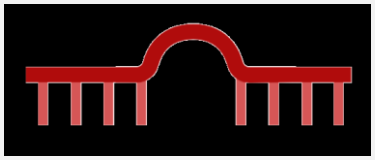


THE BRAIN IN WINTER.
TURNING OFF THE BRAIN WITH MICRORNA:

Ken Storey, Biochemistry

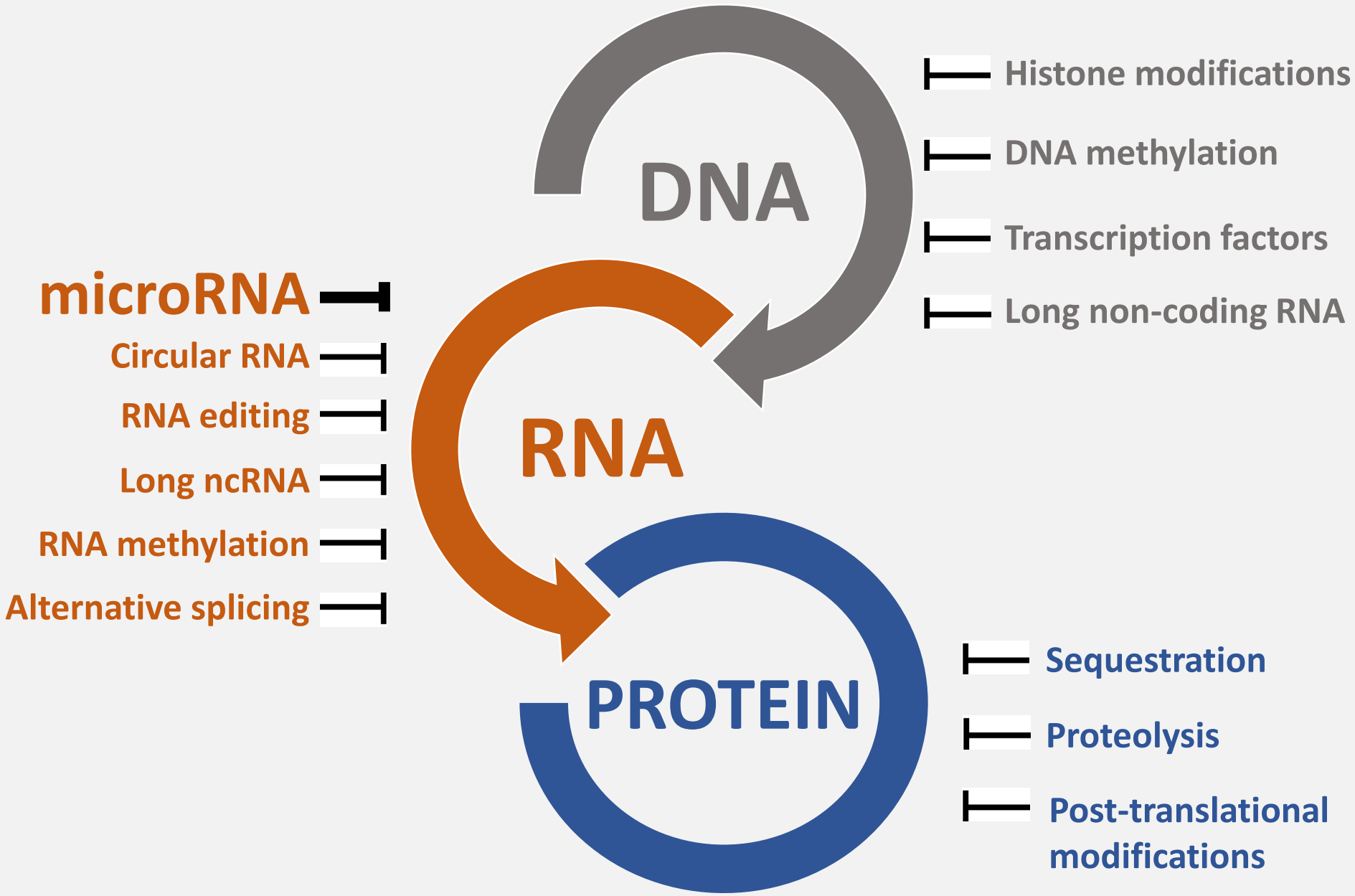
Carleton University

http://www.kenstoreylab.com/

THE PLAN OF THE TALK

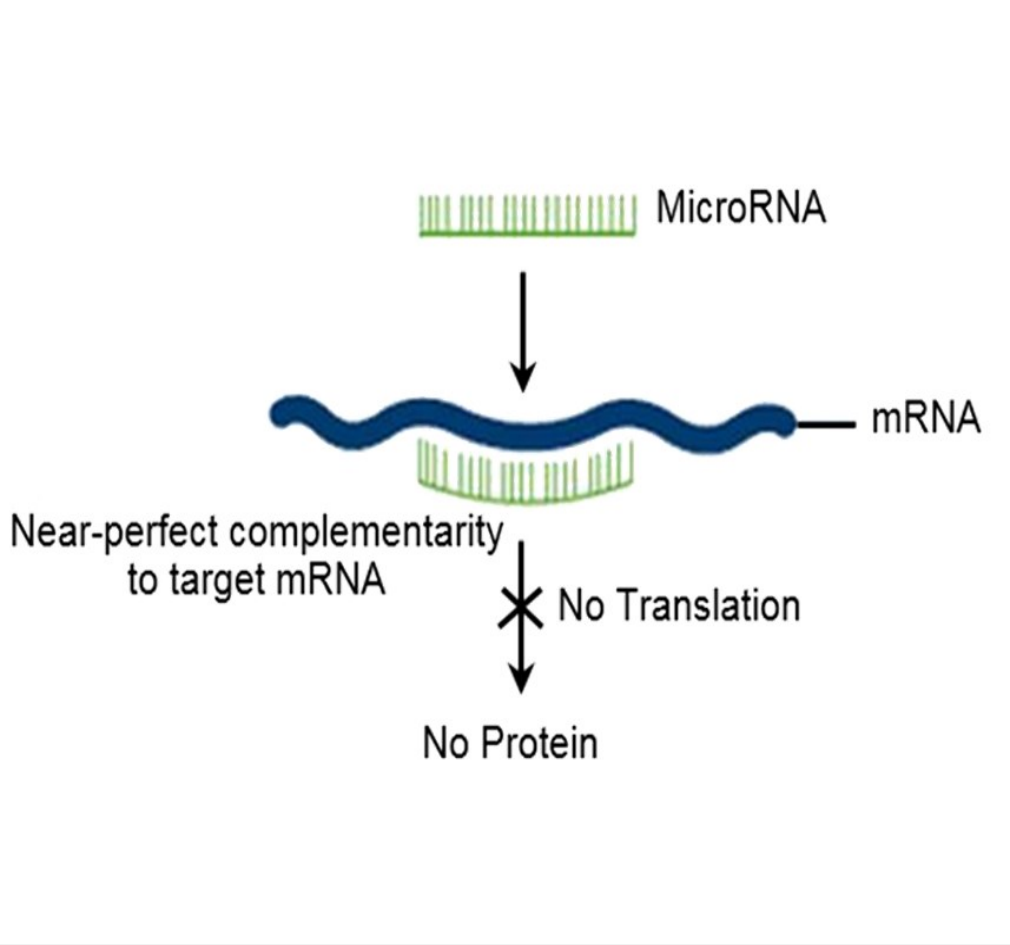
- MicroRNAs and **gene silencing**
- MicroRNAs and **animal adaptations**
- MicroRNAs and **the brain**
- MicroRNAs and **diseases and interventions**

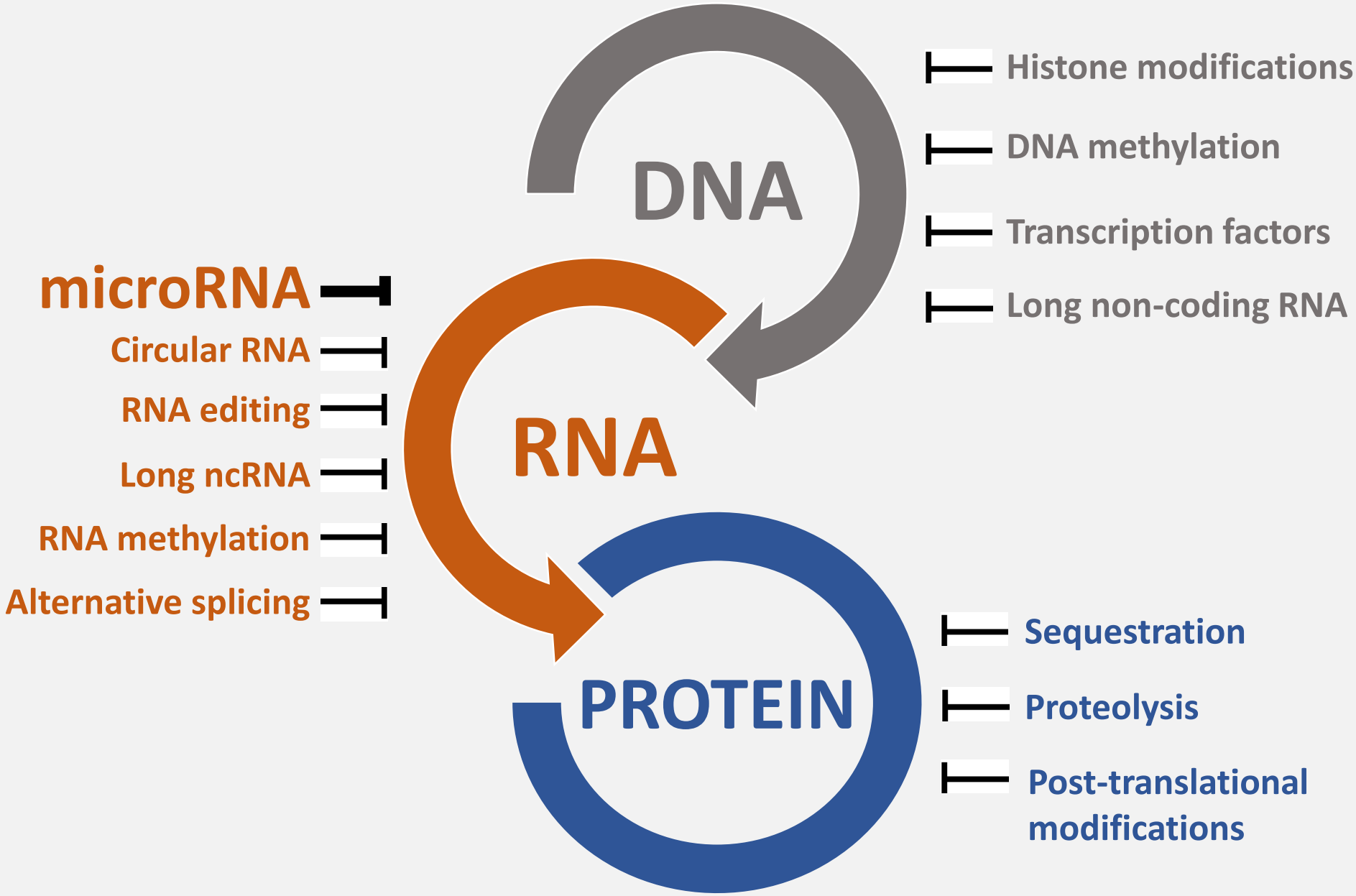
The Central Dogma


DNA
Histone modifications
DNA methylation
Transcription factors
Long non-coding RNA
RNA
microRNA
Circular RNA
RNA editing
Long ncRNA
RNA methylation
Alternative splicing
PROTEIN
Sequestration
Proteolysis
Post-translational modifications

MICRORNA?

- Short non-coding RNA molecules
- Each microRNA targets multiple gene transcripts
- Each mRNA is regulated by numerous microRNAs.




DNA
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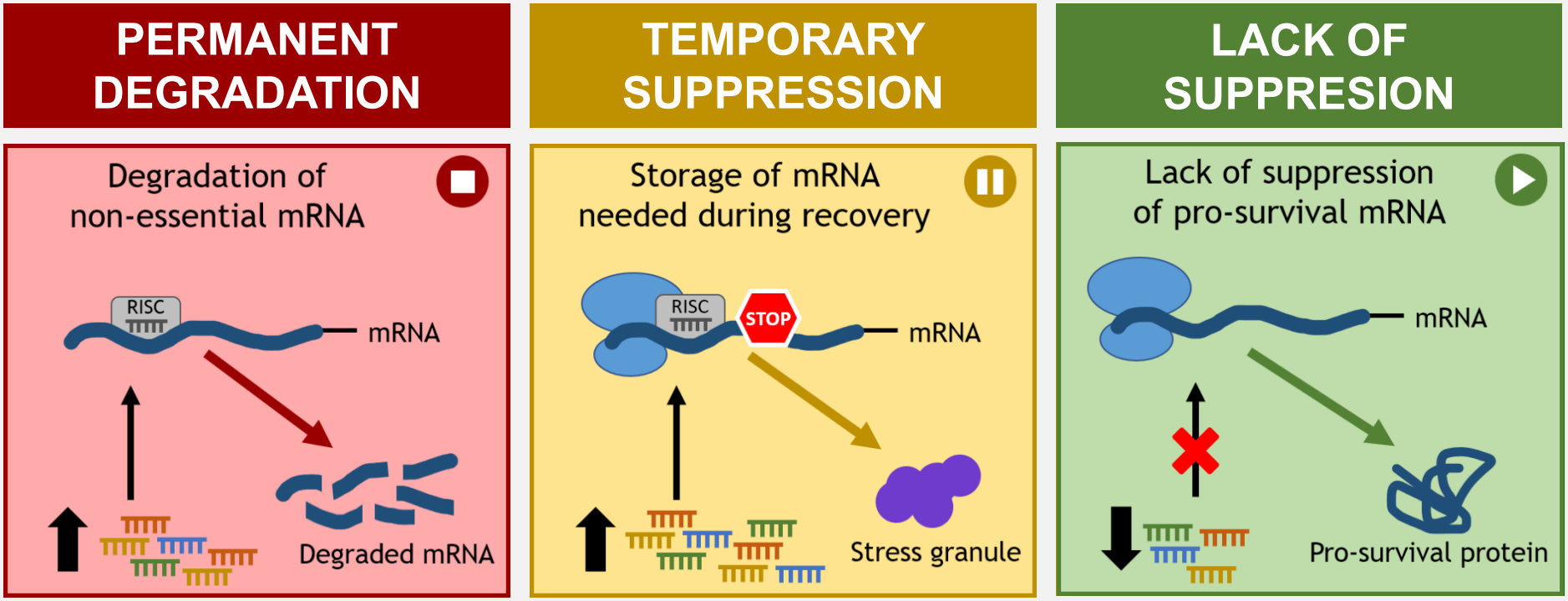
Post-translational modifications

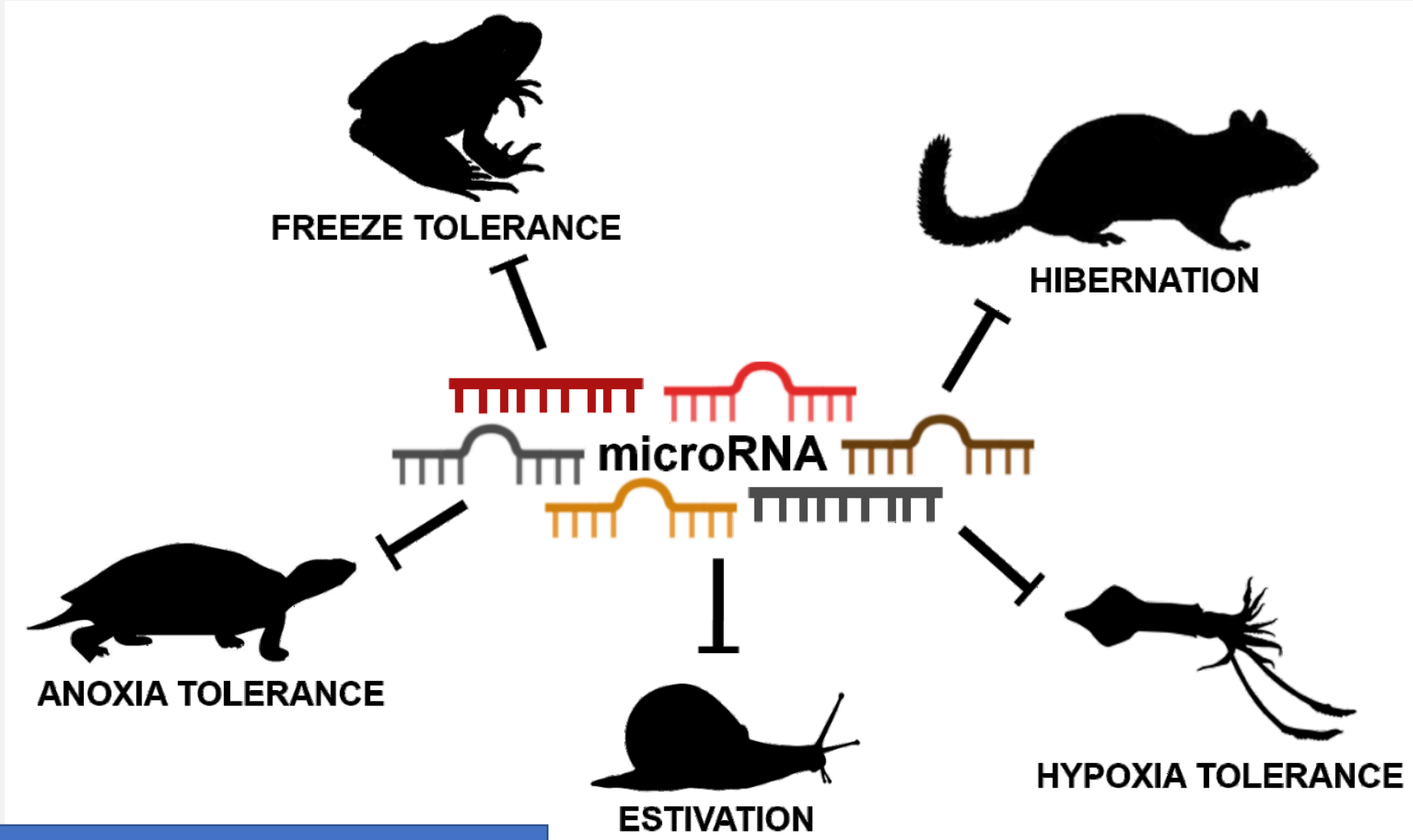
MICRORNA: MODE OF ACTION



Hadj-Moussa, Hawkins, & Storey. 2021. *miRNomics II.*

THE PLAN OF THE TALK

- MicroRNAs and **gene silencing**
- MicroRNAs and **animal adaptations**
- MicroRNAs and **the brain**
- MicroRNAs and **diseases and interventions**



MRD: Metabolic Rate Depression

Hadj-Moussa, Hawkins, & Storey. 2021. *miRNomics II.*

Extreme Brains

- Brains in Nature survive massive environmental insults.
- Frozen solid
- No Oxygen -- No problem
- Cold mammal brain …….. “I got this”
- Bottom of the ocean, dry in the desert
- Adaptation = CALL on Epigenetics & microRNA
- VERSUS
- **Human brains = NOT SO MUCH !**

Freeze tolerance

Cold

Freeze avoidance

Hibernation

Hot and Dry

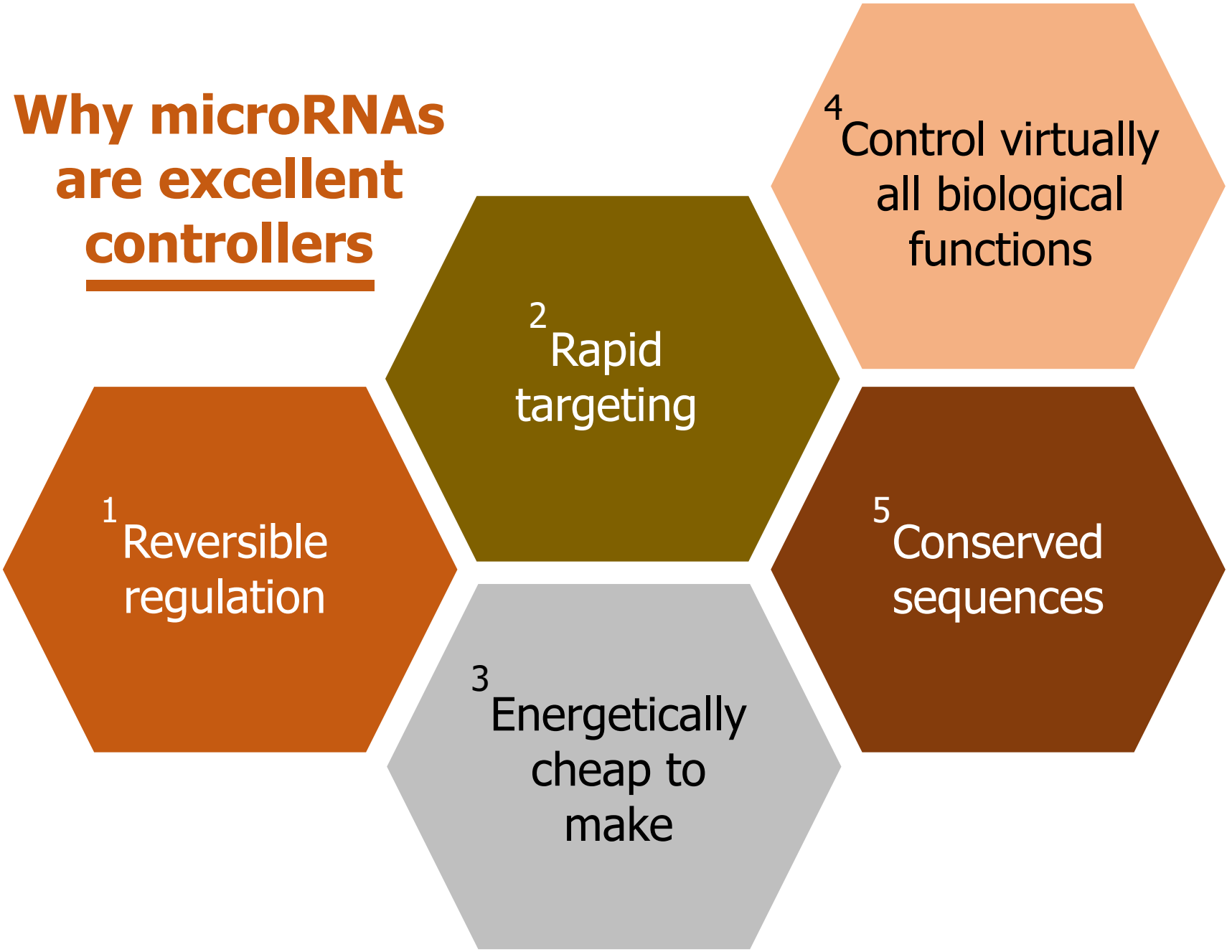
Estivation

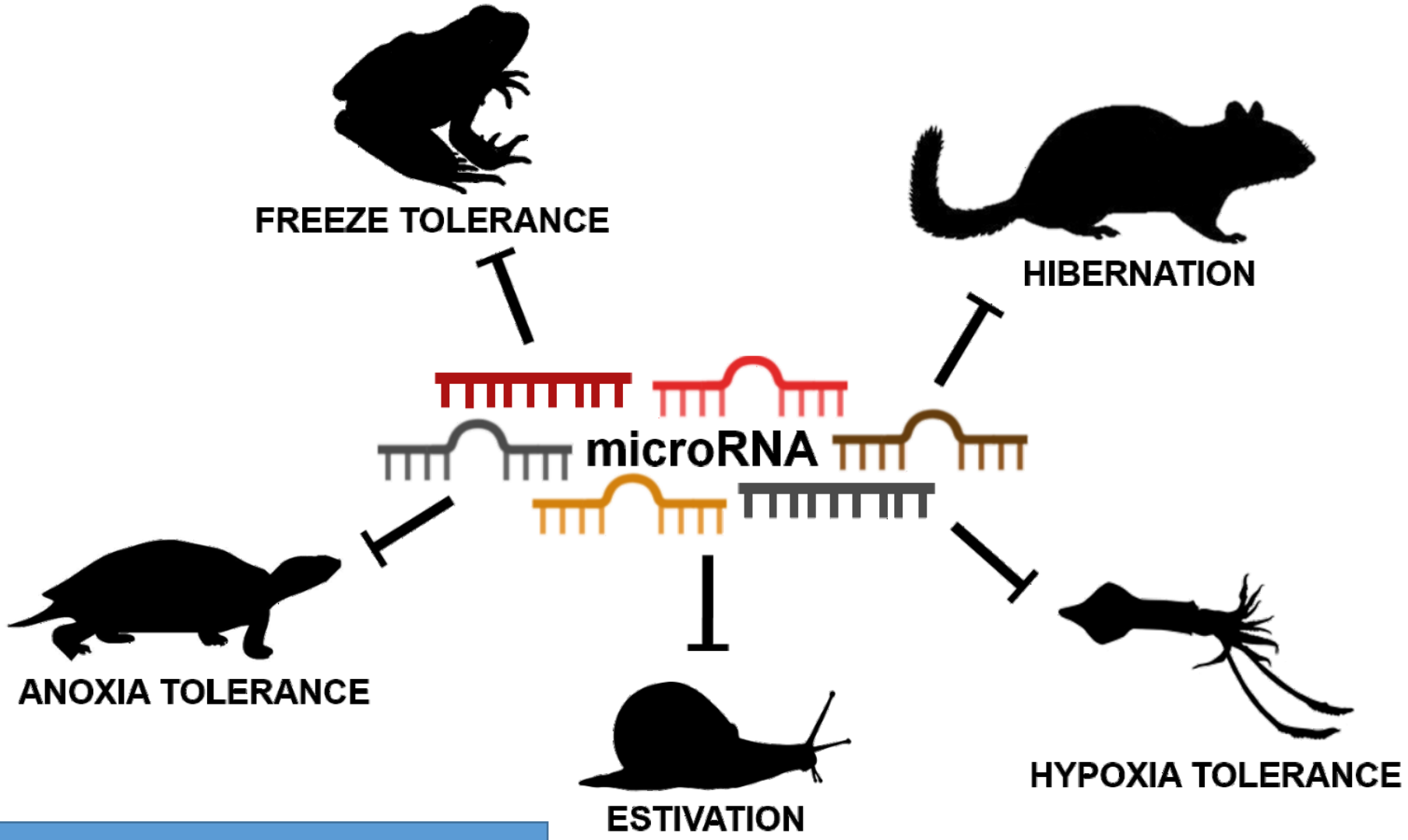
Anhydrobiosis

PAPERS:

https://pubmed.ncbi.nlm.nih.gov/?term=storey+kb&sort=date&size=100

Why microRNAs are excellent controllers





MRD: Metabolic Rate Depression

Hadj-Moussa, Hawkins, & Storey. 2021. *miRNomics II.*

METABOLIC RATE DEPRESSION

1) *TURN OFF* most genes and biological processes
2) Conserve energy usage
3) Protect from the accumulation of damaging waste products
4) Activate protective cellular defenses

Gene 544 (2014) 67-74

Identification and expression of microRNA in the brain of hibernating bats, *Myotis lucifugus*

Kyle K. Biggar, Kenneth B. Storey *

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

ARTICLE INFO

Article history:
Received 11 February 2014
Received in revised form 19 March 2014
Accepted 22 April 2014
Available online 24 April 2014

Keywords:
Non-coding RNA
Torpor
Dicer
Metabolic rate depression

ABSTRACT

Recent research has highlighted roles for non-coding RNA i7n the regulation of stress tolerance in bats. In this study, we propose that microRNA could also play an important role in neuronal maintenance during hibernation. To explore this possibility, RT-PCR was employed to investigate the expression of eleven microRNAs from the brain tissue of euthermic control and torpid bats. Results show that eight microRNAs (miR-21, -29b, -103, -107, -124a, -132, -183 and -501) increased (1.2–1.9 fold) in torpid bats, while the protein expression of Dicer, a microRNA processing enzyme, did not significantly change during torpor. Bioinformatic analysis of the differentially expressed microRNA suggests that these microRNAs are mainly involved in two processes: (1) focal adhesion and (2) axon guidance. To determine the extent of microRNA sequence conservation in the bat, we successfully identified bat microRNA from sequence alignments against known mouse (*Mus musculus*) microRNA. We successfully identified 206 conserved pre-microRNA sequences, leading to the identification of 344 conserved mature microRNA sequences. Sequence homology of the identified sequences was found to be 94.76 ± 3.95% and 98.87 ± 2.24% for both pre- and mature microRNAs, respectively. Results suggest that brain function related to the differentiation of neurons and adaptive neuroprotection may be under microRNA control during bat hibernation.

Bat Brain – Hibernate at 5°C

miRNA shows changes in cell processes

Differentiation of Neurons [neurons "grow back']

NeuroProtection ** MRD is a stress

Wood frogs <u>freeze</u> up to ~70% of body water as ice:

No heart rate

No brain activity

No breathing

Cellular and Molecular Life Sciences
https://doi.org/10.1007/s00018-018-2821-0

ORIGINAL ARTICLE

Micromanaging freeze tolerance: the biogenesis and regulation of neuroprotective microRNAs in frozen brains

Hanane Hadj-Moussa[1] · Kenneth B. Storey[1]

Received: 31 October 2017 / Revised: 8 April 2018 / Accepted: 17 April 2018

Wood frogs: freeze up to ~70% of body water as extracellular ice:

- No heart rate
- No brain activity
- No breathing

Cellular and Molecular Life Sciences (2018) 75:3635–3647
https://doi.org/10.1007/s00018-018-2821-0

Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Micromanaging freeze tolerance: the biogenesis and regulation of neuroprotective microRNAs in frozen brains

Hanane Hadj-Moussa[1] · Kenneth B. Storey[1]

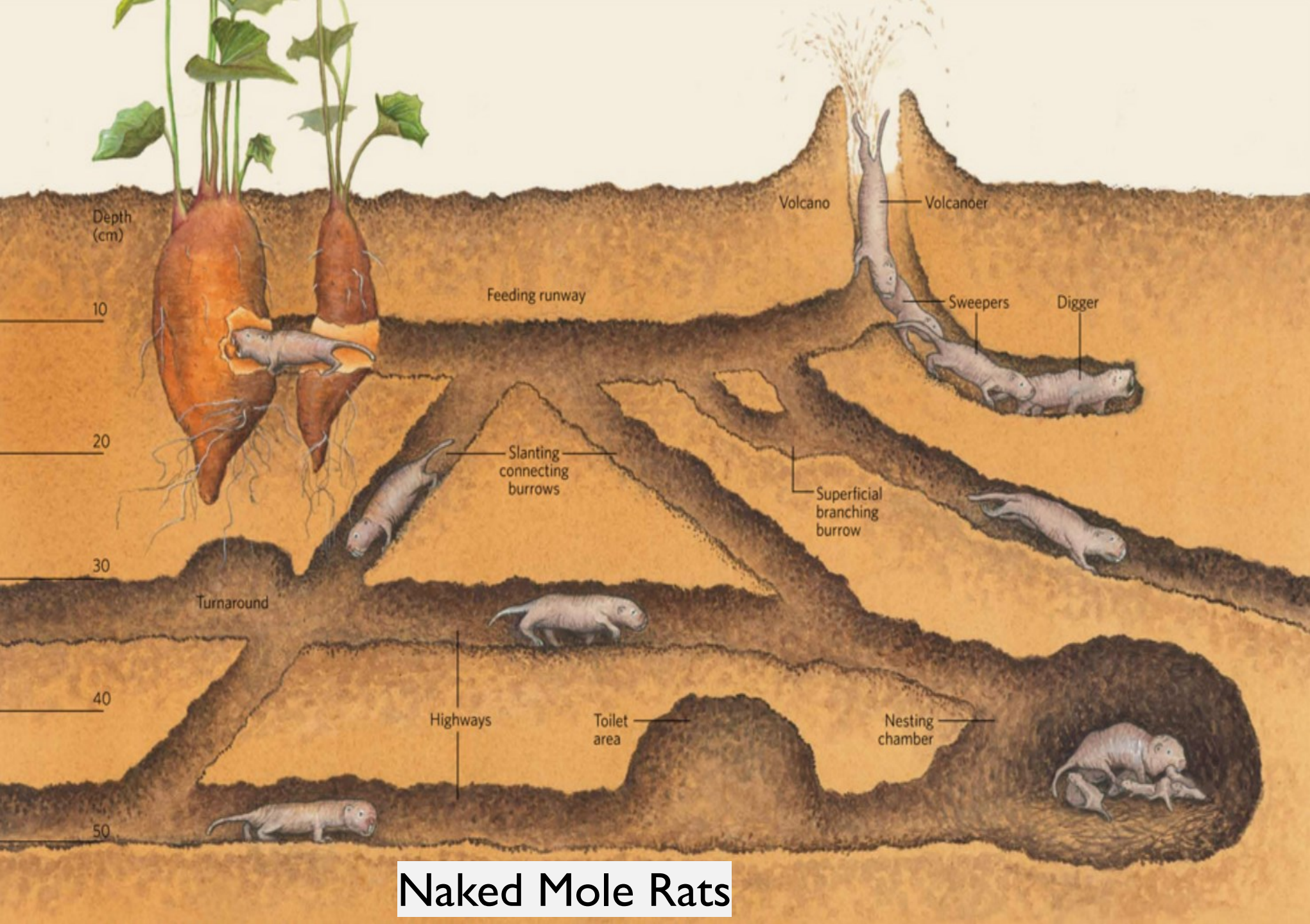
Received: 31 October 2017 / Revised: 8 April 2018 / Accepted: 17 April 2018 / Published online: 21 April 2018

Abstract

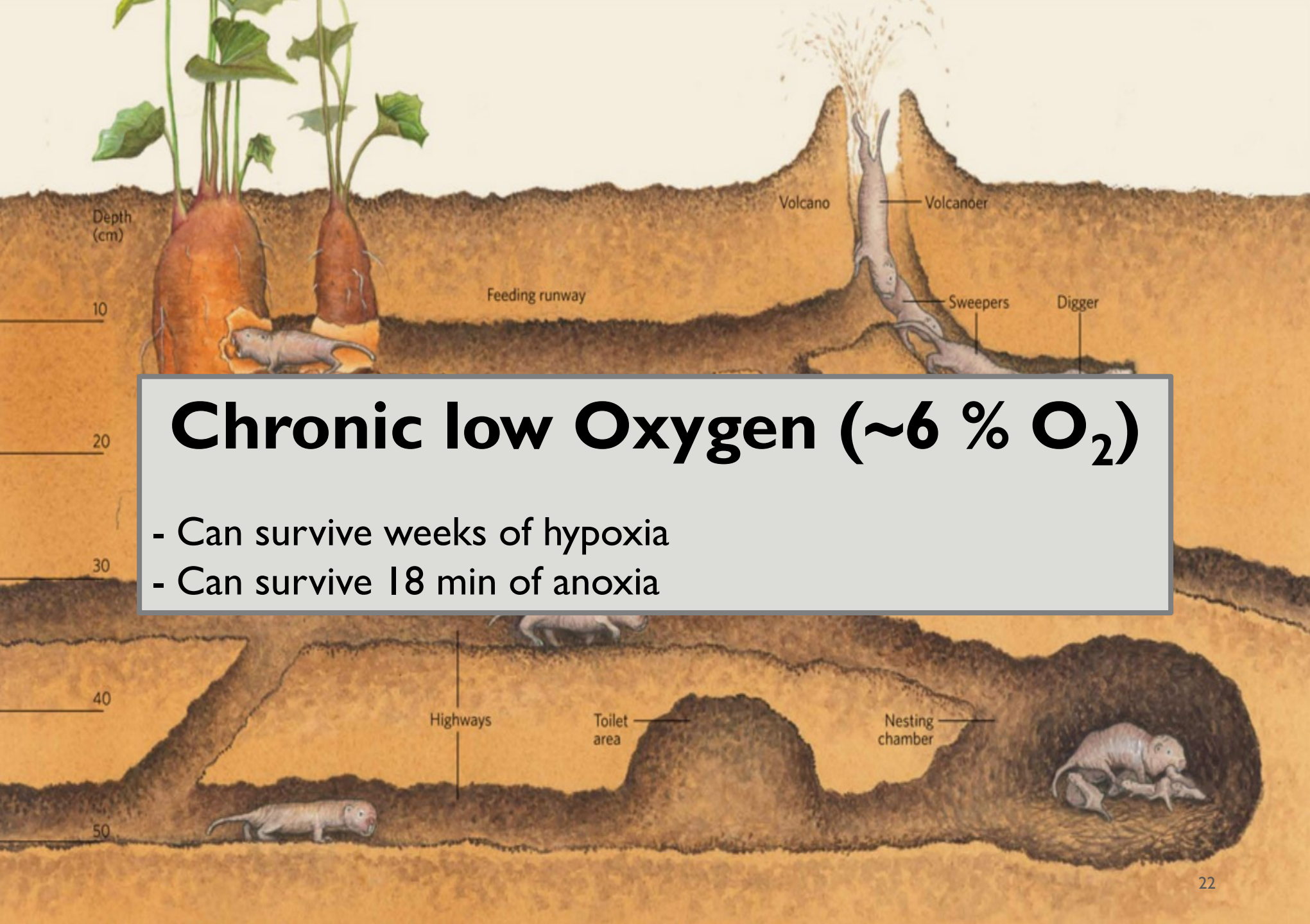
When temperatures plummet below 0 °C, wood frogs (*Rana sylvatica*) can endure the freezing of up to ~ 65% of their body water in extracellular ice masses, displaying no measurable brain activity, no breathing, no movement, and a flat-lined heart. To aid survival, frogs retreat into a state of suspended animation characterized by global suppression of metabolic functions and reprioritization of energy usage to essential survival processes that is elicited, in part, by the regulatory controls of microRNAs. The present study is the first to investigate miRNA biogenesis and regulation in the brain of a freeze tolerant vertebrate. Indeed, proper brain function and adaptations to environmental stimuli play a crucial role in coordinating stress responses. Immunoblotting of miRNA biogenesis factors illustrated an overall reduction in the majority of these processing proteins suggesting a potential suppression of miRNA maturation over the freeze–thaw cycle. This was coupled with a large-scale RT-qPCR analysis of relative expression levels of 113 microRNA species in the brains of control, 24 h frozen, and 8 h thawed *R. sylvatica*. Of the 41 microRNAs differentially regulated during freezing and thawing, only two were significantly upregulated. Bioinformatic target enrichment of the downregulated miRNAs, performed at the low temperatures experienced during freezing and thawing, predicted their involvement in the potential activation of various neuroprotective processes such as synaptic signaling, intracellular signal transduction, and anoxia/ischemia injury protection. The predominantly downregulated microRNA fingerprint identified herein suggests a microRNA-mediated cryoprotective mechanism responsible for maintaining neuronal functions and facilitating successful whole brain freezing and thawing.

FROZEN BRAIN

- MR = OFF
- Neuroprotection
- Synaptic signals
- Injury Protection
- “CryoProtection”



Naked Mole Rats



Chronic low Oxygen (~6 % O_2)

- Can survive weeks of hypoxia
- Can survive 18 min of anoxia

Hypoxic naked mole-rat brains use microRNA to coordinate hypometabolic fuels and neuroprotective defenses

Hanane Hadj-Moussa, Matthew E Pamenter, Kenneth B Storey

J. Cell Physiol. 2021. 236(7), 5080-5097. doi: 10.1002/jcp.30216.

Abstract

Naked mole-rats are among the mammalian champions of hypoxia tolerance. They evolved adaptations centered around reducing metabolic rate to overcome the challenges experienced in their underground burrows. In this study, we used next-generation sequencing to investigate one of the factors likely supporting hypoxia tolerance in naked mole-rat brains, posttranscriptional microRNAs (miRNAs). Of the 212 conserved miRNAs identified using small RNA sequencing, 18 displayed significant differential expression during hypoxia. Bioinformatic enrichment revealed that hypoxia-mediated miRNAs were suppressing energy expensive processes including de novo protein translation and cellular proliferation. This suppression occurred alongside the activation of neuroprotective and neuroinflammatory pathways, and the induction of central signal transduction pathways including HIF-1α and NFκB via miR-335, miR-101, and miR-155. MiRNAs also coordinated anaerobic glycolytic fuel sources, where hypoxia-upregulated miR-365 likely suppressed protein levels of ketohexokinase, the enzyme responsible for catalyzing the first committed step of fructose catabolism. This was further supported by a hypoxia-mediated reduction in glucose transporter 5 proteins that import fructose into the cell. Yet, messenger RNA and protein levels of lactate dehydrogenase, which converts pyruvate to lactate in the absence of oxygen, were elevated during hypoxia. Together, this demonstrated the induction of anaerobic glycolysis despite a lack of reliance on fructose as the primary fuel source, suggesting that hypoxic brains are metabolically different than anoxic naked mole-rat brains that were previously found to shift to fructose-based glycolysis. Our findings contribute to the growing body of oxygen-responsive miRNAs "OxymiRs" that facilitate natural miRNA-mediated mechanisms for successful hypoxic exposures.

NAKED MOLE RATS

***hypoxia tolerance**

Mammal champion

- **miRNA : OxymiRs**
- **Signal transduction roles**
- **Glucose transport**
- **Role of ANOXIA**

Received: 15 October 2020 | Revised: 19 November 2020 | Accepted: 1 December 2020

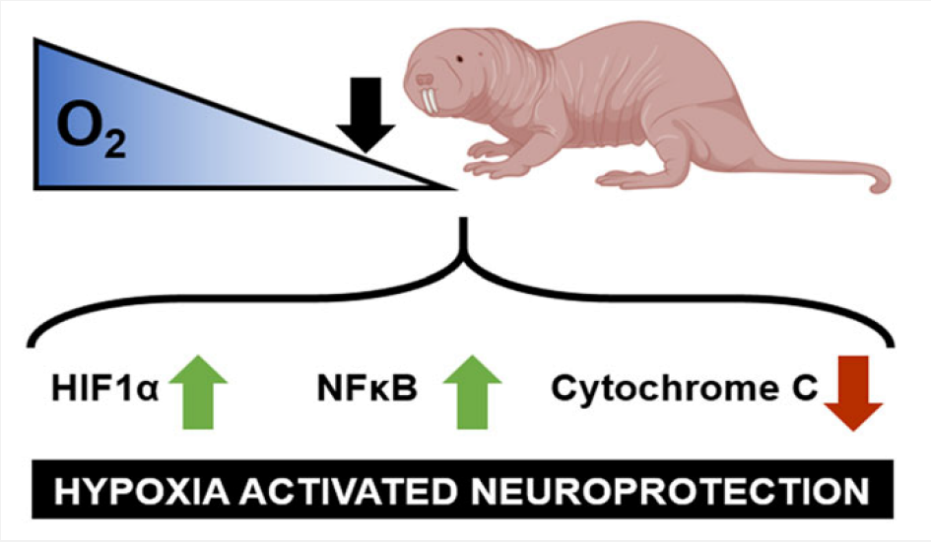
DOI: 10.1002/jcp.30216

ORIGINAL RESEARCH ARTICLE

Journal of Cellular Physiology WILEY

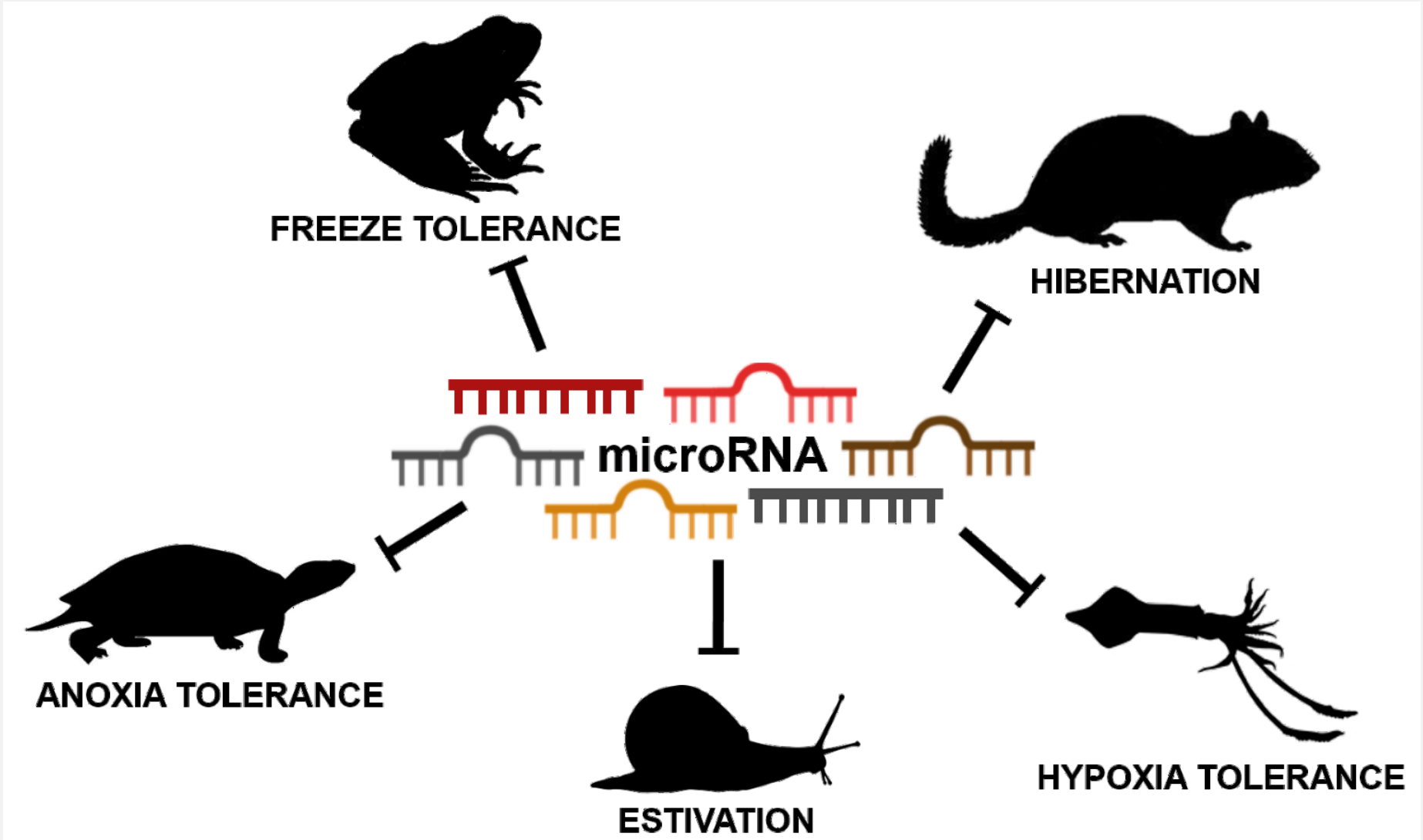
Hypoxic naked mole–rat brains use microRNA to coordinate hypometabolic fuels and neuroprotective defenses

Hanane Hadj-Moussa | Matthew E. Pamenter[2,3] | Kenneth B. Storey[1]



MicroRNAs:

- Limit carb usage in hypoxic brains
- Protect from oxidative damage
- *Turn off* non-essential energy expensive processes



MRD: Metabolic Rate Depression

Hadj-Moussa, Hawkins, & Storey. 2021. *miRNomics II.*

Review › Mol Cell Biochem. 2021 Jun;476(6):2381-2392. doi: 10.1007/s11010-021-04072-x. Epub 2021 Feb 17.

Insights from a vertebrate model organism on the molecular mechanisms of whole-body dehydration tolerance

Bryan E Luu [1 2], Liam J Hawkins [1], Kenneth B Storey [3]

Affiliations + expand

PMID: 33595794 DOI: 10.1007/s11010-021-04072-x

Abstract

Studies on the molecular mechanisms of dehydration tolerance have been largely limited to plants and invertebrates. Currently, research in whole body dehydration of complex animals is limited to cognitive and behavioral effects in humans, leaving the molecular mechanisms of vertebrate dehydration relatively unexplored. The present review summarizes studies to date on the African clawed frog (Xenopus laevis) and examines whole-body dehydration on physiological, cellular and molecular levels. This aquatic frog is exposed to seasonal droughts in its native habitat and can endure a loss of over 30% of its total body water. When coping with dehydration, osmoregulatory processes prioritize water retention in skeletal tissues and vital organs over plasma volume. Although systemic blood circulation is maintained in the vital organs and even elevated in the brain during dehydration, it is done so at the expense of reduced circulation to the skeletal muscles. Increased hemoglobin affinity for oxygen helps to counteract impaired blood circulation and metabolic enzymes show altered kinetic and regulatory parameters that support the use of anaerobic glycolysis. Recent studies with X. laevis also show that pro-survival pathways such as antioxidant defenses and heat shock proteins are activated in an organ-specific manner during dehydration. These pathways are tightly coordinated at the post-transcriptional level by non-coding RNAs, and at the post-translational level by reversible protein phosphorylation. Paired with ongoing research on the X. laevis genome, the African clawed frog is poised to be an ideal animal model with which to investigate the molecular adaptations for dehydration tolerance much more deeply.

DEHYDRATION – MRD
NAKED MOLE RATS

Prosurvival miRNA
*** Many organs**
MRD at ‘room temperature’
Antioxidant defence
Heat shock proteins
Dehydrated organs for transplant

Biochim Biophys Acta Gene Regul Mech. 2018 Jun;1861(6):586-593.
doi: 10.1016/j.bbagrm.2018.04.007. Epub 2018 May 2.

Potential role for microRNA in regulating hypoxia-induced metabolic suppression in jumbo squids

Hanane Hadj-Moussa [1], Samantha M Logan [1], Brad A Seibel [2], Kenneth B Storey [3]

Affiliations + expand

PMID: 29729419 DOI: 10.1016/j.bbagrm.2018.04.007

Abstract

At night, Humboldt squid (Dosidicus gigas) rise to the ocean's surface to feed, but come morning, they descend into the ocean's oxygen minimum zone where they can avoid predators but must deal with severe hypoxia, high pressure, and very cold water. To survive this extreme environment, squid use various adaptations to enter a hypometabolic state characterized by metabolic rate suppression by 35-52%, relative to normoxic conditions. The molecular mechanisms facilitating this metabolic flexibility have yet to be elucidated in hypometabolic squid. Herein, we report the first investigation of the role of microRNAs, a rapid and reversible post-transcriptional master regulator of virtually all biological functions, in cephalopods. We examined expression levels of 39 highly-conserved invertebrate microRNAs in D. gigas brain, mantle muscle, and branchial heart, comparing hypoxic and normoxic conditions. Hypoxia-inducible microRNAs are potentially involved in facilitating neuroprotection, anti-apoptosis, and regenerative mechanisms in brain; inhibiting apoptosis and cell proliferation while conserving energy in heart; and limiting damage by reactive oxygen species and apoptosis in muscle. Rather than orchestrate global metabolic rate depression, the majority of hypoxia-inducible microRNAs identified are involved in promoting cytoprotective mechanisms, suggesting a regulatory role for microRNA in hypoxic marine invertebrates that sets the stage for mechanistic analyses.

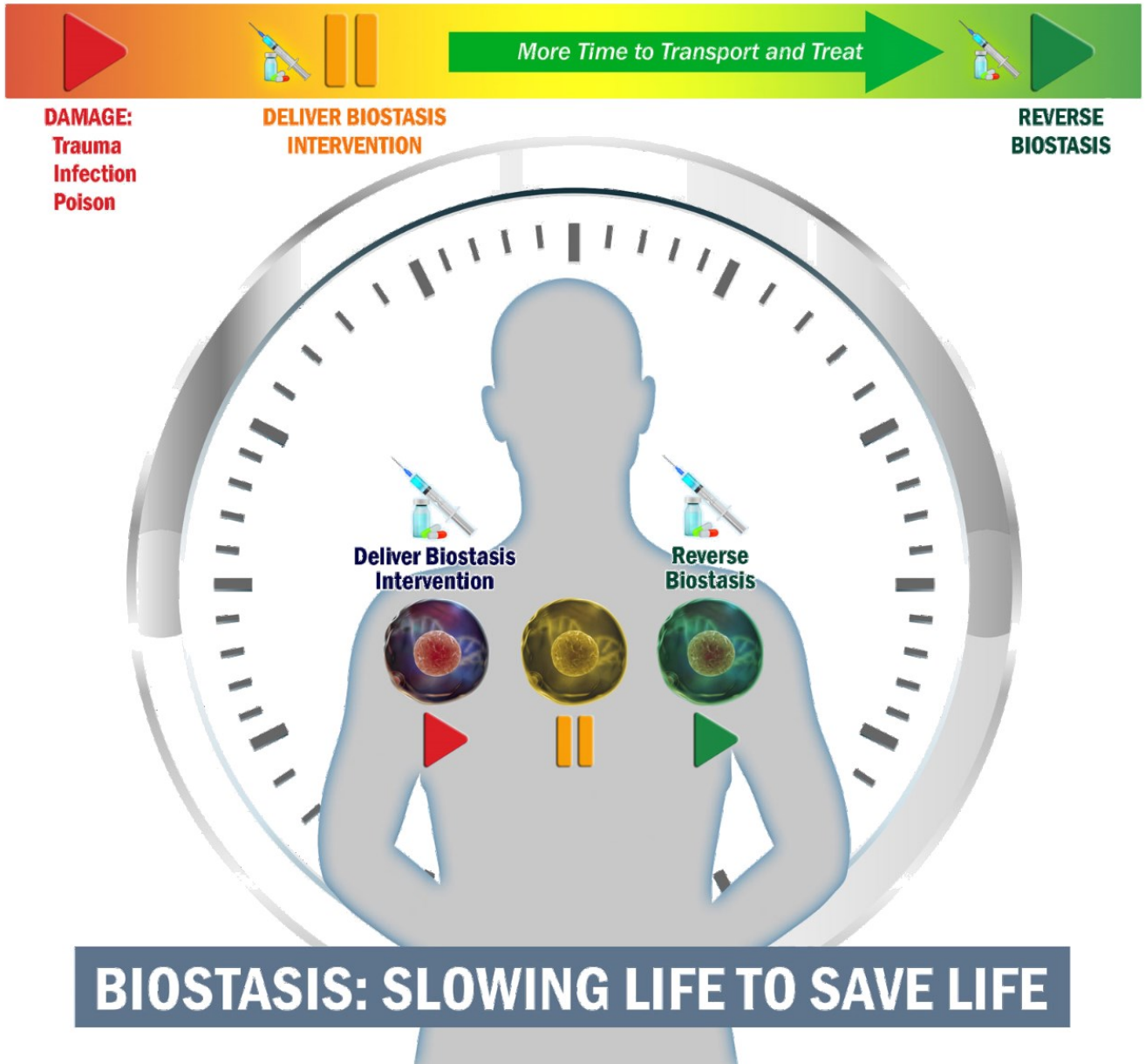
Squid have maximal MR at surface and ~ZERO MR at the bottom of the ocean .

- **This happens every evening**
  - **Aerobic at surface**
  - **Anoxic at bottom**
- **Changes happens in the brain**
  - **Regeneration paths ON**
  - **Anti-Apoptosis ON**

THE PLAN OF THE TALK

- MicroRNAs and **gene silencing**
- MicroRNAs and **animal adaptations**
- MicroRNAs and **the brain**
- MicroRNAs and **diseases and interventions**

SLOWING HUMANS DOWN



HUMAN HIBERNATION?

- Last resort
- Many trauma patients die of blood loss before treatable injuries can be fixed.
- Induce hypothermia to **10°C** in trauma (gunshot) victims
- Instead of 5-10 min to operate, doctors have over 1 hour

2018

The FEBS Journal

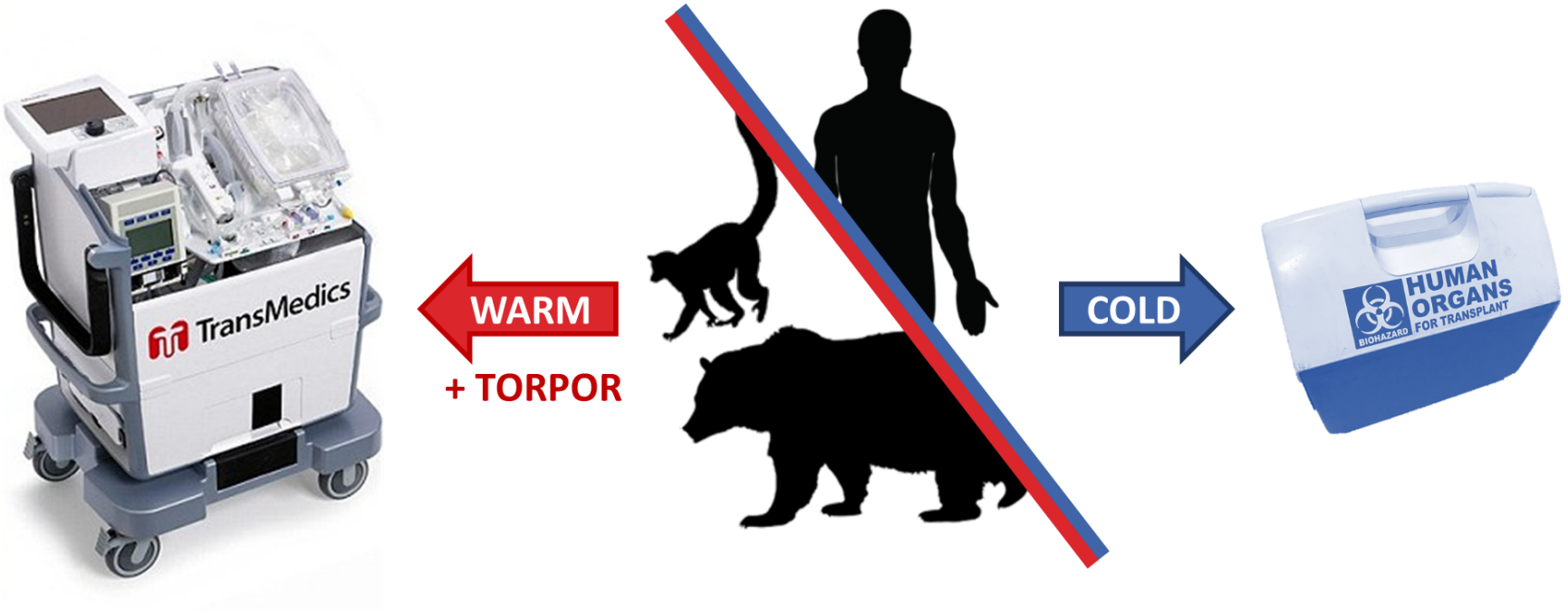
FEBS PRESS science publishing by scientists

VIEWPOINT

Bringing nature back: using hibernation to reboot organ preservation

Hanane Hadj-Moussa and Kenneth B. Storey

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



MOVE AWAY FROM → *perfuse, chill, pack in a cooler, and run*!

2018

IDEAS & SPECULATIONS

Insights & Perspectives

BioEssays

www.bioessays-journal.com

Solving Donor Organ Shortage with Insights from Freeze Tolerance in Nature

*Bryan E. Luu and Kenneth B. Storey**

Nature inspired cryoprotectants

MicroRNA to precondition donor organs

NORMOTHERMIC PRINCIPLES:

- Exploit strategies from warm hibernators to reboot **normothermic** organ preservation.
- Currently, donor organs are cooled to ~ 4°C to reduce metabolic rate, but this cooling is damaging.

WARM PERFUSIONS IMPROVE GRAFT SUCCESS:

1) Organs experience near-normal physiological conditions (e.g. keep a heart beating).
2) Continuous assessment of metabolic parameters prior to implant.
3) Infusion of substrates, nutrients, drugs, etc. as needed.
4) Organs avoid waste product accumulation.

On the long trip to Mars, can induced hibernation be used to protect the health of space travelers and to maximize extremely limited resources?

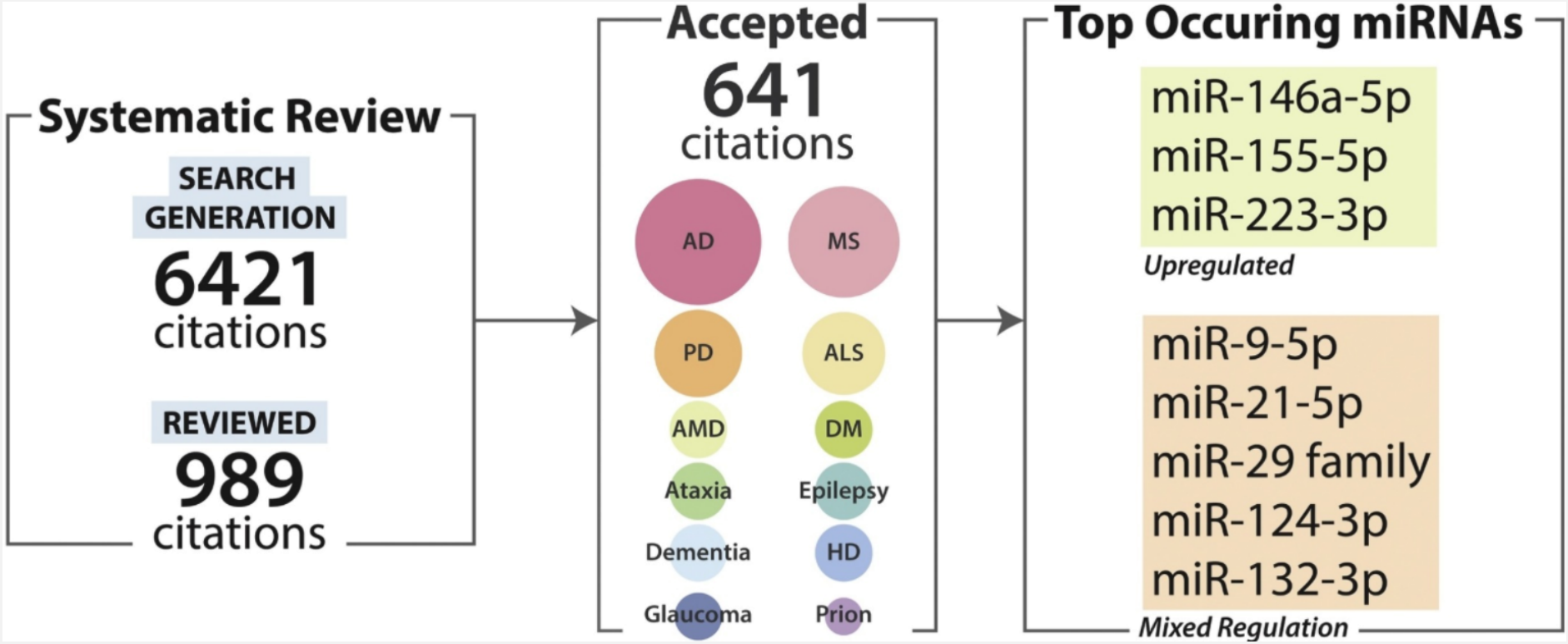
THE MEDICAL PART OF THE PLAN

- MicroRNAs and **gene silencing**
- MicroRNAs and **animal adaptations**
- MicroRNAs and **novel innovations**
- MicroRNAs -- **diseases and treatments**

Unique microRNA 'fingerprints'

- Neurodegenerative
  - Alzheimer's disease
  - Parkinson's disease
- Cancers… *all of them*
- Coronary heart disease
- Stroke
- Liver disease
- Kidney disease
- Epilepsy
- Depression
- Immune-related diseases
  - Diabetes
  - Multiple sclerosis
  - Arthritis
  - Lupus
- Viral diseases
  - HIV
  - Influenza
  - *COVID-19*

MICRORNAS IN NEURODEGENERATION



Juzwik, *et al*. 2019. *Progress in Neurobiology*.

MICRORNA BIOMARKERS

- Identify the disease, progression, stage, and genetic link
- Each disease can have one or many miRNA biomarkers that identify a health outcome
- Many microRNA biomarker trials are currently registered as phase 4 clinical trials.

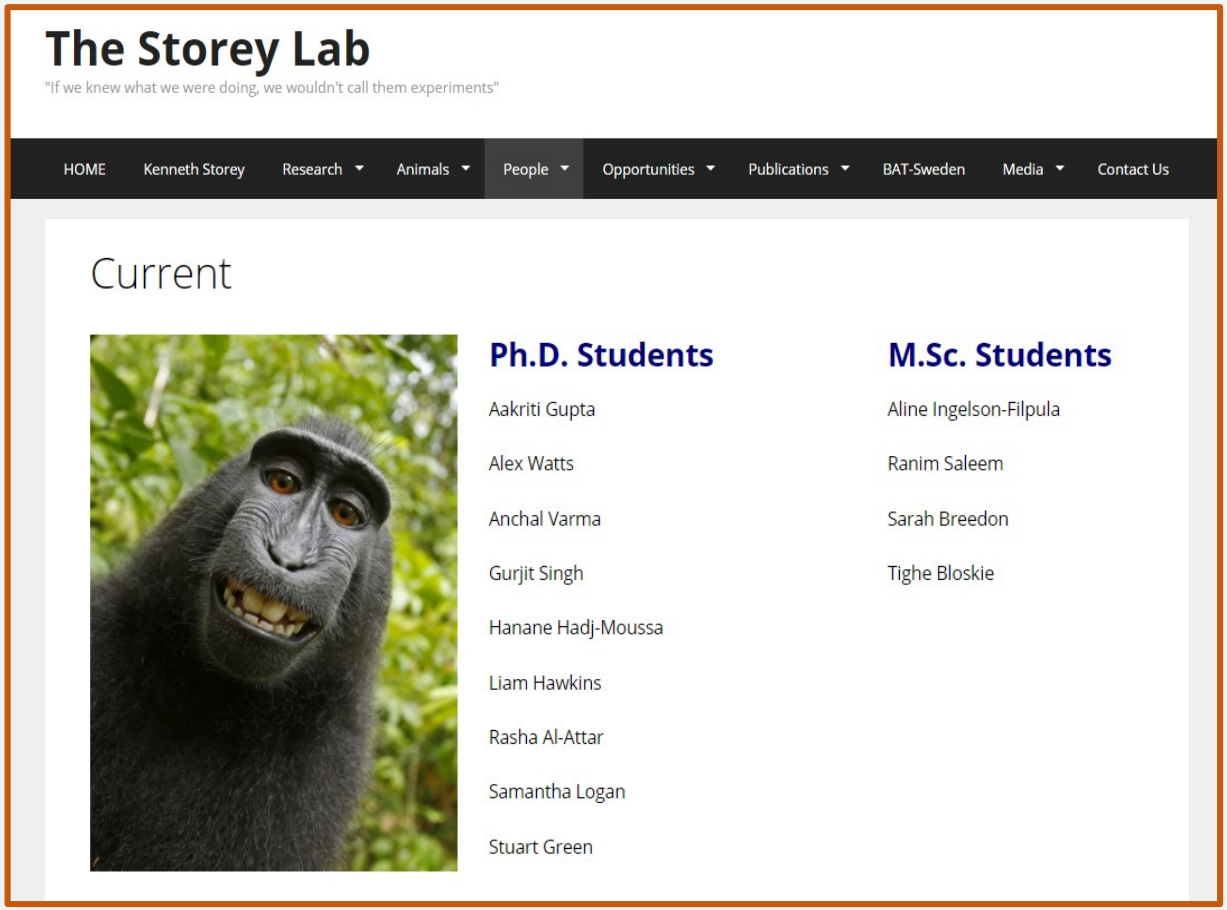
MICRORNA MEDICAL INTERVENTIONS

- Currently, no approved microRNA drugs are on the market

miRNA gene; drug name	Clinical trial number; phase status	Disease/disorder investigated
miR-34; MRX34	NCT01829971; phase 1 (terminated) NCT02862145; phase 1 (withdrawn)	Liver cancer, lymphoma melanoma
miR-92; MRG 110	NCT03603431; phase 1 (recruiting)	wound healing, heart failure
miR-16; MesomiR-1	NCT02369198; phase 1 (completed)	Mesothelioma, lung cancer
miR-122; Miravirsen	NCT02508090; phase 2 (completed) NCT02452814; phase 2 (completed) NCT01200420; phase 2 (completed) NCT01872936; phase 2 (unknown status) NCT01727934; phase 2 (unknown status) NCT01646489; phase 1 (completed)	Hepatitis C virus
miR-29; MRG-201	NCT03601052; phase 1 (recruiting)	Keloid, fibrous scar tissue formation
miR-21; RG-012	NCT02855268; phase 2 (suspended, sponsor decision) NCT03373786; phase 1 (active, not recruiting)	Alport syndrome
miR-155; Cobomarsen (MRG-106)	NCT03713320/phase 2 (recruiting) NCT03837457/phase 2 (new/not yet recruiting)	T-cell lymphoma/ mycosis fungoides

Hanna et al., 2019. *Frontiers in Genetics.*

Acknowledgements



http://www.kenstoreylab.com/

SPECIAL THANKS TO:

Jan Storey
Dr. Jing Zhang
Storey Lab members

Carleton UNIVERSITY

NSERC CRSNG

QUESTIONS?