Thesis Outline

Chapter 1 - Introduction and overview of pulsed contractility

The purpose of this chapter is to provide an overview of actomyosin-dependent cell shape changes in general, pulsatile cell shape changes in particular, to highlight some of my research questions, my approach, and the purpose of my thesis.

Section 1: Introduction to cell shape changes

1. Cell shape changes are important in a number of contexts such as cell motility, cytokinesis, polarization, and tissue morphogenesis \cite{Lecuit:2011ec, Heisenberg:2013jk}
2. Cellular-level shape changes are also used at the tissue level for processes such as bending, extension, and compression.
    a. Coordinated constriction of epithelial cells bending of epithelial sheets
    b. Cell intercalation drives tissue narrowing or elongation
    c. Collective cell migration
3. In addition to genetic and biochemical regulation, cell and tissue shape changes are regulated by mechanics \cite{Heisenberg:2013jk}
    a. Force generation is important
    b. Need adhesion to transmit forces between neighboring cells within tissues
    c. How the forces are integrated within the tissue
4. Forces are generated by cortical actin networks, which consists of meshworks of actin filaments, myosin mini-filaments, and cross-linking proteins\cite{REF}. Unlike in muscle cells, all of these components are constantly turning over and being redistributed. This allows cells to quickly reorganize cortical actomyosin

networks in response to chemical stimuli, as well as internal or external stresses \cite{REF}.

5. Cells can re-organize cortical actomyosin networks into cytokinetic rings, which divide cells; into lamellar networks that propel migrating cells forward;
6. Cell shape changes are accomplished when Force Generation can do things like split cells into two, rearrange cells within tissues, cardiac cells, re-distribute polarity factors
7. It is important to understand the interplay between biochemical signalling and patterning of actomyosin-dependent force generation in space and time. The purpose of this thesis is to understand

Section 2: Overview of the molecular details of actomyosin assembly and force generation

Section 2a :Molecular details of myosin structure and regulation

1. Non-muscle Myosin II is a hexamer. It is composed two heavy chains, two regulatory light chains, two essential light chain \cite{VicenteManzanares:2009ik}
2. Myosin is activated by Rho Kinase, and inactivated by Myosin phosphatase
3. Upon activation, myosin II Can assemble into mini-filaments
4. It's activity is thus determined by the relative balance of kinase and phosphatase activity

Section 2b: Assembly, organization and regulation of actin networks

1. Actin is a polymer that is polymerized by formins and arp-2/3
2. RhoA stimulates actin polymerization

3. Various factors regulate the stability and turnover of actin filaments
4. For example, myosin II might play a role in stabilizing actin filaments
5. Actin turnover

Section 2c: Force generation

1. Force generation occurs when myosin mini-filaments slide anti-parallel actin filaments.
2. However, force generation also depends on the architecture of the network and concentration of cross-linking proteins
3. Actomyosin based contractility can also be self-regulating, as flows can transport regulators that regulate the network \cite{Munro:2004jk}

Section 3: Overview of pulsed actomyosin contractility

Section 3a: Pulsed contractions are an important mode of contractility

1. Pulsed contractions are characterized by cycles of actomyosin assembly, contraction which leads to local deformation of the cell surface, and disassembly
2. Pulsed contractions occur in many different contexts, and can drive important morphogenetic events such as:
    a. Ventral Furrow formation, which is the invagination of the presumptive mesoderm in *Drosophila* \cite{REF}
    b. Dorsal closure in Drosophila
    c. Germband extension in Drosophila
    d. convergent extension in Xenopus \cite{REF}

e. compaction of the early mouse embryo \cite{REF}.

f. Cell shape oscillations in epithelial cells

Section 3b: Much of what we know about the importance of pulsed contractions comes from studies Drosophila

1. Although pulsed contractions were first observed in C. elegans, much of what we know about pulsed contractions has come from studies in Drosophila.
2. Apical constriction \cite{Martin}
3. Describe details of germband elongation
4. Describe the details of dorsal closure

Section 4: Models of Pulsed contractility

Pulsed contractions likely represent an excitable system. The key features of an excitable system are fast positive feedback and delayed negative feedback. This is exemplified by action potentials, calcium waves, actin waves, etc.

Section 4a: Contractile Instability

1. Contractile instability \cite{REF}
2. The positive feedback
3. The negative feedback

Section 4b: Clustering model

1. This model was proposed by Amy Maddox
2. Anillin binds to the membrane and recruits F-actin and Myosin II
3. The positive feedback occurs when local recruitment of anillin recruits actin and myosin, which bind more anillin

4. The negative feedback could occur

Section 5: Overview of my work

We know a lot about where pulsed contractions occur, the molecular components, etc, but we still don't understanding how they are initiated or terminated.

Section 5a: My approach

1. 2 color imaging
2. Genetics and pharmacological perturbations
3. Mathematical modeling (collaboration)

Section 5b: C. elegans as a model system

1. C.elegans is nice model system for many reasons including: large single cells that make it easy to do high resolution imaging, amenable to genetic and pharmacological perturbations, etc
2. The key players include RHoA, ECT-2, NOP-1, RGA3 and RGA-4, Actin, CYK-1,NMY-2, LET-502 and MEL-11, Anillin

Section 5c: The significance of this work

1. To identify the core circuit for generating pulsed contractions
2. To provide mechanistic insight into the initiation and termination of pulsed contractions
3. To show how tuning different factors affects pulse dynamics
4. To propose a new framework for pulsed contractility that spatially integrates biochemical signalling and mechanical feedback

5. To suggest that the core biochemical-mechanical feedback may be general by comparing it to other phenomena such as wound healing

Chapter 2 - Paper

Chapter 3 - Perturbation of RhoA effectors affects rho pulse dynamics

Section 1: Introduction

Previous results suggest that excitable activation of RhoA is the main driver of pulsed contractions independent of myosin or contractility. However, it is unclear whether/how factors downstream of RhoA might feedback to regulate RhoA pulsing or contraction dynamics. The purpose of this chapter is to provide evidence that factors downstream of RhoA play a role in regulating the size and spacing of RhoA pulses. Furthermore, I aim to explain these observations in the context of the conceptual framework established in chapter 2.

Section 2: Tuning F-actin dynamics regulates the size of RhoA pulses

Section 2a: Background

1. Previous work provided evidence that the RhoA GAPs RGA-3 and RGA-4 are likely candidates for providing negative feedback onto RhoA \cite{MichauxXXX}. We have shown that GFP::RGA-3 accumulates with a delay relative to RhoA, and that depletion of RGA-3 and RGA-4, RhoA no longer pulses\cite{MichauxXXX, FigureXXX}.
2. We have also shown that GFP::RGA-3 co-localizes with mCherry::LifeAct on actin filaments, which suggests that negative feedback from RGA-3 goes through

actin. Indeed, depolymerizing all of the actin also eliminated RhoA pulses. This has been observed in other systems as well \cite{Bement:2015jp}.

3. The presence of F-actin on the entire cortex suggests that RGA-3/4 could play a role as a global inhibitor, preventing accumulation of RhoA on regions of the cortex containing many actin filaments. This suggests that tears in the cortex should lead to pulses of RhoA. Indeed, there seems to be anecdotal evidence to support this {FigureXXX}. To test these ideas, I artificially exaggerated cortical tearing using RNAi against cyk-1 and pfn-1 RNAi.

Section 2b: Formin depletion affects size of RhoA pulses but not kinetics

1. CYK-1 is a formin \cite{REF}
2. CYK-1 appears to decorate actin filaments and co-accumulate with Myosin II in the early C. elegans embryo {Younan Li, personal communication, data not shown}
3. Depletion of cyk-1 by RNAi does not appear to affect RhoA pulse kinetics.
4. Pulses appear larger

Section 2c: Profilin depletion affects size of RhoA pulses (have not completed any of this)

1. Measure size of pulses
2. Measure kinetics
3. Use PIV to measure dilation vs accumulation in NMY-2::GFP;spd-5(ts)
4. Align

Section 2d: Stabilization of F-actin causes decrease in RhoA pulses

1. Depleting profilin or cyk-1 increased the size of RhoA pulses.
2. Our model would hypothesize that stabilizing actin filaments should decrease the size and/or frequency of RhoA pulses.
3. To test this, I will stabilize actin filaments with the drug Jasplakinolide, or by depleting severing by cofilin (unc-60 RNAi).

Section 3: RhoA pulses are bigger in the absence of Rho kinase

Section 3a: Background

1. Myosin II mini-filament assembly and activation is regulated by phosphorylation of its regulatory light chain by Rho kinase \cite{REF}. Recent work In Drosophila has shown that regulation of Myosin's phosphorylation state regulates medial actomyosin oscillatory activity. More specifically, recent work by Lecuit and colleagues has demonstrated that depletion of Rok eliminates the recruitment of the RhoA biosensor to the cortex.
2. It is likely that Rho kinase binds directly to RhoA \cite {REF}
3. Therefore, it is possible that through direct binding to RhoA, or indirect interactions with other factors, that Rho kinase regulates either the initiation or termination of pulsed contractions.
4. To test this, I will first look at the dynamics of GFP::LET-502 within pulsed contractions. Next, I will quantify RhoA pulse dynamics in the absence of LET-502.

3b: LET-502 co-accumulates downstream of RhoA

1. Using near-TIRF microscopy, I observed that RhoA and LET-502 have similar localization patterns.
2. Furthermore, LET-502 failed to pulse in rho-1 RNAi embryos. This suggests that LET-502 accumulation is RhoA-dependent.
3. Like the RhoA biosensor, GFP::LET-502 co-accumulates with Myosin II within pulsed contractions in P0 and AB.
4. As described in chapter 2, I determined the timing of GFP::LET-502 relative to NMY-2::RFP. I observed that….

3c: Strong depletion of LET-502 does not eliminate RhoA pulses

1. RhoA continues to pulse under conditions in which LET-502 has been strongly depleted, while Myosin II levels remained low.
2. RhoA pulse kinetics in let-502 embryos appear to be similar to those in wild-type embryos
3. RhoA pulses appear to be much larger in let-502 RNAi embryos
4. RhoA pulses in let-502 RNAi embryos appear to have a larger propensity for forming traveling waves.
5. Similar experiments were performed in the presence of the Rho kinase inhibitors H1152 and Y-27632. In both drug treatments, RhoA activation was uniformly high everywhere on the cortex.
6. These observations suggest that Rho kinase plays a role in limiting the spread of RhoA.

<u>Section 4: Anillin regulates the pulsed contractions, possibly by stabilizing actin and Myosin</u>

Section 4a: Background

1. Anillin is a multivalent scaffold that was first identified as an actin bundling protein in Drosophila \cite{REF}. Anillin has also been shown to be a key regulator of cytokinesis, where it is thought to stabilize the cytokinetic furrow \cite{REF}.
2. There are three anillin isoforms in C.elegans. ANI-1 is thought to bind Myosin II, F-actin, and active RhoA.
3. Maddox et al showed that anillin is a key regulator of contractility in the early C. elegans embryo \cite{Maddox et al}. By strongly decreasing anillin, they observed a decrease in membrane ruffling (ie pulsed contractility).
4. They also observed a decrease in Myosin II accumulation when ANI-1 was strongly depleted.
5. Maddox and colleagues hypothesized that ANI-1 was the main driver of pulsed contractions in C. elegans.

Section 4b: RhoA continues to pulse in the presence of intermediate levels of Anillin.

1. Using an RNAi feeding strain (an-1 (myo)) targeting the Myosin-binding domain of ANI-1, I observed that the RhoA biosensor and Myosin II continued to co-accumulate in pulsed contractions.
2. Quantification shows that RhoA pulses were shorter and larger than wild-type pulses.
3. However, the caveat with this experiment is that it is very hard to get a complete knockdown of anillin using the feeding strain that targets the Myosin-binding domain.

Section 4c: LET-502 continues to pulse even when Anillin has been strongly depleted

1. To get around the ani-1 (myo) incomplete RNAi phenotype, I treated worm with an RNAi feeding strain that targeted the full length anillin.
2. Since LET-502 and RhoA have similar dynamics and accumulation patterns, I used GFP::LET-502 as a proxy for RhoA activation.
3. As expected, GFP::LET-502 pulses increased in size in the absence of anillin.
4. These results suggest that Anillin is not required for the initiation or termination of RhoA pulses.

Section 4d: Measuring Actin turnover in the absence of Anillin

1. Although Anillin doesn't regulate the initiation or termination of RhoA pulses, it does appear that Anillin regulates pulsed contractions in other ways.
2. I hypothesize that Anillin stabilizes the accumulation of F-actin and Myosin within pulsed contractions
3. To test this, I will use single-molecule measurements to determine the turnover of actin filaments during individual pulsed contractions.

Chapter 4 - Conclusions and future directions

<u>Section 1: Summarize my results</u>

1. Pulsed accumulation of RhoA drives pulsed contractions in early C. elegans embryos
2. A contractile instability model can't explain pulse initiation in C.elegans
3. Myosin is not required for pulse RhoA pulses

4. Depletion of the GAPS RGA-3 and RGA-4, or F-actin, eliminates RhoA pulses
5. Depleting factors downstream of RhoA affects the size and spacing of RhoA pulses

Section 2: Future Directions for uncovering further mechanistic details of pulsed contractions

1. One question moving forward is: How does an embryo tune various factors to modify pulsatile dynamics for specific purposes.
2. Patterning of active RhoA does not appear to be independent of its regulators and effectors. Therefore a mechanism that takes into accounts the coupling between biochemical signalling and regulation and mechanics needs to be considered.
3. A good starting point would be to build an active fluids model to explore the similarities and differences between pulsed contractions in different contexts in C.elegans and Drosophila.
4. Sort out the roles of RGA-3 and RGA-4 in regulating spatial patterning of RhoA pulses. For example, how are the GAPs activated? Does binding to F-actin activate the GAPs? This would also include creating strains that express GFP-tagged fusion proteins containing the GAP domain and various membrane/F-actin binding domains?
5. RhoA activation is autocatalytic. One would expect that RhoA would form traveling waves. Further experiments should explore the extent to which factors downstream or upstream of RhoA limit RhoA's spread.

Section 3: Speculation about the generality of the underlying mechanism

1. Active rho is the key driver for pulsed contractions in C. elegans and other organisms.
2. Blebbing, wound healing, cytokinesis, tissue morphogenesis, spermatheca are also regulated by spatial regulation of RhoA activation.
3. Is there some special coupling between RhoA activation and F-actin dynamics that generate robust spatial patterns?