

Dormice functional genome annotation project: the first insights on regulation of hibernation

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Motivation and Aim: Hibernation is a phenomenon demonstrating in some groups of mammals and representing physiological adaptation for surviving seasonal severe environmental conditions. It is expressed by a long periods of a deep torpor characterized by a reduced metabolism, low body temperature and heart rate and interrupted by a short periods of an arousal. Remarkably, hibernating animals stay immobilized for a long time without significant features of hind limb muscle atrophy [1]. Edible dormouse (*Glis glis*) is a small arboreal rodent, which is superbly suitable for investigation of this phenomenon. Dormice are characterized by a long hibernation period that is 8 month and may be up to 11 month under unfavorable conditions [2]. At present time a plenty of molecular-genetic data on involvement of several molecules, including transcription factors, and signal pathways in the control of hibernation is accumulated [1, 3, 4], however, there are still no systematic data on the genome-wide promoters and enhancers activity in the tissues of a hibernating animal. The aim of present research is to identify molecular mechanisms regulating hypo-metabolic shifts and musculoskeletal adaptation during hibernation via analysis of promoter and enhancer activity in muscle of hibernator edible dormice.

Methods and Algorithms: Two groups of animals ($n = 6$) were examined: active animals ($n = 3$) kept in standard vivarium conditions with ambient temperature 23 °C and hibernated animals ($n = 3$) kept in climatic camera with ambient temperature 0 °C for a 14 days. Two types of muscles were examined: “slow” type soleus muscle and “fast” type extensor digitorum longum (EDL) muscle. For genome assembly, libraries of Illumina short pair end reads, PacBio long reads and Hi-C reads were used. For genome annotation mRNA and total RNA sequencing of muscles and lumbar spinal cord were used. For analysis of gene expression and promoter and enhancer activity CAGE-seq (Cap Analysis Gene Expression) was applied.

Results: To identify molecular mechanisms regulating hypometabolic shifts and protective musculoskeletal adaptation “Dormice functional genome annotation project” was launched. For meaningful analysis of gene expression on promoter and enhancer level a well-assembled and annotated genome of edible dormouse is required. So far there was the edible dormouse’s genome assembly submitted to GenBank by scientific group from Broad University (BU) and based on Illumina HiSeq sequencing technology.

However, we attempted to complement and improve the dormouse genome assembly by long-read sequencing on Pacific Bioscience platform and applying Hi-C chromosome conformation capture technique. Here we provide our achievements on this issue and the first systematic annotation of promoters' landscape and genes activated in different types of skeletal muscles during hibernation.

According to preliminary study 8385 CAGE clusters were determined. Differential promoter expression analysis revealed a distinct program in regulation of hibernation. m. EDL response to hibernation ~2 times higher than m. Soleus. Differential promoter expression analysis and motif analysis demonstrate that response to hibernation is different in EDL and Soleus muscles. Interestingly, differentially expressed (DE) promoters are less rich by CpG regions than others. To identify conservative promoters we performed cross-species mapping and blastn analysis, which reveals 572 mouse conservative promoters in edible dormouse.

Conclusions: Obtained data facilitate the reconstruction of the key regulatory pathways and drivers involved in the regulation of hibernation and protective musculoskeletal adaptation in edible dormice. The study provides the first systematic annotation of promoters' landscape and genes activated in "fast" and "slow" muscle types during hibernation in edible dormice.

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