Long-term objectives The long-term goal of my research is to uncover the mechanisms by which the macroscopic shape and organization of organisms arise from biochemistry and mechanics at the cellular scale. Cytoskeletal structures (actin, microtubules (MTs) and other components) provide cells with mechanical strength and influence the orientation of cell division and cell growth thereby influencing the shape and symmetries that emerge in a developing organism.

Two model systems for the development of macroscopic morphology from cellular dynamics form the core of my research program, each facilitated by collaborations with experimental labs at UBC: (A) left-right symmetry breaking in the nematode *C. elegans* early embryo and (B) the control of root growth in a member of the mustard plant family *A. thaliana*.

The proposed research requires expertise in the development of custom-built image processing tools, the translation of proposed biophysical and biochemical mechanisms into mathematical and computational models, and the analysis of model and data to draw conclusions and point toward new opportunities for experimental exploration. Previous work of mine demonstrating this expertise includes the stability of pathological dynamics in cardiac tissue [25, 27], the spatial organization and forces in eukaryotic [2, 4, 6] and bacterial [8, 10, 16, 31] cell division, and spontaneous organization of microtubules in centrosome-free contexts in an *in vitro* animal context [3, 5] and an *in vivo* plant context [12–14, 24, 32]. This previous work and the research proposed here is intrinsically cross disciplinary and requires intense and ongoing interaction between myself, my trainees and our collaborators.

A. Left-right asymmetry (chirality) in *C. elegans*

Chirality is one of the fundamental molecular and cellular properties that control animal morphogenesis, particularly the induction of larger scale organismal handedness. For example, many human organs are asymmetric along the left-right body axis. Incorrect specification of organ asymmetry – heterotaxia – can be associated with cardiac disease, aspleenia, malrotation and other pathologies.

In *C. elegans*, body handedness is specified during early embryonic cell division [28] but the mechanism by which cell division drives chiral morphogenesis remains elusive. In collaboration with the Sugioka Lab at UBC, we will use a combination of microscopy, image analysis, and mathematical and computational models to understand the earliest stages of left-right symmetry breaking in the *C. elegans* embryo. The Sugioka lab has identified the contractile ring and associated cortical flow (flow of cytoskeletal material near the cell membrane) as a critical player in the process (see Fig. 1).

This part of my research program is broken down into three projects described below, starting with (A.1) image-data analysis to determine what is happening in the embryo followed by two stages of modelling to understand (A.2) how the observed chiral cortical flow leads to multi-cellular and eventually organ chirality and (A.3) how chiral cortical flow arises from actin's molecular chirality.

Objective A.1 Develop automated software to track in 4D the position of the cytokinetic ring.

The Sugioka Lab has time series of fluorescent-image z-stacks of the early embryo including markers of actomyosin structures and the mitotic spindle. Although a qualitative kinematic description is easily extracted from such data, inferring anything about the forces acting between different structures within and between cells requires a quantitative analysis. We have developed an application that is capable of identifying the location of the contractile ring by fitting an ellipse (embedded in 3D) to the myosin signal at each z-stack of the time series. Identifying and tracking multiple rings in the z-stack is the next goal, followed by spindle and cell boundary tracking. Ideally, we will be able to identify and track cell structures through several rounds of cell division. We plan to develop a GUI for this tool which could be used for cytokinesis studies more generally.

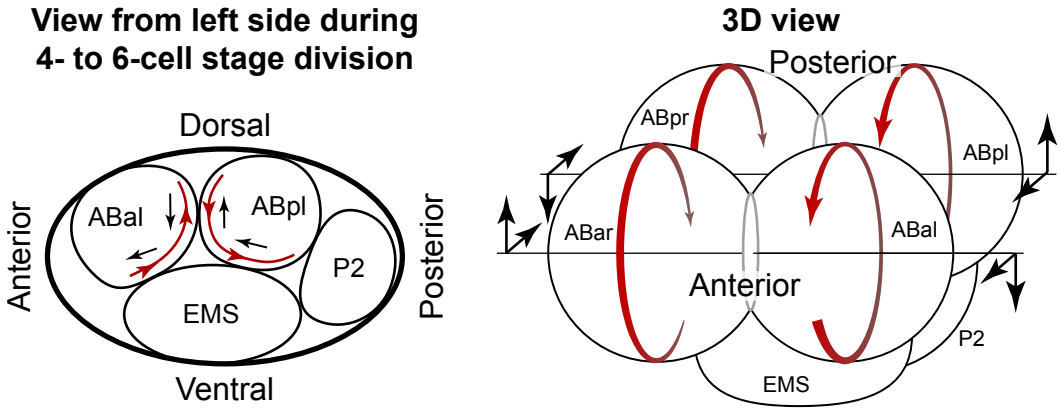

Figure 1: *C. elegans* 4-cell-stage embryos undergo asymmetric cell divisions that result in chiral blastomere arrangement. Left panel: Looking at the embryo from the left side, what will become the ABal and ABpl cells show a burst of counterclockwise cortical flow (red arrows) during division ring constriction. Where these cells contact each other and the EMS cell, transient adhesions form between cells and create friction forces, like tractor treads. The cells' treads move posteriorly relative to the EMS cell translating into an anterior force (black arrow) on both. The treads also move in opposite directions at their contact points, forcing the ABal cell down and the ABpl cell up. Right panel: 3D view of the same phenomenon. Red arrows correspond to measured cortical flow in AB cells. Black arrows on the division axis show the resulting friction forces. Left-side cells' movement is as in the left panel. Both right cells move posteriorly with ABar moving up and ABpr moving down. The orientation of the cells in response to the forces leads to a helical (chiral) configuration.

Objective A.2 Modelling transduction of chirality from intra-cellular (cortical flow) to tissue scale

Once the complete geometry of the early embryo's division machinery is available, we can analyze the movement of the parts to infer the direction and amplitude of the forces at work. Preliminary data, showing chiral cortical flow in the cells that initiate symmetry breaking at the multi-cellular scale, has allowed us to build a preliminary model (Model A.2.1) using force balance equations conceptually similar to our previous work [2, 4]. The model consists of four nodes (ABal, ABar, ABpl, ABpr) each connected by springs to their two neighbours and the EMS cell. Cortical flow (taken from data) induces friction forces at contact points. We solved the differential equations and compared the angles of ABar-ABal and ABpr-ABpl axes from dorsal and anterior views in the data and model solutions finding good agreement (see Fig. 2). As predicted by the forces in Fig. 1 (black arrows), both dorsal-view angles decrease from $\pi/2$ (right sides move posteriorly, left sides move anteriorly) and anterior-view angles go in opposite directions from zero. We had to include forces from a fixed P2 cell in order to get (just barely) the observed tilt difference between ABa and ABp cells (ABa tilts more than ABp). Although this preliminary model recapitulates the angles well enough, some of the geometric details are unrealistic (e.g. the position of the P2 cell).

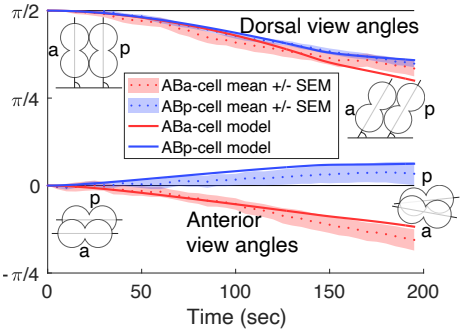

Figure 2

Recent experiments offer a simpler geometry for which Model A.2.1 may be more appropriate. In a series of *ex vivo* experiments, the Sugioka Lab removed the dividing ABa cell from the embryo

and placed it in contact with an adhesive bead. Several forms of rotation were observed that were consistent with the idea of friction forces between the cell and the bead driven by cortical rotation and adhesions. In summer of Year 1, I will recruit an undergraduate student to implement a three-node version of Model A.2.1 (ABal, ABar, bead) and compare the model solutions to the measured motion of the cell and bead.

For the *in vivo* context, there are several extensions of this model that we will explore. The first is a direct extension (Model A.2.2) which will include dynamic nodes for the EMS and P2 cell and also constrain cell movement to the interior of the ellipsoidal egg shell. Furthermore, outward forces associated with cytokinesis will also be added along the division axes. This extension can also be adapted to apply to subsequent stages of embryo development provided the orientation of the mitotic spindles of successive generations of cells can be measured and fed into the model (Model A.2.3). We will also try an alternative modelling approach by introducing forces into the phase-field model developed in [30].

Objective A.3 Modelling transduction of chirality from molecular to intra-cellular scale

Previous studies have explored the determinants of cellular chirality but these have been relatively abstract [17, 19], or have stopped short of proposing specific molecular mechanisms [20]. I propose the following mechanism and will develop a series of models to test it. At the molecular scale, shown in Fig. 3, cortical actin filaments (red) interact with each other through force-generating myosin motors (green). A motor complex sliding two filaments past each other (left pair) can induce torque in the filaments [1, 11] causing them to twist around each other (right pair).

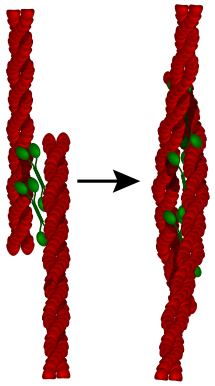

Figure 3

At low density or low motor activity or short actin lengths, this twisting might not lead to anisotropic organization but at high enough densities and sliding forces, specifically in the contractile ring, twisting filaments could wrap around each other to form a twisted rope-like structure with a consistent chirality induced by the actin filament chirality, as shown in Fig. 4. When positioned near a surface, the twisted rope would have filaments reaching out in an anisotropic configuration with interaction with the surrounding cortex being biased so as to induce chiral forces on it. These chiral forces could cause the observed chiral flow.

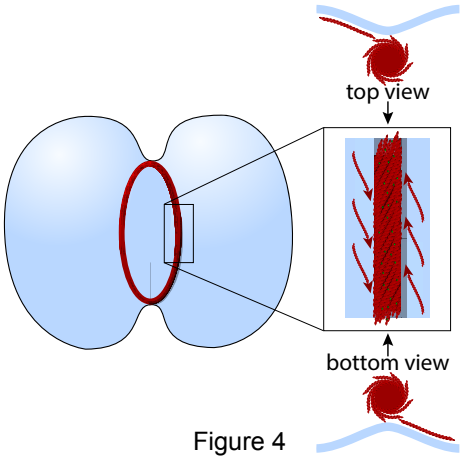

Figure 4

Recent work on interacting actin filaments [29] generalized previous software [22] to allow for twisting of filaments using the Cosserat theory of elastic rods. This approach offers a viable method for simulating the behaviour of large collections of actin filaments. It would allow us to test the hypothesis including exploring the influence of formin, myosin density/activity, actin cross-linkers and other biochemical factors on division ring organization and induction of chiral flow.

The precise details of how this mechanism might work are yet to be ellucidated and we also plan to explore in parallel ideas proposed by others, for example that invoke the torque generated by formin on growing filaments [15]. These modelling efforts will inevitably raise questions and provide predictions (e.g. dependence of cortical flow speeds on myosin activity, average actin filament length etc.) to motivate new experiments.

B. Hormonal and cytoskeletal regulation of plant growth

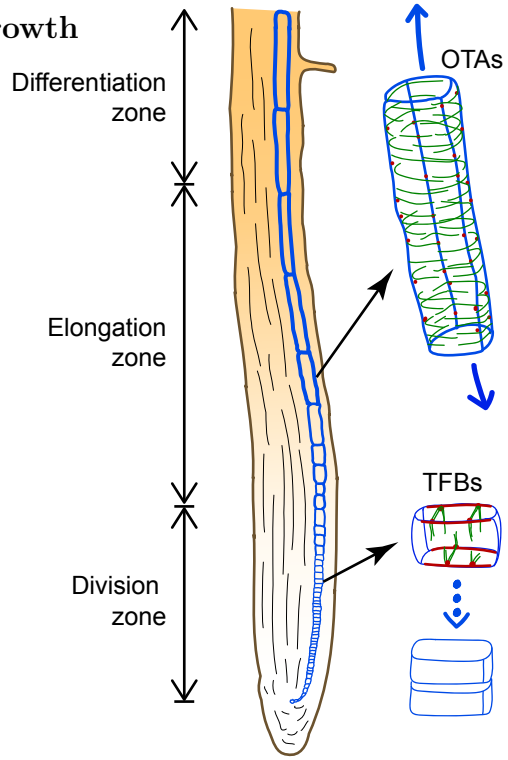

Figure 5 - modified from [20]

Plant development, especially root and stem growth, requires directional growth of cells. When tension-bearing cell wall fibers are deposited anisotropically, the weaker lateral bonds between fibers facilitate expansion transverse to the fibers. Dynamic cortically-anchored microtubules (MTs) spontaneously form into organized transverse arrays (OTAs) and facilitate such deposition. The importance of this process is demonstrated in experiments where the perturbation of MT organization results in misshapen organs and/or stunted growth [7].

The growing portion of a plant's root can be divided into three zones, from tip upward: the cell division zone, the elongation zone, and the differentiation zone (see Fig. 5). Cells in the division zone have high levels of the protein CLASP (red) which allows cortical MTs (green) to cross sharp transverse cell edges and form transfacial bundles (TFBs). TFBs suppress the formation of OTAs and promote rapid division cycles creating many small cells. In the elongation zone, CLASP levels are lower and OTAs are able to form; cell division ceases and cells undergo rapid elongation. The establishment of these zones is controlled by the hormones auxin (purple in Fig. 6), brassinosteroids (BR, orange), and cytokinins coupled to intracellular signaling networks. Through hormones, a plant can respond to environmental cues, such as low light or low water, by modulating the size of the root zones and thereby regulating root and stem growth appropriately.

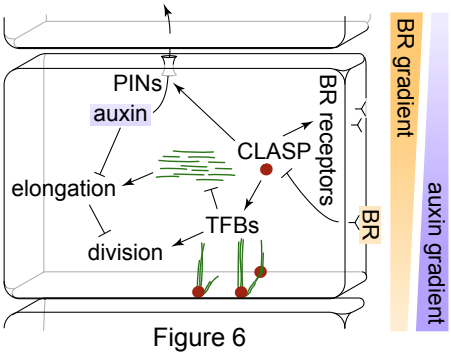

Figure 6

The goal of this part of my research program, in collaboration with the Wasteneys Lab at UBC, is to develop and test models to understand how this regulation works. The component of this regulatory network consisting of CLASP, TFBs and OTAs was the focus of previous work from our collaboration [12–14]. Fig. 6 shows a single cell in the context of the hormone gradients. It illustrates how the BR and auxin gradients influence switching between division and elongation [23]. Near the root tip, BR is low, releasing suppression of CLASP, thereby allowing CLASP to promote the formation of TFBs and thus cell cycle. CLASP also sustains the activity of PIN2, one of several auxin efflux carriers (PINs), and thus auxin transport, which plays a role in auxin gradient maintenance [18]. As a cell moves from the division zone to the elongation zone, BR increases, CLASP is suppressed and TFBs give way to OTAs, which promote elongation. Importantly, "move" here is in a relative sense because cells are dividing and elongating, which changes the position of any specific cell relative to the root tip and also the regulation of the hormone gradients that track the growing root tip.

Objective B.1 Modelling the establishment and responsiveness of root zones

In several stages, we will build a model for the growth of the root tip including the formation of the zones through the spatial and intracellular dynamics of hormones, CLASP, and PINS. The diagram in Fig. 6 shows the relevant interactions including several complex spatial phenomena

(OTAs, TFBs, cell division and elongation) abstracted as nodes. The latter will be treated as black boxes with behaviour based on previous modelling [14] and what we learn from Objective B.2.

Our first step is to build a model of a static root consisting of several columns of fixed discrete cells, each of a specified length, laid out in sequence (like the blue-outlined cells in Fig. 5) and a prescribed PIN distribution. As in [9], the model would simulate auxin flux through PINs with the goal of generating the observed auxin distribution. Adding a layer of complexity, the next step is to implement dynamics in each cell for levels of BR receptors, CLASP, PINs, and a continuous variable ϕ varying from 0 for an TFB/division state to 1 for a OTA/elongation state to capture the MT state in a highly simplified way. The goal at this stage is to have a model that can establish division and elongation zones in response to hormone profiles while still dynamically maintaining those profiles. The final model-building step is to have ϕ determine whether individual cells divide and how quickly they grow. This adds a time-dependence to the domain turning the question into a kind of discrete travelling front existence problem.

We will calibrate parameters using data on the cell size distribution along the root, growth rate, and hormone profiles. Similar data from experiments with mutants (lacking CLASP, a BR-insensitive CLASP, or lacking the BR receptor), light/dark growth conditions, and application of BR will allow us to test and refine the model. The model will provide insight into knowledge gaps and suggest new experiments that can extend our understanding of the regulation of growth.

Objective B.2 Modelling OTA formation and maintenance in elongating cells

There are still elements of the MT array organization that are not fully understood. Previous modelling of array formation showed that oriented arrays can form at a random angle via interactions between cortically constrained MTs. In a cylindrical cell with sharp edges at top and bottom, without CLASP at those edges to promote growth around them, the arrays form transverse to the cylinder axis (OTAs). However, these OTAs are local to the edges and the model predicts that in long cells, ordering fails toward the middle. It has been proposed that MTs sense tension in the cell wall, which allows the arrays to be maintained even in long cells [26]. We plan to test this hypothesis using both modelling and experiment (e.g. quantifying MT catastrophe rates as a function of orientation) as this model shortcoming poses a significant gap in our understanding of OTA maintenance throughout elongation. There are also inconsistencies between models published by different groups that PhD student Tian is currently addressing using a new computational platform for MT organization that allows more direct comparison of previous modelling assumptions.

Impact and feasibility

The modelling proposed here, blending experimental knowledge with theoretical biochemistry, biophysics and mathematics, is at the cutting edge and provides an excellent training tool for highly interdisciplinary scientists in my own and my collaborator's groups. Beyond the scientific advancement, impacts include an image analysis tool that will be shared publicly, as well as all the modelling code, especially the general OTA modelling platform, a powerful tool for reproducibility. The molecular chirality project is particular exciting because it proposes a completely new solution to a fundamental question with few existing hypotheses that can be readily tested in the lab. The other projects offer a solid theoretical grounding for existing but very new hypotheses.

The proposed work requires a broad set of skills and knowledge, many of which were central to previous work of mine. To complement my own expertise, I have established co-supervision plans with local experts Colin Macdonald (scientific computing), Miranda Cerfon-Holmes (molecular modelling), and James Feng (cellular mechanics) in addition to the mentioned lab collaborations.