TRANSCRIPTOME ANALYSIS OF ADULT MYELINATED MOTOR AXONS

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Axonal protein synthesis has been shown to play a role in developmental and regenerative growth, as well as in the maintenance of the axoplasm in steady state. Recent studies have begun to identify the mRNAs localized in axons, which could be translated locally under different conditions. Despite that now hundreds or thousands of mRNAs have been shown to be localized into the axonal compartment of neuronal cultures, knowledge of which mRNAs are localized in myelinated axons is quite limited. The purpose of this work was to characterize which mRNAs are localized in the axon of mature motor neurons.

Isolation of motor axons using a micro-dissection technique, RNA purification and deep sequencing of RNA. Data analysis was using standard pipeline to characterize transcriptomes (mapping, quantitation and biological relevance of findings).

By qPCR analysis we found that through the micro-dissection technique we obtained axoplasm reduced by 99% of glial cytoplasmic mRNA (MAG and Pmp22) and 85% of MBP mRNA, which is transported to the compact myelin. In the axoplasm we were able to identify about six hundred mRNAs. These correspond to 10% of the total obtained for whole the ventral root. The mRNAs more abundant codified for mitochondrial proteins and those associated to the cytoskeleton, but other pathways involved in plastic phenomena where also identified. Of the total of mRNA identified in myelinated axons, the 60% were present in the transcriptomic studies of in vitro cultured axons. The other 40%, are mRNAs identified for the first time in this compartment of the neuron.

This study is the first to analyze the mRNAs present in the axoplasm of myelinated motor neurons, providing new insights to understand local axonal maintenance and response capabilities of the axon in normal or pathological conditions.

Key Words: Axons, mRNA, NGS