QuanTissue Short-Visit Scientific Report

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Project outline

The aim of this project is to understand how local cell behaviors can generate the observed tissue level morphogenesis of limb bud development. Over the last few years many mathematical models have been proposed to address this question [1,2,3,4], but so far, none generated the right organ shape changes once realistic 3D initial conditions are used.

Our project (initiated during the last QuanTissue Symposium in Barcelona) is to combine the experimental data gathered by James Shape's PhD student, Gaja Lesnicar-Pucko, with a new mathematical model of limb-bud growth using CompuCell3D [5,6,7], a Glazier-Graner-Hogweg (GGH) [8,9] modeling environment developed by James Glazier's lab (Indiana University, USA). The new model will have realistic 3D initial conditions and will make use of the newly quantified cell behaviors of mesenchymal limb bud cells. The model will tell us if the spatial and temporal distributions of measured rates of cell growth (with correct division planes) and intercalation suffice to generate observed limb bud elongation. If successful, the model could either confirm the proposed mechanisms or suggest what additional ones are needed.

Purpose of the visit

Since many processes take place simultaneously at different regions of the limb bud, a close interaction between modelers and experimentalists is essential for the construction of a detailed and realistic model of this system. For this purpose, James Glazier's PhD student, Julio Monti Belmonte, was sent to Barcelona to start the development of a 3D mathematical model of the limb bud with James Sharpe and PhD student Gaja Lesnicar-Pucko, who conducted all the experimental work related to this project.

Description of the work carried out during the visit

During the visit both students worked closely together to build an elaborated 3D limb bud model. The first task was the creation of a suitable initial condition for the simulation. So far, most computer simulations of the limb are done in 2D dimensions and start from simplistic geometrical initial conditions (an exception to the rule is the latest limb bud paper from James Sharpe's lab [4]). To avoid any oversimplification and introduction of undesirable artifacts we used 3D experimental data of the limb bud at stage HH21 to create a realistic initial condition for the simulation (see Figure 1). A picture of the limb bud at stage HH24 was also converted into a simulation file for purpose of final shape comparison.

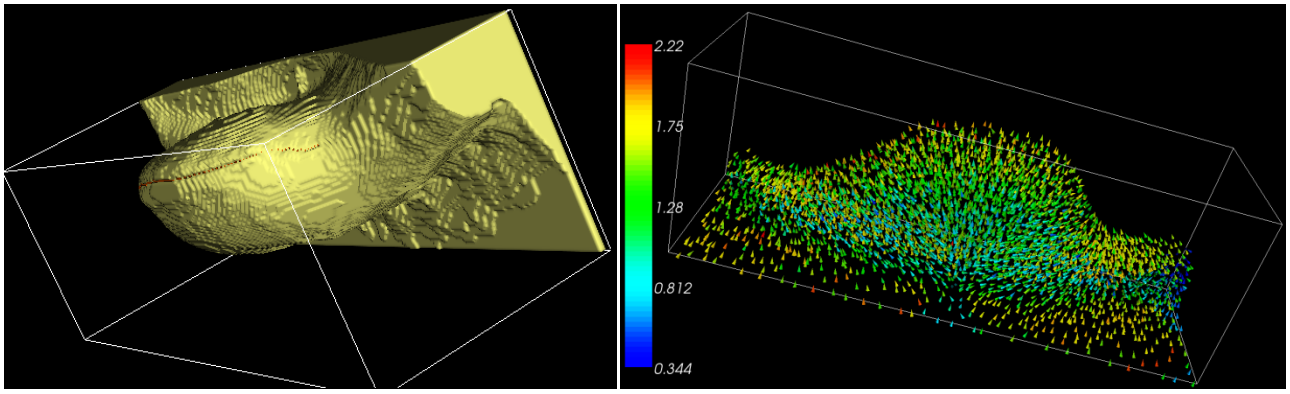

Figure 1: *Limb bud initial condition obtained from experiments (stage HH21). Left: solid volume rendering of the limb bud with the AER position indicated by the thin red line. Right: vector field showing the orientation of each cell.*

The next step was the discussion of which cell behaviors should be included in the model and what would be the best way to do it. Some cell types, like the enveloping ectoderm, were deemed non-critical and implemented with decreased level of detail, while mesoderm was defined more precisely. The total number of mesenchymal cells present in the limb bud at this stage (about 400,000) is too big to be simulated, so we decided to reduce the total number of cells by a factor of 100 for computational feasibility.

Different mechanisms and implementations of convergent-extension were also discussed and tested before being added to the model. We chose to model cell intercalation as the result of pulling forces between cells rather than surface tension minimization since the first is more compatible with the observed cell morphology. Although convergent-extension methods have already been developed for the GGH model [10,11] and other mathematical models as well [12,13], there are not any available implementations for 3D using pulling forces, so a new one had to be developed for this specific study. A quick sensitivity analysis of the method was performed to check how fast the cells intercalate as its parameters are varied (Figure 2, left) and what effects different distribution of intercalation orientations have on a given piece of tissue (Figure 2, right).

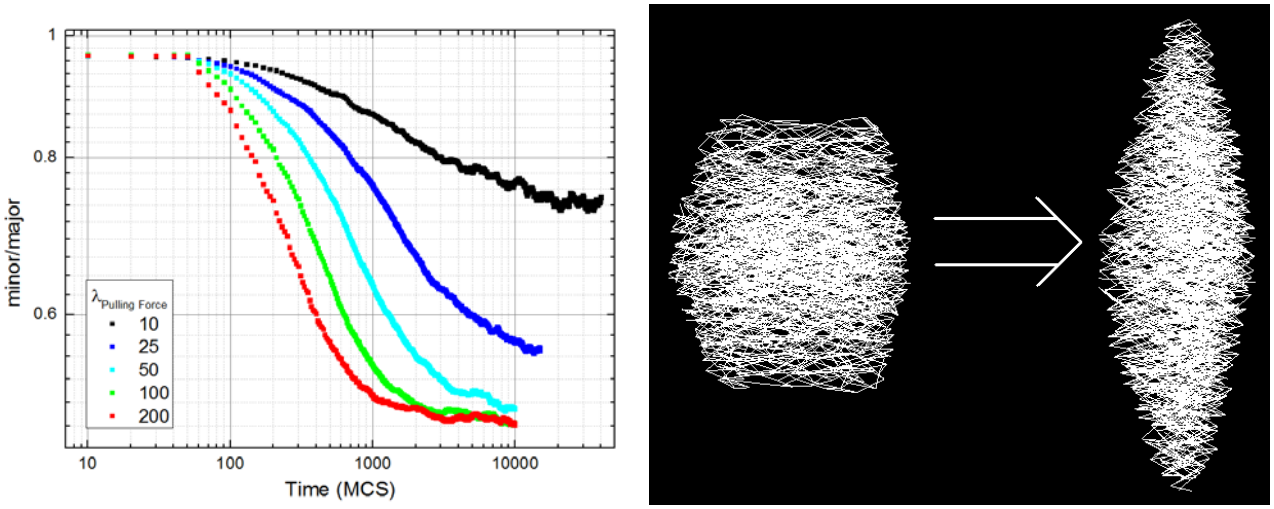

Figure 2: *3D convergent-extension model developed for the limb bud project. Left: sensitivity analysis on the strength of pulling forces between cells. Right: effect of a given set of intercalation orientation distribution on the morphology of an initially cube-shaped tissue.*

During the second week preliminary versions of the model were run and its results analyzed. Some cell behaviors, such as the orientation of the division planes and the simulation of diffusible signals, were found to be either irrelevant or unnecessary for our present purposes and were discarded. The limb bud regions where the cells divide and intercalate were also adjusted based on these preliminary results.

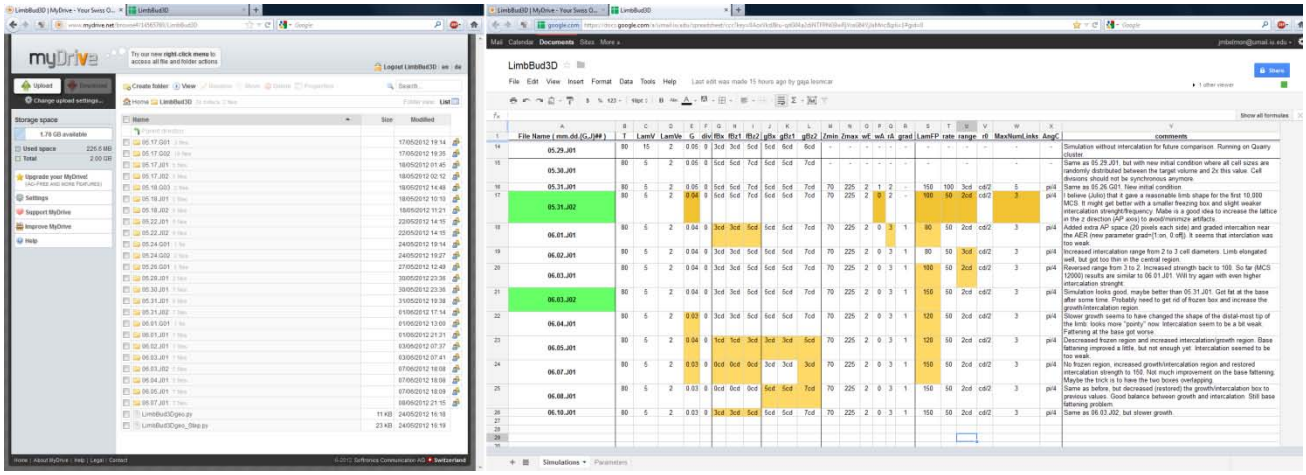

Figure 3: *File sharing system designed for tracking and discussion of simulation results. Left: deposit of all simulation files on MyDrive website. Right: Google spreadsheet with detailed description of all simulations.*

At the end of the visit an online file sharing system was set up so that the collaboration could be carried on. An account was created on the website www.mydrive.net to host the files and results (code, snapshots and videos) of all the simulations conducted during and after the visit (Figure 3, left). A shared Google spreadsheet (Figure 3, right) was also created with a detailed description of each simulation, including a list of all parameters values used and comments on the results.

Description of the main results obtained

The mathematical model showed that growth mechanisms alone, when uniformly distributed along the limb in accordance to the experimental data, fail to give the right progression of the limb bud from stage HH21 to HH24, or even to stage HH22. Even an arbitrarily division plane set to be always perpendicular to the proximal-distal axis fails to give the correct limb bud shape elongation (Figure 4, left). Furthermore, measured division planes were found to be mostly aligned in directions perpendicular to the proximal-distal axis. Together, these experimental and computational results discard the orientation of division planes as a possible correcting mechanism and confirm that some additional processes must be taking place during limb development to keep it in the correct shape.

Our results showed that addition of cell intercalation prevents the simulated growing limb from turning into a blob and helps to maintain it in an elongated shape, *i.e.*, flat in the dorso-ventral axis while elongated along the proximal-distal axis (Figure 4, right). The implementation of this new mechanism, however, is not trivial: it requires a good balance between the cells growth rate and the spatial distribution of intercalation strength and orientation.

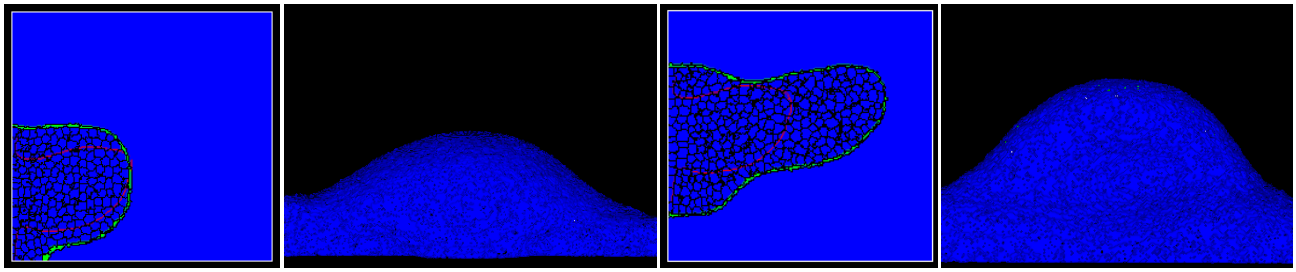

Figure 4: *Simulation snapshots (anterior-posterior cross sections and 3D dorsal views) showing limb bud growth with only proliferation (left) and with the addition of intercalation (right). On the cross sections, the red line indicates the limb bud initial shape.*

We are currently exploring the effects of variations in these parameters to get the optimal set of intercalation strength and orientation distributions. Specific perturbations of the convergent-extension movements and the resulting phenotypes will later be compared with the data. While the regulating mechanisms that set the distribution of cell orientations and pulling strengths are still unknown, we are confident that active cell intercalation is a key mechanism needed to fully explain limb bud elongation.

Projected publications and future collaboration with host institution

We are currently preparing a manuscript to be submitted for publication in a high impact journal that will contain the main results described in this report as well as supporting experimental measurements. We hope to keep the collaboration between our two groups for the near future so that we can further develop this model and possibly extend its scope for earlier and later stages of limb development.

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