Temperature 1:2, 92–93; July/August/September 2013; © 2013 Landes Bioscience

Transitioning between entry and exit from mammalian torpor

The involvement of signal transduction pathways

Shannon N Tessier and Kenneth B Storey*
Institute of Biochemistry; Department of Biology; Carleton University; Ottawa, ON Canada

Signal transduction pathways transmit information received at the cell surface to intracellular targets which direct a response. We highlight the involvement of signaling pathways in mediating transitions between mammalian torpor and euthermia and suggest these promote survival under stressors (e.g., hypothermia, ischemia-reperfusion) that would otherwise cause damage in nonhibernators.

Hibernation is an exceptional phenomenon which allows organisms to make dramatic transitions from high (~37 °C) to low body temperatures (T_b as low as –3 °C for select hibernators) without injurious effects. While the depth and length of a torpor bout varies among hibernators, there are three phases that all hibernators must survive: (1) entrance—where inhibition of mitochondrial respiration precedes drops in T_b, (2) deep-torpor—characterized by sustained, reduced metabolic rate, and (3) arousal—involving a surge in oxygen consumption as the hibernator rewarms to euthermia. Indeed, dramatic changes in physiology occur during entry and exit from torpor and it is generally accepted that each of these phases are active, controlled processes. In a recent paper,[1] we described a particular interesting trend whereby concerted changes in the phosphorylation/activation of important regulatory kinases (i.e., the mitogen-activated protein kinases, MAPKs) occurred during arousal, suggesting that signal transduction pathways are particularly important in achieving transitions between torpor and euthermia. Consequently, the present review highlights the importance of the transitory periods of hibernation (i.e., entrance and arousal), rather than the mechanisms which support the maintenance of deep-torpor. Beginning with a general description of the physiology of entrance and arousal, the involvement of signal transduction pathways and their effect on metabolism, gene expression, and the stress response will be discussed as a means of supporting dramatic temperature changes in hibernating ground squirrels and bats.

The precise environmental, physiological, and/or molecular signals which trigger entry and exit from torpor remain elusive despite intense efforts. Nonetheless, environmental cues influencing fall entrance into torpor include food shortages, seasonal changes in day length or photoperiod, temperature changes, etc. Hibernation is closely regulated by specific regions of the central nervous system. For example, the hypothalamus is responsible for altered thermoregulation, neuroendocrine control, and the timing of torpor. Enhanced brain serotonin activity may facilitate the transition into hibernation through inhibition of heat production and enhancement of heat loss. The autonomic nervous system is also likely involved in entrance and arousal since changes in heart rate, respiration, and cerebral blood flow precede changes in core T_b. Circulating endocrine factors such as insulin and ghrelin as well as seasonally regulated proteins such as the 'hibernation specific protein complex' (HPc), 'hibernation trigger' (HIT), and fibroblast growth factor 21 (FGF21) may also influence phases of hibernation.[2]

The ability of cells to respond to extrinsic and intrinsic cues depends on the transmission of signals along highly ordered signaling networks, leading to changes in cell metabolism, gene/protein expression, intracellular trafficking, etc. Consequently, signal transduction pathways are likely key players in mediating transitions between euthermic and torpid phenotypes. Two signal transduction pathways which are of particular interest include the insulin/Akt pathway and MAPKs since they exert control over a multitude of cellular pathways with established roles in hibernation (all data discussed herein is summarized in **Fig. 1**). Akt/PKB is a serine/threonine protein kinase which is activated by phosphorylation and regulates cellular processes such as glucose metabolism (via GSK3), protein translation (via TSC2, PRAS40, mTOR), and cellular survival (via Bad, FoxO). In the liver of 13-lined ground squirrels (*Ictidomys tridecemlineatus*), Akt is inactivated during deep torpor, while transitory phases such as entrance and interbout arousal showed activated Akt. This reversible activation profile was also correlated with the phosphorylation of Akt substrates—GSK3β, PRAS40—suggesting that Akt-dependent signal

Keywords: signal transduction, mammalian hibernation, entrance, arousal, stress response

Abbreviations: ERK, extracellular-signal-regulated kinase; FGF21, fibroblast growth factor 21; FoxO, forkhead box O; GSK3, glycogen synthase kinase 3; HIT, hibernation trigger; HPc, hibernation specific protein complex; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MAPKK or MEK, MAP kinase kinase; MAPKKK or MEKK, MAP kinase kinase kinase; MEF2, myocyte enhancer factor 2; mTOR, mammalian target of rapamycin; PRAS40, proline-rich Akt Substrate 40 kDa; T_b, body temperature; TF, transcription factor; TSC2, tuberous sclerosis 2

*Correspondence to: Kenneth Storey; Email: kenneth_storey@carleton.ca

Submitted: 07/01/2014
Accepted: 07/16/2014
Published Online: 07/17/2014
http://dx.doi.org/10.4161/temp.29972

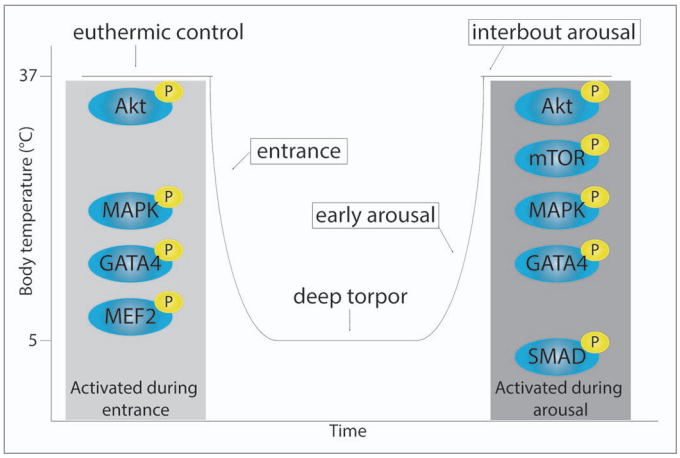

Figure 1. Summary of signal transduction pathways (e.g., Akt/PKB, MAPK) and downstream transcription factors (e.g., GATA4, MEF2, SMAD) which are responsive to entrance and arousal from torpor in ground squirrels and bats sampled from a range of tissues. Select targets show differential activation/phosphorylation levels during entrance and arousal (e.g., Akt, MAPK, GATA4), while the activation of other targets are observed in either entrance or arousal (e.g., MEF2, mTOR, SMAD). Body temperature is represented along the y-axis and time along the x-axis. P denotes a phosphate group.

transduction coordinates important cellular processes responsible for shaping entrance and arousal. This general trend was also observed in another downstream Akt-regulated protein measured in skeletal muscle, the mammalian target of rapamycin (mTOR), which demonstrated reversible control of translation with peak activation occurring in early arousal.[3]

The family of mitogen-activated protein kinases (MAPKs) are important regulators of gene expression, cell survival and apoptosis, among many others. MAP Kinases contain a three-tiered signal relay in which an activated MAP kinase kinase kinase (MAPKKK or MEKK) activates a MAP kinase kinase (MAPKK or MEK), which then activates a MAP kinase (extracellular-signal-regulated kinases, ERK; c-Jun N-terminal kinases, JNK; p38). MAPKs have also shown to be important during entrance and arousal in a range of hibernators. In the arctic ground squirrel (*Urocitellus parryii*) brain, p-ERK and p-JNK levels were high during euthermia, significantly decreased during torpor, and re-activated during arousal.[4] Data in the brain of the greater horseshoe bat (*Rhinolopus ferrumequinum*) showed JNK and ERK phosphorylation were differentially regulated during arousal, as compared with torpor.[5] Furthermore, one particular striking trend was observed in data obtained from brown adipose tissue of 13-lined ground squirrels; all MAPKs measured (p-ERK, p-p38, p-JNK) displayed relative increases in the activated form during interbout arousal, as compared with euthermic controls and/or deep-torpor.[1] While species and tissue-specific differences are expected since each tissue faces unique stresses during hibernation, these data nonetheless suggest that regulatory factors under the control of MAP-kinases are particularly important in achieving transitions between euthermia and torpor.

One particularly important type of downstream regulatory protein of signal transduction pathways include transcription factors (TFs) since they connect signaling to gene expression and have been shown to be differentially regulated during entrance or arousal. This theme is exemplified by data obtained from cardiac muscle of hibernating 13-lined ground squirrels where peak values in GATA4 TF activity occurred during entrance and arousal.[6] Moreover, the expression of downstream genes under the control of GATA4 showed concomitant increases during entrance or arousal. Data obtained from other hibernating 13-lined ground squirrel tissues also suggest transcription factors coordinate complex patterns of gene expression during the transitory phases; in skeletal muscle, peak values for the relative expression of the phosphorylated/activated form of MEF2 occurred in entrance, while the amount of phosphorylated SMAD2 and total SMAD7 protein levels were enhanced during arousal.[7] Other transcription factors which are responsive to entry and exit from torpor include those which regulate antioxidant genes since entrance and arousal mimic cycles of ischemia-reperfusion.

In summary, there is evidence to suggest that signal transduction pathways and their downstream effector proteins play an important role in coordinating entrance and arousal during mammalian hibernation. Since these molecular mechanisms occur when dramatic changes in body temperature and the hibernator's physiology are occurring, we propose that understanding the molecular mechanisms of the transitory periods are just as important as understanding torpor itself. Consequently, we eagerly await further studies in the area which aim to understand the molecular underpinnings of entry and exit into torpor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Rouble AN, et al. Mol Cell Biochem 2014; 393:271-82; PMID:24777704; http://dx.doi.org/10.1007/s11010-014-2070-y
2. Nelson BT, et al. PLoS One 2013; 8:e53574; PMID:23301087; http://dx.doi.org/10.1371/journal.pone.0053574
3. Wu CW, et al. Braz J Med Biol Res 2013; 46:1-13; PMID:23314346; http://dx.doi.org/10.1590/1414-431X20122388
4. Zhu X, et al. J Neurosci Res 2005; 80:862-8; PMID:15884016; http://dx.doi.org/10.1002/jnr.20526
5. Lee M, et al. J Neurochem 2002; 82:867-73; PMID:12358792; http://dx.doi.org/10.1046/j.1471-4159.2002.01022.x
6. Luu BE, et al. PLoS ONE 2014; (Forthcoming)
7. Brooks NE, et al. J Exp Biol 2011; 214:2522-7; PMID:21753045; http://dx.doi.org/10.1242/jeb.055764