CELL BIOCHEMISTRY AND FUNCTION
Cell Biochem Funct 2007; **25**: 759–765.
Published online 9 May 2007 in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/cbf.1416

p38^{MAPK} regulation of transcription factor targets in muscle and heart of the hibernating bat, *Myotis lucifugus*

Sean F. Eddy*,† and Kenneth B. Storey

Institute of Biochemistry and Department of Chemistry, Carleton University, Ottawa, Ont., Canada K1S 5B6

Mammalian hibernation combines a profound net metabolic rate suppression with the selective up-regulation of key genes whose protein products address specific metabolic needs of the hibernator. The signal transduction pathways and transcription factors involved in regulating hibernation-responsive gene expression are of great interest. The present study suggests an important role for the p38 mitogen-activated protein kinase (p38^{MAPK}) and selected downstream transcription factors under its control (CREB, ATF-2, Elk-1) in the metabolic response by skeletal muscle during hibernation of little brown bats, *Myotis lucifugus*. Western blotting was used to quantify both total protein and levels of the phosphorylated, active forms of p38^{MAPK}, CREB, ATF-2 and Elk-1 in both skeletal muscle and heart of euthermic and hibernating bats. The p38^{MAPK} pathway was not apparently activated in heart during torpor but skeletal muscle showed strong increases (2.2–11-fold) in the amounts of phosphorylated p38$^{T180/Y182}$, CREB^{S133}, ATF-$^{2T69/71}$ and Elk-1^{S383} in the torpid versus aroused state. By contrast both total and phosphorylated levels of Elk-1 in heart were reduced during hibernation to just 30% of the euthermic values. These data implicate p38^{MAPK} and its transcription factor targets, CREB, ATF-2 and Elk-1 in skeletal muscle maintenance during hibernation. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS — little brown bat; hibernation; metabolic arrest; signal transduction; transcription factors

INTRODUCTION

Mammalian hibernation is a survival strategy for conserving energy that otherwise would be needed to maintain a constant high body temperature (T_b) over the winter months, a time when ambient air temperatures drop and food availability is severely restricted. This energy-saving strategy is characterized by a synchronized suppression of metabolic rate, a reduction in heart rate and in the rates of all other physiological processes, a strong reduction in T_b, and a re-organization of various metabolic priorities and patterns of fuel use.[1] In ground squirrels it has been estimated that hibernation conserves about 88% of the energy that would otherwise be required to maintain euthermic T_b over the winter.[1] The core T_b of hibernating mammals typically falls to near ambient but is regulated again if outside temperatures fall to subzero values. For example, in little brown bats, *Myotis lucifugus*, the minimum core T_b seems to be about 2°C, measured both as the T_b where metabolic rate reaches its nadir and as a preferred temperature in a natural hibernaculum.[2,3]

The strong overall suppression of metabolic rate during torpor comes from a coordinated suppression of virtually all metabolic reactions. However, selected genes and proteins show enhanced expression and/or activity to accomplish specific goals. For example, all organs make a switch to a primary dependence on lipid oxidation during torpor and show metabolic adjustments that promote triacylglycerol catabolism (*e.g.* increased expression of fatty acid binding proteins, activated lipase, activation of selected transcription factors) while, at the same time, carbohydrate catabolism is inhibited (*e.g.* suppression of pyruvate dehydrogenase activity). Almost all of the metabolic

* Correspondence to: S. F. Eddy, Department of Biochemistry, K624, Women's Health Interdisciplinary Research Center, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA Tel: +1 617 638 4129, Fax: +1 617 638 4252.
E-mail: eddy@biochem.bumc.bu.edu
† Present Address: Department of Biochemistry, K624, Women's Health Interdisciplinary Research Center, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA.

Received 19 December 2006
Revised 12 February 2007
Accepted 22 February 2007

adjustments needed for hibernation can be traced back to reversible protein phosphorylation as the mechanism, which controls and coordinates the activity states of key enzymes and functional proteins. This includes the regulation of multiple enzymes of intermediary fuel metabolism, the activities of ion motive ATPases such as Na^+/K^+-ATPase[4], ribosomal initiation and elongation factors, such as eIF2α and eEF2[5,6], and the activity states of many transcription factors which, in turn, control the differential expression of genes.[7]

Signal transduction cascades are a critical part of both metabolic suppression and the reorganization of gene expression for hibernation and the differential regulation of various protein kinases and protein phosphatases has already been implicated in several aspects of metabolic control during hibernation.[7–10] Hence, an investigation of the responses during hibernation of selected transcription factors and the protein kinases that regulate them is justified.

The mitogen activated protein kinases (MAPKs) are important signalling molecules involved in relaying extracellular signals to intracellular targets, particularly in the nucleus. A recent study analyzed the responses of $p38^{MAPK}$ in five organs of Richardson's ground squirrels during hibernation, showing a strong increase in the content of active $p38^{MAPK}$ in skeletal muscle during hibernation.[8] To further determine whether activation of the $p38^{MAPK}$ signalling pathway is a general phenomenon in hibernation, the current study assesses the role of $p38^{MAPK}$ in skeletal and cardiac muscle of the hibernating little brown bat, *M. lucifugus*. Furthermore, this study addresses downstream targets of $p38^{MAPK}$ including the transcription factors CREB and ATF-2. These belong to a family of basic leucine zipper transcription factors that bind to DNA at a cyclic AMP response element (CRE) in the promoter region of selected genes.[9] In addition, we also evaluate Elk-1, a member of the ternary complex factor (TCF) family, which is known to be activated by the ERK, JNK and $p38^{MAPK}$ pathways.[10] The manuscript documents a significant role for $p38^{MAPK}$ signalling in heart and skeletal muscle during hibernation.

MATERIALS AND METHODS

Animals

Little brown bats, *M. lucifugus* (7–8 g body mass) were collected by Dr. D. Thomas on 30 November 1999 from a disused slate mine near Sherbrooke, Quebec where they had been hibernating since October (cave air temperature was 5°C). Collection aroused the bats and they remained aroused during transport to the Université de Sherbrooke. Upon arrival, 10 bats were maintained under euthermic conditions at 23–24°C air temperature; these were kept awake for a total of 48 h post-collection and were then euthanized by cervical dislocation. Ten others were placed in a cold room at 5°C and allowed to re-enter the torpor phase of hibernation; 10–12 h were required for full torpor to be re-established with a body temperature close to ambient. Hibernating animals remained torpid until sampled 36–38 h later; measured rectal temperatures at sampling were 5–6°C. Tissues from euthermic and hibernating animals were quickly excised, immediately frozen in liquid nitrogen, and then transported to Ottawa where they were stored at −80°C until analysis.

Tissue homogenization and protein preparation

Frozen tissue samples from individual animals were ground into small pieces in a mortar and pestle under liquid nitrogen. A 100 mg tissue sample was placed in 1 mL of homogenization buffer (100 mM MOPS, 25 mM HEPES, 25 mM β-glycerophosphate, 5 mM EDTA, 1 mM EGTA, 250 μM Na_3VO_4) with 1 mM phenylmethylsulfonyl fluoride added immediately prior to homogenization with a Polytron PT1000. Samples were centrifuged for 10 min at 10,000 x *g*, supernatants were removed, and soluble protein content was determined with the Coomassie blue assay using prepared BioRad reagent. A 250 μl aliquot of supernatant was mixed 1:1 (v/v) with 2× SDS-PAGE buffer (100 mM Tris–HCl, pH 6.8, 4% w/v SDS, 20% v/v glycerol, 0.4% w/v bromophenol blue, 10% v/v 2-mercaptoethanol). A volume containing 30 μg of each sample was loaded into wells of 10% (v/v) polyacrylamide gels and electrophoresed at 200 V for 1 h. Proteins were then transferred onto PVDF membrane (Pall Biosciences) as previously described.[11]

Immunoblotting

After transfer of proteins to PVDF, membranes were blocked with 5% (w/v) nonfat dried milk (NFDM) for 2 h and were then incubated at 4°C overnight with primary antibodies. Incubations were conducted in TBST (20 mM Tris base, 140 mM NaCl, 0.1% v/v Tween-20) containing 2.5% (w/v) NFDM with antibodies added at a 1:1000 (v/v) dilution. After incubation, blots were washed for 3× 15 min in TBST prior to the addition of HRP-conjugated anti-rabbit secondary antibody (1:2000 v/v dilution in TBST).

DOI: 10.1002/cbf

Secondary incubations were carried out for 2–3 h at room temperature followed by 3× 15 min washes in TBST. Blots were developed using the Western Lightning^{TM} Chemiluminescense *Plus* (NEN, Perkin-Elmer) system according to protocols provided with the system. Blots were briefly exposed to Kodak X-OMAT-AR film and developed according to the manufacturer's protocol. X-ray images were quantified using densitometric analysis software (Imagequant, Molecular Imaging). ATF-2, CREB, Elk-1, p38^{MAPK}, ATF-2$^{T69/71}$, CREB^{S133}, Elk-1^{S383}, P38$^{T180/Y183}$ antibodies raised in rabbits were purchased from Cell Technology.

Statistics

All trials were done in triplicate. Equal loading was confirmed by Coomassie blue staining of blots after use and was also used to normalize band intensities. Mean normalized band densities ± SEM for euthermic and hibernating samples were calculated and then significance testing was performed using Student's *t*-test ($p < 0.05$ unless otherwise stated). Torpid:aroused ratios were then calculated and plotted; error bars shown on the final histograms are the sum of SEM values for euthermic and hibernating trials.

Results

Because previous studies from our laboratory identified p38^{MAPK} as showing differential phosphorylation and activity during hibernation in ground squirrels,[8] we sought to determine whether this MAPK would also have a role in another type of hibernator, the little brown bat, *M. lucifugus*. Western blotting was used to determine the relative amount of active and total p38^{MAPK} in bat heart and skeletal muscle and examine a number of related proteins in the p38^{MAPK} signal transduction cascade.

Figure 1 shows total and threonine/tyrosine (T180/Y182) phosphorylated p38^{MAPK} levels in skeletal muscle and heart of aroused and torpid bats. Total p38^{MAPK} protein levels did not change in either tissue between and during the two physiological states. However, during torpor, the amount of phosphorylated active p38$^{T180/Y182}$ rose significantly in skeletal muscle. The torpid:aroused ratio for phosphorylated p38$^{T180/Y182}$ was 2.2 ± 0.4 ($n = 3$, $p < 0.05$). By contrast, there was no appreciable effect of torpor on the content of phosphorylated p38^{MAPK} in heart.

Stress induced activation of p38^{MAPK} results in activation of downstream targets, typically including the leucine zipper CREB family of transcription

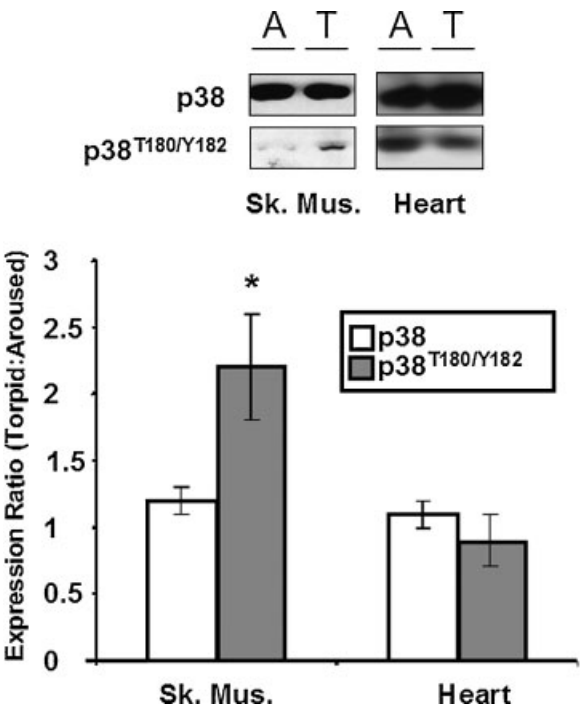

Figure 1. Total p38^{MAPK} protein levels and phosphorylated T180/Y182 p38^{MAPK} content in skeletal muscle and heart of torpid versus aroused little brown bats. Upper panel shows typical Western blots after electrophoresis of tissue samples containing 30 μg soluble protein from aroused (A) and torpid (T) bats. Histogram shows the expression ratio calculated from the mean values (±SEM, $n = 3$ independent samples) for aroused and torpid bats. *Mean value in torpor is significantly different from the corresponding aroused value, $p < 0.05$

factors that bind to CRE sites.[12,13] Hence, we next assessed the effects of torpor on CREB family members. Stress activation of CREB results from p38-mediated phosphorylation at serine 133, whereas activation of ATF-2, another CREB family member, results from phosphorylation at threonines 69 and 71.[12–15] Figure 2 shows levels of total and Serine133 phosphorylated CREB in skeletal muscle and heart of aroused and torpid bats. The amount of phosphorylated active CREB^{S133} was 2.2 ± 0.2 fold ($p < 0.001$) higher in skeletal muscle during torpor, compared with the aroused state, although total CREB protein did not change between the two states. Neither total CREB nor phosphorylated CREB^{S133} levels differed between aroused and torpid states heart, consistent with the findings for p38^{MAPK}.

The pattern of ATF-2 response during torpor was remarkably similar. The total amount of ATF-2 did not change between aroused and torpid states in skeletal

DOI: 10.1002/cbf

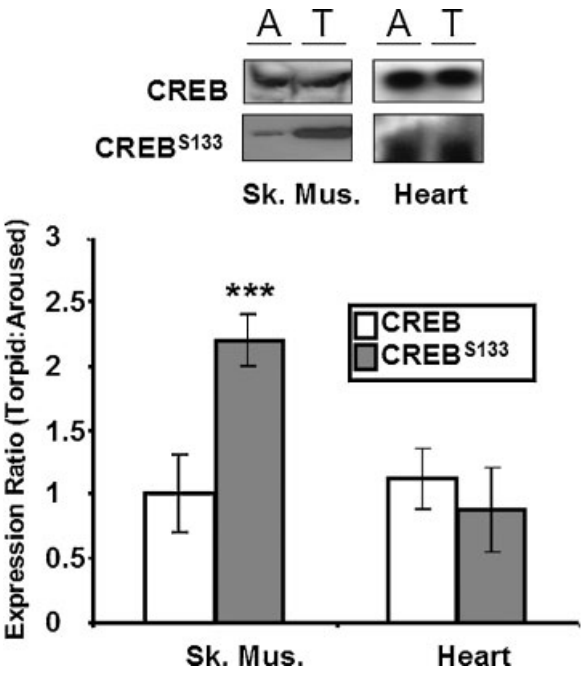

Figure 2. Total CREB protein levels and phosphorylated S133 CREB content in skeletal muscle and heart of torpid versus aroused bats. Other information as in Figure 1. ***Mean value in torpor is significantly different from the corresponding aroused value, $p < 0.001$

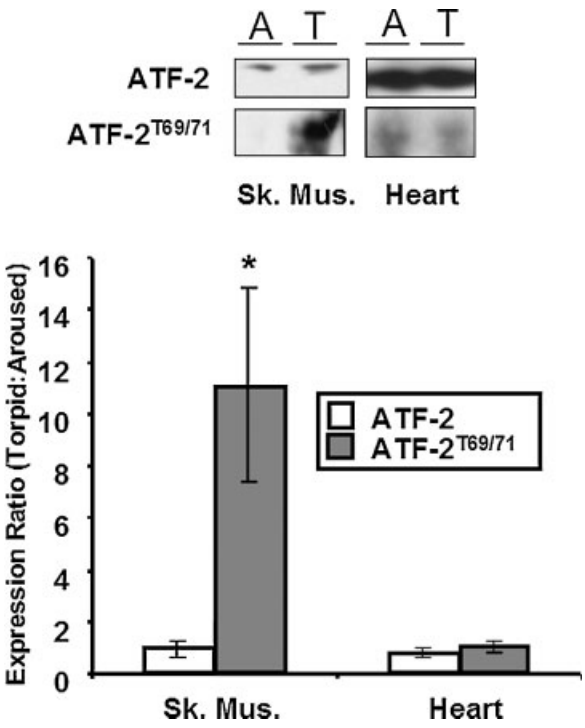

Figure 3. Total ATF-2 protein levels and phosphorylated T69/71 ATF-2 content in skeletal muscle and heart of torpid versus aroused bats. Other information as in Figure 1. *Mean value in torpor is significantly different from the corresponding aroused value, $p < 0.05$

muscle but the amount of phosphorylated ATF-2$^{T69/71}$ in muscle of torpid bats was 11.1 ± 3.7-fold ($p < 0.05$) higher than in aroused animals (Figure 3). Consistent with our previous results, there was no significant change in total or phosphorylated ATF-2$^{T69/71}$ in heart during torpor.

The response to hibernation by the ternary complex factor protein Elk-1, which is another downstream target of p38^{MAPK}, was also investigated. Total Elk-1 in skeletal muscle did not change significantly between aroused and torpid states, but a shift in banding pattern was observed (Figure 4, upper left panel), which could result from changes in phosphorylation status. Indeed, the amount of serine phosphorylated Elk-1^{S383} was 4.2 ± 1.7-fold ($P < 0.05$) higher in torpid bats (Figure 4). By contrast, total and phosphorylated levels of Elk-1 in heart were much lower in torpid versus aroused bats; the ratio torpid:aroused was just 0.3 ± 0.1 ($P < 0.005$) and 0.3 ± 0.1 ($P < 0.005$), respectively (Figure 4). Hence, the effects of torpor and arousal on Elk-1 in skeletal muscle and heart of bats were somewhat different than the patterns seen for p38^{MAPK}, CREB and ATF-2.

DISCUSSION

MAPKs play an essential role in transmitting extracellular signals to specific nuclear targets, which ultimately activate selected gene expression responses. The activation of nuclear signals occurs via a number of interactions that lead to differential phosphorylation of key intermediates in MAPK cascades and ultimately influence various transcription factors. Reversible protein phosphorylation appears to play a key role in regulating metabolism in hibernating animals,[8–10,16–20] and the present study shows that this mechanism also extends to the control of nuclear gene expression by altering activities of MAPKs and signal transduction factors that affect gene expression.

Some of the hibernation-associated changes reported in this manuscript are strikingly similar to those seen during hibernation in an evolutionarily distant rodent hibernator, the ground squirrel, *Spermophilus richardsonii*.[8] This is particularly true of the p38^{MAPK} pathway responses in skeletal muscle. Higher levels of active phosphorylated p38^{MAPK} were

DOI: 10.1002/cbf

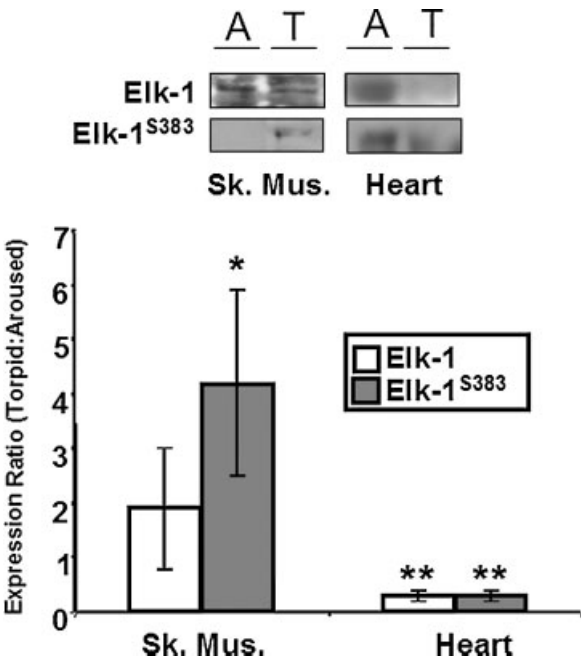

Figure 4. Total Elk-1 protein levels and phosphorylated S383 Elk-1 content in skeletal muscle and heart of torpid versus aroused bats. Other information as in Figure 1. *Mean value in torpor is significantly different from the corresponding aroused value, $p < 0.05$. **Mean value in torpor is significantly different from the corresponding aroused value, $p < 0.005$

seen in skeletal muscle from both species during torpor along with elevated amounts of the phosphorylated active forms of various downstream transcription factors controlled by p38^{MAPK} including ATF-2, CREB and Elk-1. In addition, the amount of phospho-HSP27, another protein phosphorylated downstream of the p38^{MAPK} signalling cascade, is elevated in skeletal muscle during hibernation in bats[6] and ground squirrels[8] providing further evidence for a conserved molecular mechanism in hibernating species. Active p38^{MAPK} increases in response to a variety of stresses including oxidative stress associated with ischemia reperfusion injury in rat hearts[21] and ischemia leading to up-regulation of HSP27 related kinases in rabbit hearts.[22] p38^{MAPK} activity has also been associated with cachexia and muscle wasting models, in particular, p38^{MAPK} can be activated in cultured muscle cells and muscle tissues *in vivo* leading to phosphorylation and stabilization of the transcription factor, PGC-1,[23] a co-factor that drives the formation of slow twitch muscle fibres[24] and has been shown to have increased steady state levels in hibernating bats[19] and ground squirrels.[25] Interestingly, Egginton *et al*. showed that cold exposure in hibernating Syrian hamsters displayed no atrophy to Type I, slow twitch muscle fibre cross-sectional area and composition was maintained over an 8 week time course while type II fibres displayed significant muscle atrophy.[26] Thus, we propose a model in which p38^{MAPK} stabilizes PGC-1 expression in hibernating animals, which prevents atrophy of Type I, slow twitch fibres while activating atrophic pathways in Type II muscle fibres.

CREB and ATF-2 belong to the CREB family of transcription factors, DNA binding proteins that contain leucine zippers and bind to AP-1 and CRE response elements upstream of target genes. Although they are members of the same family, they tend to act on diverse targets. CREB is involved in the activation of genes in conjunction with sterol regulatory element binding proteins. Recent evidence implicates CREB[27] and p38^{MAPK} involvement in regulating genes involved in fat metabolism and non-shivering thermogenesis such as the gene for uncoupling protein-1 (UCP-1).[28] CREB can also activate the expression of fatty acid binding protein (FABP) in cultured cells in response to insulin[29] suggesting that these factors have broad implications for hibernation biology. Since hibernators rely almost exclusively on lipid oxidation to fuel their energy needs over the winter, and since hibernator skeletal muscle shows elevated levels of *fabp* mRNA in both ground squirrels[30,11] and bats[31] during torpor, it can be postulated that the expression of a number of the genes and their protein products that are involved in the consumption and regulation of lipids would be up-regulated when animals enter torpor. These changes may be mediated by CREB and our current studies suggest the link between p38^{MAPK} activation, CREB activation, FABP expression and the physiological enhancement of lipid catabolism during hibernation.

ATF-2, another member of the CREB family of transcription factors, binds to AP-1 and CRE binding sites on DNA and is activated via phosphorylation by either p38^{MAPK} or the SAPK/JNK subfamily of MAPKs.[32] ATF-2 is known to act as a pro-survival factor in skin carcinogenesis[33] and may potentially play a protective role in preventing disuse atrophy in skeletal muscle during prolonged periods of inactivity associated with torpor.

Elk-1, is a downstream target of signalling pathways and has been described as a point of convergence for several different signals.[34,35] However, the actions of p38^{MAPK} in regulating Elk-1 are still somewhat unclear. Activation of Elk-1 via p38^{MAPK}-mediated phosphorylation at serine 383

DOI: 10.1002/cbf

leading to transcriptional activation[10] may also require $p38^{MAPK}$ cooperativity with JNK and ERK1/2.[36]

The effects of hibernation on $p38^{MAPK}$ and selected transcription factors under its control were quite different in heart than in skeletal muscle. There were no significant changes in either total or phosphorylated $p38^{MAPK}$ in heart from aroused or torpid bats. Total and phosphorylated (active) ATF-2 and CREB were also unchanged in heart of aroused versus torpid bats. The different responses of these transcription factors between heart and skeletal muscle could be due to relative differences in their functions during torpor. Elk-1 also responded differently in heart versus skeletal muscle. Both total and active Elk-1 decreased during hibernation in bat heart whereas the amount of phosphorylated active Elk-1 increased strongly in skeletal muscle during torpor. The differences in all of these parameters between the two organs probably relates to their function during torpor. Heart must continue to beat during torpor, although at a much lower rate, whereas skeletal muscle is largely inactive.

In summary, the phosphorylation and activation state of signalling molecules and transcription factors increases in skeletal muscle of *M. lucifugus* during torpor. The enhanced levels of active transcription factors demonstrated by these data may allow for an immediate increase in gene transcription followed by translation and synthesis of selected proteins during arousal or it may also assist in maintaining a subset of select genes and proteins necessary for skeletal muscle maintenance during torpor and inactivity. Alternatively, increased levels of selected mRNA transcripts may not be immediately translated, but may be kept available for rapid protein synthesis when animals enter an arousal phase. The former idea is supported by changes in known downstream target genes of activated CREB, which include hibernation-responsive regulation of UCP mRNA which was elevated during cold exposure or during torpor.[37] The data presented do not rule out the possibility that other signalling molecules play a role in hibernation, but rather provide an initial step in the study and characterization of pathways in hibernating heart and skeletal muscle of the little brown bat, *M. lucifugus*.

ACKNOWLEDGEMENTS

We are extremely grateful to Dr. D. Thomas, Université de Sherbrooke, Sherbrooke, Quebec for collecting, housing and experimentally hibernating bats for without him, this research would not be possible. Thanks to Jan Storey for critique of the manuscript. Supported by a discovery grant from the Natural Sciences and Engineering Research Council of Canada to K.B.S. and an Ontario Graduate Scholarship to S.F.E. K.B.S. holds the Canada Research Chair in Molecular Physiology.

REFERENCES

1. Wang LCH, Lee TF. Torpor and hibernation in mammals: metabolic, physiological and biochemical adaptations. In *Handbook of Physiology: Environmental Physiology*, vol., Fregley MJ, Blatteis CM (eds). Oxford University Press: New York, 1996; 507–532.
2. Hock RJ. The metabolic rates and body temperatures of bats. *Biol Bull* 1951; **101**: 289–299.
3. McManus JJ. Activity and thermal preference of the little brown bat, *Myotis lucifugus*, during hibernation. *J Mammol* 1974; **55**: 844–846.
4. MacDonald JA, Storey KB. Regulation of ground squirrel Na^+K^+-ATPase activity by reversible phosphorylation during hibernation. *Biochem Biophys Res Commun* 1999; **254**: 424–429.
5. Frerichs KU, Smith CB, Brenner M, *et al.* Suppression of protein synthesis in brain during hibernation involves inhibition of protein initiation and elongation. *Proc Natl Acad Sci USA* 1998; **95**: 14511–14516.
6. Eddy SF, McNally JD, Storey KB. Up-regulation of a thioredoxin peroxidase-like protein, proliferation-associated gene, in hibernating bats. *Arch Biochem Biophys* 2005; **435**: 103–111.
7. Storey KB. Cold ischemic organ preservation: lessons from natural systems. *J Investig Med* 2004; **52**: 315–322.
8. MacDonald JA, Storey KB. Mitogen-activated protein kinases and selected downstream targets display organ-specific responses in the hibernating ground squirrel. *Int J Biochem Cell Biol* 2005; **37**: 679–691.
9. Hai T, Hartman MG. The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. *Gene* 2001; **273**: 1–11.
10. Whitmarsh AJ, Yang SH, Su MS, Sharrocks AD, Davis RJ. Role of p38 and JNK mitogen-activated protein kinases in the activation of ternary complex factors. *Mol Cell Biol* 1997; **17**: 2360–2371.
11. Hittel D, Storey KB. The translation state of differentially expressed mRNAs in the hibernating 13-lined ground squirrel (*Spermophilus tridecemlineatus*). *Arch Biochem Biophys* 2002; **401**: 244–254.
12. Tan Y, Rouse J, Zhang A, Cariati S, Cohen P, Comb MJ. FGF and stress regulate CREB and ATF-1 via a pathway involving p38 MAP kinase and MAPKAP kinase-2. *Embo J* 1996; **15**: 4629–4642.
13. Iordanov M, Bender K, Ade T, *et al.* CREB is activated by UVC through a p38/HOG-1-dependent protein kinase. *EMBO J* 1997; **16**: 1009–1022.
14. Seko Y, Takahashi N, Tobe K, Kadowaki T, Yazaki Y. Hypoxia and hypoxia/reoxygenation activate p65PAK, p38 mitogen-activated protein kinase (MAPK), and stress-activated protein kinase (SAPK) in cultured rat cardiac myocytes. *Biochem Biophys Res Commun* 1997; **239**: 840–844.

15. Chen KD, Chen LY, Huang HL, *et al.* Involvement of p38 mitogen-activated protein kinase signaling pathway in the rapid induction of the 78-kDa glucose-regulated protein in 9L rat brain tumor cells. *J Biol Chem* 1998; **273**: 749–755.
16. Ohtsuki T, Jaffe H, Brenner M, *et al.* Stimulation of tyrosine phosphorylation of a brain protein by hibernation. *J Cereb Blood Flow Metab* 1998; **18**: 1040–1045.
17. MacDonald JA, Storey KB. Reassessment of the cold-labile nature of phosphofructokinase from a hibernating ground squirrel. *Mol Cell Biochem* 2001; **225**: 51–57.
18. MacDonald JA, Storey KB. Purification and characterization of fructose bisphosphate aldolase from the ground squirrel, *Spermophilus lateralis*: enzyme role in mammalian hibernation. *Arch Biochem Biophys* 2002; **408**: 279–285.
19. Eddy SF, Storey KB. Differential expression of Akt, PPAR-gamma, and PGC-1 during hibernation in bats. *Biochem Cell Biol* 2003; **81**: 269–274.
20. Macdonald JA, Storey KB. Temperature and phosphate effects on allosteric phenomena of phosphofructokinase from a hibernating ground squirrel (*Spermophilus lateralis*). *FEBS J* 2005; **272**: 120–128.
21. Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, *et al.* Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ Res* 1996; **79**: 162–173.
22. Kim SO, Baines CP, Critz SD, *et al.* Ischemia induced activation of heat shock protein 27 kinases and casein kinase 2 in the preconditioned rabbit heart. *Biochem Cell Biol* 1999; **77**: 559–567.
23. Puigserver P, Rhee J, Lin J, *et al.* Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPAR-gamma coactivator-1. *Mol Cell* 2001; **8**: 971–982.
24. Lin J, Wu H, Tarr PT, *et al.* Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 2002; **418**: 797–801.
25. Eddy SF, Morin P Jr., Storey KB. Cloning and expression of PPAR-gamma and PGC-1alpha from the hibernating ground squirrel, *Spermophilus tridecemlineatus*. *Mol Cell Biochem* 2005; **269**: 175–182.
26. Egginton S, Fairney J, Bratcher J. Differential effects of cold exposure on muscle fibre composition and capillary supply in hibernator and non-hibernator rodents. *Exp Physiol* 2001; **86**: 629–639.
27. Rim JS, Kozak LP. Regulatory motifs for CREB-binding protein and Nfe2l2 transcription factors in the upstream enhancer of the mitochondrial uncoupling protein 1 gene. *J Biol Chem* 2002; **277**: 34589–34600.
28. Cao W, Daniel KW, Robidoux J, *et al.* p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol* 2004; **24**: 3057–3067.
29. Klemm DJ, Roesler WJ, Boras T, Colton LA, Felder K, Reusch JE. Insulin stimulates cAMP-response element binding protein activity in HepG2 and 3T3-L1 cell lines. *J Biol Chem* 1998; **273**: 917–923.
30. Hittel D, Storey KB. Differential expression of adipose- and heart-type fatty acid binding proteins in hibernating ground squirrels. *Biochim Biophys Acta* 2001; **1522**: 238–243.
31. Eddy SF, Storey KB. Up-regulation of fatty acid-binding proteins during hibernation in the little brown bat, *Myotis lucifugus*. *Biochim Biophys Acta* 2004; **1676**: 63–70.
32. Abdel-Hafiz HA, Heasley LE, Kyriakis JM, *et al.* Activating transcription factor-2 DNA-binding activity is stimulated by phosphorylation catalyzed by p42 and p54 microtubule-associated protein kinases. *Mol Endocrinol* 1992; **6**: 2079–2089.
33. Papassava P, Gorgoulis VG, Papaevangeliou D, Vlahopoulos S, van Dam H, Zoumpourlis V. Overexpression of activating transcription factor-2 is required for tumor growth and progression in mouse skin tumors. *Cancer Res* 2004; **64**: 8573–8584.
34. Zinck R, Cahill MA, Kracht M, Sachsenmaier C, Hipskind RA, Nordheim A. Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. *Mol Cell Biol* 1995; **15**: 4930–4938.
35. Raingeaud J, Whitmarsh AJ, Barrett T, Derijard B, Davis RJ. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. *Mol Cell Biol* 1996; **16**: 1247–1255.
36. Janknecht R, Hunter T. Convergence of MAP kinase pathways on the ternary complex factor Sap-1a. *EMBO J* 1997; **16**: 1620–1627.
37. Nizielski SE, Billington CJ, Levine AS. Cold-induced alterations in uncoupling protein and its mRNA are seasonally dependent in ground squirrels. *Am J Physiol* 1995; **269**: R357–364.

DOI: 10.1002/cbf