**Final Publishable Summary Report**

Project objective

Src-family kinases play major roles in promoting adherens junctions (AJs) remodelling in development and metastasis. Their activities are

negatively regulated by C-terminal Src kinase (Csk). The host lab has recently shown that dASPP, together with Boa regulates the function of dCsk in maintaining AJs integrity in Drosophila (Langton et al., 2007 and 2009). However, the molecular nature of this interaction and the exact functions of dASPP and Boa on developing epithelia are still unknown. In this research project, we propose to address the molecular mechanism by which dASPP and Boa regulates dCsk activity and their function in epithelial growth and morphogenesis during development, as well as identify new regulators of Src signalling in epithelial cells.

A description of the work performed

The first aim of the project is to elucidate the functional relationship between dASPP, Boa and SFK pathway by biochemical assays. I have generated antibodies and expression constructs for dASPP, Boa, Csk and Src. I have performed co-immunopreciptations to investigate the interaction network among the above proteins. Additionally, I have mapped the interaction domain between dASPP and Boa, dASPP and Csk. After carefully assessing the specificity of the Csk and Src antibodies, I checked also the subcellular localization of Csk/Src in pupal retina and wing/eye discs. I have also studied whether the localisation of these proteins is interdependent by staining genetically induced mutant clones in pupal retinas.

Btk29A kinase acts downstream of SFK pathway and thus we had plan to carefully investigate its function. I have tried to create mutants of Btk29A kinase by imprecise transposon excision but was unsuccessful. Overexpression or RNAi of Btk29A in fly eyes did not give any obvious phenotype. Unfortunately, we couldn’t detect any significant binding between Src and Btk29A by co-immunoprecipitation. Thus, we decided to put this project on hold and focus on investigating the function of Boa/sec15 complex, which has been giving promising results.

The second aim of the project is to probe the relationship between the Src pathway and adherens junction(AJ) integrity in live developing *Drosophila* pupal retinas. The approaches we proposed were time-lapse imaging and FRAP (flurorescence

recovery after photobleaching) analysis of AJs in mosaic animals containing dASPP/Boa or SFK clones. In order to perform clonal analysis, we generated flies containing mcherry as a marker for FRT/FLP generated

mutant clones. Additionally, we prepared a collection of transgenic fly stock containing E-cadherin-GFP, rabs to study the dynamic of AJs, as well as vesicle trafficking. We then set up time-lapse live imaging of Drosophila pupal retinas and notums during epithelial morphogenesis using state-of-the-arts microscopes in the host institute. In collaboration with the staff from the light microscopy facilities, we also developed a method to optimize the qualities of the raw images. Currently, we are trying to finish analyzing the live-imaging and FRAP data.

The third aim of the project is to identify new members of the Boa/dASPP/Src pathway. We attempted to generate a luciferase-based dSrc activity reporter for a cell-based high throughput RNAi screen based on the state-of-the-arts split-TEV system. (Wehr et al., 2006). With the assistance from Dr. Michael Wehr, who developed the split-TV system in mammalian cell culture, we performed pilot experiments to test the sensitivity of the dSrc reporters in Drosophila cells. This revealed that the reporters were not sensitive enough to be used in a high-throughput screen. As an alternate attempt to identify novel binding partner for Boa/dASPP/Src pathway, we performed a yeast 2-hybrid screen, using Boa as a bait. We cloned a number of putative interactors and validated their binding with Boa by co-immunoprecipitations. We then showed that Boa interacts with Sec15, an exocyst component, biochemically and genetically, indicating that Boa, together with sec15, modulates polarized vesicles trafficking and affects AJ stability. In light of the above hypothesis, we then performed a series of experiment to investigate the vesicle trafficking pathway in boa mutants in vivo. We tested the function of trafficking pathway by checking the localization of markers for the different endosomal compartments and have found that the recycling endsomal pathway in Boa mutants appears to be defective. To visualize the defect of the vesicle trafficking pathway at high-resolution, together with the electron microscopy (EM) unit from the host institute, we performed EM on Boa mutant retinas and are in the progress of analysing the raw data.