WAT & BAT: Adipose Tissue

What do hibernating mammals tell us about the Elastic Limits of Adipose Metabolic Functions

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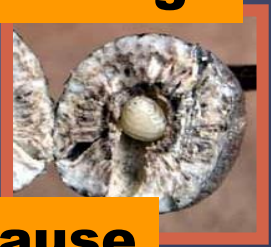
METABOLIC RATE DEPRESSION

Hibernation

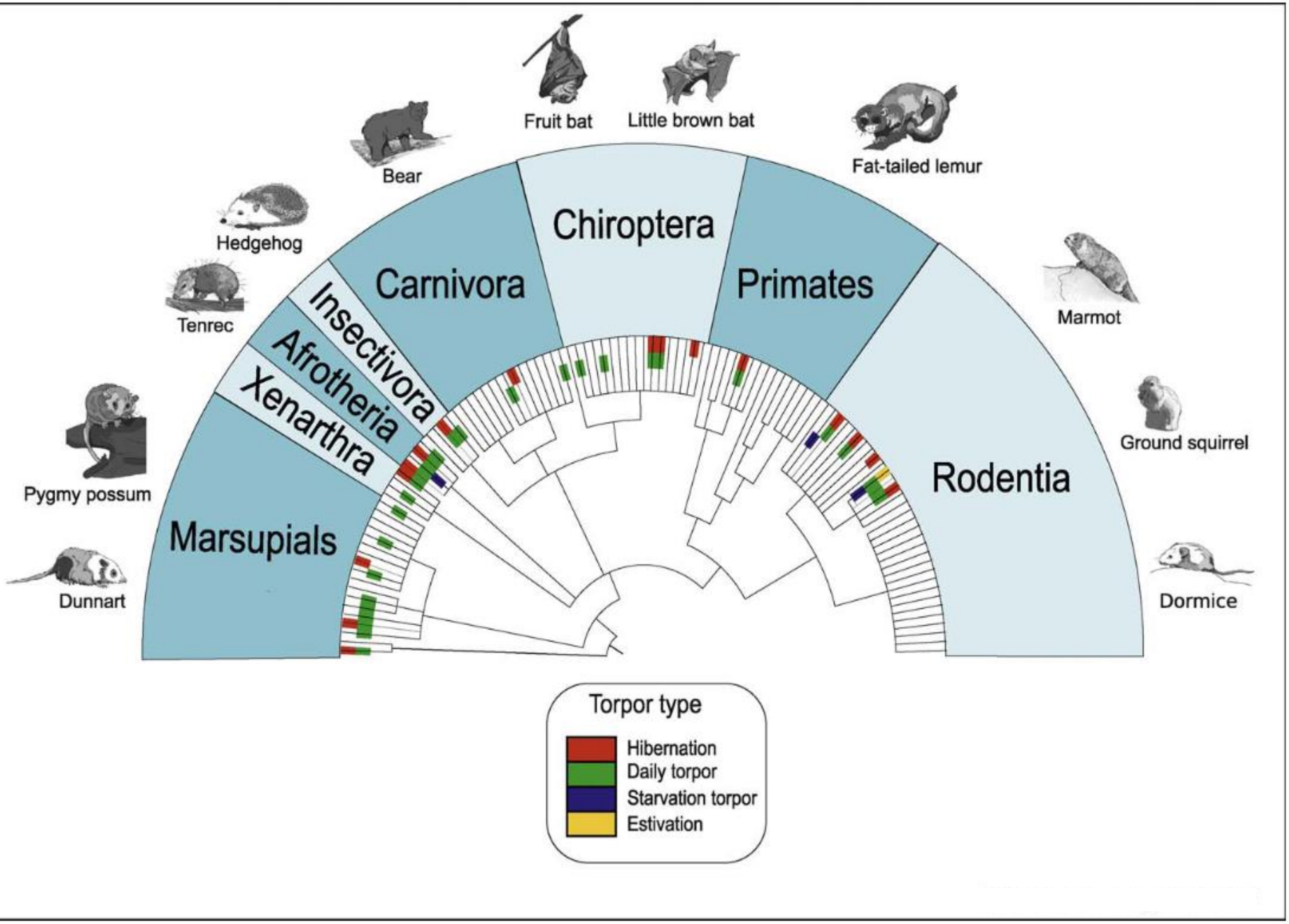
Estivation

Anoxia

Freezing



Diapause



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

HIBERNATION

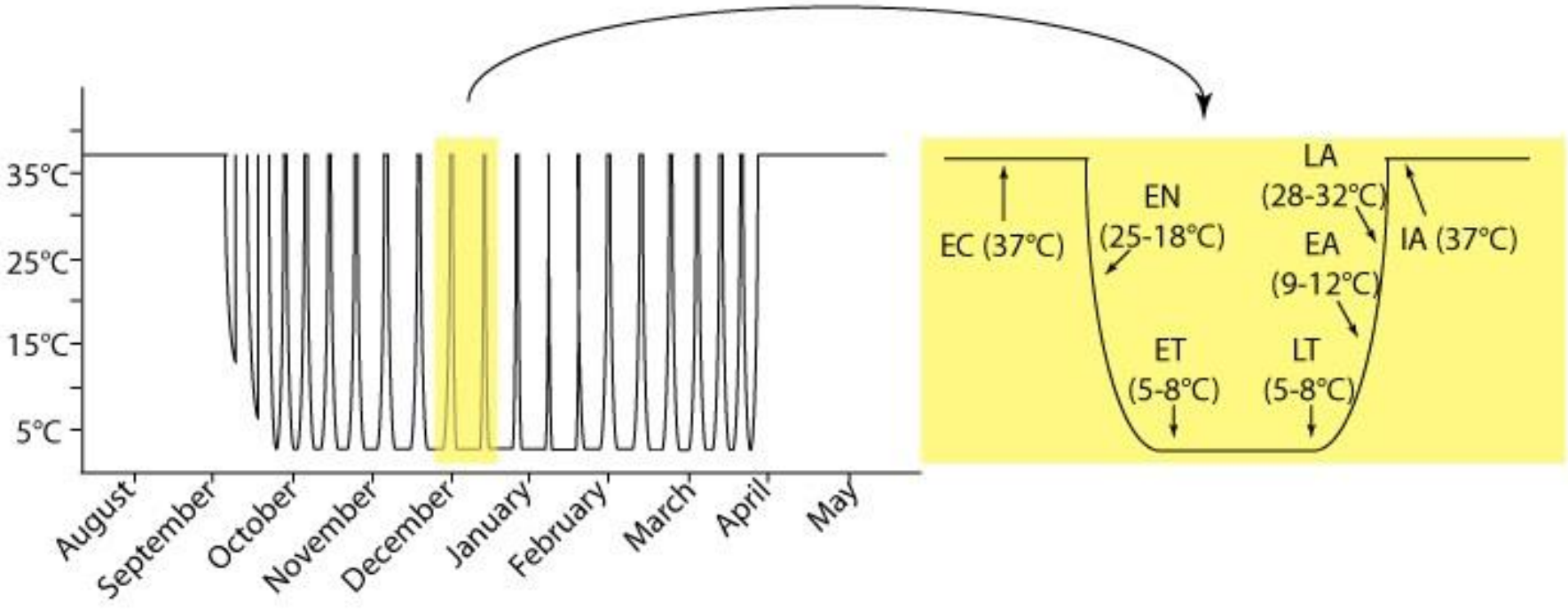
13-LINED GROUND SQUIRREL

Ictidomys tridecemlineatus

MAMMALIAN HIBERNATION

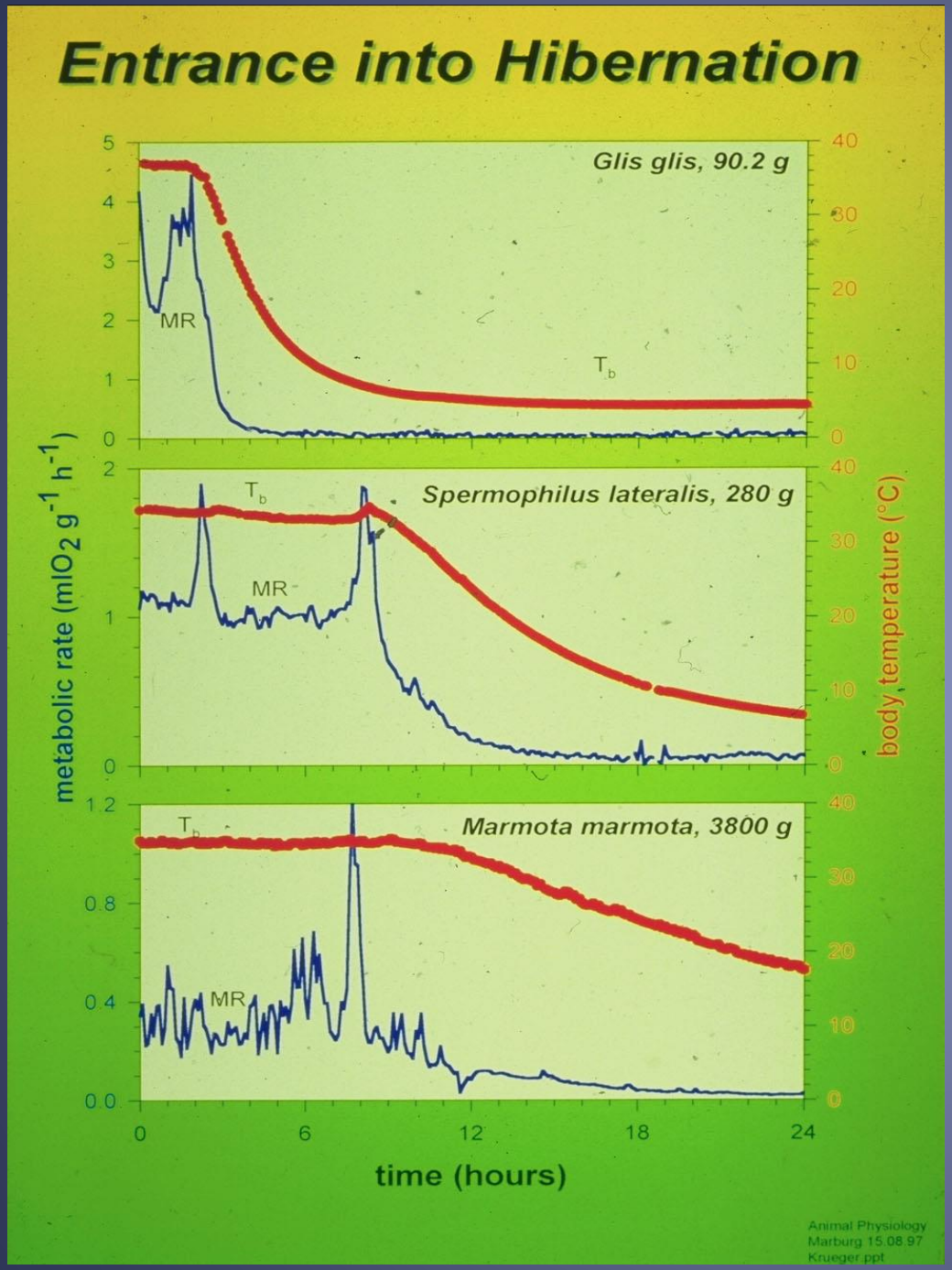
- Key characteristics :
  - metabolic rate depression (hypometabolism)
  - low body temperatures
  - Hibernation is a NATURAL model system
- Purpose is to overcome food shortages and the high energy costs of endothermic life

TORPOR-AROUSAL

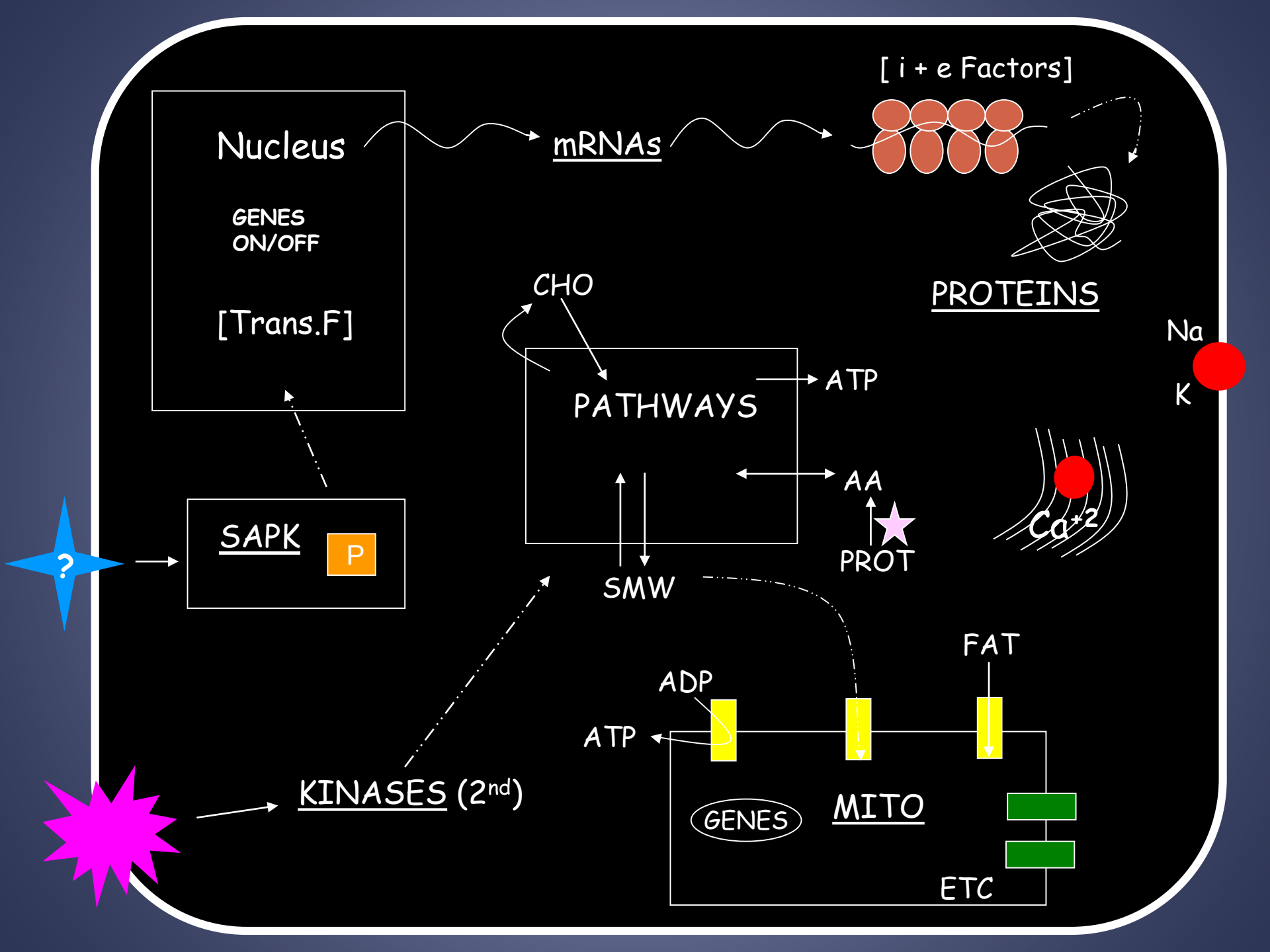


Animal studies by Dr. JM Hallenbeck and Dr. DC McMullen, NIH

Adapted from Nelson *et al.* 2009



- 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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ON/OFF
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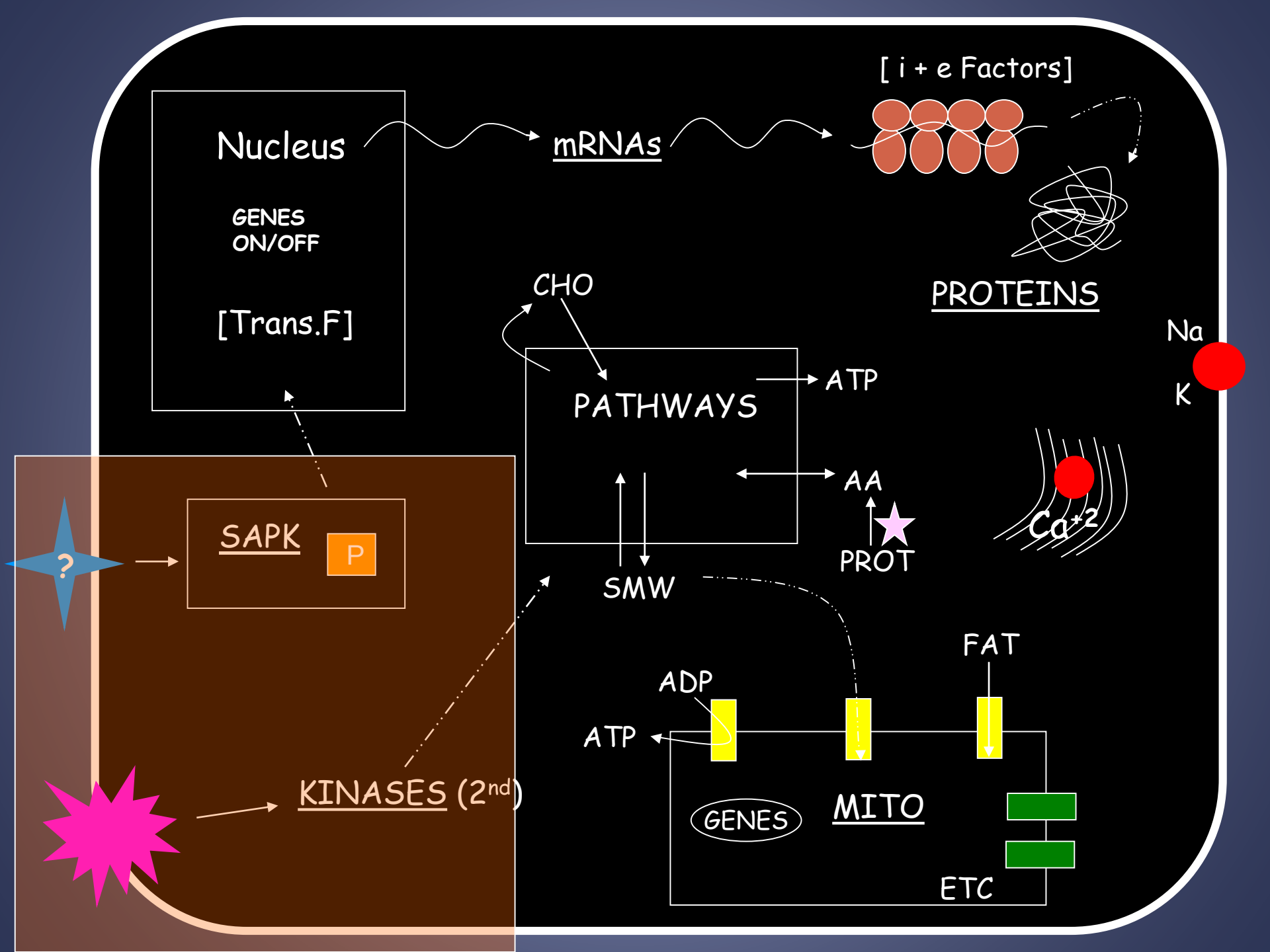
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ADP
ATP
GENES
MITO
ETC

Polysomes & translation state during 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 in line with a strong decrease (85%) in translation rate compared with euthermic kidney. There was no translational depression in brown adipose tissue during hibernation and the H isoform of fatty-acid-binding protein (H-FABP), that is up-regulated during hibernation, was increasingly abundant in the heavy polyribosome fraction isolated from the brown adipose of hibernators. This may indicate the existence of a tissue-specific mechanism for the translational control of a subset of genes that are physiologically relevant to the survival of hibernation.

Kidney:

- Disaggregation of polysomes
- 85% decrease in translation rate
- Phospho-eIF2 increases
- Synthesis of organic cation transporter (OCT2) decreased

PROTEIN SYNTHESIS CAPACITY SUPPRESSED

Brown adipose:

- Disaggregation of polysomes
- No change in translation rate
- No change in phospho-eIF2
- Synthesis of H-FABP increased

PROTEIN SYNTHESIS CAPACITY UNCHANGED

Adipocyte stress response in hibernation: BAT vs WAT

Antioxidants

Thioredoxin ↑ in BAT & WAT in torpor

SOD1 & SOD2 ↑ in WAT in torpor

No change in CAT or PRX

Heat shock proteins

Rising trend for all in BAT during arousal

HSP90 ↑ in BAT in interbout

HSP60 ↑ in WAT in entrance

Mol Cell Biochem. 2014 Aug;393(1-2):271-82. doi: 10.1007/s11010-014-2070-y. Epub 2014 Apr 29.

Characterization of adipocyte stress response pathways during hibernation in thirteen-lined ground squirrels.

Rouble AN[1], Tessier SN, Storey KB.

Author information

Abstract

To avoid the harsh conditions of winter climates, hibernating mammals undergo a systematic depression of physiological function by reducing their metabolic rate. During this process, hibernators are exposed to significant stresses (e.g., low body temperature, ischemia-reperfusion) that must be dealt with appropriately to avoid irreversible tissue damage. Consequently, we investigated the contribution of stress-responsive antioxidant enzymes, heat shock proteins, signal transduction pathways (e.g., mitogen-activated protein kinases, MAPK), and transcription factors for their role in conferring tolerance to stress in the hibernating thirteen-lined ground squirrel (Ictidomys tridecemlineatus). Using a combination of multiplex protein panels and traditional immunoblotting procedures, we have focused on these stress factors in brown adipose tissue (BAT) and white adipose tissue (WAT) over cycles of torpor-arousal since they provide the means for heat production as a result of non-shivering thermogenesis and the mobilization of critical energy reserves, respectively. We show the differential and tissue-specific regulation of stress factors including a unified upregulation of the antioxidant enzyme Thioredoxin 1 in both tissues, an upregulation of superoxide dismutase (SOD1 and SOD2) in WAT, and an increase in heat shock proteins during the transitory periods of the torpor-arousal cycle (HSP90α in BAT and HSP60 in WAT). Additionally, an upregulation of the active form of ERK1/2 and p38 in BAT and select transcription factors (e.g., CREB-1 and ELK-1) in both tissues were identified. These data provide us with greater insight into the molecular mechanisms responsible for this animal's natural stress tolerance and outline molecular signatures which define stress resistance.

Adipocyte stress response in hibernation: BAT vs WAT

MAPKs

ERK, p38, JNK ↑ in BAT in arousal

ERK, p38, JNK ↓ in WAT in torpor

- unchanged or partial recovery in WAT during arousal

Phospho-Transcription factors

p-CREB ↑ in torpor & interbout in BAT

p-Elk-1 ↑ in torpor in BAT

p-Elk-1 ↑ in torpor & arousal in WAT

Mol Cell Biochem. 2014 Aug;393(1-2):271-82. doi: 10.1007/s11010-014-2070-y. Epub 2014 Apr 29.

Characterization of adipocyte stress response pathways during hibernation in thirteen-lined ground squirrels.

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To avoid the harsh conditions of winter climates, hibernating mammals undergo a systematic depression of physiological function by reducing their metabolic rate. During this process, hibernators are exposed to significant stresses (e.g., low body temperature, ischemia-reperfusion) that must be dealt with appropriately to avoid irreversible tissue damage. Consequently, we investigated the contribution of stress-responsive antioxidant enzymes, heat shock proteins, signal transduction pathways (e.g., mitogen-activated protein kinases, MAPK), and transcription factors for their role in conferring tolerance to stress in the hibernating thirteen-lined ground squirrel (Ictidomys tridecemlineatus). Using a combination of multiplex protein panels and traditional immunoblotting procedures, we have focused on these stress factors in brown adipose tissue (BAT) and white adipose tissue (WAT) over cycles of torpor-arousal since they provide the means for heat production as a result of non-shivering thermogenesis and the mobilization of critical energy reserves, respectively. We show the differential and tissue-specific regulation of stress factors including a unified upregulation of the antioxidant enzyme Thioredoxin 1 in both tissues, an upregulation of superoxide dismutase (SOD1 and SOD2) in WAT, and an increase in heat shock proteins during the transitory periods of the torpor-arousal cycle (HSP90α in BAT and HSP60 in WAT). Additionally, an upregulation of the active form of ERK1/2 and p38 in BAT and select transcription factors (e.g., CREB-1 and ELK-1) in both tissues were identified. These data provide us with greater insight into the molecular mechanisms responsible for this animal's natural stress tolerance and outline molecular signatures which define stress resistance.

Nrf2 transcription factor & antioxidant defense

Can J Physiol Pharmacol. 2010 Mar;88(3):379-87. doi: 10.1139/Y10-017.

Heme oxygenase expression and Nrf2 signaling during hibernation in ground squirrels.

Ni Z[1], Storey KB.

Author information

Abstract

Mammalian hibernation is composed of long periods of deep torpor interspersed with brief periods of arousal in which the animals, fueled by high rates of oxygen-based thermogenesis in brown adipose tissue and skeletal muscle, power themselves back to euthermic (~37 degrees C) body temperatures. Strong antioxidant defences are important both for long-term cytoprotection during torpor and for coping with high rates of reactive oxygen species generated during arousal. The present study shows that the antioxidant enzyme heme oxygenase 1 (HO1) is strongly upregulated in selected organs of thirteen-lined ground squirrels (Spermophilus tridecemlineatus) during hibernation. Compared with euthermic controls, HO1 mRNA transcript levels were 1.4- to 3.8-fold higher in 5 organs of hibernating squirrels, whereas levels of the constitutive isozyme HO2 were unchanged. Similarly, HO1 protein levels increased by 1.5- to 2.0-fold in liver, kidney, heart, and brain during torpor. Strong increases in the levels of the Nrf2 transcription factor and its heterodimeric partner protein, MafG, in several tissues indicated the mechanism of activation of hibernation-responsive HO1 gene expression. Furthermore, subcellular distribution studies with liver showed increased nuclear translocation of both Nrf2 and MafG in torpid animals. The data are consistent with the suggestion that Nrf2-mediated upregulation of HO1 expression provides enhanced antioxidant defence to counter oxidative stress in hibernating squirrels during torpor and (or) arousal.

- **Nrf2-MafG dimer activates antioxidant response element in antioxidant genes**
- **Both proteins increase in BAT in hibernation adipose: Nrf2 by 3.5 x, MafG by 2 x**
- **No change in heme oxygenase in BAT but HO rises in liver, kidney, heart & brain**

Antioxidant defenses in hibernation: Peroxiredoxins

Arch Biochem Biophys. 2007 May 1;461(1):59-65. Epub 2007 Feb 23.

Antioxidant defense in hibernation: cloning and expression of peroxiredoxins from hibernating ground squirrels, Spermophilus tridecemlineatus.

Morin P Jr[1], Storey KB.

⊕ **Author information**

Abstract

Mammalian hibernation is characterized by prolonged torpor bouts interspersed by brief arousal periods. Adequate antioxidant defenses are needed both to sustain cell viability over weeks of deep torpor and to defend against high rates of oxyradical formation associated with massive oxygen-based thermogenesis during arousal. The present study shows that up-regulation of peroxiredoxins contributes to antioxidant defense during torpor in thirteen-lined ground squirrels, Spermophilus tridecemlineatus. Expression levels of three isozymes of the 2-Cys peroxiredoxin (Prdx) family were quantified by Western blotting, the results showing 4.0- and 12.9-fold increases in Prdx1 protein in brown adipose tissue (BAT) and heart, respectively, during hibernation compared with euthermia. Comparable increases in Prdx2 were 2.4- and 3.7-fold whereas Prdx3 rose by 3.1-fold in heart of torpid animals. Total 2-Cys peroxiredoxin enzymatic activity also rose during hibernation by 1.5-fold in heart and 3.5-fold in BAT. Furthermore, RT-PCR showed that prdx2 mRNA levels increased by 1.7- and 3.7-fold in BAT and heart, respectively, during hibernation. A partial nucleotide sequence of prdx2 from ground squirrels was obtained by PCR amplification, the deduced amino acid sequence showing 96-97% identity with Prdx2 from other mammals. Some unique amino acid substitutions were identified that might contribute to stabilizing Prdx2 conformation at the near 0 degrees C body temperatures during torpor.

- **Family of crucial intracellular antioxidants**
- **In BAT during hibernation:**
  - ***prdx2* mRNA transcripts ↑ 1.7-fold**
  - **Prdx1 protein ↑ 4-fold; Prdx2 protein ↑ 2.4-fold**
- **Total 2-Cys peroxiredoxin activity ↑ 3.5-fold**
- **Improve antioxidant defense for intense BAT thermogenesis in arousal**
- **Comparable responses seen in ground squirrel heart**

Regulation of the ER Unfolded Protein Response in Hibernators

Molecular and Cellular Biochemistry **292:** 89–98, 2006.
DOI: 10.1007/s11010-006-9221-8

Up-regulation of the endoplasmic reticulum molecular chaperone GRP78 during hibernation in thirteen-lined ground squirrels

Hapsatou Mamady and Kenneth B. Storey
Institute of Biochemistry and Department of Biology, College of Natural Sciences, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

Received 15 February 2006; accepted 1 May 2006

Abstract

Hibernating mammals endure conditions of low body temperature and oxidative stress that would be highly injurious to humans and most other mammals. Str
reticulum the glucose-regulate
evaluated the GRP78 respons
tus. Transcript levels of *grp7*
and brain of hibernating squir
GRP78 protein content, assess
1.4–1.6 fold. A 2490 bp cDN
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function under the near 0 °C
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rel brain and may be respons
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Archives of Biochemistry and Biophysics 477 (2008) 77–85
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Archives of Biochemistry and Biophysics
journal homepage: www.elsevier.com/locate/yabbi

Coping with the stress: Expression of ATF4, ATF6, and downstream targets in organs of hibernating ground squirrels

Hapsatou Mamady, Kenneth B. Storey*
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Keywords:
Activating transcription factor
Unfolded protein response
Endoplasmic reticulum stress
PERK
CREB
Spermophilus tridecemlineatus
Torpor

ABSTRACT
Perturbation of the endoplasmic reticulum (ER) protein folding apparatus via any one of several environmental or metabolic stresses rapidly triggers a complex program of cellular responses that is termed the unfolded protein response (UPR). Stresses that trigger this response in mammals can include low temperature, hypoxia, ischemia, and oxidative stress. All of these can be natural features of mammalian hibernation, and hence the UPR might be integral to long term survival in a state of cold torpor. The present study analyzes changes in gene and/or protein expression of multiple markers of the UPR in tissues of euthermic (control) versus hibernating ground squirrels, *Spermophilus tridecemlineatus*. Immunoblot analysis of ATF4 protein expression revealed strong increases of 1.9- to 2.5-fold in brown adipose tissue, skeletal muscle, and brain during hibernation. However, transcript levels of *atf4* were unchanged or lowered which suggests that ATF4 protein levels were regulated at the translational level. Subcellular localization studies showed that ATF4 translocated into the nucleus during hibernation, as did its cofactor, the phosphorylated form of CREB-1, which rose by 25- to 39-fold in nuclear extracts of brain and skeletal muscle of torpid animals. The responses of other proteins involved in the UPR including p-PERK, ATF6, GADD153, and GADD34 were also evaluated. The data suggest that ATF4 up-regulation may play an important role in coordinating gene expression responses that support the hibernating phenotype.

- Increased levels of malfolded proteins in the ER activates the UPR & raises GRP78, an ER chaperone
- In BAT during hibernation:
  grp78 transcripts 3.5-fold ↑
  GRP78 protein 1.6-fold ↑
- Similar responses in brain with control by activating transcription factor 4 (ATF4)

- ATF4 protein 1.9 & 2.5-fold ↑ in BAT & brain of hibernators
- ATF4 translocated to the nucleus & its co-activator CREB-1 ↑ by 4.3-fold & 7.4-fold in BAT & brain

PPARγ and PGC-1α in Brown and White Adipose of hibernating ground squirrels

Molecular and Cellular Biochemistry **269:** 175–182, 2005.

Cloning and expression of PPARγ and PGC-1α from the hibernating ground squirrel, *Spermophilus tridecemlineatus*

Sean F. Eddy, Pier Jr. Morin and Kenneth B. Storey
Institute of Biochemistry and Department of Chemistry, Carleton University, Ottawa, Canada

Received 2 June 2004; accepted 27 August 2004

Abstract

The peroxisome proliferator-activated receptor (PPAR) family of transcription factors play a key role in lipid metabolism and have been implicated in a number of disease states, most notably of which is obesity. Controlled regulation of lipid metabolism is a key ingredient for successful hibernation. Partial cDNA sequences for one of the PPAR proteins, PPARγ and the PPARγ co-activator (PGC-1α) have been cloned from the hibernating ground squirrel, *Spermophilus tridecemlineatus* and show differential regulation during hibernation at the mRNA level using relative RT-PCR and at the protein level via immunoblotting in brown adipose tissue (BAT), heart, skeletal muscle and white adipose tissue (WAT). The cDNA sequence for PGC-1α revealed a number of amino acid substitutions and two were worthy of note, one resulting in the loss of a potential protein kinase C (PKC) site, while another resulted in the creation of a PKC site, suggesting that PKC may be important in regulating PGC-1α. RT-PCR revealed a near 2-fold up-regulation of PPARγ in BAT and to a lesser extent (<1.5-fold) in heart and WAT, while PGC-1α displayed significantly higher levels of expression in skeletal muscle during hibernation (3.1-fold, $p < 0.005$). The protein levels of PPARγ were significantly increased in BAT and WAT (1.5 and 1.8-fold, respectively) while PGC-1α displayed significant changes in expression in heart (3.5-fold) and skeletal muscle (1.8-fold). Our current findings indicate a role for increased expression of PPARγ and PGC-1α in hibernating animals. (Mol Cell Biochem **269:** 175–182, 2005)

- PPARγ - regulates multiple enzymes/proteins of lipid metabolism
- PGC-1α – co-activator of PPARγ & regulator of mitochondria biogenesis
- PPARγ: ↑ 1.4-fold in BAT; 1.7-fold in WAT
- PGC-1α: ↑ 2.7-fold in BAT; 2.1-fold in WAT
- PPARγ data correlates with up-regulation of *a-fabp* and *h-fabp* transcripts in hibernation

Fatty acid binding proteins in hibernation: up-regulation optimizes transport to mitochondria

Biochim Biophys Acta. 2001 Dec 30;1522(3):238-43.

Differential expression of adipose- and heart-type fatty acid binding proteins in hibernating ground squirrels.

Hittel D[1], Storey KB.

Author information

Abstract

The up-regulation of heart- and adipose-type fatty acid binding proteins (H-FABPs and A-FABPs) was detected during hibernation in brown adipose tissue (BAT) of 13-lined ground squirrels, Spermophilus tridecemlineatus, using a commercial rat cDNA array. Full length cDNAs encoding H-FABPs and A-FABPs were subsequently retrieved from a BAT cDNA library. These cDNAs were used to probe Northern blots of total RNA from tissues of euthermic versus hibernating ground squirrels. H-FABP mRNA transcripts increased in BAT, skeletal muscle and heart of hibernating animals whereas A-FABP transcripts, which are normally expressed exclusively in adipose tissue, increased in both BAT and heart during torpor. It is

Biochim Biophys Acta. 2004 Jan 5;1676(1):63-70.

Up-regulation of fatty acid-binding proteins during hibernation in the little brown bat, Myotis lucifugus.

Eddy SF[1], Storey KB.

Author information

Abstract

Hibernating animals rely primarily on lipids throughout winter as their primary fuel source, thus it is hypothesized that an increase in genes and proteins relating to lipid transport will increase accordingly. The cloning and expression of heart type fatty acid-binding protein (h-fabp) from a mammalian hibernator, the little brown bat Myotis lucifugus, is presented. Northern blot analysis revealed that transcript levels of h-fabp were significantly higher during hibernation in brown adipose tissue and skeletal muscle compared with levels in euthermic bats. Similarly, heterologous probing with rat adipose type a-fabp found 3.9-fold higher levels of a-fabp transcripts in brown adipose from hibernating animals. Levels of A- and H-FABP protein were quantified in tissues of euthermic versus hibernating animals by Western blotting. A-FABP was 4-fold higher in brown adipose of hibernating, compared with euthermic bats, whereas H-FABP was significantly higher in hibernator brown adipose, heart and skeletal muscle. The present work implicates FABPs as important elements related to the hibernating state in mammals; alterations in gene and protein expression along with amino acid substitutions are shown. These likely contribute to optimizing the function of FABPs at the low body temperatures (near 0 degrees C) experienced in the hibernating state.

Ground squirrel hibernation

Heart type H-FABP: transcripts ↑ BAT, heart, skeletal muscle

Adipose type A-FABP: transcripts ↑ BAT, heart

Bat hibernation

Heart type H-FABP: transcripts ↑ BAT, skeletal muscle; protein ↑ BAT, heart, muscle

Adipose type A-FABP: transcripts ↑ BAT; protein ↑ 4-fold

AMP-activated protein kinase in BAT & WAT during hibernation

Comp Biochem Physiol B Biochem Mol Biol. 2005 Dec;142(4):374-82. Epub 2005 Oct 3.

Evaluation of the role of AMP-activated protein kinase and its downstream targets in mammalian hibernation.

Horman S[1], Hussain N, Dilworth SM, Storey KB, Rider MH.

⊕ Author information

Abstract

Mammalian hibernation requires an extensive reorganization of metabolism that typically includes a greater than 95% reduction in metabolic rate, selective inhibition of many ATP-consuming metabolic activities and a change in fuel use to a primary dependence on the oxidation of lipid reserves. We investigated whether the AMP-activated protein kinase (AMPK) could play a regulatory role in this reorganization. AMPK activity and the phosphorylation state of multiple downstream targets were assessed in five organs of thirteen-lined ground squirrels (Spermophilus tridecemlineatus) comparing euthermic animals with squirrels in deep torpor. AMPK activity was increased 3-fold in white adipose tissue from hibernating ground squirrels compared with euthermic controls, but activation was not seen in liver, skeletal muscle, brown adipose tissue or brain. Immunoblotting with phospho-specific antibodies revealed an increase in phosphorylation of eukaryotic elongation factor-2 at the inactivating Thr56 site in white adipose tissue, liver and brain of hibernators, but not in other tissues. Acetyl-CoA carboxylase phosphorylation at the inactivating Ser79 site was markedly increased in brown adipose tissue from hibernators, but no change was seen in white adipose tissue. No change was seen in the level of phosphorylation of the Ser565 AMPK site of hormone-sensitive lipase in adipose tissues of hibernating animals. In conclusion, AMPK does not appear to participate in the metabolic re-organization and/or the metabolic rate depression that occurs during ground squirrel hibernation.

- AMPK known as the energy sensor of the cell
- AMPK activity rose 3-fold in WAT but not in BAT or 3 other tissues during torpor
- Selective changes only in AMPK-activated proteins:
  - phospho-Acetyl-CoA carboxylase at Ser79 inhibitory site increased in BAT
  - phospho-eIF2at Thr56 inhibitory site increased in WAT
  - no change in AMPK phosphorylation of hormone-sensitive lipase
- Overall, AMPK does not appear to regulate changes in lipid metabolism during torpor

Mitochondrial genes, proteins & enzyme activities increased during torpor in brown adipose of little brown bats

JOURNAL OF EXPERIMENTAL ZOOLOGY 305A:620–630 (200

Differential Expression of Selected Mitochondrial Genes in Hibernating Little Brown Bats, *Myotis lucifugus*

SEAN F. EDDY*, PIER JR. MORIN, AND KENNETH B. STOREY
Institute of Biochemistry and Department of Chemistry Carleton University, Ottawa, Ont., Canada K1S 5B6

ABSTRACT High rates of non-shivering thermogenesis by brown adipose tissue accompanied by additional shivering thermogenesis in skeletal muscle provide the powerful reheating of body organs that allows hibernating mammals to return from their state of cold torpor back to euthermic function. Previous studies have suggested that changes to brown adipose mitochondria occur during hibernation and are partially responsible for its capacity for non-shivering thermogenesis. The current study shows that selected mitochondrial enzyme activities are elevated and selected genes and proteins are induced during torpor in brown adipose tissue of the little brown bat, *Myotis lucifugus*. Cytochrome oxidase activity in brown adipose tissue was more than 3-fold higher during torpor than in euthermic animals. Transcript levels of mitochondria-encoded genes, *coxII* and *nad4*, were also 3–4-fold higher during torpor, as evidenced by northern blotting. By contrast, transcripts of these genes were unchanged in skeletal muscle during torpor. Protein levels of carnitine palmitoyl transferase-1β, an enzyme embedded in the outer membrane of the mitochondria that is the rate-limiting step enzyme in β-oxidation, were also elevated by 2-fold during torpor in brown adipose but were unchanged in skeletal muscle. Cloning and sequencing of a 624 bp segment of *cpt-1β* revealed a number of amino acid substitutions in the bat protein as compared to CPT-1β from other mammals; these may be beneficial for enzyme function at low body temperatures during torpor. This study provides further evidence for a key role of mitochondria in hibernation. *J. Exp. Zool. 305A: 620–630, 2006.*

During torpor in brown adipose, compared with euthermia:

- Cytochrome oxidase activity ↑ 3-fold
- Transcripts of *coxII* and *nad4* ↑ 3–4-fold, both mitochondria-encoded genes
- 2-fold ↑ in carnitine palmitoyl transferase-1b protein

Differential expression of mitochondrial vs nuclear encoded subunits of cytochrome oxidase (complex IV) & ATP synthase (complex 5)

The Journal of Experimental Biology 205, 1625–1631 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3879

Differential expression of mitochondria-encoded genes in a hibernating mammal

Dustin S. Hittel and Kenneth B. Storey*

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

*Author for correspondence (e-mail: kenneth_storey@carleton.ca)

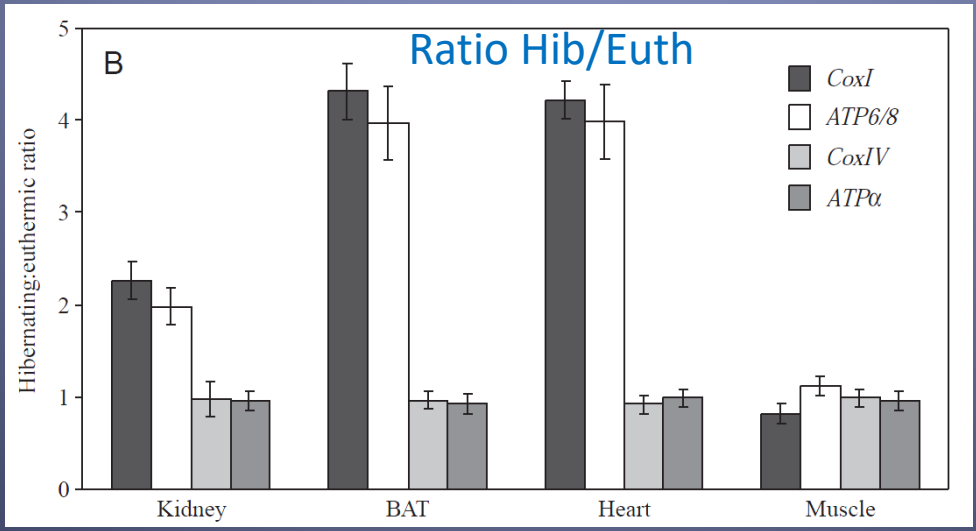
Accepted 13 March 2002

Summary

A cDNA library constructed from kidney of the thirteen-lined squirrel, *Spermophilus tridecemlineatus*, was differentially screened for genes that were upregulated during hibernation. A clone encoding cytochrome *c* oxidase subunit 1 was found and confirmed to have been upregulated by northern blotting. Differential expression of *Cox1* mRNA occurred in multiple organs during hibernation; in hibernating animals transcript levels were twofold higher in kidney and fourfold higher in heart and brown adipose tissue than in euthermic animals, but were unchanged in skeletal muscle. Transcript levels of mitochondrial-encoded ATP synthase 6/8 were similarly upregulated in these tissues whereas transcript levels of the nuclear encoded subunits *Cox4* and ATP synthase α did not change during hibernation. Immunoblot analysis revealed a 2.4-fold increase in Cox 1 protein and a slight decrease in Cox 4 protein in kidney of hibernating squirrels, compared with euthermic controls. Hibernating mammals may increase the expression of the mitochondrial genome in general, and *Cox1* specifically, to prevent or minimize the damage to the electron transport chain caused by the cold and ischemia experienced during a hibernation bout.

Key words: *Spermophilus tridecemlineatus*, hibernation, ischemia, kidney, cDNA library.

- **Increased synthesis of mitochondria-encoded subunits in BAT, kidney & heart: *cox1 & ATP6/8***
- **No change in synthesis of nuclear-encoded subunits in any tissue: *cox4 & ATPα***



Anti-apoptosis in White Adipose: Cell Preservation during Hibernation

Mol Cell Biochem. 2016 Mar 31. [Epub ahead of print]

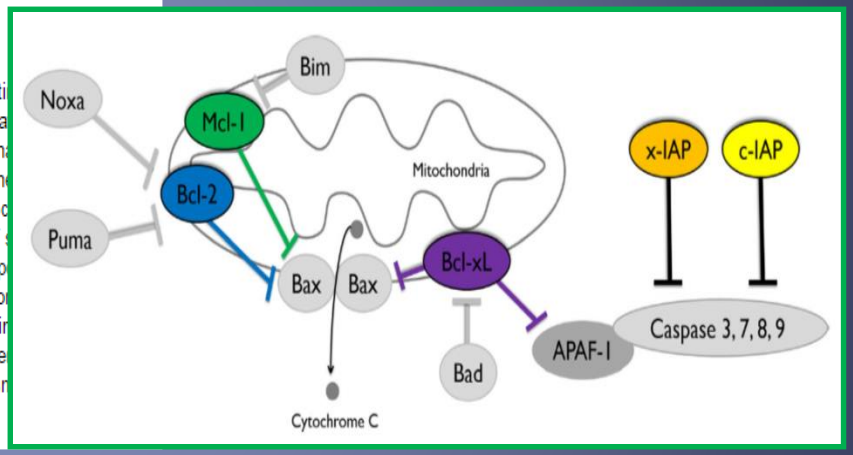
Turn down genes for WAT? Activation of anti-apoptosis pathways protects white adipose tissue in metabolically depressed thirteen-lined ground squirrels.

Logan SM[1], Luu BE[1], Storey KB[2].

Author information

Abstract

During hibernation, the metabolic rate of thirteen-lined ground squirrels (Ictidomys tridecemlineatus) can drop to <5 % of normal resti core body temperature can decrease to as low as 1-5 °C, and heart rate can fall from 350-400 to 5-10 bpm. Energy saved by hiberna squirrels to survive the winter when food is scarce, and living off lipid reserves in white adipose tissue (WAT) is crucial. While hiber energy must be used to cope with conditions that would normally be damaging for mammals (e.g., low core body temperatures, isch induce cell death via apoptosis. Cell survival is largely dependent on the relative amounts and activities of pro- and anti-apoptotic Bc proteins. The present study analyzed how anti-apoptotic proteins respond to protect WAT cells during hibernation. Relative levels of apoptotic proteins were quantified in WAT via immunoblotting over six time points of the torpor-arousal cycle. These included anti-ap family members Bcl-2, Bcl-xL, and Mcl-l, as well as caspase inhibitors x-IAP and c-IAP. Changes in the relative protein levels and/o levels were also observed for various regulators of apoptosis (p-JAKs, p-STATs, SOCS, and PIAS). Mcl-1 and x-IAP protein levels i whereas Bcl-xL, Bcl-2, and c-IAP protein/phosphorylation levels decreased signifying important roles for certain Bcl-2 family membe over the torpor-arousal cycle. Importantly, the relative phosphorylation of selected STAT proteins increased, suggesting a mechanis activation. These results suggest that an increase in WAT cytoprotective mechanisms supports survival efforts during hibernation.



Balance of pro- and anti-apoptotic protein actions determines cell survival vs death

Bcl-2 & its phospho forms (S70, T56) decline in torpor & rebound in arousal
Bcl-xL unchanged but its phospho form (S62) decreases in torpor
X-IAP, a caspase inhibitor, increases in torpor & caspase-3 decreases

These measurements in BAT are the next frontier !

Hypoxia-inducible factor-1 in brown adipose of hibernators

Biochim Biophys Acta. 2005 May 25;1729(1):32-40. Epub 2005 Mar 17.

Cloning and expression of hypoxia-inducible factor 1alpha from the hibernating ground squirrel, Spermophilus tridecemlineatus.

Morin P Jr[1], Storey KB.

⊕ Author information

Abstract

Mammalian hibernation is associated with apnoic breathing patterns and a hypoxia-hypothermia connection has been suggested as part of the mechanism by which body temperature is reduced as animals enter torpor. Hence, we hypothesized that changes in the expression of the hypoxia inducible factor (HIF-1) may potentially be involved in regulating hibernation-responsive gene targets. The expression of the alpha subunit of HIF-1 was quantified at both gene and protein levels in four organs of the thirteen-lined ground squirrel, Spermophilus tridecemlineatus. Reverse transcription-PCR showed no change in hif-1alpha transcript levels in the liver, lung, skeletal muscle or brown adipose tissue of euthermic versus hibernating animals but HIF-1alpha protein levels were elevated by 60-70% in the two organs responsive for thermogenesis (brown adipose and skeletal muscle). Furthermore, assessment of DNA binding by HIF-1 in nuclear extracts from brown adipose revealed 6-fold higher levels in hibernator tissue than in euthermic controls suggesting increased expression of HIF-1 responsive genes during hibernation. The complete nucleotide sequence of hif-1alpha from ground squirrels, the first hif-1alpha sequence amplified from a hibernating mammal, was obtained using PCR amplification and 3' and 5' RACE. Amino acid sequence analysis revealed 90-95% identity with the HIF-1alpha protein from other mammals. Several unique amino acid sequence substitutions were identified that may affect protein conformation and could possibly function to counteract low temperature effects on HIF-1alpha conformation at near 0 degrees C body temperatures during torpor.

In hibernation, compared with euthermia

- ***Hif-1*** **transcripts did not change**
- **HIF-2 protein ⇑ 1.7-fold in BAT & 1.6 fold in muscle**
- **HIF-1 mediated gene expression could increase**
- **Unique substitutions in HIF-1 protein sequence may counteract low temperature effects on conformation.**

PRINCIPLES OF MRD

Most Genes OFF !

Epigenetics refers to the study of changes in gene expression that are not dependent on gene DNA sequence.

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
- "Hiding messages"

Global DNA modifications in hibernation → Epigenetic controls

Cryobiology 69 (2014) 333–338

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Cryobiology

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

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

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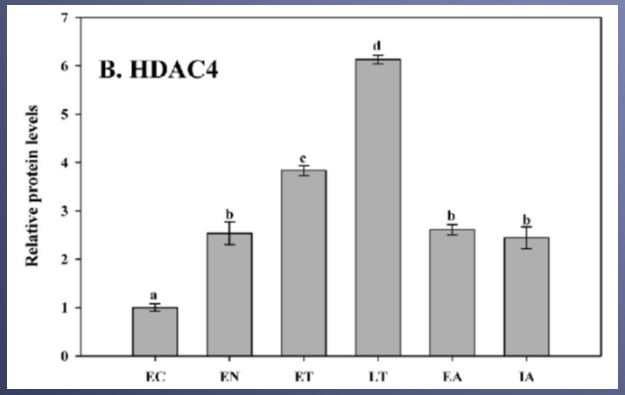
Ground squirrel hibernation
Ictidomys tridecemlineatus
Transcriptional repression
DNA methylation
Histone acetylation
HP1
MBD1

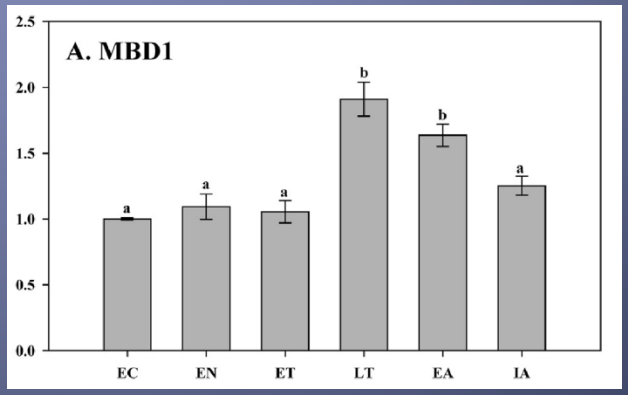
ABSTRACT

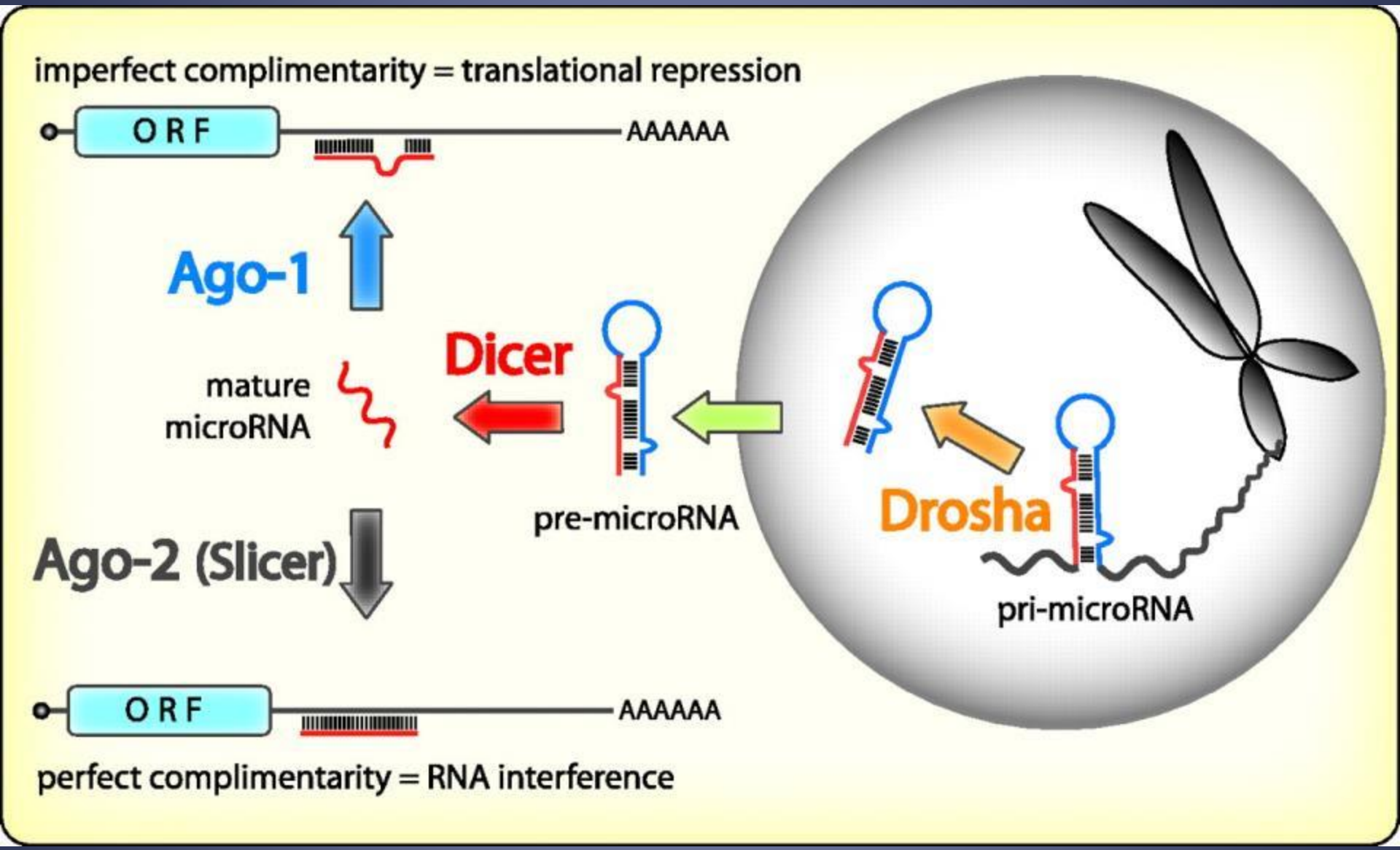
Hibernation is crucial to winter survival for many small mammals and is characterized by prolonged periods of torpor during which strong global controls are applied to suppress energy-expensive cellular processes. We hypothesized that one strategy of energy conservation is a global reduction in gene 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.

BAT responses during hibernation show global suppression of TRANSCRIPTION

- 1.7-fold ↑ in DNA methylation
- 50% ↓ in Histone H3 Lys23 acetylation = increased chromatin packing
- Strong ↑ in histone deacetylases 1 & 4
- Strong ↑ in MBD1 and HP1, co-repressors of transcription







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

MicroRNA expression in hibernation

Genomics Proteomics Bioinformatics. 2014 Dec;12(6):284-91. doi: 10.1016/j.gpb.2014.08.003. Epub 2014 Dec 16.

Expression profiling and structural characterization of microRNAs in adipose tissues of hibernating ground squirrels.

Wu CW[1], Biggar KK[1], Storey KB[2].

⊕ Author information

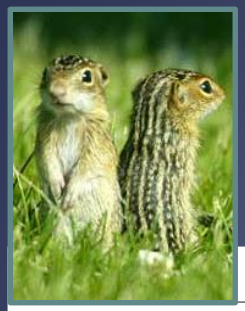
Abstract

MicroRNAs (miRNAs) are small non-coding RNAs that are important in regulating metabolic stress. In this study, we determined the expression and structural characteristics of 20 miRNAs in brown (BAT) and white adipose tissue (WAT) during torpor in thirteen-lined ground squirrels. Using a modified stem-loop technique, we found that during torpor, expression of six miRNAs including let-7a, let-7b, miR-107, miR-150, miR-222 and miR-31 was significantly downregulated in WAT ($P<0.05$), which was 16%-54% of euthermic non-torpid control squirrels, whereas expression of three miRNAs including miR-143, miR-200a and miR-519d was found to be upregulated by 1.32-2.34-fold. Similarly, expression of more miRNAs was downregulated in BAT during torpor. We detected reduced expression of 6 miRNAs including miR-103a, miR-107, miR-125b, miR-21, miR-221 and miR-31 (48%-70% of control), while only expression of miR-138 was significantly upregulated (2.91±0.8-fold of the control, $P<0.05$). Interestingly, miRNAs found to be downregulated in WAT during torpor were similar to those dysregulated in obese humans for increased adipogenesis, whereas miRNAs with altered expression in BAT during torpor were linked to mitochondrial β-oxidation. miRPath target prediction analysis showed that miRNAs downregulated in both WAT and BAT were associated with the regulation of mitogen-activated protein kinase (MAPK) signaling, while the miRNAs upregulated in WAT were linked to transforming growth factor β (TGFβ) signaling. Compared to mouse sequences, no unique nucleotide substitutions within the stem-loop region were discovered for the associated pre-miRNAs for the miRNAs used in this study, suggesting no structure-influenced changes in pre-miRNA processing efficiency in the squirrel. As well, the expression of miRNA processing enzyme Dicer remained unchanged in both tissues during torpor. Overall, our findings suggest that changes of miRNA expression in adipose tissues may be linked to distinct biological roles in WAT and BAT during hibernation and may involve the regulation of signaling cascades.

miRNA binding to mRNA transcripts = ↓ translation

with mRNAs targeted to storage or degradation

- **20 miRNAs measured**
- **BAT: miR-138 up by 3-fold; 6 miRNAs reduced**
- **BAT: silencing miR103 in mice led to ↑ expression of ß-oxidation genes**
- **BAT: silencing miR107 led to ↑ expression of uncoupling protein 2 in mice**
- **BAT & WAT: miRpath analysis indicated global changes in MAPK signaling**



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

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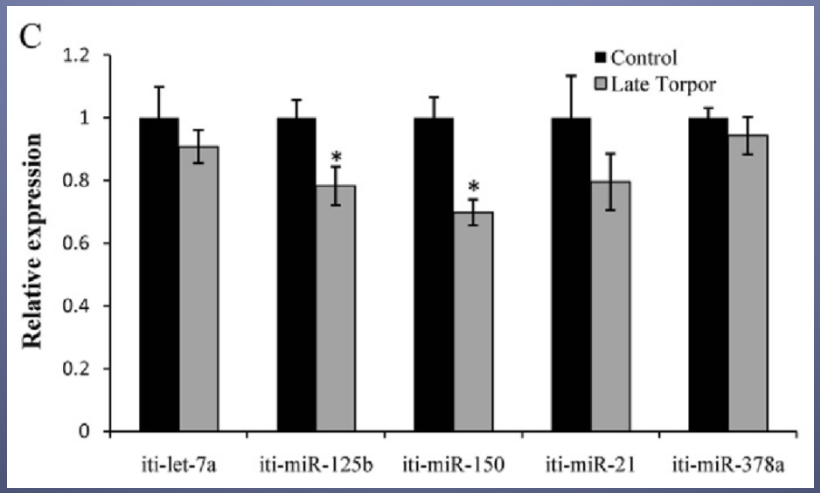
Hibernation
RT–PCR
Polyadenylation
Ictidomys tridecemlineatus
MicroRNA amplification

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 and introduced a polyadenylation step to allow amplification of all mature microRNAs from a single RNA sample. Importantly, this method retains the ability to sequence reverse transcription polymerase chain reaction (RT–PCR) products, validating microRNA-specific amplification.

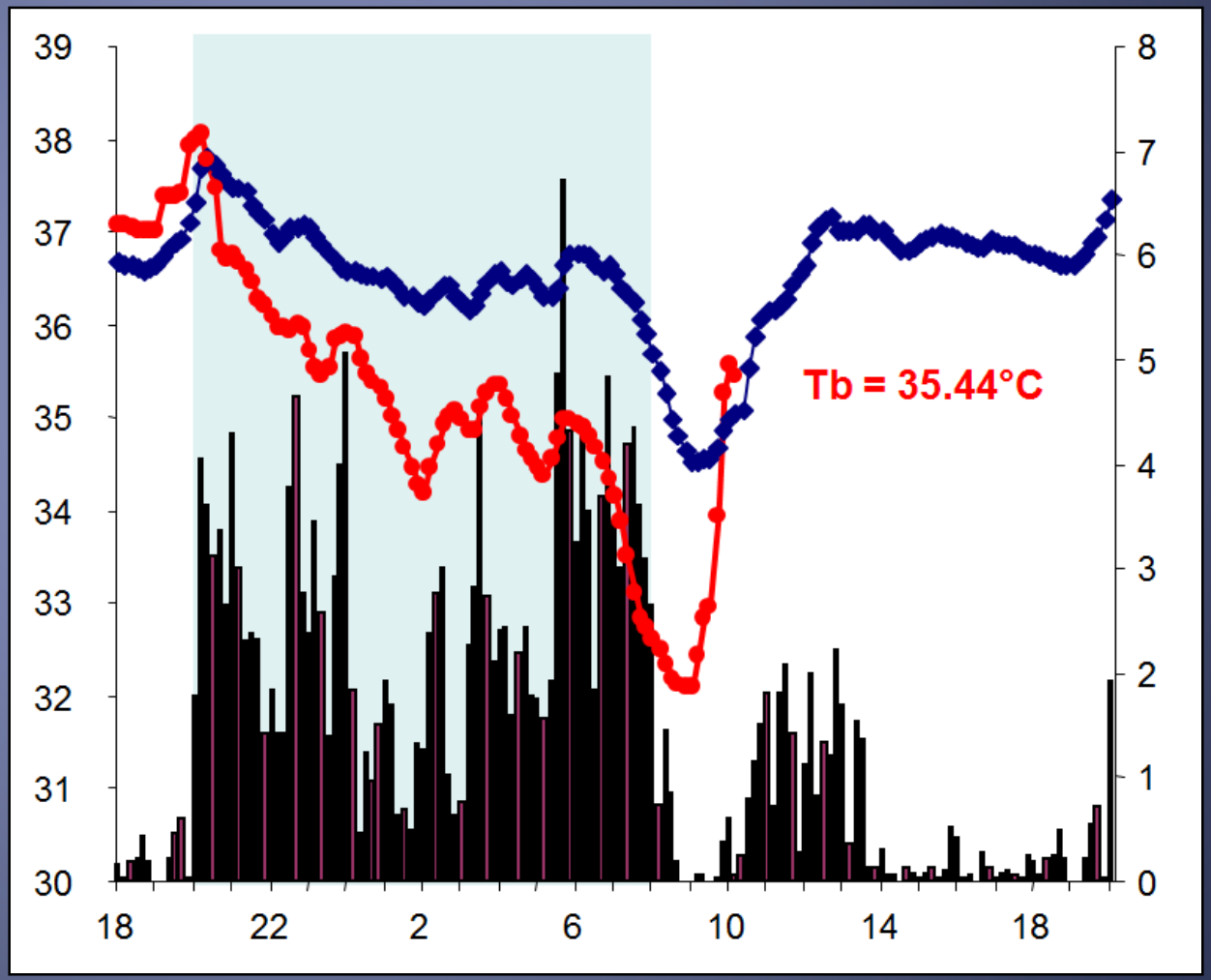
- Advanced method for microRNA expression analysis & sequencing
- Key to studies of species with non-annotated miRNAs → most comparative models
- Euthermic vs deep torpor levels of 5 miRNAs from ground squirrel brown adipose – both sequenced & quantified expression by new method



The LEMUR model

- Native to Madagascar
- Hibernate 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) that is not confounded by the additional biochemical adaptations needed for low temperatures function

PRIMATE TORPOR:
GRAY MOUSE LEMUR



Genomics Proteomics Bioinformatics 13 (2015) 77–80

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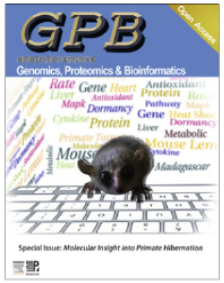


PREFACE

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

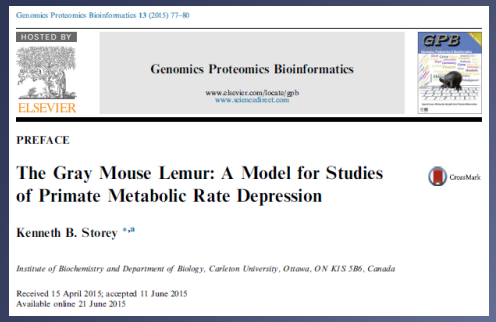
Kenneth B. Storey *,[a]

Institute of Biochemistry and Department of Biology, Carleton University,

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

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

Primate Torpor Series



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PREFACE

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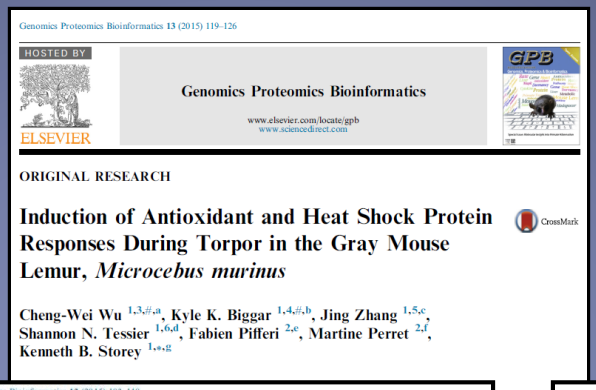
Stress response and signal transduction

Regulation of gene/protein expression

Metabolism, fuel utilization, and cytokines



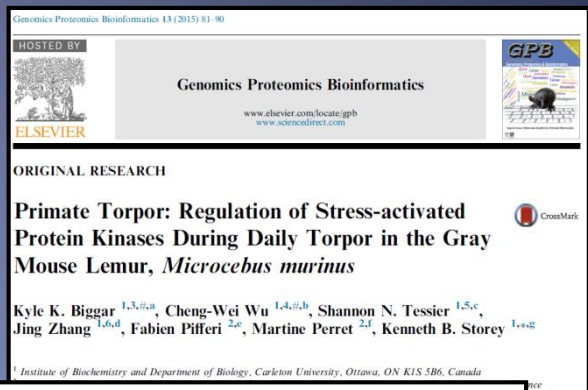
Genomics Proteomics Bioinformatics 13 (2015) 119–126

Genomics Proteomics Bioinformatics

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]



Genomics Proteomics Bioinformatics 13 (2015) 81–90

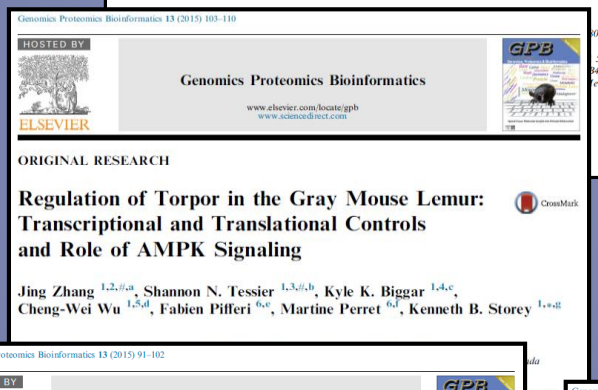
Genomics Proteomics Bioinformatics

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



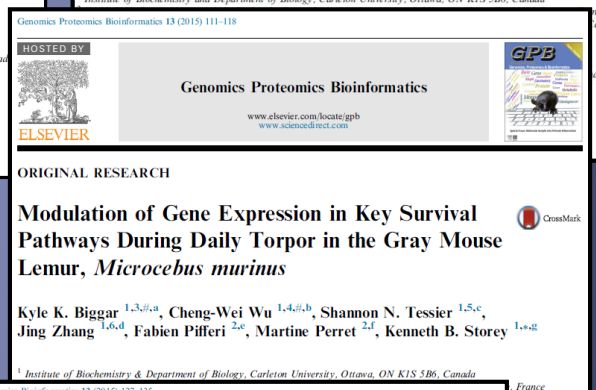
Genomics Proteomics Bioinformatics 13 (2015) 103–110

Genomics Proteomics Bioinformatics

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]



Genomics Proteomics Bioinformatics 13 (2015) 111–118

Genomics Proteomics Bioinformatics

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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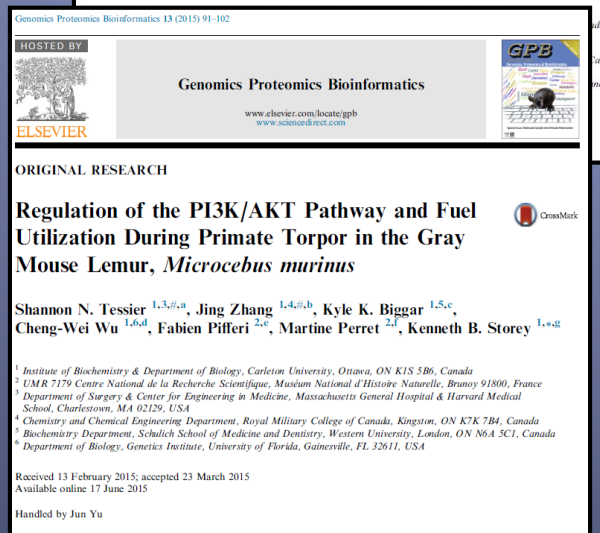

Genomics Proteomics Bioinformatics 13 (2015) 91–102

Genomics Proteomics Bioinformatics

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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Available online 17 June 2015

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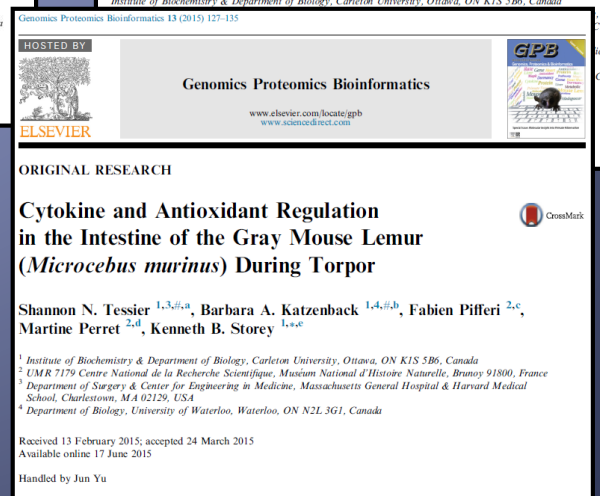

Genomics Proteomics Bioinformatics 13 (2015) 127–135

Genomics Proteomics Bioinformatics

ORIGINAL RESEARCH

Cytokine and Antioxidant Regulation in the Intestine of the Gray Mouse Lemur (*Microcebus murinus*) During Torpor

Shannon N. Tessier [1,3,#,a], Barbara A. Katzenback [1,4,#,b], Fabien Pifferi [2,c], Martine Perret [2,d], Kenneth B. Storey [1,*,e]

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INVESTIGATING CONTROL OF DAILY TORPOR IN A PRIMATE

- Closest species to man that uses hypometabolism: daily torpor or hibernation
- Enter torpor at high body temperature
- Compare aroused lemurs vs lemurs at lowest metabolic rate in torpor
- Six organs analyzed: heart, liver, kidney, skeletal muscle, brown adipose tissue, white adipose tissue
- Use Luminex multiplex, ELISA or PCR array-based methods to evaluate multiple analytes from very small tissue samples

TORPOR CONTROL BY SIGNALING CASCADES: Mitogen-activated protein kinases (MAPKs)

Luminex multiplex panels allowed assay of 12 targets simultaneously

BAT: activation of ERK ½ , p38

White adipose: MAPKs show robust activation during torpor – "awakening" this fuel storage tissue

Skeletal muscle: stress-responsive JNK & p38 activated but ERK/MEK that mediate growth responses suppressed

Liver, heart, kidney were little affected

Genomics Proteomics Bioinformatics 13 (2015) 81–90

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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*
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[3] *Biochemistry Department, Schulich School of Medicine and Dentistry, Western University, London, ON N6A 5C1, Canada*
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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 signaling pathway

- Luminex panels were used to analyze insulin & PI3K/Akt signaling and the mTOR protein synthesis pathway
- BAT : did not show changes in any of these pathways !
- Elements of Insulin/IGF receptor signaling that regulates nutrient-based anabolic /growth responses
- No change in GSK3α
- No Inhibition of carbohydrate catabolism occurred in BAT
- Almost all other tissues had KEY changes

Genomics Proteomics Bioinformatics 13 (2015) 91–102

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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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[4] *Chemistry and Chemical Engineering Department, Royal Military College of Canada, Kingston, ON K7K 7B4, Canada*
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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)
- the "energy sensor" of the cell

Heart & muscle: AMPK was activated
- switch to fatty acid oxidation in torpor
- drop in protein synthesis via mTOR inhibition

BAT: has inhibition of AMPK

Histone control of gene expression

White adipose: a decrease in phosphorylated histone H3 = a global decrease in gene transcription in torpor

BAT: no change in phosphorylation of histone H3. No global decrease in torpor

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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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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

GENE RESPONSES TO TORPOR
Adjusting key survival pathways

Array-based real-time PCR assessed 28 genes linked with ground squirrel hibernation .

MOST genes are turned *down*

Heart: some chaperone genes expressed. Key functional organ – heart must keep beating

Liver & Brown adipose: many genes showed increased expression. Key metabolic & key thermogenic organs function in a new mode in torpor.

= **Selective gene expression aids torpor**

Many less genes & fewer tissues affected in daily torpor than in long-term hibernation at cold body temperatures.

Organ preservation: identify the key processes in each organ that need adjustment

- Warm preservation may be least injurious

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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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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,

CELL PROTECTION RESPONSES TO TORPOR
Antioxidants & Chaperone proteins

Stress tolerance requires methods to preserve cell viability

Antioxidants deal with rapid changes in oxygen radicals between torpid & aroused states

Heat shock proteins protect/stabilize other proteins during torpor

Brown adipose:
- strong increases in Hsp70, Hsp90a & Superoxide Dismutase to protect this heat-generating tissue during arousal

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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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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)

INTESTINE RESPONSES TO TORPOR
Cytokines, Chemokines & Antioxidants

Pro-inflammatory cytokines & chemokines decreased in torpor
- e.g. jejunum showed strong suppression of IL-6, TNF-α, IL-12p70 & M-CSF

Anti-inflammatory cytokines did not change in torpor

Suppression of mucosal immune response in torpor is indicated

Intestine antioxidants were largely unchanged in torpor

BAT = T.B.A.

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ORIGINAL RESEARCH

Cytokine and Antioxidant Regulation in the Intestine of the Gray Mouse Lemur (*Microcebus murinus*) During Torpor

Shannon N. Tessier [1,3,#,a], Barbara A. Katzenback [1,4,#,b], Fabien Pifferi [2,c], Martine Perret [2,d], Kenneth B. Storey [1,*,e]

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KEYWORDS
Primate torpor;
Cytokines;
Chemokines;
Antioxidant enzymes;
Gut immunology

Abstract During food shortages, the gray mouse lemur (*Microcebus murinus*) of Madagascar experiences daily torpor thereby reducing energy expenditures. The present study aimed to understand the impacts of torpor on the immune system and antioxidant response in the gut of these animals. This interaction may be of critical importance given the trade-off between the energetically costly immune response and the need to defend against pathogen entry during hypometabolism. The protein levels of cytokines and antioxidants were measured in the small intestine (duodenum, jejunum, and ileum) and large intestine of aroused and torpid lemurs. While there was a significant decrease of some pro-inflammatory cytokines (IL-6 and TNF-α) in the duodenum and jejunum during torpor as compared to aroused animals, there was no change in anti-inflammatory cytokines. We observed decreased levels of cytokines (IL-12p70 and M-CSF), and several chemokines (MCP-1 and MIP-2) but an increase in MIP-1α in the jejunum of the torpid animals. In addition, we evaluated antioxidant response by examining the protein levels of antioxidant enzymes and total antioxidant capacity provided by metabolites such as glutathione (and others). Our results indicated

IS PRIMATE TORPOR THE SAME AS OTHER MAMMALIAN HIBERNATORS?

The $1,000,000 Question →
Which model will allow long term human organ preservation.

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