Metabolic Arrest, Stasis and Regeneration

Kenneth B. Storey, Carleton University, Ottawa

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0°C
NATURE’S [ NEW !] MECHANISMS

Posttranslational modifications
Epigenetics
MicroRNA
Gene suppression
Selective gene activation
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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
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.

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Available online 21 June 2015

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 $\sim 28-32^\circ C$) that is not confounded by the additional biochemical adaptations needed for low temperature hibernation in most mammals
PRIMATE TORPOR: GRAY MOUSE LEMUR

Tb = 35.44°C

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
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.
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
MAPK SIGNALING IN PRIMATE TORPOR

*p-ERK signaling is decreased in skeletal muscle, suggesting that cell growth/proliferation are suppressed during torpor
*all MAPK activated in white adipose tissue, suggesting the importance of this tissue as a source of metabolic fuel in torpor
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.
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
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
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
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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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.
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.
Liver

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.
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.
AMPK IN PRIMATE TORPOR

• 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

- 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

Figure 4  Response of p-eIF4E (Ser209) 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.
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.
Minimal changes in antioxidant metabolites during primate torpor, although enzymatic responses may be more important.
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

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 role for transcription factors. The relevance to issues in clinical medicine and atrophy resistance.

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 cell preservation strategies that support long-term viability in a hypometabolic state. These strategies, including multiple forms of post-translational modification of proteins/enzymes (Oylation), mRNA storage mechanisms, and differential expression of microRNA species have contributed new advances in understanding the range of cell functions that aid long-term viability in a torpid state. These advances have the potential to impact the treatment of cardiovascular disease and certain forms of cancer.
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
Nucleus

GENES ON/OFF

[Trans.F]

mRNAs

[ i + e Factors]

PROTEINS

Ca$^{2+}$

KINASES (2$^{nd}$)

PATHWAYS

CHO

ATP

AA

PROT

SMW

SAPK

P

KINASES (2$^{nd}$)

Nucleus

GENES ON/OFF

[Trans.F]
Primate Torpor: New Model for Banking of Human Organs?

**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
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.
Nucleus

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ADP

FAT

MITO

GENES

ETC

Na

K

Ca^2

P

?
Controlling Biological Time: Nature has the Blueprint

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

Hibernation

Estivation

Freezing

Diapause

Anoxia
Lessons in organ preservation from NATURE

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

mRNAs

GENES ON/OFF
[Trans.F]

PROTEINS

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