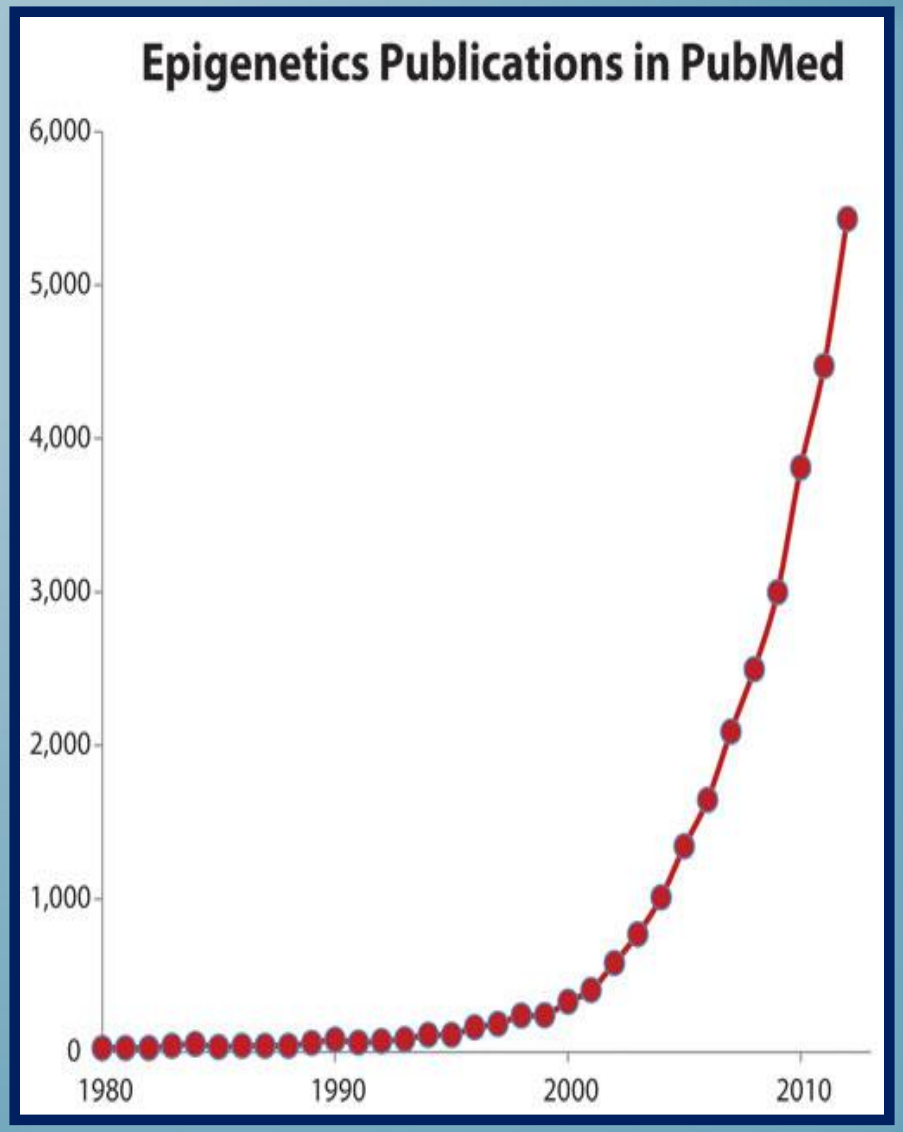

The Living Dead:
Metabolic Arrest
and the
Control of Biological Time

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

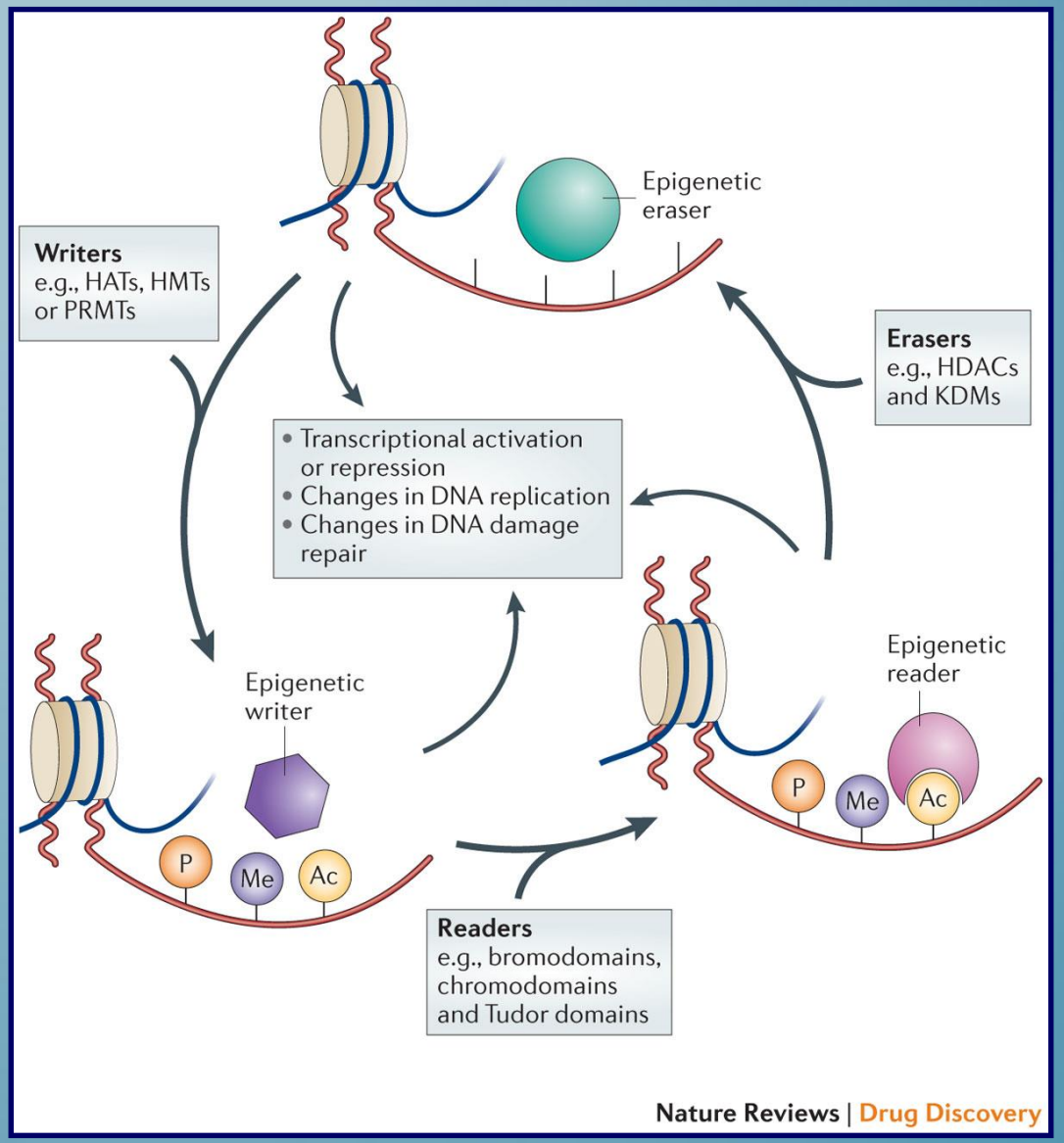
Freezing

Diapause

EPIGENETIC MECHANISMS

Master Switch: CHANGE THE READING of YOUR DNA

Turn Genes On and OFF in response to environment [Disease, Lifestyle, Interventions: Drugs]



GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

PROTEINS (ENZYMES)

No Modification

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

GENES

Transcription

Control by transcriptional regulation

RNAs

Translation

Control by translational regulation

PROTEINS (ENZYMES)

No Modification

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

DAILY TORPOR

Grey mouse lemur,
Microcebus murinus

PRINCIPLES OF METABOLIC RATE DEPRESSION

1. Most genes OFF
2. Selective gene activation
3. Epigenetic depression of gene expression

Same for ALL MRD

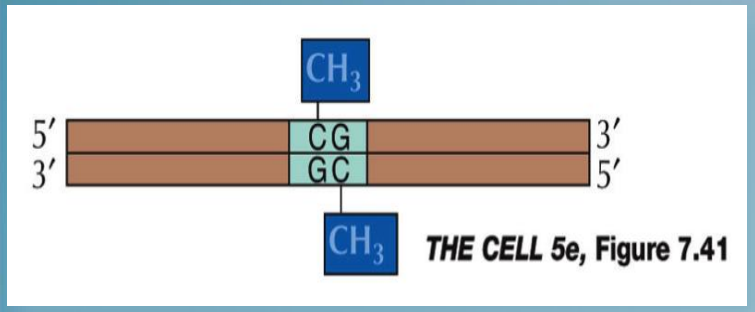
TURNING OFF GENES: ROLE OF EPIGENETICS

Epigenetics:

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

Common mechanisms:

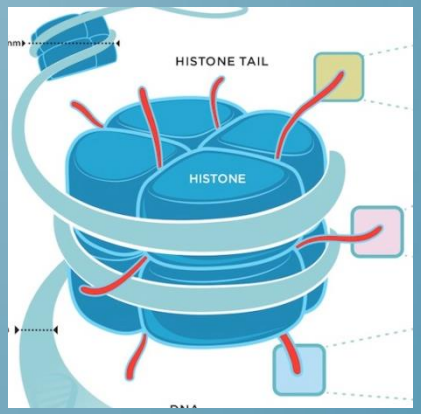
- DNA methylation
- Histone modification / histone variants
- e.g. acetylation, phosphorylation
- Regulatory non-coding RNAs
- “Hiding messages”

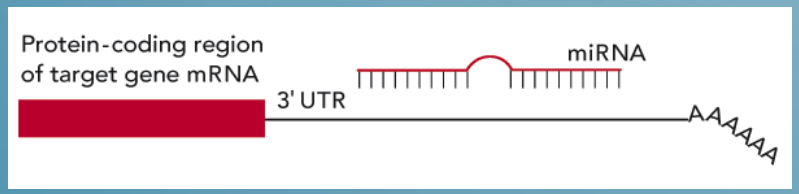


1. DNA Methylation. Methylation of cytosines at CpG dinucleotides in promoter regions. Methylation attenuates gene expression.

Epigenetics: the study of heritable changes in gene expression that do not depend on gene DNA sequence.

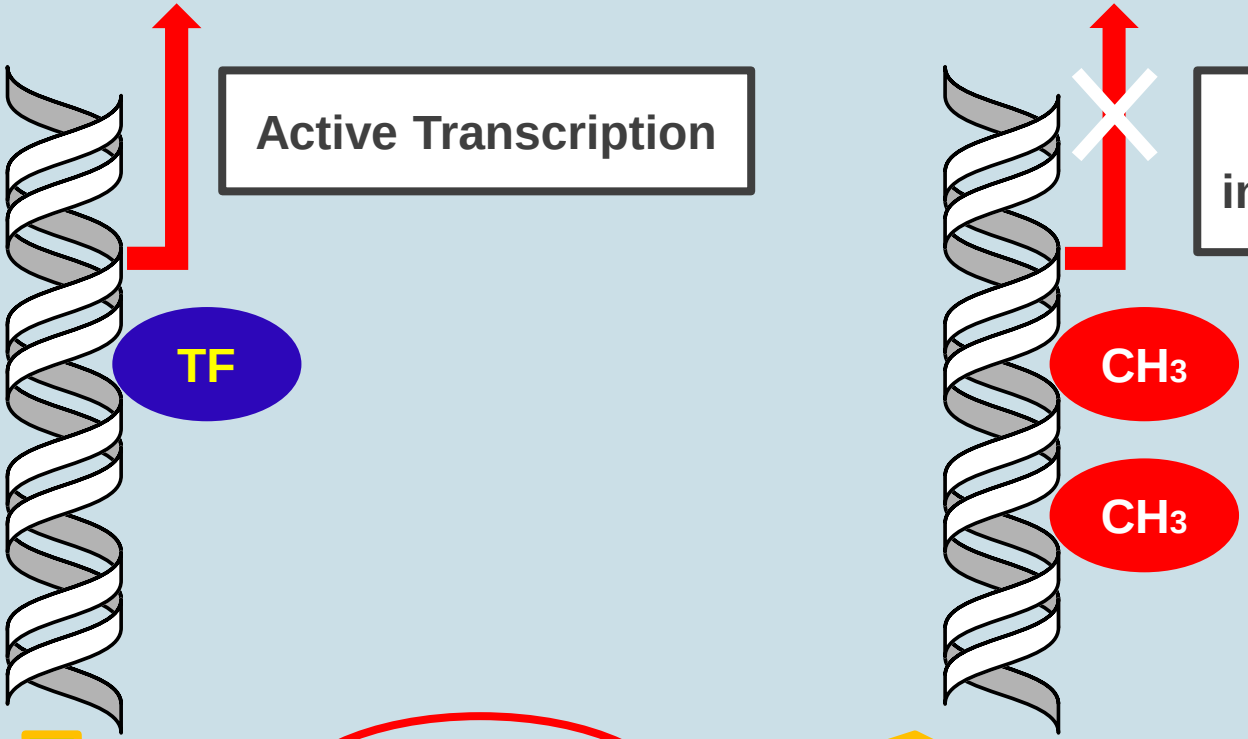
2. Histone Modification. Post-translational modifications on histone tails affect histone:DNA interactions to influence accessibility of promoter regions to transcriptional machinery.





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

DNA METHYLATION



DNMT

Hypermethylation

SAM

SAH

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

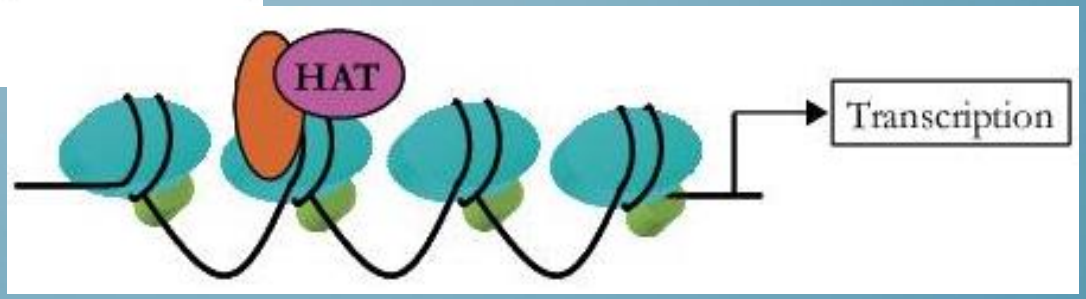
Hibernating mammals conserve energy in the winter by undergoing prolonged bouts of torpor, interspersed with brief arousals back to euthermia. These bouts are accompanied with a suite of reversible physiological and biochemical changes; however, much remains to be discovered about the molecular mechanisms 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.
J. Exp. Biol. 218: 1787-1795

Changes in DNA methylation & DNMTs restrict gene transcription during torpor

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 Deacetylases & Mammalian Hibernation

Available online at www.sciencedirect.com
ScienceDirect
CRYOBIOLOGY
ELSEVIER
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.
© 2006 Elsevier Inc. All rights reserved.

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

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.

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

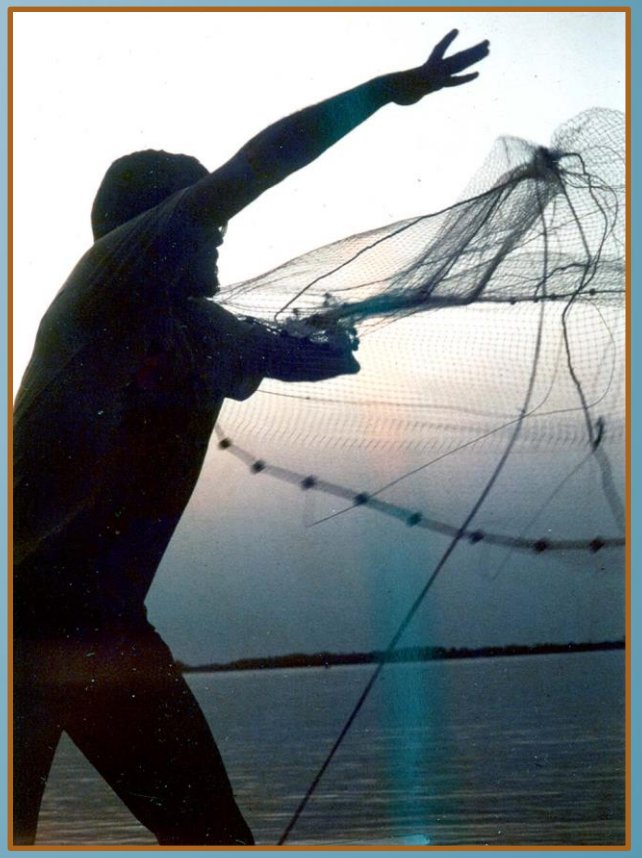
miRNAs & Dicer enzyme show organ-specific changes in mammalian hibernation

WHERE DO WE GO FROM HERE?

- *Applications of MRD research*

- *Turning it all off -- microRNA*
- *Epigenetics & adaptation*

- *Life span extension*
- *Cell cycle suppression*



NEW DIRECTIONS

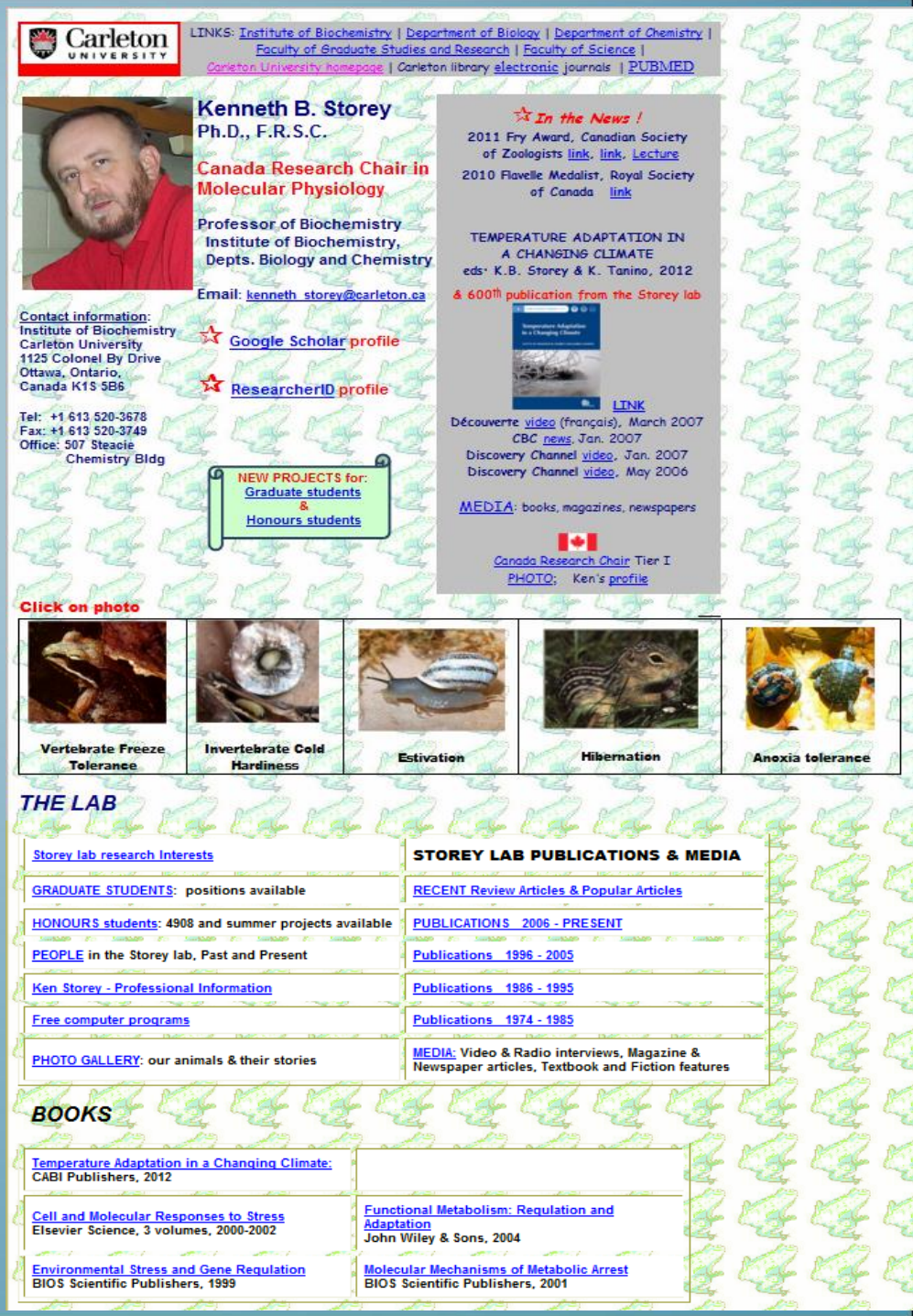
Thanks to:

C-W. Wu
K.K. Biggar
S.N. Tessier
J. Zhang
P. Morin
A. Krivoruchko
D. Hittel
Y. Maistrovski
S. Alvarado
M. Chen
J.M. Storey

Funded by NSERC Canada

NSERC CRSNG

www.carleton.ca/~kbstorey



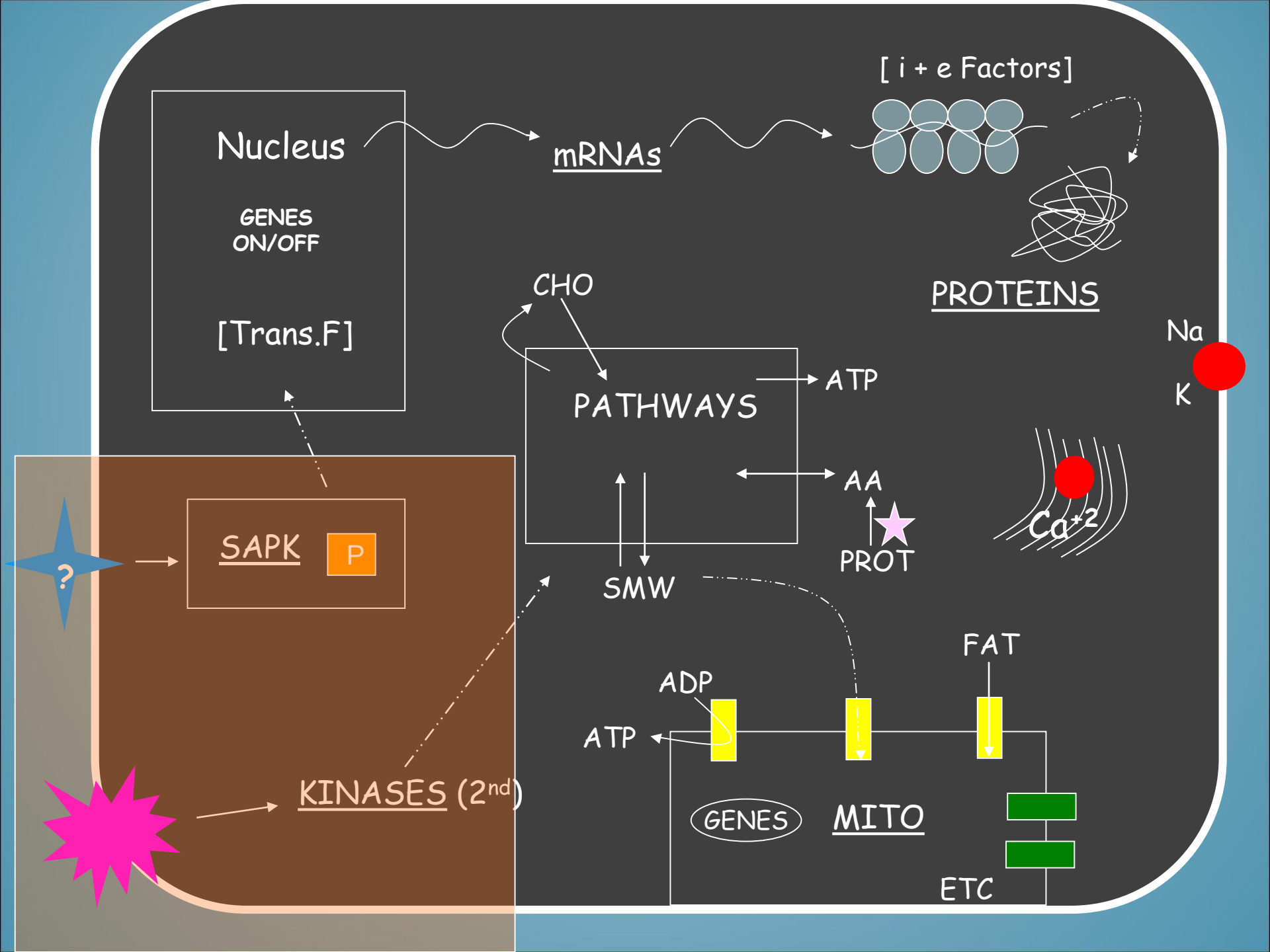
TURNING OFF GENES: ROLE OF EPIGENETICS

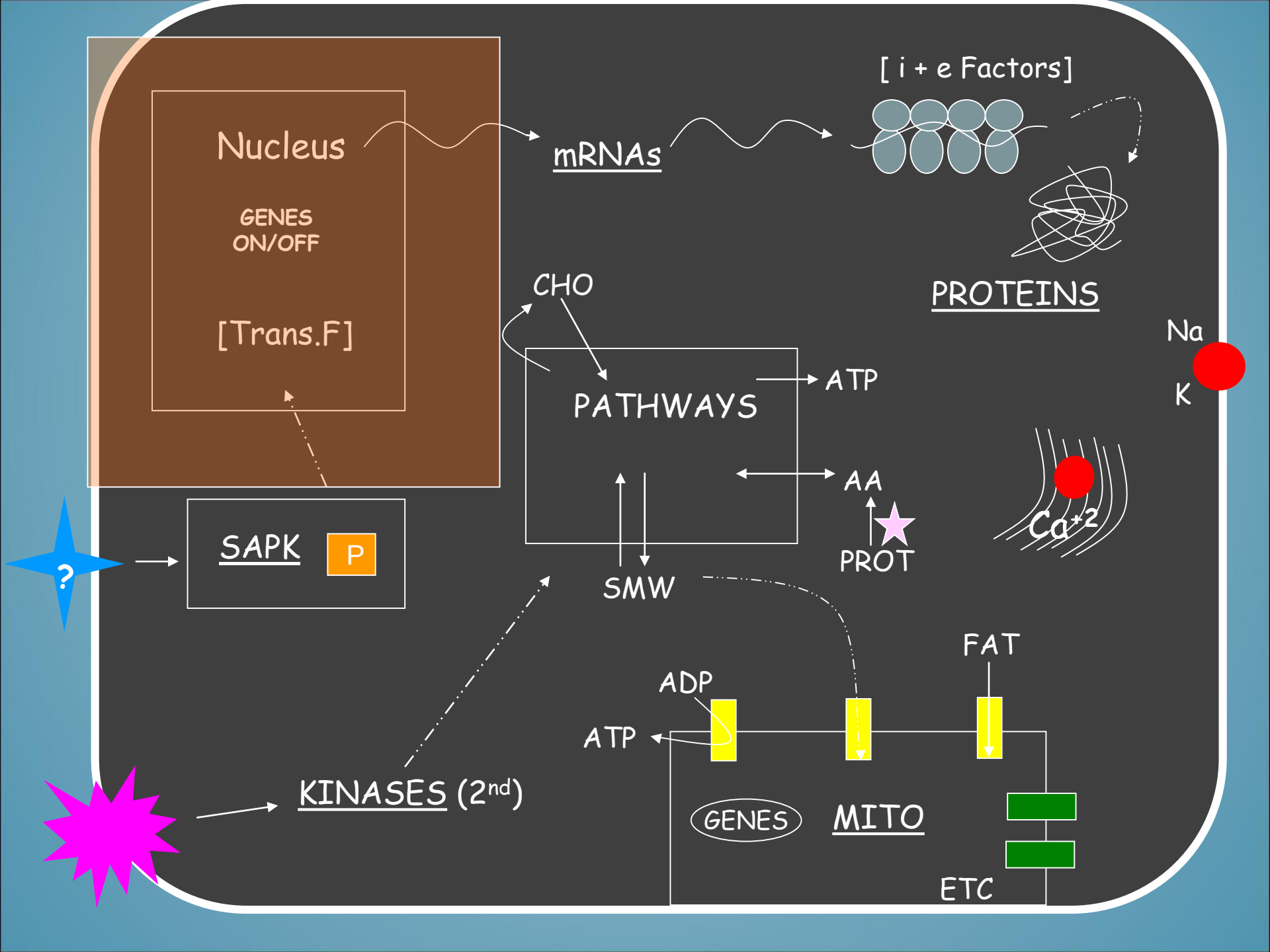
Epigenetics:

- 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


[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


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