Genomics Proteomics Bioinformatics 13 (2015) 81–90

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Genomics Proteomics Bioinformatics

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ORIGINAL RESEARCH

Primate Torpor: Regulation of Stress-activated Protein Kinases During Daily Torpor in the Gray Mouse Lemur, *Microcebus murinus*

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Received 13 February 2015; accepted 21 March 2015
Available online 18 June 2015

Handled by Jun Yu

KEYWORDS

Metabolic rate depression;
Signal transduction;
Mitogen activated protein kinase

Abstract Very few selected species of primates are known to be capable of entering torpor. This exciting discovery means that the ability to enter a natural state of dormancy is an ancestral trait among primates and, in phylogenetic terms, is very close to the human lineage. To explore the regulatory mechanisms that underlie primate torpor, we analyzed signal transduction cascades to discover those involved in coordinating tissue responses during torpor. The responses of mitogen-activated protein kinase (MAPK) family members to primate torpor were compared in six organs of control (aroused) versus torpid gray mouse lemurs, *Microcebus murinus*. The proteins examined include extracellular signal-regulated kinases (ERKs), c-jun NH_2-terminal kinases

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Peer review under responsibility of Beijing Institute of Genomics, Chinese Academy of Sciences and Genetics Society of China.

http://dx.doi.org/10.1016/j.gpb.2015.03.002

(JNKs), MAPK kinase (MEK), and p38, in addition to stress-related proteins p53 and heat shock protein 27 (HSP27). The activation of specific MAPK signal transduction pathways may provide a mechanism to regulate the expression of torpor-responsive genes or the regulation of selected downstream cellular processes. In response to torpor, each MAPK subfamily responded differently during torpor and each showed organ-specific patterns of response. For example, skeletal muscle displayed elevated relative phosphorylation of ERK1/2 during torpor. Interestingly, adipose tissues showed the highest degree of MAPK activation. Brown adipose tissue displayed an activation of ERK1/2 and p38, whereas white adipose tissue showed activation of ERK1/2, p38, MEK, and JNK during torpor. Importantly, both adipose tissues possess specialized functions that are critical for torpor, with brown adipose required for non-shivering thermogenesis and white adipose utilized as the primary source of lipid fuel for torpor. Overall, these data indicate crucial roles of MAPKs in the regulation of primate organs during torpor.

Introduction

The ability to generate internal heat and maintain a high body temperature (T_b) has huge advantages for mammals, *e.g.*, supporting advanced cognitive capabilities, speed and agility, and ability to live in cold climates [1]. However, there are high energetic costs to a life as a warm-blooded mammal. Many small mammals live "right on the edge" with the amount of food that they eat each day barely sufficient to keep them alive and warm until morning [2]. As a result, added stresses on the animal, particularly seasonal shortages of food/water and/or extreme environmental temperatures can be lethal. The solution for many small mammals is to temporarily lower their energy needs by regulating a strong suppression of their metabolic rate, causing T_b to fall, and enter either short-term daily torpor or long-term (days or even weeks) continuous hibernation [3–5].

Although alien to humans, daily torpor and hibernation occur in multiple mammalian groups including monotremes, marsupials, rodents, bats, and bears [4]. Various ground squirrel, bat, hamster, and mouse species have been main models for most of the lab-based studies of the biochemical and genetic control of the phenomena [3–5]. It is now known that torpor also occurs in a few species of primates – specifically, some lemurs that are native to Madagascar [2,6–8]. In phylogenetic terms, this indicates that the ability to enter torpor is an ancestral trait of the primate lineage, occurring among species that are very close to the human line [9].

Mouse lemurs of the *Microcebus* genus are the smallest primates in the world but among these, the gray mouse lemur, *Microcebus murinus*, is the largest (weighing around 85–110 g) [2]. Mouse lemurs are nocturnal and sleep in tree holes during the day. They are found along the entire west coast of Madagascar with other populations in the north-central and southeastern regions of the country. This land is lush and wet during the summer but cool and dry in the winter [10]. To deal with periodic food shortages, limited water supply, and cool winter temperatures, mouse lemurs employ hypometabolism [7,8,10]. Daily torpor occurs frequently in mouse lemur species and multi-day hibernation has also been reported for both *M. murinus* and *Microcebus griseorufus* [8]. Environmental factors such as photoperiod, ambient temperature, and food availability are each involved in shaping the biological rhythms of these animals that are characterized by a winter resting period, an active summer breeding season, and an autumn fattening stage [2]. Although several studies have documented the physiological responses of mouse lemur torpor/hibernation [8], this study and the others in this series are the first to explore some of the molecular mechanisms that regulate the phenomena.

Many studies of small mammal torpor and hibernation have discovered compelling commonalities. A prominent theme is a strong global suppression of metabolic rate, involving a regulated and coordinated reduction in all metabolic processes [3,11]. Moreover, fine cellular controls are needed to selectively modulate gene expression and direct specific cellular responses to meet the unique needs of individual organs [3,11]. Established models of mammalian hibernation are typically coincident with low T_b values [5]. By strongly suppressing energy-expensive cell functions and letting Tb values cool to ambient (sometimes as low as 0–5 °C), metabolic rate can often be reduced to $< 5\%$ of normal resting values in euthermia [1]. While hibernation provides an eloquent solution to seasonal shortages; it is often difficult to distinguish between the specific molecular adaptations necessary for metabolic rate depression from those that contribute to surviving cold T_b. This has been a significant area of controversy in hibernation research [12]. Interestingly, summer-active hibernating species are as susceptible to metabolic damage from hypothermia or hypoxia insults as are non-hibernating mammals, but during the winter they can easily transition into torpor, letting T_b fall to near 0 °C and displaying substantially-enhanced hypoxia/ischemia tolerance [11,12]. The lemur model is extremely attractive for studies of mammalian hypometabolism and has several advantages as a model: (a) as primates, these animals are the most closely-related species to human that exhibit natural hypometabolism, and (b) they enter torpor at high ambient temperatures so they show a "pure" form of hypometabolism that is not confounded by the additional biochemical adaptations needed by most species to adjust enzymes/proteins for low temperature function.

Studies from a range of animal models with various tolerances to different environmental stresses (freezing, anoxia, low pH, dehydration, *etc.*) have shown that global metabolic rate suppression, mediated via reversible protein phosphorylation (RPP), is crucial for survival [3]. RPP can produce major changes in the activity states of many enzymes and functional proteins, often providing on/off control. Apart from direct regulation of functional proteins, RPP is also responsible for the detection of extracellular stimuli and their propagation via intracellular signal transduction networks. The use of RPP provides a fast and coordinated mechanism to regulate the function of a wide number of cellular processes that can also be rapidly reversed once the stress is removed [13]. In particular, important targets for RPP control include proteins involved in