To be or not to be: the regulation of mRNA fate as a survival strategy during mammalian hibernation

Shannon N. Tessier · Kenneth B. Storey

Received: 10 April 2014 / Accepted: 13 April 2014 / Published online: 2 May 2014
© Cell Stress Society International 2014

Abstract Mammalian hibernators undergo profound behavioral, physiological, and biochemical changes in order to cope with hypothermia, ischemia-reperfusion, and finite fuel reserves over days or weeks of continuous torpor. Against a backdrop of global reductions in energy-expensive processes such as transcription and translation, a subset of genes/proteins are strategically upregulated in order to meet challenges associated with hibernation. Consequently, hibernation involves substantial transcriptional and posttranscriptional regulatory mechanisms and provides a phenomenon with which to understand how a set of common genes/proteins can be differentially regulated in order to enhance stress tolerance beyond that which is possible for nonhibernators. The present review focuses on the involvement of messenger RNA (mRNA) interacting factors that play a role in the regulation of gene/protein expression programs that define the hibernating phenotype. These include proteins involved in mRNA processing (i.e., capping, splicing, and polyadenylation) and the possible role of alternative splicing as a means of enhancing protein diversity. Since the total pool of mRNA remains constant throughout torpor, mechanisms which enhance mRNA stability are discussed in the context of RNA binding proteins and mRNA decay pathways. Furthermore, mechanisms which control the global reduction of cap-dependent translation and the involvement of internal ribosome entry sites in mRNAs encoding stress response proteins are also discussed. Finally, the concept of regulating each of these factors in discrete subcellular compartments for enhanced efficiency is addressed. The analysis draws on recent

research from several well-studied mammalian hibernators including ground squirrels, bats, and bears.

Keywords Mammalian hibernation · Stress tolerance · Transcriptional and posttranscriptional regulation · Translation · RNA interacting factors

Introduction

The set of genes/proteins present in a cell largely define it, and documentation of these expression signatures has helped us to understand innumerable physiological and pathological states while also having significant impacts on diagnostics, clinical care, and outcome. However, much more remains to be learned. While a global understanding of all the genes/proteins responsible for shaping a particular phenotype is an exciting goal, the real breakthrough will involve the capacity to selectively manipulate gene/protein expression profiles in order to improve health and treat disease. Dramatic advancements in our understanding of the cellular regulatory mechanisms which drive the global make-up of cells are therefore required. One particular piece of the puzzle that is needed is a better understanding of the cellular machinery responsible for directing gene and protein expression programs, including a vast array of proteins and noncoding RNAs whose complex interplay drives gene expression, mRNA transcript handling, protein synthesis, and posttranslational processing to create the final functional protein and place it in the correct subcellular destination. The expression of mammalian genes is regulated by signal transduction pathways, epigenetic factors, transcription factors and other constituents of the transcriptional machinery, mRNA processing factors, components of the translational apparatus, and the subcellular distribution of each these factors. Epigenetic factors such as DNA methylation influence the binding of transcription factors or

S. N. Tessier · K. B. Storey (✉)
Institute of Biochemistry & Department of Biology, Carleton
University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada
e-mail: kenneth_storey@carleton.ca

corepressors and posttranslational modifications of histone tails alter chromatin structure and accessibility, collectively affecting transcriptional competence (Ling and Groop 2009). The eukaryotic transcriptional machinery consists of RNA polymerase II, transcription factors, and other accessory factors which recognize specific regulatory elements in gene promoter regions in order to implement active transcription (Thomas and Chiang 2006). During transcription, pre-mRNA is processed by a vast array of proteins which orchestrate 5' capping, constitutive and alternative RNA splicing, and 3' polyadenylation which may also be influenced by regulatory elements present in the 5' and 3' UTR (Bentley 2002). The journey continues as mRNAs are shuttled from the nucleus to the cytoplasm where they interact with the translational machinery and are subject to intricate regulatory features (Proud 2002). Finally, overlaid alongside each of these steps are subcellular domains which act to enrich and localize factors into organizational centers for improved efficiency and control of mRNA fate (Mao et al. 2011).

It is becoming clear that improving our understanding of the complex influences that direct gene/protein expression programs in the context of cell stress and disease not only improves our knowledge of the natural capacity of cells to respond to stressful states but also allows us to elucidate mechanisms of context-dependent gene/protein expression with therapeutic potential. A particularly interesting model for improving our understanding of the regulation of gene/protein expression in response to environmental stress is mammalian hibernation. Hibernation is defined as a reversible state of suspended animation employed as a survival strategy to survive long winter months with limited food (Wang and Lee 1996). Seasonal hibernators cycle through periods of profound torpor (body temperatures $<5^{\circ}\text{C}$) interspersed with intermittent periods of arousal until environmental conditions return to a state that can support normal life (Wang and Lee 1996). In this capacity, mammalian hibernators endure hypothermia, ischemia-reperfusion, and restricted nutritional resources and yet transition seamlessly to and from the torpid state. The ability to induce a hibernation-like state holds immense clinical promise since it would serve as a means of lowering tissue energy needs and reduce damage caused by ischemia-reperfusion; however, it is also associated with many adverse effects in humans and most mammals. Nonetheless, hypometabolic states have been applied for treatment of stroke, heart attack, multiple organ failure, as well as brain and spinal cord injury (Lee 2008). Moreover, the ability to induce and maintain a dormant state could improve chances for survival by injured patients during transport to medical facilities, delay the onset of age-related diseases, or extend the viability of donor organs removed for transplantation. Importantly, it has been proposed that this capacity to survive during severe seasons may rely to a great extent on regulated and coordinated use of common genes/proteins rather than

specially evolved ones (Srere et al. 1992). This principle not only signifies that human cells may one day be able to take advantage of these positive survival strategies for the improvement of human health and disease, but also suggests that the principles which define a hibernating cell hold a wealth of knowledge, especially as it pertains to the regulation of gene/protein expression programs.

Mammalian hibernators are an excellent model system for uncovering mechanisms involved in mRNA fate in response to stress since they display regulatory mechanisms which act to globally reduce transcription (Morin and Storey 2006) and translation (Frerichs et al. 1998) during torpor, yet a small subset of stress-responsive pathways must be activated in order to overcome the stresses associated with the hibernating phenotype (Hittel and Storey 2001; Eddy et al. 2005a; Yan et al. 2008). Furthermore, many studies of mammalian hibernators have shown that the total pool of mRNA remains stable (Frerichs et al. 1998), and there are many examples of a discord between mRNA and protein expression profiles (Shao et al. 2010). These data suggest that mRNA substrate availability alone cannot explain the inhibition of protein synthesis nor does it provide a full understanding of the differential regulation of the hibernator proteome. Taken together, mammalian hibernators are subject to substantial transcriptional and posttranscriptional regulation, and various mechanisms have been elucidated to date (Storey and Storey 2004b). For example, work spanning a range of mammalian hibernators has identified epigenetic controls that coordinate the global suppression of transcription (Morin and Storey 2009), the involvement of transcription factors, and their role in achieving a directed cellular response to the various stresses faced as a result of hibernation (Carey et al. 2000; Cai et al. 2004; Lee et al. 2007; Mamady and Storey 2008; Morin et al. 2008b; El Kebbaj et al. 2009; Tessier and Storey 2012), microRNAs which exert reversible control over translation (Morin et al. 2008a; Liu et al. 2010; Kornfeld et al. 2012), posttranslational modifications which influence the formation of the 48S preinitiation complex (Wu and Storey 2012; Van Breukelen et al. 2004), and the disaggregation of polyribosomes during hibernation (Knight et al. 2000; Hittel and Storey, 2002; Pan and Van Breukelen 2011).

The present review evaluates multiple regulatory mechanisms involved in the control of gene/protein expression which provide the hibernating cell with the means to survive the stresses associated with cycles of torpor-arousal (e.g., hypothermia and ischemia-reperfusion), comparing data collected from several mammalian hibernators (summarized in Fig. 1). Following a description of the general principles of mammalian hibernation, the involvement of mRNA processing factors and other interacting proteins will be addressed as a means of attaining global reductions of translation during torpor and mediating a quick reversal during arousal,

protecting and stabilizing mRNA pools since there is limited capacity to replace essential biomolecules, and suggest a possible role for alternative splicing as a mechanism to expand stress tolerance. Next, the regulation of initiation and elongation translation factors as a complementary mechanism employed to achieve global reductions in translation, but also mechanisms that facilitate the evasion of cap-dependent inhibition and promote the preferential synthesis of key proteins, will be addressed. These include the importance of posttranslational modifications, protein-protein interactions, polysome disaggregation, and internal ribosomal entry sites (IRES). Finally, the subcellular organization and distribution of mRNA factors in response to hibernation will shed light on how intracellular compartments such as the nucleus and cytoplasm can be strictly regulated and allow the various components to differentially associate with cellular structures in ways that regulate their availability, activity, and net cellular response. While a host of other factors are surely involved including the importance of epigenetic factors, microRNAs and other noncoding RNAs, and stress-responsive transcription factors, the reader is directed to several excellent reviews already available on these subjects (Van Breukelen and Martin 2002; Andrews 2007; Storey and Storey 2004b, 2010; Morin and Storey 2009; Biggar and Storey 2011; Pałyga 2011; Geiser 2013).

Mammalian hibernation

The ability to enter a hypometabolic state occurs widely across the animal kingdom and, more specifically, mammalian hibernators are represented in eight different groups including monotremes (e.g., echidnas), marsupials (e.g., opossums), rodents (e.g., ground squirrels, dormice, hamsters, and jerboas), bats, shrews, insectivores (e.g., hedgehog and tenrecs), primates (e.g., lemurs), and carnivores (e.g., bears) (Storey 2010). Hibernation generally consists of multiple bouts of deep torpor, either occurring on a daily basis (e.g., Djungarian hamsters) or on the order of days (e.g., black bears) or weeks (e.g., ground squirrels), with intermittent periods of arousal during which animals rewarm to euthermic body temperature (T_b ; Wang and Lee 1996). As such, the length of time spent in deep torpor varies substantially among mammals, and, in fact, variations are also observed in the depth of torpor bouts. While an astounding 40 °C change in T_b has been observed in arctic ground squirrels with a minimal T_b as low as −3 °C (Barnes 1989), T_b in the short-beaked echidna ranges maximally from 4 to 33 °C (Grigg et al. 1989) and larger animals such as black bears display shallow body temperature ranges of only 30–36 °C (Tøien et al. 2011). A decrease in T_b passively slows basal metabolic rate and biochemical reactions; however, evidence exists for the active suppression of metabolism in a range of hibernators including

ground squirrels and bears (Chung et al. 2011; Tøien et al. 2011). In the liver of ground squirrels, decreases in mitochondrial metabolism precede drops in T_b (Chung et al. 2011), and bears demonstrate only minimal changes in T_b yet display a 75 % decrease in basal rates (Tøien et al. 2011). Hibernators also exhibit dramatic changes in heart, respiration, and organ perfusion rates which would have severe consequences for nonhibernators. In ground squirrels hibernating with a core T_b of 5 °C, heart rates decrease from 350 to 400 beats/min to just 5–10 beats/min in deep torpor, respiration rates drop from >40 breaths/min to <1 breath/min, and organ perfusion drops to <10 % of normal (Storey 2010).

Many species use only body fuel reserves to survive the winter necessitating a phase of hyperphagia prior to hibernation; however, other hibernators have food caches in their burrows (Storey 2010). Data from several hibernators suggest a strong preference for lipid catabolism and pathways which mediate carbohydrate sparing (Carey et al. 2003; Dark 2005; Shao et al. 2010). Despite reductions in oxygen delivery, hibernating cells are generally not considered hypoxic because reductions in T_b and regulated decreases in oxygen consuming cellular processes (e.g., transcription, translation, and ion pumps) reduce the demand for oxygen. A suite of molecular and biochemical mechanisms are utilized in order for a hibernating cell to achieve a new homeostatic balance whereby ATP demand versus supply are harmonized. These and other physiological parameters are rapidly reversed during periodic arousals from torpor as a 10–20-fold surge in oxygen consumption occurs within minutes when the animal rewarms to euthermia (Storey 2003). The surge in oxygen consumption during arousal increases the production of reactive oxygen species and all mammalian hibernators have developed strategies to prevent damage to cellular biomolecules (Storey 2010; Storey and Storey 2004b; Storey and Storey 2010).

mRNA processing and stability

The conversion of precursor mRNA into mature mRNA includes the processes of 5'capping, splicing, and 3' polyadenylation before export to the cytoplasm for translation. Several excellent reviews discuss mRNA processing including comprehensive descriptions of capping (Cowling 2010), splicing (Shin and Manley 2004), cleavage (Tian and Graber 2012), polyadenylation (Gruber et al. 2014), co-transcriptional mRNA processing (Bentley 2005), as well as the regulation of mRNA processing factors in response to different physiological states (Bentley 2002), external stress (Muñoz et al. 2009; Dutertre et al. 2011), and human disease (Faustino and Cooper 2003). Briefly, the 7-methyl G5'ppp5'N cap is added when RNA is about 25 bases long, and this modification is catalyzed by RNA triphosphatase, guanylyltransferase, and 7-methyltransferase acting in series (Bentley 2002). Introns are

Fig. 1 Regulatory mechanisms in control of mRNA fate during mammalian hibernation. Translation initiation and elongation factors are regulated by phosphorylation and protein-protein binding thereby reducing translational competence. Ribosomes are preferentially loaded with mRNAs containing IRES elements and this preference progresses throughout the torpor-arousal cycle, reaching the highest IRES index

during arousal. This provides a mechanism to evade cap-dependent translation. Transcription factors direct gene expression programs aimed at mitigating the various stresses associated with hibernation and a host of mRNA processing factors and RNA binding proteins have been shown to play a vital role in the nucleus, especially during the torpor-arousal transition

removed by the spliceosome which is a large, dynamic complex comprising five uridine-rich small nuclear ribonucleic particles (UsnRNPs) and many other proteins (Bentley

2002). Spliceosome assembly is assisted by serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) which play a role in splice site selection

(Kishore et al. 2010). Adding to the amazing complexity of mRNA processing, it is estimated that alternative splicing occurs in about 60–80 % of human genes (Johnson et al. 2003; Lander et al. 2001). Alternative splicing can affect the untranslated region (UTR) of mRNA that plays a major role in the control of transcript fate; therefore, alternative splicing may provide a mechanism to evade stress-induced repression of translation. Furthermore, alternative splicing may also occur within the coding sequence of mRNA, effectively diversifying the functional properties of proteins with potential for significant cellular consequences. Indeed, various forms of stress induce a large spectrum of modifications in alternative splicing (Muñoz et al. 2009) and the misregulation of pre-mRNA splicing has been associated with various human disease states (Faustino and Cooper 2003; Singh and Cooper 2012). Finally, as determined by the interaction of specific sequence elements within the pre-mRNA with a multiprotein complex, the pre-mRNA is cleaved by an endonuclease and a poly(A) tail is added to the 3' end (Gruber et al. 2014). In comparison to proteins, mRNA is significantly less stable with half-lives of different transcripts ranging from just a few minutes to 12 h (Hargrove and Schmidt 1989). Hence, multiple regulatory mechanisms have evolved that enhance mRNA stability. These involve interactions with RNA binding proteins which protect mRNA from decay pathways and sequester mRNA into cytoplasmic stress granules, structural transitions, and/or alterations to the length of the poly(A) tail (Ross 1995).

Evidence for the importance of mRNA processing factors in hibernation has been accumulated for several species including hazel and edible dormice (Malatesta et al. 2008), greater horseshoe bats (Chen et al. 2008), arctic ground squirrels (Shao et al. 2010), and thirteen-lined ground squirrels (Tessier et al. 2014). A study in hazel dormice (*Muscardinus avellanarius*) analyzed the role of transcriptional (RNA polymerase II), splicing (hnRNPs, snRNPs, and SC35), and cleavage factors (CStF and CFIm68) in liver and brown adipose tissue from euthermic, hibernating, and aroused animals (Malatesta et al. 2008). The pre-mRNA processing factors were shown to undergo intranuclear redistribution over torpor-arousal and the data indicated that pre-mRNA are preferentially stored at the splicing and cleavage stages in hepatocytes and brown adipose tissue, respectively, during torpor. Furthermore, it was observed that pre-mRNAs held at the cleavage stage in brown adipose during torpor were immediately utilized upon arousal, whereas in liver the pre-mRNAs held at the splicing stage underwent maturation before the onset of transcription. This tissue-specific response is surely attributed to differences in the functional roles of these tissues during hibernation. Brown adipose tissue is essential in the early phases of arousal whereby heat production is powered by nonshivering thermogenesis in specialized mitochondria (Milner et al. 1989). In this capacity, it is likely that the accumulation of pre-mRNA at later stages in their maturation in brown adipose would support the

drastic increase in metabolic activity required to fuel a rapid rewarming to the euthermic state. In summary, these data not only highlight a common theme in hibernating mammals whereby tissue-specific differences are often observed (Barger et al. 2003; Eddy and Storey 2007; Rouble et al. 2013), but, more importantly, places proteins involved in mRNA processing as critical factors in mediating transitions between torpor and arousal.

The involvement of mRNA processing factors in hibernation regulation, as identified from transcriptomic and proteomic studies, has also been shown in greater horseshoe bats (*Rhinolophus ferrumeuim*) and arctic ground squirrels (*Urocyon parryi*), respectively (Chen et al. 2008; Shao et al. 2010). Comparing brains from hibernating and aroused bats, Chen et al. (2008) classified the known overexpressed genes into five cellular processes including cell cycle regulation and apoptosis, the growth of neurons, signal transduction and neuroprotection, intracellular traffic, and gene expression regulation. Of particular interest, one overexpressed gene that was similar to hnRNP U isoform b was upregulated, possibly influencing pre-mRNA processing and metabolism as well as cytoplasmic-nuclear shuttling. Similarly, the differential expression of mRNA processing factors appears to be critical during torpor-arousal transitions in the liver of arctic ground squirrels; two pre-mRNA processing factors, three hnRNPs, and six splicing factors were all overexpressed in early arousal compared to late torpor, as determined using shotgun proteomics (Shao et al. 2010). Furthermore, the same study compared the shotgun proteomics data to mRNA expression data obtained previously (Yan et al. 2008) in order to identify correlations between them. While a subset of targets measured showed concomitant changes in mRNA and protein, differentially expressed proteins including those involved in protein translation and degradation, mRNA processing, and oxidative phosphorylation did not change significantly at the mRNA level, suggesting significant posttranscriptional regulation of protein expression (Shao et al. 2010). Taken together, these studies support the general importance of mRNA processing factors in three types of hibernators across different tissues, highlight a possible significant role of mRNA processing machinery in mediating transitions between torpor and arousal, and reinforce the complexity of posttranscriptional regulatory mechanisms during hibernation.

While the regulation of alternative splicing has been extensively studied in a tissue- and developmental stage-specific manner, data on the role of alternative splice variants in mammalian hibernation is very limited. Studies of the American black bear (*Ursus americanus*) aimed at the development of genomic resources was able to gain a perspective on the proportion of alternatively spliced genes through in-depth analysis of large-scale expressed sequence tags (ESTs) (Zhao et al. 2010). A total of 38,757 EST sequences were generated from cDNA libraries from 5 tissues including brain,

liver, heart, skeletal muscle, and testis using a normalization-subtraction method. To identify the splicing patterns of bear ESTs, the sequences were mapped onto the dog genome and, as a result, researchers found a total of 630 clusters (25 %) showing alternative splicing and 504 alternative splicing events were classified into 4 typical patterns. The data revealed that alternative 5'splice sites had the highest occurrence (35 %), followed by alternative 3'sites (29 %), exon skipping (24 %), and intron retention (12 %). The frequency of each category was not significantly different from the distribution of splicing events in other mammalian species but the order of each category from highest to lowest was different, e.g., in humans, the order was exon skipping (42 %), alternative 3' sites (26 %), alternative 5'splice sites (24 %), and intron retention (8 %). Although these data do not comment on the importance of alternative splicing events to torpor-arousal, other groups have begun to describe these changes.

Accumulating data have identified hibernators including arctic ground squirrels, Syrian hamsters, and black bears as models for understanding the pathobiology of neurodegenerative diseases such as Alzheimer's (Stieler et al. 2011). The pathogenesis of neurodegenerative disease is thought to be related to Tau proteins, whereby increased Tau phosphorylation is a pivotal aspect for aggregation and formation of neurofibrillary tangles (Iqbal et al. 2005). Consequently, studies with these hibernators have focused on Tau proteins and microtubule assembly/stability as well as changes in Tau phosphorylation. Tau exhibits six alternatively spliced mRNA isoforms that differ in the number of N-terminal inserts and their differential expression, in combination with protein modifications by phosphorylation, influences their affinity to microtubules. In arctic ground squirrels, mRNA isoform expression data revealed two distinct PCR products resulting from an alternative splicing event surrounding exon 10 that encodes for an additional microtubule binding repeat. Moreover, the results showed a significantly altered Tau isoform expression pattern during hibernation whereby the tau isoforms including exon 10 were significantly decreased during torpor and following arousal. Exon 10 encodes for an additional microtubule binding repeat (Stieler et al. 2011), suggesting that alternative splicing events surrounding exon 10 may alter the binding capacity of Tau with microtubules during hibernation. Similarly, results from the greater horseshoe bat, obtained from a suppression subtractive hybridization library, revealed that the bat brain calcium/calmodulin-dependent protein kinase kinase β 1 (CaMKK β 1) gene has four transcript isoforms differing primarily in exons b and d (Yuan et al. 2007). CaMKK β 1 was of particular interest because it plays an important role in calcium-mediated neurotransmission and normal brain activity (Hsu et al. 2001). Interestingly, the authors showed that the functional and nonfunctional splicing isoform of CaMKK β 1 displays distinct expression patterns between hibernating and active states whereby the nonfunctional isoform was selectively upregulated. Furthermore, the authors suggest

that this differential regulation may represent a novel neuroprotective strategy adopted by bats to avoid tissue damage during hibernation by decreasing the sensitivity of neurons to calcium influx. Consequently, these studies determined that alternative splice variants are responsive to hibernation and, moreover, suggest that changes in alternative splice variants could have significant functional consequences.

Hibernator brains are not the only tissue to show evidence of alternative splicing in response to hibernation since additional data from the liver of the greater Egyptian jerboa (*Jaculus orientalis*) and thirteen-lined ground squirrel have also recently been described. The peroxisome proliferator-activated receptor (PPAR) family of transcription factors play a key role in lipid metabolism, and controlled regulation of lipid metabolism is integral to mammalian hibernation (Eddy et al. 2005b). While PPAR γ and the PPAR γ coactivator (PGC-1 α) have been shown to respond to hibernation in thirteen-lined ground squirrels (Eddy et al. 2005b), PPAR α also plays a role in *J. orientalis* liver during hibernation (El Kebbij et al. 2009). The data showed that jerboa liver expressed an active wild-type form of PPAR α (PPAR α 1wt) and a truncated PPAR α (PPAR α 2tr), and the ratio of wt:tr was differentially regulated during hibernation. As a result of a posttranscriptional exon-skipping event, PPAR α 2tr lacks the ligand-binding domain and is thought to repress the activity of PPAR α 1wt by competing with essential coactivators (Gervois et al. 1999). During hibernation, the level of the active PPAR α 1wt did not vary significantly but PPAR α 2tr nearly disappeared so that the wt:tr ratio rose by 3.74-fold as compared with liver of active jerboas. Most recently, we described data whereby alternative TIA-1 and TIAR gene variants were detected in the liver of thirteen-lined ground squirrels (Tessier et al. 2014). T cell intracellular antigen 1 (TIA-1) and TIA1-related (TIAR) are RNA-binding proteins that play a role in pre-mRNA splicing (Minvielle-Sebastia et al. 1997; Suswam et al. 2005), translation (Sachs and Davis 1989; Kedersha and Anderson 2007), mRNA stabilization (Decker and Parker 1993), and decay (Caponigro and Parker 1995). The data revealed that TIA-1a and TIA-1b isoforms were significantly enhanced in nuclear fractions during torpor, as compared with controls, suggesting that both isoforms were responsive to hibernation. TIAR isoforms a and b also exhibited parallel changes in response to torpor; however, the subcellular distribution of these isoforms differed whereby TIARa was mostly restricted to cytoplasmic fractions. Another interesting point was the prevalence of the TIA-1b isoform relative to the TIA-1a isoform in both nuclear and cytoplasmic fractions across control and torpid conditions. Studies of the functional consequences of TIA-1 alternative processing indicate that isoform b has higher splicing activity, supporting the possibility that alternative splicing may be occurring during torpor (albeit at low levels) or that pre-mRNA preloaded with TIA-1b during

torpor may fuel the rapid reversal of the hibernating phenotype. While the abovementioned data gives us a first glimpse into the role of mRNA processing factors as significant biological processes during mammalian hibernation, we anxiously await further studies in the area since much remains incompletely understood.

Given the instability of mRNA relative to protein, the length of a torpor bout (as long as 3 weeks), and the low transcriptional rates during torpor, one could predict that gene products would become limiting during torpor. This could hold true despite low body temperatures which would be predicted to passively slow mRNA decay pathways during deep torpor. Surprisingly, data from several mammalian hibernators have shown that the total pool of mRNA remains constant through a torpor bout (Srere et al. 1995; Frerichs et al. 1998; O'Hara et al. 1999), suggesting that global reductions in degradation pathways and enhanced mRNA stability may be components of torpor. Since changes in mRNA half-life may play a direct role in mRNA abundance without any change to transcription, this regulatory mechanism could have significant consequences for torpor when energy conservation is critical. Indeed, studies with mammalian hibernators are beginning to identify mechanisms that prolong mRNA half-life and enhance stability. Pathways involved in mRNA decay include removal of the poly(A) tail prior to nucleolytic cleavage; therefore, the length of the poly(A) tail can be used as a measure of mRNA stability (Korner and Wahle 1997). As such, studies in arctic ground squirrels used the length of the poly(A) tail as a proxy for mRNA stability (Knight et al. 2000). The data revealed that poly(A) tail lengths are conserved during torpor, suggesting that mRNA is stabilized during hibernation. Further to this, when the distribution of poly(A) tail lengths were plotted as a function of torpor bout progression, the distribution pattern was consistent with the binding of poly(A)-binding protein (PABP), indicating a possible role of PABP in mRNA stability, as has been shown in other studies (Bernstein et al. 1989; Korner and Wahle 1997). While the interaction between mRNA and PABP-1 has not been fully described, we have also recently suggested a role for PABP-1 as an mRNA stabilizer in hepatocytes of thirteen-lined ground squirrels during deep torpor (Tessier et al. 2014). Our data showed that despite no observed changes in the relative expression levels of PABP-1 in nuclear fractions from euthermic versus torpid ground squirrels, RNA binding proteins including PABP-1 and TIA-1/R localized to subnuclear structures during torpor. We proposed that this nuclear localization may have a role to play in enhancing the stability of mRNA transcripts during torpor. Taken together, studies in arctic and thirteen-lined ground squirrels suggest that PABP and other RNA binding proteins may play a prominent role in enhancing mRNA stability during torpor.

Translational apparatus

Even after making it through all of the mRNA processing steps, gene transcripts still face additional checks and balances before being finally translated into a protein. A host of eukaryotic initiation factors (eIF) are involved in the regulation of translation including eIF2 α , eIF4B, eIF4E, and eIF4GI and -II which are components of the ternary and 48S preinitiation complex (Klann and Dever 2004). Regulation of translation also occurs at the elongation phase with one such mechanism focused on the translation elongation factor, eEF2 (Hizli et al. 2013). Since certain initiation and elongation factors are limiting reagents required for active translation, regulatory mechanisms which limit their accessibility may have significant effects on translational competence. Well-known regulatory mechanisms include posttranslational modifications (e.g., reversible phosphorylation) and protein-protein binding interactions that render an initiation/elongation factor unavailable to bind mRNA. For example, initiator methionyl-tRNA is introduced into the translation initiation complex by eIF2, and phosphorylation of serine 51 on the α -subunit of eIF2 inhibits this process (Sonnenberg and Hinnebusch 2009). This is because phosphorylation generates a stable eIF2-GDP complex that binds the recycling protein, eIF2B, and prevents exchange of bound GDP for GTP, a necessary step for each round of translation initiation (Baird and Wek 2012). Additional regulatory posttranslational modifications that inhibit translation include phosphorylation of eIF4E at serine 209 and modification of eEF2 by either phosphorylation at threonine 56 or ADP-ribosylation (Gingras et al. 1999; Jørgensen et al. 2006; Hizli et al. 2013). Furthermore, proteins such as 4E-binding protein-1 (4E-BP) and HSP27 can interact with eIF proteins thereby inhibiting their function and reversible phosphorylation events are one mechanism which can modulate these interactions (Gingras et al. 1999). For example, phosphorylation on threonine 45 of 4EBP, mediated by the mammalian target of rapamycin (mTOR), results in a dissociation of eIF4E:4EBP complexes, releasing the inhibitory effect of 4EBP on translation initiation (Thoreen et al. 2012). The mTOR complex (mTORC) also exerts control over translation initiation by regulating the p70 S6 kinase. Active mTORC1 phosphorylates p70S6K and activated p70S6K in turn phosphorylates the ribosomal protein S6 and eIF4B, which are components of the mRNA translation preinitiation complex (Zhang et al. 2011).

Many studies have identified differential regulation of translation initiation factors in response to cycles of torpor-arousal in mammalian hibernation (Storey and Storey 2004a). In brain of torpid thirteen-lined ground squirrels at low Tb, the rate of protein synthesis was undetectable in brain sections and, in brain extracts, was determined to be just 0.04 % of the mean rate of active euthermic squirrels (Frerichs et al. 1998). When temperature effects on translational rates were

eliminated by analyzing brain extracts from hibernators at 37 °C, net protein synthesis was still reduced by threefold as compared with euthermia. Hence, hypothermia alone could not explain the decrease in translation, implicating the involvement of additional molecular mechanisms. This was traced to controls on eIF and eEF proteins. Whereas the total amount of eIF2 α was unchanged, the phosphorylation of eIF2 α was greatly increased during torpor, suggesting a significant role for eIF2 α regulation in the suppression of translation initiation (Frerichs et al. 1998). The importance of eIF2 α regulation was also observed in the kidney of thirteen-lined ground squirrels whereby a strong decrease (85 %) in translation rate occurred during torpor (as compared to euthermia), and this correlated with a striking increase in the amount of phosphorylated eIF2 α (Hittel and Storey 2002). The opposite response was observed in brown adipose tissue where there was no translational depression during torpor and no phosphorylated eIF2 α was detected in either control or torpid animals (Hittel and Storey 2002).

To gain a more comprehensive view on translational control during hibernation, the expression of eIF2, eIF4B, eIF4E, eIF4GI and -II, and 4E-binding protein-1 (4E-BP1), -2, and -3 and phosphorylation status were assessed across the hibernation cycle in liver of golden-mantled ground squirrels (*Spermophilus lateralis*) (Van Breukelen et al. 2004). The data revealed that the total protein levels of 4E-BP was not detectable in summer active ground squirrels but present during torpid conditions (Van Breukelen et al. 2004), suggesting a significant role for 4E-BP in translational suppression during hibernation. Further to this, the same study detected changes in the phosphorylation state of 4E-BP whereby 4E-BP was hypophosphorylated during torpor (i.e., cap-dependent initiation of translation is restricted) and hyperphosphorylated during interbout arousal. Another significant regulatory locus was determined to be eIF4E which showed a 40 % greater intensity in the hyperphosphorylated form comparing winter and summer animals. Regulatory mechanisms acting to suppress protein synthesis during elongation in hibernators have also been measured; studies with ground squirrels have shown that polypeptide elongation by ribosomes were threefold longer in brain extracts from torpid animals than in control extracts (Frerichs et al. 1998). Although a comparison between three sets of active versus hibernating extracts revealed no detectable ADP ribosylation of eEF2 during hibernation (Frerichs et al. 1998), Chen et al. (2001) demonstrated that phosphorylated eEF2 (the inactive form) was increased during torpor in the brain and liver. This response correlated with an increase in the activity of eEF-2 kinase as well as with a decrease in the activity of protein phosphatase 2A which opposes eEF2 kinase (Chen et al. 2001). Taken together, these data suggest a significant role for eIF2 α , eIF4E, 4E-BP, and eEF2 phosphorylation in influencing protein synthesis and, furthermore,

demonstrates tissue-specific differences in the way in which translation is regulated during torpor.

The mTOR pathway is a master regulator of protein synthesis which exerts control over 4E-BP1 and p70 ribosomal S6 kinase (S6K1) through phosphorylation (Laplanche and Sabatini 2009). While phosphorylation of 4E-BP1 interferes with its association with eIF4E, the phosphorylation/stimulation of S6K1 results in the regulation of additional proteins such as ribosomal protein S6 leading to increases in mRNA biogenesis (Laplanche and Sabatini 2009). Data in ground squirrels over cycles of torpor-arousal demonstrated that the phosphorylation state of mTOR (Ser2448) was strongly reduced in skeletal muscle during late torpor but increased by 200 % during early arousal compared with euthermic animals, suggesting reversible control of translation over cycles of torpor-arousal (Wu and Storey 2012). Additionally, these changes correlated with the response of selected downstream substrates including p-4E-BP1 (Thr46) and p-S6 (Ser235) whereby contents dropped during torpor before returning to euthermic levels during arousal. Data in the thirteen-lined ground squirrel liver showed analogous results; phosphorylation of 4E-BP1 (Thr37/46) and S6 (Ser235/236) showed a strong reduction during torpor which was partly restored to euthermic controls during the transitory phases. This relationship between depressed protein synthesis during torpor and reversal during arousal is also supported by data from arctic ground squirrels whereby 7 eukaryotic translation initiation factors and 13 ribosomal proteins were significantly overexpressed in arousal, as compared to torpor (Shao et al. 2010).

The separation of translation complexes in linear sucrose gradients is an excellent method for measuring the overall fitness of protein synthesis. In combination with Northern and Western blotting, genome-wide microarray analysis or qRT-PCR, the constituents of polysome profiles may be analyzed in order to glean information about their regulation in response to stress. Polysome profiles have been generated for several hibernating mammals and tissues including the thirteen-lined ground squirrel brain (Frerichs et al. 1998), kidney and brown adipose tissue (Hittel and Storey 2002), arctic ground squirrel liver (Knight et al. 2000), and golden-mantled ground squirrel liver (Pan and Van Breukelen 2011). Each of these studies have independently confirmed the same principle of polysome disaggregation during hibernation (Hittel and Storey 2002), and others have extended this data to describe a return of mRNA to polysome fractions during arousal (Knight et al. 2000). Furthermore, the relationship between body temperature and polysome profiles was assessed; 18 °C was found to be the critical temperature where polysome disaggregation/reaggregation occurred, as judged by a large increase in actin mRNA and rRNA in the monosome fraction (Van Breukelen and Martin 2001). Protein constituents of monosome versus polyribosome fractions have also been measured in order to identify proteins which may

regulate ribosome transitions. Western blotting was used to assess the presence of TIA-1 and PABP-1 in the polysome profiles of kidney extracts from euthermic versus hibernating ground squirrels (Hittel and Storey 2002). TIA-1 was restricted to the monosome fractions in both situations, suggesting TIA-1 may mediate translational suppression of mRNA pools during hibernation, a hypothesis which has also been proposed in the liver of thirteen-lined ground squirrels (Tessier et al. 2014). In contrast, PABP-1 was found in monosome fractions (fractions 8–10) in extracts from euthermic animals whereas, in extracts from hibernating individuals, PABP-1 was also detected in fractions 4, 5, and 7. This transition was hypothesized to represent mRNA sequestered into untranslatable pools and, by extension, suggest the possible involvement of stress granules in the inhibition and stabilization of mRNA during hibernation (discussed further in *Subcellular compartmentation/localization* below).

While a case has been made for global decreases of cap-dependent inhibition of translation, many studies have described the importance of strategically increasing the expression of selected genes/proteins involved in regulating metabolism and cellular stress. Hence, some gene transcripts must escape these inhibitory signals. Furthermore, the mechanisms which regulate the preferential synthesis of key proteins, amongst a pool of preexisting mRNAs, are likely to be critical for survival of the challenges associated with hibernation that would be lethal to nonhibernating mammals. The answer to this lies, at least in part, with a mechanism known as an internal ribosome entry site (IRES; Pan and Van Breukelen 2011). IRES elements are nucleotide sequences that allow for translation initiation in the middle of an mRNA (they are not cap-dependent) and are typically present in mRNAs encoding stress response proteins (Fitzgerald and Semler 2009). Since organisms generally respond to stressful states by reducing global translation, these mechanisms have evolved in order to evade this response. In this capacity, Pan and Van Breukelen (2011) analyzed polysome fractions for mRNAs that are known to contain or not to contain IRES elements. These studies concluded that, during hibernation, ribosomes are preferentially loaded with mRNAs containing IRES elements and this preference progresses throughout the torpor-arousal cycle, reaching the highest IRES index during arousal. Furthermore, the same study indicated that this change in IRES preference is the result of changes in mRNA association with ribosomes instead of mRNA abundance. This observation represents a unique opportunity whereby hibernation may be used as a mechanism to resolve how this preferential ribosomal loading is mediated with implications for all forms of the stress response.

Subcellular compartmentation/localization

One prominent feature of eukaryotic cells is the ability to organize discrete subcellular compartments, enabling more efficient systems with enhanced control. To date, numerous subcellular compartments and the microenvironments therein have been identified as playing a role in the posttranscriptional regulatory program including several stress-responsive nuclear (e.g., fibrogranular material, amorphous bodies, and Cajal bodies) and cytoplasmic bodies (e.g., stress granules). Vast networks of RNA-protein and protein-protein complexes are recruited to specific sites of transcription, splicing, processing, storage, and transport (Zimber et al. 2004), resulting in their enrichment and improving the efficiency of mRNA processing. In contrast, limiting components of these large regulatory complexes may be sequestered in compartments such as the nucleolus rendering them unavailable, and effectively inhibiting unwanted cellular functions (Audas et al. 2012). Nuclear bodies respond to cell stress of many kinds including heat shock, apoptosis, senescence, heavy metal exposure, viral infection, and DNA damage, and are involved in multiple human disease pathologies such as neurodegenerative diseases and cancer (reviewed in Zimber et al. 2004; Morris 2008; Busà et al. 2010). Cytoplasmic stress granules have been shown to form in response to hypoxia (Gottschald et al. 2010), heat/cold shock (Kramer et al. 2008; Hofmann et al. 2012), oxidative stress (Emara et al. 2012), viral infection (Lindquist et al. 2010), and nutrient deprivation (reviewed in Anderson and Kedersha 2008; Jones et al. 2013). With the growing list of environmental stress conditions, viral infections, and disease states that are related to changes in the compartmentation of cellular components, it is becoming increasingly important to understand these changes in the context of gene/protein expression programs. Mammalian hibernators present a unique opportunity to explore these concepts. Importantly, these regulatory mechanisms are relevant to the hypometabolic state because this form of control allows mRNA/protein to be regulated without changes in total expression.

One particularly relevant target is transcription factors since they exert control over the transcriptional program which influences all aspects of cell survival and physiological adaptation, as well as pathological manifestation. Studies of a growing number of transcription factors have already made large strides in our understanding of the gene expression programs which facilitate the hibernation phenotype (reviewed in Storey and Storey 2004b; Morin and Storey 2009). For example, molecular pathways responsible for enhancing stress tolerance include the activation of the unfolded protein response by ATF4 and CREB-1 (Eddy and Storey 2007; Mamady and Storey 2008), modulating antioxidant capacity by Nrf2 and NF- κ B (Carey et al. 2000; Morin et al. 2008b; Allan and Storey 2012), and improving natural

tolerance of brain ischemia by FoxO (Cai et al. 2004), to name only a few (Tessier and Storey 2010; Brooks et al. 2011; Rouble et al. 2013). Transcription factors are also known to be pivotal in mediating metabolic needs during hibernation including a role for HIF-1 α (Morin and Storey 2005; Maistrovski et al. 2012), PPAR γ , and PGC-1 α (Eddy et al. 2005b). Cytoplasmic-nuclear distribution is one mechanism which regulates transcription factor activity and changes in this distribution has been established in several studies of mammalian hibernation. For example, changes in the distribution of total and phosphorylated MEF2 transcription factors (Tessier and Storey 2010) and NF-kB p50 and p65 subunits (Allan and Storey 2012) in nuclear versus cytoplasmic fractions of skeletal muscle in thirteen-lined ground squirrels showed that differential compartmentation contributes to the regulation of gene expression during hibernation. While membrane-enclosed subcellular organelles are critical to separating cellular activity in space and time, many nonmembranous structures may also play a role in the localization of transcription factors in response to hibernation. For example, CLOCK, a member of the bHLH-PAS family of transcription factors, showed intracellular redistribution during hibernation in hepatocytes of the edible dormouse whereby the protein significantly accumulated in amorphous bodies and decreased in all other cellular compartments (Malatesta et al. 2003).

The presence of nuclei containing structural components that form in response to hibernation has been observed in several tissues of the hazel dormouse (*M. avellanarius*) and the edible dormouse (*Glis glis*) (Zancanaro et al. 1993; Malatesta et al. 1994a, b, 1995, 1998, 1999, 2000; Tamburini et al. 1996). These studies have made significant strides in our understanding of nuclear dynamics in response to cycles of torpor-arousal and some general principles are described herein. Firstly, the general morphology of cell nuclei does not change substantially between euthermia and torpor (Malatesta et al. 1994a), although the cell nucleus undergoes an important structural reorganization in the hypometabolic state (Malatesta et al. 1999; Malatesta et al. 2008). In this capacity, nuclear bodies including fibrogranular material, amorphous bodies, coiled bodies, perichromatin granule-like granules, and nucleoplasmic fibrils have been shown to be correlated with hibernation (Malatesta et al. 1999). Furthermore, these nuclear structures are present across different species, although nuclear bodies were less frequent in edible versus hazel dormice and displayed morphological differences (Malatesta et al. 1999). Whereas tissue-specific differences in the distribution of nuclear bodies have been shown, most contain some mRNA splicing or processing factors (except for nucleoplasmic fibrils for which the composition remains unknown) suggesting that posttranscriptional regulatory mechanisms during torpor are quite complex. Finally, while sensitive to low body temperatures, the presence of nuclear bodies are correlated with extremely reduced metabolic rate (Malatesta et al. 1999; Malatesta et al. 2001).

Although there is compelling evidence for the advantages of studying euthermia-torpor-arousal transitions to understand the regulatory mechanisms which initiate and dissociate nuclear structures during hibernation, the exact functional role and interplay of each structure is only beginning to be understood. For example, structures including coiled bodies (CBs) and amorphous bodies (ABs) are suggested to represent storage sites for splicing factors that can be rapidly used upon arousal, nucleoplasmic fibrils are hypothesized to play a role in the dynamics of the associated structural components, and fibrogranular material (FGM) is thought to be involved in transcription and processing of pre-mRNA during hibernation (Malatesta et al. 2001). The presence of these nuclear structures is correlated with reduced metabolic rate and their reversal occurs within a few minutes of arousal, providing a mechanism for the rapid resumption of protein synthesis (Malatesta et al. 2001). We have also identified a novel subcellular body during hibernation in thirteen-lined ground squirrels (Tessier et al. 2014). This subnuclear body consists of RNA-binding proteins such as TIA-1, TIAR, and PABP-1 which are also markers of cytoplasmic stress granules. While further evidence is required to associate these hibernating-specific subnuclear foci with a specific function, we hypothesized the following possible roles: (1) the enhancement of mRNA stability, (2) sites of transcript storage while mRNA is still in the nucleus, and/or (3) selective processing of pre-mRNA essential to the stress response. Nonetheless, all of these functions likely support an overall suppression of translation during torpor, while also promoting a rapid resumption of protein synthesis when animals arouse back to euthermia.

Another relevant subnuclear structure which has been shown to undergo structural and molecular changes over torpor-arousal in hazel dormice is the nucleolus. It has been proposed that the nucleolus may act as a molecular “jail” detaining proteins from their downstream effectors (Audas et al. 2012). Proteins which are targeted to the nucleolus contain a nucleolar localization sequence (NoLS), and, using a combination of bioinformatics approaches and experimental evidence, the list of proteins detained in the nucleolus is increasing (Scott et al. 2010). In the hibernating dormouse, microscopy of the nucleolus shows many nucleoplasmic invaginations containing weakly contrasted areas of unknown nature which disappear upon arousal (Malatesta et al. 2011). These nucleolus-associated domains contain m3-G-capped snRNAs, snRNPs, hnRNPs, and the survival motor neuron protein but were devoid of polymerase I, fibrillarin, nucleolin, and the ribosomal phosphoproteins P0, P1, and P2. The authors hypothesized that nucleolus-associated domains may represent a transient storage site for those molecules involved in pre-mRNA splicing, and this would facilitate the resumption of RNA maturation by promoting the rapid reactivation of molecular trafficking from the nucleolus (Malatesta et al. 2011).

In addition to nuclear dynamics, well-studied cytoplasmic foci/granules also appear to be involved in RNA silencing during mammalian hibernation. Cytoplasmic stress granules and processing bodies are both nonmembranous, ribonucleoprotein containing structures that each recruit distinct proteins during stress which are reflective of their cellular roles. The core components of stress granules are small ribosomal subunits and translation initiation factors (eIF4E, eIF3, eIF4A, and eIFG), RNA-binding proteins (HuR, TIA-1, and TIAR), and other components of mRNA metabolism (Kedersha and Anderson 2007). By contrast, processing bodies contain components of the RNA decay machinery (DCP1/DCP2, Hedls/GE-1, SMG5/7, and UPF1) and RNA-induced silencing machinery (GW182, microRNA, and argonaute) (Kedersha and Anderson 2007). As a result, stress granules are generally considered to be sites of mRNA storage whereas processing bodies are places for mRNA degradation. Moreover, it has been proposed that mRNAs selected for degradation are passed from stress granules to processing bodies. While an attractive candidate for involvement in the hibernation state, there was no evidence of stress granules in hepatocytes of hibernating thirteen-lined ground squirrels (Tessier et al. 2014). However, given the tissue-specific mechanisms employed by each tissue to repress translation, the involvement of stress granules nonetheless remains a plausible hypothesis in other tissues, especially in the kidney as discussed above. During euthermia, Hittel and Storey (2002) found a redistribution of PABP from monosome fractions (fractions 8–10) to fractions 4, 5, and 7 during hibernation, suggesting that stress granules may play a role in the inhibition and stabilization of mRNA during hibernation. While stress granules were not verified by immunofluorescence and fluorescence microscopy, further research in the area will clarify the functional significance of data obtained from these polysome profiles.

Summary

The intracellular environment contains a huge number of merging/diverging, intersecting, and paralleling pathways with layer upon layer of regulatory mechanisms at each intersection. As presently discussed, biomolecules may be compartmentalized, allowing the cell to regulate activity in space and time and influencing the way in which biomolecules interact with the cellular environment. These events are directly correlated with the torpid state and are rapidly reversed during arousal. A case has been made for the importance of posttranscriptional controls such as pre-mRNA processing factors involved in capping, splicing, and polyadenylation, the role of RNA binding proteins and decay pathways in promoting mRNA stability, and mechanisms to globally reduce translation while also preferentially increasing the expression of stress-responsive proteins. Translation is also

controlled by kinases, such as mTOR, as well as a host of translation initiation and elongation factors which are responsive to the energetic status of the cell. Many of the regulatory mechanisms that control the conversion of a signal into a functional protein have been extensively studied in mammalian hibernation, an excellent platform for analyzing these processes because the transition into torpor represents both a huge change in metabolic state and one that is stable for days/weeks at a time. While significant advances have recently been made, further research which describes the selective regulation of genes required to support the hibernating phenotype will allow us to take advantage of these survival strategies in clinical applications.

Acknowledgments Thanks to JM Storey for editorial review of the manuscript. Thanks also to Dr. TE Audas and Dr. S Lee for their role in our collaborative paper aimed at understanding RNA binding proteins during hibernation. This work was supported by a discovery grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to KBS and the Canada Research Chairs program. SNT held an Ontario Graduate Scholarship.

References

- Allan ME, Storey KB (2012) Expression of NF- κ B and downstream antioxidant genes in skeletal muscle of hibernating ground squirrels, *Spermophilus tridecemlineatus*. *Cell Biochem Funct* 30:166–174. doi:10.1002/cbf.1832
- Anderson P, Kedersha N (2008) Stress granules: the Tao of RNA triage. *Trends Biochem Sci* 33:141–150. doi:10.1016/j.tibs.2007.12.003
- Andrews MT (2007) Advances in molecular biology of hibernation in mammals. *Bioessays* 29:431–440. doi:10.1002/bies.20560
- Audas TE, Jacob MD, Lee S (2012) Immobilization of proteins in the nucleolus by ribosomal intergenic spacer noncoding RNA. *Mol Cell* 45:147–157. doi:10.1016/j.molcel.2011.12.012
- Baird TD, Wek RC (2012) Eukaryotic initiation factor 2 phosphorylation and translational control in metabolism. *Adv Nutr* 3:307–321. doi:10.3945/an.112.002113
- Barger JL, Brand MD, Barnes BM, Boyer BB (2003) Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. *Am J Physiol Regul Integr Comp Physiol* 284:R1306–R1313. doi:10.1152/ajpregu.00579.2002
- Barnes BM (1989) Freeze avoidance in a mammal: body temperatures below 0 °C in an Arctic hibernator. *Science* 244:1593–1595. doi:10.1126/science.2740905
- Bentley D (2002) The mRNA assembly line: transcription and processing machines in the same factory. *Curr Opin Cell Biol* 14:336–342. doi:10.1016/S0955-0674(02)00333-2
- Bentley DL (2005) Rules of engagement: co-transcriptional recruitment of pre-mRNA processing factors. *Curr Opin Cell Biol* 17:251–256. doi:10.1016/j.ceb.2005.04.006
- Bernstein PS, Peltz W, Ross J (1989) The poly(A)-poly(A)-binding protein complex is a major determinant of mRNA stability in vitro. *Mol Cell Biol* 9:659–670. doi:10.1128/MCB.9.2.659
- Biggar KK, Storey KB (2011) The emerging roles of microRNAs in the molecular responses of metabolic rate depression. *J Mol Cell Biol* 3:167–175. doi:10.1093/jmcb/mjq045
- Brooks NE, Myburgh KH, Storey KB (2011) Myostatin levels in skeletal muscle of hibernating ground squirrels. *J Exp Biol* 214:2522–2527. doi:10.1242/jeb.055764

- Busà R, Geremia R, Sette C (2010) Genotoxic stress causes the accumulation of the splicing regulator Sam68 in nuclear foci of transcriptionally active chromatin. *Nucleic Acids Res* 38:3005–3018. doi:10.1093/nar/gkq004
- Cai D, McCarron RM, Hallenbeck J (2004) Cloning and characterization of a forkhead transcription factor gene, FoxO1a, from thirteen-lined ground squirrel. *Gene* 343:203–209. doi:10.1016/j.gene.2004.09.003
- Caponigro G, Parker R (1995) Multiple functions for the poly(A)-binding protein in mRNA decapping and deadenylation in yeast. *Genes Dev* 9:2421–2432. doi:10.1101/gad.9.19.2421
- Carey HV, Frank CL, Seifert JP (2000) Hibernation induces oxidative stress and activation of NK-kappaB in ground squirrel intestine. *J Comp Physiol B* 170:551–559. doi:10.1007/s003600000135
- Carey HV, Andrews MT, Martin SL (2003) Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 83:1153–1181. doi:10.1152/physrev.00008.2003
- Chen Y, Matsushita M, Nairn AC, Damuni Z, Cai D, Frerichs KU, Hallenbeck JM (2001) Mechanisms for increased levels of phosphorylation of elongation factor-2 during hibernation in ground squirrels. *Biochemistry* 40:11565–11570. doi:10.1021/bi010649w
- Chen J, Yuan L, Sun M, Zhang L, Zhang S (2008) Screening of hibernation-related genes in the brain of *Rhinolophus ferrumequinum* during hibernation. *Comp Biochem Physiol B Biochem Mol Biol* 149:388–393. doi:10.1016/j.cbpb.2007.10.011
- Chung D, Lloyd GP, Thomas RH, Guglielmo CG, Staples JF (2011) Mitochondrial respiration and succinate dehydrogenase are suppressed early during entrance into a hibernation bout, but membrane remodeling is only transient. *J Comp Physiol B* 181:699–711. doi:10.1007/s00360-010-0547-x
- Cowling VH (2010) Regulation of mRNA cap methylation. *Biochem J* 425:295–302. doi:10.1042/BJ20091352
- Dark J (2005) Annual lipid cycles in hibernators: integration of physiology and behavior. *Annu Rev Nutr* 25:469–497. doi:10.1146/annurev.nutr.25.050304.092514
- Decker CJ, Parker R (1993) A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev* 7:1632–1643. doi:10.1101/gad.7.8.1632
- Dutertre M, Sanchez G, Barbier J, Corcos L, Auboeuf D (2011) The emerging role of pre-messenger RNA splicing in stress responses: sending alternative messages and silent messengers. *RNA Biol* 8:740–747. doi:10.4161/ma.8.5.16016
- Eddy SF, Storey KB (2007) p38 MAPK regulation of transcription factor targets in muscle and heart of the hibernating bat, *Myotis lucifugus*. *Cell Biochem Funct* 25:759–765. doi:10.1002/cbf.1416
- Eddy SF, McNally JD, Storey KB (2005a) Up-regulation of a thioredoxin peroxidase-like protein, proliferation-associated gene, in hibernating bats. *Arch Biochem Biophys* 435:103–111. doi:10.1016/j.abb.2004.11.020
- Eddy SF, Morin P Jr, Storey KB (2005b) Cloning and expression of PPAR-gamma and PGC-1alpha from the hibernating ground squirrel, *Spermophilus tridecemlineatus*. *Mol Cell Biochem* 269:175–182. doi:10.1007/s11010-005-3459-4
- El Kebbab Z, Andreoletti P, Mountassif D, Kabine M, Schohn H, Dauça M, Latruffe N, El Kebab MS, Cherkaoui-Malki M (2009) Differential regulation of peroxisome proliferator-activated receptor (PPAR)-alpha1 and truncated PPARalpha2 as an adaptive response to fasting in the control of hepatic peroxisomal fatty acid beta-oxidation in the hibernating mammal. *Endocrinology* 150:1192–1201. doi:10.1210/en.2008-1394
- Emara MM, Fujimura K, Sciaranghella D, Ivanova V, Ivanov P, Anderson P (2012) Hydrogen peroxide induces stress granule formation independent of eIF2α phosphorylation. *Biochem Biophys Res Commun* 423:763–769. doi:10.1016/j.bbrc.2012.06.033
- Faustino NA, Cooper TA (2003) Pre-mRNA splicing and human disease. *Genes Dev* 17:419–437. doi:10.1101/gad.1048803
- Fitzgerald KD, Semler BL (2009) Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. *Biochim Biophys Acta* 1789:518–528. doi:10.1016/j.bbarm.2009.07.004
- Frerichs KU, Smith CB, Brenner M, DeGracia DJ, Krause GS, Marrone L, Dever TE, Hallenbeck JM (1998) Suppression of protein synthesis in brain during hibernation involves inhibition of protein initiation and elongation. *Proc Natl Acad Sci USA* 95:14511–14516. doi:10.1073/pnas.95.24.14511
- Geiser F (2013) Hibernation. *Curr Biol* 23:R188–R193. doi:10.1016/j.cub.2013.01.062
- Gervois P, Torra IP, Chinetti G, Grötzinger T, Dubois G, Fruchart JC, Fruchart-Najib J, Leitersdorf E, Staels B (1999) A truncated human peroxisome proliferator-activated receptor alpha splice variant with dominant negative activity. *Mol Endocrinol* 13:1535–1549. doi:10.1210/mend.13.9.0341
- Gingras AC, Raught B, Sonenberg N (1999) eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Ann Rev Biochem* 68:913–963. doi:10.1146/annurev.biochem.68.1.913
- Gottschald OR, Malec V, Krasteva G, Hasan D, Kamlah F, Herold S, Rose F, Seeger W, Hânze J (2010) TIAR and TIA-1 mRNA-binding proteins co-aggregate under conditions of rapid oxygen decline and extreme hypoxia and suppress the HIF-1α pathway. *J Mol Cell Biol* 2:345–356. doi:10.1093/jmcb/mjq032
- Grigg GC, Beard LA, Auger ML (1989) Hibernation in a monotreme, the echidna (*Tachyglossus aculeatus*). *Comp Biochem Physiol A Comp Physiol* 92:609–612. doi:10.1016/0300-9629(89)90375-7
- Gruber AR, Martin G, Keller W, Zavolan M (2014) Means to an end: mechanisms of alternative polyadenylation of messenger RNA precursors. *WIREs RNA* 5:183–196. doi:10.1002/wrna.1206
- Hargrove JL, Schmidt FH (1989) The role of mRNA and protein stability in gene expression. *FASEB J* 3:2360–2370. doi:10.1016/0378-1119(90)90499-h
- Hittel D, Storey KB (2001) Differential expression of adipose- and heart-type fatty acid binding proteins in hibernating ground squirrels. *Biochim Biophys Acta* 1522:238–243. doi:10.1016/s0167-4781(01)00338-4
- Hittel D, Storey KB (2002) The translation state of differentially expressed mRNAs in the hibernating 13-lined ground squirrel (*Spermophilus tridecemlineatus*). *Arch Biochem Biophys* 401:244–254. doi:10.1016/s0003-9861(02)00048-6
- Hizli AA, Chi Y, Swanger J, Carter JH, Liao Y, Welcker M, Ryazanov AG, Clurman BE (2013) Phosphorylation of eukaryotic elongation factor 2 (eEF2) by cyclin A-cyclin-dependent kinase 2 regulates its inhibition by eEF2 kinase. *Mol Cell Biol* 33:596–604. doi:10.1128/mcb.01270-12
- Hofmann S, Cherkasova V, Bankhead P, Bukau B, Stoecklin G (2012) Translation suppression promotes stress granule formation and cell survival in response to cold shock. *Mol Biol Cell* 23:3786–3800. doi:10.1091/mbc.E12-04-0296
- Hsu LS, Chen GD, Lee LS, Chi CW, Cheng JF, Chen JY (2001) Human Ca²⁺/calmodulin-dependent protein kinase kinase β gene encodes multiple isoforms that display distinct kinase activity. *J Biol Chem* 276:31113–31123. doi:10.1074/jbc.M011720200
- Iqbal K, Alonso AC, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I (2005) Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta* 1739:198–210. doi:10.2174/978160805722113010006
- Johnson JM, Castle J, Garrett-Engle P, Kan Z, Loecher PM, Armour CD, Santos R, Schadt EE, Stoughton R, Shoemaker DD (2003) Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* 302:2141–2144. doi:10.1126/science.1090100
- Jones BL, VanLoosen J, Kim MH, Miles SJ, Dunham CM, Williams LD, Snell TW (2013) Stress granules form in *Brachionus manjavacas* (Rotifera) in response to a variety of stressors. *Comp Biochem Physiol A Mol Integr Physiol* 166:375–384. doi:10.1016/j.cbpa.2013.07.009

- Jørgensen R, Merrill AR, Andersen GR (2006) The life and death of translation elongation factor 2. *Biochem Soc Trans* 34:1–6. doi:10.1042/bst0340001
- Kedersha N, Anderson P (2007) Mammalian stress granules and processing bodies. *Methods Enzymol* 431:61–81. doi:10.1016/S0076-6879(07)31005-7
- Kishore S, Lubner S, Zavolan M (2010) Deciphering the role of RNA-binding proteins in the post-transcriptional control of gene expression. *Brief Funct Genom* 9:391–404. doi:10.1093/bfpg/elq028
- Klann E, Dever TE (2004) Biochemical mechanisms for translational regulation in synaptic plasticity. *Nat Rev Neurosci* 5:931–942. doi:10.1038/nrn1557
- Knight JE, Narus EN, Martin SL, Jacobson A, Barnes BM, Boyer B (2000) mRNA stability and polysome loss in hibernating Arctic ground squirrels (*Spermophilus parryi*). *Mol Cell Biol* 20:6374–6379. doi:10.1128/mcb.20.17.6374-6379.2000
- Korner CG, Wahle E (1997) Poly(A) tail shortening by a mammalian poly(A)-specific 3'-exoribonuclease. *J Biol Chem* 272:10448–10456. doi:10.1074/jbc.272.16.10448
- Kornfeld SF, Biggar KK, Storey KB (2012) Differential expression of mature microRNAs involved in muscle maintenance of hibernating little brown bats, *Myotis lucifugus*: a model of muscle atrophy resistance. *Genom Proteome Bioinforma* 10:295–301. doi:10.1016/j.gpb.2012.09.001
- Kramer S, Queiroz R, Ellis L, Webb H, Hoheisel JD, Clayton C, Carrington M (2008) Heat shock causes a decrease in polysomes and the appearance of stress granules in trypanosomes independently of eIF2(alpha) phosphorylation at Thr169. *J Cell Sci* 121:3002–3014. doi:10.1242/jcs.031823
- Lander ES, Linton LM, Birren B et al (2001) Initial sequencing and analysis of the human genome. *Nature* 409:860–921. doi:10.1038/35057062
- Laplante M, Sabatini DM (2009) mTOR signaling at a glance. *J Cell Sci* 122:3589–3594. doi:10.1242/jcs.051011
- Lee CC (2008) Is human hibernation possible? *Annu Rev Med* 59:177–186. doi:10.1146/annurev.med.59.061506.110403
- Lee YJ, Miyake S, Wakita H, McMullen DC, Azuma Y, Auh S, Hallenbeck JM (2007) Protein SUMOylation is massively increased in hibernation torpor and is critical for the cytoprotection provided by ischemic preconditioning and hypothermia in SHSY5Y cells. *J Cereb Blood Flow Metab* 27:950–962. doi:10.1038/sj.jcbfm.9600395
- Lindquist ME, Lifland AW, Utley TJ, Santangelo PJ, Crowe JE Jr (2010) Respiratory syncytial virus induces host RNA stress granules to facilitate viral replication. *J Virol* 84:12274–12284. doi:10.1128/JVI.00260-10
- Ling C, Groop L (2009) Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes* 58:2718–2725. doi:10.2337/db09-1003
- Liu Y, Hu W, Wang H, Lu M, Shao C, Menzel C, Yan Z, Li Y, Zhao S, Khaitovich P, Liu M, Chen W, Barnes BM, Yan J (2010) Genomic analysis of miRNAs in an extreme mammalian hibernator, the Arctic ground squirrel. *Physiol Genomics* 42A:39–51. doi:10.1152/physiolgenomics.00054.2010
- Maistrovski Y, Biggar KK, Storey KB (2012) HIF-1 α regulation in mammalian hibernators: role of non-coding RNA in HIF-1 α control during torpor in ground squirrels and bats. *J Comp Physiol B* 182: 849–859. doi:10.1007/s00360-012-0662-y
- Malatesta M, Zancanaro C, Martin TE, Chan EKL, Almaric F, Luhrmann R, Vogel P, Fakan S (1994a) Cytochemical and immunocytochemical characterization of nuclear bodies during hibernation. *Eur J Cell Biol* 65:82–93
- Malatesta M, Zancanaro C, Martin TE, Chan EKL, Almaric F, Luhrmann R, Vogel P, Fakan S (1994b) Is the coiled body involved in nucleolar functions? *Exp Cell Res* 211:415–419. doi:10.1006/excr.1994.1106
- Malatesta M, Zancanaro C, Tamburini M, Martin TE, Fu X-D, Vogel P, Fakan S (1995) Novel nuclear ribonucleoprotein structural components in the dormouse adrenal cortex during hibernation. *Chromosoma* 104:121–128. doi:10.1007/BF00347694
- Malatesta M, Zancanaro C, Marcheggiani F, Cardinali A, Rocchi MBL, Capizzi D, Vogel P, Fakan S, Gazzanelli G (1998) Ultrastructural morphometrical and immunocytochemical analyses of the exocrine pancreas in a hibernating dormouse. *Cell Tissue Res* 292:531–541. doi:10.1007/s004410051082
- Malatesta M, Cardinali A, Battistelli S, Zancanaro C, Martin TE, Fakan S, Gazzanelli G (1999) Nuclear bodies are usual constituents in tissues of hibernating dormice. *Anat Rec* 254:389–395. doi:10.1002/(SICI)1097-0185(19990301)254:3<389::AID-AR10>3.0.CO;2-E
- Malatesta M, Gazzanelli G, Battistelli S, Martin TE, Almaric F, Fakan S (2000) Nucleoli undergo structural and molecular modifications during hibernation. *Chromosoma* 109:506–513. doi:10.1007/s004120000102
- Malatesta M, Luchetti F, Marcheggiani F, Fakan S, Gazzanelli G (2001) Disassembly of nuclear bodies during arousal from hibernation: an in vitro study. *Chromosoma* 110:471–477. doi:10.1007/s004120100166
- Malatesta M, Baldelli B, Rossi L, Serafini S, Gazzanelli G (2003) Fine distribution of CLOCK protein in hepatocytes of hibernating dormice. *Eur J Histochem* 47:233–240
- Malatesta M, Biggiogera M, Baldelli B, Barabino SM, Martin TE, Zancanaro C (2008) Hibernation as a far-reaching program for the modulation of RNA transcription. *Microsc Res Tech* 71:564–572. doi:10.1002/jemt.20587
- Malatesta M, Zancanaro C, Biggiogera M (2011) Immunoelectron microscopic characterization of nucleolus-associated domains during hibernation. *Microsc Res Tech* 74:47–53. doi:10.1002/jemt.20872
- Mamady H, Storey KB (2008) Coping with the stress: expression of ATF4, ATF6, and downstream targets in organs of hibernating ground squirrels. *Arch Biochem Biophys* 477:77–85. doi:10.1016/j.abb.2008.05.006
- Mao YS, Zhang B, Spector DL (2011) Biogenesis and function of nuclear bodies. *Trends Genet* 27:295–306. doi:10.1016/j.tig.2011.05.006
- Milner RE, Wang LC, Trayhurn P (1989) Brown fat thermogenesis during hibernation and arousal in Richardson's ground squirrel. *Am J Physiol* 256:R42–R48
- Minvielle-Sebastia L, Preker PJ, Wiederkehr T, Strahm Y, Keller W (1997) The major yeast poly(A)-binding protein is associated with cleavage factor IA and functions in premessenger RNA 3'-end formation. *Proc Natl Acad Sci USA* 94:7897–7902. doi:10.1073/pnas.94.15.7897
- Morin P Jr, Storey KB (2005) Cloning and expression of hypoxia-inducible factor 1 alpha from the hibernating ground squirrel, *Spermophilus tridecemlineatus*. *Biochim Biophys Acta* 1729:32–40. doi:10.1016/j.bbaexp.2005.02.009
- Morin P Jr, Storey KB (2006) Evidence for a reduced transcriptional state during hibernation in ground squirrels. *Cryobiology* 53:310–318. doi:10.1016/j.cryobiol.2006.08.002
- Morin P Jr, Storey KB (2009) Mammalian hibernation: differential gene expression and novel application of epigenetic controls. *Int J Dev Biol* 53:433–442. doi:10.1387/jfdb.082643pm
- Morin P Jr, Dubuc A, Storey KB (2008a) Differential expression of microRNA species in organs of hibernating ground squirrels: a role in translational suppression during torpor. *Biochim Biophys Acta* 1779:628–633. doi:10.1016/j.bbaggm.2008.07.011
- Morin P Jr, Ni Z, McMullen DC, Storey KB (2008b) Expression of Nrf2 and its downstream gene targets in hibernating 13-lined ground squirrels, *Spermophilus tridecemlineatus*. *Mol Cell Biochem* 312: 121–129. doi:10.1007/s11010-008-9727-3
- Morris GE (2008) The Cajal body. *Biochim Biophys Acta* 1783:2108–2115. doi:10.1016/j.bbammr.2008.07.016
- Muñoz MJ, Pérez Santangelo MS, Paronetto MP, de la Mata M, Pelisch F, Boireau S, Glover-Cutter K, Ben-Dov C, Blaustein M, Lozano JJ, Bird G, Bentley D, Bertrand E, Kornbliht AR (2009) DNA damage

- regulates alternative splicing through inhibition of RNA polymerase II elongation. *Cell* 137:708–720. doi:[10.1016/j.cell.2009.03.010](https://doi.org/10.1016/j.cell.2009.03.010)
- O'Hara BF, Watson FL, Srere HK, Kumar H, Wiler SW, Welch SK, Bitting L, Heller HC, Kilduff TS (1999) Gene expression in the brain across the hibernation cycle. *J Neurosci* 19:3781–3790
- Palyga J (2011) Genetic and epigenetic regulation in hypometabolism. In: Nowakowska A, Caputa M (eds) *Hypometabolism: strategies of survival in vertebrates and invertebrates*. Research Signpost, Kerala, pp 183–202
- Pan P, Van Breukelen F (2011) Preference of IRES-mediated initiation of translation during hibernation in golden-mantled ground squirrels, *Spermophilus lateralis*. *Am J Physiol Regul Integr Comp Physiol* 301:R370–R377. doi:[10.1152/ajpregu.00748.2010](https://doi.org/10.1152/ajpregu.00748.2010)
- Proud CG (2002) Control of the translational machinery in mammalian cells. *Eur J Biochem* 269:5337. doi:[10.1046/j.1432-1033.2002.03289.x](https://doi.org/10.1046/j.1432-1033.2002.03289.x)
- Ross J (1995) mRNA stability in mammalian cells. *Microbiol Rev* 59:423–450
- Rouble AN, Hefler J, Mamady H, Storey KB, Tessier SN (2013) Anti-apoptotic signaling as a cytoprotective mechanism in mammalian hibernation. *Peer J* 1:e29. doi:[10.7717/peerj.29](https://doi.org/10.7717/peerj.29)
- Sachs AB, Davis RW (1989) The poly(A) binding protein is required for poly(A) shortening and 60S ribosomal subunit-dependent translation initiation. *Cell* 58:857–867. doi:[10.1016/0092-8674\(89\)90938-0](https://doi.org/10.1016/0092-8674(89)90938-0)
- Scott MS, Boisvert FM, McDowall MD, Lamond AI, Barton GJ (2010) Characterization and prediction of protein nucleolar localization sequences. *Nucleic Acids Res* 38:7388–7399. doi:[10.1093/nar/gkq653](https://doi.org/10.1093/nar/gkq653)
- Shao C, Liu Y, Ruan H, Li Y, Wang H, Kohl F, Goropashnaya AV, Fedorov VB, Zeng R, Barnes BM, Yan J (2010) Shotgun proteomics analysis of hibernating arctic ground squirrels. *Mol Cell Proteomics* 9:313–326. doi:[10.1074/mcp.M900260-MCP200](https://doi.org/10.1074/mcp.M900260-MCP200)
- Shin C, Manley JL (2004) Cell signaling and the control of pre-mRNA splicing. *Nat Rev Mol Cell Biol* 5:727–738. doi:[10.1038/nrm1467](https://doi.org/10.1038/nrm1467)
- Singh RK, Cooper TA (2012) Pre-mRNA splicing in disease and therapeutics. *Trends Mol Med* 18:472–482. doi:[10.1016/j.molmed.2012.06.006](https://doi.org/10.1016/j.molmed.2012.06.006)
- Sonenberg N, Hinnebusch AG (2009) Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136:731–745. doi:[10.1016/j.cell.2009.01.042](https://doi.org/10.1016/j.cell.2009.01.042)
- Srere HK, Wang LC, Martin SL (1992) Central role for differential gene expression in mammalian hibernation. *Proc Natl Acad Sci USA* 89:7119–7123. doi:[10.1073/pnas.89.15.7119](https://doi.org/10.1073/pnas.89.15.7119)
- Srere HK, Belke D, Wang LCH, Martin SL (1995) Alpha 2-macroglobulin gene expression during hibernation in ground squirrels is independent of acute phase response. *Am J Physiol* 37:R1507–R1512
- Stieler JT, Bullmann T, Kohl F, Tøien Ø, Brückner MK, Härtig W, Barnes BM, Arendt T (2011) The physiological link between metabolic rate depression and tau phosphorylation in mammalian hibernation. *PLoS ONE* 6:e14530. doi:[10.1371/journal.pone.0014530](https://doi.org/10.1371/journal.pone.0014530)
- Storey KB (2003) Mammalian hibernation: transcriptional and translational control. In: Roach RC, Wagner PD, Hackett PH (eds) *Hypoxia: through the lifecycle*. Kluwer Academic/Plenum Publishers, New York, pp 7–24
- Storey KB (2010) Out cold: biochemical regulation of mammalian hibernation—a mini-review. *Gerontology* 56:220–230. doi:[10.1159/000228829](https://doi.org/10.1159/000228829)
- Storey KB, Storey JM (2004a) Mammalian hibernation: biochemical adaptation and gene expression. In: Storey KB (ed) *Functional metabolism: regulation and adaptation*. John Wiley & Sons, New Jersey, pp 442–471
- Storey KB, Storey JM (2004b) Metabolic rate depression in animals: transcriptional and translational controls. *Biol Rev Camb Philos Soc* 79:207–233. doi:[10.1017/S1464793103006195](https://doi.org/10.1017/S1464793103006195)
- Storey KB, Storey JM (2010) Metabolic rate depression: the biochemistry of mammalian hibernation. In: Makowski GS (ed) *Advances in clinical chemistry*. Academic, Burlington, pp 77–108
- Suswam EA, Li YY, Mahtani H, King PH (2005) Novel DNA-binding properties of the RNA-binding protein TIAR. *Nucleic Acids Res* 33:4507–4518. doi:[10.1093/nar/gki763](https://doi.org/10.1093/nar/gki763)
- Tamburini M, Malatesta M, Zancanaro C, Martin TE, Fu X-D, Vogel P, Fakan S (1996) Dense granular bodies: a novel nucleoplasmic structure in hibernating dormice. *Histochem Cell Biol* 106:581–586. doi:[10.1007/BF02473273](https://doi.org/10.1007/BF02473273)
- Tessier SN, Storey KB (2010) Expression of myocyte enhancer factor-2 and downstream genes in ground squirrel skeletal muscle during hibernation. *Mol Cell Biochem* 344:151–162. doi:[10.1007/s11010-010-0538-y](https://doi.org/10.1007/s11010-010-0538-y)
- Tessier SN, Storey KB (2012) Myocyte enhancer factor-2 and cardiac muscle gene expression during hibernation in thirteen-lined ground squirrels. *Gene* 501:8–16. doi:[10.1016/j.gene.2012.04.004](https://doi.org/10.1016/j.gene.2012.04.004)
- Tessier SN, Audas TE, Wu CW, Lee S, Storey KB (2014) The involvement of mRNA processing factors TIA-1, TIAR, and PABP-1 during mammalian hibernation. *Cell Stress Chaperones*. doi:[10.1007/s12192-014-0505-8](https://doi.org/10.1007/s12192-014-0505-8)
- Thomas MC, Chiang CM (2006) The general transcription machinery and general cofactors. *Crit Rev Biochem Mol Biol* 41:105–178. doi:[10.1080/10409230600648736](https://doi.org/10.1080/10409230600648736)
- Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM (2012) A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* 485:109–113. doi:[10.1038/nature11083](https://doi.org/10.1038/nature11083)
- Tian B, Graber JH (2012) Signals for pre-mRNA cleavage and polyadenylation. *Wiley Interdiscip Rev RNA* 3:385–396. doi:[10.1002/wrna.116](https://doi.org/10.1002/wrna.116)
- Tøien Ø, Blake J, Edgar DM, Grahn DA, Heller HC, Barnes BM (2011) Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* 331:906–909. doi:[10.1126/science.1199435](https://doi.org/10.1126/science.1199435)
- Van Breukelen F, Martin SL (2001) Translational initiation is uncoupled from elongation at 18 °C during mammalian hibernation. *Am J Physiol Regul Integr Comp Physiol* 281:R1374–R1379
- Van Breukelen F, Martin SL (2002) Invited review: molecular adaptations in mammalian hibernators: unique adaptations or generalized responses? *J Appl Physiol* 92:2640–2647. doi:[10.1152/japplphysiol.01007.2001](https://doi.org/10.1152/japplphysiol.01007.2001)
- Van Breukelen F, Sonenberg N, Martin SL (2004) Seasonal and state-dependent changes of eIF4E and 4E-BP1 during mammalian hibernation: implications for the control of translation during torpor. *Am J Physiol Regul Integr Comp Physiol* 287:R349–R353. doi:[10.1152/ajpregu.00728.2003](https://doi.org/10.1152/ajpregu.00728.2003)
- Wang LCH, Lee TF (1996) Torpor and hibernation in mammals: metabolic, physiological, and biochemical adaptations. In: Fregley MJ, Blatteis CM (eds) *Handbook of physiology: environmental physiology*. Oxford University Press, New York, pp 507–532
- Wu CW, Storey KB (2012) Regulation of the mTOR signaling network in hibernating thirteen-lined ground squirrels. *J Exp Biol* 215:1720–1727. doi:[10.1242/jeb.066225](https://doi.org/10.1242/jeb.066225)
- Yan J, Barnes BM, Kohl F, Marr TG (2008) Modulation of gene expression in hibernating arctic ground squirrels. *Physiol Genomics* 32:170–181. doi:[10.1152/physiolgenomics.00075.2007](https://doi.org/10.1152/physiolgenomics.00075.2007)
- Yuan L, Chen J, Lin B, Liang B, Zhang S, Wu D (2007) Up-regulation of a non-kinase activity isoform of Ca(2+)/calmodulin-dependent protein kinase kinase beta1 (CaMKKbeta1) in hibernating bat brain. *Comp Biochem Physiol B Biochem Mol Biol* 146:438–444. doi:[10.1016/j.cbpb.2006.12.005](https://doi.org/10.1016/j.cbpb.2006.12.005)
- Zancanaro C, Malatesta M, Vogel P, Osculati F, Fakan S (1993) Ultrastructural and morphometrical analyses of the brown adipocyte nucleus in a hibernating dormouse. *Biol Cell* 79:55–61. doi:[10.1016/0248-4900\(93\)90263-E](https://doi.org/10.1016/0248-4900(93)90263-E)
- Zhang J, Tessier SN, Storey KB (2011) PI3K-Akt regulation as a molecular mechanism of the stress response during aerobic dormancy. In: Nowakowska A, Caputa M (eds) *Hypometabolism: strategies of survival in vertebrates and invertebrates*. Research Signpost, Kerala, pp 147–182
- Zhao S, Shao C, Goropashnaya AV, Stewart NC, Xu Y, Tøien Ø, Barnes BM, Fedorov VB, Yan J (2010) Genomic analysis of expressed sequence tags in American black bear *Ursus americanus*. *BMC Genomics* 11:201. doi:[10.1186/1471-2164-11-201](https://doi.org/10.1186/1471-2164-11-201)
- Zimber A, Nguyen QD, Gespach C (2004) Nuclear bodies and compartments: functional roles and cellular signalling in health and disease. *Cell Signal* 16:1085–1104. doi:[10.1016/j.cellsig.2004.03.020](https://doi.org/10.1016/j.cellsig.2004.03.020)