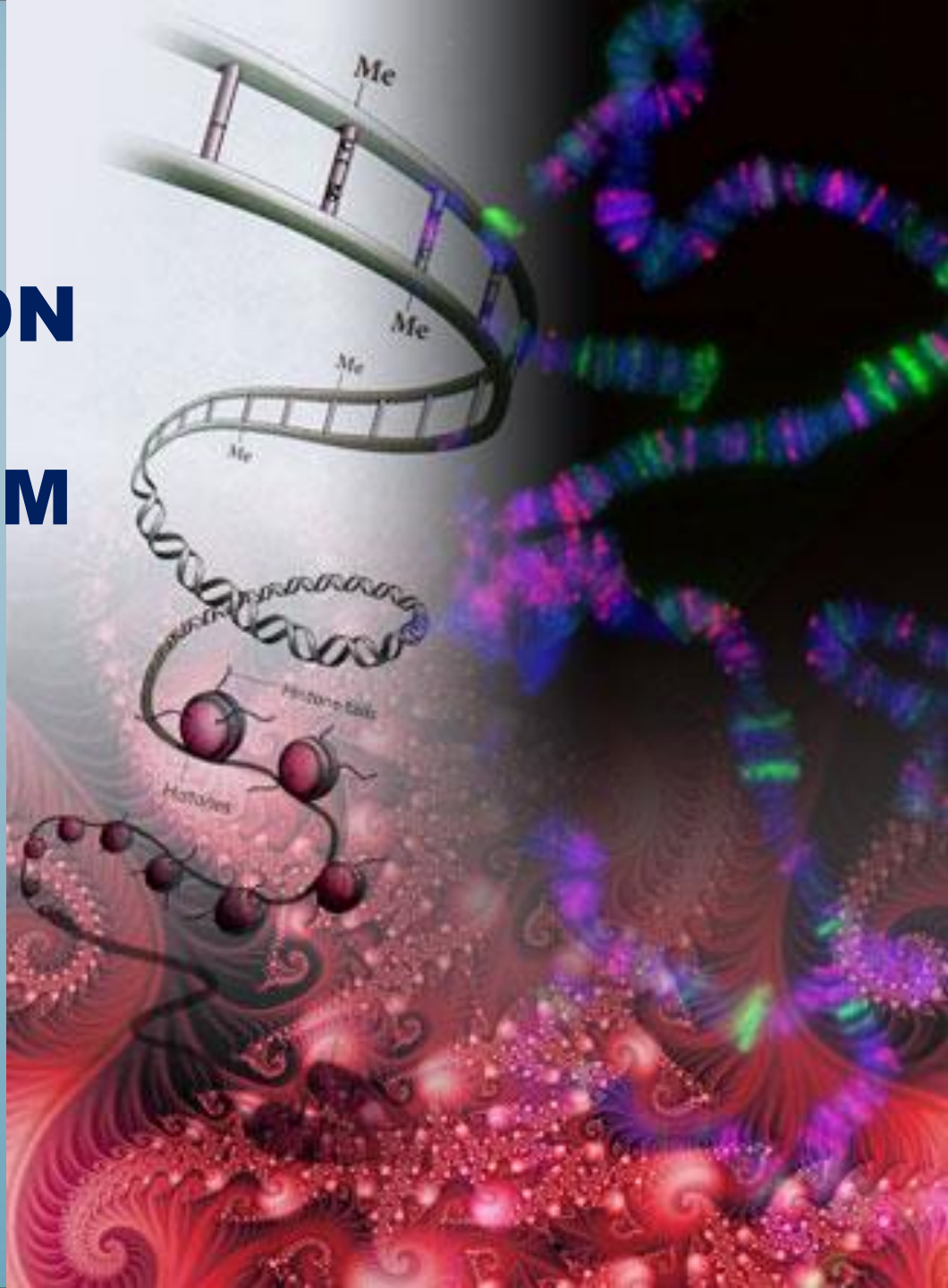
EPIGENETICS, GENE REGULATION & HYPOMETABOLISM

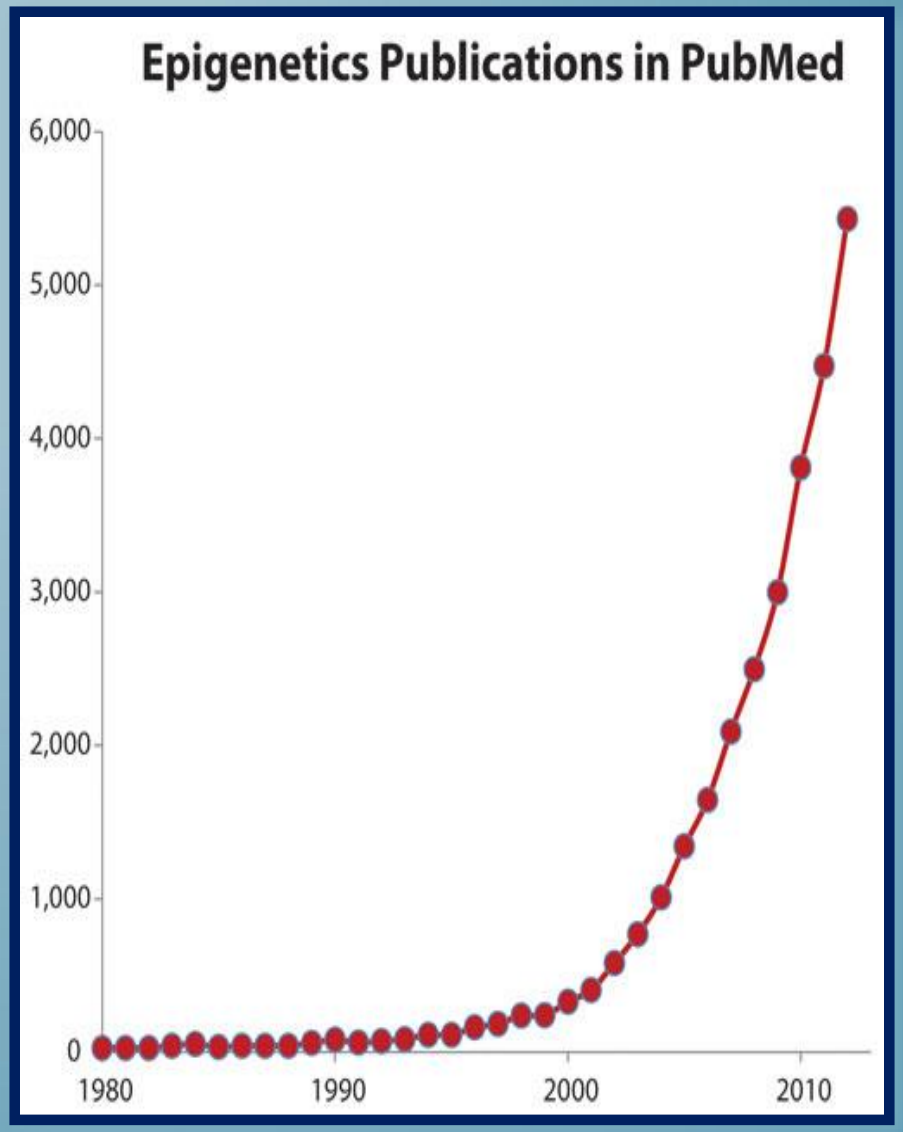


Kenneth B. Storey
Carleton University

www.carleton.ca/~kbstorey

EPIGENETIC RESEARCH

The number of publications in the field increased dramatically in the last 10 years.



Genetic Engineering and Biotechnology News Feb 1, 2013 (Vol. 33, No. 3)

METABOLIC RATE DEPRESSION

Hibernation

Anoxia

Estivation

Freezing

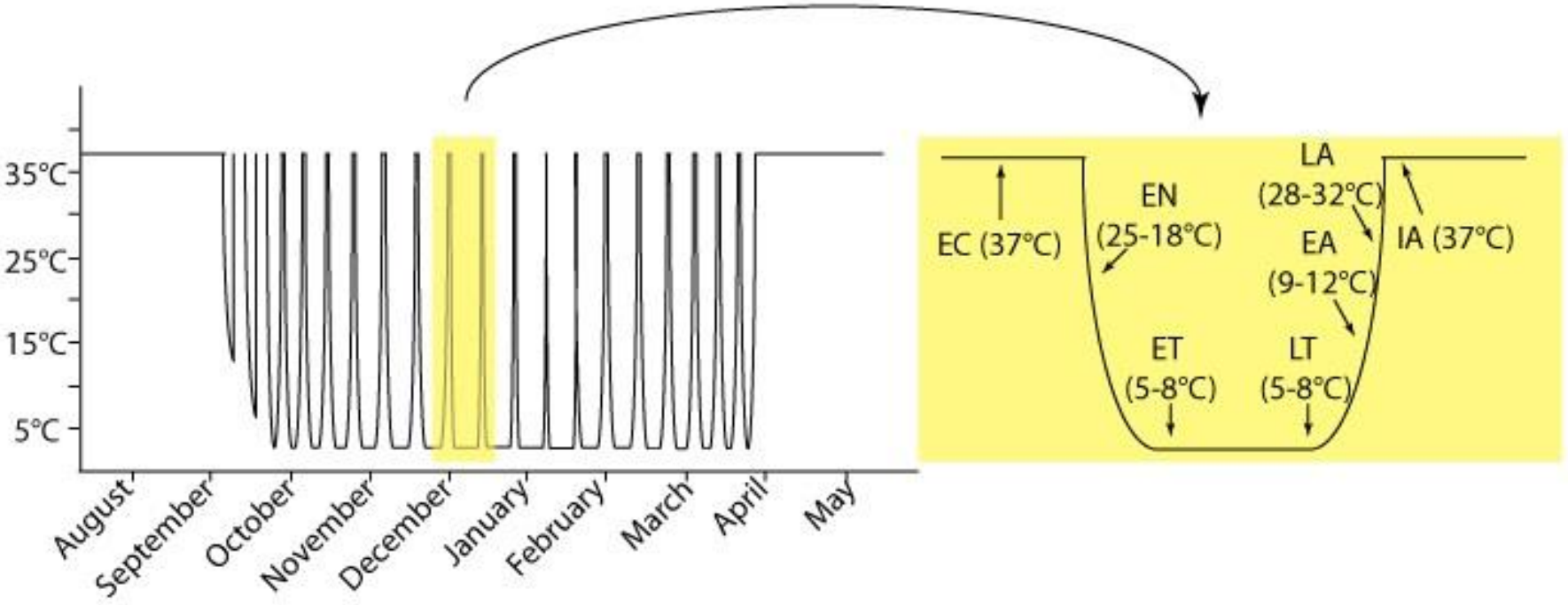
Diapause

MAMMALIAN HIBERNATION

- Key characteristics :
  - metabolic rate depression (hypometabolism)
  - low body temperatures
  - Hibernation is a NATURAL model system

- Purpose is to overcome food shortages and the high energetic costs of endothermy (warm-blooded)

TORPOR-AROUSAL



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

Figure adapted from Nelson *et al.* 2009

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 TOLERANCE

Painted turtle
Chrysemys picta

Red-eared turtle
Trachemys scripta elegans

Periwinke
Littorina littorea

FREEZE TOLERANCE

Wood frog
Rana sylvatica

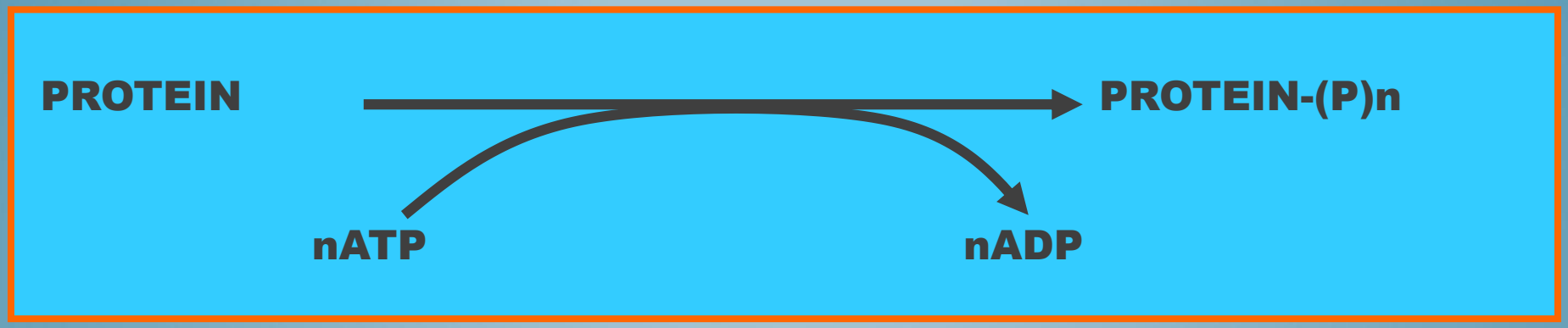
PRINCIPLES OF MRD

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

Same for ALL systems

www.carleton.ca/~kbstorey

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)

Phospho / de-Phospho

PATHWAY CONTROLS :

- ALL PATHWAYS, REGULATION IN MINUTES,
- REVERSED BY PROTEIN PHOSPHATASES
- METABOLIC COST = <1 % TOTAL ENERGY
- MANY NEW ENZYME TARGETS DISCOVERED

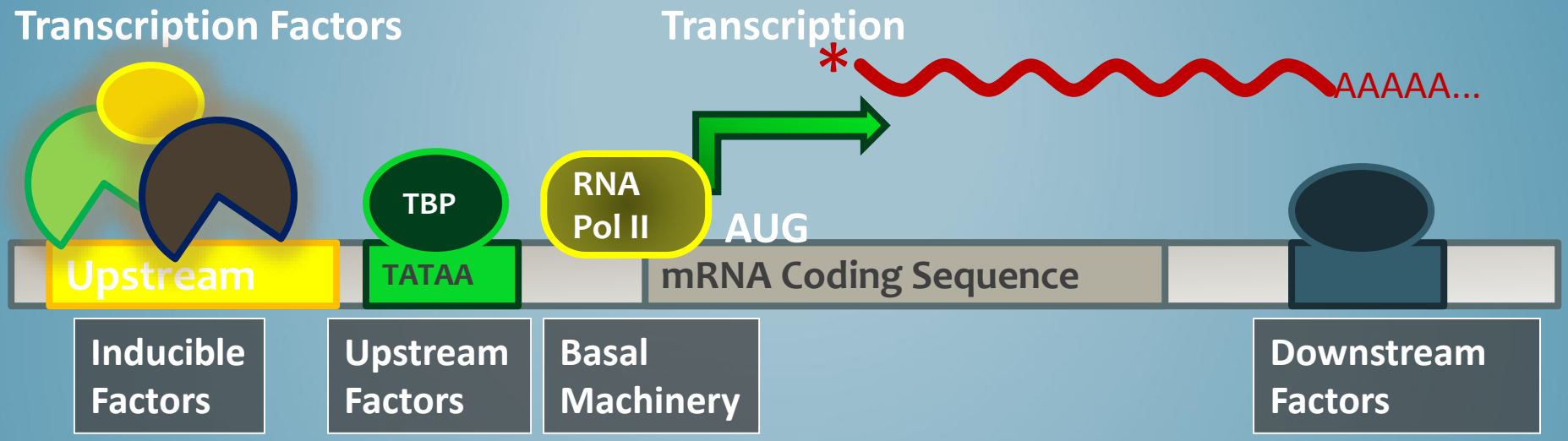
- Glycolysis (GP, GS, PFK, PK)
- Fat synthesis (ATP-CL, ACC)
- CHO fuel use (PDH)
- Translation (eIF2α, eEF2)
- Ion pumps (NaK, Ca-ATPase)

PRINCIPLES OF MRD

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

Same for ALL systems

Regulation of Gene Transcription



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

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

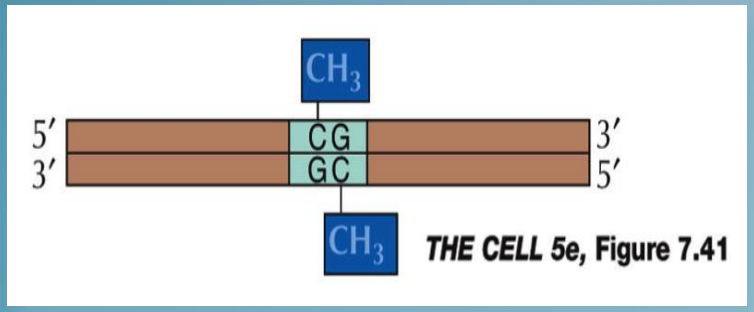
- *ATF (Glucose Regulated Proteins)*
- *HIF (O_2), HSF (Hsp)*
- *NFkB (IkB-P), Nrf-2, NRF-1*
- *PPAR, PGC, RXR, chREBP, CREB-P*
- *STAT, SMAD, p53-P, HNF, AP (1,2)*

- *Methods: EMSA, CHiP*

PRINCIPLES OF MRD

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

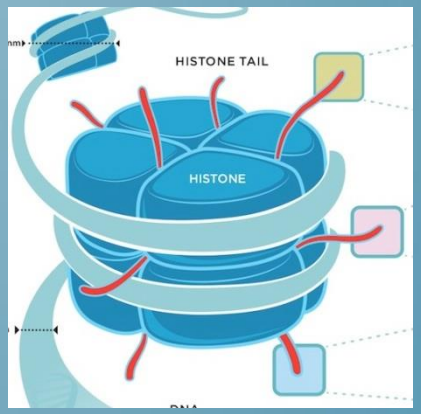
Same for ALL systems

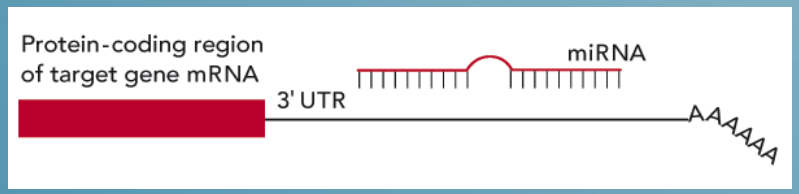


1. DNA Methylation. Methylation of cytosines at CpG dinucleotides in promoter regions. Methylation attenuates gene expression.

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

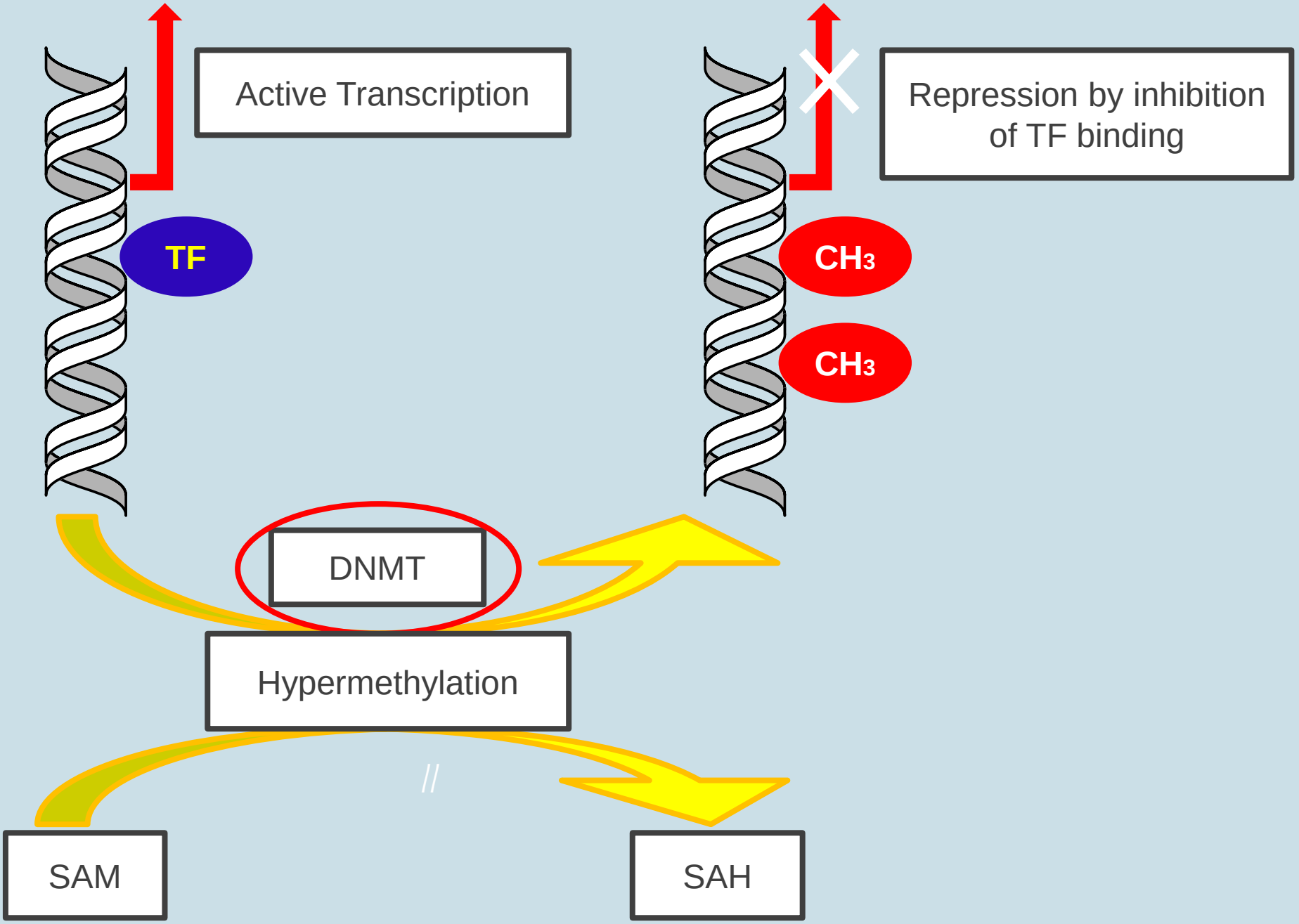
2. Histone Modification. Post-translational modifications on histone tails affect histone:DNA interactions to influence accessibility of promoter regions to transcriptional machinery.



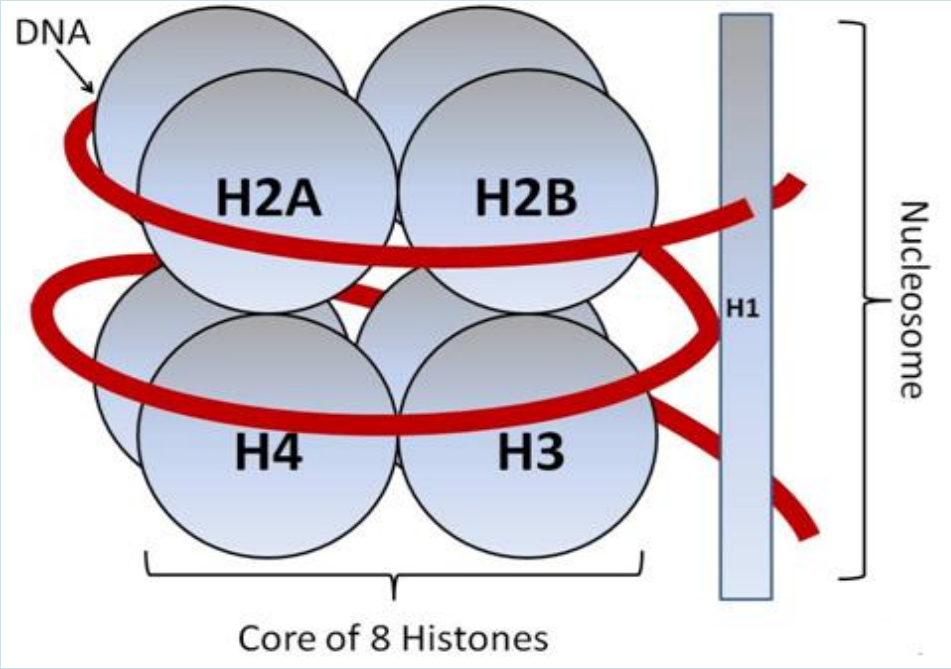


3. Non coding RNAs. microRNAs base-pair with complementary sequences in mRNA to achieve translational repression or target degradation

DNA METHYLATION



HISTONE MODIFICATION

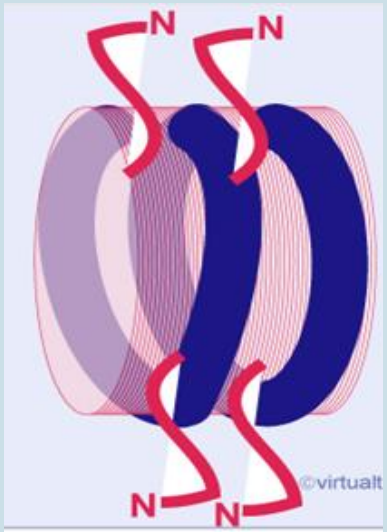


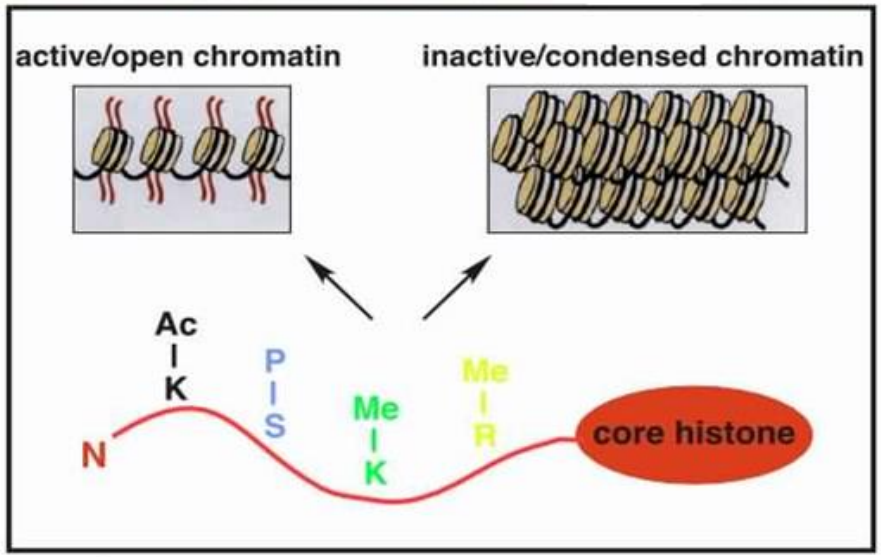
Nucleosome:

- Made of 8 histone proteins (Octamer) + linker protein
- Chromatin wraps around the histones ("beads on a string")

3 main types of modifications:

- Methylation
- Acetylation
- Phosphorylation

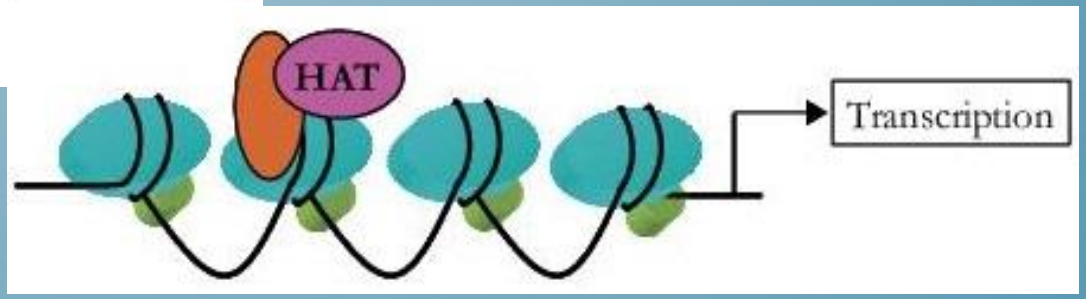




Higher compaction = Lower expression

THE "HISTONE CODE"

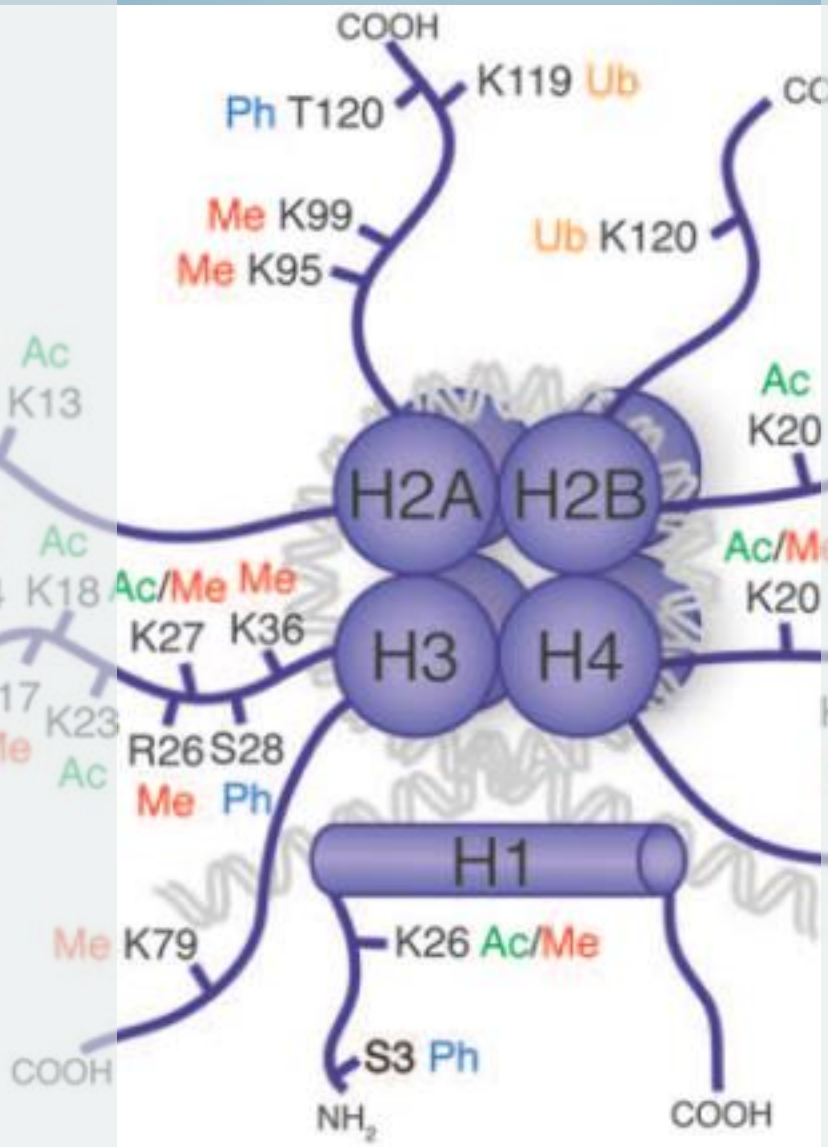
This code is maintained by:
"WRITERS," enzymes that can methylate and acetylate
"ERASERS," enzymes that can demethylate and deacetylate
"READERS," enzymes that recognize, bind and recruit other proteins to the modifications



The recruited proteins then act to alter chromatin structure to promote or repress transcription.

HISTONE MODIFICATIONS

H3-Acetyl-Lys27
H3-Trimethyl-Lys4
H3-Monomethyl-Lys27
H3-Monomethyl-Arg8
H3-Dimethyl-Lys27
H3-Dimethyl-Arg8
H3-Di/Trimethyl-Lys27
H3-Acetyl-Lys9
H3-Trimethyl-Lys27
H3-Panmethyl-Lys9
H3-Phospho-Ser28
H3-Monomethyl-Lys9
H3-Phospho-Ser31
H3-Dimethyl-Lys9
H3-Acetyl-Lys36
H3-Trimethyl-Lys9
H3-Dimethyl-Lys36
H3-Phospho-Ser10
H3-Trimethyl-Lys36
H3-Phospho-Ser28
H3-Dimethyl-Arg2
H3-Monomethyl-Lys18



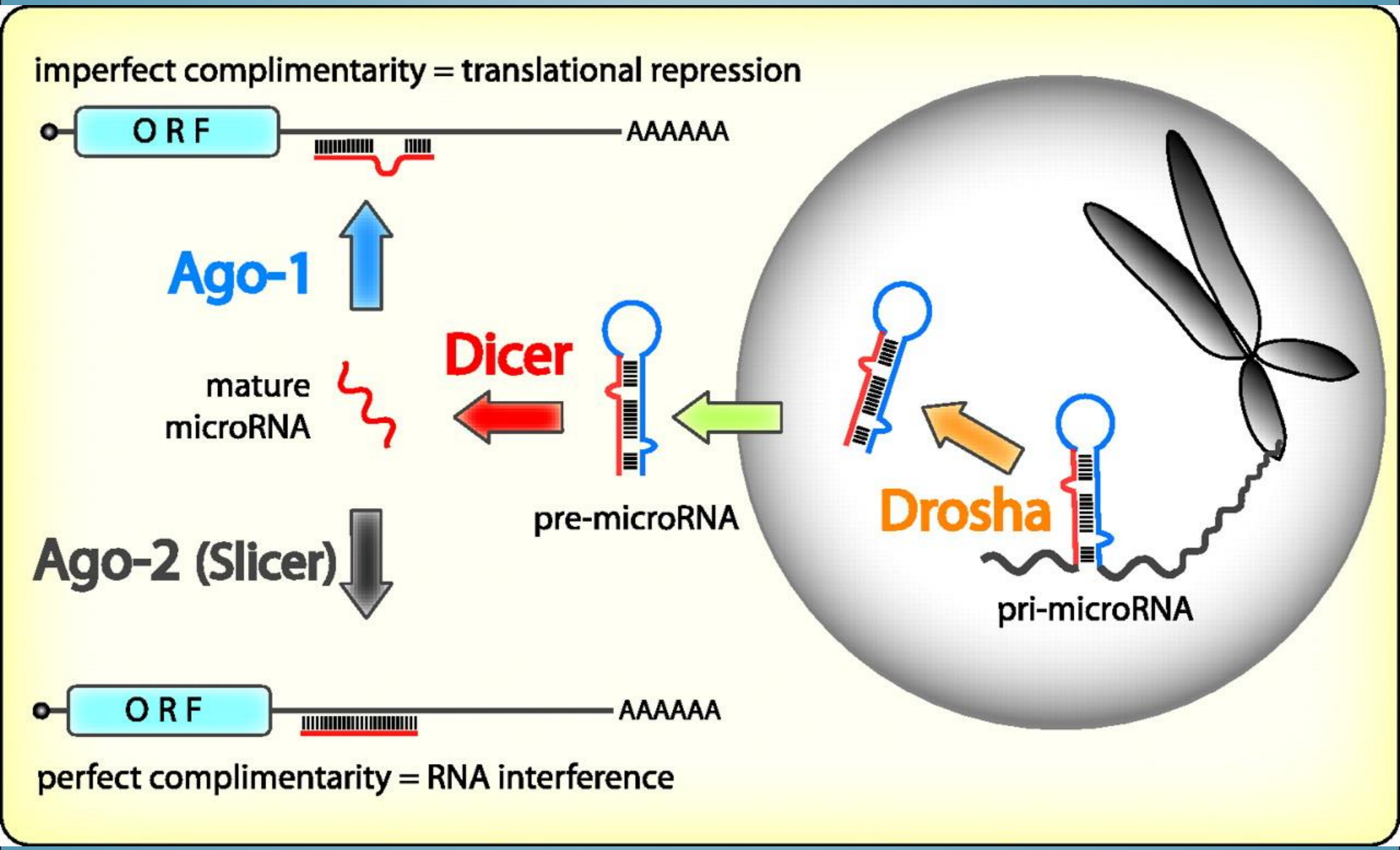
H3-Monomethyl-Lys79H3-Phospho-Thr3
H3-Acetyl-Lys23
H3-Dimethyl-Lys79
H3-Acetyl-Lys4
H3-Monomethyl-Lys23
H3-Monomethyl-Lys122
H3-Monomethyl-Lys4
H3-Dimethyl-Lys23
H3-Dimethyl-Lys4
H3-Phospho-Thr45
H3-Phospho-Thr11
H3-Acetyl-Lys56
H3-Acetyl-Lys14
H3-Monomethyl-Lys56
H3-Dimethyl-Lys14
H3-Dimethyl-Lys56
H3-Dimethyl-Arg17
H3-Acetyl-Lys64
H3- Acetyl-Lys18
H3-Acetyl-Lys79
ETC……

EPIGENETIC MODIFICATION: NON-CODING RNAs

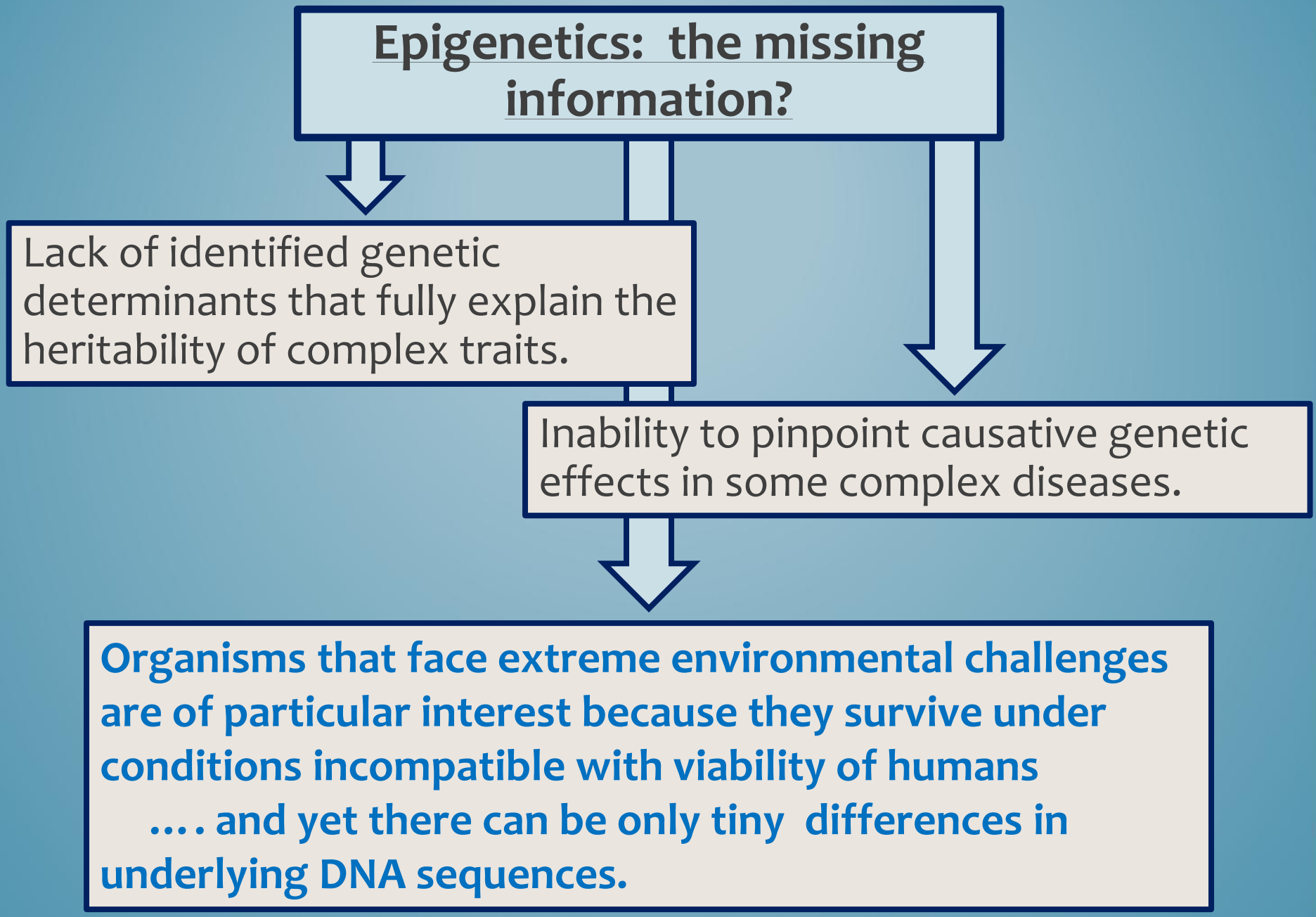
A non-coding RNA is a functional RNA molecule that is not translated into a protein.

siRNAs, microRNAs (~22 nucleotides; fine tune gene expression)

A mechanism for post-transcriptional gene regulation.



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


Epigenetics: the missing information?
Lack of identified genetic determinants that fully explain the heritability of complex traits.
Inability to pinpoint causative genetic effects in some complex diseases.
Organisms that face extreme environmental challenges are of particular interest because they survive under conditions incompatible with viability of humans
…. and yet there can be only tiny differences in underlying DNA sequences.

TURNING OFF GENES: ROLE OF EPIGENETICS

Epigenetics:

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

Common mechanisms:

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

Global changes in methylation of gene promoters to reduce transcription rates

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

Transcription and translation are ATP-expensive

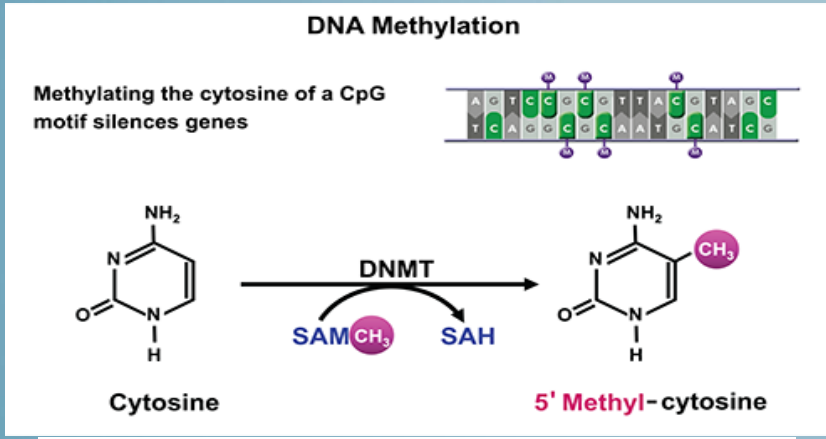
Epigenetic modifications could alter rates of transcription/translation to produce energy savings in hypometabolism

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

Other post-transcriptional controls can apply –

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

DNA Methylation & Gene Silencing



http://pubs.niaaa.nih.gov/publications/arcr351/6-16.htm

Changes in DNA methylation modifies gene transcription

Affects: development, disease, phenotypic plasticity, seasonal changes, behaviour, etc.

Integr Comp Biol. 2014 Jul;54(1):68-76. doi: 10.1093/icb/icu034. Epub 2014 May 10.

The dynamic nature of DNA methylation: a role in response to social and seasonal variation.

Alvarado S[1], Fernald RD[2], Storey KB[2], Szyf M[3].

Author information

Abstract

An organism's ability to adapt to its environment depends on its ability to regulate and maintain tissue specific, temporal patterns of gene transcription in response to specific environmental cues. Epigenetic mechanisms are responsible for many of the intricacies of a gene's regulation that alter expression patterns without affecting the genetic sequence. In particular, DNA methylation has been shown to have an important role in regulating early development and in some human diseases. Within these domains, DNA methylation has been extensively characterized over the past 60 years, but the discovery of its role in regulating behavioral outcomes has led to renewed interest in its potential roles in animal behavior and phenotypic plasticity. The conservation of DNA methylation across the animal kingdom suggests a possible role in the plasticity of genomic responses to environmental cues in natural environments. Here, we review the historical context for the study of DNA methylation, its function and mechanisms, and provide examples of gene/environment interactions in response to social and seasonal cues. Finally, we discuss useful tools to interrogate and dissect the function of DNA methylation in non-model organisms.

Alvarado, S., Fernald, R.D., Storey, K.B., and Szyf, M. 2014.

DNA Methylation & Mammalian Hibernation

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

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

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

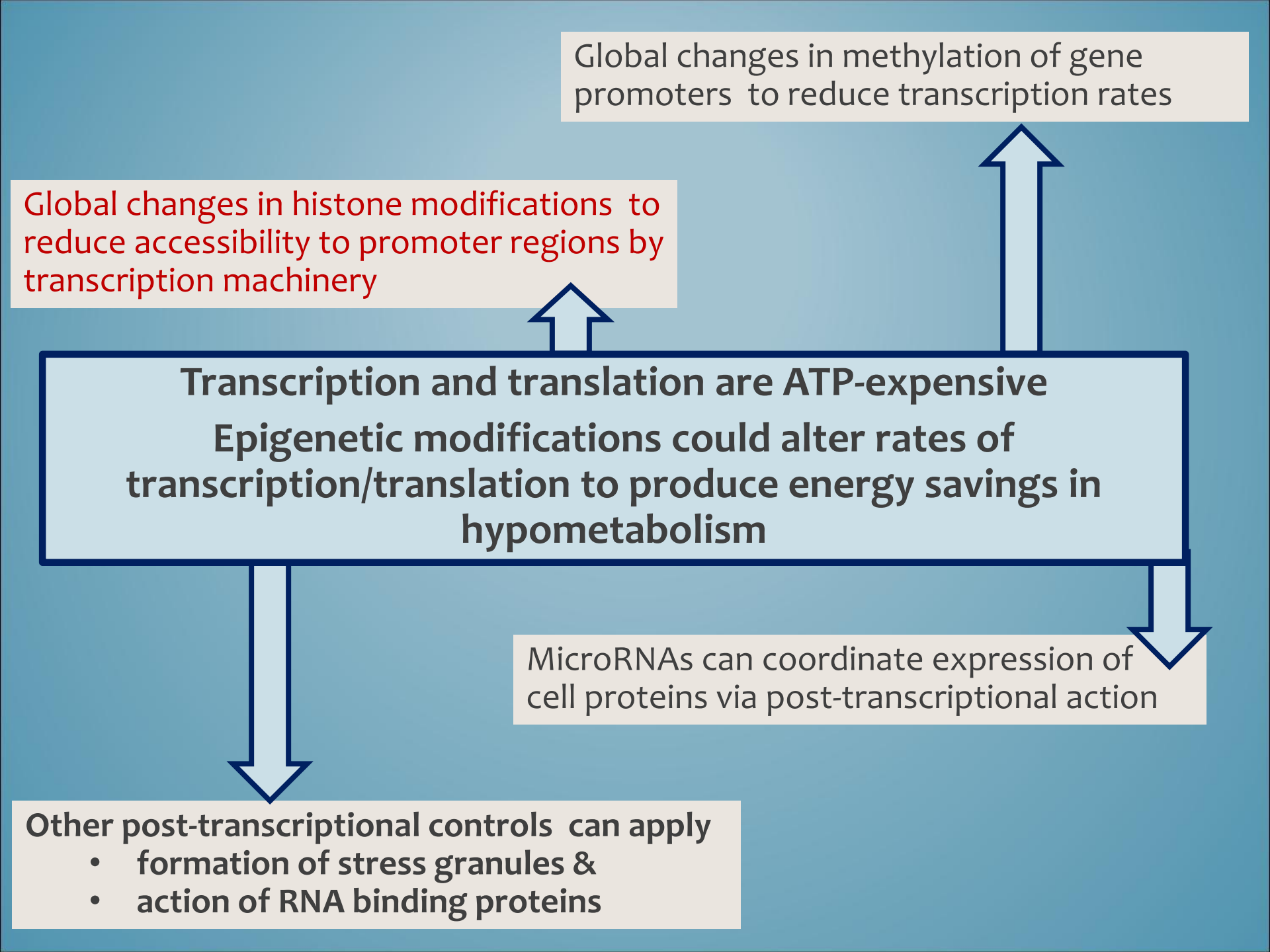
Author information

Abstract

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

Alvarado, S., Mak, T., Liu, S., Storey, K.B., and Szyf, M. 2015. in press

Changes in DNA methylation & DNMTs restrict gene transcription during torpor


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

Histone Deacetylases & Mammalian Hibernation

Available online at www.sciencedirect.com
ScienceDirect
CRYOBIOLOGY
Cryobiology 53 (2006) 310–318
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 a result of coordinated suppression of multiple energy expensive metabolic processes. Gene transcription is one of these and the present study examines mechanisms of transcriptional control that could contribute to lowering the rate of gene expression in torpor. Histone deacetylases (HDAC) have been linked to gene silencing and measured HDAC activity was 1.82-fold higher in skeletal muscle of hibernating thirteen-lined ground squirrels, *Spermophilus tridecemlineatus*, compared with euthermic controls. Western blotting also showed that HDAC1 and HDAC4 protein levels were 1.21-and 1.48-fold higher, respectively, in muscle from torpid animals. Histone H3 was also evaluated by Western blotting. Total histone H3 was unchanged but two forms of covalently modified histone H3 that are associated with active transcription (phosphorylated Ser 10 and acetylated Lys 23) were significantly reduced by 38–39% in muscle during hibernation. Finally, RNA polymerase II activity was measured using a PCR-based approach; activity in muscle from hibernating squirrels was only 57% of the euthermic value. These data support an overall decrease in transcriptional activity in skeletal muscle of hibernating animals that is accomplished by multiple molecular mechanisms.

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

Transcription Suppression in Hibernation

- 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

Histone Deacetylases & Anoxia Tolerance

Mol Cell Biochem (2010) 342:151–161
DOI 10.1007/s11010-010-0479-5

Epigenetics in anoxia tolerance: a role for histone deacetylases

Anastasia Krivoruchko · Kenneth B. Storey

010/ Accepted: 17 April 2010 / Published online: 1 May 2010
ness Media, LLC. 2010

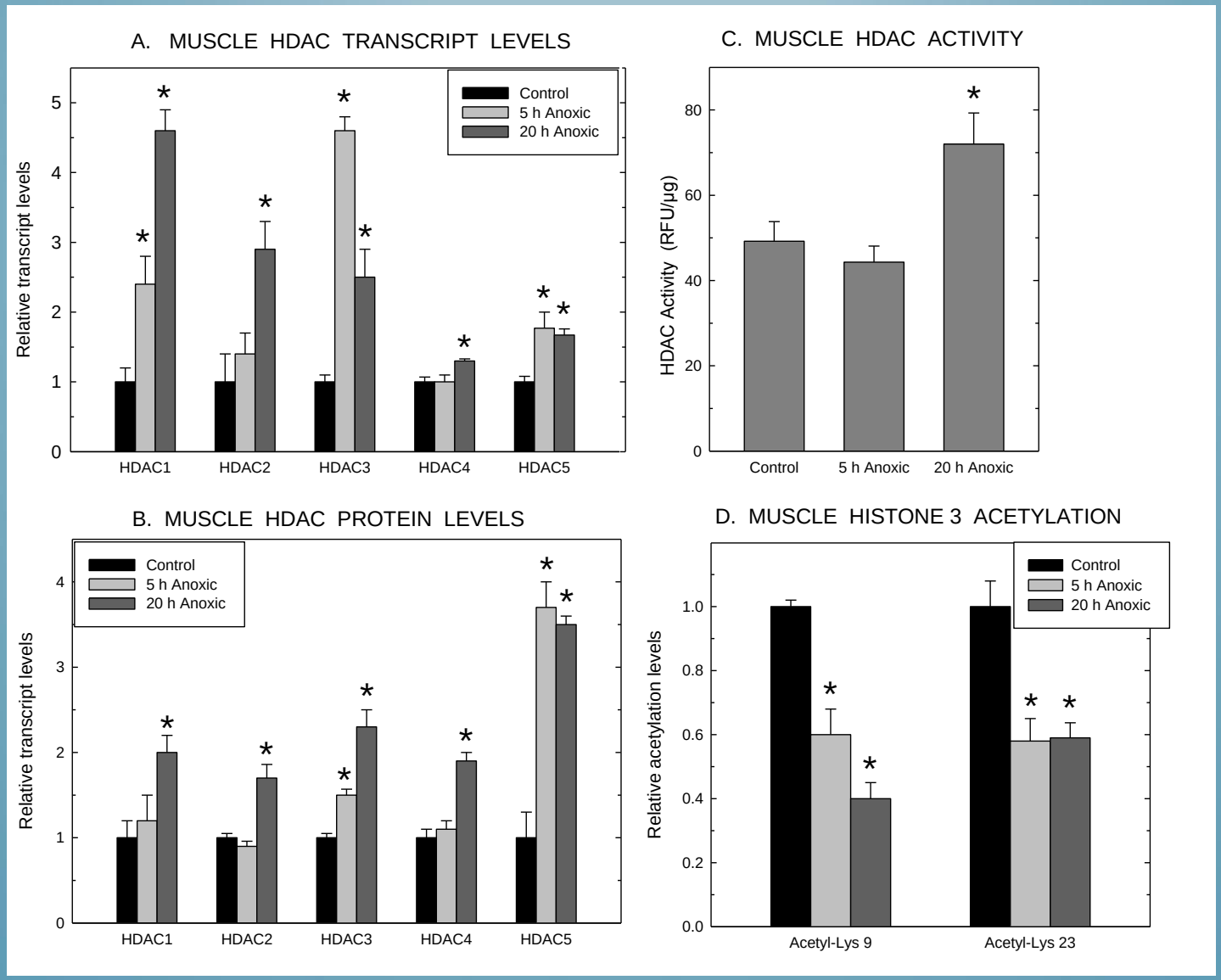
rtance of epigenetics has been estab-
ological processes but the relevance of
nism to animal survival of low oxygen
been examined. To establish whether
s could be involved in natural anoxia
tolerance, we have examined the anoxia-responsive expres-
sion of the transcriptional silencers, histone deacetylases
(HDACs), in tissues of a unique model for anoxia tolerance,

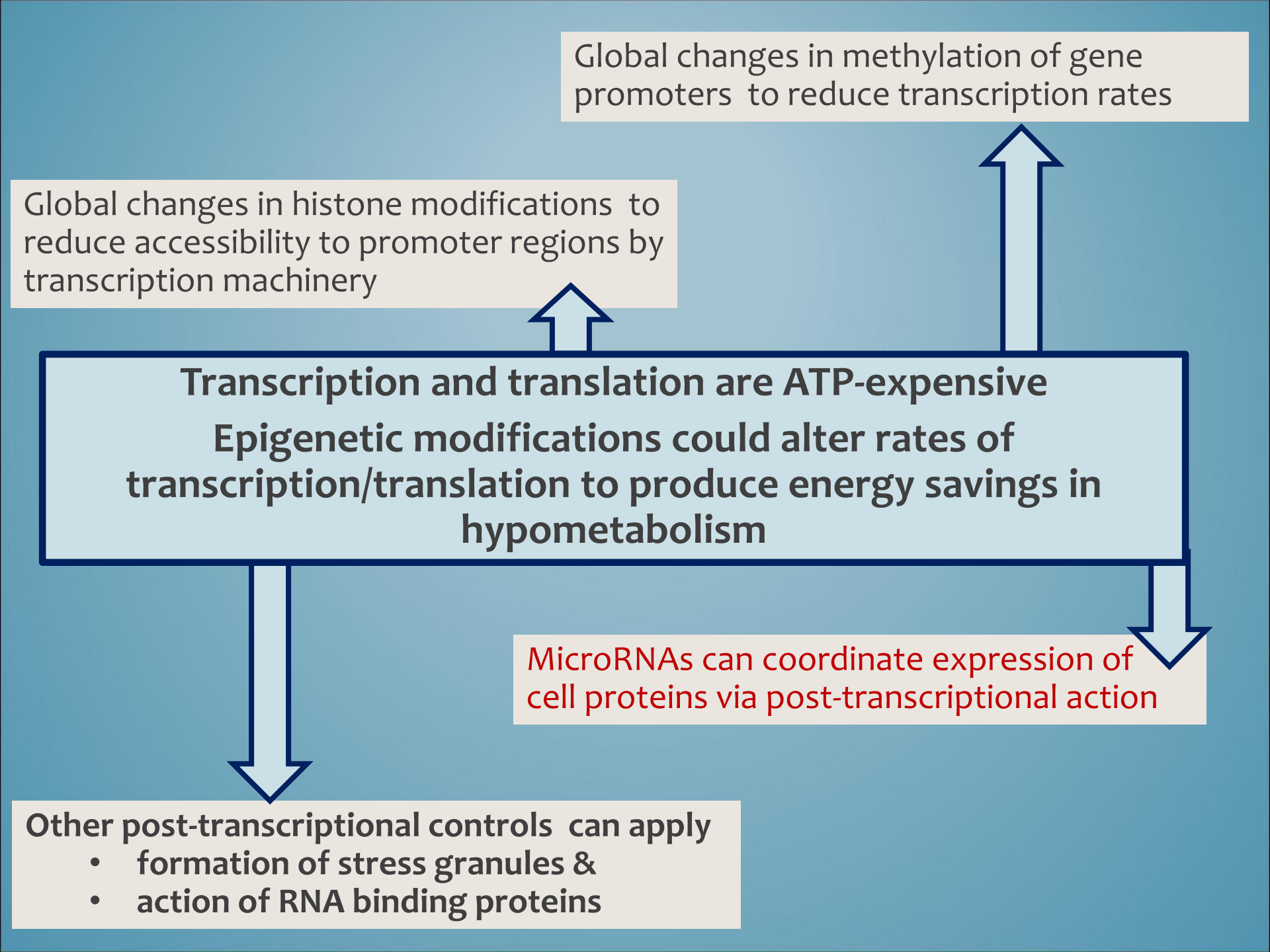
by contributing to
this hypometaboli

Keywords *Trac*
Anoxia tolerance
Epigenetics · Hist

Histone deacetylases are involved in natural anoxia tolerance

HDAC Responses to anoxia in turtle white muscle

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MicroRNAs can coordinate expression of cell proteins via post-transcriptional action
Other post-transcriptional controls can apply
• formation of stress granules &
• action of RNA binding proteins

Turning it all off

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

Review

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

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 p… cell cycle ar… ous disease… attack in hu…

Biochimica et Biophysica Acta 1779 (2008) 628–633

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

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

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

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
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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 during hibernation but decreased by 60% in kidney. These data are the first report that differential regulation

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

ARE MicroRNAs 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)

MicroRNAs and Regulation of the Cell Cycle

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 focus of numerous biochemical studies. When anoxia tolerant turtles are faced with periods of oxygen deprivation, numerous physiological and biochemical alterations take place in order to facilitate vital reductions in ATP consumption. Such strategies include reversible post-translational modifications as well as the implementation of translation and transcription controls facilitating metabolic depression. Although it is clear that anoxic survival relies on the suppression of ATP consuming processes, the state of the cell cycle in anoxia tolerant vertebrates remain elusive. Several anoxia tolerant invertebrate and embryonic vertebrate models display cell cycle arrest when presented with anoxic stress. Despite this, the cell cycle has not yet been characterized for anoxia [illegible] clinical implications. Uncontrollable [illegible]ebrate tissues. Consequentially, the mo- [illegible]ces. This review article will discuss the [illegible] of the retinoblastoma pathway, the mo- [illegible] possibility of translational

Cell Cycle 11:9, 1705-1713; May 1, 2012; © 2012 Landes Bioscience

SPECIAL FOCUS REPORT

Evidence for cell cycle suppression and microRNA regulation of cyclin D1 during anoxia exposurein turtles

Kyle K. Biggar and Kenneth B. Storey*

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

Key words: *Trachemys scripta elegans*, anoxia, microRNA, cyclin, metabolic rate depression

The red-eared slider turtle (*Trachemys scripta elegans*) has a well-developed natural tolerance for oxygen deprivation that derives from biochemical adaptations, including anoxia-induced suppression of metabolic rate. We hypothesized that mechanisms that suppress ATP-expensive cell cycle activity would contribute significantly to establishing the hypometabolic state during anaerobiosis. Cyclin D1 is a critical regulator of the G_1 phase of the cell cycle and is regarded as key to initiating cell proliferation. The relative protein expression of cyclin D1 was analyzed in both whole-cell and nuclear fractions of liver, kidney and skeletal muscle from turtles exposed to 5 or 20 h of submergence anoxia. Expression of cyclin D1 in both total and nuclear fractions decreased significantly under anoxia in liver and kidney as compared [illegible] in muscle. The relative phosphorylation state of cyclin D1 [illegible]sues. Since phosphorylation of threonine 286 is necessary for [illegible]ternative mechanism is responsible for cyclin D1 suppression [illegible]nange under anoxia in any tissue, so a post-transcriptional [illegible] of *cyclin D1* showed the presence of both an AU-rich region [illegible]*A-15a*. Levels of both microRNAs increased in liver and kidney [illegible]microRNA inhibition of mRNA translation as the mechanism [illegible]e anoxic turtle.

Anoxia elevated miR-16-1 & miR-15a to suppress cyclin D1 protein, a key regulator of cell cycle initiation

MicroRNAs and estivation

Gene 529 (2013) 269–275

Contents lists available at ScienceDirect

Gene

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

Dehydration mediated microRNA response in the African clawed frog *Xenopus laevis*

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

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

ARTICLE INFO

Article history:
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Keywords:
Metabolic rate depression
Amphibian
Iuminex
Non-coding RNA

ABSTRACT

Exposure to various environmental stresses induces metabolic rate depression in many animal species, an adaptation that conserves energy until the environment is again conducive to normal life. The African *Xenopus laevis*, is periodically subjected to arid summers in South Africa, and utilizes en hypometabolic state of estivation as a mechanism of long term survival. During estivation, frogs deal with substantial dehydration as their ponds dry out and *X. laevis* can endure >30% loss of it We hypothesize that microRNAs play a vital role in establishing a reversible hypometabolic state a to dehydration stress that is associated with amphibian estivation. The present study analyzes whole body dehydration on microRNA expression in three tissues of *X. laevis*. Compared to cont *miR-1*, *miR-125b*, and *miR-16-1* decreased to 37 ± 6, 64 ± 8, and 80 ± 4% of control levels during in liver. By contrast, *miR-210*, *miR-34a* and *miR-21* were significantly elevated by 3.05 ± 0.45, 2.1 1.36 ± 0.05-fold, respectively, in the liver. In kidney tissue, *miR-29b*, *miR-21*, and *miR-203* wer 1.40 ± 0.09, 1.31 ± 0.05, and 2.17 ± 0.31-fold, respectively, in response to dehydration whereas *miR-34a* were elevated in ventral skin by 1.35 ± 0.05 and 1.74 ± 0.12-fold, respectively. Bioinfor of the differentially expressed microRNAs suggests that these are mainly involved in two processe sion of solute carrier proteins, and (2) regulation of mitogen-activated protein kinase signaling. Th first report that shows a tissue specific mode of microRNA expression during amphibian dehydrati evidence for microRNAs as crucial regulators of metabolic depression.

Dehydration led to differential expression of microRNAs in *X. laevis* organs

MicroRNAs and freeze tolerance

Cryobiology 59 (2009) 317–321

Contents lists available at ScienceDirect

Cryobiology

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

MicroRNA regulation below zero: Differential expression of miRNA-21 and miRNA-16 during freezing in wood frogs ☆

Kyle K. Biggar, Adrian Dubuc, Kenneth Storey *

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

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Received 1 July 2009
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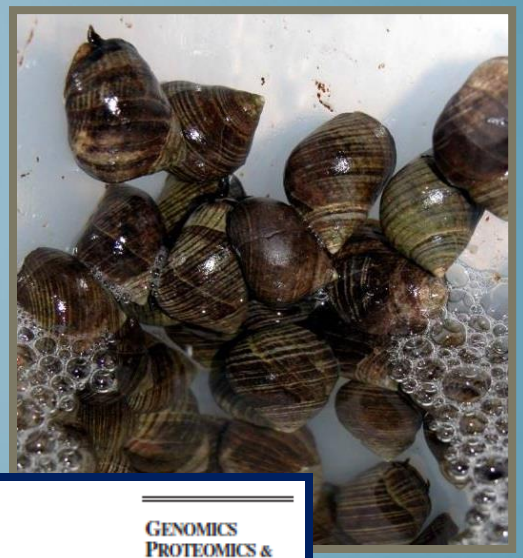
Keywords:
Rana sylvatica
Vertebrate freeze tolerance
MicroRNA
Post-transcriptional control of gene expression
Metabolic rate depression
Anti-apoptosis
Cell cycle control
Dicer

ABSTRACT

Natural freeze tolerance depends on numerous biochemical adaptations that address the multi
imposed on cells by freezing and preserves viability by suppressing energy-expensive cell func
frozen state. We hypothesized that microRNAs, small non-coding RNA transcripts that bind to m
act to establish rapid biological controls that aid the reorganization of metabolic priorities for fr
vival. Selected microRNA species (miR-16 and miR-21) were evaluated using RT-PCR in liver and sk
cle of wood frogs (*Rana sylvatica*) comparing controls (5 °C acclimated) with animals frozen for 24
Levels of miR-21 increased significantly during free
respectively. MiR-16 transcripts also rose significa
skeletal muscle. Protein levels of Dicer, a type III R
in the cytoplasm, were unchanged in liver and dec
the first report of differential regulation of microR
mechanism for rapid, yet reversible, gene silencing

miRNAs & Dicer enzyme show organ-specific changes in freeze tolerant frogs

Invertebrate microRNAs: new method for detection & amplification



Analytical Biochemistry 416 (2011) 231–233

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Notes & Tips

Amplification and sequencing of mature microRNAs in uncharacterized animal models using stem–loop reverse transcription–polymerase chain reaction

Kyle K. Biggar, Samantha F. Kornfeld, Kenneth B. Storey*

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

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Article history:
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ABSTRACT

Expression of mature microRNA (miRNA) tr
model systems but is difficult to evaluate u

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SciVerse ScienceDirect

Genomics Proteomics Bioinformatics 10 (2012) 302–309

GENOMICS PROTEOMICS & BIOINFORMATICS

www.elsevier.com/locate/gpb

Original Research

MicroRNA Regulation in Extreme Environments: Differential Expression of MicroRNAs in the Intertidal Snail *Littorina littorea* During Extended Periods of Freezing and Anoxia

Kyle K. Biggar[#,*], Samantha F. Kornfeld[#], Yulia Maistrovski, Kenneth B. Storey

Institute of Biochemistry & Department of Biology, Carleton University, Ottawa, ON K1S 5B6, Canada

Received 26 July 2012; revised 11 September 2012; accepted 12 September 2012
Available online 8 October 2012

Abstract

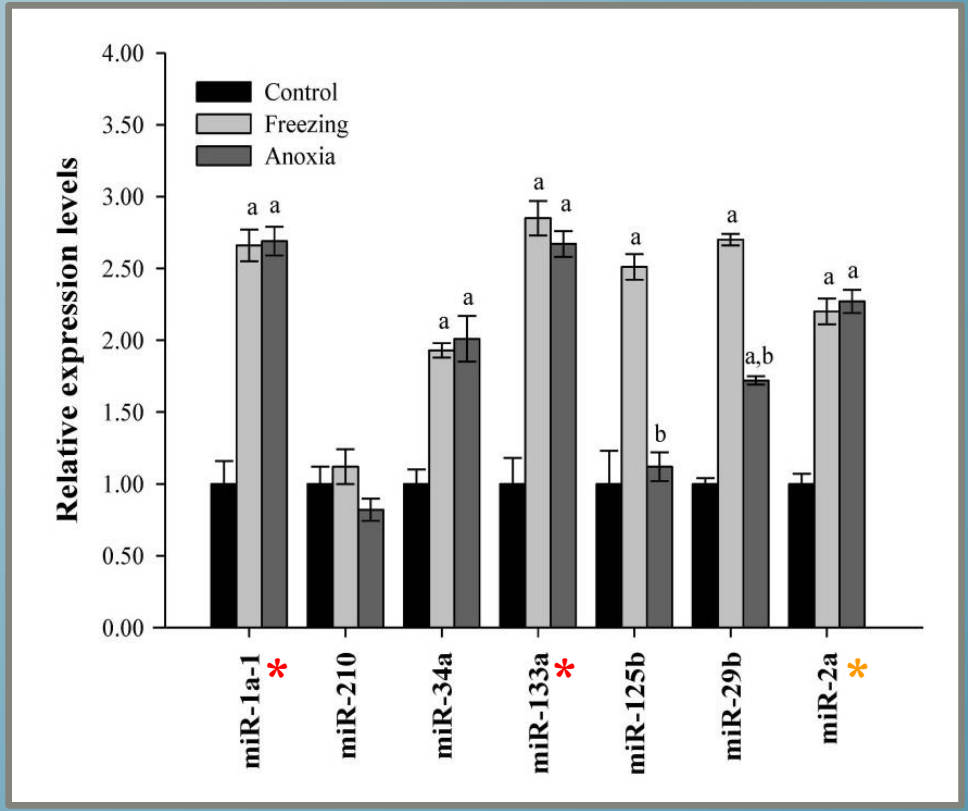
Several recent studies of vertebrate adaptation to environmental stress have suggested roles for microRNAs (miRNAs) in regulating global suppression of protein synthesis and/or restructuring protein expression patterns. The present study is the first to characterize stress-responsive alterations in the expression of miRNAs during natural freezing or anoxia exposures in an invertebrate species, the intertidal gastropod *Littorina littorea*. These snails are exposed to anoxia and freezing conditions as their environment constantly fluctuates on both a tidal and seasonal basis. The expression of selected miRNAs that are known to influence the cell cycle, cellular signaling pathways,

MicroRNAs respond to anoxia & freezing in intertidal snails

MicroRNAs in *Littorina littorea* FOOT MUSCLE: Up-regulated by Freezing & Anoxia

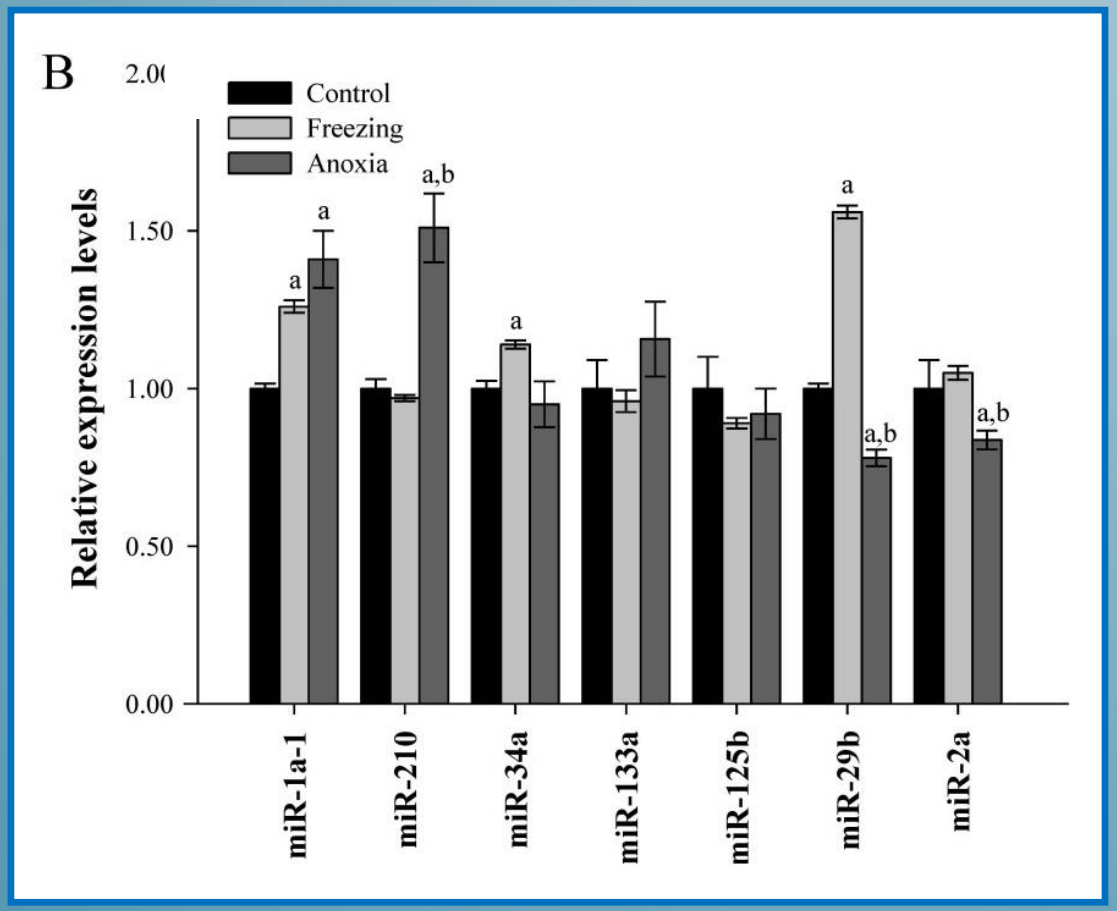
miR-1a-1* & miR-133a*
- myocyte proliferation & differentiation
-regulate *Mef2a* and *Gata4*, Tfs that promote muscle maintenance

miR-2a*
- anti-apoptotic action by targeting the pro-apoptotic protein, *Reaper*



Biggar, Kornfeld & Storey, 2011. Anal. Biochem. 416, 231-3.
Biggar, Kornfeld, Maistrovski & Storey, 2012. Genom. Proteom. Biotech. in press

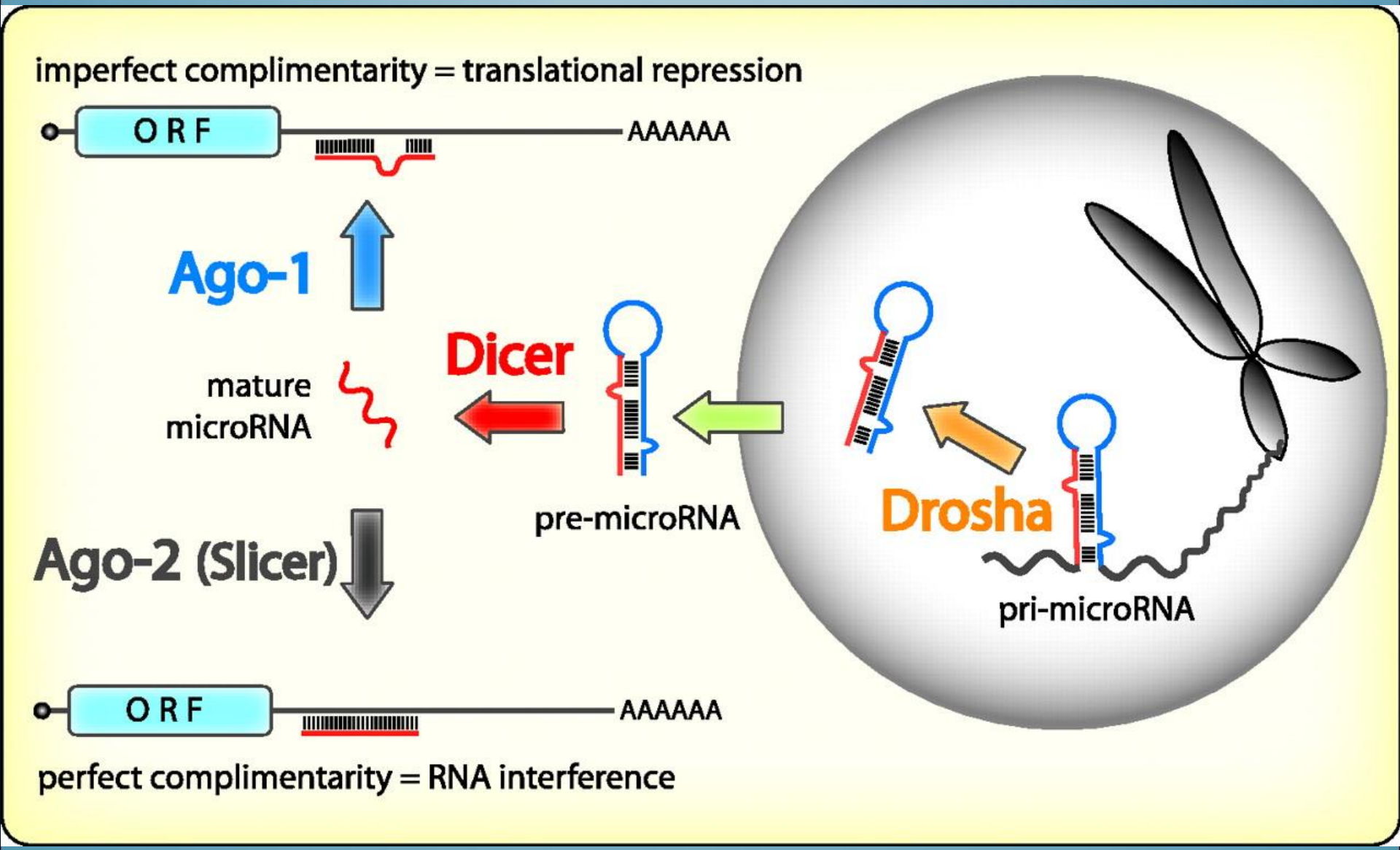
Micro RNAs in *Littorina littorea* HEPATOPANCREAS: Up-regulated by Freezing & Anoxia



Major changes:

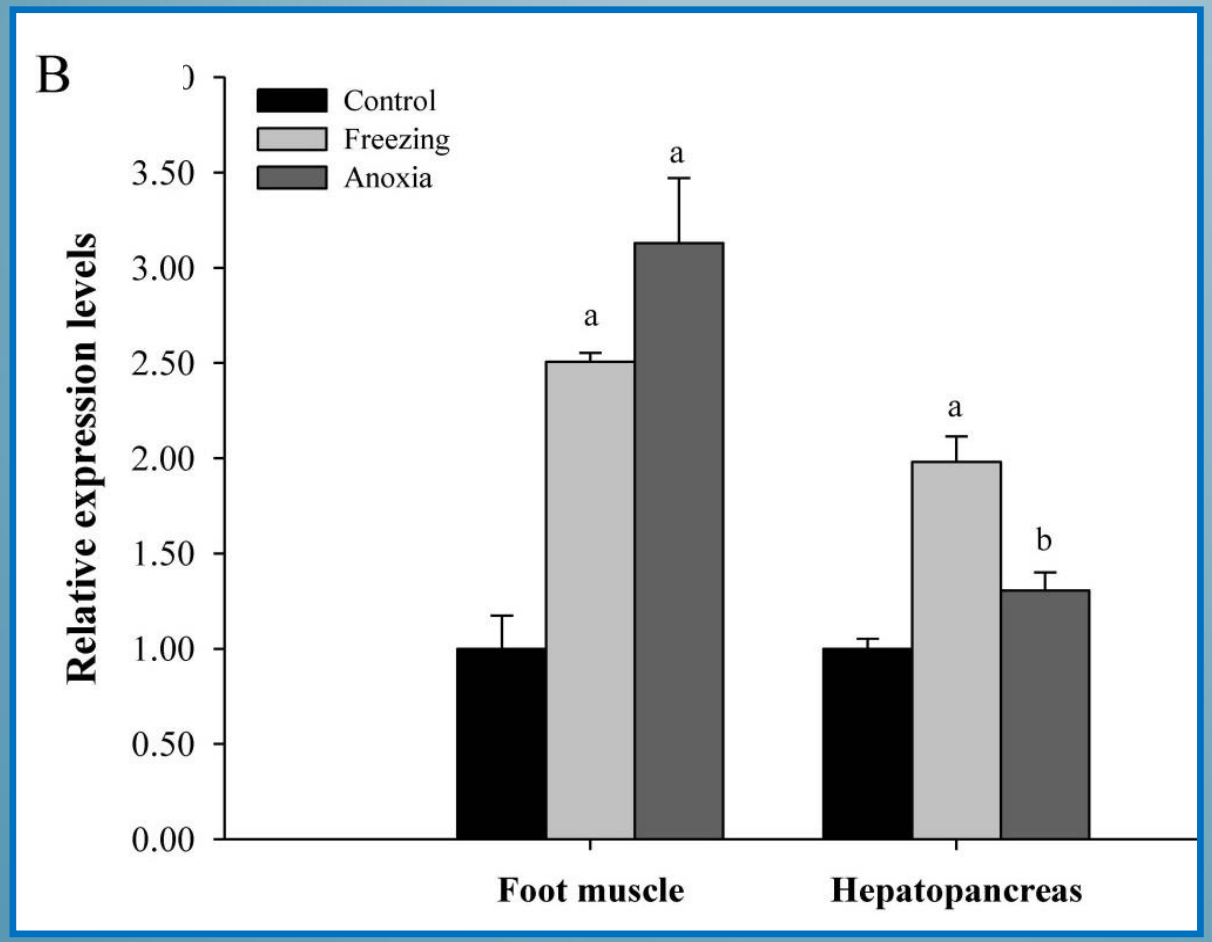
- miR-1a-1 up in freeze & anoxia (like in foot)
- miR-210 up in anoxia
- miR-29b up in freeze

MICRO RNA: Drosha & Dicer



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

DICER ENZYME IN *L. littorea* TISSUES



Dicer protein increased in both freezing & anoxia (immunoblots)

Elevated miRNA processing

MicroRNAs and estivation

Marine Genomics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Genomics

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

Short communication

Large-scale identification and comparative analysis of miRNA expression profile in the respiratory tree of the sea cucumber *Apostichopus japonicus* during aestivation

Muyan Chen [a,*], Kenneth B. Storey [b]

[a] Fisheries College, Ocean University of China, Qingdao, PR China
[b] Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

ARTICLE INFO

Article history:
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Available online xxxx

Keywords:
miRNA
Sea cucumber
Aestivation

ABSTRACT

The sea cucumber *Apostichopus japonicus* withstands high
metabolic rate and entering a state of aestivation. We hyp
could provide important post-transcriptional regulation of
over mRNA translation. The present study analyzed profiles
tree using Solexa deep sequencing technology. We identi
miRNAs specific to sea cucumber. Animals sampled during
uous torpor) were compared with animals from a non-aest
aestivation and returned to an active state). We identified

OPEN ACCESS Freely available online

PLOS ONE

High-Throughput Sequencing Reveals Differential Expression of miRNAs in Intestine from Sea Cucumber during Aestivation

Muyan Chen[1]*, Xiumei Zhang[1], Jianning Liu[2], Kenneth B. Storey[3]

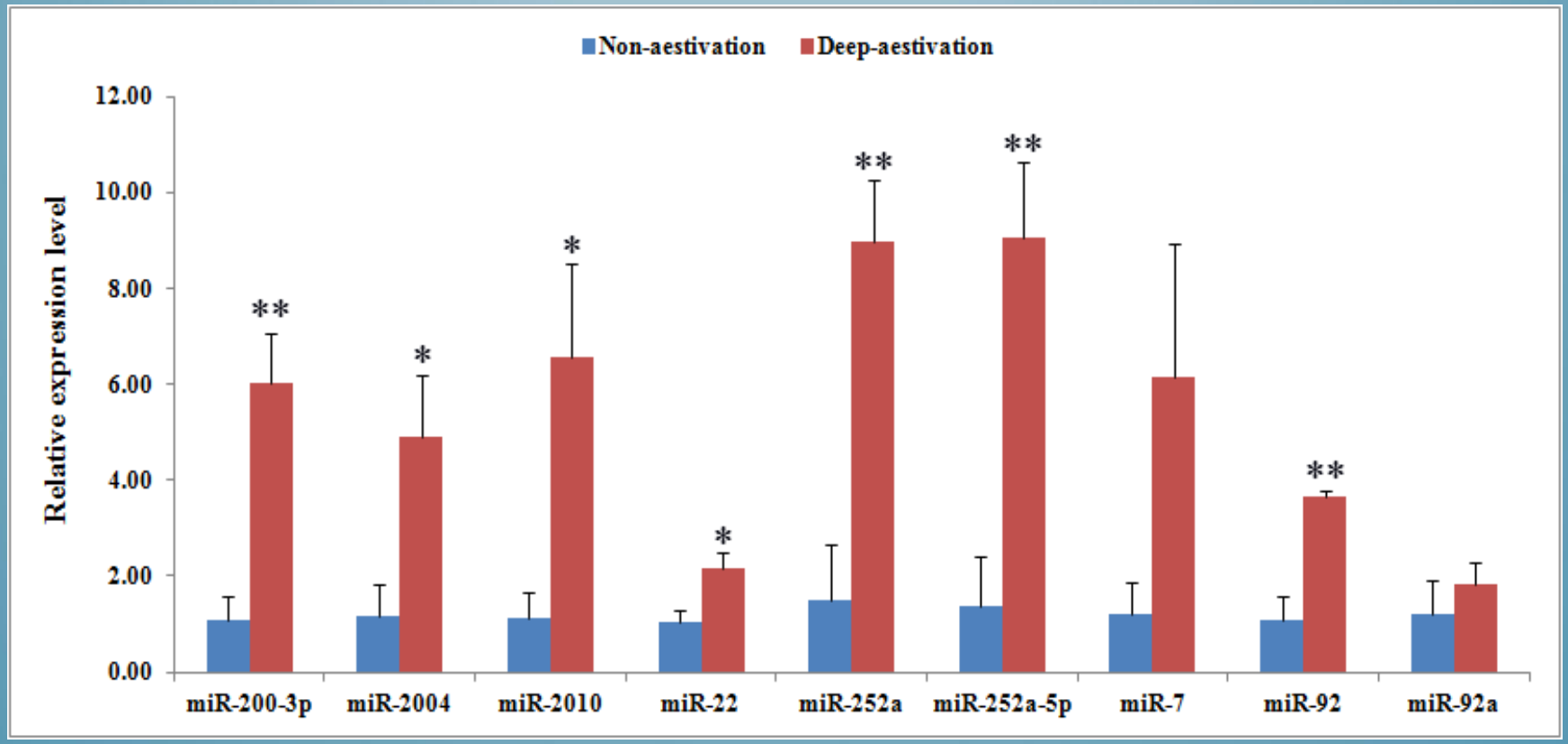
1 Fisheries College, Ocean University of China, Qingdao, PR China, **2** LC-BIO CO., LTD. Hangzhou, PR China, **3** Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada

Abstract

The regulatory role of miRNA in gene expression is an emerging hot new topic in the control of hypometabolism. Sea cucumber aestivation is a complicated physiological process that includes obvious hypometabolism as evidenced by a decrease in the rates of oxygen consumption and ammonia nitrogen excretion, as well as a serious degeneration of the intestine into a very tiny filament. To determine whether miRNAs play regulatory roles in this process, the present study analyzed profiles of miRNA expression in the intestine of the sea cucumber (*Apostichopus japonicus*), using Solexa deep sequencing technology. We identified 308 sea cucumber miRNAs, including 18 novel miRNAs specific to sea cucumber. Animals sampled during deep aestivation (DA) after at least 15 days of continuous torpor, were compared with animals from a non-aestivation (NA) state (animals that had passed through aestivation and returned to the active state). We identified 42 differentially expressed miRNAs [RPM (reads per million) > 10, |FC| (|fold change|) ≥1, FDR (false discovery rate) <0.01] during aestivation, which were validated by two other miRNA profiling methods: miRNA microarray and real-time PCR. Among the most prominent miRNA species, miR-200-3p, miR-2004, miR-2010, miR-22, miR-252a, miR-252a-3p and miR-92 were significantly over-expressed during deep aestivation compared with non-aestivation animals. Preliminary analyses of their putative target genes and GO analysis suggest that these miRNAs could play important roles in global transcriptional depression and cell differentiation during aestivation. High-throughput sequencing data and microarray data have been submitted to GEO database.

Tissue specific over-expression of selected microRNAs in estivation

Aestivation in sea cucumbers: Differential expression microRNAs in intestine



Histone Modification & Hypoxia in Ocean Squid

Reduced phosphorylation or acetylation of histone tails to suppress gene expression under hypoxia

photo: Scott Cassell

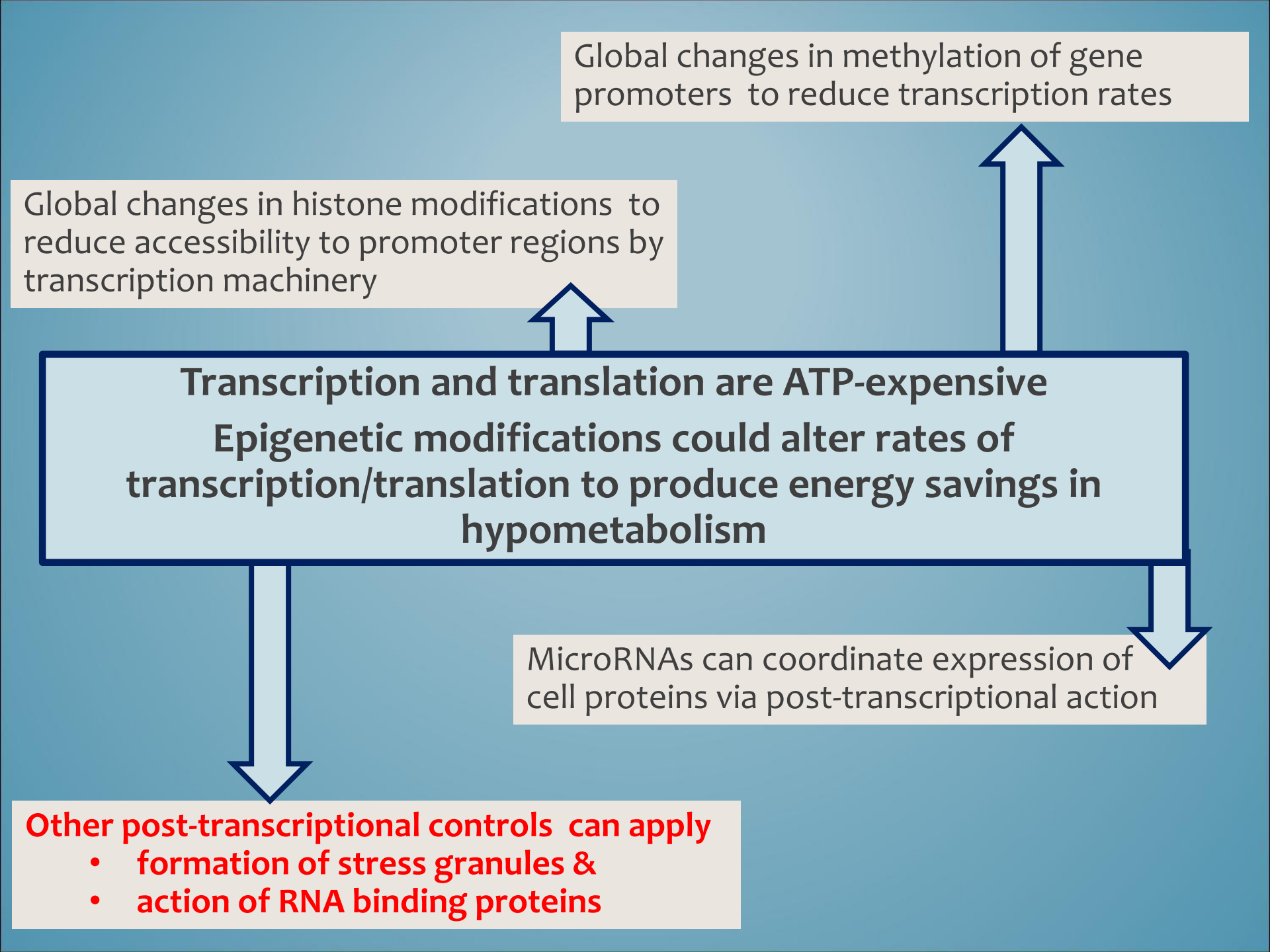
Metabolic suppression during protracted exposure to hypoxia in the jumbo squid, *Dosidicus gigas*, living in an oxygen minimum zone

Brad A. Seibel[1,*], N. Sören Häfker[2], Katja Trübenbach[3], Jing Zhang[4], Shannon N. Tessier[4], Hans-Otto Pörtner[2], Rui Rosa[3] and Kenneth B. Storey[4]

ABSTRACT

The jumbo squid, *Dosidicus gigas*, can survive extended forays into the oxygen minimum zone (OMZ) of the Eastern Pacific Ocean. Previous studies have demonstrated reduced oxygen consumption and a limited anaerobic contribution to ATP production, suggesting the capacity for substantial metabolic suppression during hypoxic exposure. Here, we provide a more complete description of energy metabolism and explore the expression of proteins indicative of transcriptional and translational arrest that may contribute to metabolic suppression. We demonstrate a suppression of total ATP demand under hypoxic conditions (1% oxygen, P_{O_2}=0.8 kPa) in both juveniles (52%) and adults (35%) of the jumbo squid. Oxygen consumption rates are reduced to 20% under hypoxia relative to air-saturated controls. Concentrations of arginine phosphate (Arg-P) and ATP declined initially, reaching a new steady state (~30% of controls) after the first hour of hypoxic exposure. Octopine began accumulating after the first hour of hypoxic exposure, once Arg-P breakdown resulted in sufficient free arginine for substrate. Octopine reached levels near 30 mmol g^{-1} after 3.4 h of hypoxic

J. Exp. Biol. 217:2555-68; 2014


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

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

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

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

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

Author information

Abstract

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

Polysome profiles and mammalian hibernation

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

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

Hittel D[1], Storey KB.

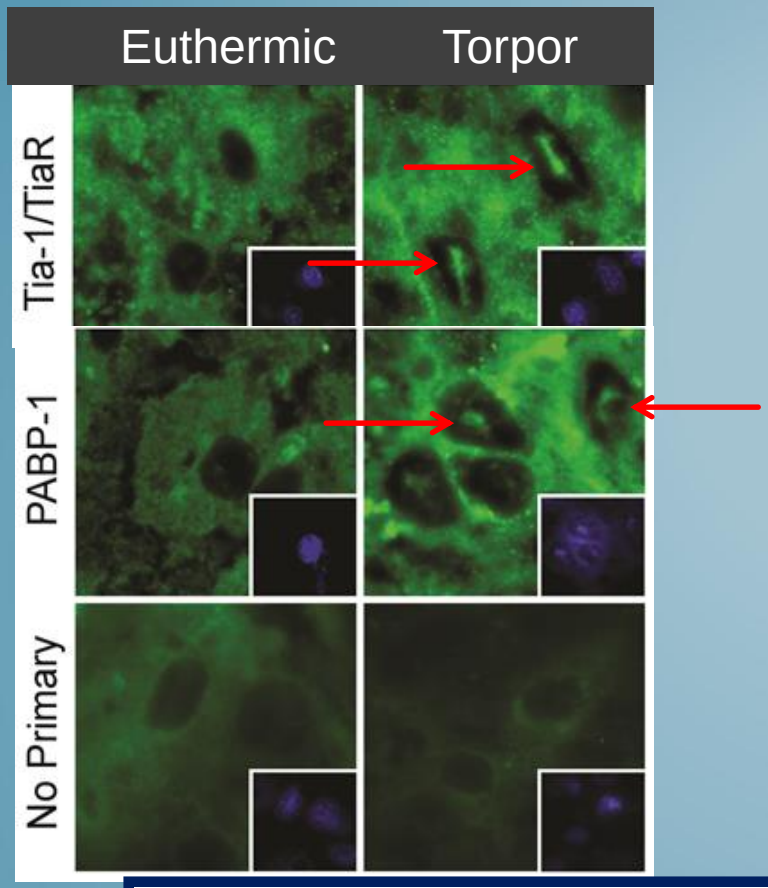
Author information

Abstract

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

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

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



RNA binding proteins & hibernation

Subnuclear structures formed with TIA & PABP greatly increased during torpor

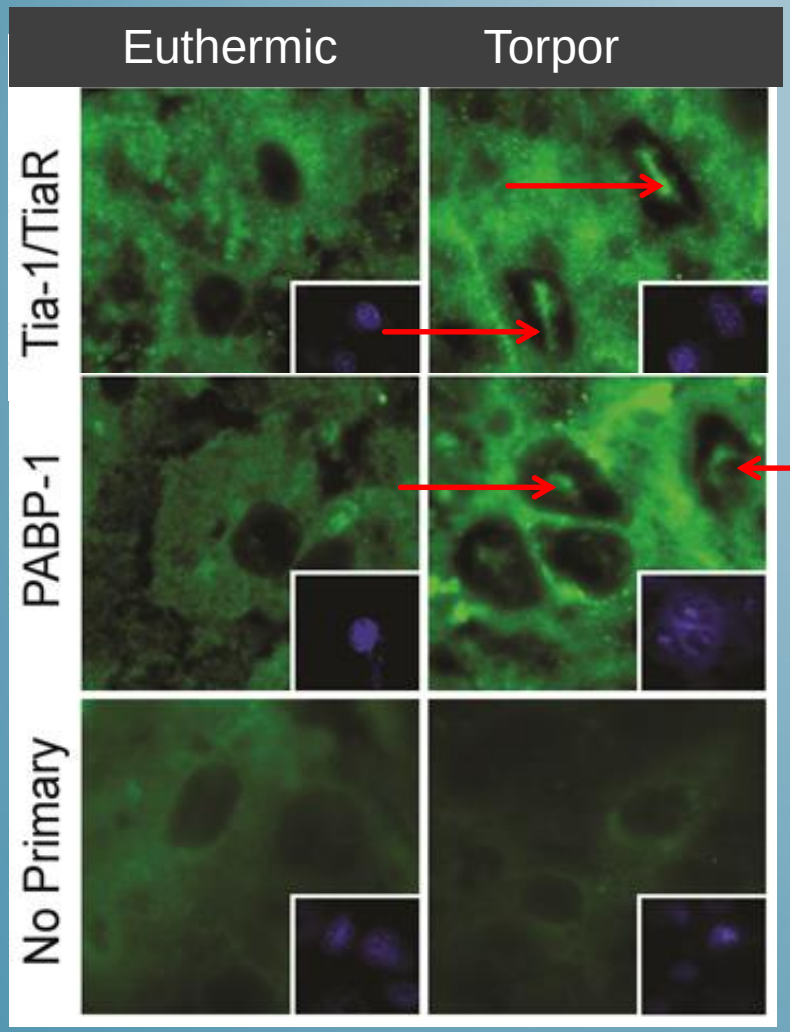
Cell Stress and Chaperones 2014; 19(6):813-25.

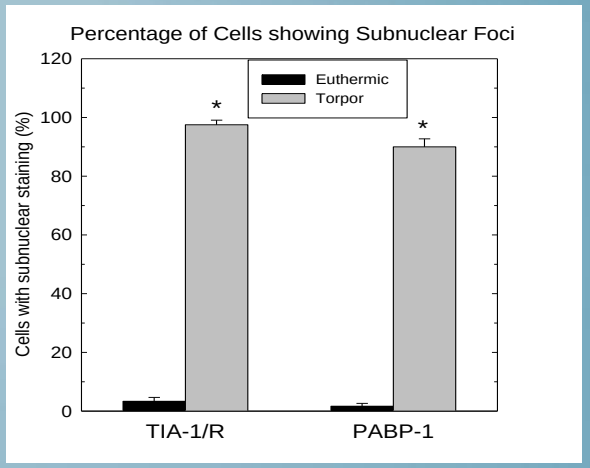
ORIGINAL PAPER

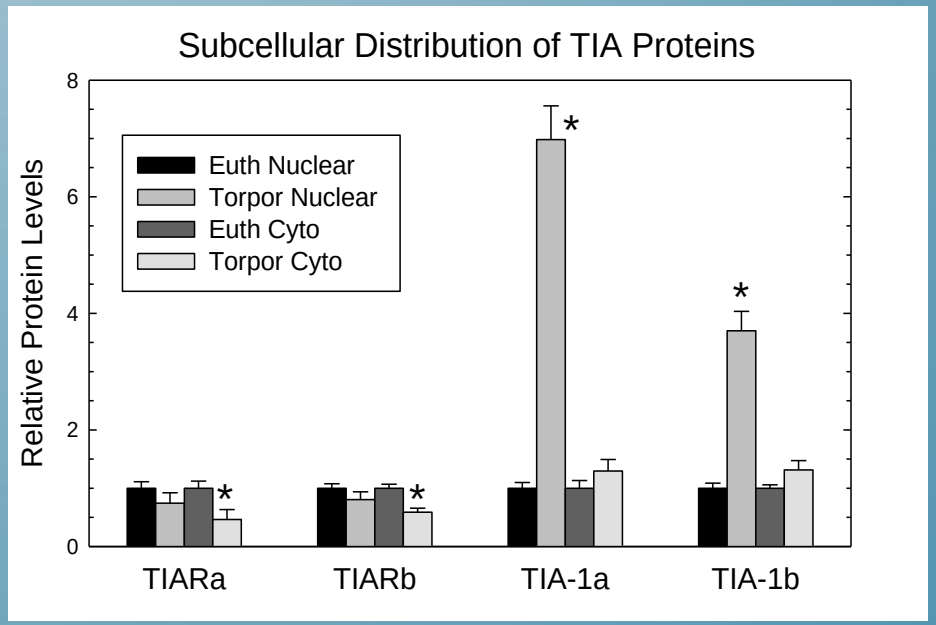
The involvement of mRNA processing factors TIA-1, TIAR, and PABP-1 during mammalian hibernation

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

RNA Binding Proteins & Mammalian Hibernation

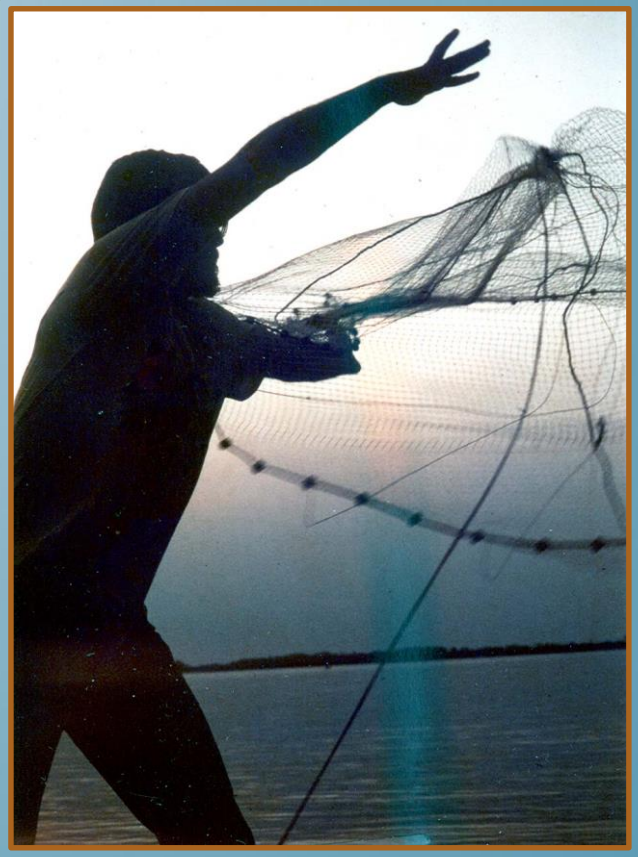






WHERE DO WE GO FROM HERE?

- *Applications of MRD research*
- *Novel phosphorylations*
- *Atrophy, hypertrophy*
  -- autophagy for survival
- *Turning it all off -- microRNA*
- *Epigenetics & adaptation*
- *Life span extension*
- *Antioxidant Defense*
- *Cell cycle suppression*
- *Unity through evolution*



NEW DIRECTIONS

NEW DIRECTIONS - TBA

Big Science Edition:

1. GENOMES: Sequence all the genes to feel better !

The western painted ***turtle genome****, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage.*

Shaffer HB,...Storey KB, et al., Genome Biol. 2013 14(3): R28

2. Protein 2D: What about the Proteins

OMICS – Proteomics

Genomic tools to discover biochemical adaptations

Genome Biol. 2013 Mar 28;14(3):R28. [Epub ahead of print]

The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage.

Shaffer HB[1], Minx P, Warren DE, Shedlock AM, Thomson RC, Valenzuela N, Abramyan J, Amemiya CT, Badenhorst D, Biggar KK, Borchert GM, Botka CW, Bowden RM, Braun EL, Bronikowski AM, Bruneau BG, Buck LT, Capel B, Castoe TA, Czerwinski M, Delehaunty KD, Edwards SV, Fronick CC, Fujita MK, Fulton L, Graves TA, Green RE, Haerty W, Hariharan R, Hernandez O, Hillier LW, Holloway AK, Janes D, Janzen FJ, Kandoth C, Kong L, de Koning AJ, Li Y, Literman R, McGaugh SE, Mork L, O'Laughlin M, Paitz RT, Pollock DD, Ponting CP, Radhakrishnan S, Raney BJ, Richman JM, St John J, Schwartz T, Sethuraman A, Spinks PQ, Storey KB, Thane N, Vinar T, Zimmerman LM, Warren WC, Mardis ER, Wilson RK.

Author information

Abstract

BACKGROUND: We describe the genome of the western painted turtle, Chrysemys picta bellii, one of the most widespread, abundant, and well-studied turtles. We place the genome into a comparative evolutionary context, and focus on genomic features associated with tooth loss, immune function, longevity, sex differentiation and determination, and the species' physiological capacities to withstand extreme anoxia and tissue freezing.

RESULTS: Our phylogenetic analyses confirm that turtles are the sister group to living archosaurs, and demonstrate an extraordinarily slow rate of

of the painted turtle to withstand complete anoxia and partial freezing appears to be associated

ntify candidate genes for future functional analyses. Tooth loss shares a common pattern of

genes with birds, although the rate of accumula

erally reflect phylogeny rather than convergenc

show signatures of strong natural selection, i

Painted turtles:

- Adults endure extreme anoxia
- Hatchlings are freeze tolerant

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S. Alvarado
M. Chen
J.M. Storey

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NSERC CRSNG

www.carleton.ca/~kbstorey