Metabolic Arrest, Stasis and Regeneration

Kenneth B. Storey, Carleton University, Ottawa

www.carleton.ca/~kbstorey

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

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

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

In Vitro Preservation Technology

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

Nature's BioPreservation [Organs-OFF]

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

NATURE'S [NEW !] MECHANISMS

Posttranslational modifications

Epigenetics

MicroRNA

Gene suppression

Selective gene activation

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Low	Squirrel	√
	Bat	√
_____0°C_____		
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	Turtle	√

Nature's [NEW !] Mechanisms

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In Vitro Preservation Technology

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

METABOLIC RATE DEPRESSION

Hibernation

Estivation

Anoxia

Freezing

Diapause

METABOLISM IN HIBERNATION

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

ATP turnover ↓ to <5% of normal

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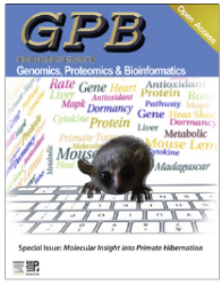


PREFACE

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

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

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

PRIMATE HIBERNATION

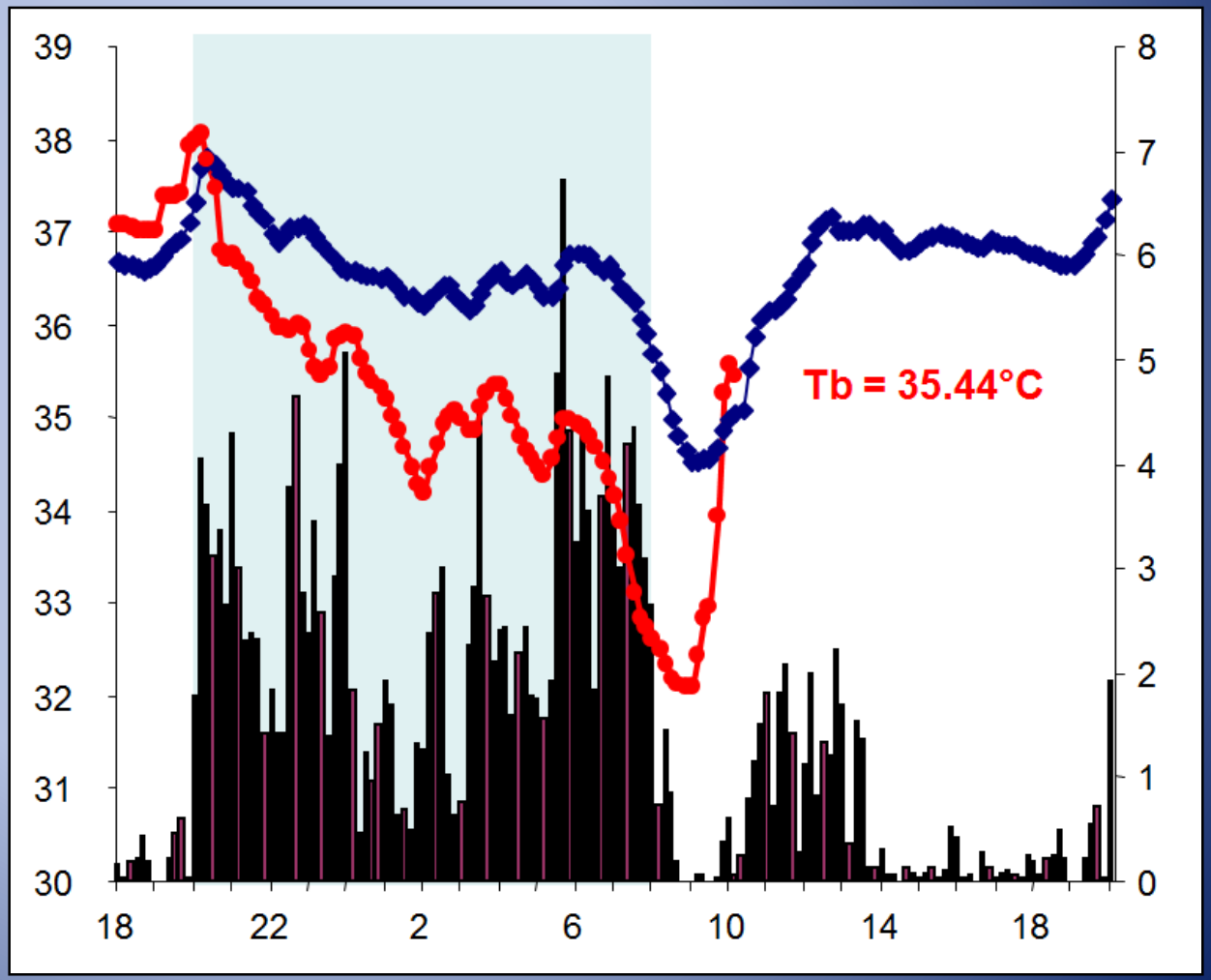
Gray Mouse Lemur

Madagascar - western dry forests

LEMUR model

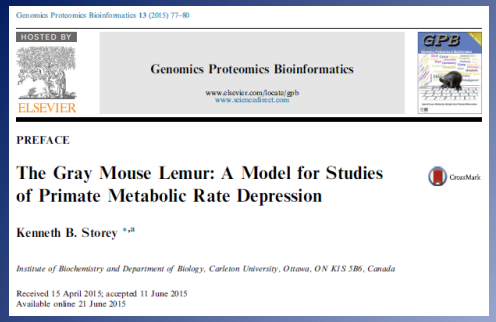
- Native to Madagascar
- Hibernate to deal with chronic food shortages in the dry season
- The most closely related species to man that exhibit natural hypometabolism
- Enter torpor at high ambient temperatures (T_b may only fall to ~28-32°C) that is not confounded by the additional biochemical adaptations needed for low temperature hibernation in most mammals

PRIMATE TORPOR: GRAY MOUSE LEMUR



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

Primate Torpor Series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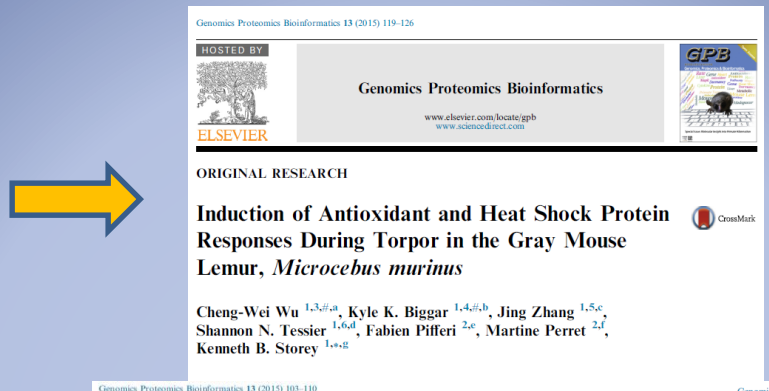
Stress response and signal transduction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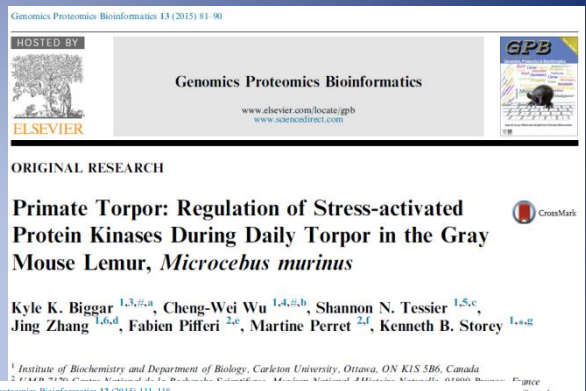
Cheng-Wei Wu [1,3,#,a], Kyle K. Biggar [1,4,#,b], Jing Zhang [1,5,c], Shannon N. Tessier [1,6,d], Fabien Pifferi [2,e], Martine Perret [2,f], Kenneth B. Storey [1,*,g]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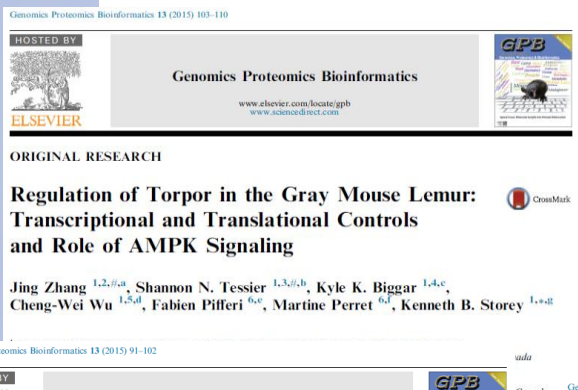
Regulation of gene/protein expression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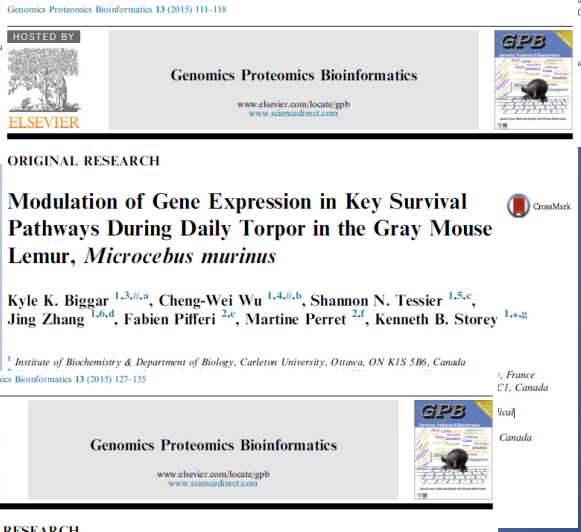
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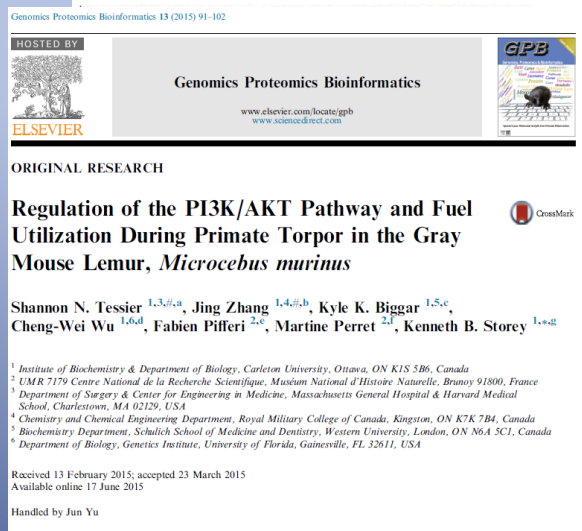
Metabolism, fuel utilization, and cytokines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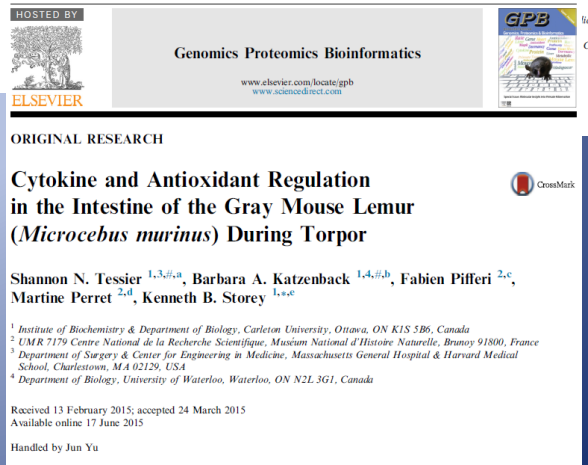
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ORIGINAL RESEARCH

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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TORPOR CONTROL BY SIGNALING CASCADES:
Insulin signaling pathway

Luminex panels were used to analyze insulin & PI3K/Akt signaling and the mTOR protein synthesis pathway

Elements of Insulin/IGF receptor signaling were inhibited in skeletal muscle and white adipose indicating suppression of nutrient-based anabolic /growth responses

Heart showed strong activation of GSK3α indicating a key role for this kinase in cardiac metabolic responses to torpor

Inhibition of carbohydrate catabolism occurred at pyruvate dehydrogenase in skeletal muscle

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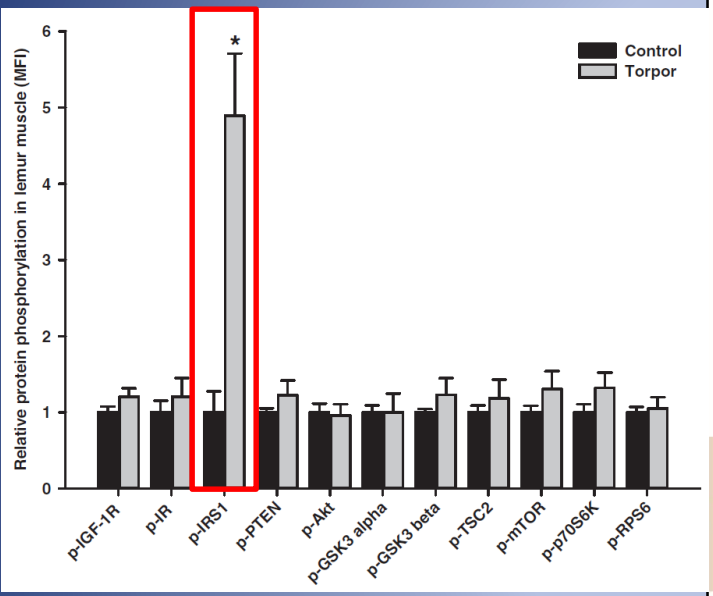
KEYWORDS
Insulin signaling pathway;
PI3K/AKT;
mTOR;
GSK3;
Pyruvate dehydrogenase;
Metabolic rate depression

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,

Insulin / AKT



*general inhibition of insulin signaling in most tissues, except liver

*tissue specific response

The regulation of the PI3K/AKT pathway during primate torpor in the grey mouse lemur, *Microcebus murinus*.

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

Luminex multiplex panels assayed
12 targets simultaneously in 6 tissues
- High throughput / high efficiency
- Multiple targets analyzed in 1 sample

Total protein & phospho-protein (active form) compared for ERK, MEK, JNK & p38

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

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

Liver, heart, kidney, brown adipose were little affected

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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]

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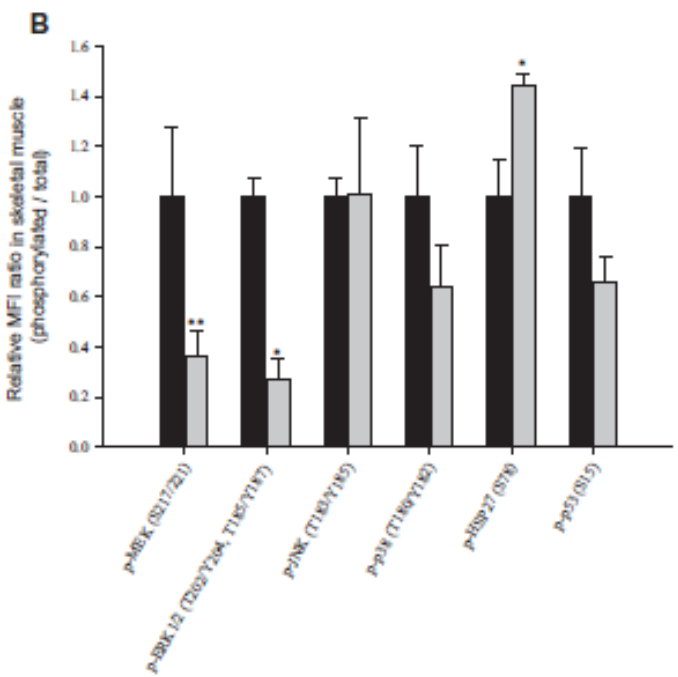
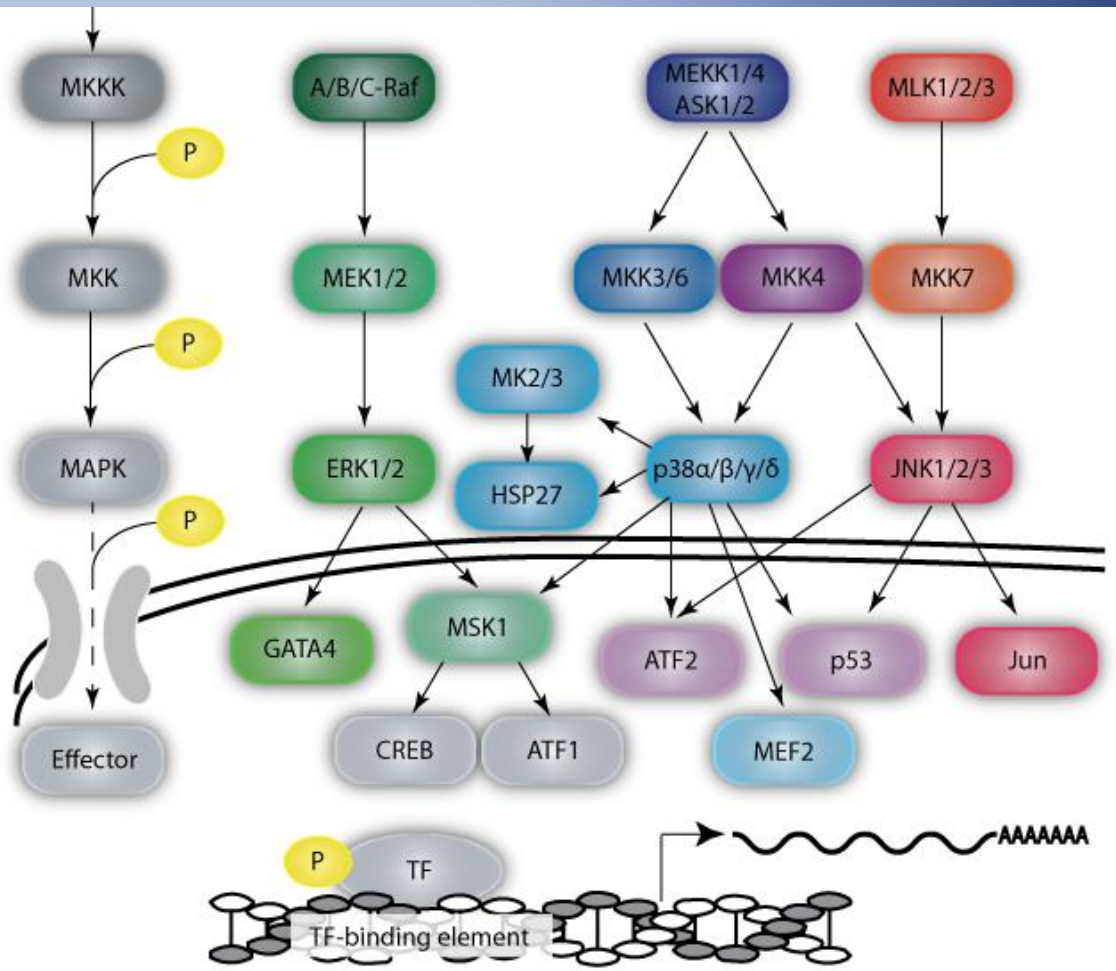
KEYWORDS
Metabolic rate depression;
Signal transduction;
Mitogen activated protein kinase

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

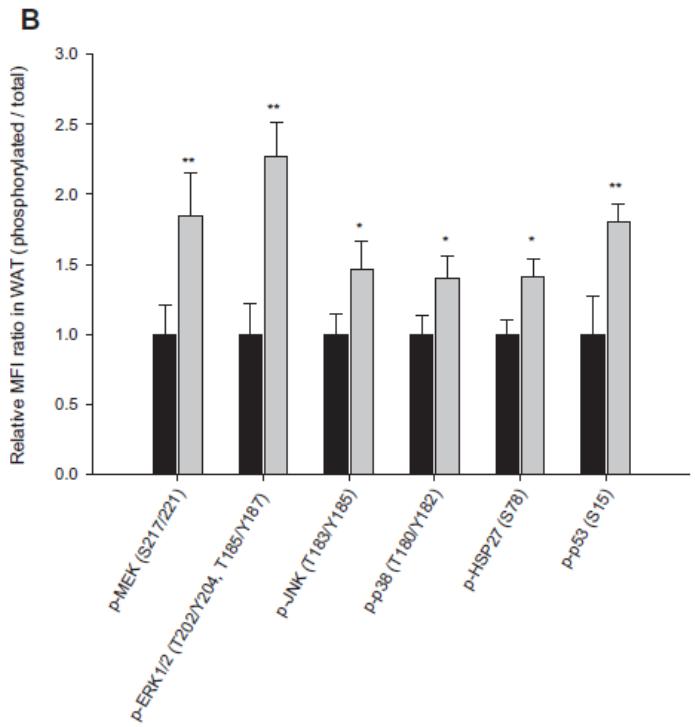
MAPK SIGNALING IN PRIMATE TORPOR



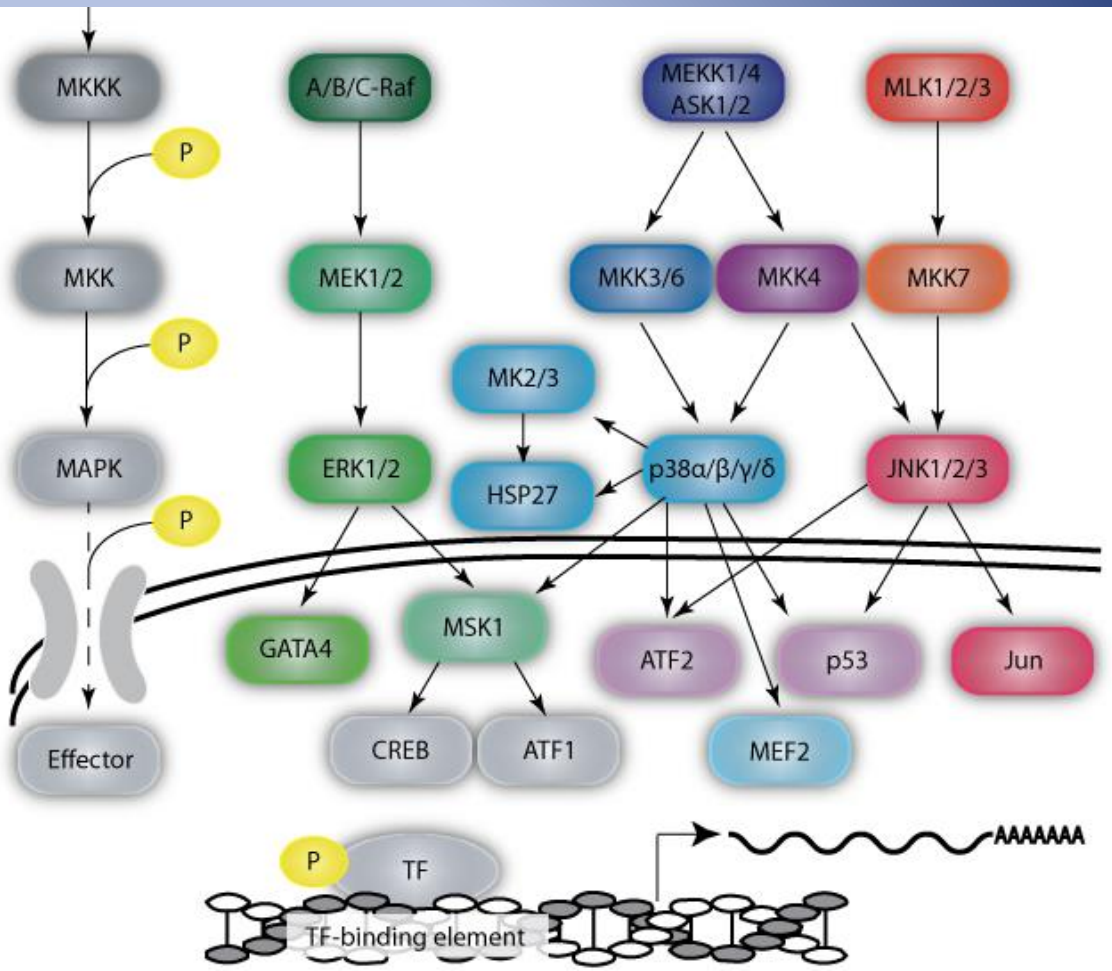


*p-ERK signaling is decreased in skeletal muscle, suggesting that cell growth/proliferation are suppressed during torpor

MAPK SIGNALING IN PRIMATE TORPOR



*all MAPK activated in white adipose tissue, suggesting the importance of this tissue as a source of metabolic fuel in torpor



TORPOR CONTROL : AMPK signaling & gene/protein synthesis

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

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

Histone control of gene expression

White adipose: showed a strong decrease in phosphorylated histone H3 aiding a global decrease in gene transcription in torpor

Heart: showed increased acetylation of histone H3 suggesting selective increases in gene transcription -- perhaps modulating heart performance in torpor

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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

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

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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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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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

Genomics Proteomics Bioinformatics 13 (2015) 127–135

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ORIGINAL RESEARCH

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

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[2] *UMR 7179 Centre National de la Recherche Scientifique, Muséum National d'Histoire Naturelle, Brunoy 91800, France*
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Handled by Jun Yu

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

SUMMARY: THE LEMUR MODEL

Overall, this group of studies illustrates;

- conservation during lemur torpor of many of the basic regulatory parameters of metabolic rate depression that are found across phylogeny
- the power of a multiplex approach to biochemical analysis
- new features of torpor such as cytokine responses by the immune system in intestine.

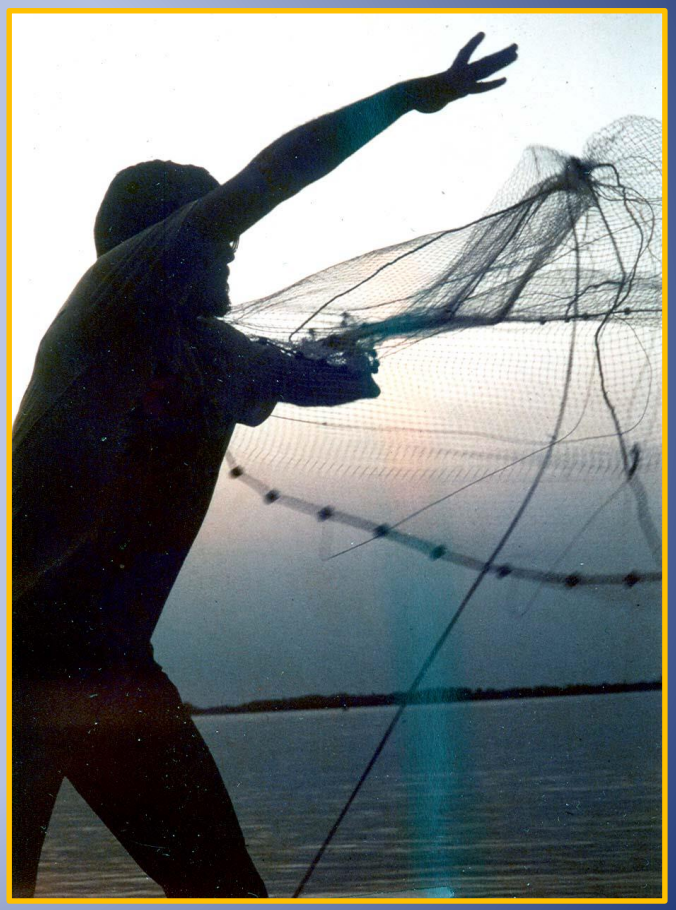
This validates the use of the lemur model and “warm temperature torpor.”

The stage is set for in-depth studies of the genomics/proteomics of lemur torpor that will lead to identification of the critical elements of torpor induction and control **that could be applied to improve human organ preservation**.

Where do we go from here?

Nature's Tools for MRD

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



NEW DIRECTIONS

Mammalian Torpor & Hibernation

- J. Storey
- S. Eddy
- D. Hittel
- J. MacDonald
- A. Fahlman
- P. Morin
- C. Holden
- H. Mehrani
- J. Ni
- M. Hapsatou
- K. Abnous
- A. Krivoruchko
- R. Bell
- S. Tessier
- C-W. Wu
- J. Zhang
- K. Biggar
- Y. Maistrovski Biggar
- S. Brooks
- C. Frank
- J. Hallenbeck
- D. Thomas
- A. Rubstov
- J. Stewart

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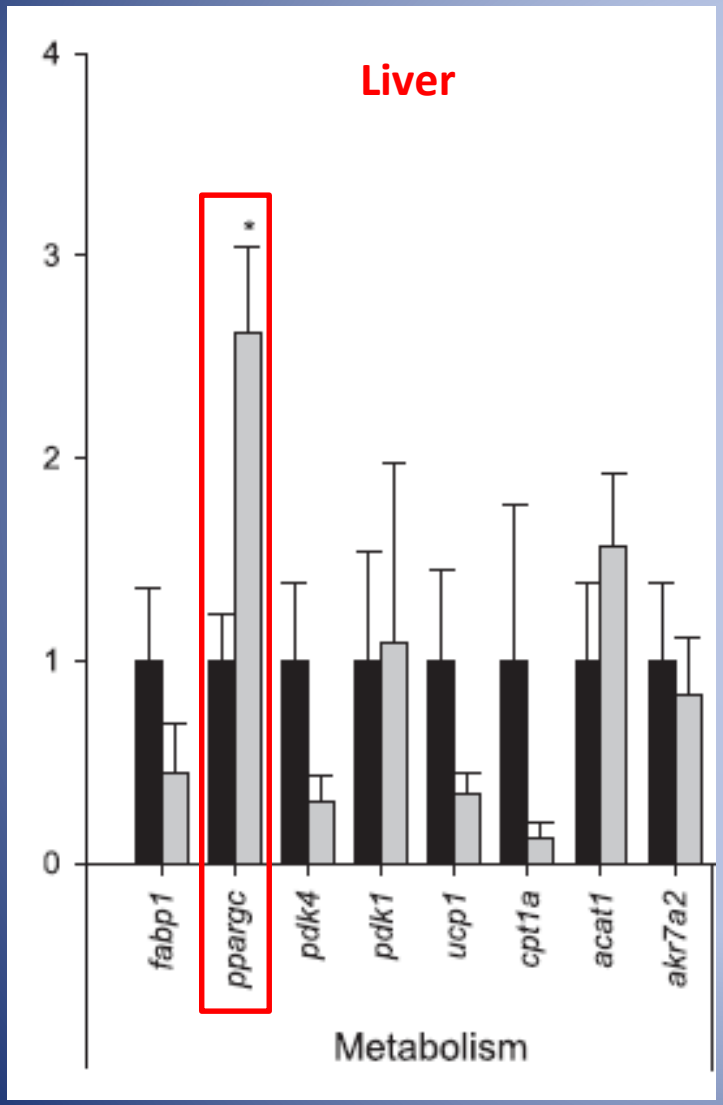
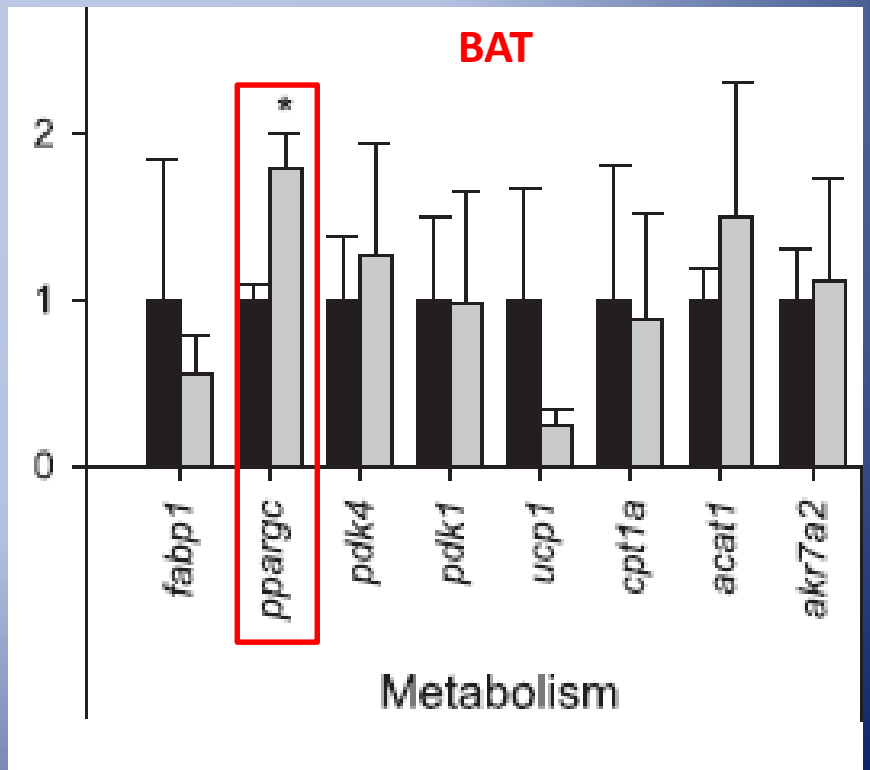
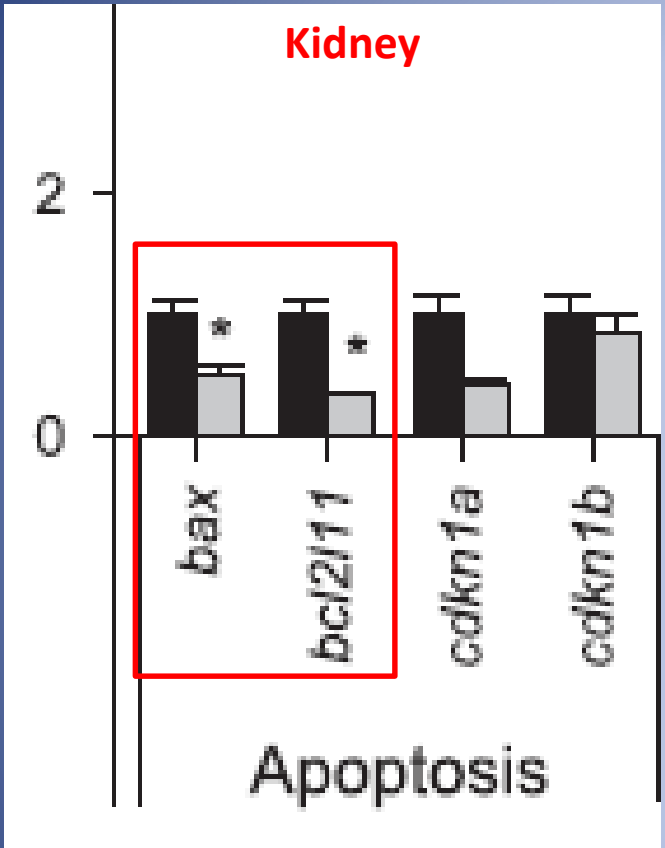
TRANSCRIPTOMICS: METABOLISM



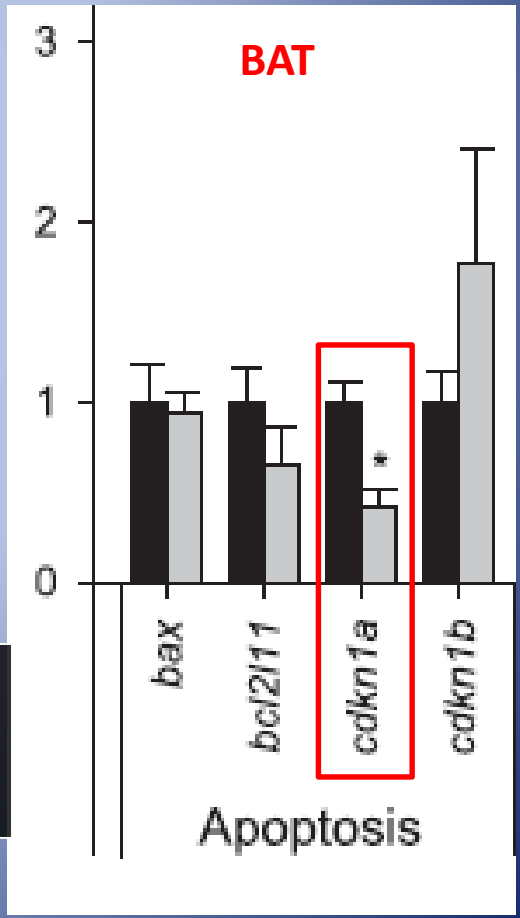
The increase in *ppargc* expression may function to increase lipid catabolism in BAT, while shutting down pyruvate oxidation in the liver.



TRANSCRIPTOMICS: APOPTOSIS

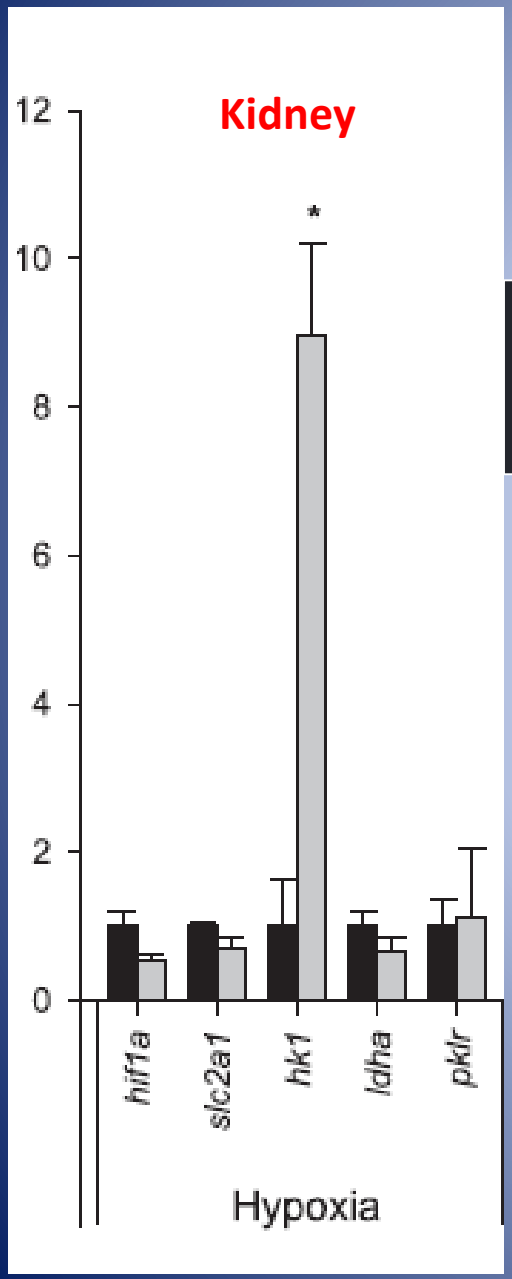


The pro-apoptotic genes, bax and bcl2l11, were significantly decreased in kidney.



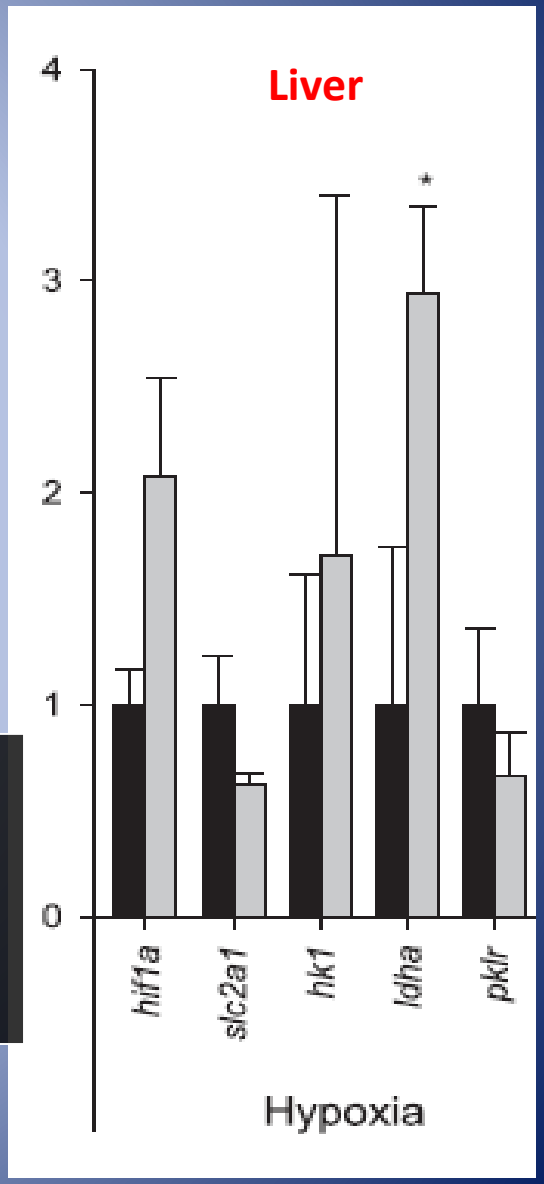
Genes associated with cell cycle progression are modulated during lemur torpor.

TRANSCRIPTOMICS: HYPOXIA

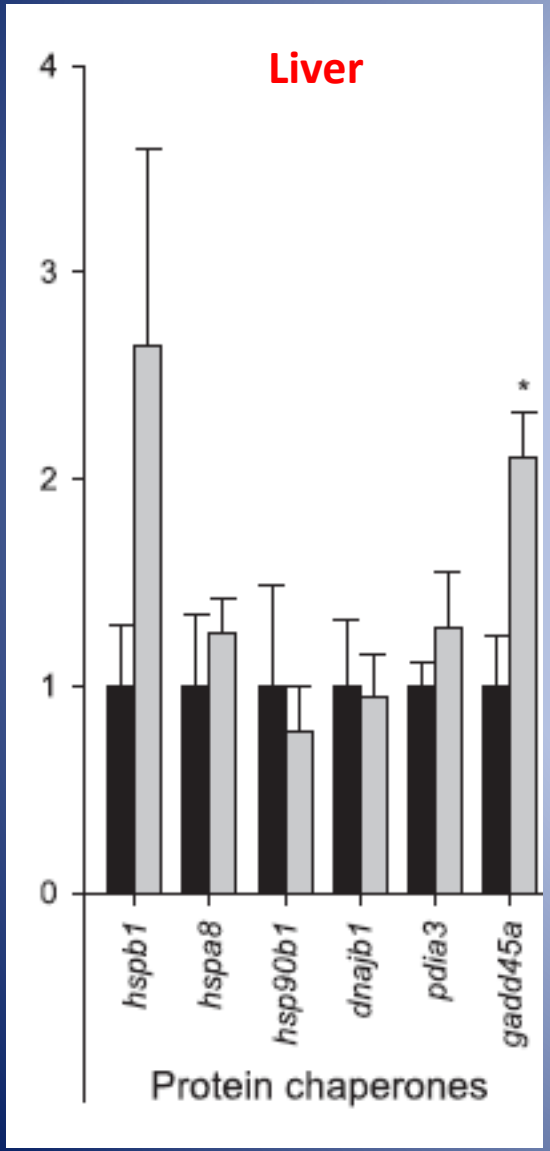


Upregulation of hexokinase may represent a change in glycolytic flux.

Lactate dehydrogenase was upregulated which may allow for the continued functioning of glycolysis during low oxygen conditions.

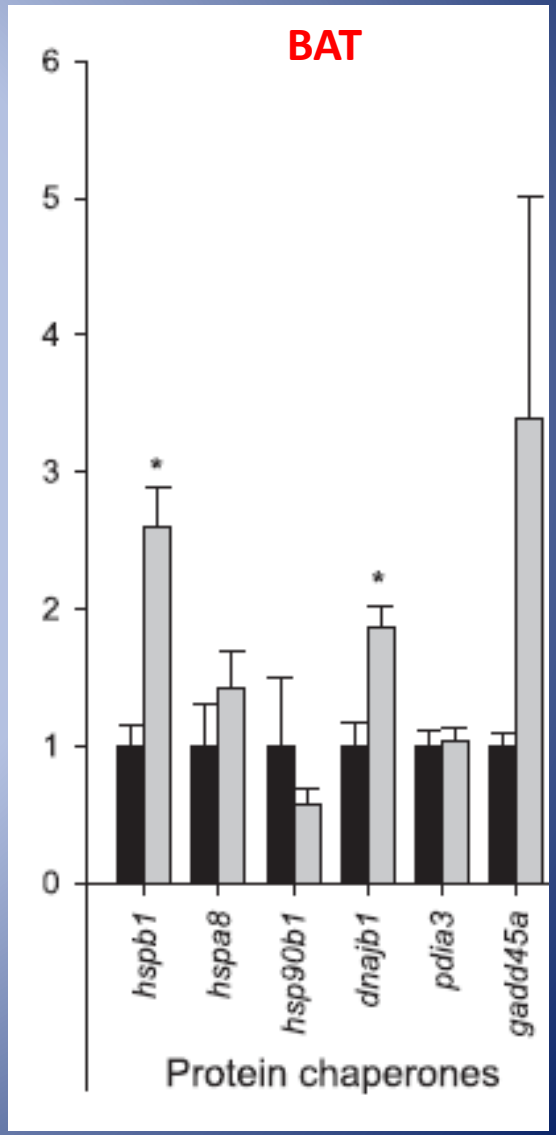


TRANSCRIPTOMICS: PROTEIN CHAPERONES



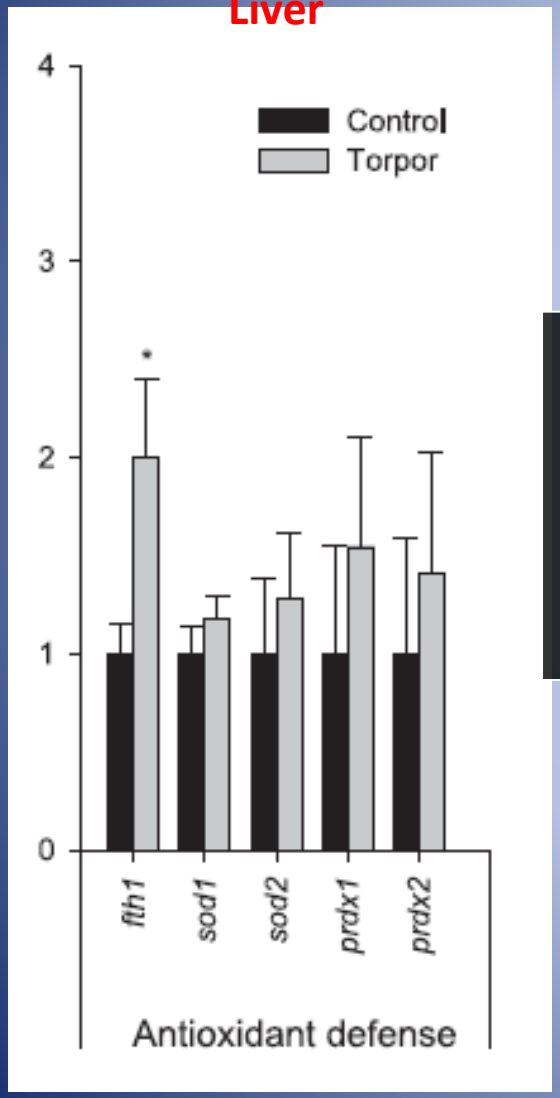
GADD45α, an integral component of the stress Response, Is typically involved in arresting the cell cycle.

The expression of two chaperones-encoding proteins were elevated in BAT, possibly because of the physiological role that this tissue plays during torpor.



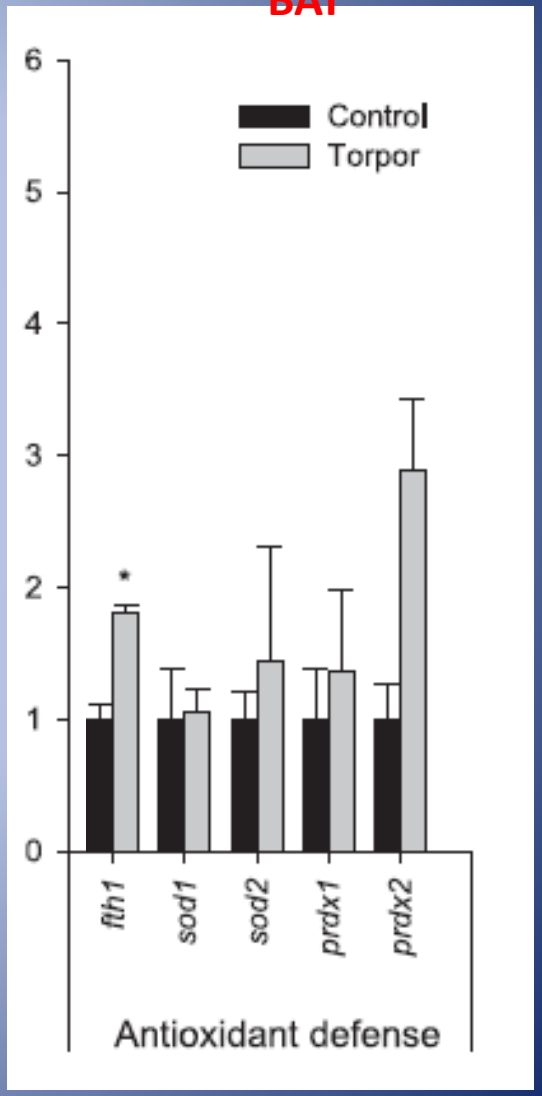
TRANSCRIPTOMICS: ANTIOXIDANT RESPONSE

Liver



The increased fth1 expression suggests a key role for ferritin in the torpid lemur for iron storage as one mechanism for protection from iron catalyzed oxidative damage.

BAT



AMPK IN PRIMATE TORPOR

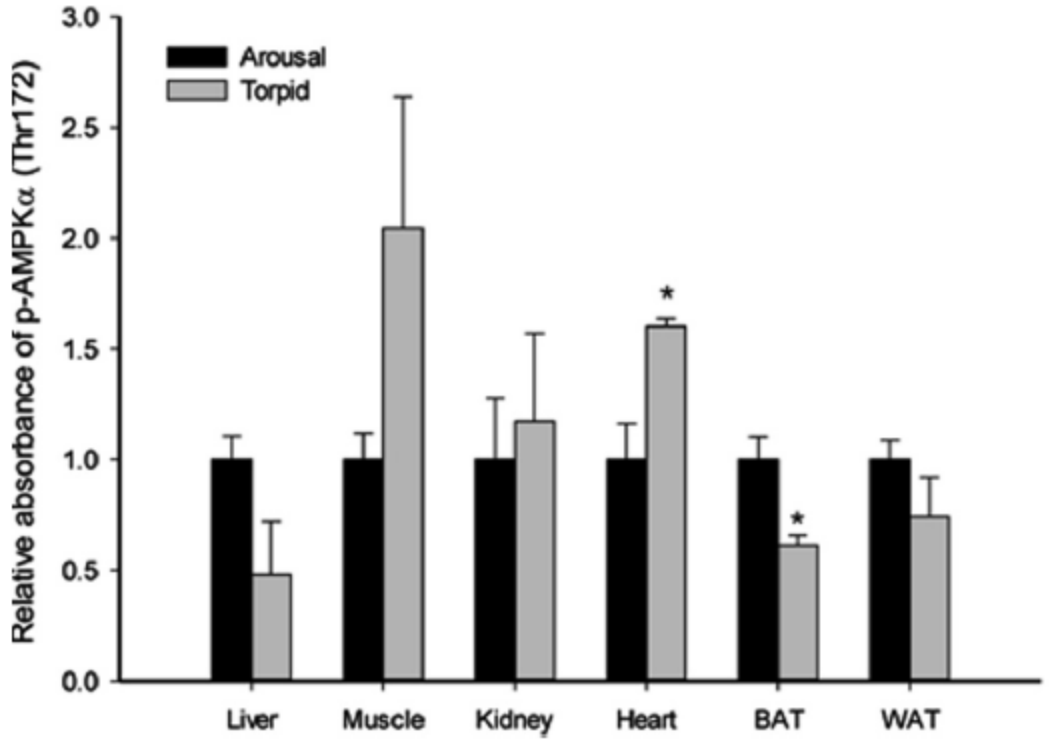


Figure 1 Response of p-AMPKα (Thr172) to daily torpor in various lemur tissues

- AMPK is often considered as the cellular energy sensor
- Enhanced activity in the heart may be to promote fatty acid uptake or inhibit translation
- Given that BAT is responsible for non-shivering thermogenesis, it is possible that BAT must retain some level of protein synthesis activity in the hypometabolic state

REGULATION OF TRANSLATION IN PRIMATE TORPOR

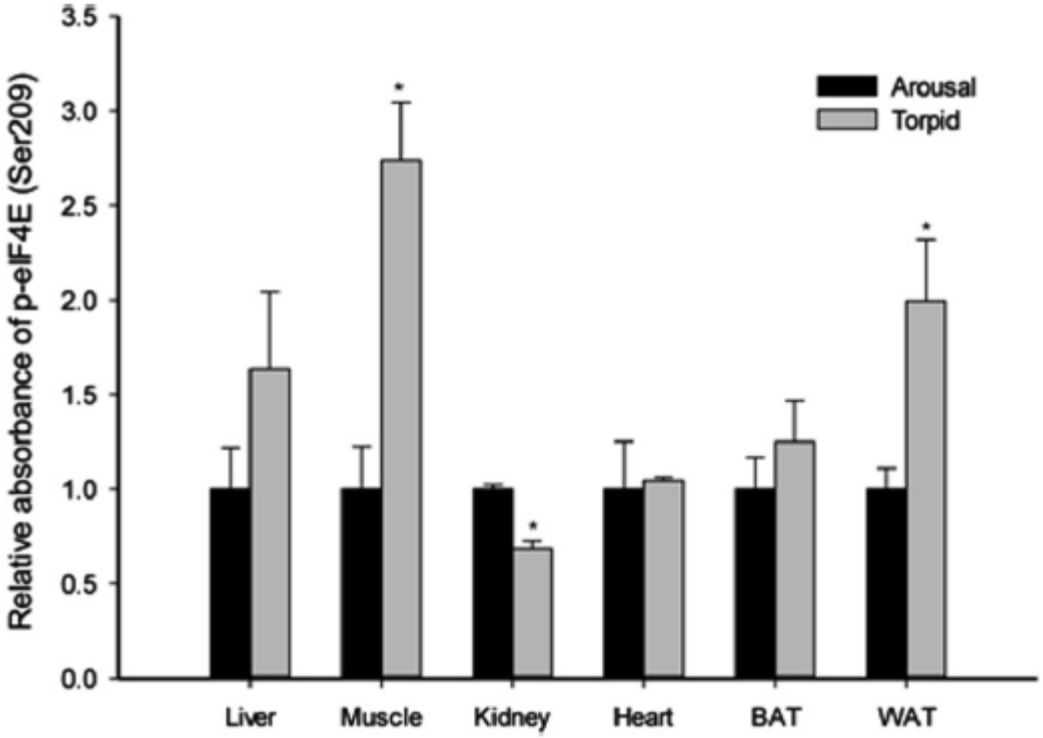


Figure 4 Response of p-eIF4E (Ser209) to daily torpor in various lemur tissues

- There was no change in commonly regulated translation factors (e.g. p-eIF2α and p-4EBP)
- Evidence of reduced translation in kidney
- However, increases in p-eIF4E occurred in muscle and WAT, suggesting increased translation

REGULATION OF TRANSCRIPTION IN PRIMATE TORPOR

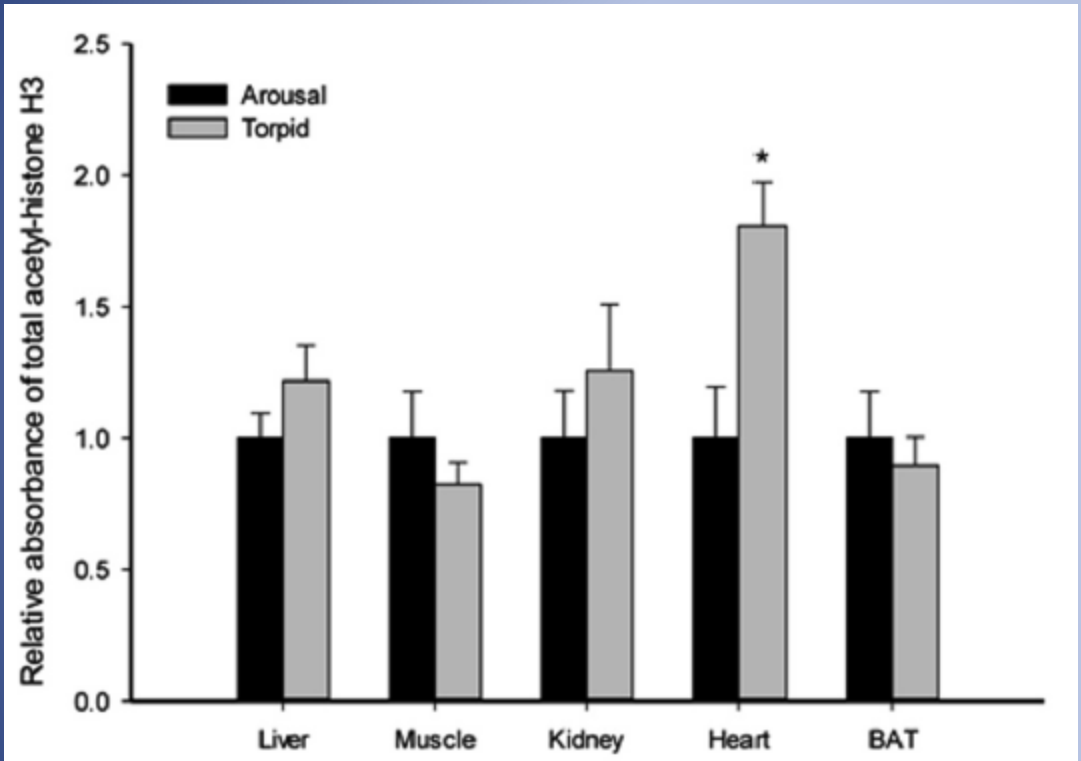
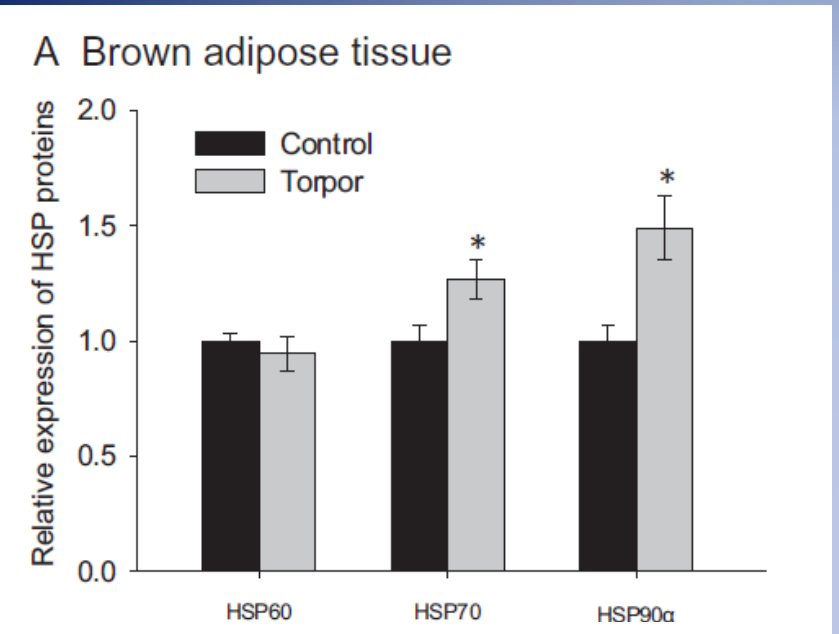
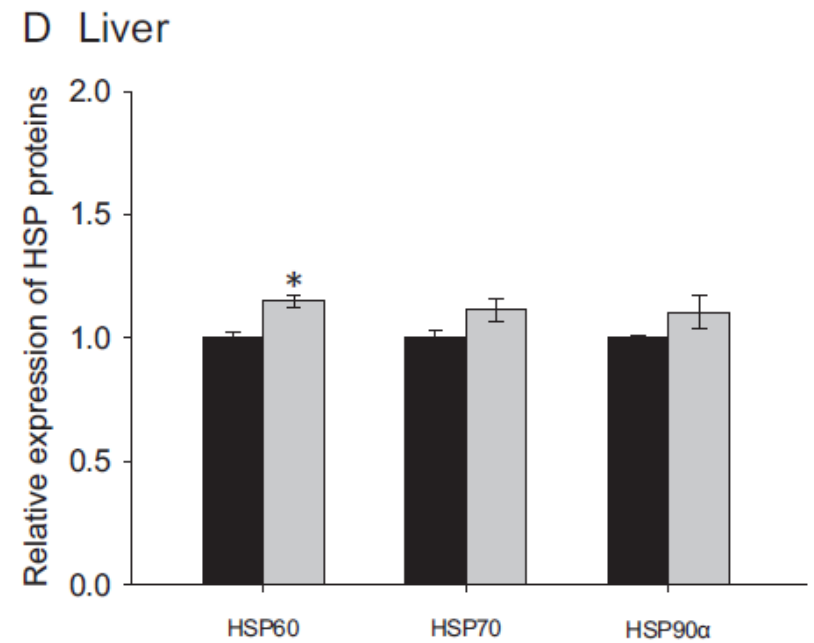


Figure 6 Response of total acetyl-histone H3 to daily torpor in various lemur tissues

- Histone acetylation leads to transcriptional activation by opening up chromatin structure to facilitate binding of the transcriptional apparatus
- We observe evidence of transcriptional activity in the heart during torpor

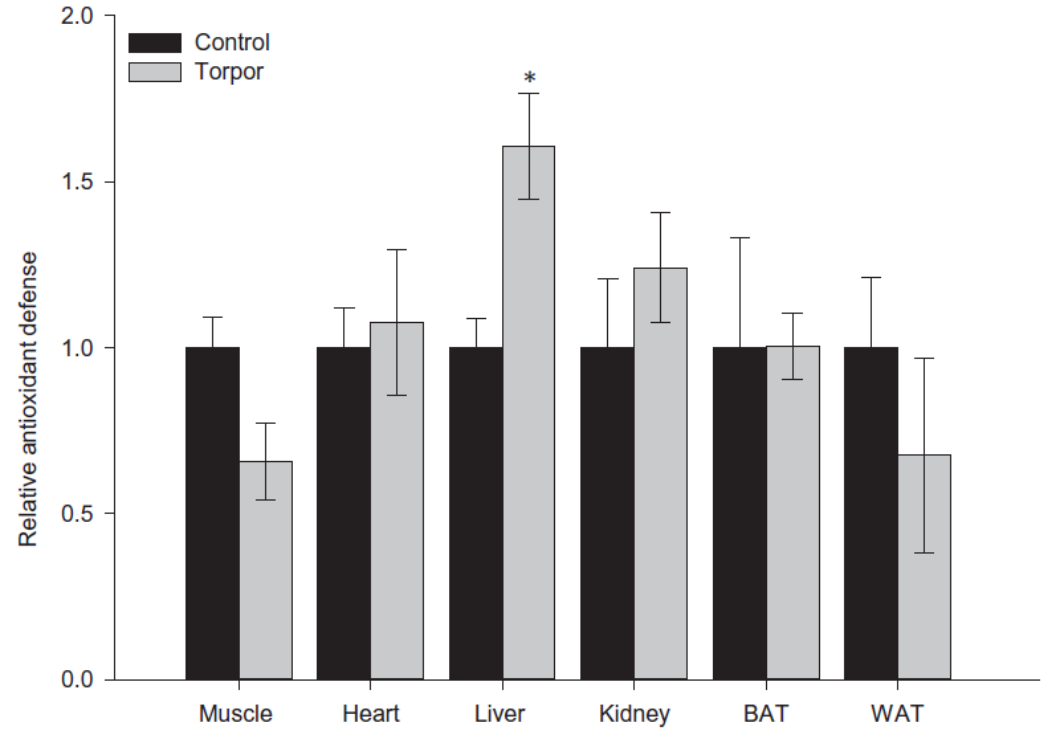
STRESS RESPONSE IN PRIMATE TORPOR





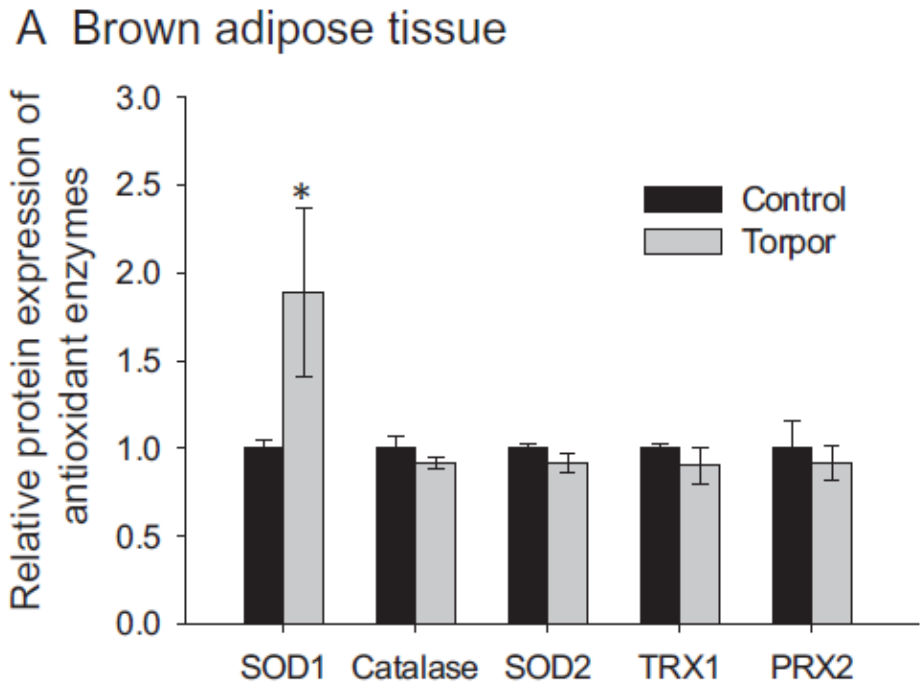
- Protein chaperone expression only increased in BAT and Liver
- No changes in other tissues
- Perhaps relatively higher temperature of lemur torpor does not require global increases in protein chaperone expression

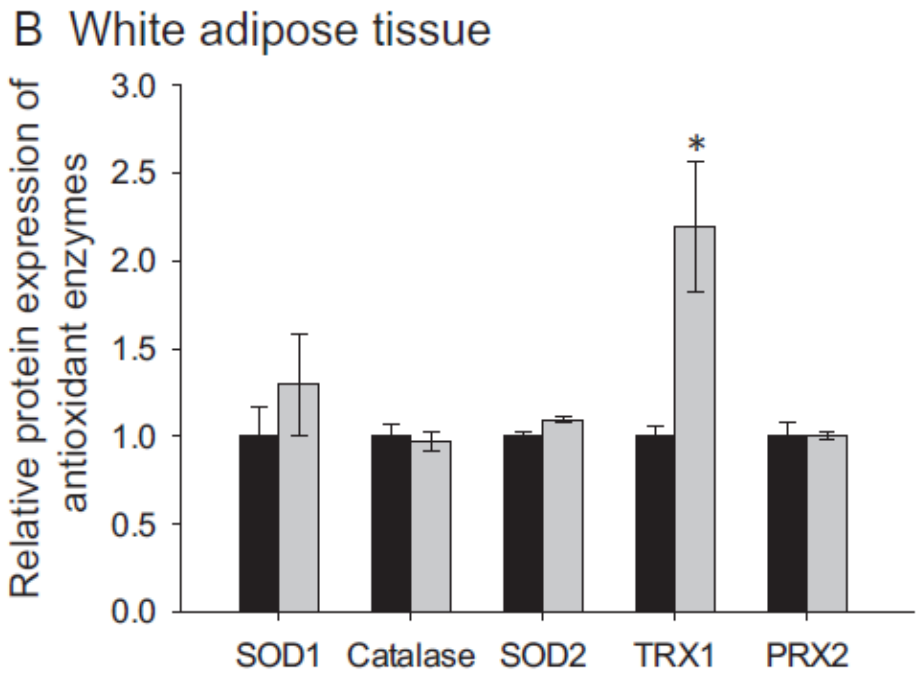
STRESS RESPONSE IN PRIMATE TORPOR



Minimal changes in antioxidant metabolites during primate torpor, although enzymatic responses may be more important.

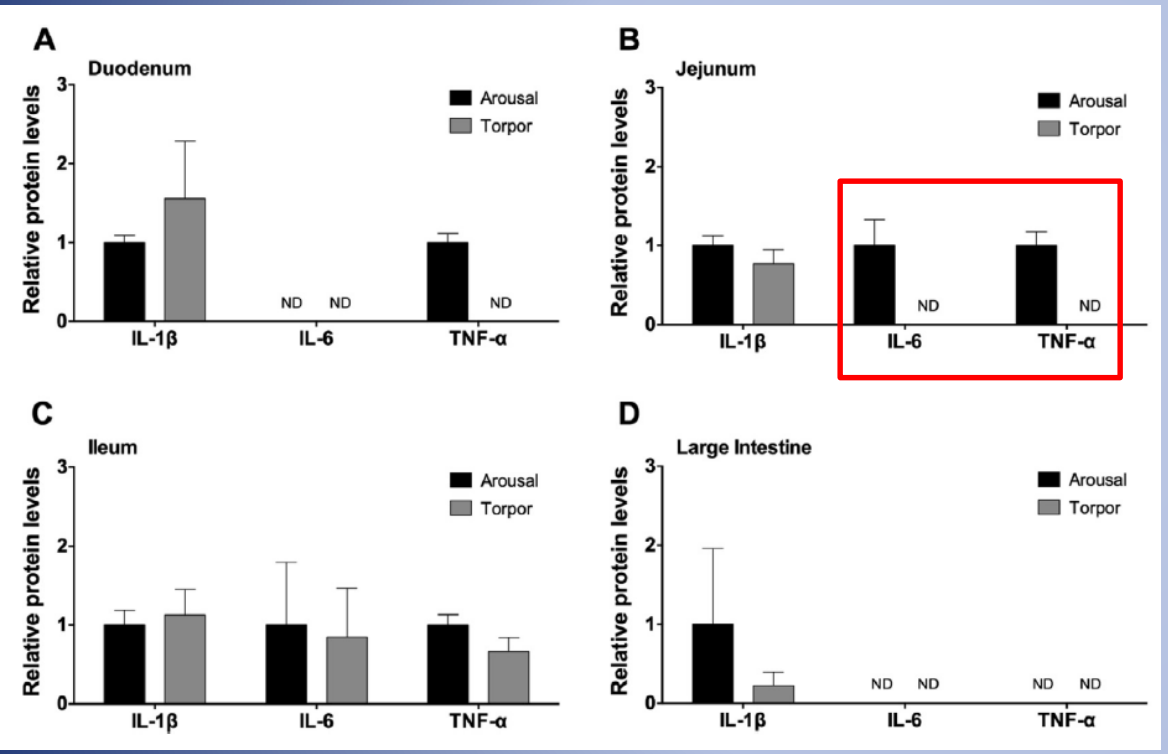
STRESS RESPONSE IN PRIMATE TORPOR





- Also, minimal relative changes in protein expression, although post-translational modifications may significantly change enzyme activity despite constant protein expression.

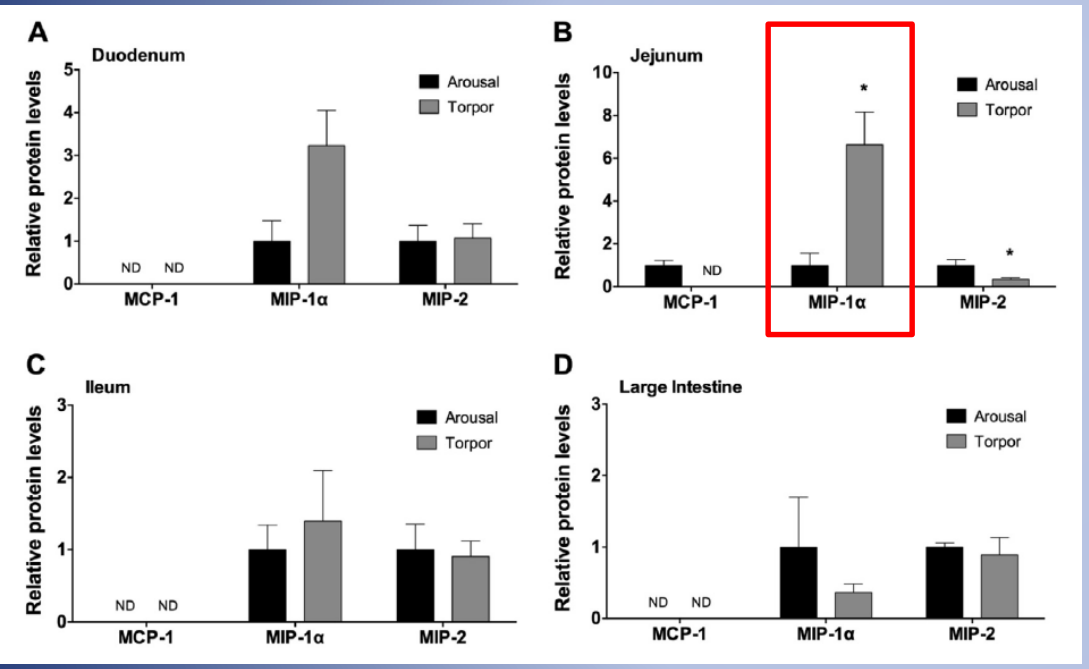
INTESTINAL CYTOKINES IN PRIMATE TORPOR



Pro-inflammatory cytokines;

- In the jejunum, protein levels of the pro-inflammatory cytokines were greatly reduced, suggesting suppression of the mucosal immune response during torpor.

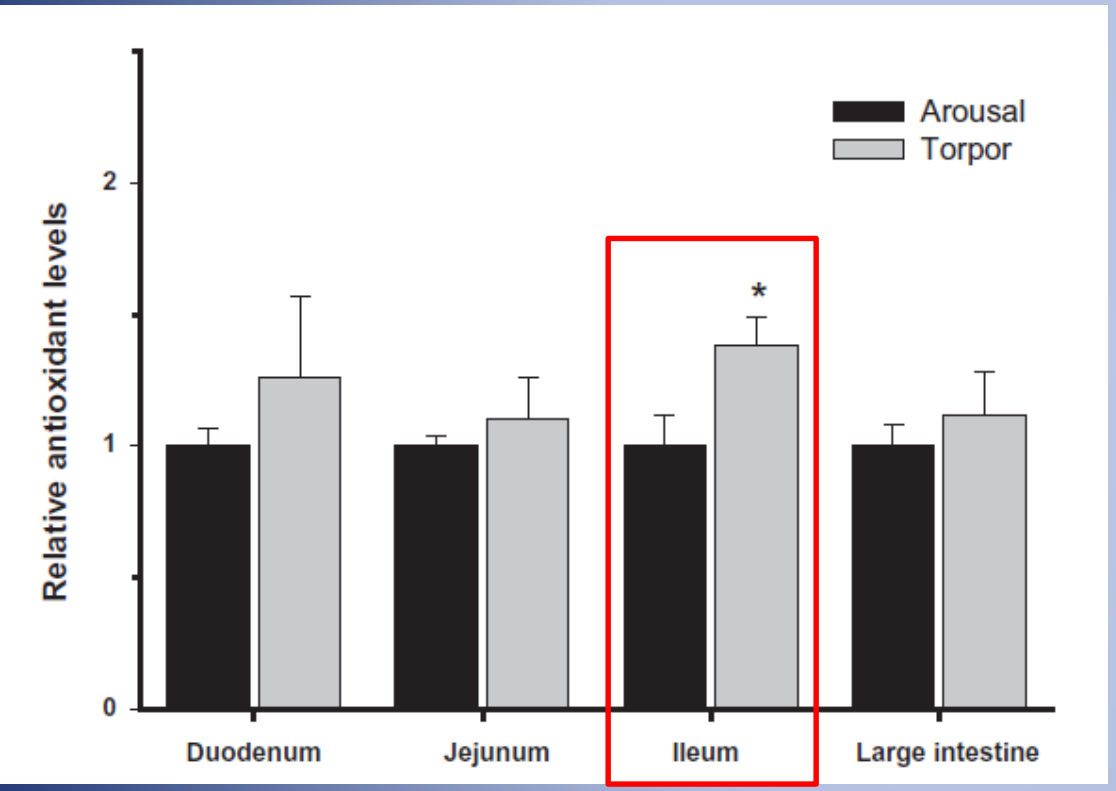
INTESTINAL CHEMOKINES IN PRIMATE TORPOR



Chemokines;

- Recruit immune cells to the site of infection or control cell migration into tissues
- The increased levels of MIP-1α in the jejunum may suggest the recruitment of WBCs to the intestine

INTESTINAL ANTIOXIDANTS IN PRIMATE TORPOR



- Minimal changes in antioxidant metabolites
- No changes in protein expression, although post-translational modifications may significantly change enzyme activity

Hibernation and medicine

Primates !!

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

Metabolic rate depression: the biochemistry of mammalian hibernation.

Storey KB, Storey JM.

Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada. kenneth_storey@carleton.ca

Abstract

During winter hibernation, small mammals fall into long periods of deep cold torpor where metabolic rate is suppressed 90% and core body temperature can fall to near 0 degrees C. Studies with hibernators illustrate the molecular regulatory mechanisms that coordinate the suppression of metabolic functions during torpor, reprioritize energy use, and preserve/stabilize macromolecules to support long-term viability during cold torpor. This review explores mechanisms including posttranslational modification of proteins, differential regulation of enzymes, global suppression of transcription and translation including a ro
transcription factors. The
relevant to issues in clini
and atrophy resistance.

Gerontology. 2010;56(2):220-30. Epub 2009 Jul 14.

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

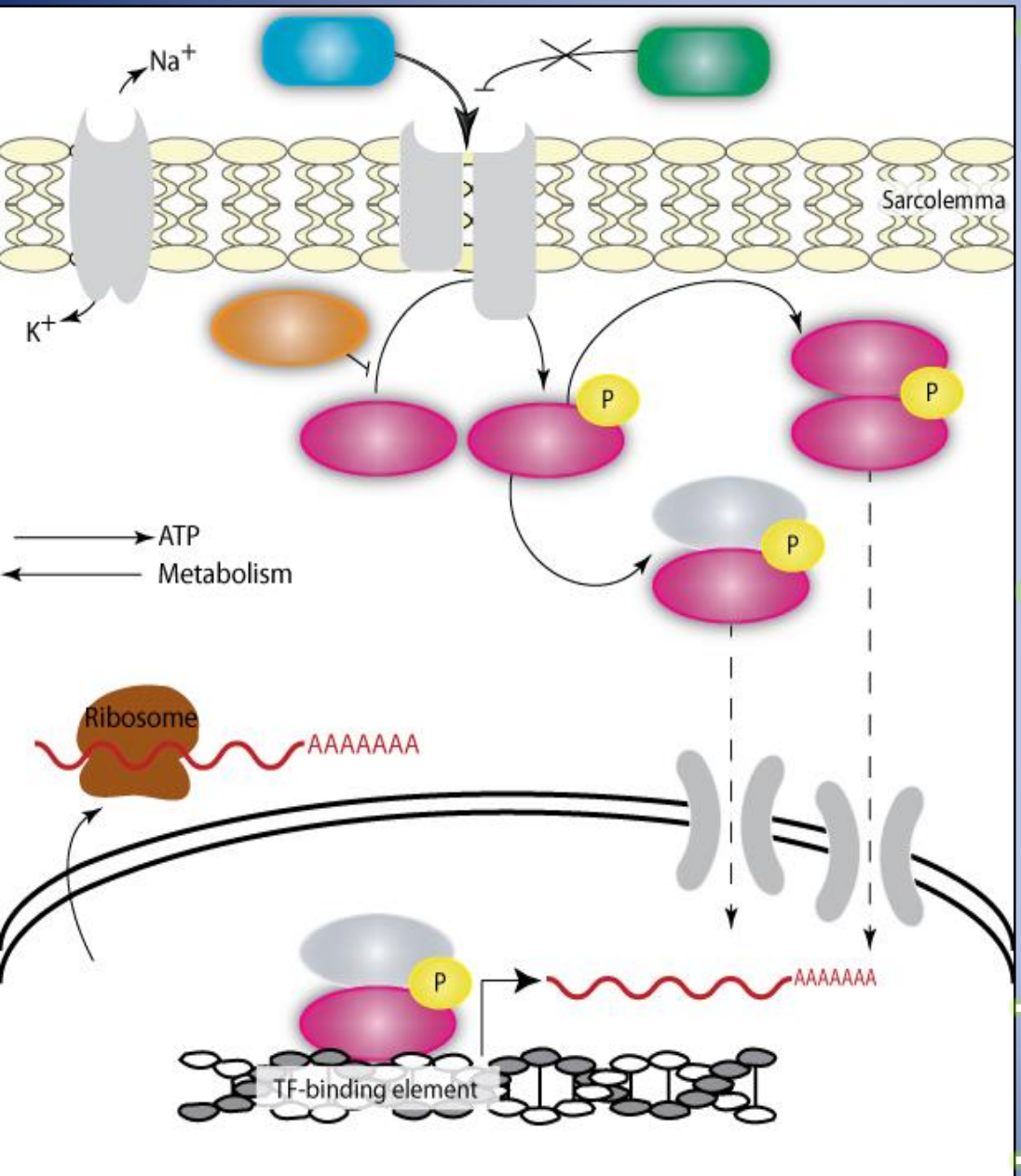
Storey KB.

Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada. kenneth_storey@carleton.ca

Abstract

Hibernating mammals offer an intriguing example of natural torpor and illustrate the regulatory mechanisms that control
e cell preservation strategies that support long-term viability in a hypometabolic state. These
proving the hypothermic preservation of human organs for transplant, and guidelines that
r as an intervention strategy in human medicine. Recent advances in hibernation research
contribute to metabolic depression by orchestrating the global suppression of ATP-
ation including multiple forms of post-translational modification of proteins/enzymes
lOylation), mRNA storage mechanisms, and differential expression of microRNA species.
also contributed new advances in understanding the range of cell functions that are
ut some critical preservation strategies that aid long-term viability in a torpid state. These
perones and the implementation of the unfolded protein response, and the enhancement of
s) to control the actions of extracellular proteases in clotting and inflammation responses.

Our Experimental Approach



Proteomics

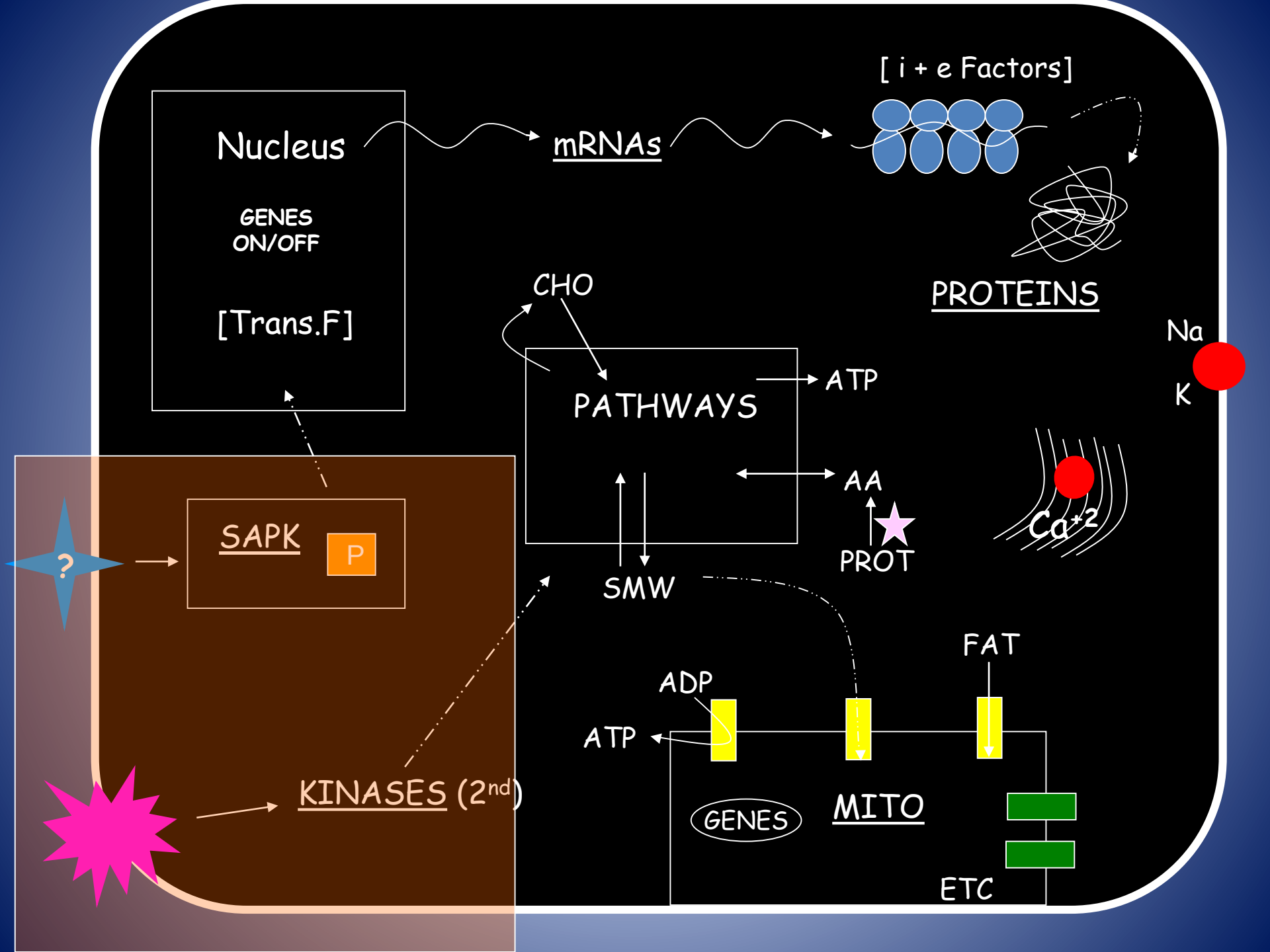
Metabolomics

Transcriptomics

Genomics/Epigenetics

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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GENES
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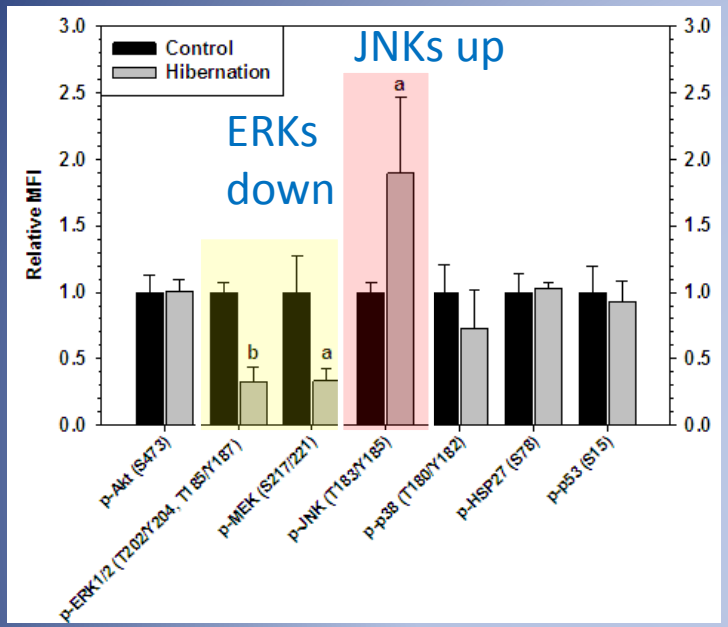
ETC

Primate Torpor: New Model for Banking of Human Organs?



Gray mouse lemur

Novel model
- **both Daily Torpor and Seasonal Hibernation**
- **torpor in mild climate -- body temperature may fall only a few degrees**

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

Metabolic rate depression & ORGAN PRESERVATION

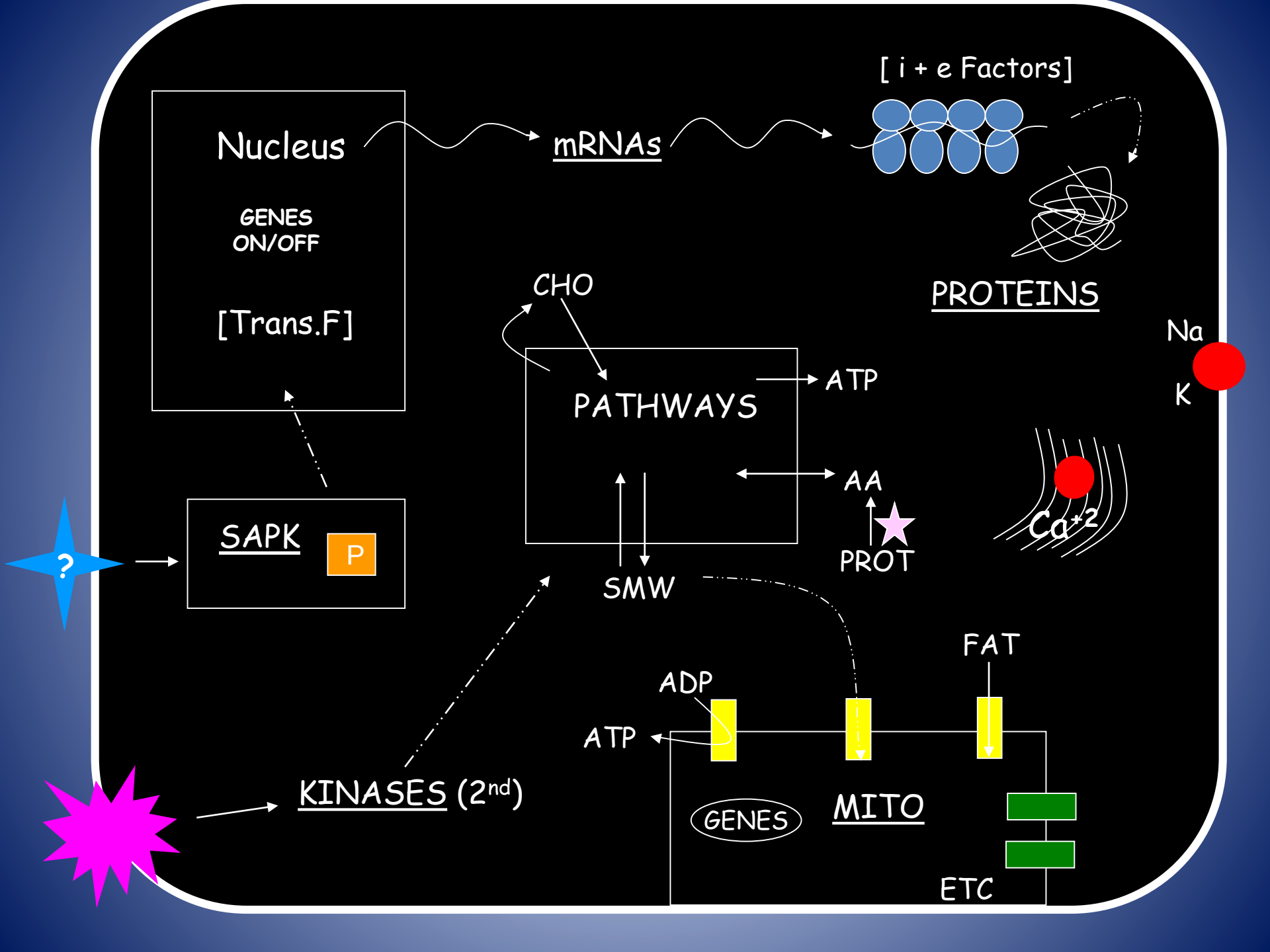
SHUT IT DOWN

- Survive hypometabolism (<5 % of normal)
- Resist ischemia-reperfusion
- Survive with restricted nutritional resources
- Regulatory mechanisms enhance cell preservation
- Perform seamless transitions to / from the hypometabolic state

ORGAN PRESERVATION: is it TANGIBLE?

Natural hypometabolic states occur in species from seven orders of mammals, suggesting that the phenotype arises from a ***genotype present in all mammals, including humans***.

Key model systems that endure parallel stresses are invaluable tools in the field of organ preservation.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KINASES (2nd)
ADP
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FAT
GENES
MITO
ETC

Controlling Biological Time: Nature has the BluePrint

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

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

Freezing

Diapause

Lessons in organ preservation from NATURE

Kenneth B. Storey, Carleton University, Ottawa

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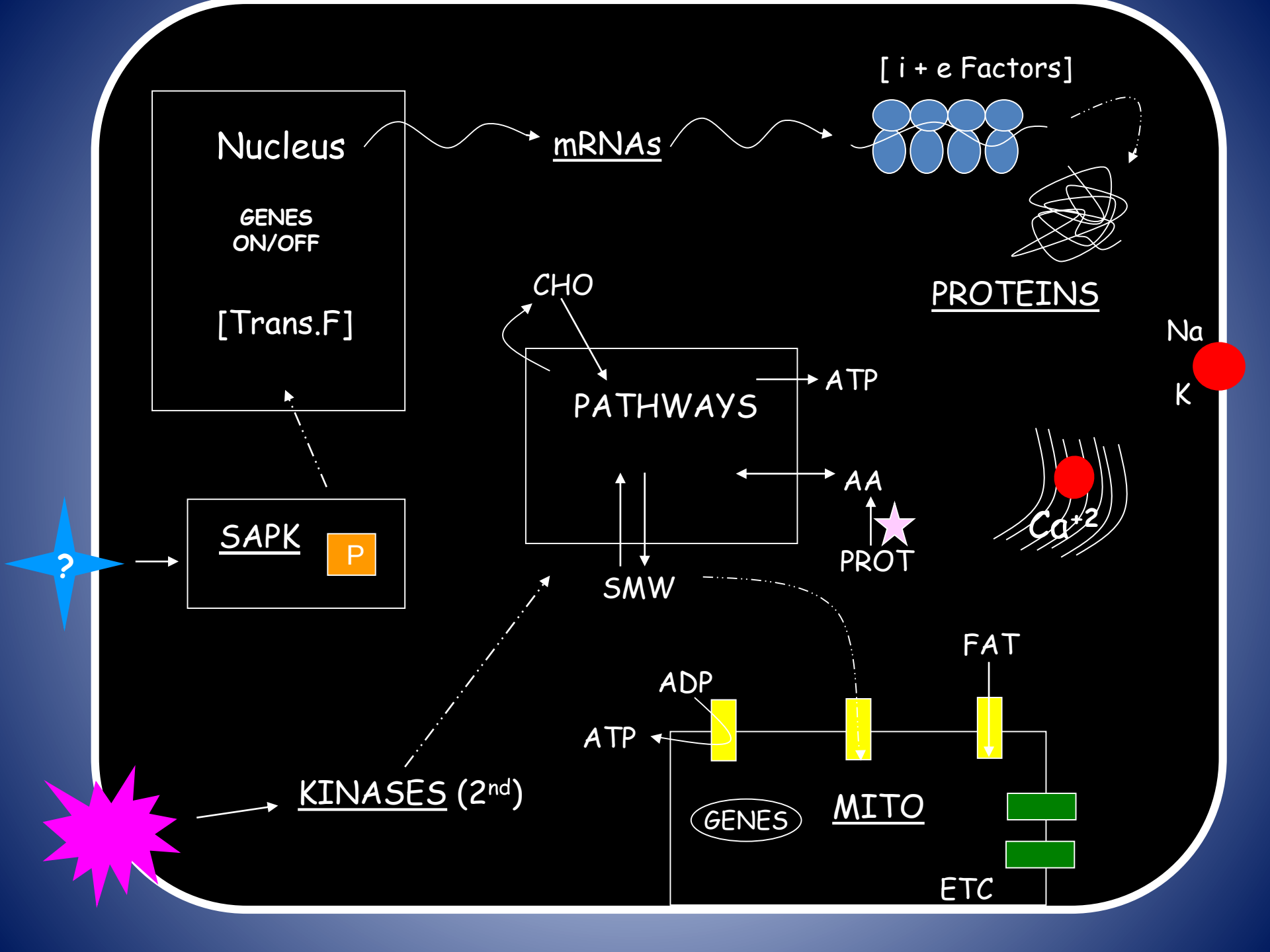
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FAT
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Metabolic rate depression & ORGAN PRESERVATION

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