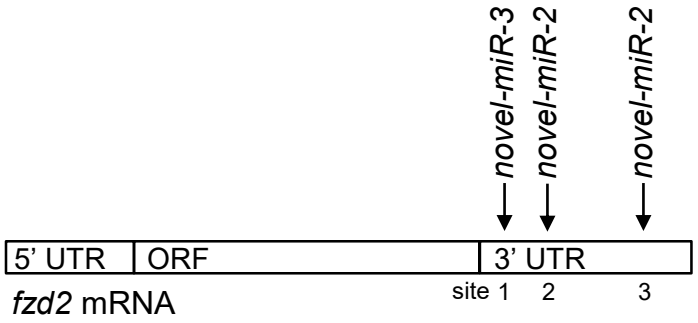
Institute of Biochemistry and Department of Biology, Carleton University, Ottawa

Received 15 April 2015; accepted 11 June 2015
Available online 21 June 2015

Gray mouse lemur, *Microcebus murinus*
- Native to Madagascar

Overview: Fewer cellular changes needed when torpor is at higher body temperature !

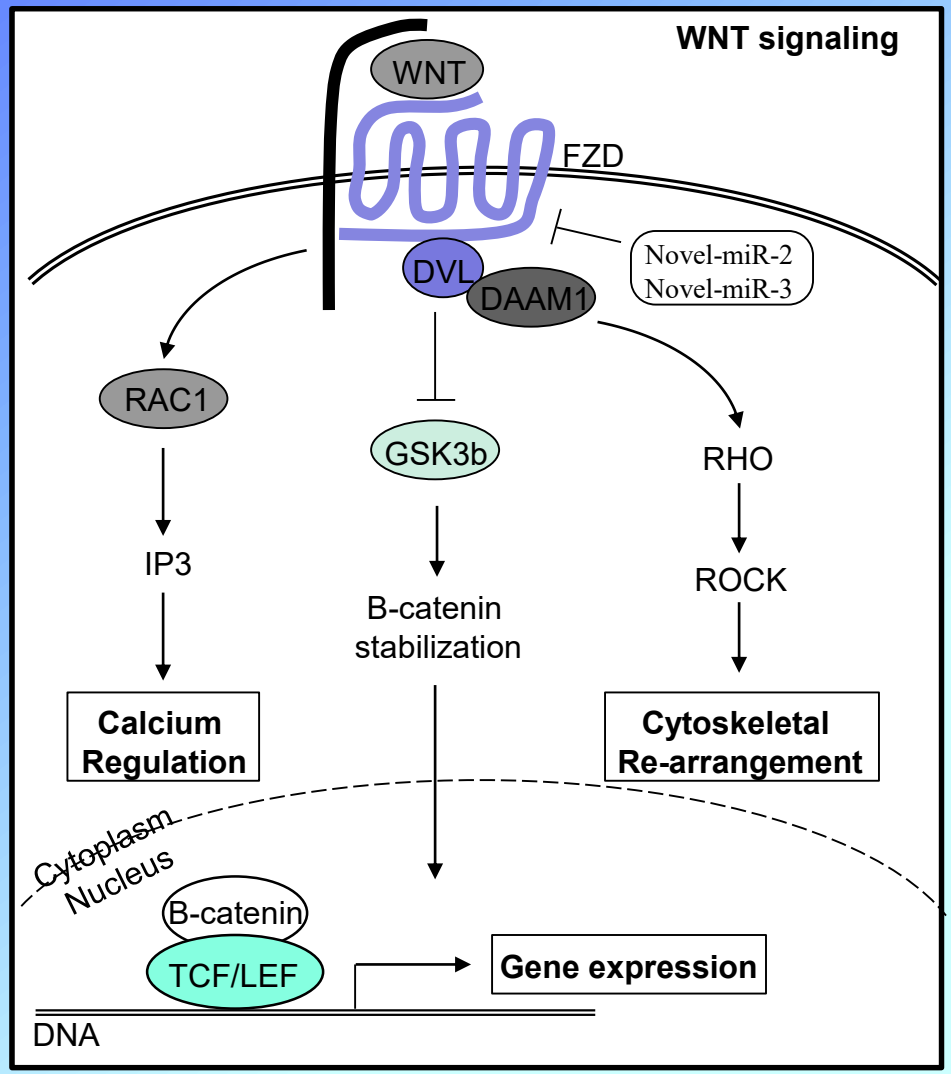
LEMUR HEART miRNA



Site 1
```
target 5' U   U      CUGAU         C 3'
            GUG GCGGCA     UUGUCAAGA
            CAC CGUCGU     GGCAGUUCU
miRNA 3'  U   U      C             U 5'
mfe: -28.7 kcal/mol
```

Site 2
```
target 5' G      GUU  A           3'
            UUGUCG   UC   GAUUUUUGU
            GACGGU   AG   CUAAAAACA
miRNA 3'  A           GAC         5'
mfe: -18.5 kcal/mol
```

Site 3
```
target 5'   A               G 3'
              UCGUUCU GAUUUUUG
              GGUAGGA CUAAAAAC
miRNA 3' AGAC       C        A 5'
mfe: -19.3 kcal/mol
```

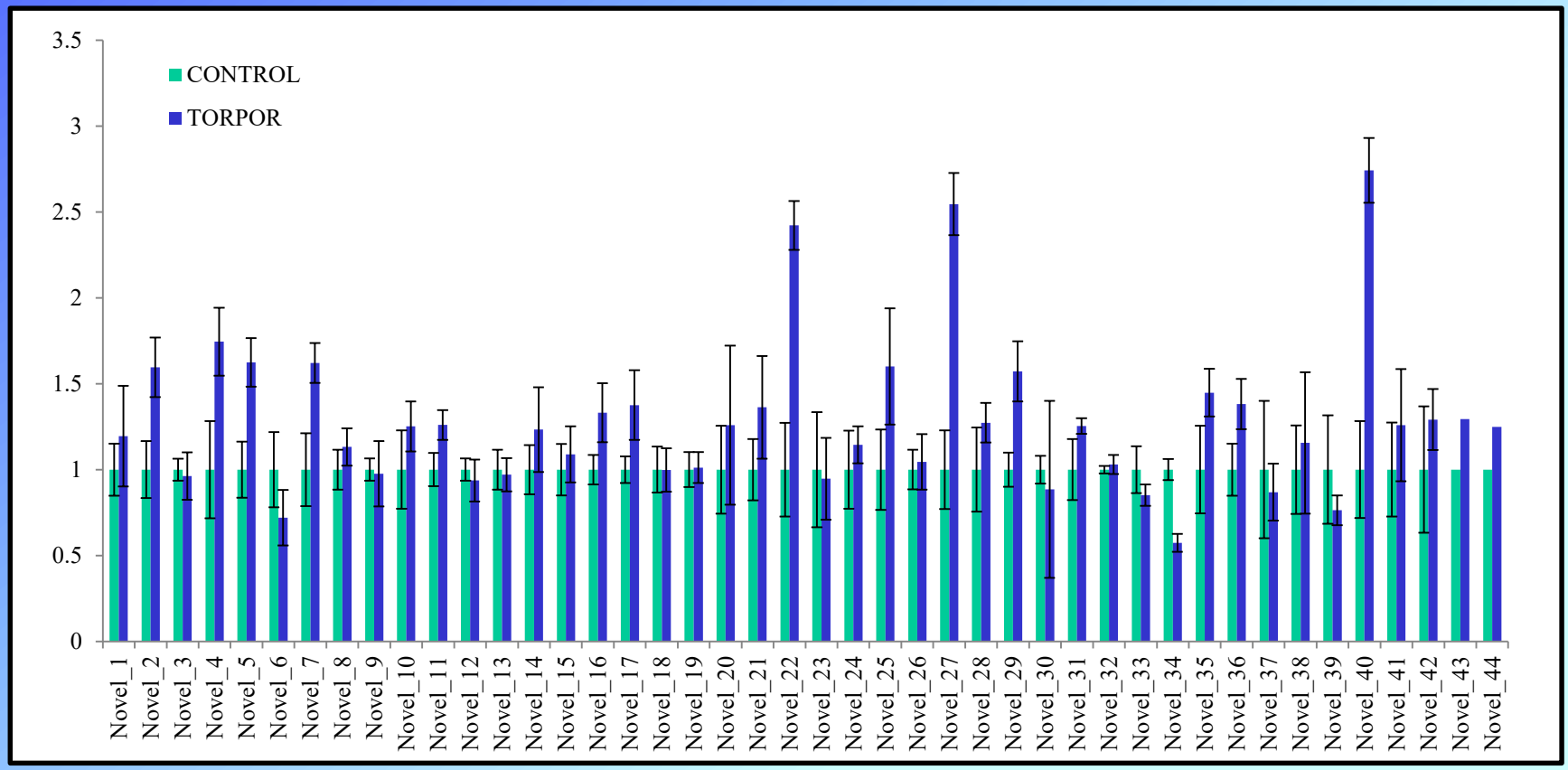


Identification and target prediction of two novel microRNAs that increase during torpor in lemur heart

LEMUR microRNA - Liver

- 16 microRNAs significantly increased during torpor
- 30 microRNAs significantly decreased
- Pathway mapping shows control over cell cycle and cell survival

- 44 Novel miRNAs discovered



TORPOR CONTROL BY SIGNALING CASCADES

Mitogen-activated protein kinases (MAPKs)

Luminex multiplex panels allowed assay of 12 targets simultaneously

Heart: activation of JNK only

Liver: MAPKs , no change !

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ORIGINAL RESEARCH

Primate Torpor: Regulation of Stress-activated Protein Kinases During Daily Torpor in the Gray Mouse Lemur, *Microcebus murinus*

Kyle K. Biggar [1,3,#,a], Cheng-Wei Wu [1,4,#,b], Shannon N. Tessier [1,5,c], Jing Zhang [1,6,d], Fabien Pifferi [2,e], Martine Perret [2,f], Kenneth B. Storey [1,*,g]

[1] *Institute of Biochemistry and Department of Biology, Carleton University, Ottawa, ON K1S 5B6, Canada*
[2] *UMR 7179 Centre National de la Recherche Scientifique, Muséum National d'Histoire Naturelle, 91800 Brunoy, France*
[3] *Biochemistry Department, Schulich School of Medicine and Dentistry, Western University, London, ON N6A 5C1, Canada*
[4] *Department of Biology, Genetics Institute, University of Florida, Gainesville, FL 32611, USA*
[5] *Department of Surgery & Center for Engineering in Medicine, Massachusetts General Hospital & Harvard Medical School, Charlestown, MA 02129, USA*
[6] *Chemistry and Chemical Engineering Department, Royal Military College of Canada, Kingston, ON K7K 7B4, Canada*

Received 13 February 2015; accepted 21 March 2015
Available online 18 June 2015

Handled by Jun Yu

KEYWORDS
Metabolic rate depression;
Signal transduction;
Mitogen activated protein kinase

Abstract Very few selected species of primates are known to be capable of entering torpor. This exciting discovery means that the ability to enter a natural state of dormancy is an ancestral trait among primates and, in phylogenetic terms, is very close to the human lineage. To explore the regulatory mechanisms that underlie primate torpor, we analyzed signal transduction cascades to discover those involved in coordinating tissue responses during torpor. The responses of mitogen-activated protein kinase (MAPK) family members to primate torpor were compared in six organs of control (aroused) versus torpid gray mouse lemurs, *Microcebus murinus*. The proteins examined include extracellular signal-regulated kinases (ERKs), c-jun NH_2-terminal kinases

TORPOR CONTROL BY SIGNALING CASCADES

Insulin signalling pathway

- **Luminex panels used to analyze insulin & PI3K/Akt signaling and mTOR protein synthesis pathway**
- **Heart: GSK3α increase**
- **Liver: IR increase**

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ORIGINAL RESEARCH

Regulation of the PI3K/AKT Pathway and Fuel Utilization During Primate Torpor in the Gray Mouse Lemur, *Microcebus murinus*

Shannon N. Tessier [1,3,#,a], Jing Zhang [1,4,#,b], Kyle K. Biggar [1,5,c], Cheng-Wei Wu [1,6,d], Fabien Pifferi [2,e], Martine Perret [2,f], Kenneth B. Storey [1,*,g]

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Received 13 February 2015; accepted 23 March 2015
Available online 17 June 2015

Handled by Jun Yu

KEYWORDS
Insulin signaling pathway;
PI3K/AKT;
mTOR;
GSK3;
Pyruvate dehydrogenase;
Metabolic rate depression

Abstract Gray mouse lemurs (*Microcebus murinus*) from Madagascar present an excellent model for studies of torpor regulation in a primate species. In the present study, we analyzed the response of the insulin signaling pathway as well as controls on carbohydrate sparing in six different tissues of torpid versus aroused gray mouse lemurs. We found that the relative level of phospho-insulin receptor substrate (IRS-1) was significantly increased in muscle, whereas the level of phospho-insulin receptor (IR) was decreased in white adipose tissue (WAT) of torpid animals, both suggesting an inhibition of insulin/insulin-like growth factor-1 (IGF-1) signaling during torpor in these tissues. By contrast, the level of phospho-IR was increased in the liver. Interestingly, muscle,

TORPOR CONTROL

AMPK signaling & gene/protein synthesis

AMP-activated protein kinase (AMPK) is the "energy sensor" of the cell

Heart: AMPK activated

- **switch to fatty acid oxidation in torpor**

Liver: AMPK decrease & protein synthesis control at eIF4E

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ORIGINAL RESEARCH

Regulation of Torpor in the Gray Mouse Lemur: Transcriptional and Translational Controls and Role of AMPK Signaling

Jing Zhang [1,2,#,a], Shannon N. Tessier [1,3,#,b], Kyle K. Biggar [1,4,c], Cheng-Wei Wu [1,5,d], Fabien Pifferi [6,e], Martine Perret [6,f], Kenneth B. Storey [1,*,g]

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Received 13 February 2015; accepted 21 March 2015
Available online 17 June 2015

Handled by Jun Yu

KEYWORDS
Posttranslational modification;
Histone H3;
Ribosomal initiation factors;

Abstract The gray mouse lemur (*Microcebus murinus*) is one of few primate species that is able to enter daily torpor or prolonged hibernation in response to environmental stresses. With an emerging significance to human health research, lemurs present an optimal model for exploring molecular adaptations that regulate primate hypometabolism. A fundamental challenge is how to effectively regulate energy expensive cellular processes (*e.g.*, transcription and translation) during transitions

CELL PROTECTION RESPONSES TO TORPOR

Antioxidant enzymes & Chaperone proteins

Stress tolerance thought to require Antioxidant defences and Heat shock proteins

Neither Heart nor Liver show changes in HSPs or antioxidants

Genomics Proteomics Bioinformatics 13 (2015) 119–126

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ORIGINAL RESEARCH

Induction of Antioxidant and Heat Shock Protein Responses During Torpor in the Gray Mouse Lemur, *Microcebus murinus*

Cheng-Wei Wu [1,3,#,a], Kyle K. Biggar [1,4,#,b], Jing Zhang [1,5,c], Shannon N. Tessier [1,6,d], Fabien Pifferi [2,e], Martine Perret [2,f], Kenneth B. Storey [1,*,g]

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Received 13 February 2015; accepted 24 March 2015
Available online 17 June 2015

Handled by Jun Yu

KEYWORDS

Heat shock proteins;
Antioxidant capacity;
Primate hypometabolism;
Stress response

Abstract A natural tolerance of various environmental stresses is typically supported by various cytoprotective mechanisms that protect macromolecules and promote extended viability. Among these are antioxidant defenses that help to limit damage from reactive oxygen species and chaperones that help to minimize protein misfolding or unfolding under stress conditions. To understand the molecular mechanisms that act to protect cells during primate torpor, the present study characterizes antioxidant and heat shock protein (HSP) responses in various organs of control (aroused)

GENE RESPONSES TO TORPOR: ADJUSTING KEY SURVIVAL PATHWAYS

Array-based PCR of 28 genes linked with hibernation.
MOST genes turned *down*

Heart: some genes increase expression. Key function – heart must keep beating

Liver: increased expression of multi-genes. Function via novel miRNA = **Selective gene expression aids torpor**

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ORIGINAL RESEARCH

Modulation of Gene Expression in Key Survival Pathways During Daily Torpor in the Gray Mouse Lemur, *Microcebus murinus*

Kyle K. Biggar [1,3,#,a], Cheng-Wei Wu [1,4,#,b], Shannon N. Tessier [1,5,c], Jing Zhang [1,6,d], Fabien Pifferi [2,e], Martine Perret [2,f], Kenneth B. Storey [1,*,g]

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Received 13 February 2015; accepted 20 March 2015
Available online 17 June 2015

Handled by Jun Yu

KEYWORDS
Daily torpor;
Primate hypometabolism;
PPAR gamma coactivator;
Ferritin;
Chaperone proteins

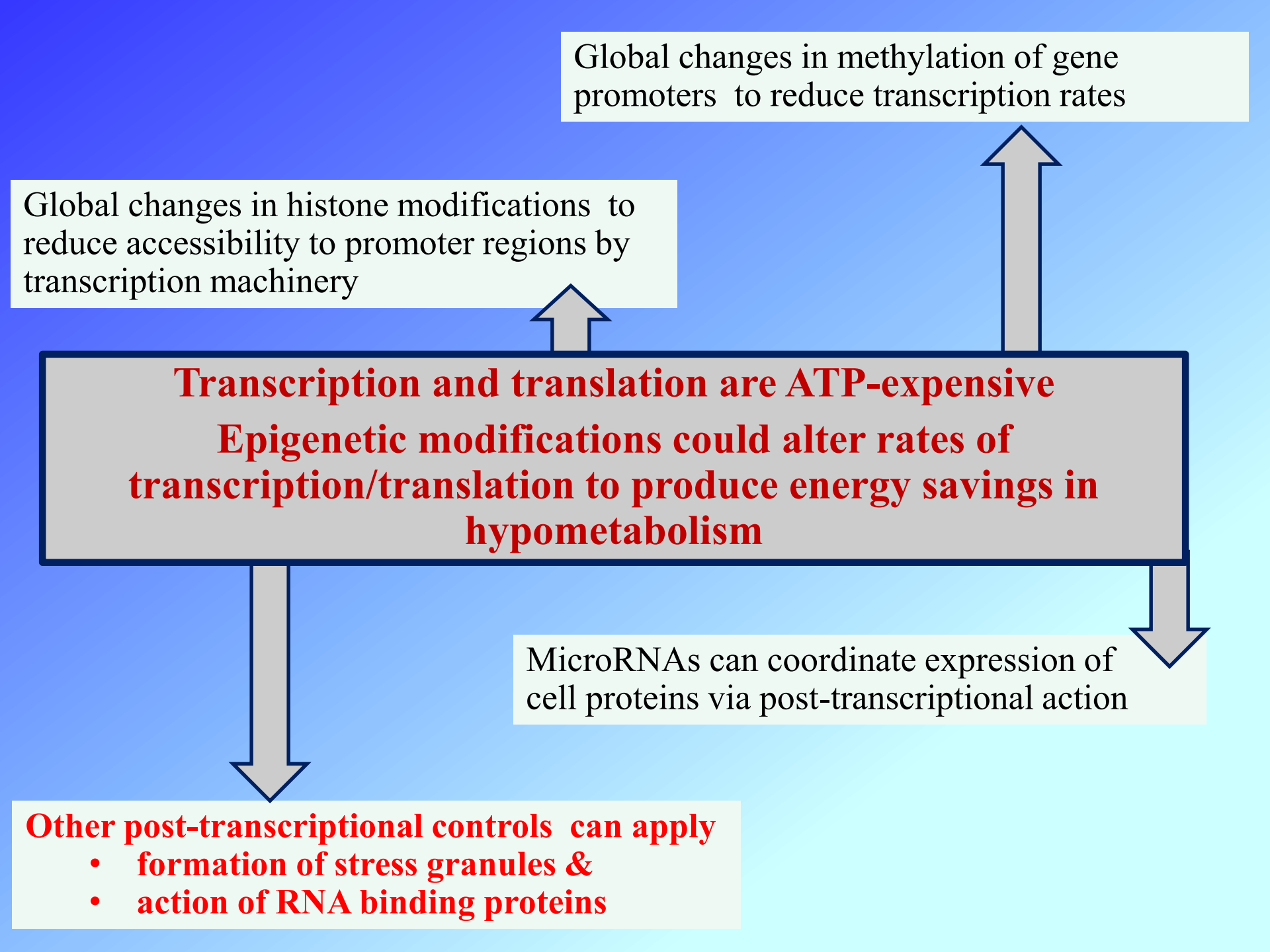
Abstract A variety of mammals employ torpor as an energy-saving strategy in environments of marginal or severe stress either on a daily basis during their inactive period or on a seasonal basis during prolonged multi-day hibernation. Recently, a few Madagascar lemur species have been identified as the only primates that exhibit torpor; one of these is the gray mouse lemur (*Microcebus murinus*). To explore the regulatory mechanisms that underlie daily torpor in a primate, we analyzed the expression of 28 selected genes that represent crucial survival pathways known to be involved in squirrel and bat hibernation. Array-based real-time PCR was used to compare gene expression in control (aroused) versus torpid lemurs in five tissues including the liver, kidney,

PRIMATE TORPOR: Shutting down primates, LIKE YOU !!

The $1,000,000 Question →
What will allow for long term human organ preservation?

- **Many less genes & fewer tissues affected in RT torpor than in long-term hibernation at cold body temperatures.**
- **<u>Organ preservation</u>: identify the key processes in each organ that need adjustment**

- Warm preservation may be least injurious


Global changes in methylation of gene promoters to reduce transcription rates
Global changes in histone modifications to reduce accessibility to promoter regions by transcription machinery
Transcription and translation are ATP-expensive
Epigenetic modifications could alter rates of transcription/translation to produce energy savings in hypometabolism
MicroRNAs can coordinate expression of cell proteins via post-transcriptional action
Other post-transcriptional controls can apply
• formation of stress granules &
• action of RNA binding proteins

Non-coding RNA: MicroRNA & Antisense RNA regulate HIF-1α in hibernation

J Comp Physiol B. 2012 Aug;182(6):849-59. doi: 10.1007/s00360-012-0662-y. Epub 2012 Apr 13.

HIF-1α regulation in mammalian hibernators: role of non-coding RNA in HIF-1α control during torpor in ground squirrels and bats.

Maistrovski Y[1], Biggar KK, Storey KB.

Author information

Abstract

A potential role for non-coding RNAs, miR-106b and antisense hypoxia inducible transcription factor-1 (HIF-1α), in HIF-1α regulation during mammalian hibernation was investigated in two species, the thirteen-lined ground squirrel (Ictidomys tridecemlineatus) and the little brown bat (Myotis lucifugus). Both species showed differential regulation of HIF-1α during hibernation. HIF-1α protein levels increased significantly in skeletal muscle of both species when animals entered torpor, as well as in bat liver. HIF-1α mRNA levels correlated with the protein increase in bat skeletal muscle and liver but not in squirrel skeletal muscle. Antisense HIF-1α transcripts were identified in skeletal muscle of both hibernators. The expression of ...le of torpid bats compared with euthermic contr... ...translation in this tissue during torpor. The exp... ...F- ...oth skeletal muscle and liver of bats and in grou... ...el post-transcriptional mechanisms of HIF-1α re... ...isms are conserved in two divergent mammali...

Polysome profiles and mammalian hibernation

Arch Biochem Biophys. 2002 May 15;401(2):244-54.

The translation state of differentially expressed mRNAs in the hibernating 13-lined ground squirrel (Spermophilus tridecemlineatus).

Hittel D[1], Storey KB.

Author information

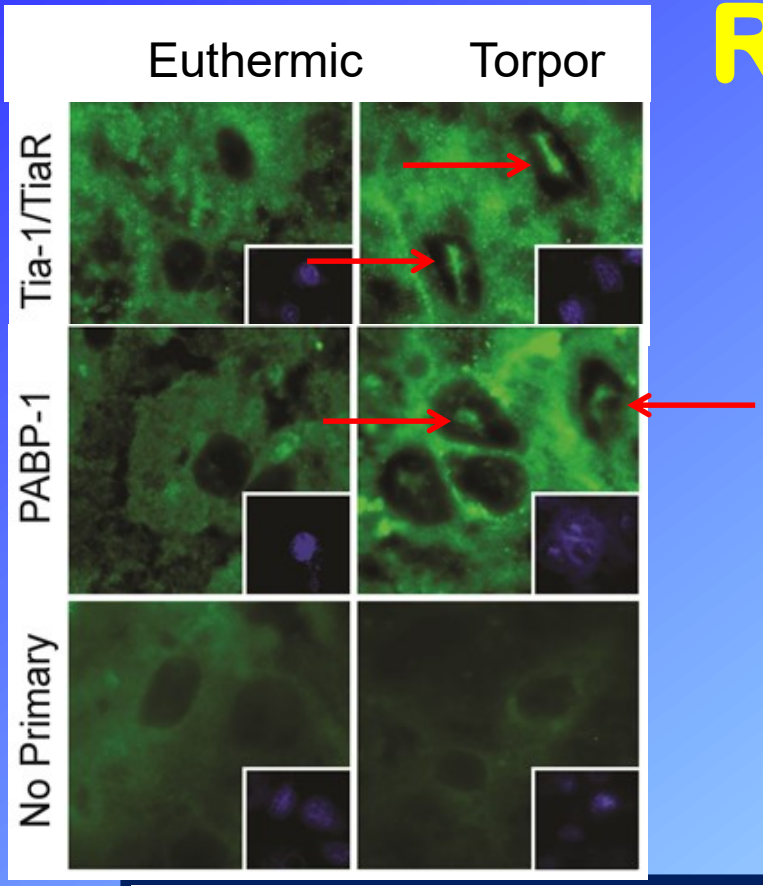
Abstract

The translation state of differentially expressed mRNAs were compared in kidney and brown adipose tissue of the hibernating ground squirrel, Spermophilus tridecemlineatus. Polysome analysis revealed a striking disaggregation of polyribosomes during hibernation and the redistribution of Cox4 (cytochrome c oxidase subunit 4) and Oct2 (organic cation transporter type 2) transcripts into monosome and mRNP fractions of kidney cytoplasmic extracts. Additionally, OCT2 protein levels decreased in kidney of hibernating animals i
rate compared with euthermic kidney. There was no translational depression in brown adipose tissue
-binding protein (H-FABP), that is up-regulated during hibernation, was increasingly abundant in the
the existence of a tissue-specific mechanism for the
ernation.

Polysomes dissociate & mRNA moves to monosome & RNP fractions during torpor

Brown adipose retains polysomes & translation of key proteins e.g. FABP

RNA binding proteins & hibernation



Subnuclear structures formed with TIA & PABP proteins are greatly increased during torpor

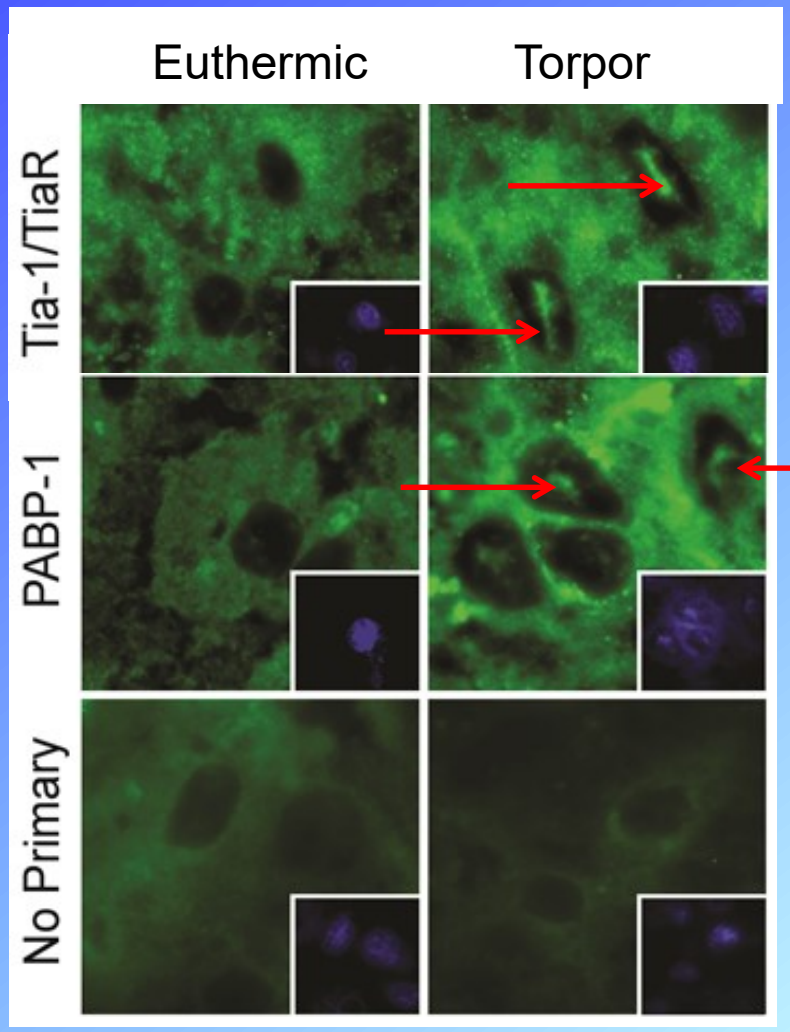
Cell Stress and Chaperones
DOI 10.1007/s12192-014-0505-8

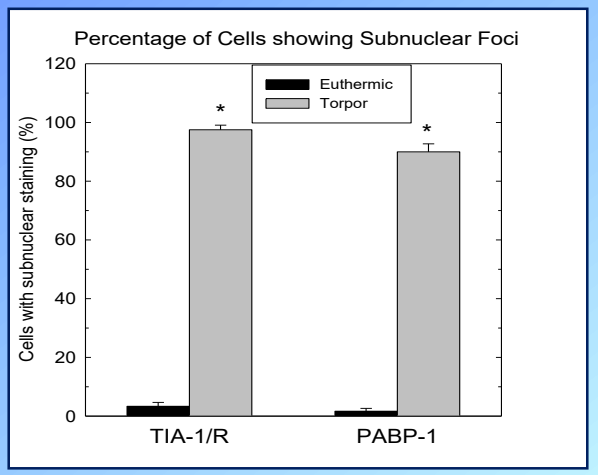
ORIGINAL PAPER

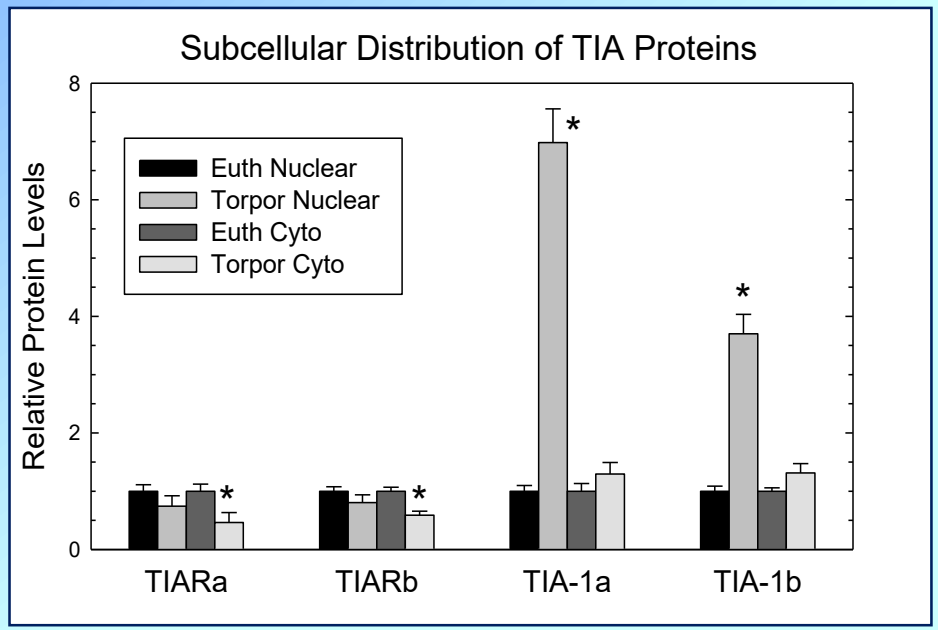
The involvement of mRNA processing factors TIA-1, TIAR, and PABP-1 during mammalian hibernation

Shannon N. Tessier · Timothy E. Audas · Cheng-Wei Wu · Stephen Lee · Kenneth B. Storey

RNA Binding Proteins & Mammalian Hibernation







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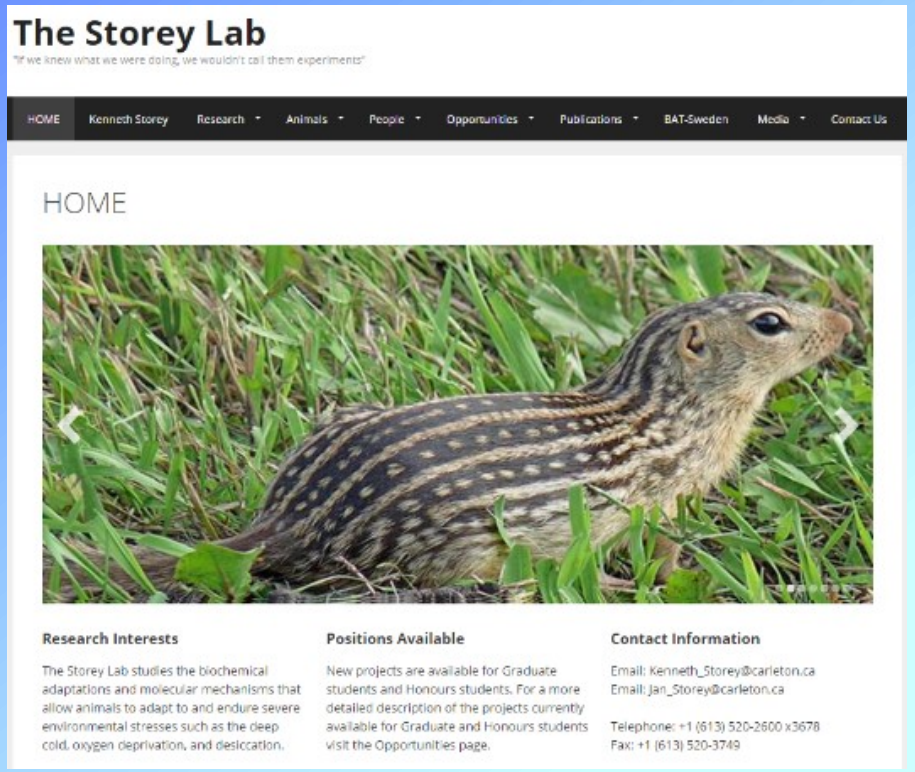
S. Wijenayake

J. Hallenbeck
D. Thomas
S. Brooks
M. Rider
M. Perret
F. Pifferi
J.M. Storey



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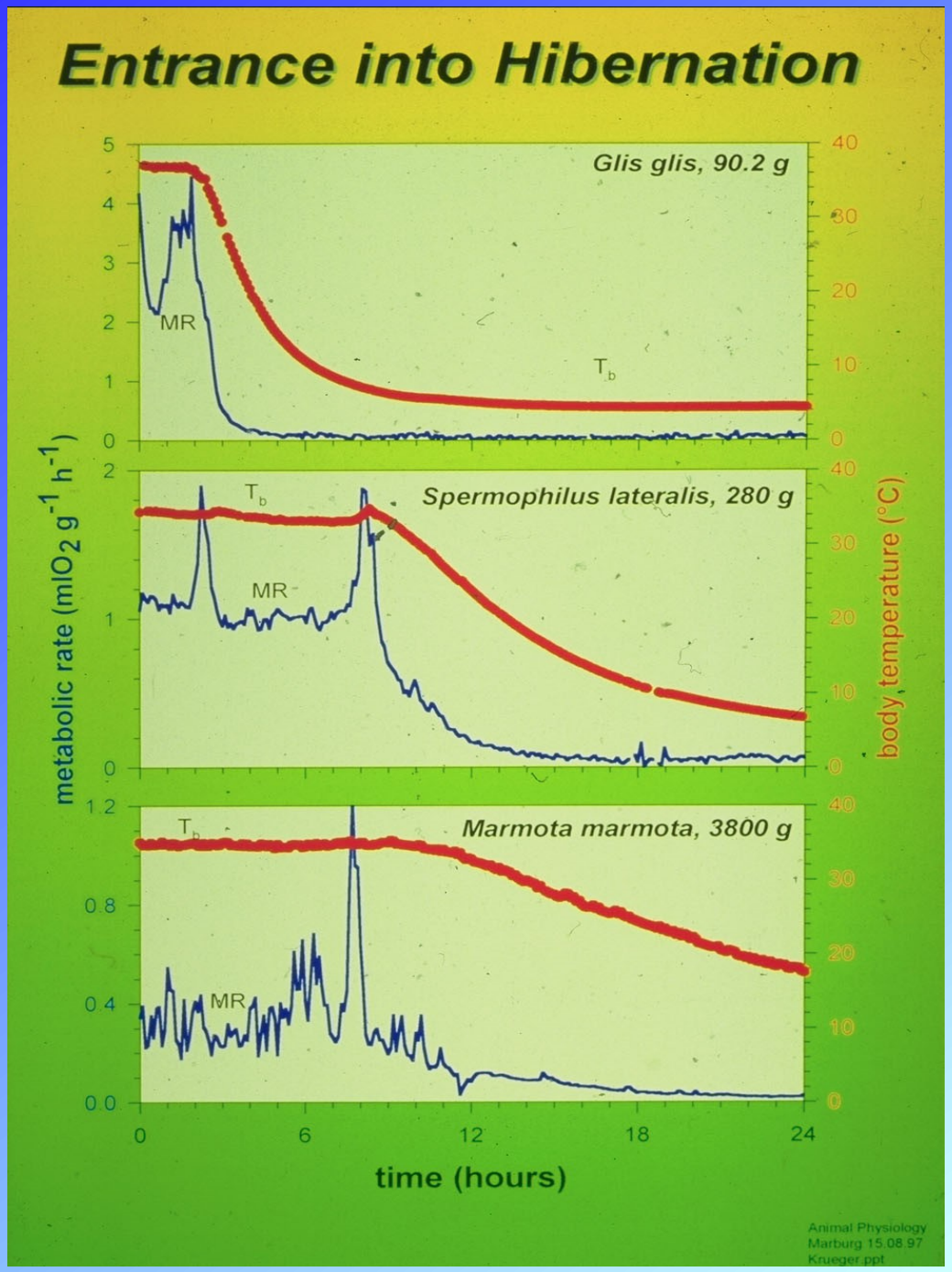

- Metabolism inhibited causing Tb to fall
- Metabolic rate falls to <5% of normal
- Smaller animals cool down faster
- Q_{10} values up to 15
- Reversible in arousal
- Torpor bout duration 4 days to 2 weeks

PRINCIPLES OF HIBERNATION

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

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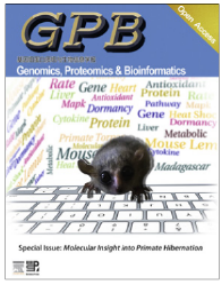

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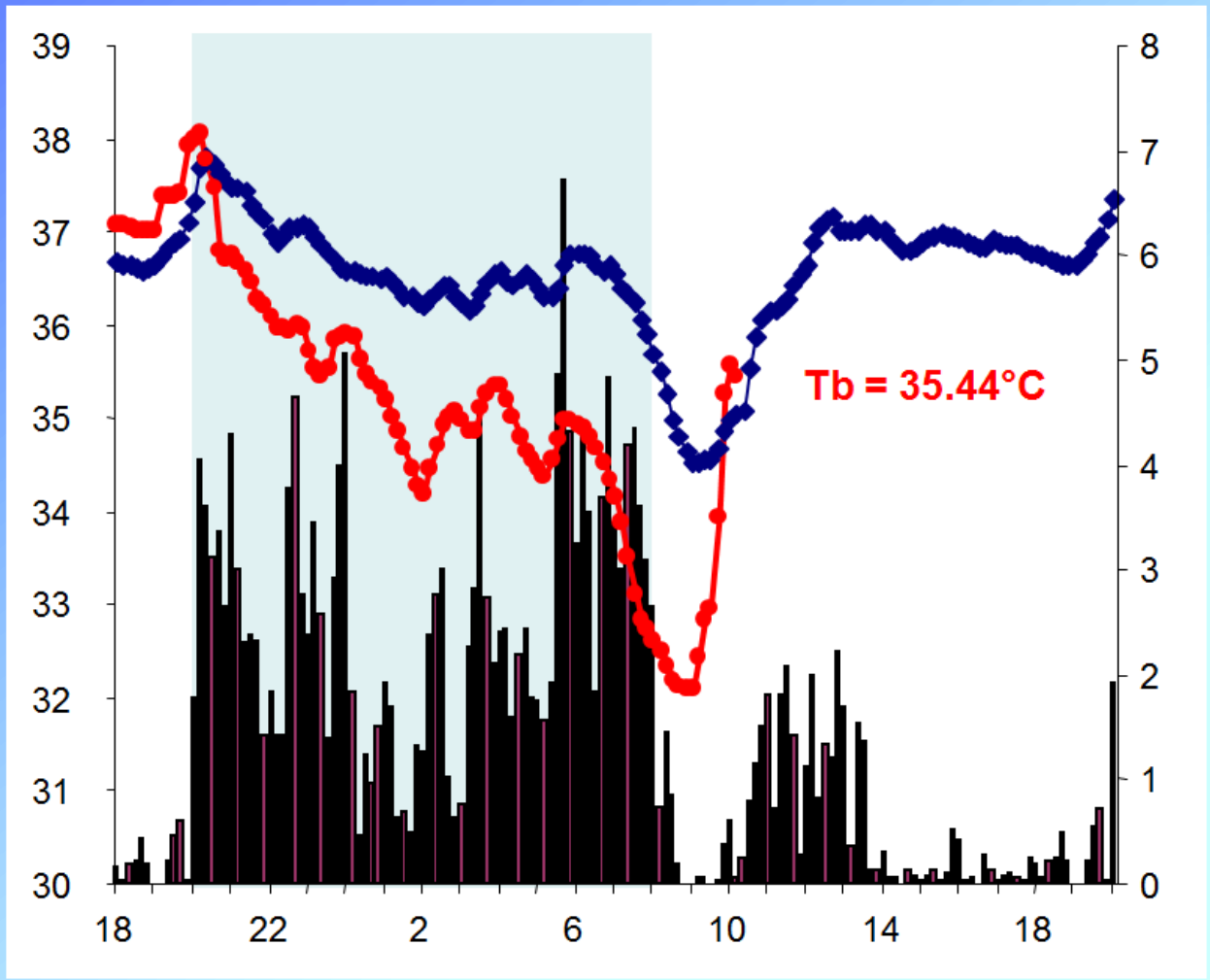
Storey lab: 6 paper series in Genom. Proteom. Bioinform. 2015 [open access]

Gray mouse lemur, *Microcebus murinu*
- Native to Madagascar

PRIMATE TORPOR: GRAY MOUSE LEMUR



http://www.wild-facts.com/2010/wild-fact-676-real-estate-hog-grey-mouse-lemur/