HEP Internship project 3:

Unraveling the function of band 3 "tyrosine" phosphorylation during erythropoiesis and in erythrocytes.

Introduction: Erythropoiesis is a process occurring in the bone marrow which end point is the production of erythrocytes (red blood cells). During erythropoiesis the erythrocyte precursor undergoes significant changes among which are cytoskeleton and membrane remodeling, expulsion of the nuclei and degeneration of organelles like mitochondria and the golgi apparatus.
We are interested in a multi-protein complex, the band-3 macro-complex present in the erythrocyte membrane. Among its different functions the complex links the intracellular cytoskeleton to the membrane thereby increasing the rigidity of the red cells and partly dictating the bio-concave shape of red cells ensuring optimal surface exposure to the extra cellular environment to maximize gas exchange. Red cells membranes need to be flexible to be able to pass through small capillaries; hence the rigidity of the membrane needs to be regulated. Band-3 is a target for several kinases and phosphatases, moreover hyper phosphorylation of band-3 is observed in several hematological disorders like thalassemia and sickle cell disease. However, the functionality of band-3 phosphorylation is yet to be established. As band-3 involved in linkage of plasma membrane to the cytoskeleton, phosphorylation of band-3 can play role in dynamic regulation of this linkage and therefore the shape of the cell.

Aim: This projects aims to study the molecular mechanisms of band-3 phosphorylation in order to unravel the signaling networks involved in band-3 phosphorylation and its role in the physiology of red cell.
It is believed that band-3 is phosphorylated on four Tyrosine residues: Tyr8, Tyr21, Tyr359 and Tyr904. In this project we will mutate band-3 on each of this residue and in combinations, transfect 293T cells with mutated band-3 and study the effect of introduced mutations on the phosphorylation status of band 3. We are also interested in the role of the band-3 phosphorylation in membrane-cytoskeleton reorganization during erythropoiesis. For that we will transduce primary cultured human erythroblasts with the mutated band-3 constructs whilst knocking down the endogenous band-3 with a short hairpin and follow the effect of band-3 mutation during erythroblast expansion and differentiation.

Techniques: This project involves cell culture, site directed mutagenesis technique, sequencing, creation of lentiviral constructs, RNA interference, virus work, Western Blotting (SDS-PAGE), flow cytometry, image stream, isolation of membrane proteins and red cell membrane preparation.

Duration: 6 – 9 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, Emile van den Akker, either by e-mail: e.vandenakker@sanquin.nl or phone: (+31) 20-512 7004.