Research Networking Programmes

Short Visit Grant ☒ or Exchange Visit Grant ☐

(please tick the relevant box)

Scientific Report

The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.

Proposal Title: The role of septate junctions in epithelial morphogenesis in the Drosophila epidermis

Application Reference N°: 6839

1) Purpose of the visit

Simple epithelial tissues have the extraordinary capacity to resolve wounds in a rapid and efficient manner through a resealing mechanism conserved across species. This process involves dramatic cellular rearrangements and the assembly of a contractile actomyosin cable at the edge of a wound. Our recent data points to a key role of Septate Junctions (SJs) during wound closure in the Drosophila embryonic epidermis. SJs are essential cell junctions present in epithelial tissues in invertebrates but their functions during epithelial morphogenesis remain largely unknown. By analyzing embryonic phenotypes of mutants for different SJ components, we found that epidermal cells show altered cell shapes and defective cell rearrangements, suggesting SJs have a role in regulating the mechanical properties of the epidermis and consequently the response of the epithelium to injury.

Our goal with this visit was to determine whether the epidermis of SJ mutants has altered physical properties. One of the most reliable methods used to investigate this in an living organism is by laser ablating cell boundaries and then analysing their response to the perturbation (Farhadifar et al 2007, Rauzi et al 2008, Mayer et al

2010). Since our group currently lacks the laser set-up required to perform these experiments, we teamed up with the group of Carl-Philipp Heisenberg at the Institute of Science and Technology in Austria (IST), who are experts in this field and have all the technical resources necessary to perform these experiments (Behrndt et al 2012, Campinho et al 2013). In this visit our plan was thus to measure the response of epidermal cells to ablation in both wild type and SJ mutant embryos and thereby determine whether these junctions have an influence in the tension and/or in the stiffness of the tissue.

2) Description of the work carried out during the visit

In order to perform this work I teamed up with Martin Behrndt, a PhD student in the Heisenberg Lab, an expert in the laser ablation system and on the analysis of the obtained data. I also had the great help of the group of Daria Siekhaus at the IST in order to grow and keep the Drosophila strains used in this work.

To determine the role of SJs in regulating mechanical properties of the epidermis, we compared wild type embryos to mutant embryos that lack the function of one of the core components of SJs, Kune, an essential transmembrane protein of the Claudin family. Both control and kune mutant embryos expressed fluorescent markers for E-cadherin (Ubi-Ecad-GFP) to visualize cell boundaries and adherens junctions, and for Actin (Cherry-Moesin) that worked as an alternative marker and as a means to detect any wound response of the ablated cells (Behrndt et al 2012).

We performed laser ablation experiments using a UV laser cutter set-up as previously described (Behrndt et al 2012), on both wild type and kune mutant embryos at stage 15 of embryonic development. At this stage of development SJs are fully functional in the wild type in contrast to kune mutants. It is also at this stage that we observe major cell shape and wound healing defects in kune mutants. During the first week of experiments, we optimized the settings for image accquisition and laser ablation. Once we had all the parameters optimized, we performed laser cuts in epidermal cells at the level of the adherens junctions by applying 50 UV pulses to 2 equidistant sites in a 2 µm-long line, perpendicular to the junction. Images were acquired every 1 second for at least 1 minute. We performed laser ablation on at least 20 cells of each genotype (wild type and kune mutants).

It is known that cortical tension is proportional to the intial recoil velocity of the cortex (Mayer et al 2010). To determine whether tension is affected in kune mutants, we manually measured the initial displacement of each junction using Fiji software (Schindelin et al 2012) and then calculated the initial recoil velocity considering the first 2 seconds after ablation. In addition, we investigated whether kune

mutants have differences in the stiffness of the tissue. This property can be determined by examining how the recoil velocity decays over time. This decay in the recoil velocity is exponential and the associated decay time constant is inversely proportional to the stiffness of the cortex (Mayer et al 2010). Therefore, we will fit an exponential decay curve for each ablated junction and calculate the respective decay time constant, and compare wild type to kune mutant embryos.

3) Description of the main results obtained

We are currently still quantifying and analysing all the data obtained in this visit, therefore, we cannot yet conclude whether tension and/or stiffness are affected in kune mutants. However, our preliminary observations seem to indicate that the initial recoil velocity is higher in kune mutants than in wild type, which would support our hypothesis that SJs regulate the mechanical properties of the embryonic epidermis.

4) Future collaboration with host institution (if applicable)

We hope to continue the colaboration with Carl-Philipp Heisenberg's lab during this project. Depending on the outcome of the analysis of the data obtained in this visit, we might need to obtain more measurements for each of these genotypes in order to conclude whether the SJ component Kune regulates tension and/or stiffness of in epidermal cells.

In case we do not find any differences in the mechanical properties of the tissue between wild type and kune mutants, we plan to visit again the Heisenberg lab to perform laser ablation experiments of the actomyosin cable that forms at the wound edge upon injury. Our previous data shows that the stability of this contractile cable is defective in SJ mutants, leading to the failure in wound contraction and closure. Thus it would be highly relevant to determine whether in the mutants the tension at the level of the actin cable is affected. For that we would also have to perform laser cutting experiments on the actin cable and determine its initial recoil velocity.

5) **Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)***

The results of these experiments will be part of a manuscript in preparation on the role of Septate Junctions during wound healing in Drosophila.

6) Other comments (if any)

References

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