Research Networking Programmes

Short Visit Grant ☐ or Exchange Visit Grant ☒

(please tick the relevant box)

Scientific Report

Scientific report (one single document in WORD or PDF file) should be submitted online within one month of the event. It should not exceed eight A4 pages.

Proposal Title: Computational model of trabeculation of the ventricular myocardium

Application Reference N°: 4185

1) Purpose of the visit

Translate cell adhesion, local proliferation rates, cell polarity, and endo-myocardial signalling into a Cellular Potts model for trabecular growth in order to identify how these genetic, cellular and mechanic factors interact and regulate trabeculation.

2) Description of the work carried out during the visit

Trabeculation, the process in which trabeculae become apparent and grow into a framework of muscular strands is a crucial process in heart development. The first trabeculae appear like ridges perpendicular to the blood flow and increase the surface area of the myocardium that is in contact with the cardiac lumen. This in turn allows an increase of muscle mass without need of a coronary circulation. To be able to create a simulation of this process we needed to create a model of the initial situation. We decided to base this initial situation on the primitive ventricle just before the process of trabeculation started. In Zebrafish and Mouse, respectively, this primitive ventricle exists out of a smooth one or two cell layer thick myocardial wall, a layer of cardiac jelly and a lining of endocardium separating the myocardium and jelly from the cardiac lumen.

We started with a 2D model in which it would be faster to test the role of the variables used in the simulation. This was done by making a virtual cross section perpendicular to the future trabecular ridges using a custom written Matlab script. In this initial situation we created a 2D lattice in which the endocardium and the myocardium were modelled as

a single layer of cells (in cross section appearing as a row of cells). Between the endocardium and the myocardium a layer of cardiac jelly was created to separate the endocardium and myocardium. At two locations endocardial protrusions were created forming a connection between the myocardium and endocardium. This model was composed out of approximately 60 cells of about 100 pixels each. In this initial model we defined adhesion properties between all cell types. By choosing and testing the adhesion values carefully I assured that the tissue conformation remained intact. An additional adhesive property, the N-Cadherin density was set specifically for myocardial cells. This density could be set for individual cells allowing individual myocardial cells to be less adhesive to the rest of the ventricular wall which in turn should enable these cells to leave the epithelial like organisation of the ventricular wall. However, with extensive testing this simulation appeared not to be able form trabeculae within reasonable time.

The cause for this failure is probably that, in the case of a 2D model, the cells neighbouring the cell with decreased adhesion are not connected to each other and therefore not able to push the cell out of the wall. To test whether this was the case we modified the initial situation to have a layer of myocardium which was two cells thick. In this case cells were indeed able to leave the wall. This however, is still quite different from the actual 3D situation were the neighbouring cells are forming a ring around the less adhesive cell which makes extrusion of this cell much more efficient.

At this point we decided to switch to a 3D organisation were we now modelled the endocardium and the myocardium as single layer sheets of cells. This model consisted out of approximately 450 cells of around 125 voxels each. By reducing the cell diameter to approximately 5 voxels, the total lattice size was a very modest 150x50x45 voxels enabling relatively fast simulations. Drawback of the reduction the size of cells is that cell shapes are less evident. This initial 3D situation resulted in a very regular organisation of cells. Therefore to get a more natural arrangement of cells the target volume and corresponding surface of each cell was randomly changed within Compucell.

In the first 3D model, we selected single cells in the myocardium to divide. These cells had a slightly decreased cell adhesion with their myocardial neighbours which made it easier to leave the myocardial sheet. We increased the target volume and surface gradually. These dividing cells got elongated perpendicular to the ventricular wall, extending into the cardiac jelly. When the cells doubled in volume this triggered a division along their major axes. By division over the major axes the cells divided within the plane of the wall, as was observed by the host laboratory. After division the cells remain to have a slightly smaller N-cadherin density and one of the cells would then leave the myocardial wall and form the first sign of a trabecule.

Although it is likely that dividing cells have reduced cell adhesion because of the remodelling of their cytoskeleton during cell division, we had problems combining this with the knowledge that proliferation in the wall is dependent on notch signalling while notch expression is highest in the endocardium just next to the base of the trabecules. Furthermore, I visited the lab of Didier Stainier (Max-Planck-Institut für Herz- und Lungenforschung, Bad Nauheim) who shared live imaging data on cell behaviour during Zebrafish heart maturation (unpublished, confidential data). These data made it less likely that the dividing cells were the cells which would leave the wall.

A second 3D model was significantly more complex by making proliferation in the ventricular wall dependent on notch signalling. An important requirement of the implementation of the notch signalling is that activated notch in the endocardium shows a lateral inhibition pattern combined with a gradient from high near the ventricular wall to low more distant from the wall. A recent publication from Sprinzak et al (2010, Nature) showed a model which calculated notch activity of a cell based on notch and delta transcription and on the expression of delta and notch within the cell and in its neighbouring cells. This model was able to produce both a notch activity gradient as well as a lateral inhibition pattern. Therefore we decided to implement the set of Ordinary differential equations (ODEs) Sprinzak et al published in the Systems Biology Markup Language (SBML). The most recent version of Compucell is able to solve such ODEs. We then assigned these ODEs to all endocardial cells.

To create the gradient of delta expression observed by Grego-Bessa et al (Dev Cell 2007) we implemented a Delta Inducing Signal (DIS) from the myocardium. This signal was modelled to be secreted by all myocardial cells creating a gradient from the myocardium to the lumen. This resulted in a higher DIS in the endocardial protrusions then in the endocardium more distal from the ventricular wall. Notch transcription was defined homogeneous throughout the endocardium as was observed by Grego-Bessa et al. This combination resulted in a gradual expression of activated notch in a lateral inhibition pattern. Activated notch in turn leads to expression of Nrg1 which is able to act as a paracrine signal.

When the amount of Nrg1 signal exceeds a threshold this would trigger a cell to prepare for cell division by growing and reducing its N-cadherin density. A neighbouring cell is reducing its N-cadherin density even further causing the dividing cell to take the place of its neighbour which is then pushed out of the wall. This extrusion happens with multiple cells next to the endocardial protrusions. In this model we defined an additional property of the cells, the target length. Before extrusion myocardial cells had a target length aiming at a round cell. We changed the target length of the extruded cells causing them to elongate. These cells were connecting to each other forming the trabecular ridges.

In parallel to the modelling mouse embryos ranging from day 8.5 to day 9.5 post fertilization were collected by the host. The hearts of these embryos were used for whole mount staining of cell membranes, nuclei, endocardium and a proliferation marker (pHH3 or Ki67). The hearts are mounted on slides and images were acquired using confocal microscopy.

To be able to assess cell shapes and proliferation relative to the endocardial protrusions and trabecular ridges, I developed segmentation and quantification tools in Matlab. These tools had to deal with the fact that nuclei in the early heart are often situated very close together and then appear to be fused in the confocal images. In short, I developed a method which first segments the complete nuclear volume using a low intensity threshold on the local maxima of the stack and then uses a higher intensity threshold to identify the cores of the nuclei. These cores are then used as seeds for a watershed segmentation within the total nuclear volume. This separates many of the fused nuclei. The segmentation of the whole cells is done by using the nuclei as seed in the membrane stained image set and again perform a watershed segmentation. This results in segmented cells with their corresponding nuclear volume and staining density. The distribution of

the nuclear volumes in the segmented cells showed sharp peaks at a nuclear volume of 1, 2 and 3 nuclei. Because mononucleated cells are expected, the nuclear volume could therefore be used as a quality check for the segmentation. Cell size, elongation, roundness were computed for all cells and their positivity for the proliferation marker was determined. These individual cell properties were then visualized using Amira.

3) Description of the main results obtained

We created a computational model of early trabecule formation. In this model we included cell shape parameters involving target volume, target surface and length. Cell adhesion between all cell types and adherence by N-cadherin was specified for each myocardial cell. We implemented a notch signalling from the endocardium to the myocardium which is in turn activated by a delta-inducing signal from the myocardium. Notch signalling is then triggering myocardial cells to divide which pushes other myocardial cells out of the epithelial-like organisation of the ventricular wall. These extruded cells elongate and connect with each other to form a trabecular ridge.

Mouse embryos ranging from day 8.5 to day 9.5 post fertilization were used for whole mount staining of cell membranes, nuclei, endocardium and a proliferation marker (pHH3 or Ki67). This resulted in a collection of confocal stacks from which cell shapes and proliferation patterns can be determined during the first day of trabeculation.

To enable quantitative comparison between the in silico simulated morphology to the in vivo observations tools were created to automatically segment single cells and measure cell shape and proliferative properties within those cells.

4) Future collaboration with host institution (if applicable)

The project will be continued in collaboration with the host institution. A setup will be created to systematically test a large range of model variables. This setup will be used to simulate loss- and gain-of-function mutations. The results will be compared to transgenic mouse models.

Skype meetings are held on a regular basis to discuss progress.

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)***

A computational model of cardiac trabeculation, de Boer et al, to be prepared

6) Other comments (if any)