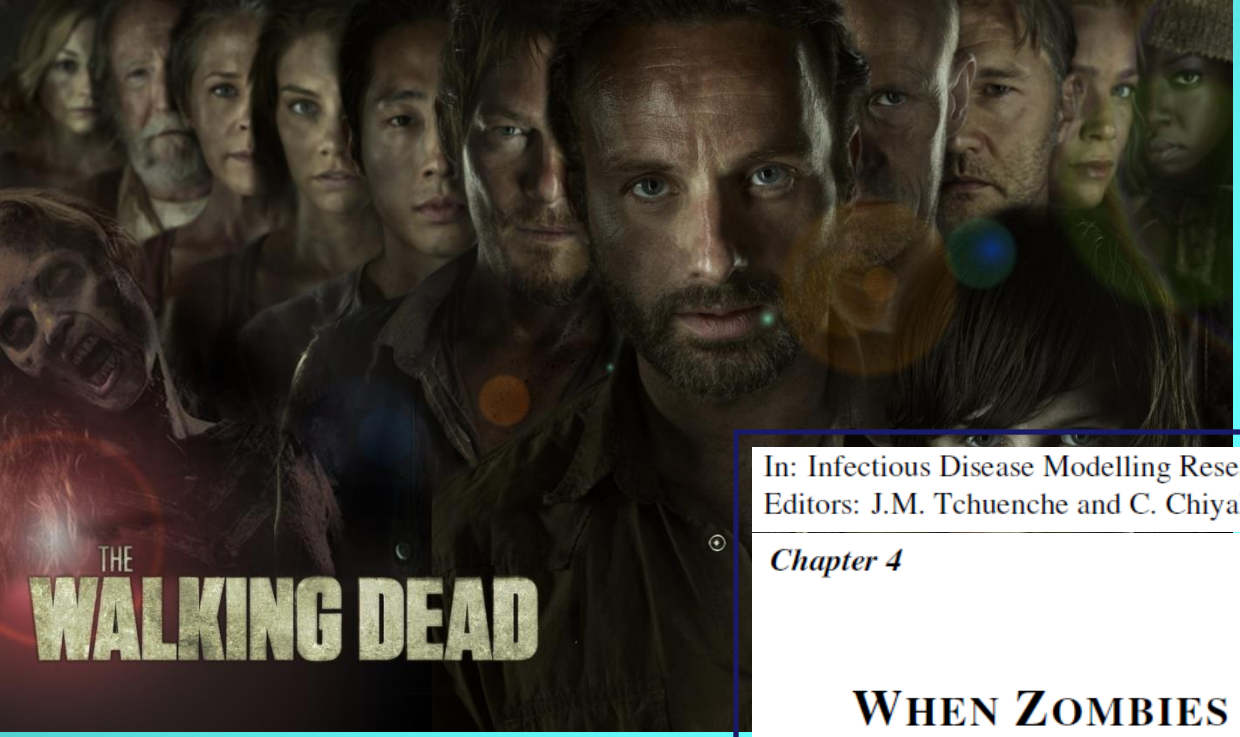

The Living Dead:
Metabolic Arrest
and the
Control of Biological Time



In: Infectious Disease Modelling Research Progress ISBN 978-1-60741-347-9
Editors: J.M. Tchuenche and C. Chiyaka, pp. 133-150

Chapter 4

WHEN ZOMBIES ATTACK!: MATHEMATICAL MODELLING OF AN OUTBREAK OF ZOMBIE INFECTION

Philip Munz[1,*], *Ioan Hudea*[1,†], *Joe Imad*[2,‡], *Robert J. Smith?*[3,§]

Abstract

Zombies are a popular figure in pop culture/entertainment and they are usually portrayed as being brought about through an outbreak or epidemic. Consequently, we model a zombie attack, using biological assumptions based on popular zombie movies. We introduce a basic model for zombie infection, determine equilibria and their stability, and illustrate the outcome with numerical solutions. We then refine the model to introduce a latent period of zombification, whereby humans are infected, but not infectious, before becoming undead. We then modify the model to include the effects of possible quarantine or a cure. Finally, we examine the impact of regular, impulsive reductions in the number of zombies and derive conditions under which eradication can occur. We show that only quick, aggressive attacks can stave off the doomsday scenario: the collapse of society as zombies overtake us all.

HIBERNATION

13-LINED GROUND SQUIRREL
Ictidomys tridecemlineatus

HIBERNATION

Little Brown Bat
Myotis lucifugus

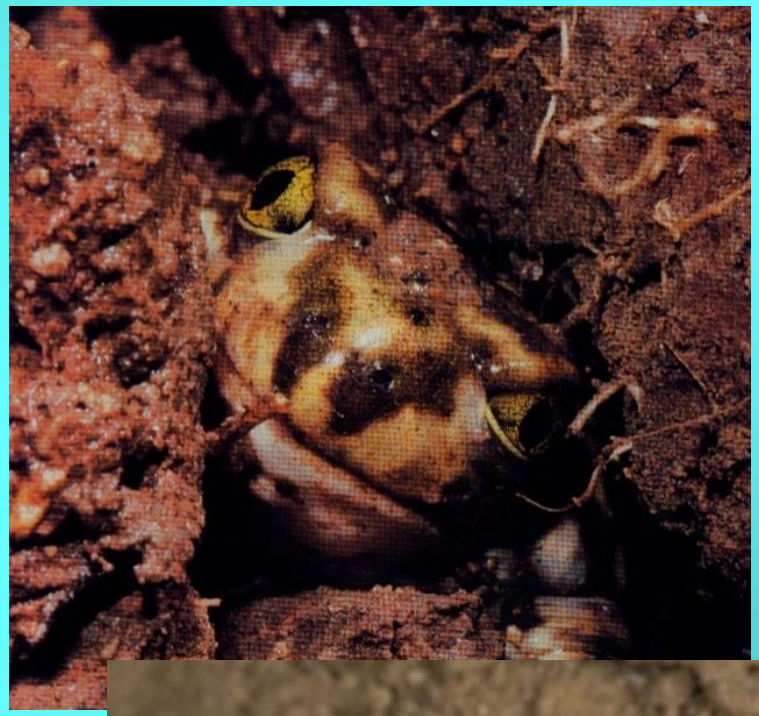
DAILY TORPOR

Grey mouse lemur,
Microcebus murinus

ESTIVATION

Milk snail
Otala lactea

ESTIVATION



Spadefoot toad
Scaphiopus holbrookii

ANOXIA

Painted turtle
Chrysemys picta

Periwinke
Littorina littorea

Red-eared turtle
Pseudemys scripta

FREEZING

Wood frog
Rana sylvatica

MAMMALS ON ICE:

Metabolic Rate Reduction

www.carleton.ca/~kbstorey

Model Hibernators

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

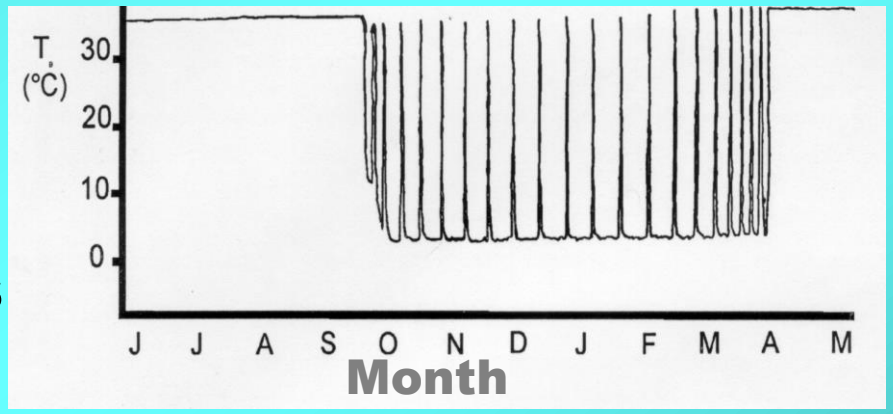
***Ictidomys tridecemlineatus*, 13-lined ground squirrel**

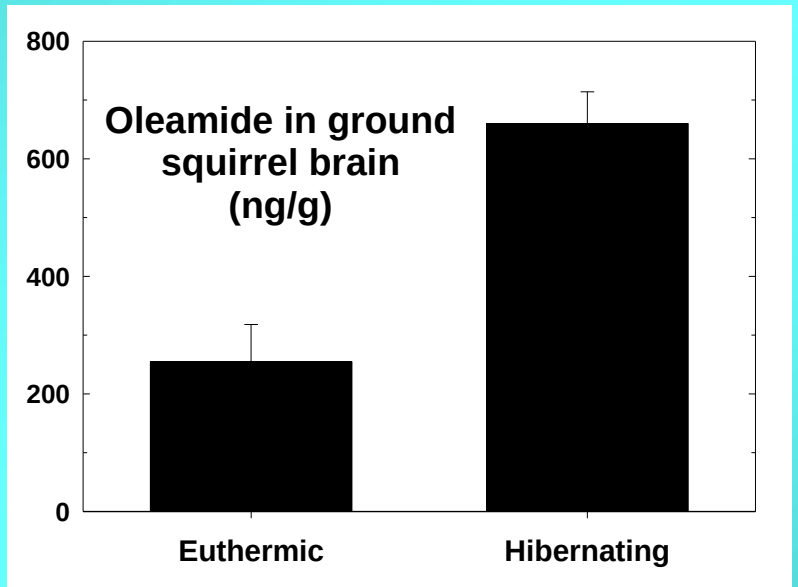
***Myotis lucifugus*, little brown bat**

- Seasonal phenomenon
- Pre-hibernation hyperphagia
- Gain up to 40% of body mass
- Need polyunsaturated fats
- Find hibernaculum: dark, near 0°C

What happens?

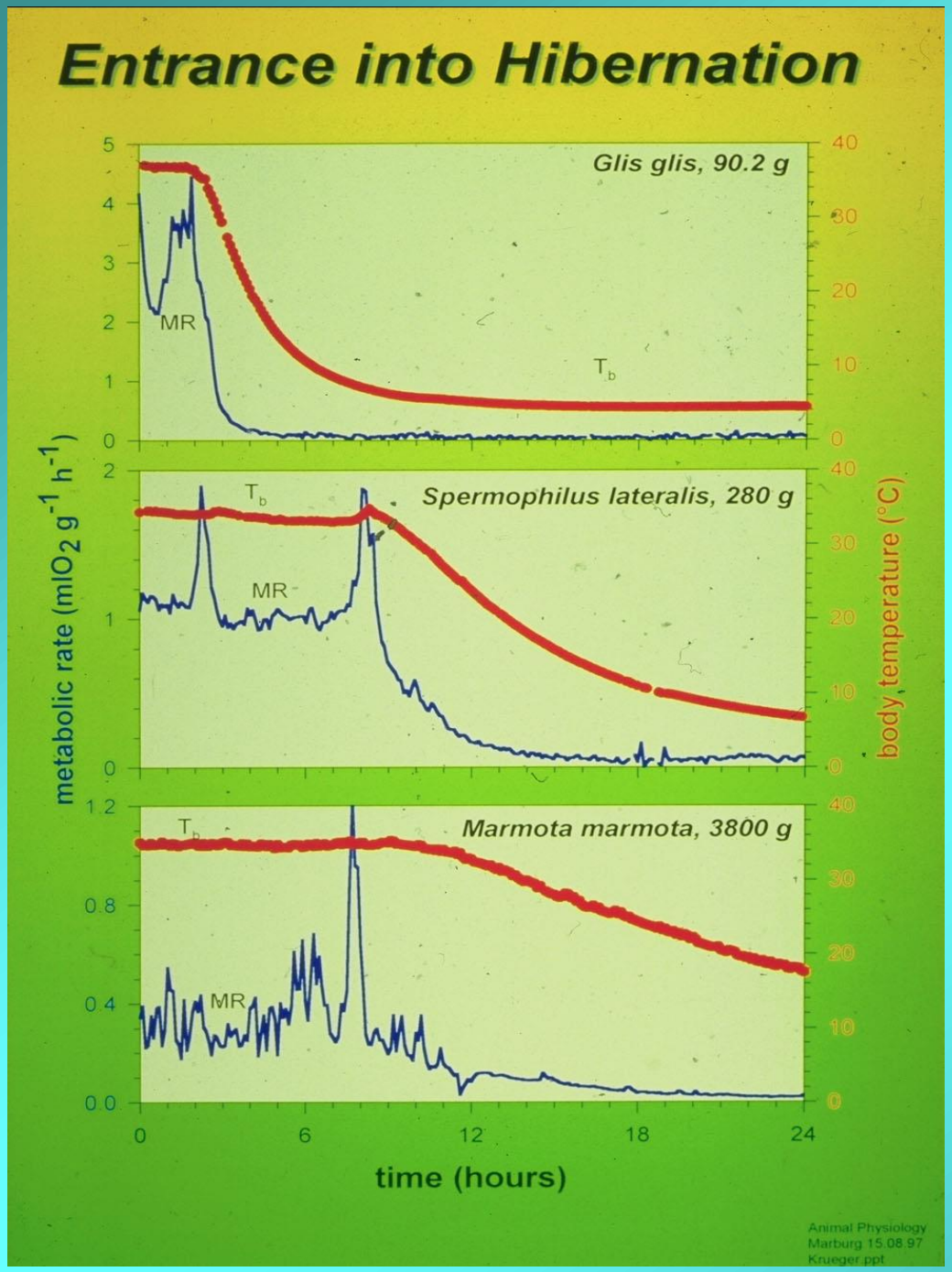
- drop in body temperature
- reduced heart rate
- apnoic breathing
- some muscle atrophy
- periods of torpor lasting weeks
- non-REM sleep
- oleamide increases in brain





- suppression of carbohydrate oxidation
- RQ of 0.7 = lipid oxidation

Stewart JM, Boudreau NM, Blakely JA & Storey KB. 2002. J. Thermal Biol. 27, 309-315.



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

PRINCIPLES OF HIBERNATION

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

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

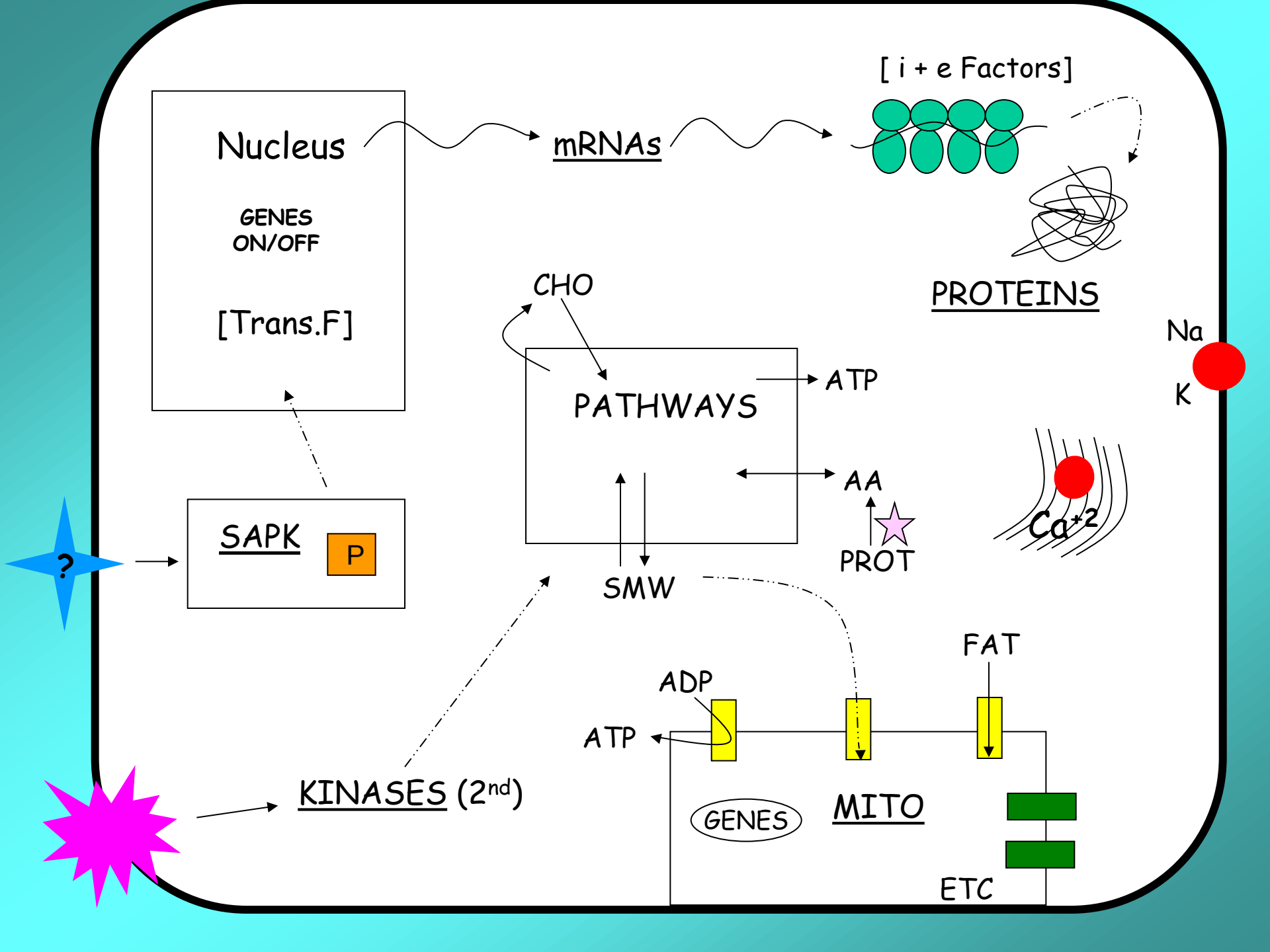
Freezing

Diapause

PRINCIPLES OF HIBERNATION

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

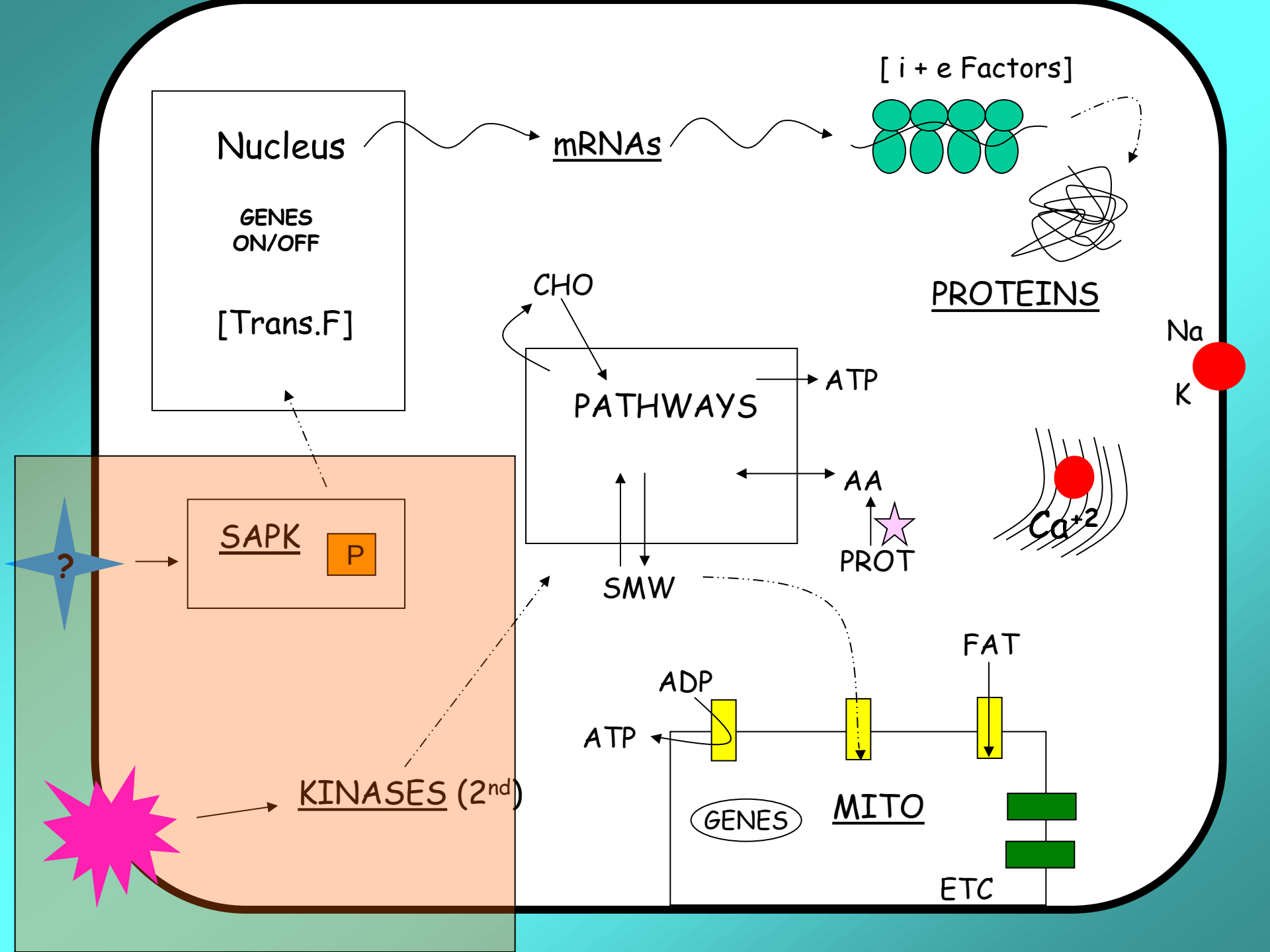
Same as with ALL MRD


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

METABOLISM IN HIBERNATION

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

ATP turnover ↓ to <5% of normal

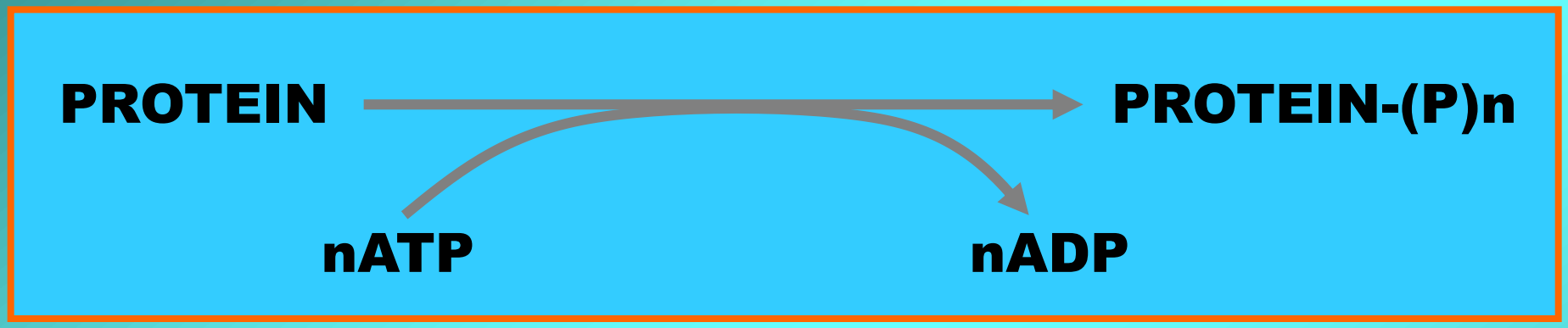

[i + e Factors]
Nucleus
GENES
ON/OFF
[Trans.F]
mRNAs
PROTEINS
CHO
PATHWAYS
ATP
AA
PROT
SMW
Na
K
Ca+2
SAPK
P
?
KINASES (2nd)
ADP
ATP
FAT
GENES
MITO
ETC

Metabolic Rate Depression CHANGES

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

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

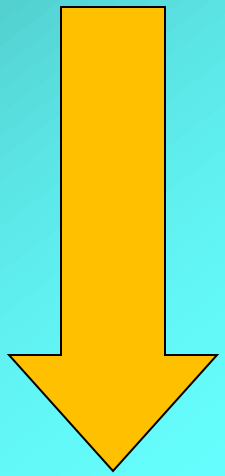
PROTEIN KINASES



- Covalent modification by phosphorylation
- Families of protein kinases: PKA (cAMP), PKG (cGMP), CaM (Ca^{2+}), PKC (Ca^{2+}, PL, DG)
- SAPKs : daisy chain phosphorylations
- Regulation via interconversion of active vs subactive forms of protein substrates
- p38, ERK (1/2), JNK, AMPK, AKT (mTOR)

PATHWAY CONTROL IN HIBERNATION

Phospho / de-Phospho



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

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

Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 467 (2007) 10–19
ABB
www.elsevier.com/locate/yabbi

Regulation of skeletal muscle creatine kinase from a hibernating mammal

Khalil Abnous, Kenneth B. Storey *

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Received 29 May 2007, and in revised form 9 July 2007
Available online 22 August 2007

Abstract

Control over skeletal muscle energetics is critical in hibernation to sustain viability over weeks of cold torpor and to support shivering thermogenesis during arousal. Creatine kinase (CK)[1] has a key role in muscle energetics and this study analyzes muscle CK from ground squirrels, *Spermophilus richardsonii*. CK activity was ~20% lower during hibernation than in euthermia, as was CK protein whereas CK mRNA was reduced by ~70%. Hibernator CK showed reduced affinity for ATP and creatine, compared with euthermic CK. Incubations that promoted endogenous protein kinase or phosphatase action, coupled with ion exchange chromatography to separate high and low phosphate forms, showed that soluble CK from euthermic squirrels was a mix of phosphorylated and dephosphorylated forms whereas only phospho-CK was detected in hibernating animals. High and low phosphate CK forms had different affinities for ATP and creatine substrates but did not differ in stability to urea denaturation. About 20–25% of CK was bound to the insoluble fraction of muscle and bound CK showed different kinetic responses to kinase and phosphatase treatments.

1. **Novel Phospho-Enzymes: BioInformatics + Phospho-analysis**
2. **^{32}P-ATP labeling studies**
3. **Purification / Properties**
4. **Structure / Function**
5. **Phospho-sites**

Posttranslational Modification: The Next Generation

Novel Phosphorylation Control of

CK, GDH, Hexokinase, G6PDH, LDH, NADP-IDH, α-GPDH, AMPD, GAPDH, FBPase, Antioxidant enzymes

PTMs: Acetylation, Methylation, SUMOylation, etc.

MRD CHANGES

Few ‘SAP’ kinases activated

- **Gene ‘inactivation’ (⇩ mRNA)**

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

TURNING OFF GENES: Role of Epigenetics

Epigenetics:

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

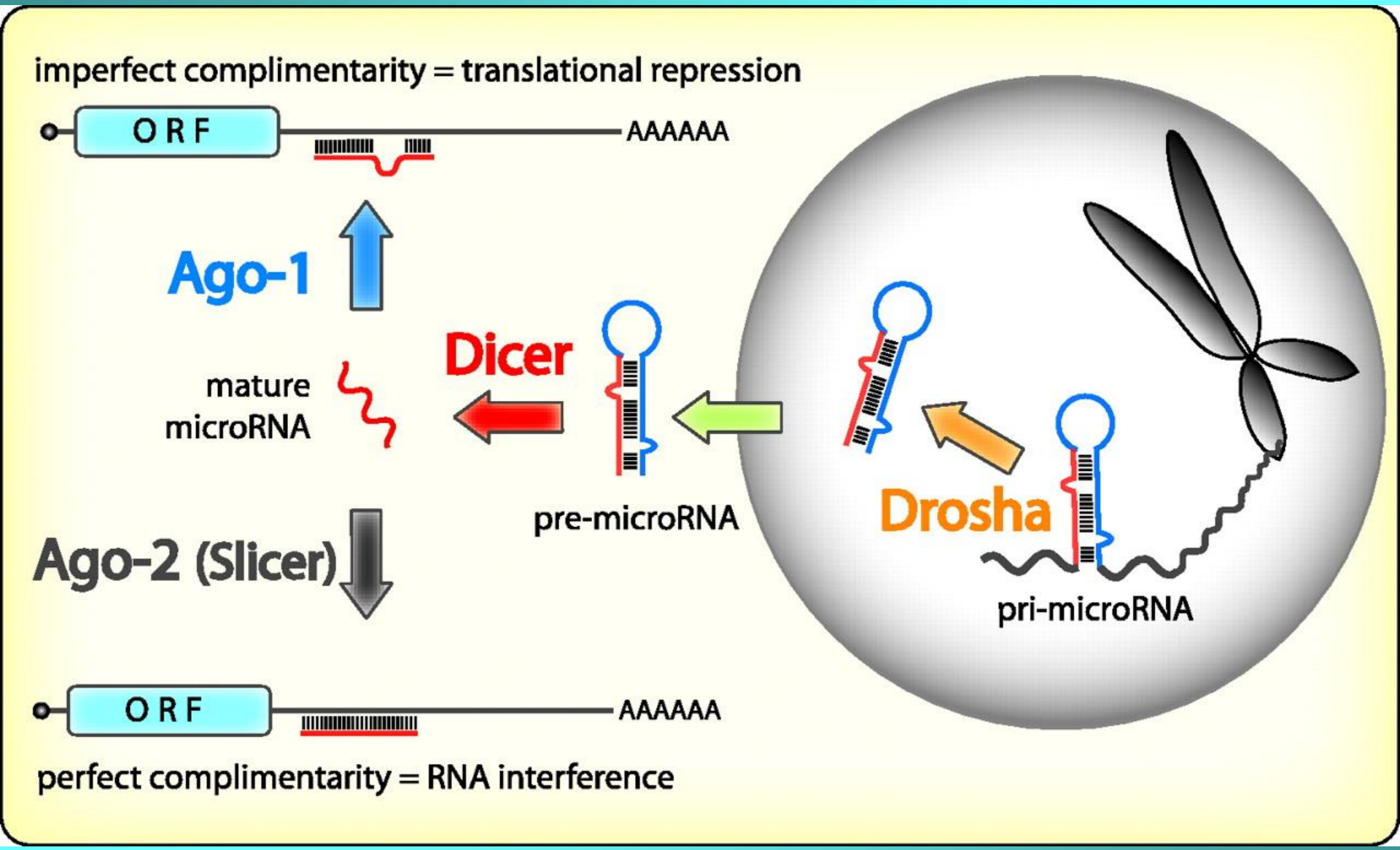
Common mechanisms:

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

Regulatory non-coding RNAs

microRNA

- Small RNAs ~22 nucleotides in length
- Highly conserved across species
- Bind to 3’ UTR of mRNAs
- Could be 1000, affect 60 % of genes
- Disease involvement
- Act to :
  - Block translation of mRNA
  - Target mRNA for degradation



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

Are miRNAs differentially regulated in hibernators?

- **Yes!** **Selected miRNAs were regulated in heart, muscle & kidney of hibernating 13-lined ground squirrels**

miRNA	Fold change	Process in higher vertebrates
Mir-1	2.0	Myogenesis
Mir-133a	2.4	Myogenesis
Mir-206	2.6	Myogenesis
Let-7	2.0	Cell cycle
Mir-26	2.4	Hypoxia
Mir-451	2.6	Erythropoiesis

(Morin, Dubuc & Storey, 2008, Biochim Biophys Acta 1779:628-633)

Turning it all off

Journal of Molecular Cell Biology Advance Access published December 21, 2010
doi:10.1093/jmcb/mjq045 *Journal of Molecular Cell Biology* (2010), 1–9 | 1

Review

The emerging roles of microRNAs in the molecular responses of metabolic rate depression

Kyle K. Biggar and Kenneth B. Storey*

Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
* Correspondence to: Kenneth B. Storey, Tel: +613-520-3678; Fax: +613-520-3749; E-mail: kenneth_storey@carleton.ca

Metabolic r
estivation, a
bolic states
organisms
likely drive
bolism and
marily from
examples f
response to
studies hav
decrease pr
cell cycle ar
ous disease
attack in hu

Biochimica et Biophysica Acta 1779 (2008) 628–633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Differential expression of microRNA species in organs of hibernat squirrels: A role in translational suppression during torpor

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

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

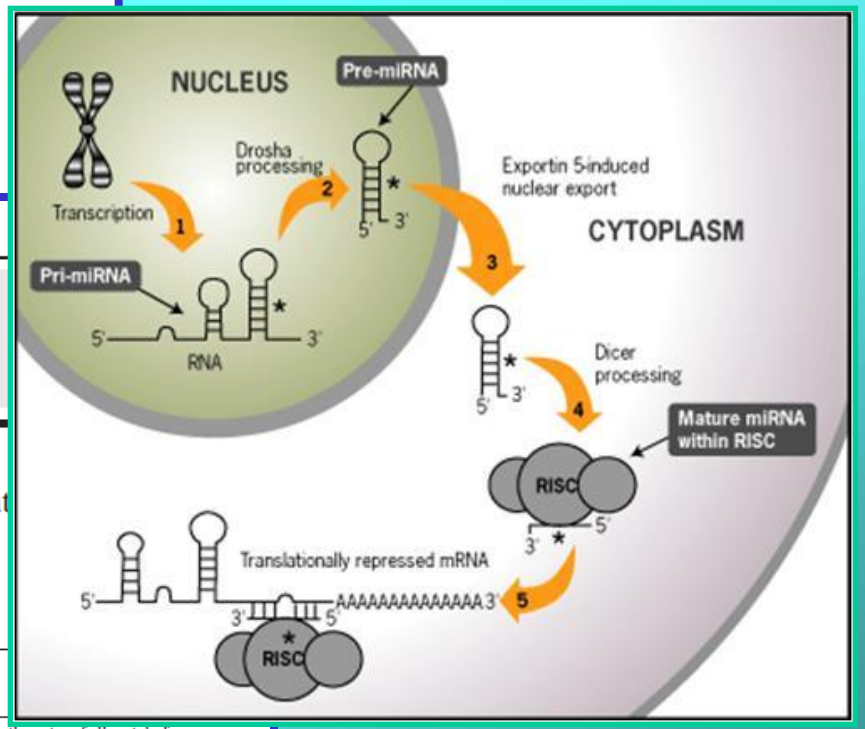
ARTICLE INFO

Article history:
Received 25 April 2008
Received in revised form 17 July 2008
Accepted 28 July 2008
Available online 5 August 2008

Keywords:
MicroRNA
Hibernation
Spermophilus tridecemlineatus
Dicer
Reversible control of translation

ABSTRACT

Mammalian hibernation includes long periods of profound torpor where the rates of all metabolic processes are strongly suppressed in a reversible manner. We hypothesized that microRNAs (miRNAs), small non-coding transcripts that bind to mRNA, could play a role in the global suppression of mRNA translation when animals enter torpor. Selected miRNA species (4–9 of the following: mir-1, mir-24, mir-15a, mir-16, mir-21, mir-122a, mir-143, mir-146 and mir-206) were evaluated in four organs of euthermic versus hibernating ground squirrels, *Spermophilus tridecemlineatus* using RT-PCR. Levels of mir-24 transcripts were significantly reduced in heart and skeletal muscle of torpid animals as were mir-122a levels in the muscle. Mir-1 and mir-21 both increased significantly in kidney during torpor by 2.0- and 1.3-fold, respectively. No changes were found for the four miRNA species analyzed in liver. Protein levels of Dicer, an enzyme involved in miRNA processing were also quantified in heart, kidney and liver. Dicer protein levels increased by 2.7-fold in heart



METABOLIC RATE DEPRESSION

Hibernation

Estivation

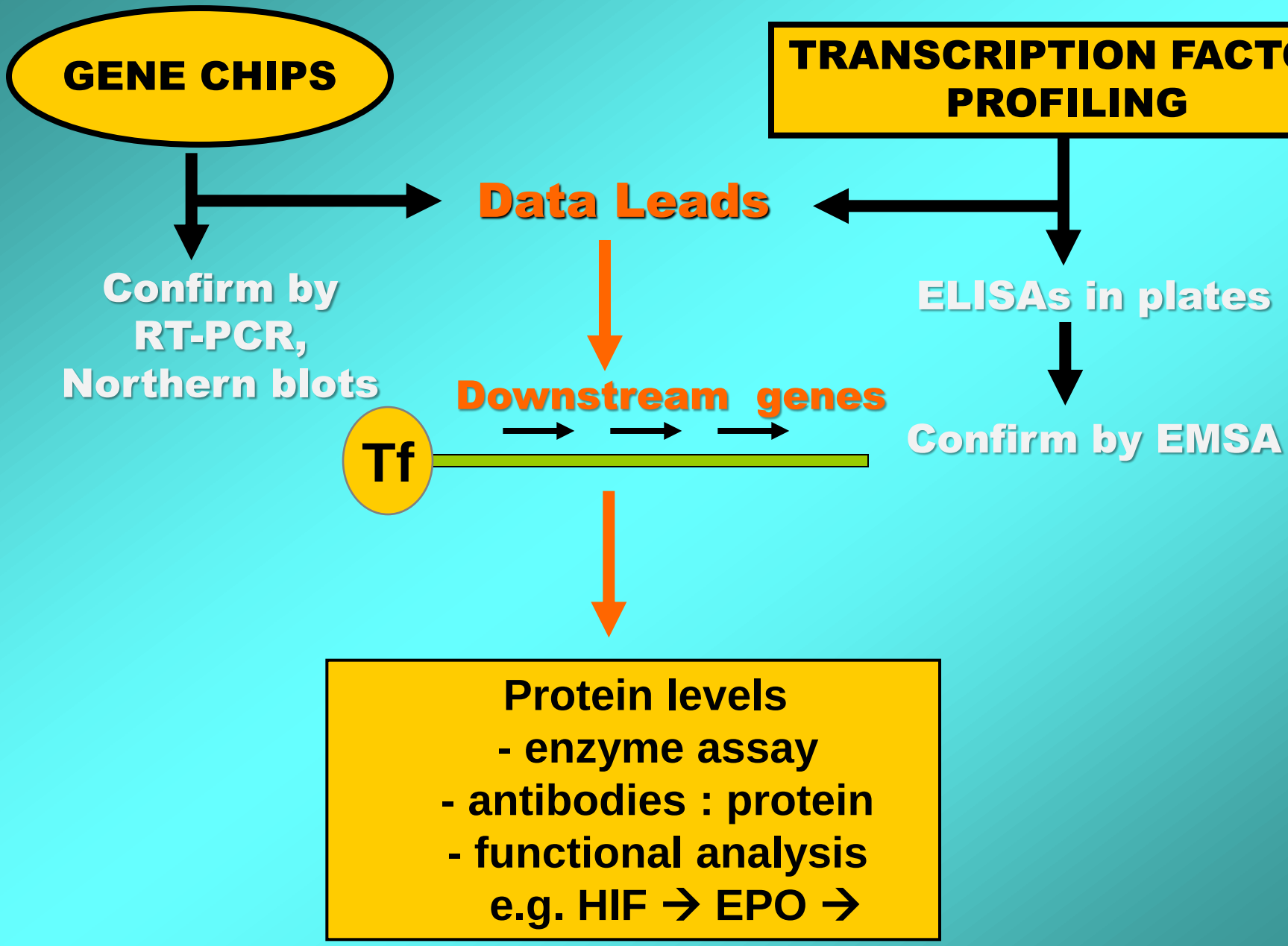
Anoxia

Freezing

Diapause

MRD CHANGES

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

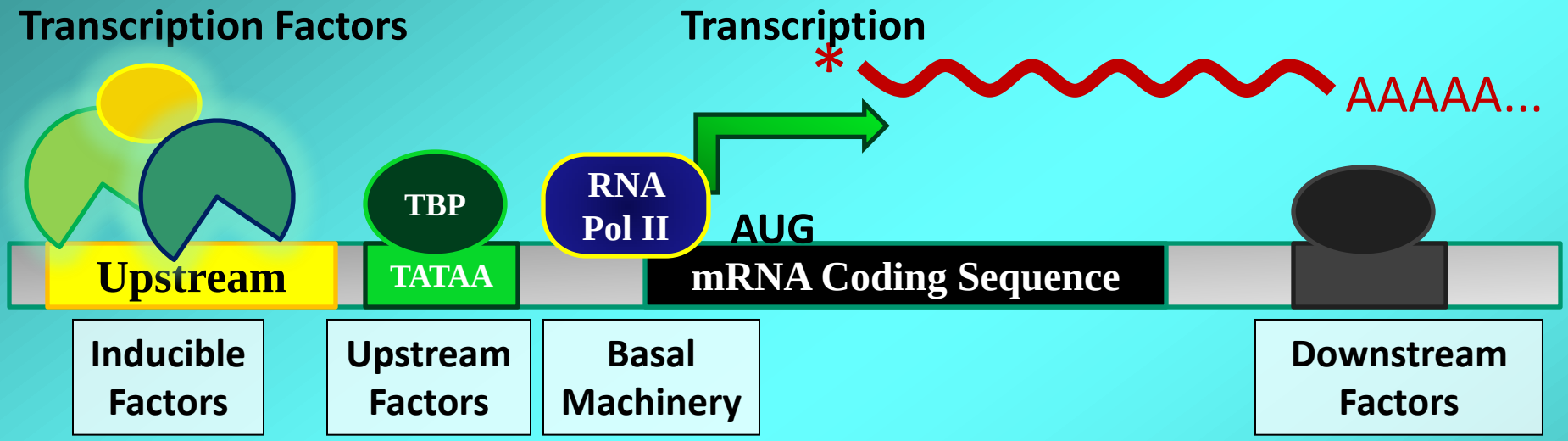

GENE CHIPS
TRANSCRIPTION FACTOR PROFILING
Data Leads
Confirm by RT-PCR, Northern blots
ELISAs in plates
Downstream genes
Tf
Confirm by EMSA
Protein levels
- enzyme assay
- antibodies : protein
- functional analysis
e.g. HIF → EPO →

GENE CHANGES IN HIBERNATION

- **Some Activated :**
  - **Mitochondrial Genes**
  - **AOE**
  - **FABP, CPT, etc.**
  - **Shock proteins (GRP, HSP)**
  - **Transcription factors**

- **DNA Chip ~1-2%** ⬆

Regulation of Gene Transcription



10 **Beyond gene chips: transcription factor profiling in freeze tolerance**

In: Hypometabolism in Animals: Hibernation, Torpor and Cryobiology (Lovegrove, B.G., and McKechnie, A.E., eds.) University of KwaZulu-Natal, Pietermaritzburg, pp. 101-108.

KENNETH B. STOREY

Institute of Biochemistry, Carleton University, Ottawa, Canada K1S 5B6; kenneth_storey@carleton.ca

Abstract

The Wood Frog, *Rana sylvatica*, is one of several terrestrially hibernating anurans that display natural freeze tolerance. The multifaceted biochemical responses to the cellular stresses imposed when ~65% of total body water is converted to extracellular ice have

TRANSCRIPTION FACTORS

- ATF (Glucose Regulated Proteins)
- HIF (O_2), HSF (Hsp)
- NFkB (IkB-P), Nrf-2, NRF-1
- PPAR, PGC, RXR, chREBP, CREB-P
- STAT, SMAD, p53-P, HNF, AP (1,2)

- Methods: EMSA, CHiP

Where do we go from here?

Nature's Tools for MRD

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

NEW DIRECTIONS

Novel Enzyme Controls

Available online at www.sciencedirect.com
ScienceDirect
Archives of Biochemistry and Biophysics 467 (2007) 10–19
ABB
www.elsevier.com/locate/yabbi

Regulation of skeletal muscle creatine kinase from a hibernating mammal

Khalil Abnous, Kenneth B. Storey *

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Received 29 May 2007, and in revised form 9 July 2007
Available online 22 August 2007

Abstract

Control over skeletal muscle energetics is critical in hibernation to sustain viability over weeks of
thermogenesis during arousal. Creatine kinase (CK)[1] has a key role in muscle energetics and this stu
squirrels, *Spermophilus richardsonii*. CK activity was ~20% lower during hibernation than in euther
mRNA was reduced by ~70%. Hibernator CK showed reduced affinity for ATP and creatine, compa
that promoted endogenous protein kinase or phosphatase action, coupled with ion exchange chrom
phosphate forms, showed that soluble CK from euthermic squirrels was a mix of phosphorylated a
only phospho-CK was detected in hibernating animals. High and low phosphate CK forms had diff
substrates but did not differ in stability to urea denaturation. About 20–25% of CK was bound to
bound CK showed different kinetic responses to kinase and phosphatase treatments.

Comparative Biochemistry and Physiology, Part B 157 (2010) 310–316
Contents lists available at ScienceDirect
Comparative Biochemistry and Physiology, Part B
journal homepage: www.elsevier.com/locate/cbpb

Regulation of liver glutamate dehydrogenase by reversible phosphorylation in a hibernating mammal

Ryan A.V. Bell, Kenneth B. Storey *

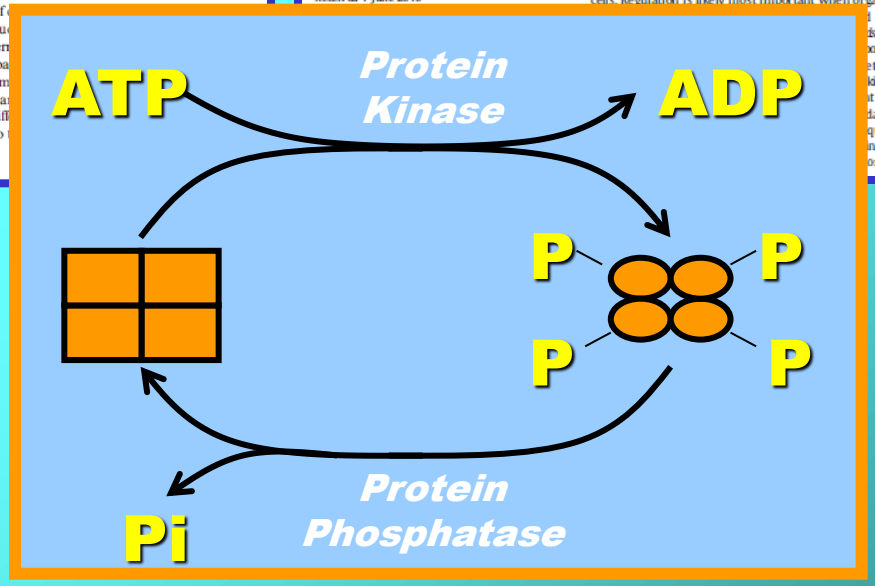
Institute of Biochemistry and Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, Canada K1S 5B6

ARTICLE INFO

Article history:
Received 1 June 2010

ABSTRACT

Glutamate dehydrogenase (GDH) is a key enzyme that links amino acid and carbohydrate metabolism in cells. Regulation is likely most important when organisms are confronted with extreme stresses such as the
associated with winter. Many small mammals, such as
sonii, cope with these conditions by hibernating. Animals
olic rate is greatly suppressed, body temperature drops to
t from fixed internal body stores of fuels. To investigate
kinetic properties of GDH were analyzed in liver from
t differences in V_{max}, K_m glutamate, K_a ADP and inhibition
data suggested an activation of the glutamate-oxidizing
quent experiments suggested that the molecular basis of
n phosphorylation state of GDH between euthermia and
osphorylated and activated when animals transition into



Epigenetics in Adaptation miRNA [PLUS]

Mol Cell Biochem. 2010 Sep;342(1-2):151-61. Epub 2010 May 1.

Epigenetics in anoxia tolerance: a role for histone deacetylases.

Krivoruchko A, Storey KB.

Institute of Biochemistry, Carleton University, Ottawa, ON, Canada. krivoruchko@gmail.com

Abstract

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

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Cryobiology 53 (2006) 310–318

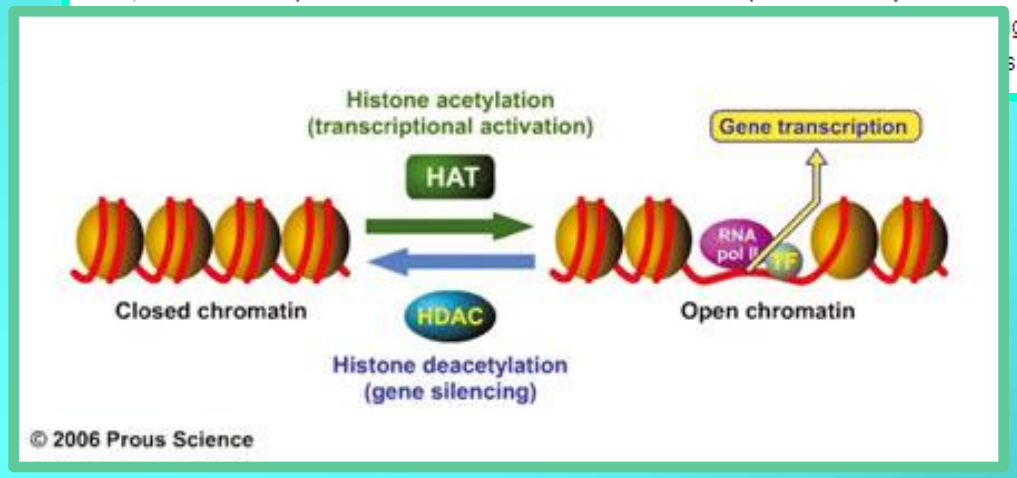
CRYOBIOLOGY

www.elsevier.com/locate/ycryo

Evidence for a reduced transcriptional state during hibernation in ground squirrels ☆

Pier Jr Morin*, Kenneth B. Storey

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6



Life span extension

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

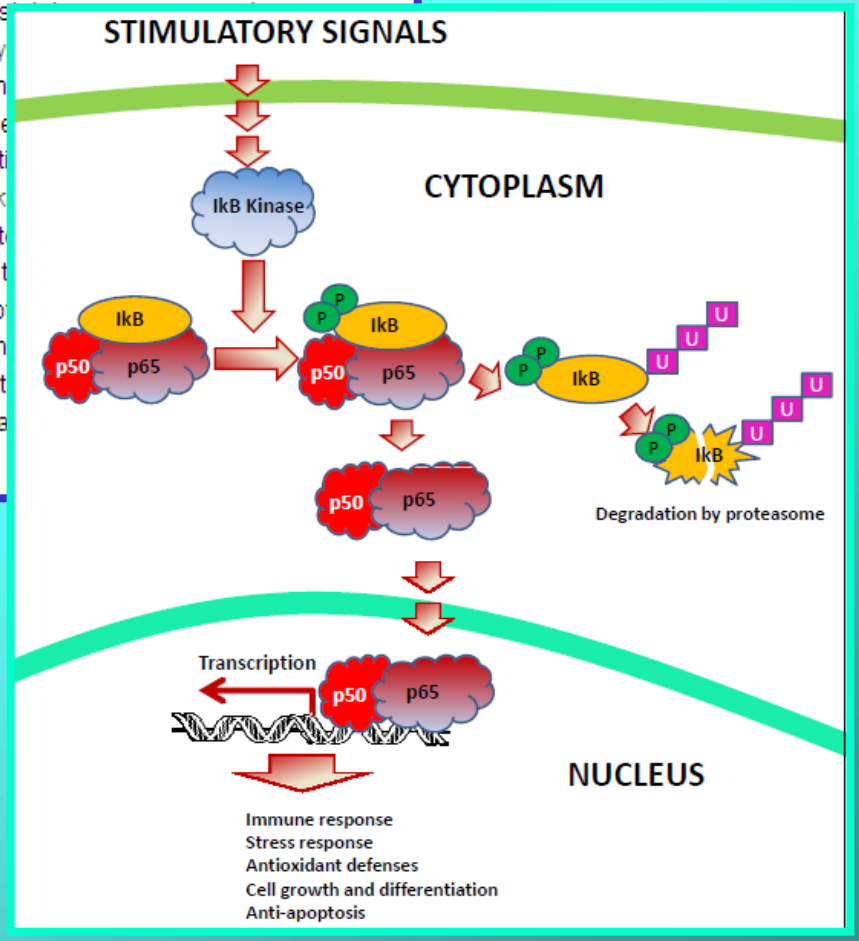
Forever young: mechanisms of natural anoxia tolerance and potential links to longevity.

Krivoruchko A, Storey KB.

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

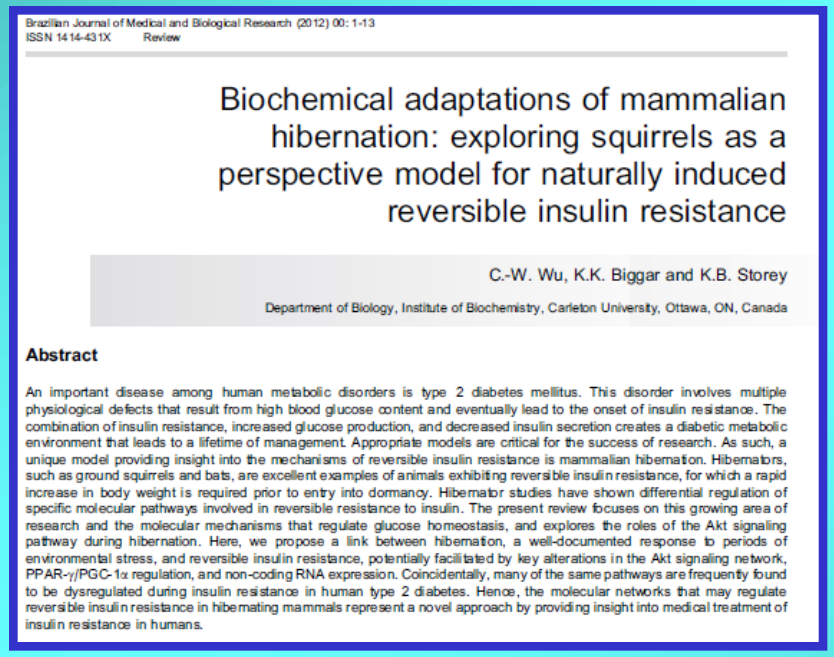
Abstract

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



Hibernators as a model for metabolic disease?

Type 2 Diabetes mellitus



Brazilian Journal of Medical and Biological Research (2012) 00: 1-13
ISSN 1414-431X Review

Biochemical adaptations of mammalian hibernation: exploring squirrels as a perspective model for naturally induced reversible insulin resistance

C.-W. Wu, K.K. Biggar and K.B. Storey

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

Abstract

An important disease among human metabolic disorders is type 2 diabetes mellitus. This disorder involves multiple physiological defects that result from high blood glucose content and eventually lead to the onset of insulin resistance. The combination of insulin resistance, increased glucose production, and decreased insulin secretion creates a diabetic metabolic environment that leads to a lifetime of management. Appropriate models are critical for the success of research. As such, a unique model providing insight into the mechanisms of reversible insulin resistance is mammalian hibernation. Hibernators, such as ground squirrels and bats, are excellent examples of animals exhibiting reversible insulin resistance, for which a rapid increase in body weight is required prior to entry into dormancy. Hibernator studies have shown differential regulation of specific molecular pathways involved in reversible resistance to insulin. The present review focuses on this growing area of research and the molecular mechanisms that regulate glucose homeostasis, and explores the roles of the Akt signaling pathway during hibernation. Here, we propose a link between hibernation, a well-documented response to periods of environmental stress, and reversible insulin resistance, potentially facilitated by key alterations in the Akt signaling network, PPAR-γ/PGC-1α regulation, and non-coding RNA expression. Coincidentally, many of the same pathways are frequently found to be dysregulated during insulin resistance in human type 2 diabetes. Hence, the molecular networks that may regulate reversible insulin resistance in hibernating mammals represent a novel approach by providing insight into medical treatment of insulin resistance in humans.

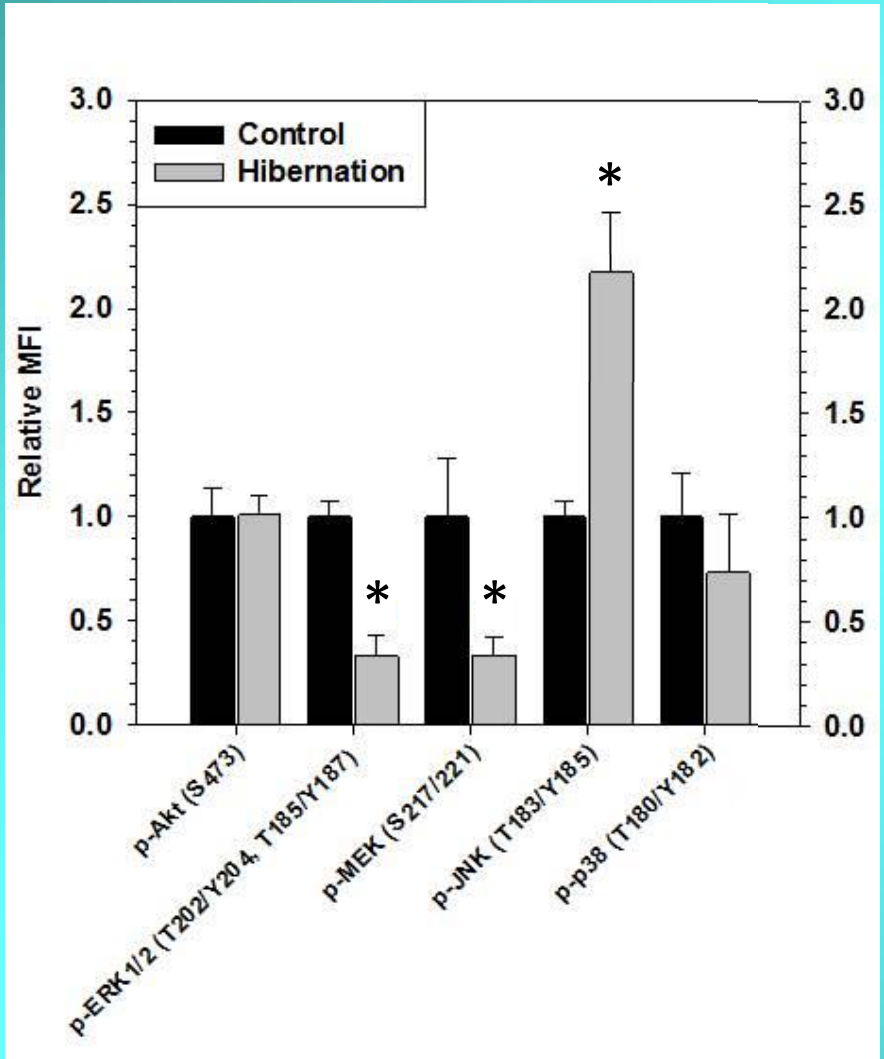
- **Hibernators show reversible insulin resistance**
- **Rapid weight gain, hyperinsulinemia during entry into hibernation, reversed in deep torpor**
- **Modifications to similar pathways: Glucose transport, Akt activity, PPAR-γ signaling**

PRIMATE HIBERNATION !!

Gray Mouse Lemur

Madagascar - western dry forests

MRD in Primate hibernation [Stress Kinases]

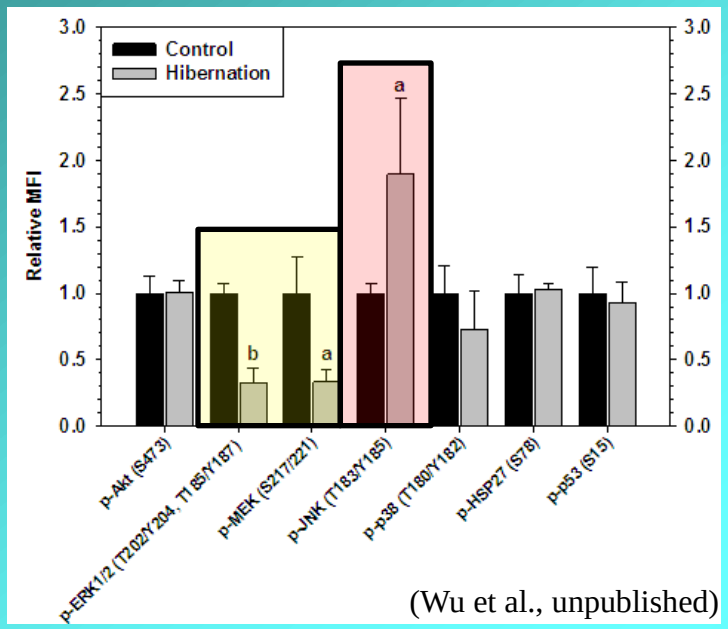


Biggar KK, Wu CW, Tessier SN, Zhang J & Storey KB

Gray Mouse Lemur

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

Primate Hibernation



(Wu et al., unpublished)

Gray mouse lemur

- Novel model of hibernation
  - Short term torpor in mild climate (Tb falls to ~20°C)
- Reduction of ERK signaling
- Activation of JNK stress response

Hibernation and medicine

Primates !!

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

Metabolic rate depression: the biochemistry of mammalian hibernation.

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
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.

Unity through Evolution

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

Review

An Overview of Stress Response and Hypometabolic Strategies in *Caenorhabditis elegans*: Conserved and Contrasting Signals with the Mammalian System

Benjamin Lant and Kenneth B. Storey ✉

Institute of Biochemistry, Carleton University, Ottawa, Ont., Canada

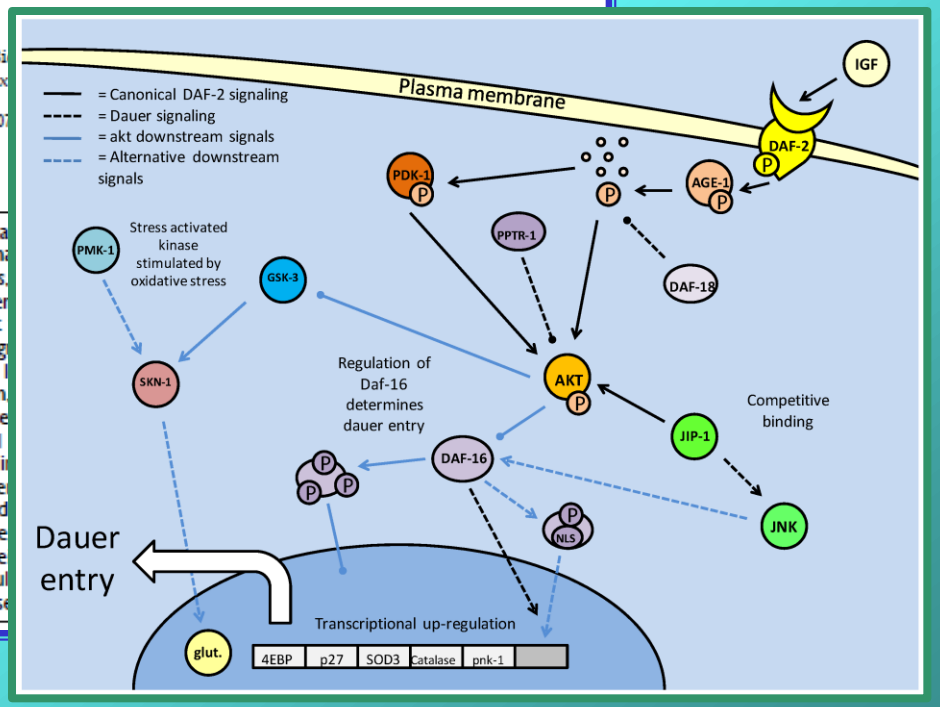
✉ Correspondence to: Kenneth B. Storey, Institute of Bi
Ottawa, Ont. K1S 5B6, Canada. Tel. +1 613 520 3678, Fax

Received: 2009.09.11; Accepted: 2009.11.25; Published: 2010.01.0

Abstract

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

WWCeD

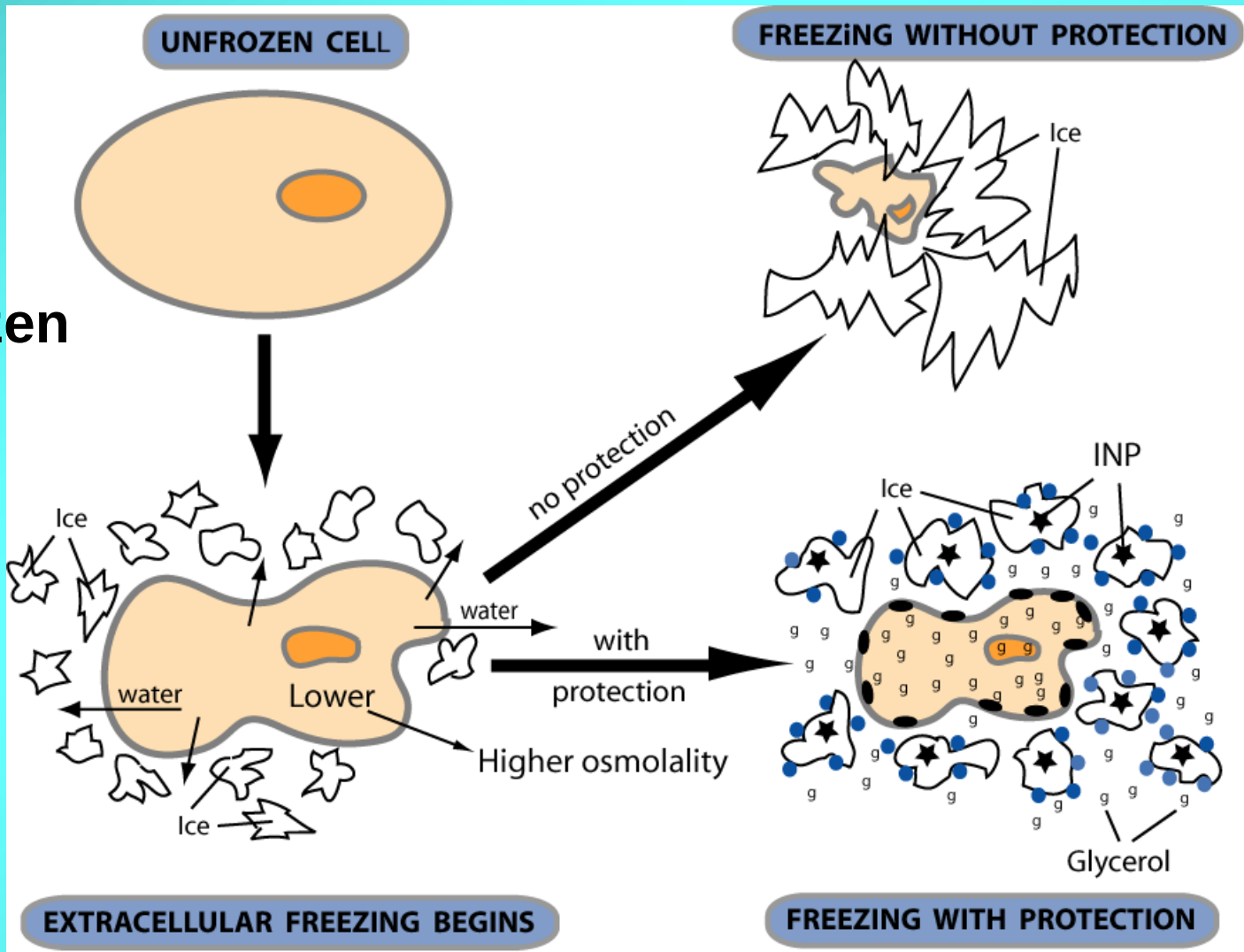


The (Real) Living Dead

WOOD FROG
Rana sylvatica

SURVIVING FREEZING

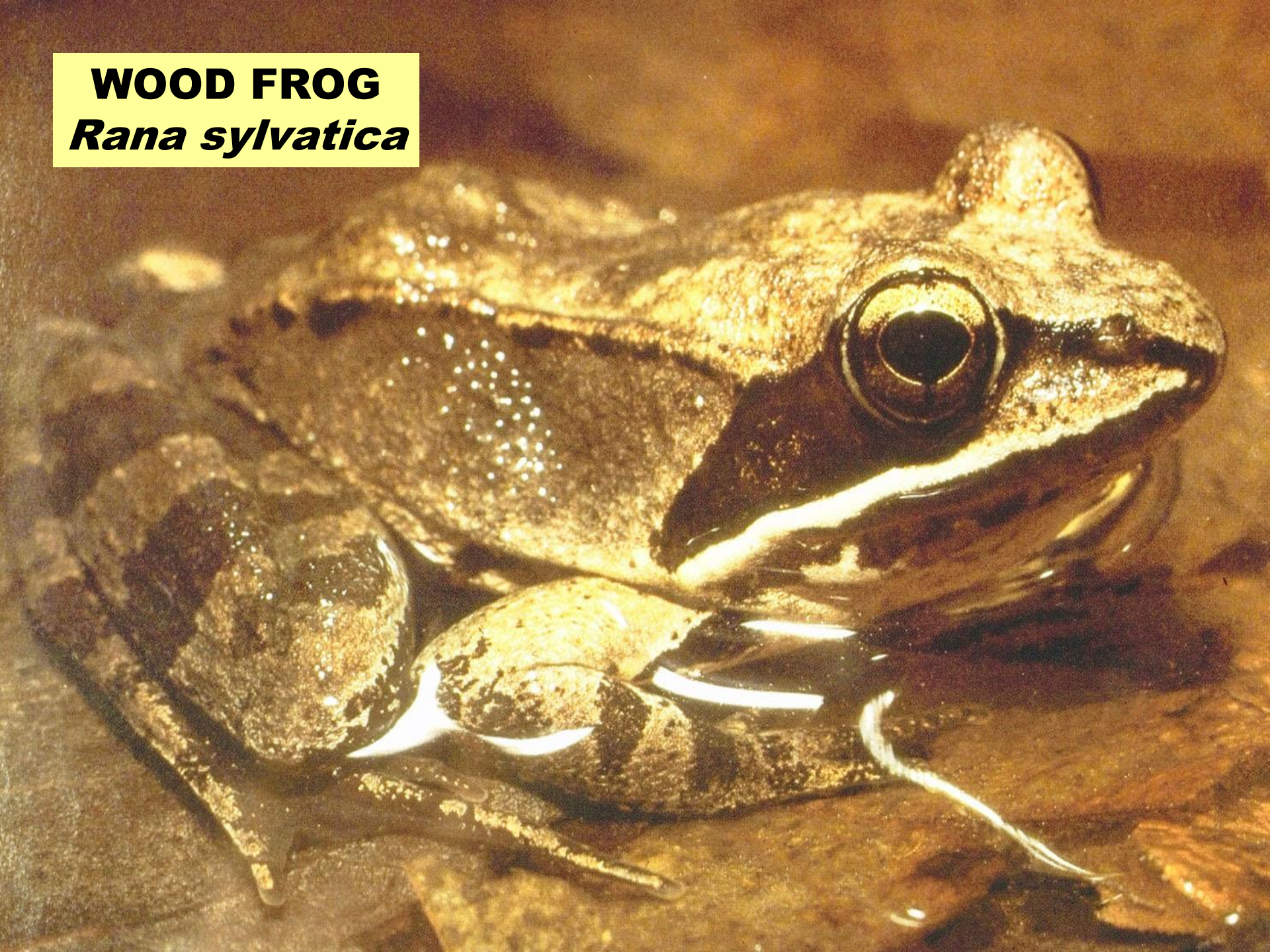
- Extracellular freezing only
- Up to 70% of body water frozen
- High ‘polyols’
- Acclimation required
- Glucose
- Glycerol
- Sorbitol



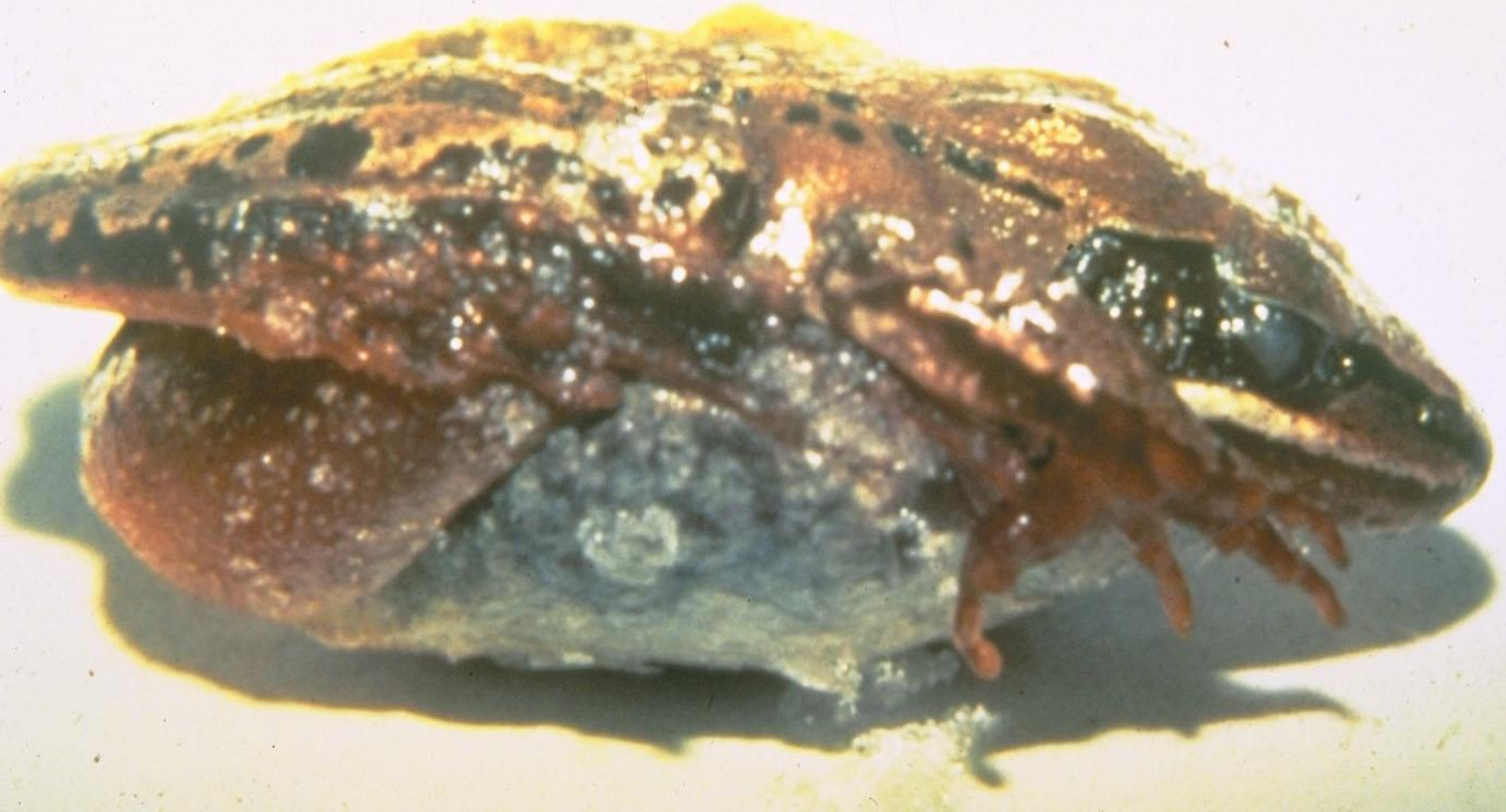
FREEZE TOLERANT ANIMALS

- Insects (many)
- Intertidal molluscs & barnacles
- Amphibians & Reptiles
  - Frogs (6 species)
  - Hatchling turtles
  - Garter snakes
  - Lizards (some)

VERTEBRATE FREEZE TOLERANCE


WOOD FROG
Rana sylvatica

WOOD FROG
Rana sylvatica



WINTER HYPOMETABOLISM

Hibernation

Anoxia

Freezing

Diapause

FREEZING (MRD)

1. Stress Kinases ON
2. All Cell Processes OFF
3. Enzymes : Modified & “Off”
4. Micro RNA
5. Genes turned OFF – Epigenetics
6. Key Genes ON

THE FUTURE ??

FROZEN IN TIME

Woman put in deep freeze in 1959 revived by doctors and given life-saving heart surgery

A WOMAN WHO should have died in 1959 has been saved by modern heart surgery — after spending the last 30 years in a deep freeze!

At the age of 26, Helene Chapparal had been placed in a state of suspended animation by doctors who lacked the medical know-how to repair her ailing heart.

That history-making experiment recently became a success when physicians in Lyon, France revived the frozen woman and corrected her heart deformity using newly-developed surgical techniques!

Dr. Maurice Fougeres, 58, headed the team of doctors who brought Helene back to life. He had also been present in 1959 when she was frozen.

"I had just graduated from medical school," he says. "I was invited to attend a top secret experimental procedure.

"I walked into the lab and was stunned to see the greatest medical minds of that time clustered around a very beautiful woman lying on an operating table."

The young doctor listened in amazement as the chief surgeon explained that the woman suffered from heart disease and had only hours to live.

The untested procedure called for her body to be frozen, and then revived when medical science had developed a cure for her ailment.

"She was placed in a tub surrounded by dry ice," recalls Dr. Fougeres. "An artificial respirator kept her breathing until the last possible second, in order to avoid sudden shock which would have killed her.

● *HELENE WAS put in freezing tank similar to one above used by New Cryonics Society*

Storage room

"When her body temperature neared the freezing point, her blood was removed and her veins were filled with a glycerol and water solution. She was then covered in foil and sealed inside a coffin-like tube containing liquid nitrogen at minus 320 degrees Fahrenheit."

Helene's frozen body was moved to a hospital storage room, where it remained for the next 30 years.

Recently, doctors became confident that modern surgery could easily repair Helene's heart problem. Most of the older physicians who had been present when the woman was frozen had died, so Dr. Fougeres was asked to head the medical team to revive her.

"We began by warming the patient's blood and reinjecting it into her defrosted body," he notes. "Electrical stimulation was applied to her heart and brain.

"I guess deep down I didn't believe it was possible, so when that first, faint heartbeat was detected, I nearly jumped out of my skin.

"She lay there unconscious, but very much alive. She had not aged, and was as beautiful as on that day in 1959."

After several hours, Helene was sent into an operating room, where surgeons repaired her heart defect. Two days later she woke up from her deep sleep for the first time.

● *HELENE CHAPPARAL spent 30 years in deep freeze*

● *DR. FOUGERES was present when Helene was frozen and also when she was brought back to life*

Scientists revive frozen human brain!

Soviet scientists revived and held a conversation with the brain of a man who fell into an icy Siberian crevasse in 1921 and remained frozen until the discovery of the body earlier this year.

In a report to the science journal *Mind and Body*, Dr. Alexei Kolosov conceded that his conversation with the brain was short but called it groundbreaking because it proved that human tissue and intelligence can survive long-term freezing.

Organ linked to computer and answers stunned docs' questions

The Moscow-based expert said the brain was removed from the body and linked to a computer prior to his attempt to communicate with it.

It then answered yes or no to six simple questions before it lapsed into a coma and died.

"We did not raise the dead but we came very, very, close," said the expert.

"For a few minutes the brain waves were nearly as active as those of a living man.

"Their movement during questioning indicates that the brain was aware and even eager to answer.

"We asked such things as: 'Are you comfortable? Do you know where you are? Do you remember your name?'

"By comparing the brain waves running through our computer it is clear that it responded yes or no to each question. It was simply a very stunning piece of research."

In a related experiment, Dr. Kolosov and his colleagues were able to restore temporary heart and lung function to the frozen man's body after the brain had been removed, the journal said.

"We originally thought about attempting to revive body and mind as a whole," said Dr. Kolosov. "But we decided that our chances of success were greater if we separated the two."

The frozen man was identified as Boris Chakovsky, a Siberian laborer who was reported missing and presumed dead after he disappeared in 1921.

"His papers were still in his pockets when his body was recovered from the ice last February," said Dr. Kolosov.

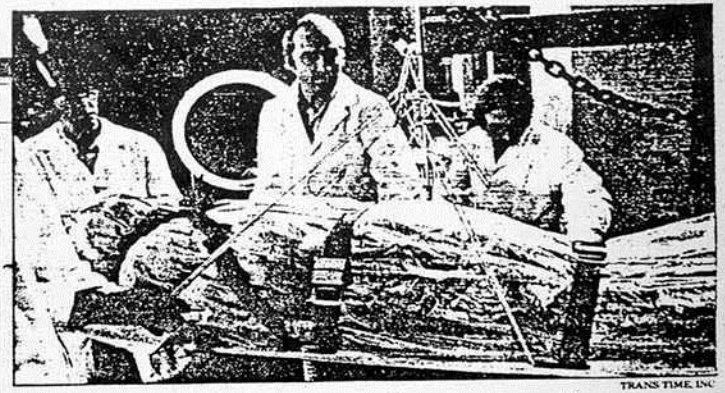
"We imagine he slipped or was pushed into the crevasse, couldn't get out and froze within minutes," he continued.

"Otherwise he would have suffered brain damage that would have made it impossible for us to communicate with him, even for a few seconds."

SCIENCE

Heaven Can Wait a While

A woman loses her head



TRANS TIME, INC.

Frozen in time: *Technicians prepare a corpse for cryonic preservation*

The question of life after death has always been an iffy one, and a handful of optimists aren't taking any chances. Dora Kent, 83, was confined to a nursing home with severe arthritis and a degenerative brain disease. But her devoted son Saul, 48, who had lived with her most of his life, didn't want his mother to disappear forever. So he apparently retained the services of Alcor Life Extension Foundation. Alcor cut off his mother's head and froze it in liquid nitrogen, against the unlikely day when science might be able to bring it back to life and attach it to a new body. But the Riverside County, Calif., coroner's office now suspects Dora Kent may not have been legally dead when she was beheaded. And last week a search of the Alcor facility revealed weapons and explosives, one intact frozen corpse and six heads frozen in hope of eventual reunion with other bodies. But none of the heads was Dora's.

The suspicions of the coroner's office were first aroused by Dora Kent's death certificate, which said she died, unattended, at a "residence." But the address listed was that of the Alcor facility, and officials there admitted she had been brought in alive and died on their premises. Alcor officials now acknowledge that the woman's death should have been certified by an outside authority before they proceeded to remove her head. (Her body was cremated.) According to deputy coroner Rick Bogan, however, no tests were performed to establish "brain death," a requirement of California law before any organs or body parts can be taken.

Ice price: Cryonics, the attempted preservation of human life by storing bodies at subfreezing temperatures, had a minor vogue in the self-centered '70s, and the American Cryonics Society claims a current membership of 100 people who have committed themselves to being frozen after death. The remains of about 15 people are believed to be on ice at three storage centers in California and Michigan. The price for the promise of coming back to the 21st century can be as high as $125,000.

Cryonics is not to be confused with cryobiology, a recognized branch of medical research that studies the effects of very low temperatures on living tissue. Advances in this field now allow the freezing of blood, corneas, bone marrow, sperm and even embryos for use at a later date. But so far, cryobiologists have been unable to make use of frozen organs such as the pancreas, kidneys or heart in transplant surgery. That's why they dismiss the claims of the body freezers. "There is no scientific basis on which to say anyone can be frozen and then reanimated," maintains Arthur Rowe, former president of the Society for Cryobiology and editor of its journal.

Even if the Riverside authorities do locate Dora Kent's wayward head, bringing it back to life might not be a good idea: she'd probably die again—of shock. The elderly woman, it turns out, may not have had any plans to be frozen (let alone decapitated); according to Bogan, the consent forms were signed only by her son.

JEAN SELIGMANN *with* MARK MILLER *in Washington and* LINDA BUCKLEY *in Los Angeles*

Coroner: Woman's death murder; head then frozen

RIVERSIDE, Calif. (AP) — A coroner has reclassified as homicide the death of an elderly woman whose head was surgically removed and frozen in hopes that she could some day be brought back to life with a new body.

"We're saying this was an 83-year-old lady that was ill and pushed over the edge by the use of a drug," Supervising Deputy Coroner Dan Cupido said Tuesday.

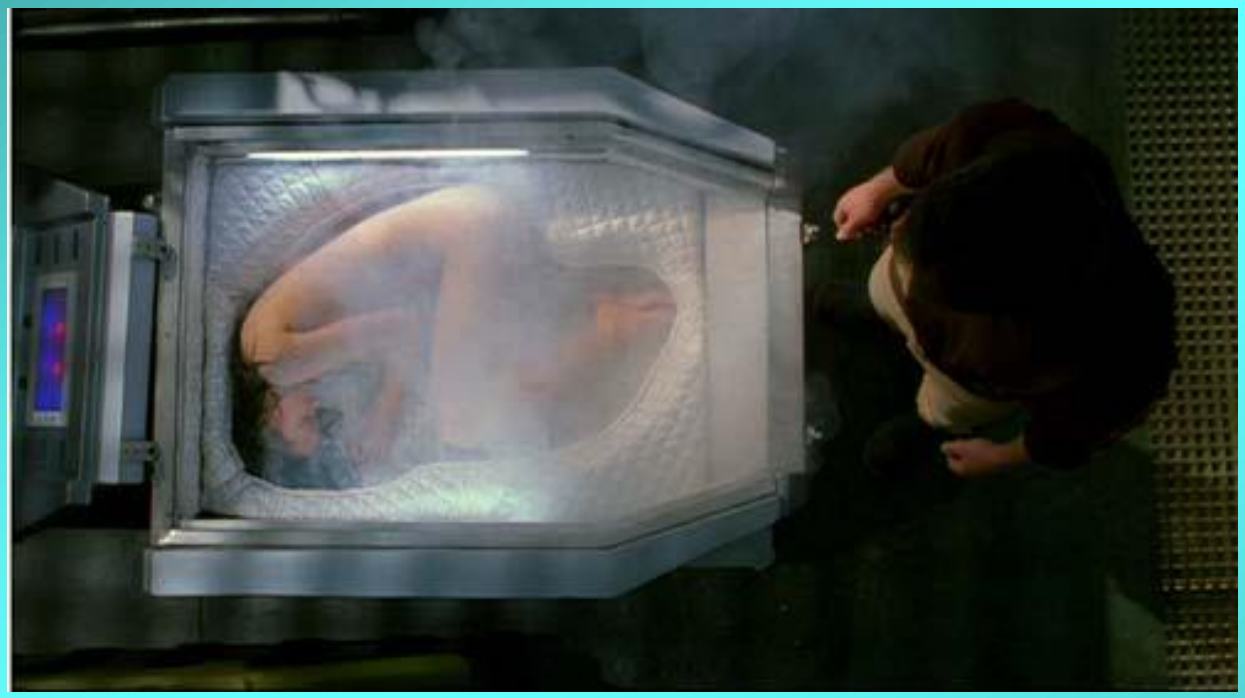
Dora Kent's death last December at the Alcor Life Extension Foundation laboratory in Riverside has been under investigation since January.

Deputy District Attorney Curt Hinman said he is "fairly confident charges will be filed," but he did not say who would be charged, when, or specify the charges.

The woman's original death certificate indicated she died from pneumonia in the absence of a doctor, which prompted the coroner's office to investigate whether she was alive when the procedure to remove her head was started.

Authorities seeking to force Alcor to produce the frozen head have been blocked by an injunction.

CRYONICS: TIME



TIME:

- **For BRAIN DEATH from oxygen lack in heart attack or stroke is 2-5 minutes**
- **For corpse to have cryonics started**
- **For cooling a human body from 37°C down to 5°C**

LIQUID NITROGEN

CRYONICS: DAMAGE

DAMAGE:

- **FREEZING a human in dry ice from 5°C to -80°C**
- **FREEZING a human from -80°C to -196°C by immersion in liquid nitrogen**

SOLUTION ???
HEAD PREPARATION

NOT


I would turn in my grave..
.. if I weren't
CRYOGENICALLY FROZEN!
ICANHASCHEEZBURGER.COM

FREEZING HUMANS

DOES IT WORK ?

A. Liquid Nitrogen Storage (-196°C)

- frogs only to -20°C (cell destruction)
- fragility/crush (neurons)

B. Frozen Liquid Expands !

C. Bits and Bobs …….

D. Time to Preservation (oxygen lack, neurons)

E. You've paid UPFRONT for "forever" !

F. Legal implications (thawed by your kids)

FREEZING HUMANS

Is it correct to freeze humans and then bring them back in the future for "eternal life"?

A. Who would be chosen for this (costly) procedure?

B. How would we pay for re-animation and re-integration into society?
- for 20 subjects
- for 2000 subjects
- for 2 billion subjects

C. Spiritual / Religious implications

D. Legal implications

Dr. Ken Storey
Institute of Biochemistry
Carleton University
Ottawa, Canada

www.carleton.ca/~kbstorey

Metabolic Rate Depression

- J. STOREY
- S. EDDY
- D. HITTEL
- J. MacDONALD
- A. FAHLMAN
- P. MORIN
- C. HOLDEN
- H. MEHRANI
- J. NI
- M. HAPSATOU
- S. TESSIER
- M. WU
- S. BROOKS
- C. FRANK
- J. HALLENBECK
- D. THOMAS
- A. RUBTSOV
- J. STEWART

NSERC CRSNG

Funded by
NSERC Canada

www.carleton.ca/~kbstorey

LIFE IN THE COLD

www.carleton.ca/~kbstorey

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

Freezing

Diapause

ORGANS FOR TRANSPLANT

1. Scientific Solutions

A. IMMEDIATE: extend the viability of removed organs by hours/days

B. FUTURE:

- freeze organs to create organ banks
- stem cell research - grow new organs

C. FAR FUTURE:

- cloning of tissues (one cell --> organ)
- artificial tissues (from non-cell sources)

D. XENOTRANSPLANTS

- Dangers and risks?

E. Clone "NEAR-HUMANS" for parts:

- Society plus science (+/- embryos)
- Have your own clone, just in case?
- The rights of a clone?

ORGANS FOR TRANSPLANT

Tens of thousands wait for a few organs
- **who decides?**
- **should you be able to pay for an organ?**

2. Society Solutions: Dollars, Science, Morals

A. SELL ORGANS: **$$ from rich to poor people**
Organs from poor to rich people
Morally correct? How to regulate?

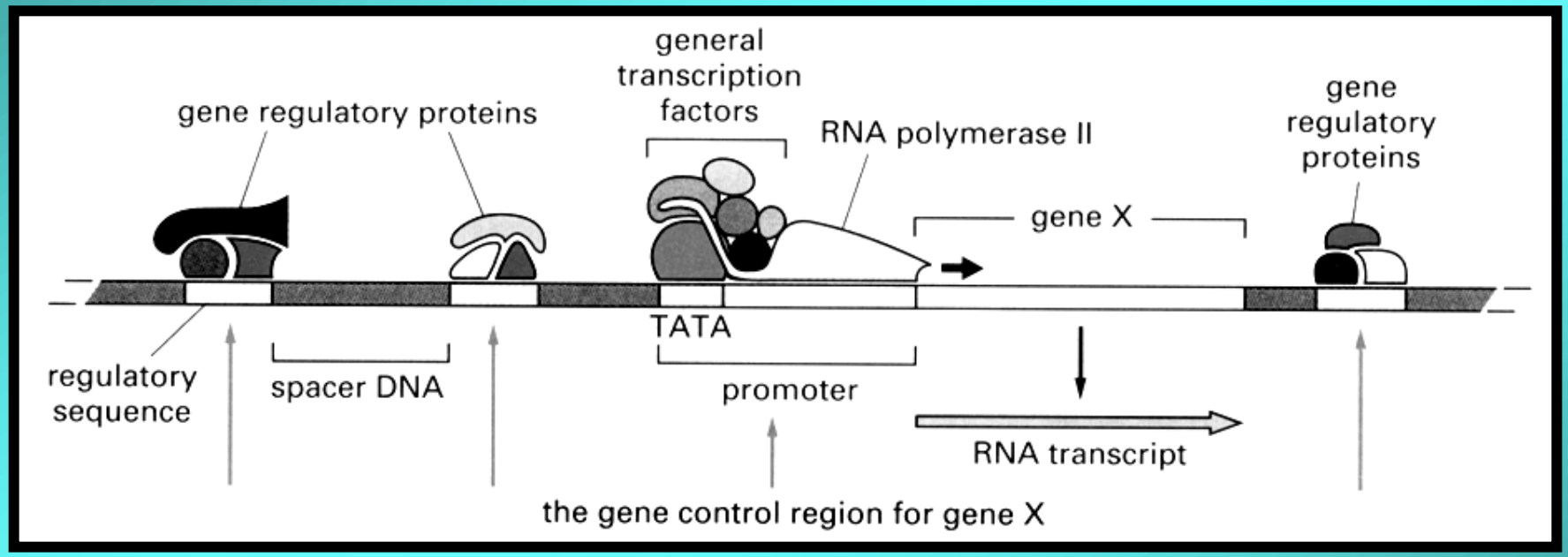
B. Get organs by "PRESUMED CONSENT"
- **Will doctors revive or harvest?**
- **How dead do you have to be?**
- **Religious / spiritual implications**

CRYOPRESERVED HUMAN TISSUES

- SPERM
- EMBRYOS
- SKIN
- CORNEA
- VEINS
- BLOOD CELLS
- HEART VALVES
- TEETH, BONE
- BONE MARROW
- PANCREATIC TISSUE
- THYROID TISSUE
- PARATHYROID TISSUE
- FETAL TISSUES (some)
- **RAT LIVER**

THE FUTURE ??

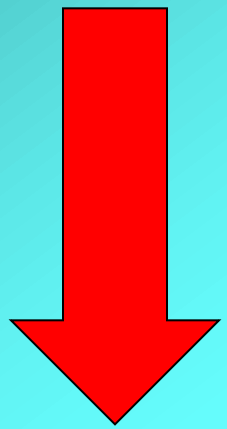
CONTROL REGION OF A TYPICAL EUKARYOTIC GENE



Epigenetics = OFF) :

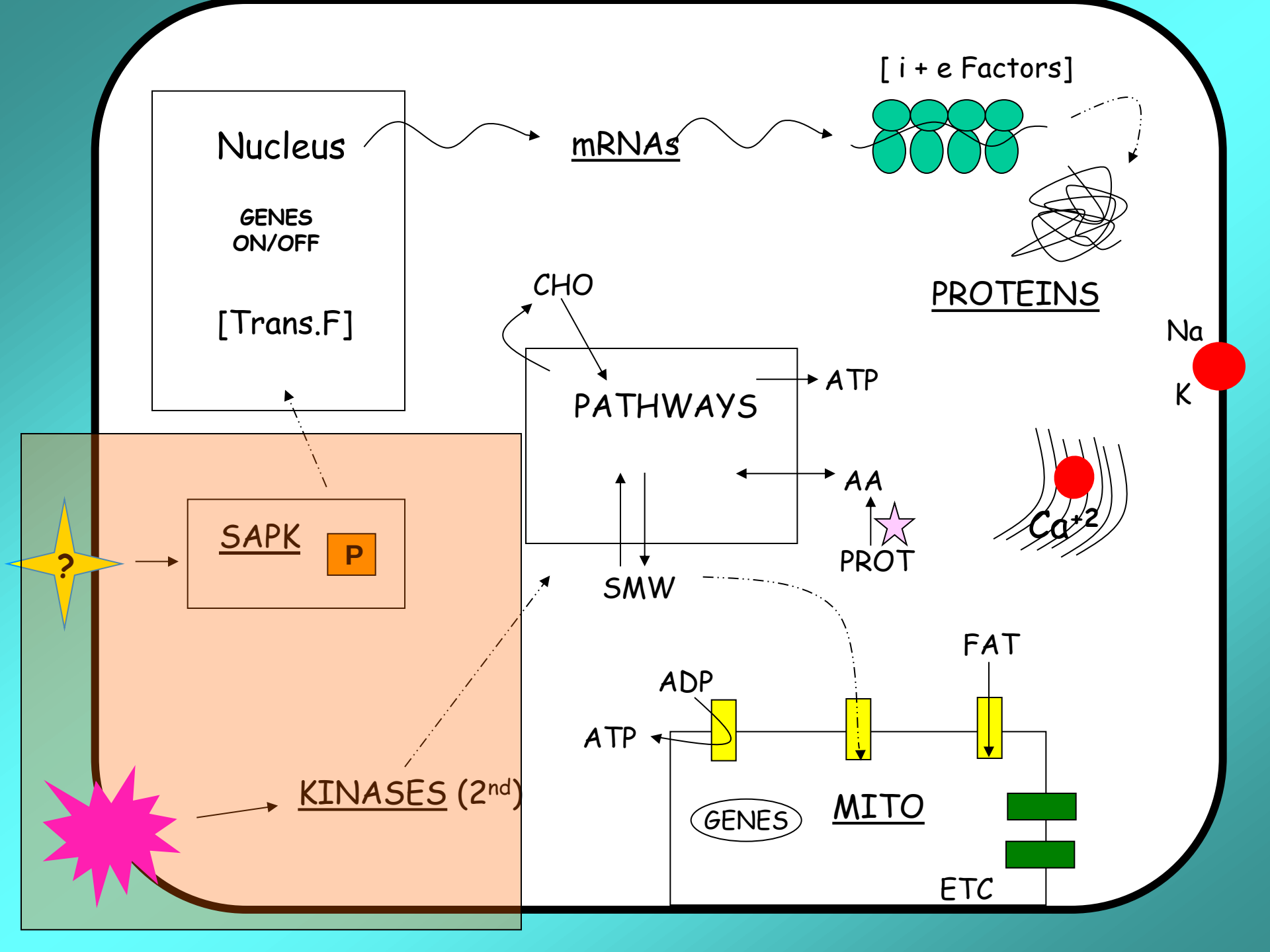
- **microRNA**
- **phospho-RNA Polymerase**
- **Histones modified**
- **HDAC / HAT changes**

CELL PROCESSES



- DNA/RNA synthesis
- Protein synthesis
- Fuel metabolism
- Ion pumping
- Work done

ATP turnover ↓ to <5% of normal


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

FREEZE INDUCED CHANGES

- **Few ‘SAP’ kinases activated**

- **Results: p38, AMPK, JNK, ERK, PKA, PKC, PERK, Fuel Pathways**
- **Protein Phosphatases (1, 2A, 2C)**

Zhang J, Tessier SN, Storey KB. 2011. *In:* Hypometabolism: Strategies of Survival in Vertebrates and Invertebrates. (Nowakowska, A. and Caputa, M., ed.), Research Signpost. pp. 147-182.

FREEZE INDUCED CHANGES

Metabolic Depression :

- **Few 'Stress' kinases activated**

- **Gene 'inactivation' (mRNA)**
- **Few Genes activated**

Dieni CA, Storey KB. 2011. Comp. Biochem. Physiol. B 159, 236-243
Dieni CA, Storey KB. 2010. J. Comp. Physiol. B 180, 1133-1142.
Dieni CA, Storey KB. 2009. Comp. Biochem. Physiol B 152, 405-412.

FREEZE INDUCED GENE CHANGES

* Few Genes activated
  - Frozen Life !

- Transcription factors
  - shock proteins
  - antioxidant enzymes

cDNA Arrays
- Methods
- Materials
- Sources

DNA Libraries

Carleton UNIVERSITY

LINKS: Institute of Biochemistry | Department of Biology | Department of Chemistry | Faculty of Graduate Studies and Research | Carleton University homepage | Carleton library electronic journals | PUBMED | Safety - MSDS Online | Storey lab Chemical List

Kenneth B. Storey
Ph.D., F.R.S.C.

Canada Research Chair in Molecular Physiology

Professor of Biochemistry
Institute of Biochemistry,
and Departments of
Biology and Chemistry

Email: kenneth_storey@carleton.ca

ISI Highly Cited Researcher
Click to view record

Contact information:
Institute of Biochemistry
College of Natural Sciences
1125 Colonel By Drive
Ottawa, Ontario, Canada
K1S 5B6

Tel: +1 613 520-3678
Fax: +1 613 520-2569
Office: 508 Steacie
Chemistry Bldg

New book

FUNCTIONAL METABOLISM: REGULATION & ADAPTATION
edited by K.B. Storey, Wiley-Liss

LINK

In the News !

Diabetes theory, **NY Times** May 2005
Suspended animation, US News & World Report, May 2005
DISCOVER magazine, Feb. 2005
Carleton U. magazine, Winter 2005
Washington Post feature, Dec. 2004

Links: book, magazine and newspaper articles ** Ken in fiction ** Wood frog fiction ** Ontario high school texts

Canada Research Chair Tier 1;
PHOTO; Ken's profile

THE LAB

Research Interests	Professional Information
Positions available	NEW Reviews & Popular Articles
Lab personnel, Past and Present	RECENT PUBLICATIONS 1996 - Present
Free computer programs	Publications 1986 - 1995
Array-it - Info on running cDNA arrays	Publications 1974 - 1985
PHOTO GALLERY -- our animals & their stories	Book, magazine, newspaper articles & Fiction

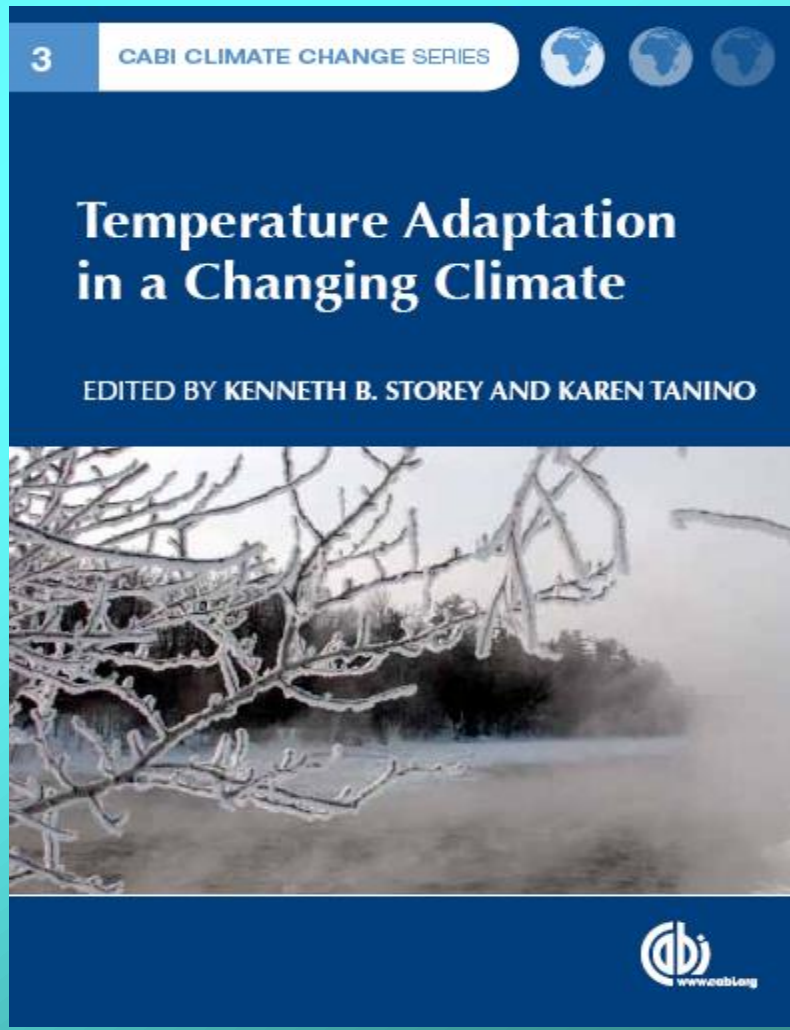
Molecular Adaptation to Climate Change:

Challenges for Amphibians & Reptiles

Freezing survival

Estivation

Anoxia tolerance



Garter snake, *Thamnophis sirtalis*

Painted turtle hatchlings

Chrysemys picta marginata

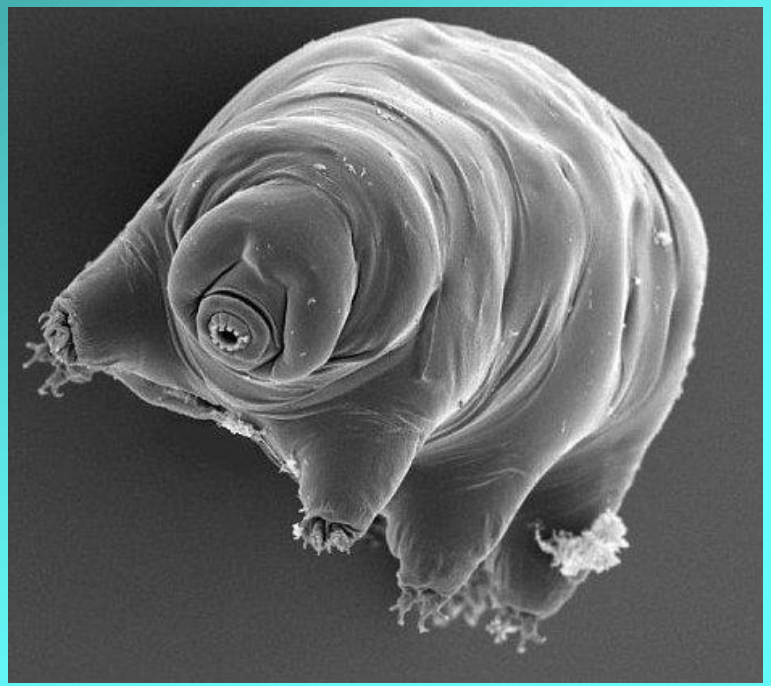
Box turtle, *Terrapene carolina*


GRAY TREE FROG
Hyla versicolor

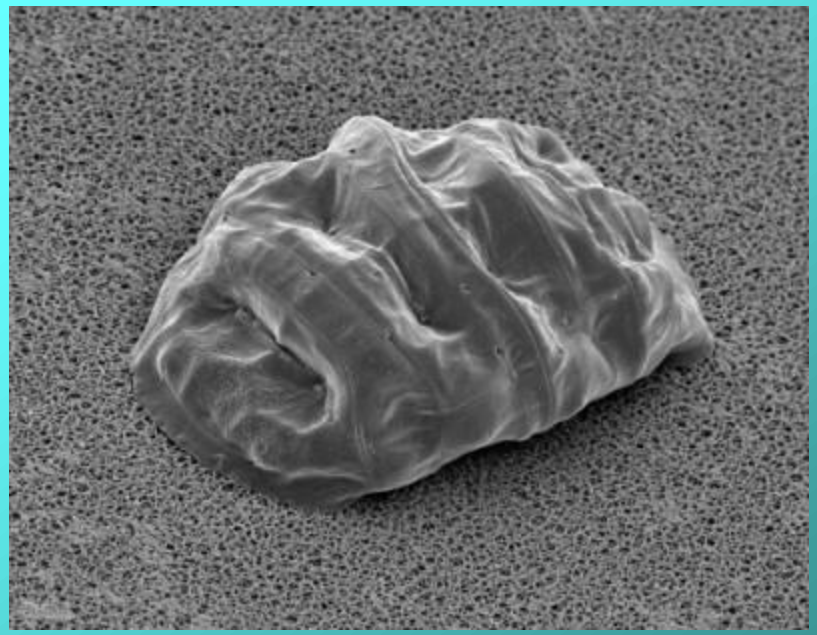
CHORUS FROG
Pseudacris triseriata

SPRING PEEPER
Pseudacris crucifer

ANHYDROBIOSIS



Tardigrade



Atrophy – Hypertrophy

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

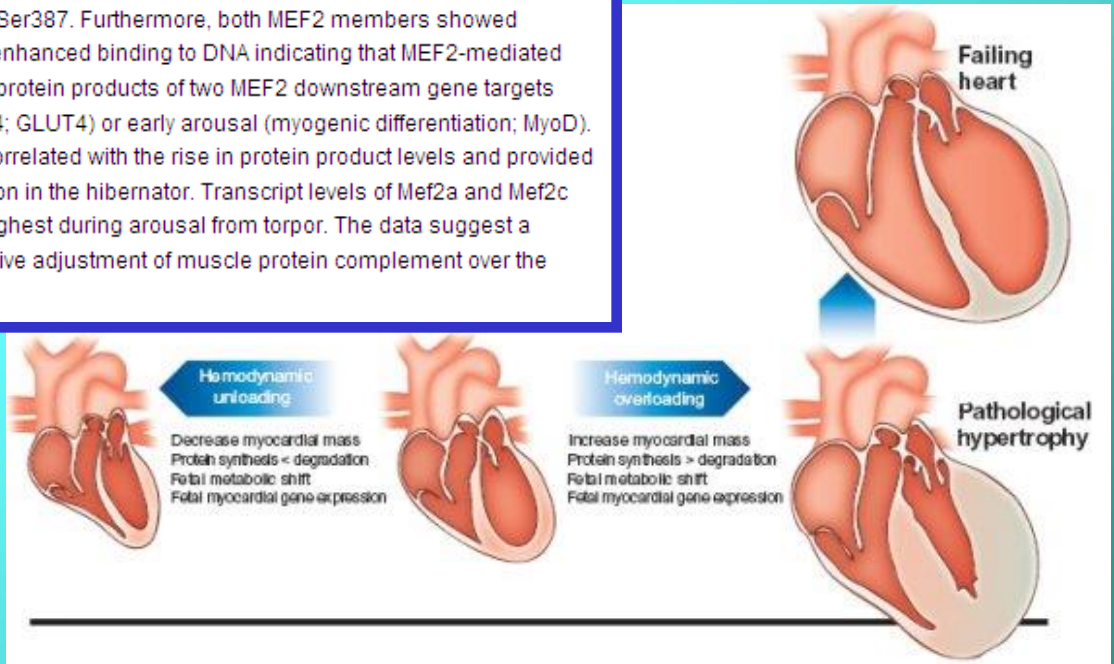
Expression of myocyte enhancer factor-2 and downstream genes in ground squirrel skeletal muscle during hibernation.

Tessier SN, Storey KB.

Institute of Biochemistry & Department of Biology, Carleton University, Ottawa, ON, Canada.

Abstract

Myocyte enhancer factor-2 (MEF2) transcription factors regulate the expression of a variety of genes encoding contractile proteins and other proteins associated with muscle performance. We proposed that changes in MEF2 levels and expression of selected downstream targets would aid the skeletal muscle of thirteen-lined ground squirrels (Spermophilus tridecemlineatus) in meeting metabolic challenges associated with winter hibernation; e.g., cycles of torpor-arousal, body temperature that can fall to near 0°C, long periods of inactivity that could lead to atrophy. MEF2A protein levels were significantly elevated when animals were in torpor (maximally 2.8-fold higher than in active squirrels) and the amount of phosphorylated active MEF2A Thr312 increased during entrance into torpor. MEF2C levels also rose significantly during entrance and torpor as did the amount of phosphorylated MEF2C Ser387. Furthermore, both MEF2 members showed elevated amounts in the nuclear fraction during torpor as well as enhanced binding to DNA indicating that MEF2-mediated gene expression was up-regulated in torpid animals. Indeed, the protein products of two MEF2 downstream gene targets increased in muscle during torpor (glucose transporter isoforms 4; GLUT4) or early arousal (myogenic differentiation; MyoD). MyoD mRNA transcript levels correlated with the rise in protein product levels and provided MEF2-mediated gene expression in the hibernator. Transcript levels of Mef2a and Mef2c erns with levels of both being highest during arousal from torpor. The data suggest a gene transcription in the selective adjustment of muscle protein complement over the



Unavoidable metabolic costs

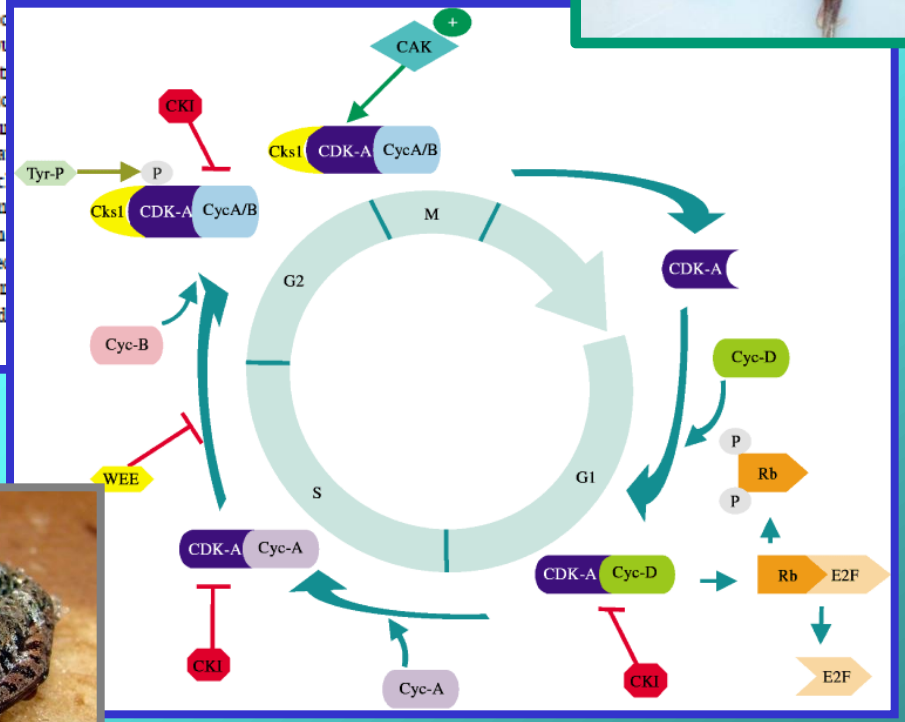
Current Genomics, 2009, *10*, 573-584

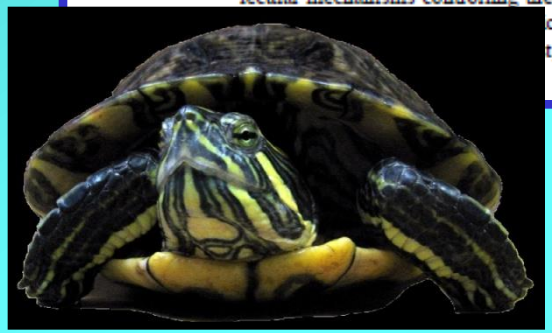
Perspectives in Cell Cycle Regulation: Lessons from an Anoxic Vertebrate

Kyle K. Biggar and Kenneth B. Storey*

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

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





Available online at www.sciencedirect.com
ScienceDirect
Cryobiology 53 (2006) 310–318
CRYOBIOLOGY
www.elsevier.com/locate/ycryo

Evidence for a reduced transcriptional state during hibernation in ground squirrels ☆

Pier Jr Morin*, Kenneth B. Storey

Institute of Biochemistry and Department of Chemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ont., Canada K1S 5B6

Received 14 March 2006; accepted 4 August 2006
Available online 18 September 2006

Abstract

During mammalian hibernation, metabolic rate can be reduced to <5% of the euthermic rate as suppression of multiple energy expensive metabolic processes. Gene transcription is one of these examines mechanisms of transcriptional control that could contribute to lowering the rate of gen Histone deacetylases (HDAC) have been linked to gene silencing and measured HDAC activity skeletal muscle of hibernating thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, co controls. Western blotting also showed that HDAC1 and HDAC4 protein levels were 1.21-and 1. tively, in muscle from torpid animals. Histone H3 was also evaluated by Western blotting. Total hist but two forms of covalently modified histone H3 that are associated with active transcription (pho acetylated Lys 23) were significantly reduced by 38–39% in muscle during hibernation. Finally, RNA was measured using a PCR-based approach; activity in muscle from hibernating squirrels was only value. These data support an overall decrease in transcriptional activity in skeletal muscle of hibe accomplished by multiple molecular mechanisms.

Transcription Suppression in Hibernator Muscle

- Phospho-Histone H3 (Ser10) levels reduced
  * Inhibits transcription
- Histone Deacetylase activity increased 80%
- Acetyl-Histone H3 (Lys23) levels reduced
  * Both inhibit transcription *
- HDAC 1 & 4 protein levels increased
- RNA Polymerase II activity Decreased

GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

No Modification

PROTEINS (ENZYMES)

Control by proteases

Degradation

INACTIVE ENZYME

Covalent modification

Control by post-translational modification

FUNCTIONAL ENZYMES

Inhibition and Activation

Control at level of enzyme function

ACTIVE ENZYMES