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REVIEW

Molecular interactions underpinning the phenotype of hibernation in mammals

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ABSTRACT

Mammals maintain a constant warm body temperature, facilitating a wide variety of metabolic reactions. Mammals that hibernate have the ability to slow their metabolism, which in turn reduces their body temperature and leads to a state of hypothermic torpor. For this metabolic rate reduction to occur on a whole-body scale, molecular interactions that change the physiology of cells, tissues and organs are required, resulting in a major departure from normal mammalian homeostasis. The aim of this Review is to cover recent advances in the molecular biology of mammalian hibernation, including the role of small molecules, seasonal changes in gene expression, cold-inducible RNA-binding proteins, the somatosensory system and emerging information on hibernating primates. To underscore the importance of differential gene expression across the hibernation cycle, mRNA levels for 14,261 ground squirrel genes during periods of activity and torpor are made available for several tissues via an interactive transcriptome browser. This Review also addresses recent findings on molecular interactions responsible for multi-day survival of near-freezing body temperatures, single-digit heart rates and a slowed metabolism that greatly reduces oxygen consumption. A better understanding of how natural hibernators survive these physiological extremes is beginning to lead to innovations in human medicine.

KEY WORDS: Ground squirrel, Hypothermia, Physiology, Seasonal adaptation, Torpor, Transcriptome

Introduction

Mammals walk, swim and fly through Earth's biosphere by performing chemical reactions in their bodies. These reactions take place in a warm cellular environment that is stably maintained independent of the temperature of the animal's surroundings. This internal body temperature is optimized for molecular interactions that support cellular function, and hence mammalian life. After millions of years of evolution, mammals have settled on a body temperature of around 37°C to carry out biochemical reactions ranging from DNA replication to glycolysis. However, some mammals gain survival advantages by temporarily reducing physical activity, metabolic rate and body temperature – a phenomenon known as torpor. Torpor allows mammals to cope with environmental stressors, such as a lack of food and water, and is just one component of a seasonal adaptation known as hibernation.

Hibernation can be viewed as series of physiological adaptations in various tissues and organs that give an animal the ability to survive climatic extremes. The sum of these adaptations results in a radical departure from the normal physiological homeostasis seen in most mammals. This Review covers recent advances in the molecular biology of hibernation, with a focus on molecular interactions underpinning the hibernation phenotype. Specific topics include the torpor–arousal cycle, the role of small molecules, changes in gene expression, cold-inducible RNA-binding proteins, the somatosensory system and emerging information on hibernating primates. This new information not only is beginning to explain how natural hibernators survive physiological extremes that would be lethal to most mammals, but also identifies molecular mechanisms that may prove useful to human medicine.

Hibernation torpor–arousal cycles

Hibernation is used by a wide phylogenetic range of mammals (reviewed in Melvin and Andrews, 2009), and is generally characterized by long bouts of hypothermic torpor that are regularly interrupted by brief normothermic interbout arousals (IBAs). Fig. 1 shows the body temperature of a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) as measured by a surgically implanted transmitter over the course of 1 year. In this small rodent, hibernation is characterized by 7- to 10-day torpor bouts at body temperatures of 5–7°C, separated by short (<24 h) IBAs at 37°C, over a period of 5–6 months. Among hibernating mammals, torpid body temperatures and the periodicity of IBAs can vary depending on the species, from a low of −3°C interspersed with IBAs every 3–4 weeks in Arctic ground squirrels (*Urocitellus parryii*; Barnes, 1989) to a relatively warm hibernating body temperature of 28°C with no IBAs in Malagasy common tenrecs (*Tenrec ecaudatus*; Treat et al., 2018).

IBAs are a mystery of hibernation, but their importance is underscored by the fact that they occur in the vast majority of hibernating mammals. The rapid rewarming phase of an IBA initially involves non-shivering thermogenesis that originates in a specialized heater organ unique to mammals called brown adipose tissue (reviewed in Ballinger and Andrews, 2018). At first glance, it appears counterintuitive that a seasonal adaptation that has evolved to conserve energy also requires intense bursts of energy to regularly drive arousals. Many ideas have been proposed to justify the existence of IBAs, but growing evidence indicates that these brief periods of normothermia allow for resumption of transcription (van Breukelen and Martin, 2002) and translation (van Breukelen and Martin, 2001), activate a dormant immune system to combat pathogens (Prendergast et al., 2002), and provide a time of molecular replenishment, replacement and repair (D'Alessandro et al., 2017; Wiersma et al., 2018). Indeed, it makes sense that turnover and synthesis of macromolecules such as RNA and protein are more efficient, and proceed at a faster rate, at warmer body temperatures.

For a mammal to survive long-term hypothermia is quite a feat, but to also survive the rapid physiological shifts of the torpor–IBA cycle requires tremendous physiological resilience. Hibernators have evolved resilience in their nervous and cardiovascular systems

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Journal of Experimental Biology (2019) 222, jeb160606. doi:10.1242/jeb.160606

List of symbols and abbreviations

5′-AMP	5′-adenosine monophosphate
ADAR	adenosine deaminase acting on RNA
BHB	D-beta-hydroxybutyrate
CHD9	chromodomain helicase DNA binding protein 9
CIRBP	cold-inducible RNA-binding protein
eQTL	expression quantitative trait loci
GABA	gamma-aminobutyric acid
Gln	glutamine
Glu	glutamate
GWAS	genome-wide association scan
H1F0	H1 histone family member 0
H_2S	hydrogen sulfide gas
HDAC	histone deacetylase
HIST1H1C	histone cluster 1 H1 family member C
HIST1H1E	histone cluster 1 H1 family member E
IBA	interbout arousal
iPSCs	induced pluripotent stem cells
MCT1	monocarboxylic acid transporter 1
NMR	nuclear magnetic resonance
$p27^{Kip1}$	cyclin-dependent kinase inhibitor p27
PDK4	pyruvate dehydrogenase kinase 4
RBM3	RNA binding protein motif 3
SRSF5	serine/arginine-rich splicing factor 5
T1AM	3-iodothyronamine
TCA cycle	tricarboxylic acid (aka Krebs) cycle
TRPM8	transient receptor potential cation channel subfamily M 8
TRPV3	transient receptor potential vanilloid 3
TRPV4	transient receptor potential vanilloid 4

to protect the brain and heart, the two organs most sensitive to reduced blood flow (referred to as ischemia; see Glossary) and reperfusion injury (see Glossary), which can be caused by the resumption of blood flow. Molecular interactions contributing to the hibernation phenotype in the brain and heart are shown in Tables 1 and 2, respectively, and illustrate the variety of mechanisms that support the function of these two critical organs during the physiological extremes of hypothermic torpor and rapid arousal. Mechanisms by which hibernators avoid injury during both torpor and arousal are of great biomedical interest because of applications in the areas of traumatic brain injury, myocardial infarction, organ preservation, hemorrhagic shock and stroke.

Role of small molecules in the hibernation phenotype

Hibernation is a whole-body phenotype involving reductions in oxygen consumption, metabolic reactions, core body temperature, neural activity and heart rate. Accumulation and depletion of specific metabolites and other small molecules can change whole-animal physiology so that these physiological extremes are attainable and survivable. In this section, I will describe studies that show how changes in the abundance of a handful of unremarkable organic molecules can have a profound influence on the hibernation phenotype.

Metabolomic screens

Because small molecules are constantly and rapidly modified in living cells, simultaneous screening of multiple metabolites can provide insight into how various metabolic pathways give rise to the hibernation phenotype. Metabolomic profiling of plasma by the groups of Carey (Nelson et al., 2010) and Martin (D'Alessandro et al., 2017; Epperson et al., 2011) has provided a comprehensive view of seasonal changes in the populations of small molecules that circulate in the blood, and hence bathe the cells of hibernating ground squirrels. The large body of data collected on the changing levels of 266 metabolites throughout the year by D'Alessandro et al. (2017) has provided information that sheds light onto a variety of mechanisms that potentially regulate the hibernation phenotype. Specifically, IBAs appear to be a time for removal of potentially toxic metabolic waste that has accumulated in the blood during torpor, as well as replenishment of metabolic substrates such as free fatty acids and amino acids. Plasma samples collected from nine different time points across the hibernation cycle show fluctuations in several molecules including: the basic amino acids lysine and arginine, one-carbon metabolism intermediates, and the sulfur-containing amino acids methionine and cysteine (D'Alessandro et al., 2017). However, metabolic markers associated with reperfusion injury, such as succinate and fumarate, showed little change, supporting the hibernator's natural avoidance of this pathology.

Nuclear magnetic resonance of small molecules in the brain of living hibernators

Seasonal changes in the levels of small molecules are most accurately measured in living animals over a period of months. By

Glossary

Excitotoxicity
Neuronal pathology caused by excess glutamate in the extracellular space that overstimulates excitatory glutamate receptors. Overactivated glutamate receptors result in high levels of Ca2+ entering the cells, eventually leading to neuronal death.

Hyperphagic
Feeding behavior where animals consume relatively large amounts of food in a defined period of time. In Schwartz et al. (2015b), hyperphagic ground squirrels were defined as animals eating at least 20 g of food per day and were observed to have an average final daily consumption of 26.92±0.74 g day^{-1} at the beginning of September.

Hypophagic
Feeding behavior where animals consume relatively small amounts of food in a defined period of time. In Schwartz et al. (2015b), hypophagic ground squirrels, that were previously hyperphagic, were defined as animals eating less than 10 g of food per day and were observed to have an average final daily consumption of 3.41±1.06 g day^{-1} at the end of September.

Ischemia
Reduction in blood flow and oxygen delivery to living tissue. Deprivation of oxygen can damage tissues and organs. Some organs, such as the heart and brain, are more sensitive to ischemic damage than others.

Isoelectric brain
A state in which an electroencephalogram (EEG) readout indicates no brain electrical activity. The isoelectric readout, sometimes referred to as a flat EEG, is often indicative of deep coma or brain death in humans. In natural hibernators, this electro-cerebral inactivity is due to living neurons and synaptic networks that are dormant during hypothermic torpor.

Induced pluripotent stem cells
Eukaryotic cells that are originally acquired from living animals and then selectively 'reprogrammed' in culture so that they can differentiate into a variety of different cells and tissues.

Reperfusion injury
Resumption of blood flow to ischemic tissue resulting in damage due to a sudden increase in oxygen delivery. Reperfusion of an ischemic cell generates reactive oxygen species, which can damage cell membranes, exacerbate inflammatory processes and cause deleterious modifications of DNA and proteins.

Scaffold
Long assemblies of sequenced DNA fragments that represent the primary sequence in the genome where the sequence originates. A single chromosome may be represented by many scaffolds or just one scaffold. Improvements in sequence assembly of the thirteen-lined ground squirrel genome increased the previously longest scaffold from 58.28 to 73.92 Mb, and 539 original draft assembly scaffolds were reduced to 33 scaffolds (Grabek et al., 2017, preprint).

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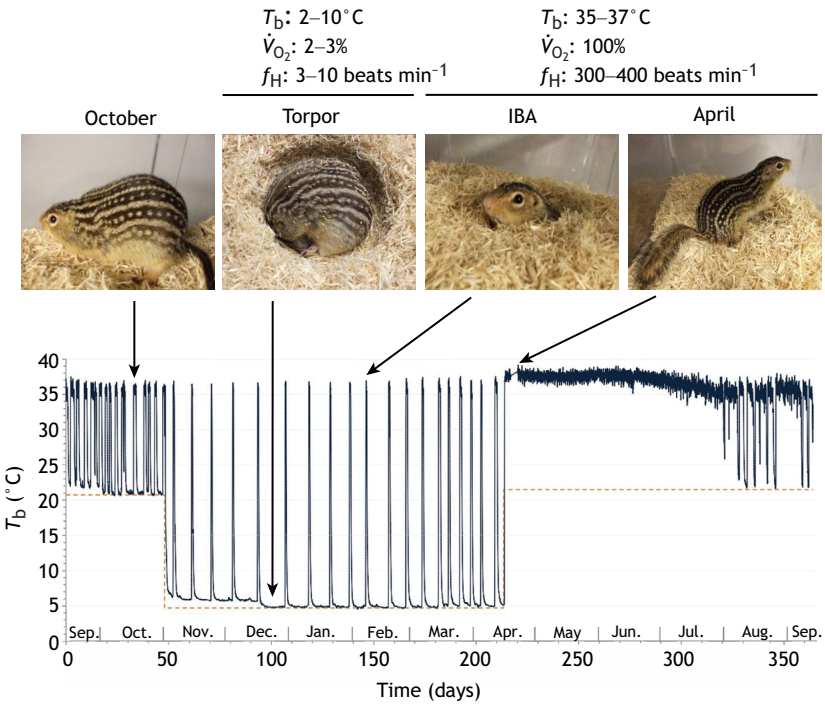

Fig. 1. Full-year body temperature tracing of a thirteen-lined ground squirrel. Core body temperature (solid line) of a thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) measured by a surgically implanted transmitter over the course of 1 year. The animal was maintained in an environmental chamber with food at a room temperature (dashed line) of 21–23°C from mid-April to late October. Food was removed on 1 November and room temperature was maintained at 5°C from early November to mid-April. Body temperature (T_b) measurements show the extensive use of torpor prior to lowering room temperature, as shown previously (Russell et al., 2010). Representative photographs and physiological measurements of T_b, oxygen uptake rate ($\dot{V}_{O_2}$) and heart rate (f_H; beats min^{-1}) are shown above the indicated points on the graph. IBA, interbout arousal. The *y*-axis represents T_b in °C, and the *x*-axis represents time in days. Modification of figure originally published in Cooper et al. (2016).

placing living hibernators in a high-field (9.4 Tesla) magnet with a 31 cm bore, Henry et al. (2007) employed proton (^{1}H) nuclear magnetic resonance (NMR) to simultaneously measure the level of 18 different small molecules in the brains of thirteen-lined ground squirrels throughout the hibernation season. This non-invasive method was used to determine the concentration of small organic molecules in specific brain regions of the same animals at four different activity points from October through March: autumn active, torpor, IBA and spring active.

Among the notable findings of this longitudinal study was that the brain phosphocreatine:creatine ratio was increased by over two-fold (143%) in torpid versus normothermic autumn-active animals, and remained increased (+83%) during IBA (Henry et al., 2007). This increase in energy-storing phosphocreatine at the expense of creatine is consistent with a sharply reduced ATP demand in the isoelectric brain (see Glossary) during torpor (Walker et al., 1977). The high phosphocreatine:creatine ratio during torpor is likely to

Table 1. Molecular interactions underpinning the hibernation phenotype in the brain

Molecular interaction	Putative phenotype	Reference
Higher (>2-fold) respiration rates through complex I of the electron transport chain	Enhanced oxidative capacity of mitochondria during torpor and IBAs	Ballinger et al., 2017
Increased conversion of creatine to phospho-creatine during torpor	Energy conservation – preserve brain cell ion gradients	Henry et al., 2007
Increased GABA and reduced Gln and Glu levels	Neuroprotection by avoiding excitotoxicity	Henry et al., 2007; Osborne and Hashimoto, 2008
Increase in MCT1 at blood–brain barrier	Enhanced transport of BHB into brain	Andrews et al., 2009
Preferred catabolism of BHB over glucose	Fat-derived BHB provides non-lactate generating fuel source	Andrews et al., 2009
Production of melatonin during IBAs	Antioxidant that offers protection from reperfusion injury	Schwartz et al., 2015a; Tan et al., 2005
Low-temperature RNA editing	Means of diversifying RNA pool during torpor	Riemondy et al., 2018
Increased expression of RBM3	Recovery of synaptic connections lost during torpor	Peretti et al., 2015

BHB, D-beta-hydroxybutyrate; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; IBA, interbout arousal; MCT1, monocarboxylic acid transporter 1; RBM3, RNA binding protein motif 3.

Table 2. Molecular interactions underpinning the hibernation phenotype in the heart

Molecular interaction	Putative phenotype	Reference
Conversion of phospho-cofilin 2 to cofilin 2	Preserve ATP and inhibit apoptosis	Grabek et al., 2011
Upregulation of *CHRM2*	Slows heart rate	Grabek et al., 2017, preprint; Vermillion et al., 2015a
Hibernation-specific expression of pancreatic lipase	Low-temperature lipolysis in cardiomyocytes	Andrews et al., 1998; Squire et al., 2003
Upregulation of *PDK4*	Blocks carbohydrate oxidation	Andrews et al., 1998; Buck et al., 2002
Increase in SCOT1 (OXCT1) protein	Enzyme that catalyzes rate-limiting step in ketone metabolism	Grabek et al., 2011; Russeth et al., 2006
Increases in ATP2A2 and SLC8A1 proteins	Enhanced Ca^{2+} handling required for low-temperature cardiomyocyte function	Heinis et al., 2015

ATP2A2, ATPase Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} Transporting 2 (aka SERCA2A); CHRM2, cholinergic receptor, muscarinic 2; PDK4, pyruvate dehydrogenase kinase 4; SCOT1, succinyl CoA transferase; SLC8A1, Solute Carrier Family 8 Member A (aka NCX1).

Journal of Experimental Biology (2019) 222, jeb160606. doi:10.1242/jeb.160606

conserve energy to preserve ion gradients in brain cells, allowing the cells to withstand extreme changes in physiology during what Frerichs (1999) called a 'reversible cellular arrest'.

A striking observation of Henry et al. (2007), with implications for neuroprotection, was that the total concentration of γ-aminobutyric acid (GABA, the main inhibitory neurotransmitter) was increased during torpor (+135%), and both glutamine (Gln) and glutamate (Glu, the main excitatory neurotransmitter) concentrations were decreased by 54% and 17%, respectively. Osborne and Hashimoto (2008) also looked at total tissue content of neurotransmitter amino acids in hibernating Syrian hamsters (*Mesocricetus auratus*), and their results agreed with those of Henry et al. (2007), showing that GABA globally increased in the brain during torpor, and Glu decreased. The increase in GABA and decrease in Glu in both ground squirrel and hamster brains suggests that a coordinated decrease in excitatory neurotransmission, and a simultaneous increase in inhibitory neurotransmission, acts as a means of neuroprotection by avoiding excitotoxicity (see Glossary) during torpor (Table 1).

Ketones as a preferred fuel during torpor

During summer, many hibernating mammals are hyperphagic (see Glossary) as they accumulate and store fat in white adipose tissue prior to the onset of hibernation. As autumn approaches, thirteen-lined ground squirrels transition to a hypophagic (see Glossary) period as food consumption drops by an average of 55% in 3 weeks despite a warm ambient temperature and free access to food (Schwartz et al., 2015b). When food is no longer available, and hibernation begins, fatty acids stored in white adipose tissue become the animal's primary fuel source. The conversion of fatty acids into shorter ketone molecules in the liver provides a secondary fuel that circulates in the blood and is catabolized by several tissues including the heart and brain (Andrews et al., 2009).

Serum levels of the fat-derived ketone D-β-hydroxybutyrate (BHB) are highest during deep torpor (Krilowicz, 1985; Rauch and Behrisch, 1981), and exist in a reciprocal relationship with the serum concentration of glucose in the thirteen-lined ground squirrel (Andrews et al., 2009). The concentration of the ketone transporter monocarboxylic acid transporter 1 (MCT1) is increased at the blood–brain barrier as animals enter hibernation (Andrews et al., 2009). These observations raised the interesting question of whether carbon utilization is altered during hibernation, especially in the normally glucose-dependent brain. To address this question, ^{13}C-labeled BHB and glucose were injected into torpid ground squirrels, and their uptake and metabolism were measured by high-resolution NMR in both brain and heart extracts at several different body temperatures, ranging from 7 to 38°C, during arousal from torpor (Andrews et al., 2009).

Resolution of ^{13}C-labeled metabolites showed that both BHB and glucose enter the brain and heart, but their metabolism varies greatly. After entering the brain, glucose is largely ignored by the metabolic machinery, as it remains in an unmodified form, even at warm body temperatures following arousal, suggesting little or no glycolytic activity during hibernation (Fig. 2). Minimization of glucose catabolism during torpor limits lactic acid production and therefore reduces potential damage from lactic acidosis in the brain during periods of reduced blood flow. In contrast to glucose, labeled carbons from BHB enter the tricarboxylic acid (TCA) cycle without generating lactic acid (Table 1). In the heart, both glucose and BHB are transported into the organ, but only ^{13}C from BHB enters the heart TCA cycle (Fig. 2). This result shows that fuel selection is controlled at the level of individual metabolic pathways in hibernating thirteen-lined ground squirrels, and that seasonally

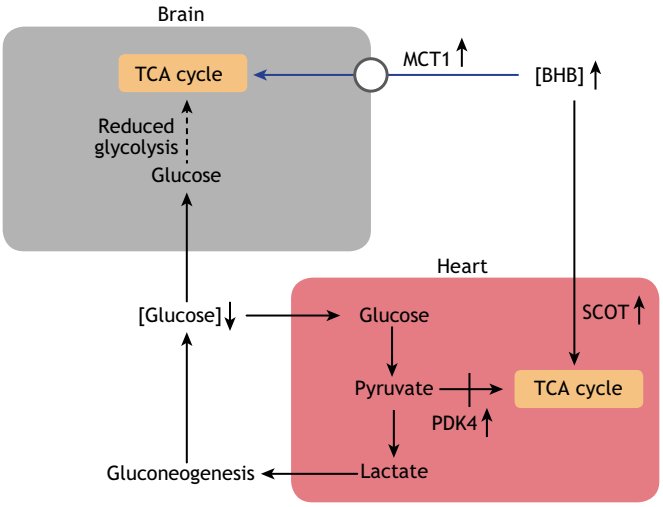

Fig. 2. Model showing beta-hydroxybutyrate utilization and glucose conservation in the brain and heart during hibernation. During torpor, the serum concentration of the D-stereoisomer of beta-hydroxybutyrate (BHB) is increased, and the concentration of glucose is reduced. The figure shows the metabolism of BHB and glucose, and indicates that BHB is the preferred fuel during hibernation, based on previous work (Andrews et al., 2009; Buck et al., 2002; Russeth et al., 2006). Long solid arrows indicate active metabolic pathways, dashed arrows indicate pathways with reduced activity during hibernation, and a solid block across a line indicates pathway stoppage. Short vertical arrows pointing up indicate an increase in concentration or activity. Short vertical arrows pointing down indicate a decrease in concentration or activity. The open circle on the edge of the brain compartment signifies that MCT1 is located at the blood–brain barrier. MCT1, monocarboxylic acid transporter 1; PDK4, pyruvate dehydrogenase kinase 4; SCOT, succinyl CoA transferase; TCA cycle, tricarboxylic acid cycle. Model was originally presented in Andrews et al. (2009).

induced adaptive mechanisms give rise to the strategic utilization of BHB during hibernation (Andrews et al., 2009).

Melatonin as a potent antioxidant in hibernators

After long periods of hypothermia and slow heart rates during torpor, hibernating mammals produce melatonin when they arouse (Florant et al., 1984; Larkin et al., 2003; Stanton et al., 1986). Melatonin is an ancient molecule that is found in cyanobacteria (Manchester et al., 2015), and its importance in regulating circadian rhythms in mammals is well characterized (reviewed in Reiter et al., 2014). However, melatonin also has a structure that can effectively scavenge and reduce free radicals (Reiter et al., 2017), suggesting that it plays a protective role in avoiding reperfusion injury upon arousal from torpor (Tan et al., 2005). Schwartz et al. (2015a) used a melatonin receptor antagonist to show that melatonin signaling also contributes to neuroprotection and optimal mitochondrial function in the brain during arousal from torpor in thirteen-lined ground squirrels (Table 1). The increase in melatonin during arousal, combined with the non-lactic acid-generating metabolism of BHB, led to the development of a hibernation-based therapy for hemorrhagic shock composed of BHB and melatonin (Klein et al., 2010; Perez de Lara Rodriguez et al., 2017). This therapy for profound blood loss has also been tested effectively in the large animal pig model (Mulier et al., 2012; Wolf et al., 2017b), and can be produced in a lyophilized form (Wolf et al., 2018).

Torpor-inducing molecules

Over 10 years ago, a handful of small to medium-sized molecules were found to induce hypothermia and torpor in non-hibernating

mice (reviewed in Andrews, 2007). Included in this group were hydrogen sulfide gas (H_2S), 5′-adenosine monophosphate (5′-AMP), 3-iodothyronamine (T1AM) and the stomach hormone ghrelin. Some of these molecules are considered to have clinical importance because of their role in metabolic rate reduction and hence protection from ischemia and reperfusion injury (Bouma et al., 2012; Wolf et al., 2017a). A recent study shows the mechanism by which exogenous H_2S induces mouse hypothermia may actually be the result of hypoxia (Hemelrijk et al., 2018), as hypoxic conditions are generated by the original experimental combination of 80 ppm H_2S with sub-normoxic 17.5% O_2 (Blackstone et al., 2005). In contrast, mice exposed to 80 ppm H_2S under normoxic (21% O_2) conditions did not exhibit a reduction in body temperature when compared with normoxic controls (Hemelrijk et al., 2018).

With respect to natural hibernation, 5′-AMP and adenosine have received considerable attention as molecules with the potential to induce torpor, but their mode of action in hibernators has not been fully elucidated. Important findings have included: (1) seasonal entrance into torpor is mediated by adenosine A(1) receptors in Arctic ground squirrels (Jinka et al., 2011; Olson et al., 2013); (2) 5′-AMP metabolism by AMP deaminase 2 in the summer, and its availability to activate AMP kinase in the winter, serves as a switch that governs fat metabolism in the liver of hibernating thirteen-lined ground squirrels (Lanaspa et al., 2015); and (3) induction of a torpor-like state by 5′-AMP does not depend on H_2S in Syrian hamsters (Dugbartey et al., 2015). However, at the time of this Review, no single molecule, or combination of molecules, has been shown to successfully induce and maintain hibernation in naturally hibernating mammals.

Seasonal changes in gene expression

To study the function of individual genes in hibernating mammals, tissue-specific overexpression of cloned sequences has been accomplished using adenoviral vectors in the liver of ground squirrels (Nelson et al., 2013), and more recently in the heart of marmots (Zhao et al., 2018). In the -omics era, multiple screening methods have been used to monitor the expression of large sets of genes to better understand the cumulative effect of differential gene expression on hibernation. These strategies can be applied to a wide variety of animals and adaptations throughout the circannual cycle (reviewed in Schwartz and Andrews, 2013), but this approach is often limited to a single organ or tissue, providing only a partial understanding of changes in whole-animal physiology. In this section, I will present a compilation of transcriptomic, proteomic and genomic studies that provide insight into how the differential expression of genes contributes to the hibernation phenotype in the thirteen-lined ground squirrel.

Hibernation transcriptome browser

Transcriptomic approaches have identified the expression of genes during various seasons and activity states in thirteen-lined ground squirrel brown adipose tissue and white adipose tissue (Hampton et al., 2013), cortex and hypothalamus regions of the brain (Schwartz et al., 2013), bone marrow (Cooper et al., 2016), and heart and skeletal muscle (Vermillion et al., 2015a). The aggregate data from all seven of these tissues are now available as an online resource. The *Ictidomys tridecemlineatus* transcriptome browser contains information on the mRNA levels for 14,261 genes and can be found at https://d.umn.edu/~mhampton/GB18.html. At this website, n=3 for each data point, and RNA measurements are shown on the y-axis as the mean of the upper-quartile normalized counts of sequence reads from four collection points (torpor, IBA, and active animals during April and October; see Fig. 1). By pointing the cursor at the individual data bars, this interactive browser provides the user with the mean±s.e.m. number of RNA reads. In addition, it also contains links to Uniprot and NCBI Gene browsers for each mRNA.

Upon inspection of the combined transcriptome data, only 10 genes show statistically significant differential expression in the majority of tissues, with eight genes (*CLK1*, *AMD1*, *BANP*, *PNRC1*, *TSC22D3*, *ABCA*, *SRSF6* and *MID1IP1*) showing mRNAs at their lowest level during torpor, and two genes (*HIST1H1C* and *RBM3*) showing mRNA levels that are significantly higher during torpor. Many other genes show differential expression, but statistical significance between any two activity states may be limited only to specific tissues. Proteogenomic studies have merged the transcriptome data with large-scale iTRAQ-based proteome data to compare the seasonal mRNA levels with levels of corresponding proteins in ground squirrel heart (Vermillion et al., 2015b), skeletal muscle (Anderson et al., 2016) and mitochondria from brown adipose tissue (Ballinger et al., 2016). In addition, extensive proteomic studies from the Martin group have been described for liver (Hindle et al., 2014), brown adipose tissue (Hindle and Martin, 2014), forebrain (Hindle and Martin, 2013) and kidney (Jani et al., 2012).

Upregulation of genes encoding chromatin-associated proteins

One of the two genes showing significant global upregulation during torpor is also thought to play a broad non-specific role in gene expression. *HIST1H1C* (Fig. 3A) encodes a histone H1 variant referred to as H1.2. H1 histones are bound to DNA between nucleosomal core particles, and are often involved in the inactivation of proximal genes (reviewed in Hergeth and Schneider, 2015). Histone H1.2 has been shown to direct the genome-wide association of the pRb tumor suppressor protein with chromatin in the form of a H1.2–pRb complex that promotes transcriptional repression and facilitates cell cycle arrest (Munro et al., 2017). Earlier studies of hibernating mammals have shown cessation of mitotic activity during torpor (Kruman et al., 1988; Popov et al., 2011); however, it is unknown whether this stoppage is due to a cell cycle regulatory event or low body temperature.

Two other histone H1-coding genes show upregulation in every tissue during torpor (although this upregulation only reaches statistical significance in a subset of the tissues examined). These genes are *HIST1H1E* (Fig. 3B), which shows a significant increase in bone marrow, and *H1F0* (Fig. 3C), which is significantly increased during torpor in heart, skeletal muscle and bone marrow (Vermillion et al., 2015a; Cooper et al., 2016). An overall increase in three different H1 sub-types may suggest increased chromatin compaction, resulting in a general repression of transcriptional activity during hibernation.

Certain histone deacetylases (HDACs), which also have been reported to have a repressive effect on gene activity, show their highest mRNA levels during hibernation in heart (*HDAC11*; Vermillion et al., 2015a), skeletal muscle (*HDAC10*; Vermillion et al., 2015a) and bone marrow (*HDAC7*; Cooper et al., 2016). Post-translational modification of histones has recently been shown to be responsive to torpor–arousal cycles, and specific acetylation sites have been identified on thirteen-lined ground squirrel histones H2B and H3 (Tessier et al., 2017). Hypothetically, a general repression of gene activity owing to chromatin structure could play a role in the overall reduction in physiological activity that occurs during torpor. However, the role of chromatin in regulating gene expression in hibernating mammals remains largely unexplored.

Journal of Experimental Biology (2019) 222, jeb160606. doi:10.1242/jeb.160606

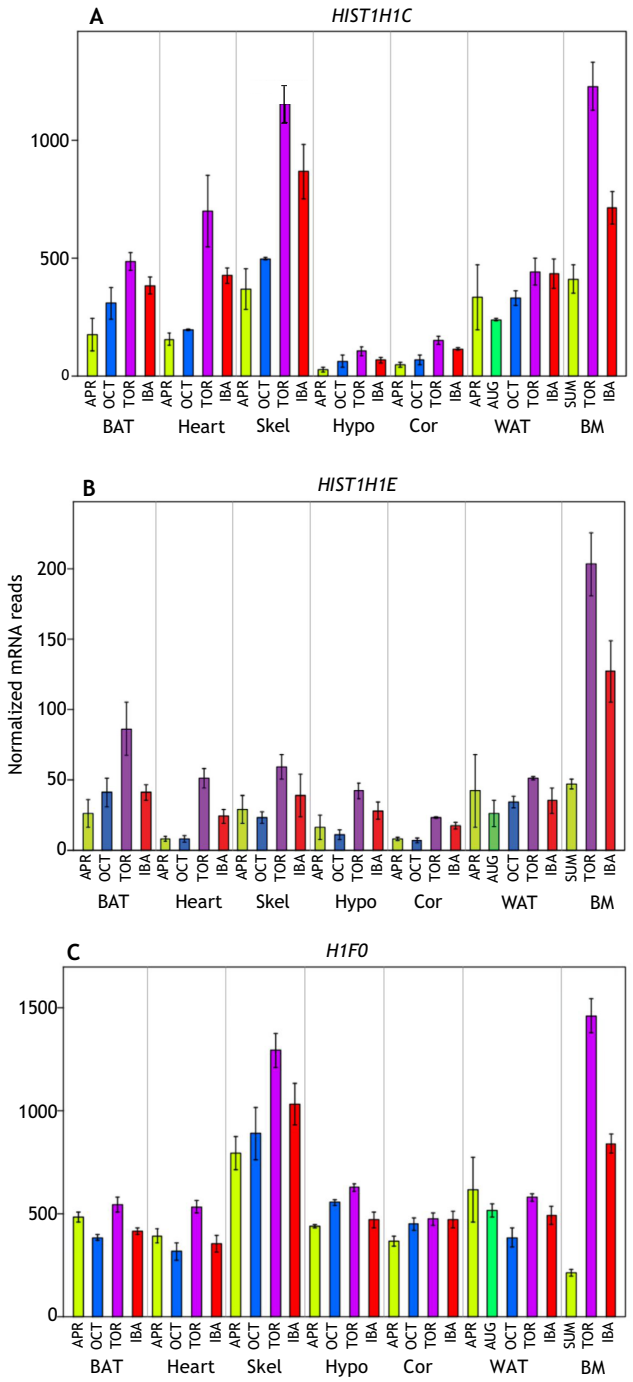

Fig. 3. Seasonal mRNA expression patterns of histone H1 variants in seven tissues from the thirteen-lined ground squirrel. (A) *HIST1H1C*, (B) *HIST1H1E* and (C) *H1F0* mRNA levels were determined in previous studies of brown and white adipose tissue (Hampton et al., 2013), cortex and hypothalamus regions of the brain (Schwartz et al., 2013), bone marrow (Cooper et al., 2016), and heart and skeletal muscle (Vermillion et al., 2015a). Measurements shown on the *y*-axis are means±s.e.m. (*n*=3) of the upper-quartile normalized counts of reads from four collection points [April, October, torpor and interbout arousal (IBA)], plus August for white adipose tissue; and only three collection points for bone marrow. APR, April; BAT, brown adipose tissue; BM, bone marrow; Cor, brain cortex; HIST1H1C, histone cluster 1 H1 family member C; HIST1H1E, histone cluster 1 H1 family member E; H1F0, H1 histone family member 0; Hypo, hypothalamus; OCT, October; Skel, skeletal muscle; Tor, torpor; SUM, summer in July; WAT, white adipose tissue.

Low-temperature RNA editing

A gene encoding another nuclear protein, chromodomain helicase DNA binding protein 9 (CHD9), shows significant upregulation during hibernation in thirteen-lined ground squirrel heart and skeletal muscle (Vermillion et al., 2015a), and brain cortex (Schwartz et al., 2013). Interestingly, the *CHD9* mRNA in the brain cortex undergoes RNA editing during torpor (Riemondy et al., 2018), starting at body temperatures <23°C and continuing with multiple A-to-G editing events throughout a torpor bout despite the uninterrupted low body temperature. A-to-I RNA editing by the ADAR family of deaminases also occurs at low body temperatures during torpor and provides an additional post-transcriptional mechanism to diversify the brain RNA pool during hibernation (Riemondy et al., 2018). Diversifying the coding capacity of an existing mRNA during torpor has the advantage of modifying the amino acid sequence of a protein at body temperatures that prevent *de novo* RNA synthesis (van Breukelen and Martin, 2002).

Hibernation as a function of genomic architecture

Genomes from a divergent group of mammalian hibernators have been sequenced with varying levels of coverage (Villanueva-Canas et al., 2014). This includes the recently improved coverage of a human-sized hibernator, the American black bear (*Ursus americanus*) (Srivastava et al., 2018), and the genome of one of the largest known hibernating mammals, the grizzly bear (*Ursus arctos* ssp. *horribilis*) (Taylor et al., 2018). The 200 Mammals Project is nearing completion (L. B. Goodman, Broad Institute, personal communication), and includes the genome sequences of at least 15 hibernating mammals, including the meadow jumping mouse (*Zapus hudsonius*). The inclusion of *Z. hudsonius* is important because this small North American rodent has the potential to serve as a genetic model for mammalian hibernation owing to captive breeding programs, short generation time, multiple litters per year, and the use of photoperiod to control breeding and hibernation (W. Israelsen, UT Southwestern, personal communication).

Coverage of the thirteen-lined ground squirrel genome has improved with technology, from the original 2X coverage in 2006 (reviewed in Andrews, 2007) to the 495X coverage in 2011 (WGS Project AGTP01). A combination of this high-coverage genome, extensive transcriptome data and *in vivo* measurements of animal physiology has provided Grabek and colleagues with sufficient data to examine genomic architecture as a driver of the hibernation phenotype (Grabek et al., 2017, preprint). After first increasing the contiguity of the sequenced genome, the authors compared their new and improved assembly with individual genomic sequences of 153 ground squirrels collected at precisely defined time points and body temperatures – many of which were measured by surgically implanted dataloggers. Using genome-wide association scans (GWAS), they identified genetic variants significantly associated with the onset of autumn entrance into torpor (Grabek et al., 2017, preprint).

GWAS variants and expression quantitative trait loci (eQTL), along with heart transcriptome analysis (Hampton et al., 2011; Vermillion et al., 2015a), identified three loci that were highly significant for the onset of torpor (Grabek et al., 2017, preprint) that contained genes whose expression is significantly upregulated in the heart during hibernation (Fig. 4). These include genes for cholinergic receptor, muscarinic 2 (*CHRM2*; *P*=3.20E–05); HLA class II histocompatibility antigen, DP beta 1 chain (*HLA-DPB1*; *P*=3.70E–05); and Zinc finger and BTB domain-containing protein 22 (*ZBTB22; P*=3.5E–04). Both HLA-DPB1 (identified by GWAS)

Journal of Experimental Biology (2019) 222, jeb160606. doi:10.1242/jeb.160606

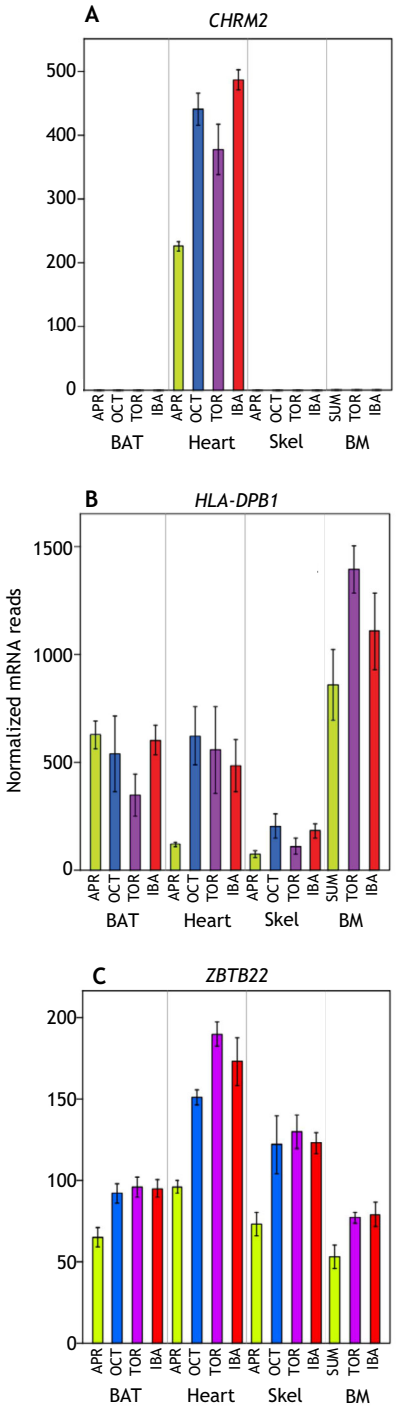

Fig. 4. Expression of genes identified as GWAS variants or expression quantitative trait loci (eQTL) that are important for onset of autumn entrance into torpor. Genes featured in this figure were identified by Grabek et al. (2017, preprint). Levels of specific mRNAs in organs of mesodermal origin are shown. (A) *CHRM2*, (B) *HLA-DPB1* and (C) *ZBTB22* mRNA levels were determined in previous studies of BAT (Hampton et al., 2013), bone marrow (Cooper et al., 2016), and heart and skeletal muscle (Vermillion et al., 2015a). Measurements shown on the *y*-axis are means±s.e.m. (*n*=3) of the upper-quartile normalized counts of reads from the four collection points (April, October, torpor and IBA), and three collection points for bone marrow. APR, April; BAT, brown adipose tissue; BM, bone marrow; CHRM2, cholinergic receptor, muscarinic 2; HLA-DPB1, HLA class II histocompatibility antigen, DP beta 1 chain; IBA, interbout arousal; OCT, October; Skel, skeletal muscle; Tor, torpor; SUM, summer in July; ZBTB22, Zinc finger and BTB domain-containing protein 22.

and ZBTB22 (identified by eQTL) are located on the same scaffold (see Glossary). The increase in *CHRM2* expression (Fig. 4A) is logical because this receptor reduces the heart rate (Table 2) by slowing depolarization and reducing the atrial contractile force. However, the roles of the HLA-DPB1 and ZBTB22 gene products during hibernation in the heart are not obvious, as HLA-DPB1 is a component of the immune system and ZBTB22 is presumably a nucleic acid binding protein. Fig. 4 shows the expression of these genes in the heart, plus three other organs of mesodermal origin (brown adipose tissue, skeletal muscle and bone marrow), during periods of normal activity in April and October, and during hibernation periods of torpor and IBA.

Overall, Grabek et al. (2017, preprint) represents an important first step in showing that GWAS and eQTL studies have the potential to uncover genes that are important for the hibernation phenotype. As the genomes of other hibernators are better defined, this approach will become a powerful means to identify genes that control hibernation in mammals.

Molecular response to changing temperatures

Detecting seasonal changes in environmental temperature and surviving a profound decrease in body temperature require molecular interactions that respond and function during these transitions. Despite a general depression in physiological activity, a group of specific genes and proteins has evolved to respond to the onset of lower temperatures and therefore contribute to the hibernation phenotype. This section examines new findings that shed light on molecular sensitivity to changing temperatures – both in the surrounding environment and within the body itself.

Cold-inducible RNA-binding proteins

RNA binding protein motif 3 (RBM3) is a well-established cold-induced RNA-binding protein that facilitates protein synthesis under conditions that would normally be too cold for optimal translation (Dresios et al., 2005). In addition to *RBM3*, two other RNA-binding proteins, cold-inducible RNA-binding protein (*CIRBP*) and serine/arginine-rich splicing factor 5 (*SRSF5*), show elevated gene expression in thirteen-lined ground squirrels during hibernation. Both genes are cold-inducible, with *CIRBP* showing a significant increase during torpor in skeletal muscle (Vermillion et al., 2015a) and bone marrow (Cooper et al., 2016), and *SRSF5* showing a significant increase in heart and skeletal muscle (Vermillion et al., 2015a), and brain cortex (Schwartz et al., 2013). CIRBP plays an important role in the cold-inducible arrest of cell division in mouse cells by enhancing the translation of cyclin-dependent kinase inhibitor p27 ($p27^{Kip1}$) through its binding of the 5′UTR of the $p27^{Kip1}$ mRNA (Roilo et al., 2018).

RBM3 not only shows significant global upregulation in thirteen-lined ground squirrels, but it also shows winter induction in multiple tissues of golden-mantled ground squirrels (Williams et al., 2005), and in the heart and liver of hibernating American black bears (Fedorov et al., 2011). The fact that *RBM3* expression is also upregulated in rat skeletal muscle subjected to conditions of disuse atrophy (Dupont-Versteegden et al., 2008) suggests that the RBM3 protein in hibernating mammals may be involved in the preservation of muscle mass during prolonged periods of inactivity during torpor.

Using wild-type mice, Peretti et al. (2015) found that expression of *RBM3* in the brain is required for plasticity and recovery of synaptic connections that are lost when mouse body temperature is lowered to 16–18°C for 45 min. *RBM3*-mediated neuroprotection was seen when mice re-warmed after hypothermia, but did not occur in *RBM3*-knockdown mice, indicating the importance of the RBM3

protein rather than some other hypothermic effect on neuroprotection (Peretti et al., 2015). This observation in mice resembles the neuronal plasticity seen during hibernation, as neurons in the brain of hibernators shrink during hypothermia, but rapidly grow back to their original size in 2–3 h during arousal (Magarinos et al., 2006; von der Ohe et al., 2006, 2007). Upregulation of the *RBM3* gene in numerous tissues and organs throughout the body of natural hibernators is a strong indicator of the importance of RBM3 in enhancing protein synthesis under cold conditions, and therefore promoting animal survival during hypothermic extremes.

Role of the somatosensory system and temperature-sensitive TRP channels

A critical aspect of successful hibernation is the ability to sense a change of temperature in both the environment (external) and the body (internal). Changes in external temperatures can indicate seasonality and therefore signal the beginning or end of the hibernation season. Sensing internal temperature is important for establishing body temperature minimums and maximums throughout hibernation. The somatosensory system helps mammals respond and react to temperature extremes. During torpor, somatosensory neurons from thirteen-lined ground squirrels are maintained in a semi-active state rather than their function being completely shut down. This partially active state during torpor seems to be mediated by the altered activity of voltage-gated sodium channels (Hoffstaetter et al., 2018b), and appears to provide rapid restoration of full sensory function when the animal arouses.

A growing body of evidence shows that transient receptor potential (TRP) channels play an important role in a variety of sensory modalities, including thermosensitivity (reviewed in Hoffstaetter et al., 2018a). Among the temperature-sensitive channel proteins, TRPV1, TRPV3, TRPV4 and TRPM8 may be important for the hibernation phenotype. For example, a commonality among the cold-inducible genes encoding RNA-binding proteins *RBM3*, *CIRBP* and *SRSF5* is that their cold-inducible expression in mammalian tissue culture cells is dependent on the transient receptor potential vanilloid 4 (TRPV4) channel protein (Fujita et al., 2017).

TRPV4 is a temperature-activated cation channel that is activated in response to non-noxious warmth in human cells (>34°C), but the channel is closed at cooler temperatures (reviewed in Vriens et al., 2014). In human tissue culture cells, cold induction of *RBM3*, *CIRBP* and *SRSF5* was suppressed by a TRPV4-specific antagonist, or by reducing the level of TRPV4 protein using a TRPV4-specific shRNA (Fujita et al., 2017). Interestingly, an inhibitor that selectively blocks the ion channel activity of the TRPV4 protein had no effect on its ability to induce the expression of *RBM3*, *CIRBP* and *SRSF5* at the cold-inducible temperature of 32°C (Fujita et al., 2017). This finding suggests that the TRPV4 protein, but not its ion channel activity, is necessary for induction of these three cold-inducible RNA-binding proteins. A more recent report (Fujita et al., 2018) suggests that in addition to TRPV4, both TRPV3 and TRPM8 proteins, but not their ion channel activities, are also necessary for the cold induction of *RBM3*, *CIRBP* and *SRSF5* (Fig. 5).

Unlike the majority of mammalian species, hibernators need to reduce the thermal sensitivity of their somatosensory neurons so they can adapt to a wide range of core body temperatures such as the 40°C range from +37 to −3°C in Arctic ground squirrels (Barnes, 1989). Despite similarities in the primary structure of specific TRP proteins from different mammals, the group of Gracheva and Bagriantsev found that slight alterations in the amino acid sequence

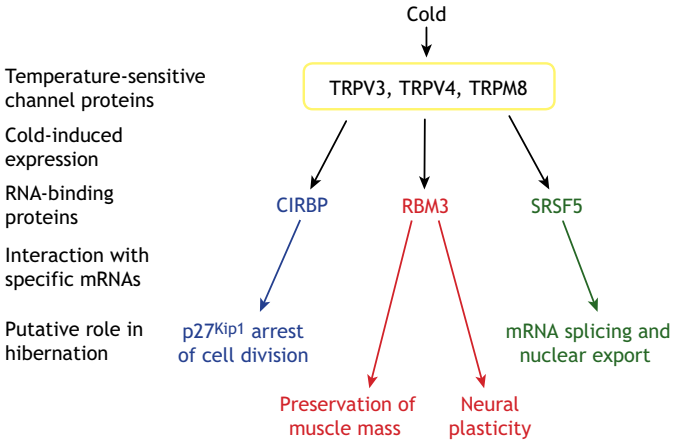

Fig. 5. Model showing cold induction of RNA-binding proteins and their putative role in hibernation. Colder temperatures induce the expression of three RNA-binding proteins in mammalian tissue culture cells via the influence of temperature-sensitive channel proteins TRPV3, TRPV4 and TRPM8. The putative roles of cold-inducible RNA-binding proteins CIRBP, RBM3 and SRSF5 during hibernation are based on their function in various mammalian systems described in the text (see Molecular response to changing temperatures, Cold-inducible RNA-binding proteins). CIRBP, cold-inducible RNA-binding protein; $p27^{Kip1}$, cyclin-dependent kinase inhibitor p27; RBM3, RNA binding protein motif 3; SRSF5, serine/arginine-rich splicing factor 5; TRPV3, transient receptor potential vanilloid 3; TRPV4, transient receptor potential vanilloid 4; TRPM8, transient receptor potential cation channel subfamily M 8.

of TRPV1 and TRPM8 in hibernators can result in very different sensitivities to extreme temperatures (Laursen et al., 2016; Matos-Cruz et al., 2017).

TRPM8 in rats is activated at temperatures below 26°C, and therefore helps to define the cold temperature tolerance of an animal. However, in thirteen-lined ground squirrels and Syrian hamsters, the TRPM8 cold sensitivity is reduced to account for a temperature range that can go below 10°C. With this reduced sensitivity, these hibernators do not perceive cold temperature as uncomfortable. Matos-Cruz et al. (2017) found that the region of the TRPM8 protein that accounts for reduced sensitivity is within the core transmembrane domain. The authors pinpointed the precise location of the reduced cold sensitivity in the ground squirrel TRPM8 to a mere six amino acids in the transmembrane core. Mutating these six ground squirrel amino acids so that they are the same as that in rat TRPM8 resulted in rat-level sensitivity to the cold (Matos-Cruz et al., 2017). This elegant study showed that changing the cold sensitivity of TRPM8 is achieved by changing only six of the 1104 amino acids of this channel protein.

At the other end of the mammalian temperature spectrum, TRPV1 is a heat-sensitive ion channel of the somatosensory system that is responsive to temperatures greater than 40°C in rats and mice. Laursen et al. (2016) explored the heat tolerance of the TRPV1 channel using a heat-tolerant mammal (Bactrian camel) and a cold-tolerant mammal (thirteen-lined ground squirrel). Unlike the heat sensitivity of rat and mouse TRPV1, both the camel and ground squirrel protein showed little response over a 22 to 46°C temperature ramp, indicating diminished heat sensitivity and a correlation between the ability of squirrels and camels to cope with hot environments (Laursen et al., 2016). Surprisingly, the diminished heat sensitivity of both camel and ground squirrel TRPV1 could be converted to rat-level sensitivity by just a single amino acid change in the cytosolic N-terminus of the TRPV1 protein. Overall, these studies show the amazing functional plasticity of transient

Journal of Experimental Biology (2019) 222, jeb160606. doi:10.1242/jeb.160606

receptor potential channels TRPV1 and TRPM8, and indicate that modest amino acid sequence differences of these proteins in thirteen-lined ground squirrels greatly reduce their heat and cold sensitivity.

Hibernator induced pluripotent stem cells to study cold adaptation

Hypothermia is used in various medical procedures, but the ability to study the effects of hypothermia on living cells is often limited by insufficient cell growth and viability at low temperatures. Of course, this limitation could be remedied with the use of mammalian cells that thrive under low-temperature conditions, such as those from a natural hibernator. Induced pluripotent stem cells (iPSCs; see Glossary) have become an enormously important biomedical tool for studying disease and discovering drugs (reviewed in Avior et al., 2016), and now have been successfully produced and propagated from a hibernating mammal (Ou et al., 2018). These ground squirrel iPSCs show remarkable cell viability and function at an incubation temperature of 4°C. Moreover, using these cells, researchers have identified pharmacological strategies to rectify cold-temperature-associated deficiencies in cells from normothermic mammals, such as microtubule destruction, mitochondrial hyperpolarization and lysosomal membrane permeabilization (Ou et al., 2018). This revolution in iPSC culturing conditions has the potential to prolong the shelf life of organs used for transplantation, and might allow the identification of new medicines that improve the survival of patients who have fallen victim to traumatic brain injury, myocardial infarction and stroke.

Primate hibernators

Earlier this century, the first hibernating primate to be identified and characterized, the fat-tailed dwarf lemur (*Cheirogaleus medius*), was found on the island of Madagascar (Dausmann et al., 2004, 2005). Although this tropical location does not experience the subzero temperatures experienced by many of the Northern Hemisphere hibernators, it does have a dry winter season during which food and water become scarce. Since that initial discovery, four dwarf lemur species in the genus *Cheirogaleus* have been identified as true obligate hibernators that hibernate for 5–7 months during the Madagascan winter (reviewed in Blanco et al., 2018). These small prosimians were initially found to hibernate in tree holes, but at least one species has also been found to hibernate underground (Blanco et al., 2013), similar to rodent hibernators in much colder locations. In addition to the four known lemur species, another hibernating primate, the pygmy slow loris (*Nycticebus pygmaeus*), has since been identified in Vietnam and shows brief torpor bouts of up to 63 h (Ruf et al., 2015). Three other primates, the African lesser bushbaby [*Galago moholi* (Nowack et al., 2010)], the grey mouse lemur [*Microcebus murinus* (Faherty et al., 2017)] and the reddish-gray mouse lemur [*Microcebus griseorufus* (Kobbe and Dausmann, 2009)], have also been shown to use torpor and heterothermy (Fig. 6).

Lemurs split from the human lineage of primates approximately 58–63 million years ago, and somehow made the long, deep-water crossing from Africa to Madagascar at least 40 million years ago (Godinot, 2006). The four *Cheirogaleus* species occupy vastly different Madagascan habitats, ranging from cold and wet rainforests in the east, to hot and dry deciduous forests in the southwest part of the island. During hibernation, these small primates show physiological changes in heart rate and O_2 consumption that resemble those of ground squirrels, with recorded body temperatures as low as 9–10°C (reviewed in Blanco et al., 2018). More importantly, certain gene expression

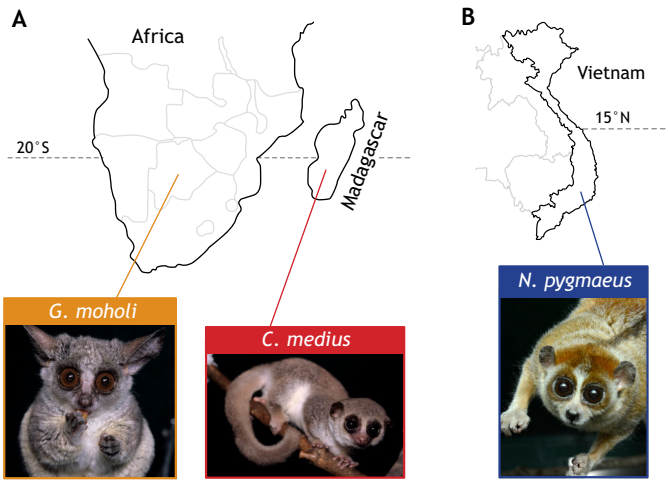

Fig. 6. General geographic range and photographs of three primate species that hibernate or use heterothermy and torpor. (A) Map of southern Africa and Madagascar pointing to the native range of the seasonal obligate hibernator, the fat-tailed dwarf lemur (*Cheirogaleus medius*, red box), and a small prosimian that is capable of employing torpor under adverse conditions, the African lesser bushbaby (*Galago moholi*, orange box). (B) Map of Vietnam pointing to the native range of an Asian hibernating primate, the pygmy slow loris (*Nycticebus pygmaeus*, blue box). Photographs were provided by David Haring, staff photographer of the Duke Lemur Center.

patterns in white adipose tissue of hibernating lemurs in captivity (Faherty et al., 2016) and in the wild (Faherty et al., 2018) are similar to those seen in the well-characterized hibernators of the Northern Hemisphere.

Gene expression similarities of long-studied hibernating rodents with two different lemur species strengthens the argument that studying mammalian hibernation in ground squirrels has applicability to human health. For example, the gene encoding pyruvate dehydrogenase kinase 4 (*PDK4*) is upregulated during hibernation in both *C. medius* and *C. crossleyi*. PDK4 is a protein kinase that phosphorylates the pyruvate dehydrogenase complex and hence promotes fat catabolism by shutting down the flow of glycolytic carbon into the TCA cycle (Fig. 2 and Table 2). This fundamental gatekeeper of glucose metabolism was initially found to be expressed during hibernation in thirteen-lined ground squirrels (Andrews et al., 1998; Buck et al., 2002), and has since been shown to play a major role in various aspects of human health, such as nutrition, exercise, obesity, diabetes and cancer (reviewed in Jeoung, 2015; Zhang et al., 2014). *PDK4* is just one gene that is differentially expressed in hibernators, but its overall importance in human health underscores the biomedical potential of understanding molecular interactions in hibernating mammals.

Future directions

Hibernation is a radical departure from normal physiological homeostasis; therefore, it is amazing that it occurs over such a wide phylogenetic range of mammals. Despite the fact that most mammalian species do not hibernate, this extreme phenotype is built upon genes and biochemical processes that are shared by all mammals. This commonality suggests that certain aspects of the phenotype can be duplicated in non-hibernators. Placing a human in a state of suspended animation has been proposed as a futuristic means of conserving resources during long-term space travel, but on a shorter time scale, studying how individual tissues and organs adapt to physiological extremes in hibernating rodents can be used to inform the design of pharmaceuticals now.

This Review covers many of the molecular details of hibernation that we know to date, but it also reveals what we do not know. Future investigations are likely to include: mechanistic aspects of low-temperature enzyme activity; the role of epigenetics in hibernation-related gene expression; development of genetic models such as *Zapus*; and GWAS identification of genes associated with the induction and maintenance of torpor. Currently, medical innovations derived from environmental adaptations of animals in the wild are understudied and possibly underappreciated. However, this avenue of inquiry holds tremendous potential for the future of human medicine. This future is starting to be realized with a hibernation-inspired therapy for hemorrhagic shock, hibernator stem cells used to correct cold-temperature deficiencies in cells from normothermic mammals, and our growing knowledge of primates that use hibernation on an annual basis.

Conclusions

Over the past decade, the research community has begun to develop a better understanding of the molecular interactions that serve as the mechanistic basis of hibernation in mammals. With this knowledge comes the potential for applying hibernation strategies for the improvement of human health. Minimizing disuse atrophy in muscles and bones, extending the time of organ preservation, improving treatment of trauma and hemorrhagic shock, developing new strategies to combat obesity and preventing debilitating reperfusion injury following myocardial infarction and stroke are some examples of situations where hibernation strategies can contribute to human medicine.

In many respects, natural hibernators represent the mammalian equivalent of suspended animation. Unlike the many cold-blooded vertebrate and invertebrate species whose body temperatures change in step with the surrounding environment, hibernating mammals retain the ability to return their body temperature to 37°C despite the temperature of their surroundings. This ability requires resilience and endurance: resilience to survive rapid and extreme physiological changes that occur with great regularity over a period of months, and endurance to maintain living cells, tissues and organs for days and weeks despite profound reductions in body temperature, oxygen consumption and heart rate.

Harnessing this resilience and endurance will require an understanding of how it occurs. As this Review attempts to show, we now have a foothold on many hibernation processes, but we still have a long way to go. This journey of discovery will occur much quicker with significant investment in hibernation research by governmental agencies. When this paradigm shift of sufficiently funding research using ‘non-model’ organisms eventually does occur, we will be in a much better position to develop therapies for the betterment of human health based on what we learn from hibernating mammals.

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Competing interests

The author is a co-inventor of technology derived from Klein et al. (2010), and is an advisor for Fauna Bio Inc.

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