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## ACCEPTED MANUSCRIPT

# MAP kinase signalling and Elk1 transcriptional activity in hibernating thirteen-lined ground squirrels.

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#### **Abstract**

*Background:* The thirteen-lined ground squirrel (*I. tridecemlineatus*) becomes hypometabolic during hibernation; characterized by reductions in energy expensive processes like transcription during periods of low temperature and starvation. We were interested in elucidating the mechanisms of transcriptional control by studying the MAPK pathway and downstream transcription factors in skeletal muscle. We were also interested in how environmental factors, such as body temperature, that fluctuate during hibernation, can affect transcriptional regulation.

*Methods:* Protein abundance of MAPKs and effectors were quantified using immunoblotting and Luminex® multiplex assays. DNA-protein interaction (DPI)-ELISA was used to quantitatively assess the binding of transcription factors to DNA promoter sequences under environmental conditions which are reflective of those during torpor.

*Results:* JNK2/3 showed increases in protein abundance during early arousal. The transcription factor, Elk1, showed increases in phosphorylation of S383 (which is indicative of increased activity) during arousal that accompany decreases in total protein levels. Further analysis on the relative binding of this transcription factor to its consensus sequence during euthermia and torpor showed that its binding is significantly different when environmental conditions such as temperature, [urea], and [Ca<sup>2+</sup>] were changed.

*Conclusion:* We show evidence of Elk1 regulation during hibernation, and its activity could be influenced by environmental factors such as temperature, [urea] and [Ca<sup>2+</sup>], as well as post-translational modifications via JNK2/3.

*General Significance: I. tridecemlineatus* has natural mechanisms of transcriptional regulation during hibernation through phosphorylation and changes to the cellular environment. We identify regulation of JNK and Elk1 in the skeletal muscle of hibernating *I. tridecemlineatus*.

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