DIVISION OF CELL PROLIFERATION (ADJUNCT)

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The aim of this adjunct division, started in June 1998, is to understand the basic rules by which elaborate neural circuits develop and function. With less than 10^5 neurones, and subject to powerful molecular and genetic techniques, the brain of the fruit fly *Drosophila melanogaster* is a good model system for investigating the whole of an easily-accessible nervous system that shares certain of the architectural and functional features of the more complex vertebrate brains. Second year of the five-year term, and with new research staff and graduate students, we started a large-scale screening to find strains useful for this purpose.

I. Comprehensive identification and developmental tracing of brain cells

A comprehensive and detailed anatomical knowledge of the brain is a prerequisite for 1) analysing the phenotypes of nervous system-related mutants, 2) identifying the cells that express the cloned genes, 3) understanding the way information is processed in the brain, and 4) devising computer models that simulate brain functions. In spite of the hundred years of efforts using Golgi and other anatomical techniques, however, the circuit structure of higher order associative regions of the brain is still essentially unresolved. Moreover, traditional neuroanatomy tends to focus only on the mature adult brain, leaving the developmental processes largely uninvestigated. Since many nervous system-related mutants show structural defects, however, understanding the role of the responsible genes require detailed basic knowledge about when and how the brain structure is formed. Thus, “developmental neuroanatomy” becomes all the more important in the age of molecular cloning.

The GAL4 enhancer-trap system, which is widely used for mutagenesis and gene cloning of *Drosophila*, is also a powerful tool for obtaining a vast array of transformant strains that label specific subsets of brain cells. We screen such lines from a stock of 4500 GAL4 strains made by the “NP consortium”, a joint venture of eight Japanese *Drosophila* laboratories organised by us. After initial pilot experiments, we employed a two-stage screening process. In the first step, all the lines are crossed with the flies carrying the UAS-*GFP* transgene, which fluoresces only in the cells where GAL4 expression is active. The patterns of the GAL4-expressing cells are recorded from freshly dissected, unfixed adult brain tissue using a high-speed confocal microscope. Photographs of between 20 and 100 optical sections are taken. As of December 1999, ca. 50000 photographs depicting 1612 of the total 4500 strains have been accumulated in a computer database. In the second step, useful lines are selected from the database, and fixed and clealised brain specimens at various developmental stages are subjected to confocal serial sectioning with a conventional confocal microscope and to three-dimensional reconstruction with a UNIX workstation.

Among the 1612 lines screened, 98 % showed GAL4 expression in the brain, suggesting that most of the genes in the genome are expressed in at least some portion of the brain cells. Among them, less than 20 % showed expression in a small enough number of cells for which identification is feasible. In many such strains, the expression pattern changes drastically during neurogenesis. Only a few percent of the whole strains label identical neurons throughout larval, pupal and adult stages. These lines are found valuable to trace the development, path finding and synapse formation processes of the labelled cells.

Although the long-term aim of this project is to identify as many neurones and glial cells as possible to get the comprehensive overview of the fly brain structure, at the initial stage a few brain regions are chosen for intensive study. The first target is to identify projection interneurons that connect lower-level sensory neuropile and higher-order associative regions. These fibres convey olfactory, gustatory, auditory and visual sensory information. As for visual pathways, for example, five novel types of projection neurones have already been identified by our screen.

The other target is to reveal the formation process of one of the highest-order associative centres called the “central complex”. In this area, fibres from various brain regions converge to form the sophisticated array circuit structure, providing a good model system for studying the molecular mechanisms of path finding and circuit formation. We have identified several strains that label central complex neurons from rather early stages and are tracing the developmental processes in more detail (Fig. 1).

II. Tools for comprehensive cell lineage analysis of the brain

Combining the flippase-FRT recombination induction system and GAL4-UAS expression activation system, we have previously developed a novel technique, the “FRT-GAL4 system”, with which one can label a small number of neural stem cells at any desired developmental stage and reveal the projection patterns of their progeny at a later period. Though this system proved useful for revealing the clonal architecture of several brain regions, the performance of the transformant lines used for activating the *flippase* gene was not optimal, showing a high level of spontaneous recombi-

nation. To solve this problem, we generated and screened ca. hundred new lines and obtained several that show no background *flippase* expression and sharp heat shock-induced activation. Using these lines, an attempt to make a comprehensive map of the lineage-related circuit structures of the fly brain is underway.

III. Analysis of genes for path finding processes in the adult brain development

Although many genes related to the neuronal path finding processes have been identified and analysed in *Drosophila*, their functions have been studied primarily in the simple motor neurons and the ventral nerve cord of embryos. Little has been investigated about their functions when neurones compose highly complicated circuits of the adult brain.

The latest genetic mosaic system, the "MARCM (mosaic analysis with a repressible cell marker) system", can generate labelled clones with mutant genotype whereas surrounding wild-type cells remain unlabelled. This is a powerful technique for studying the function of a gene in the path finding processes of identified fiber projection. Using this system, we analysed the function of the gene *trio*, which encodes a Dbl family protein. As a model system for analysing its possible role in path finding controle of developing axons, we focused on the brain region called mushroom bodies (MB), whose clonal structure has been revealed in great detail by us and other investigators.

An MB is made by four specific stem cells (neuroblasts) and thus consists of four clones. When one of them were labelled without introducing *trio* genotype, the neurones of the clone extended their axons through the peduncle and arborised into the vertical and medial lobes (Fig. 2A). In contrast, neurons of the *trio* mutant clones appeared to navigate normally through the peduncle till the approximate region of the two lobes, but there they either stalled or are misrouted around the end of the peduncle, forming only sparse arborisation into the lobes (Fig. 2B). On the other hand, branching in the calyx appeared unaffected. These suggest that *trio* plays an essential role in projection and arborisation of axons from the peduncle into lobes.

IV. Contribution to the science community

As a joint venture with German and US research groups, we maintain *Flybrain*, a web-based image database of the *Drosophila* nervous system (http://flybrain.nibb.ac.jp). Over 2000 images has already been stored and served worldwide. Another database maintained here is *Jfly*, which is intended to help the exchange of information among Japanese-speaking *Drosophila* researchers (http://jfly.nibb.ac.jp). Archives of research-related discussions, images and experimental protocols, as well as meetings and job announcements, are provided.

Publication List:

Awasaki, T., Saito, M., Sone, M., Suzuki, E., Sakai, R., Ito, K., Hama, C. The Drosophilla Trio plays an essential role in patterning of axons by regulating their directional extention . *Neuron*. **in press**.

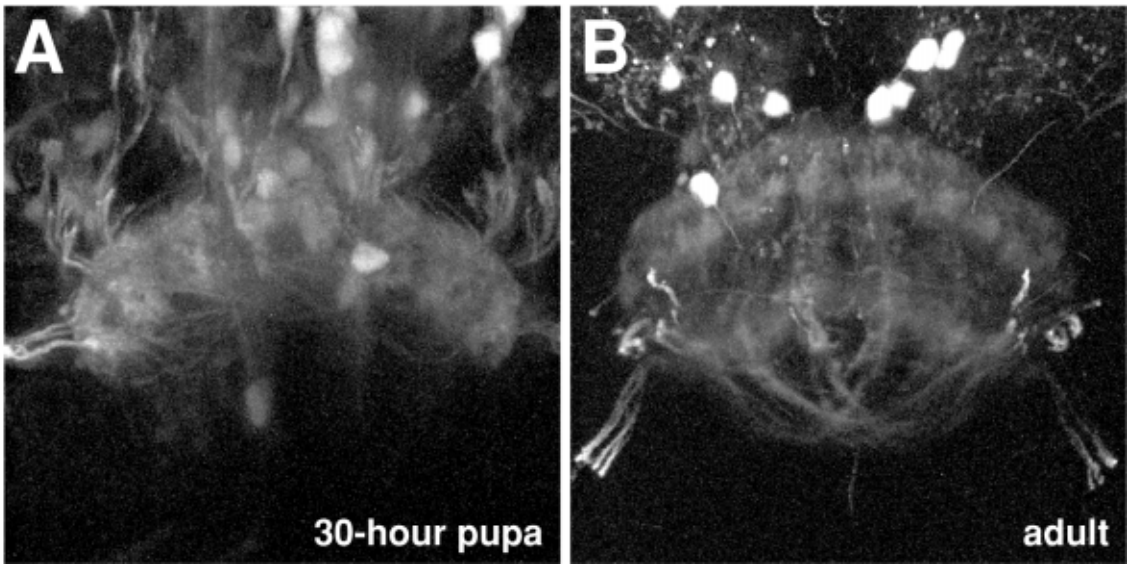

Fig. 1 Development of fibres innervating the central complex
A: In early pupae, only upper part of the fan-shaped body region of the central complex is formed. B: The adult fan-shaped body shows two layers of tangential connections.

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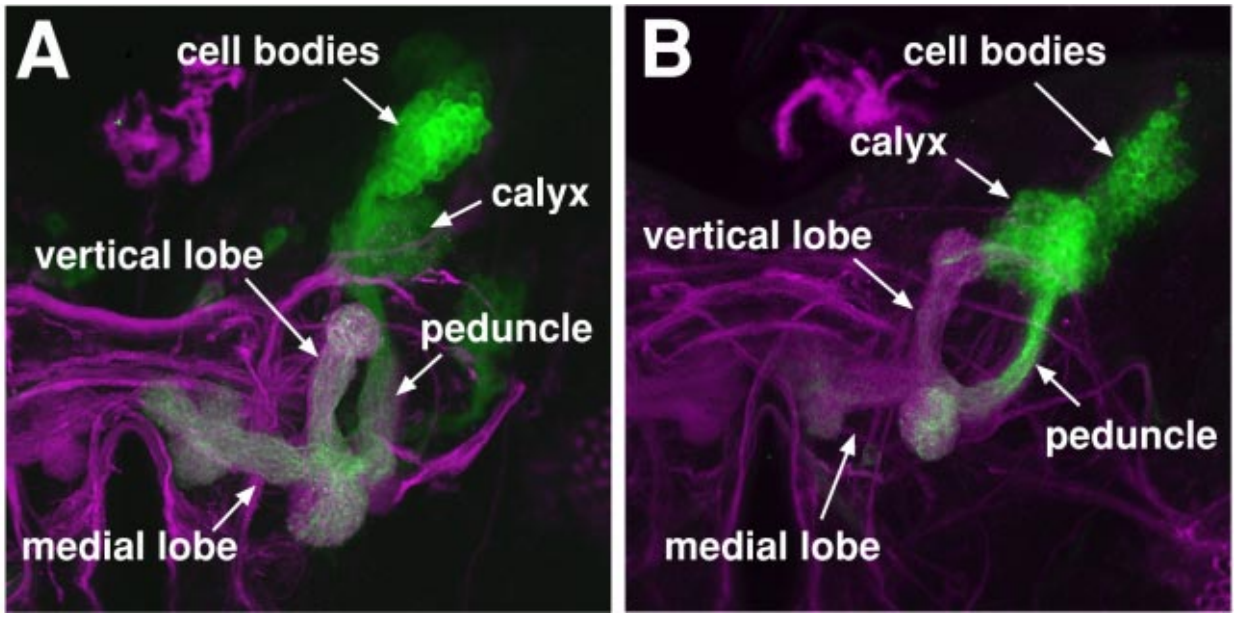

Fig. 2 *trio* clones in the mushroom bodies (MB)
A: The MB consists of neurons produced by four stem cells. One of the four wild-type clones is made to express *CD8* reporter gene and labelled with anti-CD8 antibody (green). Fibres from all the four clones, together with some other tracts, are labelled with anti-FasII (purple). B: In the *trio* mutant clone (green), neurones extend axons in the peduncle but exhibit poor projection into the vertical and medial lobes. Anti-FasII labelling (purple) shows that the other three clones form essentially normal MB structure.