Title of Project : Identification of Molecular Programs That Generate Neuronal Circuit Diversity

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Research Area : Comprehensive Fields, Neuroscience

Keyword : Neural Development and Its Abnormality, Axon Guidance, Neuronal Identity

【Purpose and Background of the Research】

Neuronal circuit diversity, which underlies the ability of an animal to accomplish its behavioral repertoires and higher brain function, is generated through a stepwise series of phases in the neuronal differentiation program. During development, neuronal fate is first specified by the expression of unique sets of transcription factors within individual cells, followed by neuronal subclass-specific axon pathfinding directed by these transcriptional codes.

In this research project, to elucidate the molecular programs that direct the neuronal circuit diversification, we will focus on the differentiation program employed by commissural neurons, the axon guidance of which has been best characterized in the field. Specific aims are as follows. Aim1: To identify axon guidance receptors and receptor-associated cofactors, the expression of which is regulated by the transcription factors expressed specifically in commissural neuron subclasses. Aim2: To unmask the molecular nature of axon guidance reprogramming upon crossing the midline by focusing on the regulation of selective sorting of guidance receptors on pre- and post-crossing segment of commissural axons, together with the analysis of post-translational modifications of guidance receptors.

【Research Methods】

In aim1, we will use *in vivo* electroporation system for gain-of-function and loss-of-function of genes of interest in mice in order to evaluate whether candidate transcription factors, which have been screened by our previous research in the context of their unique expression in commissural neuron subclasses, play a role in axon guidance especially after crossing the midline. In addition, we will explore guidance receptors acting specifically in commissural neuron subclasses using DNA microarrays in combination with *in vivo* electroporation in mice. Next, in aim2, we will investigate the molecular basis for axon guidance reprogramming using mouse explant cultures that recapitulate the molecular attributes of pre- and post-crossing segment of commissural axons. This will be further combined with cell-imaging techniques provided by laser scanning confocal microscope to analyze in depth.

【Expected Research Achievements and Scientific Significance】

The molecular mechanisms that regulate axon guidance during development will become applicable to the better understanding of axon regeneration mechanism. As an extension for clinical applications, it is also essential to have the knowledge not just from the level of gene expression but also from the level of functions of expressed proteins. In this context, the unique strategies employed in our project could pave the way for this direction, and will have potentials to gain novel unanticipated insights into the molecular pathways that contribute critically to the generation of neuronal circuit diversity. This achievement is also expected to have an impact on neighboring fields.

【Publications Relevant to the Project】

・Shirasaki R, Lewcock JW, Lettieri K, Pfaff SL. FGF as a Target-Derived Chemoattractant for Developing Motor Axons Genetically Programmed by the LIM Code. ***Neuron*** 50, 841-853, 2006.
・Shirasaki R, Pfaff SL. Transcriptional Codes and the Control of Neuronal Identity. ***Annu. Rev. Neurosci.*** 25, 251-281, 2002.
・Shirasaki R, Katsumata R, Murakami F. Change in Chemoattractant Responsiveness of Developing Axons at an Intermediate Target. ***Science*** 279, 105-107, 1998.

【Term of Project】 FY2009-2013

【Budget Allocation】 72,900 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.fbs.osaka-u.ac.jp/~neurobiol/shirasaki/