Genetic networks guiding heart valve development

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The molecular mechanisms that guide the development of the atrio-ventricular canal (AVC) are not well understood but are of immense clinical importance, since defects in the development of the AVC, in particular cardiac valve malformations, affiliate to the most common congenital heart defects and often lead to early embryonic death or severe cardiac dysfunction later on. In a forward genetic screen, we recently isolated a zebrafish mutant which displays a pathologically developed atrio-ventricular canal accompanied by absent endocardial cushions, the precursors of the cardiac valves. We find by positional cloning that the phenotype is caused by a missense mutation in an important transcriptional regulator.

The main goal of this scientific tandem project (6 month stay in the lab of Prof. Conlon) is to elucidate the specific role of this transcriptional regulator in the control of AVC formation in the zebrafish heart.

(1) Detailed morphological, structural and molecular characterization of the mutant embryos including *in vivo* microscopy, histology and ultrastructural analysis, RNA-antisense *in situ* hybridization studies, qRT-PCR, Western Blots and immunostainings at the defined stages. Development and definite morphology of the AVC will be further determined by high-resolution confocal microscopy using mutant embryos crossed with a transgenic zebrafish line expressing GFP in myocardial and mCherry in endocardial cells (Tg(mlc2::rasGFP/flk1::rasCherry)). In addition, development of endocardial cells will be visualized using the AV endocardial cell-specific Alcama (Dm-grasp) antibody.

(2) Molecular and biochemical evaluation of the impact of the mutation on transcriptional activity (Luciferase assays, EMSA), subcellular localization (*in vitro* and *in vivo*) and the physical association to known interaction partners (Co-IP, pulldown assay).

(3) Finally, the PhD student will screen for downstream components that are regulated by the transcriptional regulator and are involved in controlling AVC development. To do so, transcriptional profiles of mutant zebrafish will be assessed by heart-specific microarrays (Affymetrix® Zebrafish Gene 1.0 ST Array) and validated by qRT-PCR. The *in vivo* role of selected dysregulated genes, in particular their role in the formation of the AVC, will be analyzed by reverse genetics approaches such as gene inactivation (Morpholino-knock-down) and over-expression studies (transient or stable) in zebrafish.