氏　　名	Eszter Toth
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審査組織	グローバル教育院
学位論文題目	Single-cell nanobiopsy reveals compartmentalization of mRNA in neuronal cells (シングルセルナノバイオプシーによる神経細胞の mRNA 局在に関する研究)

	（職名）	（学位）	（氏名）
主　　査	筑波大学教授	農学博士	馬場 忠
副　　査	筑波大学教授	農学博士	深水 昭吉
副　　査	筑波大学准教授	博士（医学）	渋谷 和子
副　　査	筑波大学教授（グローバル教育院）	Ph.D.	Bela Gyurcsik

論文の要旨
Abstract of thesis

In highly polarized neuronal cells, compartmentalization of mRNA and local protein synthesis is implemented in rapid, remarkably precise, local responses to external stimuli, allowing tight and accurate regulation of subcellular composition and content. This phenomenon implies that at each sub-cellular compartment, the amount of specific proteins depends on the local rate of translation rather than the absolute amount of mRNA. So far, analysis of mRNA species in dendrites and axons has revealed thousands of transcripts that are differentially localized. Some sequence motifs in the 5'- and 3'-untranslated regions of mRNAs have been found to regulate the localization of transcripts to neuronal processes in the translationally repressed state during mRNA trafficking. Moreover, both the mRNA transport and the local translation are involved in different aspects of neuronal homeostasis, including growth cone guidance, axon maintenance, injury response, and synapse and memory formation. Altered mRNA transport and translation is known to occasionally result in devastating consequences, including mental retardation or neurodegenerative disease, such as amyotrophic lateral sclerosis.

Comparative subcellular transcriptome analysis of neurons has faced many technical limitations. For instance, to detect genes specific for the axons or dendrites, it is required to separate the neurites

from somatic cells. The separation is achieved either by culturing neurons in compartmentalized chambers, microdissection of specific brain areas where the cells have highly ordered, uniform arrangement, e.g. the CA1 region of the hippocampus, or by laser microdissection and glass micropipette aspiration of neurites of cultured neurons. Currently available techniques, including *in situ* hybridization, bulk microarray, and RNA sequencing, impose a tradeoff between spatial resolution and multiplexing; only a few kinds of transcripts are visualized by *in situ* hybridization at a time, whereas when tissue, cells or whole neurites are harvested for multiplexed microarray or RNA sequencing, all spatial information is lost. In addition, previous studies used different cell types for axonal and dendritic transcriptome analysis, making data comparison very difficult. There was no available method for multiplexed, neurite transcriptome analysis at the single-cell-level. A label-free, single-cell nanobiopsy platform has recently been developed based on scanning ion conductance microscopy (SICM), which uses electrowetting within a quartz nanopipette to extract cellular material from living cells with minimal disruption of the membrane and cellular milieu. Using the electron microscopic measurements and geometrical calculations, the volume is estimated to be approximately 50 fL corresponding to almost 1% of the volume of a cell.

In this thesis, the applicant utilizes several methods for data analysis, including Gene Ontology Enrichment Analysis and Self-Organizing Maps to interpret the single-nanobiopsy RNA-sequencing results and the precise mRNA localization patterns in neuronal cells. The subcellular mRNA pools showed a great mosaicism, and the cell regions fundamentally differed from each other in terms of the mRNA compositions. Neuronal cell bodies indicated the enrichment for transcripts encoding proteins involved in transcriptional regulation and protein transport, while neurites were enriched in genes related to protein synthesis, protein targeting to endoplasmic reticulum, and mRNA metabolism. In addition to the previously identified transcripts, the applicant found a new set of mRNAs that specifically localize to neurites, including mRNAs encoding proteins that were previously believed to localize exclusively to the nucleus. Thus, this thesis provides evidence that single-neuron nanobiopsy studies can deepen our understanding of mRNA compartmentalization, and open the possibility to study the molecular mechanism for specific neuronal functions, cellular circuitry, neuronal growth, and network formation.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant used the nonobiposy technique to aspirate mRNAs spatially compartmentalized in a single neuronal cell derived from human iPS cells, and successfully identified transcripts from cell bodies and neurites. However, it remains unclear whether such transcripts are translated into proteins, and are functionally active in these compartments. To address these questions, further studies are required using the nonobiposy technique in combination with mass spectrometer analysis.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on March 12, 2018. The applicant provided an overview of dissertation, addressed questions, and comments raised during Q&A session. All committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.