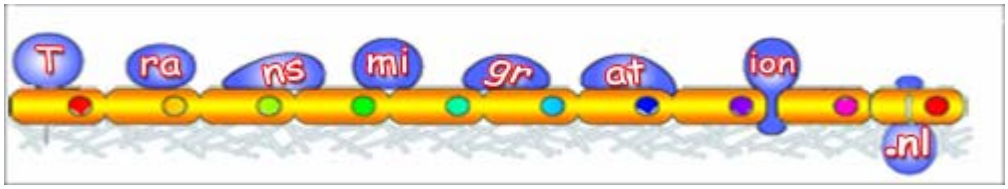
MCB Internship project 3:



VE-cadherin distribution at cell-cell junctions

Introduction: The vascular endothelium separates tissues from the circulation and regulates the extravasation of fluids, plasma proteins and leukocytes. Changes in the structural integrity of the endothelial monolayer are essential for inflammatory responses, but can result in vascular leakage when induced inappropriately. In vascular endothelial cells, the junctional protein VE-cadherin is particularly important for the maintenance of inter-endothelial cell contacts. Like other cadherins, VE-cadherin mediates calcium-dependent homophilic adhesion. In addition, within the cell, VE-cadherins provide sites for attachment of the cytoskeleton by interacting with several cytoplasmic proteins, called catenins. The mechanisms by which these catenins, including β-catenin and p120-catenin, regulate VE-cadherin function and distribution are not clear. In this project, the distribution of the VE-cadherin complex will be further investigated using state-of-the-art immunofluorescent microscopy.

Aim: To study the dynamics of the VE-cadherin complex, several fusion proteins will be generated. Using GFP (green fluorescent protein), the distribution of VE-cadherin and the catenins will be followed in real-time using the confocal laser scanning microscope. Next to GFP, the green-to-red photoswitchable fluorescent protein Dendra will be used. This protein switches irreversibly from green to red when exposed to intense 405 or 488nm. By fusing this protein to VE-cadherin and the catenins, the distribution of the local VE-cadherin complex can be followed in time.

Techniques: Students will be trained to generate fusion proteins using molecular biology, including PCR and cloning of constructs. In addition, expression will be checked using Western blotting and SDS-PAGE. Also, the student will be trained to operate the confocal laser scanning microscopy to make real-time recordings of VE-cadherin and catenin distribution. To challenge the endothelial cell-cell junctions, calcium-switch assays and thrombin will be used.

Duration: at least 6 months. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Jaap van Buul, either by e-mail: j.vanbuul@sanquin.nl or phone: 020-5121219.