### Project Proposal  A*STAR Graduate Scholarship (Overseas)

<table>
<thead>
<tr>
<th>Project Title:</th>
<th>The dynamics of epithelial cell junction formation – regulation and role of Cdc42.</th>
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<tbody>
<tr>
<td><strong>Starting date:</strong></td>
<td>From September 2014</td>
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<tr>
<td><strong>Supervisors:</strong></td>
<td>Ed Manser &amp; Vania Braga</td>
</tr>
</tbody>
</table>

### Project Description

The integrity of epithelial cell junctions underlies many important aspects of tissue development and homeostasis. As well as being transducers of mechanical force in tissues cell-cell junctions recruit and are dynamically regulated by protein signaling complexes. These signaling scaffolds make connections via downstream signaling enzymes such as protein kinases and small GTP proteins of the Rho family (1). Such pathways help to maintain the polarity of epithelial cells, and allow switching of cell behaviour in response to changes in cell status (for example after injury). The cell-cell adhesion complexes directly connect to microtubules, intermediate filaments and the actin cytoskeleton.

This project aims to investigate how Cdc42 is activated at cell-cell junctions, and the way in which the spatio-temporal control of Cdc42 activation drives maturation of cell–cell contacts. Studies indicate that multiple Cdc42 activators are needed for the formation and stability of cell-cell junctions (1). This includes FGD1-related Cdc42-GEF (FRG or Frabin), Tuba, and ARHGEF4 (also called ASEF). Early junctions are thought to require Nectin-Afadin complexes (2) which then recruit FRG (and perhaps other Cdc42-GEFs) needed to form and sustain typical E-cadherin junctions (AJs).

The project will focus on three classes of Cdc42 activator (above) to investigate their relative contributions to early and late stages of epithelial junction formation in immortalized human keratinocytes. In order to understand how these proteins function in a complex, keratinocytes or alternate epithelial cell lines expressing GFP-BirA tagged Cdc42-GEFs will be generated. Using this new method allows for proteomic analysis (3) of nearby proteins (to the BirA fusion) under cellular conditions of choice (for example with or without extracellular calcium). The local protein composition of cell adhesion complex containing the Cdc42-GEF will be analysed using SILAC (stable Lys/Arg isotope labelling of cells). With quantitative ms/ms mass-spectrometry it is possible to identify protein complexes (in their native state) without the need to recover the protein interactions intact. The project will establish the nature of the compartment in which RhoGEF operate at various stages of cell-cell junction formation, and the protein interactions that contribute to Cdc42 activation.

Understanding the way in which epithelial cells establish and maintain cell-cell junctions is an important goal, and both laboratories have track record in understanding cell signaling that involves small GTP binding proteins of the Rho family (cf. the polarity protein Cdc42). The Manser lab is particularly interested in the metazoan specific Cdc42 effector PAK4, and the role of this protein kinase at cell-cell junctions.

### References.

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