On contractile actomyosin waves and their role in junctional remodeling during epithelial constriction

Angughali Aheto Sumi

Thesi Doctoral UPF / 2016

Director Thesi

Jérôme Solon

Departament Cell and Developmental Biolgy

Universitat Pompeu Fabra Barcelona

Contents

Abstract (English)

Epithelial tissues undergo extensive remodeling during embryonic development. Recent studies have revealed that, in a number of developmental processes, epithelial remodeling is associated with pulsations of individual cell surface areas and cortical actomyosin flows.

During *Drosophila* dorsal closure, the amnioserosa (AS), a contractile tissue covering the dorsal region of the embryo, shows contractile pulsations and regular actomyosin flows during the reduction of its apical surface area. The biophysical mechanism driving these shape pulsations as well as the role of contractile actomyosin waves in epithelial contraction and dorsal closure still remains unclear.

In this project, we developed a biophysical model for cell shape oscillations that is based on intrinsic properties of the cell: cortex turnover, active contractility by force producing molecules and cell elasticity. We show that coupling these three key ingredients is sufficient for generating stable oscillations. Further, within this framework we were also able to generate waves by coupling the oscillating units and introducing a diffusion term to account for exchange of force producing molecules between the units.

Next, we investigated the role for these contractile actomyosin waves in tissue remodeling. We developed a novel technique that allowed us to

apply mechanical stretch on the AS tissue and study the response of cells to such stress. With this method, we were able to arrest the pulsatile contractions and actomyosin flows in AS cells. We show that this arrest is associated with the relocalisation of actin and myosin from the medial region of the cells towards the adherens junctions to maintain junction integrity upon stretch. This relocalisation of myosin directly correlates with the junctional strain and does not occur in cells that have excessive membrane material as a consequence of endocytosis inhibition. In the latter case, cells continue pulsing and seem to be "insensitive" to stretch.

Upon stretch release, myosin relocalises to the medial area of the cell and pulsations resume. This indicates that cells can switch between two states depending on tension: one in which cells exhibit shape oscillations associated with contractile actomyosin pulses and waves, and the other where cell shape is stabilised with myosin preferentially localised at the cell junctions. Further, following release from long duration ($> 10mins$) stretch application, cell junctions were highly wrinkled. Strong and consistent localisation of myosin waves at these regions led to straightening and reduction of junctional lengths. Moreover, during dorsal closure, AS cells constantly reduce their areas while maintaining junctions of consistent thickness and length relative to area. This is not the case where endocytosis is blocked or myosin activity is down-regulated.

Our results not only shed light on fundamental physical properties of the actomyosin cortex, in particular they also indicate a role of myosin contractile waves in junctional remodeling during AS cell constriction.

Abstract (Spanish)

Los tejidos epiteliales llevan a cabo una remodelación extensiva durante el desarrollo embrionario. Estudios recientes han revelada que, en un sinnumero de procesos de desarrollo embrionario, la remodelación epitelial se asocia con pulsaciones de áreas en células individuales y con flujos corticales de actomiosina.

Durante el cierre dorsal de Drosophila, la amnioserosa (AS), un tejido contractil que cubre la región dorsal del embrión, se observan pulsaciones contráctiles en células individuales y flujos regulares de actomiosina durante la reducción de la superficial apical celular. Al día de hoy, no se conoce el mecanismo biofísico que produce estas pulsaciones celulares ni y el papel que tienen las oscilaciones contráctiles de actomiosina en el epitelio del cierre dorsal embrionario.

En este proyecto, se desarrolló un modelo biofísico para entender estas oscilaciones celulares. El modelo se basa en propiedades intrínsecas de la célula como la rotación de la corteza celular, la contractilidad activa mediante moléculas productoras de fuerza y la elasticidad celular. Utilizando éste modelo, se muestra que acoplando estas tres propiedades clave es suficiente para generar oscilaciones celulares estables. Además, dentro de este marco, se han generado oscilaciones mediante el acoplamiento de varias unidades oscilantes y la introducción de un término de difusión

para considerar el intercambio de moléculas productoras de fuerza entre las unidades.

A continuación, se investigó el papel de estas oscilaciones contráctiles de actomiosina en la remodelación de tejidos. Como resultado, se desarrolla una técnica innovadora que permite aplicar extensión mecánica al tejido de AS y estudiar la respuesta celular ante tal estrés. Con este método, se pueden detener las pulsaciones contráctiles y los flujos de actomiosina en células de la AS. Se muestra que este arresto celular está asociado con la relocalización de actina y miosina de la región central de las células hacia las uniones adherentes intercelulares para mantener su integridad durante la extension epitelial. Esta relocalización de miosina se correlaciona directamente con la tensión en uniones intercelulares y no ocurre en células en las que el reciclaje cellular a través de endocitosis se ha bloqueado. El resultado es un exceso en la acumulación de membrana plasmática en células oscilantes que no responden a la extension epitelial.

Tras liberar al tejido de la extension epithelial, la miosina se relocaliza a la área central de las células y las pulsaciones continuan. Esto indica que las células pueden cambiar entre dos estados según la tension aplicada: uno dónde las células muestran oscilaciones asociadas con pulsaciones contráctiles de actomiosina, y otra donde la forma celular se establece con la localización preferente de miosina en las uniones inter-

celulares. Además, tras liberar el tejido de una extensión de alta duración (>10mins), las uniones intercelulares sufrieron corrugaciones. La localización consistente de oscilaciones de miosina en las regions corrugadas, resulta en una extension y reducción en la longitud de las uniones intercelulares. Además, durante el cierre dorsal, las células de la AS reducen sus areas constantemente, mientras mantienen uniones intercelulares de espesor consistente y longitud relativa a su área. Esto no es el caso cuando la endocitosis se bloquea o la actividad de miosina se reduce.

Nuestros resultados no solo muestran las propiedades fundamentales de la corteza cellular de actomiosina, también indican el papel de oscilaciones contráctiles de miosina en la remodelación de uniones intercelulares durante la constricción de la AS.

To Ipu, Iza and Matt

Acknowledgments

This is probably the best opportunity I will ever get to express my gratitude, in writing, to all the people who have helped me get to where I am today, so I intend to be rather comprehensive.

First, I would like to thank my supervisor Jerome Solon, for accepting me to be a part of your wonderful lab, for giving me the freedom to explore and obtain experiences and skills that will certainly help in my future endeavours as a scientist. Thank you for carefully going through my thesis and helping me shape it into its final form. Your supportive and understanding nature both at work and outside has helped me to reach the point where I stand today.

I thank Kai Dierkes for teaching me and helping me to grasp concepts that were crucial in understanding the physics and math part of my project. Thank you Kai, for your constant support and care throughout the 4 years that you worked with us, and even after finishing your work at the lab to go off adventuring. Thank you for all those tea times and chats, and for making sure I stayed motivated (for a lot of things). Thank you so much for all the time you spent reading my thesis and improving it with your insightful comments and suggestions. You're a brilliant, wonderful human being.

I am grateful to my Thesis Committee members - Jordi Casanova,

Manuel Mendoza and Matthieu Louis. Thank you for giving your time to check my progress throughout the years, and for your interest in my project and helping me focus on the important topics.

I would like to thank Guillaume Salbreux for collaborating with us on the AS oscillation project. Your contribution was of immense help in publishing the results from this work, and I am proud to be on this paper.

Next, my gratitude goes to Julien Colombelli, for letting me use your laser cutter and the spinning disk (your facility is impressive!). The experiments carried out on your laser cutter allowed me to obtain data that were important for testing our model. Thank you for giving your time to make sure things were working fine and for your kind and hospitable nature.

I thank the ALMU facility members - Timo, Arate, Raquel and Javi, for your help and support in using the microscopes. Thank you for bearing with my numerous reports and complaints about issues with the equipments.

A special and massive thanks to folks at the HR department, especially Gloria, Nuria, Marta and Juan. Thank you so much for all your helps with administrative work. Without people like you, life would have been much more difficult as a foreigner.

I have always felt that I am fortunate when it comes to people that I have to associate with. Throughout all these years, somehow, I have constantly found myself surrounded by kindhearted, caring people. This has been true again in terms of the members that I have had to share the lab with. I'm glad to be getting this chance to express my gratitude. First of all, thank you Arturo, for teaching me all that I needed to know about fly handling and imaging embryos, microinjection and other skills that allowed me to become an independent experimentalist. Thank you for all the time you came with me to IRB for my cutting experiments. I remember when I first arrived at the lab, your warm smile made me feel instantly welcome and comfortable. Thank you Petra for helping me with the fly crosses and recombinations. Thank you too for always checking on me, to make sure everything was going smoothly for me - at work, and outside of work. Juanjo, thank you for always having the time to talk with me whenever I was feeling lost at work. Thank you for helping me write the first draft that kick-started this whole thesis writing adventure, and then helping me wrap it up by editing the Spanish abstract. Thank you Thomas for always having suggestions for improvement - be it in presenting lab meetings or talks, or data analyses. I also want to mention Laure here - it was really nice to have shared the lab with you. Thank you for all the times that you helped me with things related to work and

outside.

To the two other students in the lab - Pez and Nat, who became my best friends. Thank you for all the “tea time?”, “scienceing” in various places and “piggies fun time”. Life at the lab wouldn’t have been the same without you guys. Thank you Nat for all your girly advices, for always accompanying me when I needed a Spanish translator, and for always making me feel better by saying “piggyyy” when I wasnt being my best or happiest self. Your zest for life brightened and enlivened the lab. Thank you Pez, you always had time to talk with me, and boy! was there a lot of talking to do! You helped me through all the difficult times - work-related and personal. It seems that your compassion and kindness is unfathomable. I love you both and I’m glad to have been, and to be one of the 3 piggies forever.

Next, I would like to thank the lovely people in the hood who made things easier (and still do) during the toughest of my days : Andrea, Carmen, Ceecee, Cheryl, Dakayla, Krystal, Leah, Marcelle, Nikki, Shaun and Sheena. I especially thank and admire Marcelle, Carmen, Cheryl and Andrea. Thank you for all the laughter, sharing and listening. As we always say, “... and it doesn’t cost money! ”.

A big thanks to Rishi for helping with the Spanish abstract. Thank you too for not minding when for weeks I took up the couch as my writing,

eating and napping/sleeping area - and for, as you put it "becoming a part of the furniture" in the common space.

Matt, thank you for your constant support and encouragement, for cheering me on through these years and throughout the thesis writing process. Thank you for the love and happiness that you've brought into my life, and for being with me and helping me get through difficult times.

Apu Hukuto, oshikimthi for encouraging me to go for that BTech degree, that was the first stepping stone that eventually led me here.

Ipu and Iza, thank you for always having faith in me and letting me fly unfettered. Surely, it required a lot of strength and courage to send me Imphal when I was but thirteen, in order that I be exposed to the outside world and be independent (and learn how to make budgets!). In many of the letters I have written to both of you since my time in Imphal when you introduced to me the art of writing letters, I have said you're the most wonderful parents anyone could ask for. I say that now too, and I mean it more than ever. It is because of your efforts that I am now living this good life. Akiqheu lono oko shikimthi.

List of Figures

List of Tables

List of abbreviations

AJ: Adherens junction

AS: Amnioserosa

cAMP: Cyclic adenosine monophosphate

DC: Dorsal closure

DRhoGEF2*: Drosophila* Rho guanine nucleotide exchange factor 2

ECM: Extracellular matrix

GBR: Germband retraction

MbsN300: Constitutively active form of the myosin-binding subunit of Myosin Light Chain (MLC) phosphatase

mCherry-Moe: Moesin (actin binding protein) tagged with Cherry

MLCK: Myosin light chain kinase

MLCKct: Constitutively active form of Myosin light chain kinase

PAC: Photoactivated adenylase cyclase

Rab5DN: Dominant negative Rab5

ROCK: Rho-associated coiled coil-containing kinase

Sqh-Cherry and Sqh-GFP: *Drosophila* spaghetti squash tagged with Cherry and GFP. Sqh gene encodes regulatory myosin light chain (RMLC) of myosin II

Tom-cadh: Cadherin tagged with Tomato

ZA: Zonula adherens

Chapter 1 - Introduction

1 Introduction

This thesis is an outcome of an interdisciplinary work involving biology and physics. Our group is interested in the biomechanics of morphogenesis and my project was focused on two topics: first, biophysical mechanisms driving epithelial cell shape pulsations, which is presented in Chapter 2 and second, the role of contractile actomyosin waves in maintaining junctional homeostasis of constricting epithelial cells during dorsal closure, presented in Chapter 3.

This chapter (1) starts with a basic overview of relevant biology - a description of the epithelium, followed by the molecular and physical properties of the cell cortex and the roles it plays in cell shape changes during morphogenesis. Next, I introduce the adherens junctions and its connection with the cell cortex, a cross-talk that is crucial in the maintenance of tissue integrity and homeostasis - be it during embryogenesis or in a fully formed animal. The chapter ends with a brief description of the fruit fly and the tissue that we chose as our model - the amnioserosa.

1.1 Epithelia : structure and role in embryonic development

The term "epithelia" was first introduced by the anatomist Frederik Ruysch in 1703, to describe the cellular tissue he observed while dissecting the lips of a cadaver. He showed that several parts of the body

have tissues made up of many small nipple-like protrusions and coined the term with Greek roots *epi* (on top of) and *thele* (nipple) [MacCord, 2012]. Now we know that epithelia line the body and organ surfaces of animals and take part in various physiological functions including digestion, excretion, reproduction, leukocyte trafficking and hormonal signaling. The epithelium is one of the four basic types of animal tissue (the other three being connective, muscle and nervous tissues). Further, epithelia is broadly classified into three types depending on their morphologies: squamous, columnar or cuboidal which can be one cell thick (simple) or two or more cells thick (stratified). There are also several other types of modified epithelia such as ciliated that are hair-like and pseudo stratified where the ends do not all meet the surface and specialize in secretion (Figure 1.1.1).

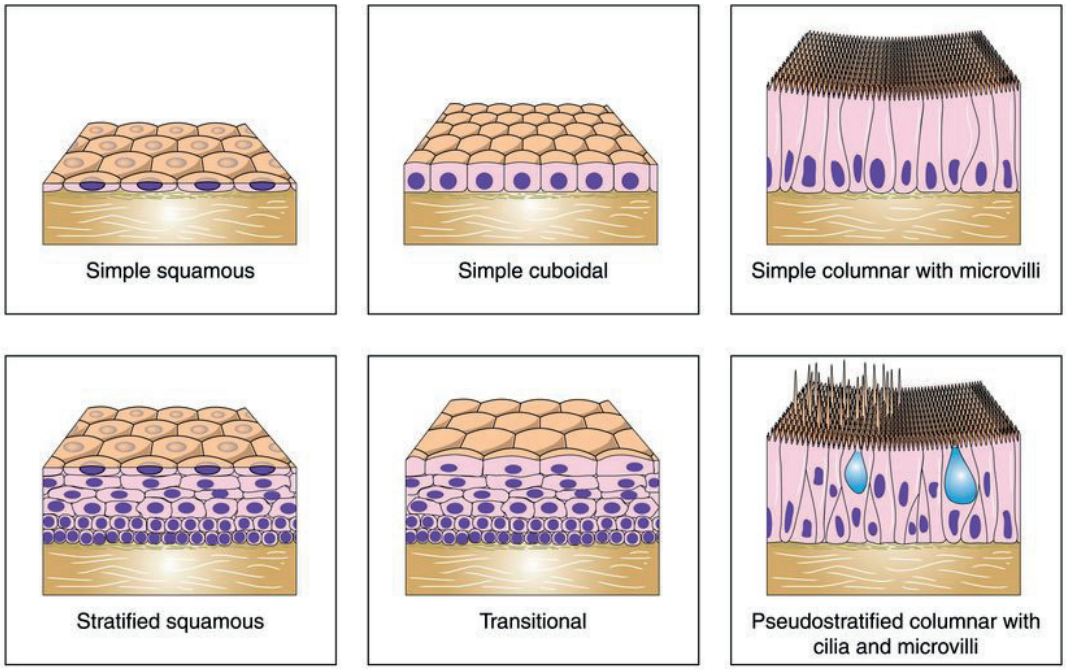


Figure 1.1.1: Different types of epithelial cells. Source: [D'Onofrio, 2009]

Classic textbook description and illustration of the epithelium gives the reader an impression that this tissue is static. However, live cell and tissue imaging show that the epithelium is highly dynamic and undergoes dramatic rearrangement and remodelling during embryonic development. Hence, the epithelium, in conjunction with another tissue type, the mesenchyme, forms almost every part of the animal - from the skin and hair to the digestive system. The maintenance and regulation of organ function by the epithelium further continues throughout the life of the animal. A key feature that is necessary for epithelial cells to carry out crucial functions and generate different cell types for morphogenesis is

the apico-basal plasma membrane polarity. This means that the apical and basolateral domains are structurally and functionally different: the apical domain is exposed to the outside, while the basolateral contacts the interior and cells are held together by an adhesion complex. In *Drosophila* embryos, this polarity is established and maintained by three polarity complexes.

Two complexes determine the apical domain: the first consists of the Crumbs (Crb) and Stardust (Std, Pals) proteins [Tepass and Knust, 1993] and the second consists of Bazooka (Baz, Par-3), Par-6 and atypical protein kinase C (aPKC) [Müller and Wieschaus, 1996]. At the basolateral side, the domain is determined by the proteins Lethal giant larvae (Lgl), Discs large (Dlg), and Scribble (Scrib) [Bilder and Perrimon, 2000].The adherens junctions that connect the cells are composed of E-cadherin, Armadillo (Arm, β-catenin), and α-catenin [Oda et al., 1993, Tepass and Knust, 1993].

The importance of apico-basal polarity in epithelial cells become apparent when we examine the various processes that occur during embryo morphogenesis. First, the polarization of the cytoskeletal components enables these cells to undergo apical constriction which is necessary for gastrulation and tubulogenesis [Sawyer et al., 2010]. Next, formation of columnar and squamous epithelia depend on lateral shrinkage. Further,

epithelial cells are able to orient their mitotic spindles such that they divide parallel to or perpendicular to the sheet depending on whether to increase cell numbers or generate daughter cells to form stratified epithelia [Lechler and Fuchs, 2005, Legoff et al., 2013, Bosveld et al., 2016]. Moreover, during embryogenesis, epithelial cells transition to mesenchymal cells (EMT) and back to epithelial form (MET) such as during organogenesis of kidney. The establishment and maintenance of apical-basal polarity is crucial for the latter transition [Lim and Thiery, 2012, Nelson, 2009].

As described above, epithelial cells go through extensive remodelling and rearrangement in order to develop from a clump of cells into a fully formed animal with highly complex and diversely functional organs. Such a process requires tissue shape changes including elongation, lengthening, bending and branching. How are these shape changes controlled? The field of morphogenesis has been dominated by biochemical and genetic studies and much is known in terms of the genetics involved in the patterning and sculpting of the embryo (reviewed in [Scott, 2000, Fraser and Harland, 2000]), however, it is clear that the shape of a cell and any change that occur in its geometry is determined by a balance of physical forces that are exerted by as well as received at the cell surface. These forces can arise mainly through three ways: first, active forces

generated from within the cell through contractile cytoskeletal material or by changing osmostic pressure via opening and closing of ion/water channels. Second, force exerted on the cell surface by neighboring cells or through the ECM and third, force generated by the plasma membrane due to changes in its protein contents through trafficking.

Among these however, the key mechanism by which cells derive their forces seem to be the through the active contraction of the actomyosin cortex as we will see in the following sections.

1.2 Role of cortical forces in cell shape changes and tissue morphogenesis

In this section, we review the basic biological background for the key components of the cell that are involved in producing force and maintaining adhesions between cells within the tissue. The focus will be on the actomyosin network and its molecular and physical properties. We will then briefly look at how cortical forces enable cells and tissues to change their geometry. Taking advantage of the advancement in high resolution microscopy techniques, we have begun to acquire spatial and temporal information about cellular components at resolutions that are better than ever, and this has revealed strikingly dynamic features of the cortical network. This will be discussed towards the end of the section.

1.2.1 Molecular components of the cortex

Actin was first discovered in non-muscle cells by Straub in 1942 and has since then been considered as the key regulator of cell shape changes and force generation during cell migration and cytokinesis, possibly by a tight spatiotemporal regulation of its mechanical property [Pollard, 1976, Clarke and Spudich, 1977, Stossel, 1978] Actin filaments are semi-flexible polymers with persistence length (Lp) $\sim$ 17 uM [Gittes et al., 1993] ; Lp is the distance over which the filament buckles under thermal forces and increases with increasing stiffness. They are $\sim$ 7nm in diameter and are built from globular actin monomers. The filaments have a fast (plus) and slow (minus) growing end with a higher critical monomer concentration at the minus end ($\tilde{}0.6\ uM\ vs\ \tilde{}0.1\ uM$). When the concentration of the monomers is higher than the critical concentration of the filament end, the monomers bind and grow by polymerisation, whereas when their concentration is lower than critical levels, the monomers unbind from the filament. Due to the difference in critical actin concentrations at the two ends, the filaments grow rapidly at plus ends and shrink at the minus ends leading to an asymmetric growth while the filament length stays constant. This allows for the polymerised monomers to push forward to generate force and is known as tread-milling [Lodish et al., 2000, Cooper, 2000, Ananthakrishnan et al., 2006].

Actin filaments can assemble into a variety of structures such as networks and bundles that are essential for numerous cellular processes (Figure 1.2.2). These structures appear to be tuned to the requirements of the cellular activity such as cell migration and protrusion (lamellipodia and filopodia), adhesion (lamella and stress fibres) and shape changes (cortex).

The cell cortex is a thin layer of actin-rich network immediately below the plasma membrane. Electron microscopy of mammalian cell cultures and dictyostelium estimates the cortex to be 50 - 100nm, using electron microscopy [Hanakam et al., 1996, Charras et al., 2006] while in mitotic HeLa cells it was found to be approximately 190nm [Clark et al., 2013]. The initiation and regulation of the cortex depend on a myriad of proteins, of which the nucleator protein Arp2/3 is most well known. The filament length is controlled by capping proteins such as capZ that bind to the ends and prevent polymerisation, whereas severing proteins such as gelsolin and severin cut the filaments into fragments. Further, proteins that promote depolymerisation such as Cofilin (Twinstar in *Drosophila*) also regulate filament growth. Within the cortex, actin filaments are tightly cross-linked into a mesh by proteins such as α-actinin, fimbrin and filamin as well as contractile proteins (myosins, tropomyosin, tropomodulin). This mesh in turn is tethered to the plasma membrane by

linker proteins of the ezrin-radixin-moesin (ERM) family [Salbreux et al., 2012, Ananthakrishnan and Ehrlicher, 2007].

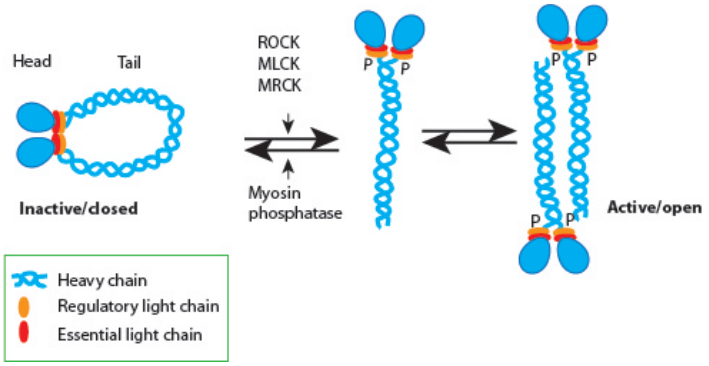


Figure 1.2.1: Myosin structure and activation: Non-muscle myosin II is phosphorylated by various kinases that induce conformational changes whereby it unfolds from a closed and inactive state to assemble into active bipolar mini-filaments that bind to actin filaments. Figure modified from Munjal and Lecuit [2014].

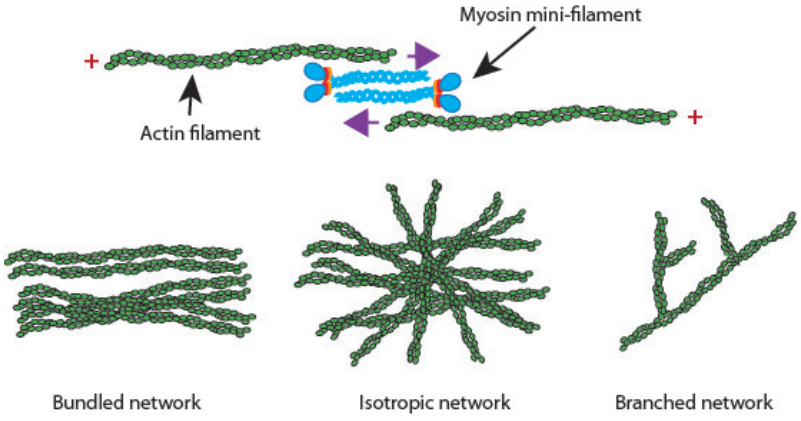


Figure 1.2.2: Actin organisation and force transmission: Myosin mini-filaments pull on actin filaments in anti-parallel direction to generate contractility. Actin filaments can organise into different network with the help of cross-linkers. Figure modified from Alberts et al. [1994] and Munjal and Lecuit [2014].

Myosins are a superfamily of proteins that have the ability to generate mechanical work with energy released by ATP hydrolysis. The myosin family have at least nine groups, however, they are generally classified

into myosin-I and myosin-II depending on whether they form monomers or dimers. Myosin-I are monomeric and incapable of forming filaments and are found abundantly in non-muscle cells. Myosin-II are two-headed dimers that can form filaments and are intensively studied in muscle cells however they are also found in non-muscle cells.

Non-muscle Myosin-II forms a hexamer consisting of 2 heavy chains (MHC), 2 essential light chains (ELC) and 2 regulatory light chain (RLC) [Hartman and Spudich, 2012] . The N-terminal domain of the heavy chain (head region) contains actin and ATP binding sites and is responsible for its motor activity, while the C-terminal folds into an a-helical coiled-coil domain that mediate the formation of tail-tail homodimers (Figure 1.2.1)[Niederman and Pollard, 1975]. Myo-II ATPase hydrolyses ATP, releasing phosphate and energy that induces a powerstroke to move the actin filament to which the myosin is attached, whereas ADP dissociation and ATP replacement leads to its detachment from actin. The fraction of time spent by a myosin on the filament in this force-generating state is called duty ratio.

Contractile activity is achieved by formation of mini-filaments composed of several myosin hexamers that are bound tail-tail that pull on actin filaments in anti-parallel direction as shown in Figure 1.2.2 [Mahajan and Pardee, 1996], and the activity of these mini-filaments is reg-

ulated by phosphorylation. The RLC is phosphorylated at highly conserved residues conserved residues (T18 and S19 in mammals, T20 and S21 in *Drosophila*) by several kinases including ROCK (Rho-associated coiled coil-containing kinase, activated by RhoA), MRCK (myotonic dystrophy kinase-related Cdc42-binding kinase, activated by Cdc42) and MLCK (myosin light chain kinase, activated by Ca2+) Reviewed in Matsumura, 2005. Myosin heavy chains are also phosphorylated by MHCK in Dictyostelium, or casein kinase II (CK II) and protein kinase C (PKC) in mammalian cells [Egelhoff et al., 1993, Craig et al., 1983]. Further, inhibition of myosin phosphatases by kinases such as ROCK also regulate myosin activity [Kimura et al., 1996].

Myosin mini-filaments and actin form a network whose contractility depends on several factors. This includes myosin ATPase rate, duty ratio, mini-filament assembly rate, actin filament organization (filament density, levels of branched vs un-branched filaments) as well as dynamics of actin cross-linking proteins [Levayer and Lecuit, 2012].

1.2.2 Physical properties of the cortex

As described in the previous section, the cellular cortex at the molecular level is composed of actin filaments cross-linked by proteins such as α-actinin and myosin II, forming a continuous mesh that is linked to the

membrane by linker proteins. The microscopic organisation and dynamics of these cortical components gives rise to the mechanical properties of the cell.

All constituents of the cortex undergo constant turnover thereby imparting a viscous fluid-like property to the cortex. This is because actin polymerisation and depolymerisation as well as the dissociation of myosin and cross-linkers dissipate stress over longer timescales (tens of minutes) and allows the network to remodel. At short timescales of milliseconds to seconds it behaves like an elastic material, generating high stress within the network. The turnover dynamics of these constituents have been measured in numerous systems using fluorescence recovery after photobleaching (FRAP), a process in which fluorescent molecules are permanently bleached in a region of interest. The rate of fluorescence recovery determines mobility of proteins and the analysis of FRAP results can indicate the presence of binding interactions and immobile fractions which reveals the dynamics of the protein of interest. Using this method, it has been shown that the entire cortex turns over in about 1 minute. However, analyses specific for the dynamics of myosin and actin cross-linkers have estimated their turnover to be 5-10 times faster than actin, See Table 1.2.1 (Reviewed in [Salbreux et al., 2012]). This suggests that the timescale for viscoelastic relaxation of the cortex depends on the

dynamics of the cross-linkers rather than actin itself. The response of the cortex to perturbation is characterised by an elastic modulus that relates network deformation to applied stresses. Experiments involving deformation of fibroblasts by stretching with optical forces revealed that cortical shear modulus is in the order of 220 Pa [Ananthakrishnan et al., 2006]. Further, upon deformation by indentation, such as those induced by atomic force microscopy (AFM), the contractile tension of the cortex generates an elastic response that is independent of the actual cortex elasticity. This response is termed "cortical stiffness" and could depend on cortical tension, elastic modulus of the cortex as well as cell geometry. Using AFM to measure the stiffness of fibroblasts grown on polyacrylamide gels of varying shear moduli, it was found that these cells change their cytoskeletal organisation to match the stiffness of the substrate - increasing stiffness of substrate led to increased stiffness of the cortex [Solon et al., 2007].

Cell type	Protein	Turnover half-time	Reference
Pig Kidney Epithelial cells (LLCPK1) during cytokinesis	Actin	45 s (polar cortex) 26 s (contractile ring)	Murthy and Wadsworth [2005]
Normal Rat Kidney cells, anaphase and telophase	Actin	15 s	Guha et al. [2005]
Dictyostelium, contractile ring	Myosin II	7 s	Yumura [2001]
Drosophila S2 cells	Myosin II	6 s (metaphase) 14 s (anaphase)	Uehara et al. [2010]
Normal Rat Kidney cells	Alpha-actinin (cross-linker)	8s (equator in cytokinesis) 19s (poles in cytokinesis)	Mukhina et al. [2007]
Dictyostelium	Dynacortin (cross-linker)	0.45s (interphase) 0.98s (equator in cytokinesis) 0.51 s (poles in cytokinesis)	Reichl et al. [2008]
Dictyostelium	Fimbrin (cross-linker)	0.26s (interphase) 0.58s (equator in cytokinesis) 0.31 s (poles in cytokinesis)	Reichl et al. [2008]
Dictyostelium	Cortexillin-I (cross-linker)	3.3s (interphase) 5.4s (equator in cytokinesis) 4.51 s (poles in cytokinesis)	Reichl et al. [2008]

Table 1.2.1: Turnover of cortical components measured by FRAP. Source: Salbreux et al. [2012]

The next property of the cortex is active tension that is generated by actomyosin contractility which is due to motor proteins such as myosin. Cortical tension on the other hand is the total mechanical tension of the cortex, which is the force per unit length exerted on a local section

of the cortex by the surrounding network. A number of methods have been developed to estimate cortical tension and includes atomic force microscopy (AFM) [Krieg et al., 2008], laser ablation [Mayer et al., 2010], micropipette aspiration [Evans and Yeung, 1989], and cell compression between plates [Yoneda and Dan, 1972]

Cortical tension has been estimated to be _30pN/mm in blood granulocytes and zebrafish embryo progenitor cells [Evans and Yeung, 1989, Krieg et al., 2008], while in *D. discoideum* cells it can be up to several thousands pN/mm [Schwarz et al., 2000, Dai et al., 1999]. Myosin-II has been identified as the major contributor to cortical tension. This is achieved by assembly of unipolar MyoII hexamers into bipolar filaments that are composed of up to 10 heads in either side [Vicente-Manzanares et al., 2009]. The force per head is estimated to be around 3.5 pN and with a duty ratio of 0.2-0.4 [Kovács et al., 2007], a typical mesh of cortex can exert tension around 100 pN/mm [Salbreux et al., 2012].

1.2.3 Cortical forces controlling cell shape changes during epithelial morphogenesis

In invertebrate models, key morphological events that are now well characterized include gastrulation, germband extension and retraction, dorsal closure and ommatidia formation in *D. melanogaster*, ventral closure and embryonic elongation *C. elegans*. Fairly well studied morphogenetic

events in vertebrates include cardiac patterning in zebrafish and gastrulation in X. laevis. From these studies we find, that in general, five types of epithelial remodeling take place: cell intercalation, rotation, invagination, extension and tube formation, constriction and elongation as shown in Figure 1.2.3 [Quintin et al., 2008].

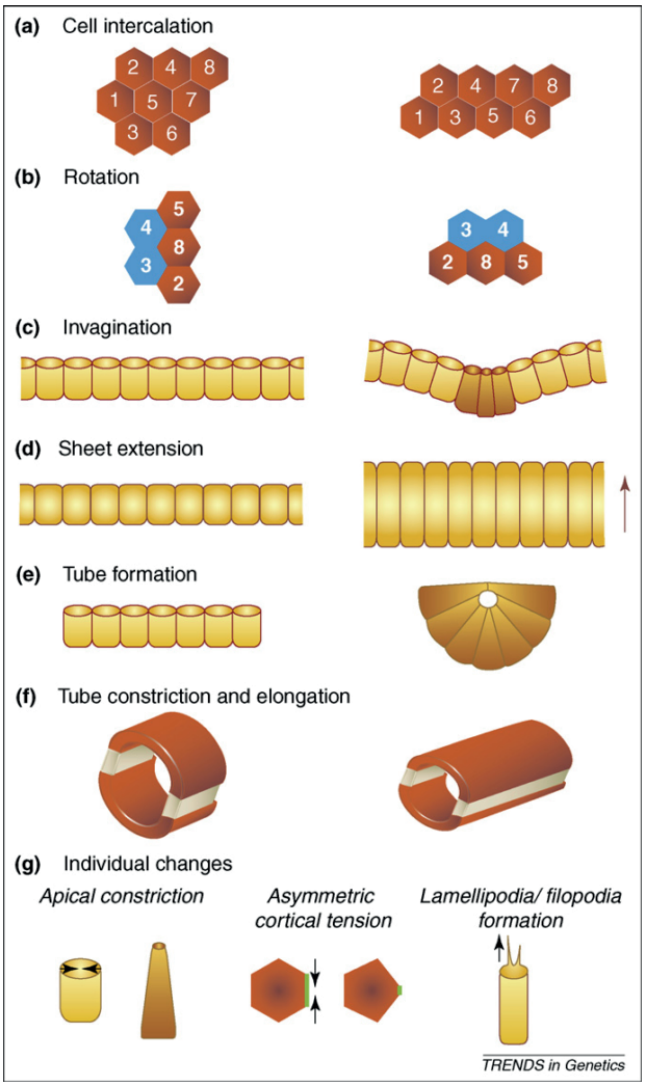


Figure 1.2.3: Types of epithelial remodeling during morphogenesis. (a) Cell intercalation - cells change their relative position through an irreversible planar polarised remodeling of their junctions. (b) cell rotation - cells rotate relative to neighbouring cells through asymmetric junctional shortening between rotating an non-rotating cells (c) Invagination - apical regions of some cells constrict which also shorten their apico-basal length while neighbouring cells maintain their shape. (d) Sheet extension - a large group of cells elongate in the same direction by intercalation through planar polarised junctional remodeling. (e) Tube formation - This is achieved by a group of cells undergoing apical constriction which is followed by sealing of the external cells leading to lumen formation. (f) Tube constriction and elongation - cells that have formed a tube decrease their circumferential length while maintaining their position leading to elongation of the tube. (g) Types of cell shape changes driving the above processes. Figure source: [Quintin et al., 2008]

In each of these shape changes and remodeling events, the key force producing molecule is the motor protein non-muscle myosin II that drive apical constriction and asymmetric or symmetric junctional shrinkages. At this point we will briefly look at each type of epithelial remodeling and studies that show the role of cortical forces in generating these events.

Cell intercalation occurs during gastrulation and neurulation and has been fairly well studied in germband extension of *Drosophila* embryo. Bertet et al reported for the first time the mechanism by which cell intercalation drives polarised epithelial remodeling, they found that cell junctions parallel to the dorso-ventral axis, the "vertical junctions" shrank their length thereby bringing four cells in contact that then expanded parallel to the antero-posterior. This allowed more cells to form new junctional contacts. They found that the shrinkage of the "vertical junctions" was driven by the enrichment of myosin II [Bertet et al., 2004].

Next, cell rotational movement has been observed in the *Drosophila* egg chamber [Cetera et al., 2014, Montell, 2003], and in the developing compound eye of *Drosophila* Fiehler and Wolff [2007]. In the latter process, a group of 800 cells rotate 90 degrees within the epithelium, these cells are the precursors to the ommatidia (units of the compound eye). Fiehler et al showed that myosin II localised in the interface between the rotating and non-rotating cells and is essential for driving this rotational

movement.

The next type of tissue movement- invagination is driven by apical constriction associated with the enrichment of myosin II in the apico-medial region of the cell. It has been studied in the internalisation of the C.elegans during which endodermal precursor cells ingress from the ventral surface to the interior of the embryo, leaving a gap, followed by the closing of the gap through convergence of the neighbouring cells. This ingression is driven by apical contraction that is dependent on myosin II contraction [Lee and Goldstein, 2003, Lee et al., 2006]. A similar study has been done in the *Drosophila* embryo as well, during mesoderm invagination where the same mechanism of myosin dependent apical constriction leads to tissue invagination [Sweeton et al., 1991, Martin et al., 2010].

Epithelial tissues elongate either by the concerted elongation of the constituent cells in the same direction or by the spatial arrangment of these cells. A good example of a system that has been studied for such tissue movement is dorsal closure in *Drosophila* embryo. Following germand retraction, a gap is left on the ventral side of the embryo, this gap is sealed shut by the movement of epidermis from either side of the gap. The leading edge of the epidermal cells accumulate actin and myosin to form a supracellular cable which has been shown to be one of the forces

at play for a succesful closure [Franke et al., 2005, Kiehart et al., 2000, Hutson et al., 2003].

The last two types of tissue remodeling, tube formation and elongation, are essential for forming all the major organs in an adult animal, these major organs being made up of an extensive network of tubes. The *Drosophila* embryo has proven to be an excellent model for studying the formation and maintenance of tubes in processes including tracheal development [Shaye et al., 2008] as well as salivary gland formation. The process mediating the formation of the secretory tubes in the salivary glands is apical constriction and the role of Rho, an upstream regulator of myosin has been implicated during the constriction of the apical membrane [Letizia et al., 2011, Chung and Andrew, 2008].

In conclusion, we have seen that much of the work done on epithelial remodeling has revealed the central role of actomyosin contractility in driving cell and tissue shape changes. Now, the dynamics of the cellular machinery responsible for these forces can be studied at different temporal and spatial resolutions. In cases where the cellular dynamics are studied at low temporal resolution, say at > 60 s period, the outputs that we obtain would show the machinery to be in more or less a steady state distribution, and this would still be sufficient to explain the changes in cells and tissues. However, in recent years, the advancement in high

resolution (temporal and spatial) live imaging has allowed us to capture the dynamics of supramolecular structures that make up the cortex, at timescales of $<$ 1s. This has revealed dynamic properties of the intracellular components, including the cortical network. One such example is the pulsatile behaviour of the cortex that involves constant assembly and disassembly of the networks that also travel across the apical surface of the cell [Martin et al., 2009, Solon et al., 2009, Gorfinkiel and Blanchard, 2011, Levayer and Lecuit, 2013]. The next section will cover the topic on how these fluctuating and contractile actomyosin play a role in cell shape changes.

1.2.4 Cell shape changes driven by pulsatile actomyosin contractility

Cortical flows and its potential involvement in processes such as cell migration and division was first reviewed in the late 80s [Bray and Whe, 1988]. Later it was described in *C. elegans* during pseudo-cleavage, a process which is important for generation of asymmetry in the zygote at first cleavage [Hird and White, 1993] and these flows were shown to facilitate the transport of cell-fate determinants towards the anterior domain and leads to the establishment of embryonic polarity [Munro et al., 2004]. It was followed by similar observations in the *Drosophila* embryo during mesoderm invagination in which pulsed myosin II accumulation in the

cell apico-medial region coincided with constriction of the cell surface followed by a stabilisation phase[Martin et al., 2009], and during germband elongation where myosin II flows allow shrinkage of the junctions leading to elongation of the cells at the antero-posterior axis [Rauzi et al., 2010]. Figure 1.2.4 shows how myosin II flows drive cell constriction and junctional shrinkage that were described in the works of Martin et al and Rauzi et al.

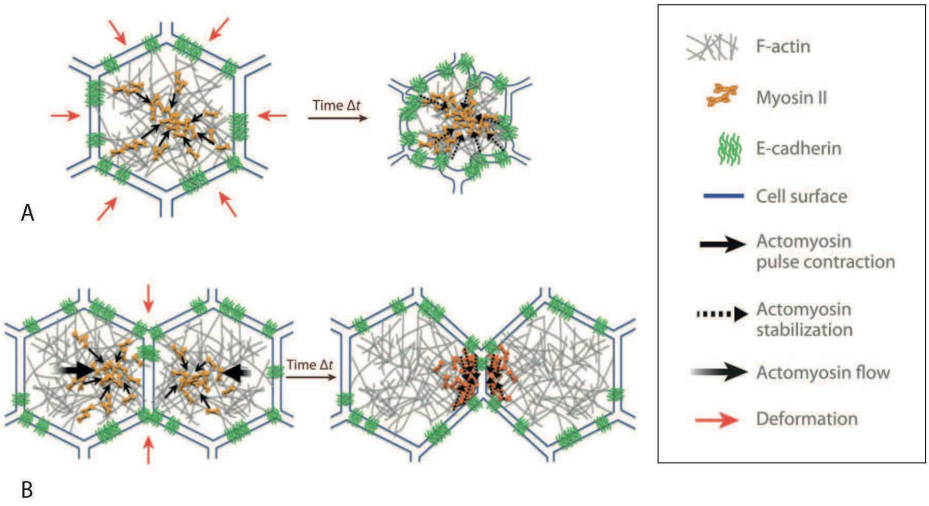


Figure 1.2.4: Contractile actomyosin flows drive apical constriction (A) and junctional shrinkage (B). Red arrows show the deformation caused by pulsatile myosin contractions pulling on cadherin during time interval dt, that leads either to an isotropic cell constriction (A) or anisotropic shrinkage in one junction of the cell (B). Figure adapted from [Lecuit et al., 2010]

Next, it was described in later stages in *Drosophila* embryo during dorsal closure (DC) - a process in which a gap at the dorsal region of the embryo is sealed by an epithelial sheet that progress from either side of the opening. This gap is covered by amnioserosa (AS) cells that

exhibit pulses and flows of the actomyosin network, which coincide with the pulsatile contraction of the cells. As closure proceeds, a supracellular actin cable also forms at the leading edges of the advancing epidermis. It was suggested that the AS pulsations provide active force while the supracellular cable sustains the force in a ratchet-like mechanism thus driving the whole process [Solon et al., 2009, Blanchard et al., 2010, David et al., 2010].

This flow and pulsing behaviour of actomyosin has also been observed during developmental processes in other systems such as the *Xenopus laevis* embryo during convergent extension [Skoglund et al., 2008], *Drosophila* follicle cells during egg chamber elongation [He et al., 2010] and more recently in the mouse embryo during compaction [Maitre et al., 2015]. In all these cases, there is a general characteristic of actomyosin flows and pulses that is similar, however there are differences in certain aspects such as the amplitude and period of oscillation, domains where it occurs (apical or basal), and whether or not it is connected to a ratchet. This suggests there is likely to be a tissue specific regulation of an intrinsic oscillator that determines whether oscillations would occur in some cells and at some developmental stages and not others.

In any case, the fact that actomyosin fluctuations are widespread leads to numerous open questions. What is the mechanism/s driving these

pulses? What causes the actomyosin contractions and flows to be pulsed rather than continuous? Is there an essential role for these pulse in morphogenesis or is it simply an epi-phenomenon of other cellular activities? There are still no clear answers to these questions and the key aims for this project were to investigate the mechanisms that could be driving pulsatile cell oscillations, and eventually to find if there are any essential roles for these pulsations.

1.3 Tissue integrity and homeostasis during morphogenesis

1.3.1 Role of adherens junctions in maintaining tissue integrity

In previous sections, we have looked at cell shape changes driven by actomyosin contractility during epithelial morphogenesis. How do cells maintain contact and transmit forces across epithelial sheets in order for tissue remodeling to occur?

Animal tissues, whether during morphogenesis or in adults, need to be resilient and dynamic at the same time. Mechanically strong adhesive forces are required for the tissue to maintain stability and structural integrity under external stress, while still dynamic in order to form and establish new contacts during cell rearrangements renewal and wound healing. Adherens junctions (AJ) complexes are responsible for providing these features to epithelial cell sheets during morphogenesis and

maintenance of tissue integrity [Halbleib and Nelson, 2006].

Epithelial cadherins (E-cadherin) are the main component of the AJs. Cadherins or "calcium-dependent adherent proteins" require calcium for their activity as the name suggests; calcium is important for rigidifying and stabilising the cadherin complexes that are formed with other proteins [Cailliez and Lavery, 2005]. AJ cadherins are known as classical cadherins as they were the first to be identified among the cadherin superfamily [Gallin, 1998]. The E-cadherin extracellular domains maintain homophilic contacts between adjacent cell while the intracellular domain is highly conserved and interacts with the catenins. p120 catenin and β-catenin (homolog of *Drosophila* Armadillo) bind to the intracellular domains, while β-catenin binds to α-catenin to form the cadherin-catenin complex. The cadherin-catenin complex in turn binds to actomyosin network and actin-associated proteins such as α-actinin, vinculin and formin-1 [Kozlov and Bershadsky, 2004, Perez-Moreno and Fuchs, 2006]. Another integral component of the AJ is the nectin-afadin complex (Echinoid-Canoe complex in *Drosophila*) [Wei et al., 2005, Sawyer et al., 2009]. Apart from these proteins, Stehbens et al showed that cadherins also interact with microtubules (MTs); blocking MT activity affected concentration and accumulation of cadherin in the junctions. MTs dynamics were also required for myosin II activation, which in turn

is necessary for cadherin focal adhesions [Stehbens et al., 2006]. Figure 1.3.1 is a simple representation of the cadherin-catenin complex and its interaction with the cytoskeletal components.

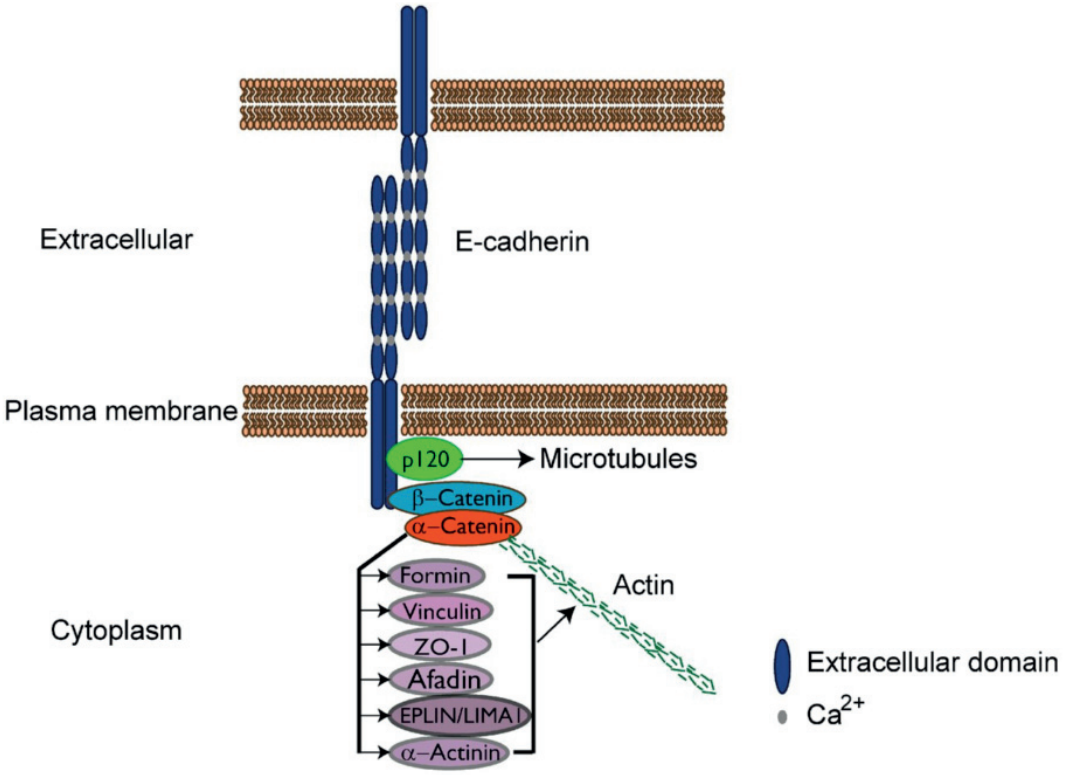


Figure 1.3.1: The E-cadherin-catenin complex. E-cadherins form trans-homophilic interactions and for this Ca2+ ions are required to rigidify the extracellular domains. The intracellular domain has binding sites for p120 catenin and β-catenin. Microtubules are linked to the cadherin through p120, while α-catenin binds to β-catenin. This is where various actin binding proteins as well as actin filament itself interact with the cadherin -catenin complex. Figure taken from: [Baum and Georgiou, 2011]

A key feature of AJs is that it undergoes constant assembly and disassembly, which is important for tissue integrity during morphogenesis involving movements such as intercalation, folding and bending. Further, this feature has to be maintained during processes such as cell divi-

sion, cell death and wound healing. The dynamic nature of AJs depends on its turnover through endocytosis of cadherin that can occur through either caveolin-mediated [Akhtar and Hotchin, 2001], macropinocytosis-like pathways [Bryant et al., 2007], or clathrin-mediated pathways [Kowalczyk and Nanes, 2012]. Clathrin-mediated endocytosis which was first reported in MDCK cells by Le et al ([Le et al., 1999]. During this process, adaptor protein complexes bind to target proteins and recruit endocytic machinery eventually clustering into clathrin-coated pits. These pits are then scissioned from the plasma membrane by a dynamin-mediated process to form endocytic vesicles. Following internalisation, the proteins are either recycled back to the plasma membrane or degraded by lysosomes.

In *Drosophila* there are two classical cadherins, DE-cadherin (encoded by shotgun) [Tepass et al., 1996] and DN-cadherin [Iwai et al., 1997]. A number of studies have shown that DE-cadherin is expressed during *Drosophila* oogenesis ([Godt and Tepass, 1998]) and embryogenesis ([Tepass and Knust, 1993]) and is crucial for the maintenance of the epithelia. Development was arrested as early as oogenesis when maternal and zygotic shg were removed, while using germline clones with weak shg alleles resulted in severe epithelial defects. More recently, Wang et al showed an important role of DE-cadherin in early embryogenesis including blastoderm formation, gastrulation and neurulation [Wang

et al., 2004]. For their study, they used several DE-cadherin constructs with varying levels of modifications to the cadherin domains which were then driven by different drivers including maternally expressed drivers, heatshock-Gal4 and daughterless-Gal4. The most severe phenotype was the absence of the epithelial blastoderm while the less severely affected embryos underwent cellularisation with gastrulation defects. Gastrulation typically consists of invagination, ingression and delamination which are highly dependent on cells adhesiveness and their cytoskeleton. In cadherin mutant embryos, these movements were highly defective and development arrested, suggesting a role of cadherin in maintaining tissue integrity during large scale tissue remodeling and rearrangment. Based on a number of studies, it is beginning to be clear that much of the force that act upon cell-cell adhesions are in fact generated by neighboring cells themselves where they pull on each other and the adhesion system mechanically couples the contractile machinery of neighboring cells, effectively turning the tissue into a supracellular contractile network [Fernandez-gonzalez et al., 2009, Rauzi et al., 2008, Martin et al., 2010]. Thus there is an apparent connection between the AJ and the cytoskeleton which will be discussed in the next section.

1.3.2 Cross-talk between adherens junctions and cytoskeleton

As discussed in section 1.2, non-muscle cells derive their contractile force mainly from the actomyosin machinery. The contractile machinery is believed to be coupled to the cadherin rich cell junctions to mediate large scale cellular rearrangements. This is based on the observation that depletion of myosin II leads to perturbed morphogenetic movements, such as during *Drosophila* germband extension [Bertet et al., 2004]. Large scale tissue movements and remodeling suggest that cells are mechanically coupled through a mechanotransductive system. How are these forces transmitted? A recent study using Förster resonance energy transfer (FRET)-based molecular tension sensor revealed that mechanical tension is transmitted through the E-cadherin cytoplasmic domain in epithelial cells. Further, the actomyosin cytoskeleton exert tension on E-cadherin and this tension requires αE-catenin and the catenin-binding domain of E-Cadherin [Borghi et al., 2012]. Next, a cell doublet assay involving the suspension of a pair of cells in a non-adhesive microwell showed that the recruitment of E-cadherin clusters at the cell-cell contact region depends on actin dynamics whereby the slow down of actin turnover led to an increase of E-cad sequestration at the junction. This study also showed that myosin II contractility is able to modulate the turnover of actin, which in turn regulates E-cad recruitment and immo-

bilisation at AJs [Engl et al., 2014]. The role of myosin contractility in modulating actin turnover has also been shown in neuronal growth cones [Medeiros et al., 2006], as well as in *in vitro* experiments [Haviv et al., 2008, Reymann et al., 2012]. A study by Leerberg et al suggests that the recruitment of cadherin to cell junctions by actomyosin dynamics could be related to conformational changes in the proteins involved such as α-actinin whose vinculin binding sites are exposed upon stretching, that then allows actin to bind [Leerberg et al., 2014]. This shows an important role of actomyosin network and its rheological properties in regulating cell-cell adhesion, and its influence on the cadherin junctions to maintain tissue integrity.

In addition, the role of myosin and its upstream regulators in cadherin localisation and endocytosis has been studied in *Drosophila* germband extension (GBE). This process is driven by cell intercalation during which cells exchange neighbours through planar polarised junction remodelling that is facilitated by shrinkage of vertical junctions (cell junctions that are oriented along the dorso-ventral axis of the *Drosophila* embryo). This junction shrinkage occurs due to anisotropic actomyosin flows from the apico-medial region to the vertical junctions . Cadherin localisation is planar polarised, whereby it is less abundant in these shrinking 'vertical' junctions. This planar polarised localisation of cadherin is due to

up-regulation of clathrin and dynamin-mediated endocytosis, and the recruitment of the endocytic machinery is driven by the *Drosophila* Dia (diaphanous) the formin homolog that polymerises actin, and Myo-II that are activated by Rho1 which is in turn activated by RhoGEF2 [Levayer et al., 2011, Levayer and Lecuit, 2013]. Apart from this, other proteins that have been found to play a role in the integrity of the cadherin-based cell-cell junctions include anillin [Ciara C. Reyes, 1 Meiyan Jin, 2 Elaina B. Breznau, 1 Rhogelyn Espino, 2 Ricard Delgado-Gonzalo et al., 2014], p120-catenin [Davis et al., 2003], the small GTPase Rap1 [Price et al., 2004] and Shroom3 [Nishimura and Takeichi, 2008].

The cross-talk of adherens junctions and the cytoskeleton has also been shown to be necessary for wound healing to maintain tissue integrity thereby ensuring the survival of the animal. Epithelial tissues have remarkable ability to repair wounds, especially in the embryo, where the epidermis heals rapidly without inflammation or forming scar tissue [Rowlatt, 1979, Lorenz and Adzick, 1993]. During wound healing, a supracellular cable is formed at the wound margin, this cable is made up of filamentous actin and non-muscle myosin II [Martin and Lewis, 1992, Brock et al., 1996]. The cable constricts and coordinates the cells surrounding the wound [Fernandez-Gonzalez and Zallen, 2013, Wood et al., 2002]. Along with the assembly of the actomyosin networks, cell junc-

tional components are depleted at the regions where the wound meets the surrounding cells and are redistributed at specific points of the wound. Junction components that are downregulated at the wound margins include E-cadherin [Brock et al., 1996], α-catenin [Wood et al., 2002] and β-catenin [Zulueta-Coarasa et al., 2014]. This junctional rearrangment is thought to facilitate actomyosin cable formation that eventually drives wound healing [Brock et al., 1996, Carvalho et al., 2014]. Quite recently, in 2015, Hunter et al carried out wounding experiments in the *Drosophila* embryo, and showed that the endocytic machinery accumulates at wound margins within a few minutes. They found that endocytic activity is important for polarised E-cadherin depletion, actomyosin cable formation, actin protrusions and rapid wound closure [Hunter et al., 2015].

This indicates that the cross-talk of adherens junctions and the cytoskeleton is necessary for multiple events; be it tissue remodeling involving cell rearrangment or during wound healing. Further, this relationship between AJ dynamics and actomyosin network may also be at play in other processes such as metastasis, where it has been proposed that in metastatic cells, the downregulation of E-cadherin allows the cell to detach from neighbouring cells and become invasive [Yang et al., 2006, Lombaerts et al., 2006].

To conclude this section, large scale cell shape changes and tissue

movements required for complex morphogenetic processes basically rely on two key mechanical properties: contractility and cell-cell adhesion. Further, subcellular organization of the actomyosin cortex is tightly regulated - temporally and spatially depending on the type of tissue movement and the developmental stage. In all epithelial tissues, even in those that otherwise seem stable, AJ require constant turnover, and this inherent plasticity is required for juntional integrity as the tissue undergoes a variety of shape changes. We have observed in the amnioserosa, this interplay between the actomyosin network and the adherens junctions. An observation that might hold the key to finding the role of contractile pulsations in this tissue. The details of the study will be presented in Chapter 3 of this thesis.

1.4 *Drosophila* melanogaster and the amnioserosa tissue as a model system

1.4.1 The fruit fly

In the early 1910s, T.H Morgan was looking for a suitable organism to carry out evolutionary studies and after careful consideration, chose *Drosophila*, commonly known as the fruit fly. A key feature that was advantageous was the relatively small size of the fly and the ease with which it could be reared to produce a large number of offsprings in a

short amount of time. Morgan began breeding his flies as an experimental approach to studying evolution, but the appearance of a sex-linked mutation in his stock turned his attention to the material basis of sex determination and inheritance. His success with the fly kick-started the "experimentalist approach" which went on to dominate the field of biology in the 20th century, and won him the first ever Nobel prize in genetics in 1933. Since then, other great biological discoveries have resulted from studies using *Drosophila* as a model, including the 1946 Nobel won by Hermann Muller for "the discovery of the production of mutation by means of x-ray irradiation, and in 1995, it was Christiane Nusslein-Volhard Eric F. Wieshcaus and Edward B. Lewis who were awarded for "discoveries concerning the genetic control of early embryonic development".

In the past few decades, *Drosophila* has become a popular model not only in genetic studies but also in many other areas largely due to the following key reasons: there are few to no restrictions on their use in the laboratory owing to the minimal ethical and safety issues related with it. The flies are easy to maintain, have modest dietary and spatial requirements, and are robust against pathogens. At 25°C it takes approximately 10 days for the embryo to develop into a fully grown adult. Each female lays up to 100 eggs per day and can continue doing so up to 20 days. Ex-

perimental observation and manipulation can be easily done throughout most developmental stages: from the embryo, to the larva and to an adult [Stocker and Gallant, 2008]. Next, it has only 4 chromosomes which can be visualised in the polytene chromosomes of the larval salivary gland. Genetic manipulation is easy whereby various genes can be mutated and the resulting phenotypes simply observed under the stereomicroscope by looking at features such as wings, eyes, bristles as well as body shape and colour (Reviewed in St Johnston, 2002).

Perhaps the most impressive tool developed was the UAS/Gal4 system for targeted gene expression in *Drosophila*. This system has made *Drosophila* one of the most genetically amenable metazoan and attracted researchers from diverse areas of studies. Drosophilists across the globe share their animals freely, and thousands of lines are deposited in official stock centres: Bloomington, Indiana, and Kyoto, Japan. A large collection of RNAi lines are maintained in Vienna, Austria, and the *Drosophila* Genomics Resource Centre houses cDNA clones, cell lines and microarrays.

The only drawback of *Drosophila* is that it is not possible to freeze them for storage and revived for later use, be it in the form of embryos, larvae or pupae, and thus have to be constantly turned over to new vials every month. However the benefits far outweigh this drawback.

1.4.2 The amnioserosa tissue

In this project, we focus on a specific tissue in the *Drosophila* embryo called the amnioserosa (AS) in order to study cell shape changes, tissue integrity and adherens junction homeostasis during development. Following germband retraction - a process that occurs at later stages of embryogenesis in *Drosophila*, a large gap is left on the dorsal side of the embryo. This gap is covered by the amnioserosa - a single layer of extraembryonic squamous epithelial tissue. Through a process called dorsal closure (DC), a number of co-ordinated tissue movements and forces work together to close this gap. The AS is considered to be an essential force generator in the process of closure by losing volume [Saias et al., 2015], delamination [Toyama et al., 2008] as well as undergoing constriction as the epidermis from either side of the gap move forward to seal the gap. An interesting feature of these cells is that their surface areas show regular contraction and expansion which correlate with the activity of the actomyosin cortex which constantly assemble and disassemble as well as travel through the surface like waves. Much has been documented about the behaviour of these oscillations and various methods of perturbation carried out in order to understand better the mechanism that drive the dynamics, as well as to find out the implications such characteristic might have on the process of dorsal closure [Kiehart et al., 2000, Solon et al.,

2009, Gorfinkiel et al., 2009, Hutson et al., 2003, Toyama et al., 2008]. However, key questions remain to be answered, including mechanism/s underlying such behaviour and whether or not these fluctuations have a developmental role.

This project focuses on these two aspects: first, the biophysical mechanism driving the area oscillation and second, the role that contractile actomyosin waves play in dorsal closure. In Chapter 2, I will present our hypothesis for the first question; and briefly describe the biophysical model that was developed based on experimental data and subsequently published in PRL [Dierkes et al., 2014]. The work described in Chapter 2 was carried out in collaboration with biophysicists Dierkes, K. who did his post-doctorate research in our lab, and Salbreux, G. who works in Francis Crick Institute, London, UK, for which they developed the biophysical model to describe the mechanism driving cellular shape oscillations. In order to test our model we perturbed the AS tissue using chemical, molecular and mechanical methods. Subsequently, we developed a simple tool for stretching the AS cells and captured *in vivo,* cell junctions and cortical dynamics at high temporal and spatial resolution. The results of this experiment implicated a role for contractile actomyosin waves in maintaining homeostasis of cell junctional length during dorsal closure, which will be described in Chapter 3 of this thesis.

Chapter 2: Biophysical mechanisms driving cell shape pulsations

2 Biophysical mechanisms driving cell shape oscillations

Introduction

Oscillation is the repetitive and periodic variation of some measure or quantity between two or more states, or around a central value which is often a state of equilibrium. Oscillations are found everywhere, in physical, chemical and biological systems and can be simple, damped and driven, coupled as well as continuous or sustained. We will briefly look at the main characteristics of an oscillator such as amplitude, displacement, period and frequency for which we will consider a simple harmonic oscillator - a block of mass m attached to a spring. The block is located at an equilibrium position, x = 0, and slides on a frictionless horizontal surface. Displacement from this position results in oscillation around x = 0 with a periodic motion that repeats with a time period T, (Figure 2.0.1, a). The frequency is $\frac{2\pi}{T}$ and the amplitude (A) is the maximum displacement from x = 0. The oscillator has two degrees of freedom - position (x) and velocity (v) and these two variables span a two-dimensional space called phase space. A simple harmonic oscillator is periodic and therefore its trajectory is a closed curve, an ellipse (Figure 2.0.1, b, red line). Harmonic oscillators continue indefinitely without energy input, however in real systems energy is dissipated in the form of heat and the oscillations

decay over time as the mass goes back to its equilibrium position x = 0 Figure 2.0.1 (c). In phase space this trajectory is a spiral that ends at (x,v) = (0,0). Damped oscillators can persist when there is a constant input of energy leading to sustained oscillations. These types of oscillations follow a limit cycle which is an isolated closed trajectory into which neighbouring trajectories spiral either towards or away (Figure 2.0.1(b); blue dashed line).

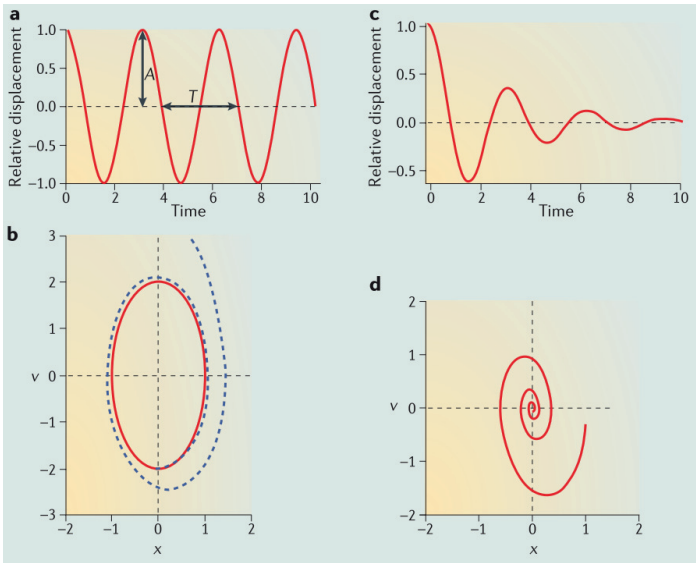


Figure 2.0.1: Basic characteristics of oscillation. (a) Plot showing trajectory of a simple harmonic oscillator around its equilibrium position (x = 0), with an amplitude (A) and period (T). (b) Trajectory of a harmonic oscillator in phase space (red line), while the blue dashed-line shows the limit cycle of a sustained oscillator which is a phase space into which other trajectories spiral into it. (c) Plot shows trajectory of a damped oscillator over time and in phase space (d). Figure source [Lenz and Søgaard-Andersen, 2011]

In biological systems, oscillations are ubiquitous and are observed in all organisms at widely varying time scales of periods ranging from mil-

liseconds to months or years [Goldbeter, 2008] and spatial scales involving sub-cellular machineries to large group of organisms. Oscillations emerge as a consequence of complex and dynamic interactions between the internal components of a biological system and their collective behaviour. Biochemical oscillations occur in the context of metabolism, signaling and development and control various aspects of cellular processes ranging from signaling, motility, growth, division and death. The following sections will highlight studies that have described oscillations in biological systems, narrowing it down to those driven by the cytoskeletal networks. We will then focus on a specific type of oscillation - the regular contraction and expansion of the surface area of epithelial cells in developing embryos. From section 2.2 onwards we will introduce our model system - the amnioserosa (AS) which is found in late stage *Drosophila* embryo, and our hypothesis for the oscillation in the AS cells which is based on cell cortex contractility and turnover. The model we developed will be discussed in section 2.5 and in later sections, we will present a detailed description of various methods we employed to test our model.

2.1 Oscillations in biological systems

Spontaneous oscillations have been observed in a variety of biological systems such as the fibrillar flight muscles in insects that oscillate at a

rhythm which is not synchronised with the nervous impulses that activate them [Pringle, 1978]. Partial activation of skinned skeletal and cardiac muscle fibers *in vivo* also generate spontaneous oscillations [Ishiwata et al., 2007]. Further, non-muscular motor systems also show oscillations, for instance during asymmetric cell division of the *C. elegans* zygote [Pecreaux et al., 2006], as well as in the antennal hearing organs of the *Drosophila* adult [Göpfert et al., 2005]. A study on bullfrog sacculus revealed the oscillation of hair bundles which are sensory element found in the hearing organs of vertebrates. These oscillations were found to be driven by interactions between motors likely to be myosin-1c or myosin-VIIa and actin filaments as well as $Ca2+$ feedback through mechanically gated channels [Martin et al., 2003].

Gene expression is determined by transcription factors which are themselves genetic products and this leads to a complex network of genetic interactions that can result in oscillatory instabilities. The most commonly studied genetic oscillators are the circadian clock [Dunlap, 1999] and cell cycle oscillators [Tyson et al., 2001]. Oscillations have also been observed during vertebrate segmentation, in which specialised mesoderms called somites give rise to various parts such as skeletal muscles and dermis. The formation of somites is oscillatory whereby the segmentation clock - driven by Wnt and Notch genes- runs in somite precursors and

stops when the precursors reach a specific maturation stage. This temporal oscillation gives rise to a periodic patterning of somite boundaries [Pourquié, 2003, Herrgen et al., 2010]. Next, exposure of various cell types to radiation damage lead to the oscillation in the levels of p53, a tumour suppresor; it is speculated that the purpose of this oscillation is to ensure the repair of the DNA in stressed cells without the risk of an irreversible biological outcome due to excessive p53 activation [Lev Bar-Or et al., 2000].

So far, the best approach to understanding the basis of biological oscillations has been to focus on general mechanisms and use simplified models. For instance, in the case of genetic oscillations, the simplest feedback oscillator is made up of a single gene, its product and the resulting mRNA. If transcription of the mRNA is inhibited by the gene, and there is a time delay between the start and end of translation, the expression of this gene can become oscillatory [Monk, 2003]. Such time-keeping mechanisms that generate patterns are thought to confer robustness and reproducibility to the organism, and aid in evolutionary flexibility to adopt new expression patterns without having to make dramatic structural changes [Moreno-Risueno and Benfey, 2011].

2.1.1 Oscillations driven by cytoskeletal networks

Motor proteins are specialized macromolecules that have the ability to generate force through ATP hydrolysis. These motors are attached to filamentous proteins such as actin and microtubules that serve as tracks. In normal conditions of ATP level and temperature, these motors generate constant force and maintain normal velocity along the tracks in a direction that is dependent on the polarity of the track [Winkelmann et al., 1995] However, in some cases, these motors can generate oscillatory motions such as in the insect wings as mentioned in the previous section [Pringle, 1978]. In the late 90s, Jülicher et al developed a theoretical model to describe the physical mechanism for motor oscillations. They showed that a collection of motors acting on an elastic element generally had a tendency to spontaneously oscillate without the need for a chemical oscillator. In their model, they were also able to predict the characteristic properties of myofibril oscillations [Jülicher and Prost, 1997]. More recently, Placais et al showed experimentally, that a simple system consisting of a single actin filament and few tens of myosin proteins can indeed generate spontaneous oscillations when subjected to an elastic load [Plaçais et al., 2009].

Microtubules are closely associated with the actomyosin network in terms of structure and function. Indeed, the contractile oscillatory be-

haviour of the cell cortex was first observed in cultured tissue cells by Bornens et al while studying the role of microtubules in polarity of human lymphoblasts (KE37). In motile cells undergoing polarised movement, a constriction ring forms near the centrosome and the cytoplasm flows from the ring towards the membrane in the direction of the movement. However, in the absence of microtubules, this ring is unstable and moves back and forth across the cell while the protrusion of the membrane shifts from one end to another in a periodic manner. This oscillatory activity correlates with the flow of cortical myosin and the periodic assembly of actin [Bornens et al., 1989]. Next, a study showed that deploymerisation of microtubules in spreading fibroblast and epithelial cells led to rhythmic contractions of the cell body. Two mechanisms were suggested to be playing a role in driving these morphological oscillations : Rho mediated and calcium activated contractile pathways [Pletjushkina et al., 2001]. The former study was done on cells grown in suspension and the latter on spreading cells, both of which are situations where the adhesion is weak and thus could reflect actomyosin dynamics as a consequence of the absence of adhesion. Moreover, in both studies the actomyosin distribution was visualised on fixed cells. To address this, a similar study was carried out on adherent cells in conditions where these cells could not attach to the substrate. It was revealed that removal of microtubules

induced oscillation in entire cells as well as cell fragments, and that the oscillations were driven by actomyosin contractility [Paluch et al., 2005].

Next, with the advancement in live imaging technology, tissues in developing embryos could be imaged in vivo at better temporal and spatial resolutions. This led to many interesting observations of individual cell dynamics and properties of intracellular constituents during collective tissue movements associated with morphogenesis. One of these observations was the fluctuations in the cell areas with corresponding actomyosin dynamics - the regular contraction and expansion of cell areas and the assembly and disassembly of the actomyosin cortex. A closer look at the cells revealed propagation of local actomyosin contraction through the cell surface in a wave-like manner. Such dynamic wave-like behaviour of actomyosin has been observed in more than one developmental process in the *Drosophila* embryo including germband extension and dorsal closure [Solon et al., 2009, Gorfinkiel et al., 2009, David et al., 2010, Rauzi et al., 2010, Levayer and Lecuit, 2013] as well pulsatile contractions without waves in mesoderm invagination [Martin et al., 2009]. Moreover, similar observations have been made in *Drosophila* follicle cells [He et al., 2010], Zebrafish gastrulation [Behrndt et al., 2012] as well as in protruding Chinese Hamster Ovary (CHO) cells [Driscoll et al., 2015].

2.1.2 Mechanism of cell shape oscillation

The mechanism/s driving cell shape oscillation has been investigated by several groups and it appears to involve different molecular networks such as PAR complex [David et al., 2010, 2013], ion channels Hunter [2012] as well as the actomyosin contractility [Paluch et al., 2005].

It has been found that PAR proteins Baz, Par-6 and aPKC play a role in regulating actomyosin networks in oscillating AS cells. Through live imaging, it was shown that Baz and Par-6/aPKC regulate different phases of myosin assembly and disassembly cycle in which Baz promotes pulse duration, whereas Par-6 and aPKC promote lull times between pulses.

Further, ion channels seem to play a role in certain circumstance in which cell contraction is induced by uncaged Ca2+ or endogenous Ca2+ flashes that are found in the AS cells. Increasing Ca2+ by microinjection of GsMTx4 (a toxin that specifically inhibits mechanically gated channels) led to contraction of cells. Moreover, two candidate channel subunits encoded by ripped pocket (rpk) and dtrpA1 were identified. RPK is a DEG/ENaC subunit expressed during *Drosophila* embryogenesis while dTRPA1 is a Ca2+-permeable TRP channel subunit required for larval thermosensing and locomotion. RNAi knockdown of these subunits partially phenocopied the effect of GsTMx4 suggesting that ion channels

regulate ion flux in cells, leading to Ca2+-dependent cell contractility.

Finally, properties of the actomyosin networks themselves can lead to sustained oscillations which was shown in a study carried out in Human KE37 and Murine L929 cells [Paluch et al., 2005]. In this case, oscillation was triggered by local disruption of actin cortex with latrunculin A and cytocholasin D as well as by flowing a jet of culture medium on the cell membrane with a micropipette that led to stress induced cortical breakage. According to their hypothesis, cortical disruption leads to the formation of a hole in the membrane with an accumulation of actomyosin at the rim and a small region devoid of actin and myosin (Figure 2.1.1 b), from this region a growing bulge (bleb) forms (Figure 2.1.1 c) that is gradually enriched by actin monomers and a new shell forms through the recruitment of actin (Figure 2.1.1 d, e, f). This is followed by the redistribution of myosin to the new shell with a time delay that depends on the formation of the actin network to which the myosin II can bind (Figure 2.1.1 g). The concentration of myosin II however, remains higher at the point where the constriction ends (Figure 2.1.1 h) and this leads to a local inhomogeneity in the stress, thus favouring new breakage and bulging (Figure 2.1.1 i) [Paluch et al., 2005].

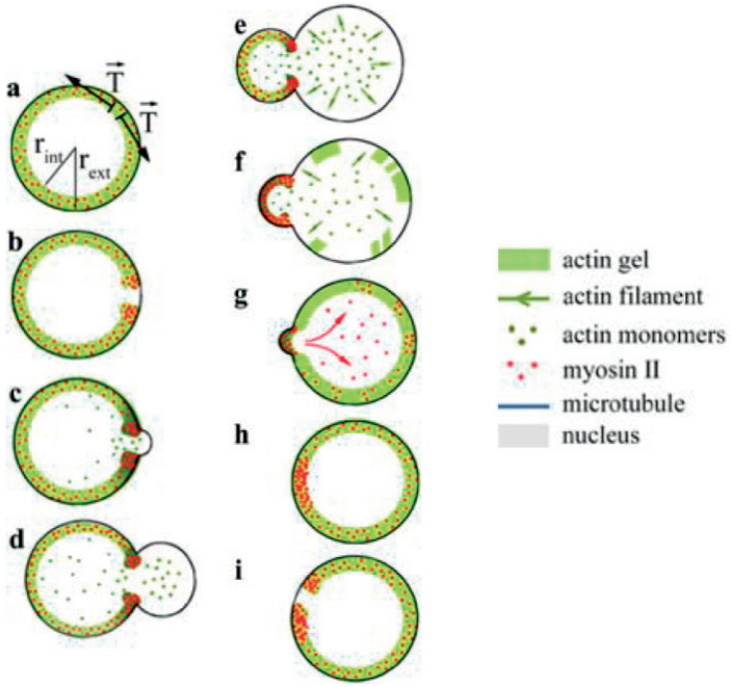


Figure 2.1.1: Mechanism for cell shape oscillations in cell fragments proposed by Paluch et al. [2005]

2.2 How are cell shape oscillations driven by actomyosin activity?

2.2.1 Turnover: an intrinsic property of the actomyosin cortex

The protein constituents of the cortex undergo constant turnover during which actin filaments polymerise and depolymerise, while myosin and cross-linker proteins bind and unbind from the actin filaments. Apart from this, actin binding proteins such as gelsolin, cofilin and vinculin also contribute to the turnover of actin filaments. This allows for the stress to be dissipated over longer timescales (tens of minutes) and the network

to remodel, such that at these timescales the network behaves like a viscous fluid. However, at short timescales of milliseconds to seconds there is high stress within the network, imparting an elastic property to the cortex. Through FRAP experiments, it has been shown that the entire cortex turns over in about 1 minute while myosin and actin cross-linkers turnover is 5-10 times faster than actin (Reviewed in [Salbreux et al., 2012]). This suggests that the timescale for viscoelastic relaxation of the cortex depends on the dynamics of the cross-linkers rather than actin itself.

A recent study has shown that coupling cortex turnover and cell elasticity is sufficient for generating cell shape oscillations. This work was carried out in dividing HeLa and L929 fibroblasts that exhibited cell shape oscillations with cytoplasmic flows between the two poles. During these oscillations, the fluorescence intensity of cortical Lifeact and MRLC were anti-correlated with the size of the poles, indicating cortex density increase with contraction of poles. In order to describe the mechanism for these oscillations, they developed a model based on the competition between cortex turnover and contractility. The mechanism being that if polar contraction is faster than cortex turnover, polar contraction would lead to the accumulation of cortex. The contraction is slowed down by elasticity and tension at the contracting pole is decreased due to cortex

turnover. This leads to the increase in tension in the opposite pole and reversal of oscillation [Sedzinski et al., 2011]. Our model for shape oscillations in the amnioserosa (AS) cells is based on a similar hypothesis involving cell cortex turnover, active contractility and cell elasticity.

With regards to the shape oscillations in the AS cells, a number of key questions remain to be answered are: First, what is the biophysical mechanism that drive these oscillations? and second, is there a role for these oscillations? A few speculations have been made regarding the role of these fluctuations: it might be a way to ensure the equilibrium of the apical tension between neighboring cells, or it could simply be an epiphenomenon of the dynamics of actin and myosin [Blanchard et al., 2010]. Another idea was that these oscillations could be facilitating the process of dorsal closure, however, down-regulating myosin II activity and effectively abolishing these fluctuations led to a closure rate that was in fact faster than that of WT embryos [Saias et al., 2015]. This motivated us to carry out further investigation in order to find a plausible role for this cellular behaviour, keeping in mind that it has been observed in numerous developmental processes, and likely to be found in more in the near future.

2.3 Amnioserosa cells and their shape oscillations

The amnioserosa (AS) tissue serves as a good model for studying contractile epithelial cells and their cytoskeletal and junctional dynamics during developmental processes involving tissue constriction. The large size and location of these cells make it easy for imaging, moreover, availability of Gal4 drivers for this tissue allows for genetically perturbing cells specifically in the AS. We have taken advantage of these properties to study cell shape oscillation and whether they have a developmental role.

AS cells are specified during axis specification at early oogenesis [O'Connor et al., 2006, Ray et al., 1991]. Figure 2.3.1 shows lateral view (A-D) and dorsal view (E-G) of the embryo during developmental stages 8 - 15. Amnioserosa is highlighted in green.

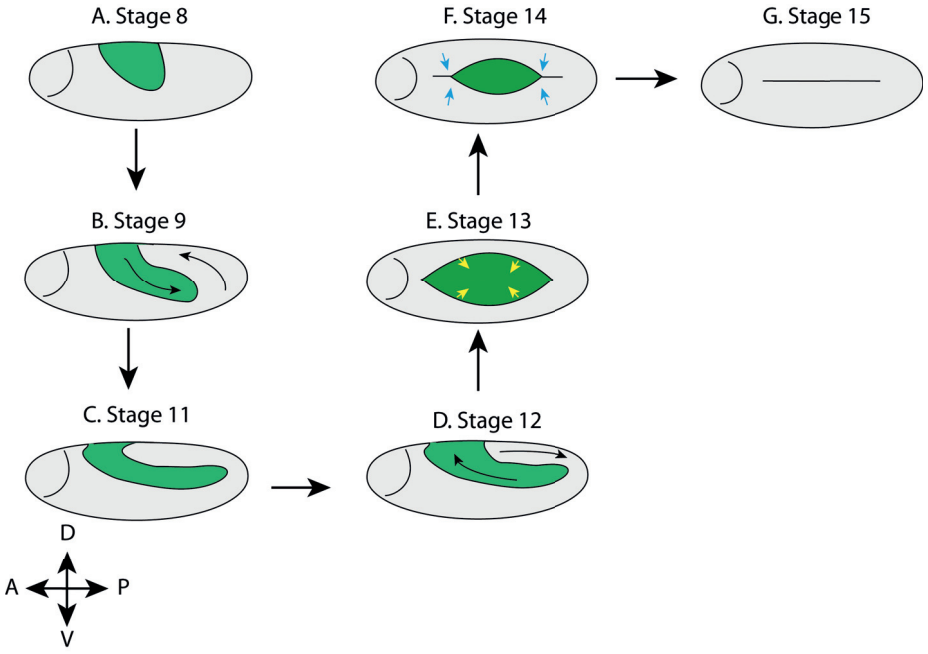


Figure 2.3.1: Stages 8-15 of *Drosophila* embryo development showing the movement of the AS (highlighted in green). Lateral view (A-D) and dorsal view (E-G). Embryonic axes are A = anterior, P = posterior, D = dorsal, V = ventral. (A) Germband extension starts partly stretching the AS. (B) Late germband extension, AS cells are highly elongated at this stage. (C) Germband retraction starts, AS still elongated and ready to extend. (D) Germband retraction continues as AS begins to extend (E) Dorsal closure begins and AS cells begins to constrict (F) Mid dorsal closure, AS cells begin to delaminate and invaginate as epidermis progresses and fuse along the two canthi (edges) (G) End of closure, the gap is sealed and AS cells undergo apoptosis.

During germband extension the AS tissue stretches along the flanks of the embryo and becomes flattened by the advancing germband (Figure 2.3.1 A, B). At this stage, AS cells actively elongate and change from columnar to squamous shape in a process called rotary cell elongation whereby the microtubule bundles that are initially oriented apicobasally elongate and rotate 90 degrees such that the apical ends are now pointing towards the direction of elongation. Such movement and

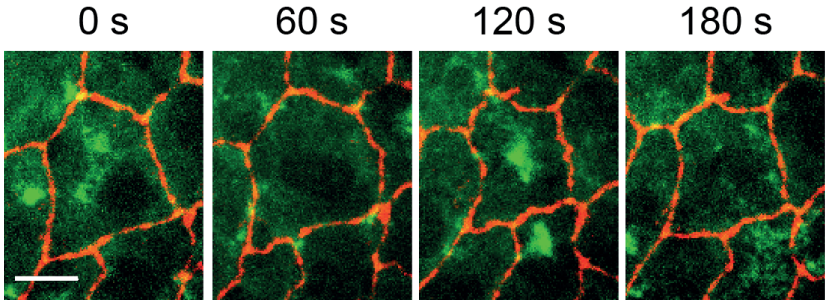
redirection of the microtubules is mediated by cortical actin and myosin [Pope and Harris, 2008]. During the next stage the germband begin to retract towards the posterior region and the AS cells shorten and become squamous and roughly hexagonal, occupying the ovoid gap left by the retracted germband (Figure 2.3.1 D, E). This gap is eventually sealed through a process known as dorsal closure (DC) which involves the co-ordination of various forces and tissue movements. The amnioserosa is considered to be one of the key players in this process during which cells lose volume and undergo constriction, as well as show regular oscillations of their surface areas. The quantification of these oscillations and the cortical dynamics that correlate with these oscillations are provided in the following section.

2.4 Results

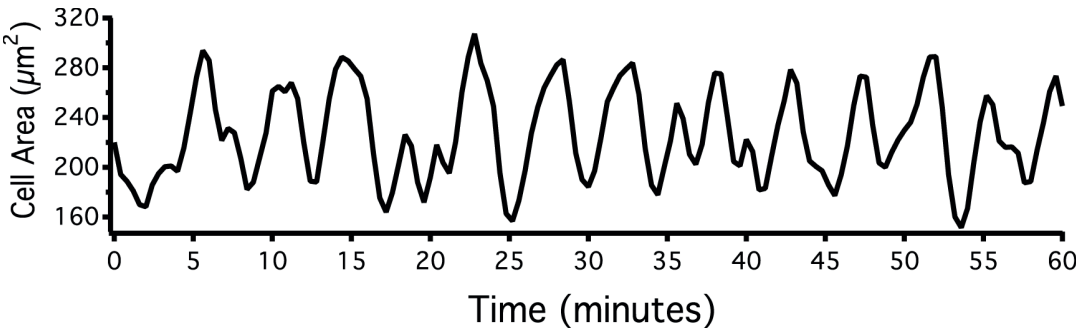
2.4.1 Actomyosin network in the AS cell apical surface display pulsed contraction and relaxation

A typical amnioserosa cell during dorsal closure is ∼10-20 microns across the apical surface and ∼10 microns at the transversal side. Throughout DC, AS cells show regular oscillation of their surface areas with a period of ∼230 seconds [Solon et al., 2009]. To capture cell and cytoskeletal dynamics we carried out live imaging of embryos expressing GFP- tagged

myosin and Tomato-tagged cadherin. From the images obtained, cell outlines were segmented and tracked with a semi-automated software Packing Analyzer [Aigouy et al., 2010] from which data such as area, perimeter and myosin intensity over time were extracted. Figure 2.4.1a and Movie_01 shows time lapse images of a representative amnioserosa oscillating over time in which the contraction of the cell area can be observed to coincide with the accumulation of a contractile myosin pulse. Plotting the cell area over time shows the regularity of these shape oscillations (Figure 2.4.1b).



(a) Snapshots of an oscillating AS cell. Adherens junctions are visualised by expressing Tomato E-Cadherin (red) and myosin is visualised with Sqh-GFP (green).

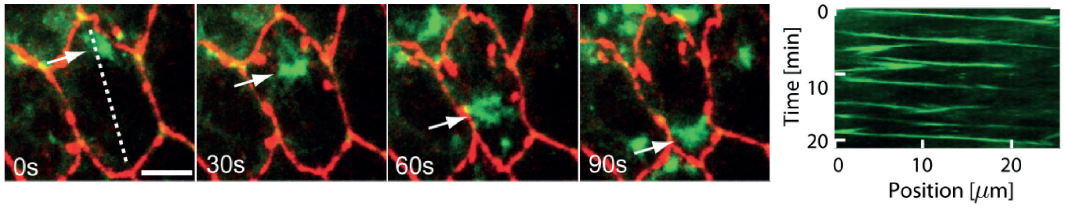


(b) Plot of area of a representative amnioserosa (AS) cell over time

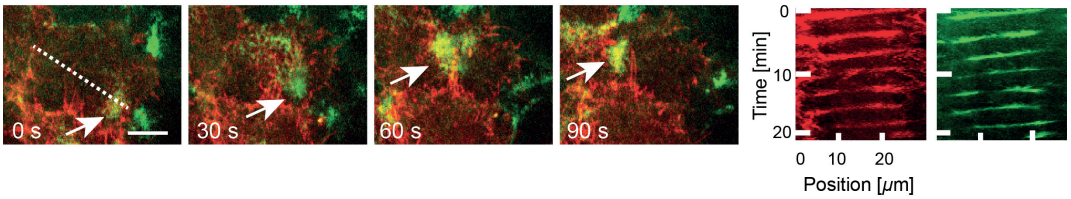
Figure 2.4.1: Area oscillation of a single AS cell

2.4.2 Quantification of contractile actomyosin waves

Next, the pulsed contractions and relaxations of myosin in these cells were also observed to propagate through the cells in a wave-like manner (Figure 2.4.2a, white arrows). A kymograph was obtained by drawing a line along the path of the wave (Figure 2.4.2a, white dashed line), and the slope of the lines in the kymograph allowed us to measure the velocity of contractile waves. We carried out this measurement in a subset of cells exhibiting unidirectional propagation (N=15 cells) and the average velocity of a pulse was found to be $13.4 \pm 4.6\ \mu m/min$ [Dierkes et al., 2014]. The behaviour of both actin and myosin were analysed from live images of cells co-expressing mcherry moesin (an actin binding protein) and Squash-GFP (myosin regulatory light chain) which showed similar dynamics (Figure 2.4.2 b, c, Movie_02).



(a) Left: Timelapse images showing myosin pulse (white arrows) traveling through an amnioserosa cell. Cell junctions are visualised with Tomato E-cadherin (red) and Myosin with Sqh-GFP. Right: Kymograph of myosin intensity along the dashed line in left figure [Dierkes et al., 2014]



(b) Left: Timelapse images show traveling actin and myosin pulse (white arrows) in an amnioserosa cell. Actin is visualised with Mcherry Moe (red) and Myosin with Sqh-GFP. Right: Kymograph of actin and myosin intensity along the dashed line in figure on the right

Figure 2.4.2: Contractile actomyosin wave in a single cell and kymograph of myosin and actin intensites.

2.5 Biophysical model of AS cell oscillation based on cortex turnover

This part of the project was carried out in collaboration with Kai Dierkes, a post-doctorate in the lab and Guillaume Salbreux (Francis Crick Institute in London, UK). The results of this work were subsequently published in Physical Review Letters, the paper for which is attached at the end of this chapter.

2.5.1 Minimal contractile unit

A minimal, generic model for cell shape oscillation was developed by

Salbreux, G. and Dierkes, K., which is based on cortex turnover and contractility - similar to the model proposed by Sedzinski et al [Sedzinski et al., 2011] (described in Section 2.2).

For this, we consider a system consisting of three elements:

1. A contractile element that is spatially homogeneous and is made up of constituents that are undergoing constant renewal.

2. An elastic element

3. A viscous damper which acts as the dissipative element of the system.

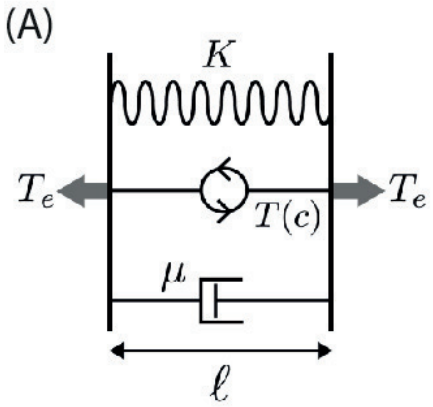


Figure 2.5.1: Schematic of a minimal model (A) for a mechanochemical oscillator. Figure from [Dierkes et al., 2014]

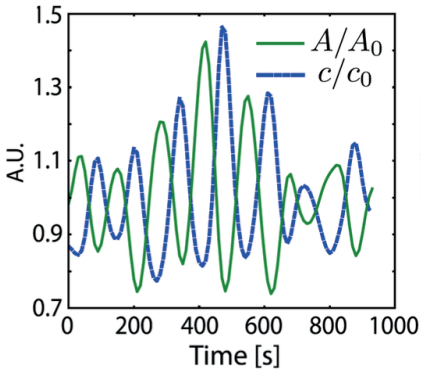
The contractile element represents a contractile network that exchanges force- producing molecules (for example: myosin motor proteins) with a reservoir. The concentration (c) of the force producing molecules follows the dynamic equation:

$$\frac{dc}{dt} = -\frac{1}{\tau}(c - c_0) - \frac{c}{l}\frac{dl}{dt} \tag{1}$$

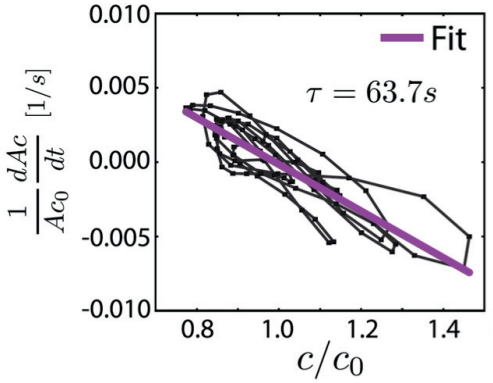
where l is the length of the contractile element. Here, the dynamics of concentration c is due to: (i) binding and unbinding of the force producing molecules with respective rates $k_{on} = c_0/\tau$ and $k_{off}c = c/\tau$, and (ii) a term related to matter conservation, which gives rise to the second term on the right-hand side of $Eq.(1)$.

In order to test whether the dynamics observed in AS cells is consistent with $Eq.(1)$, the cell surface area of the amnioserosa cells and the myosin intensity within those particular cells were measured. It has been shown previously that the period of oscillations in AS cells is $\sim 230s$ [Solon et al., 2009].

Our measurements indicated that the oscillation in myosin concentration is slightly shifted with respect to cell area as shown in Figure 2.5.2a. $Eq.(1)$ predicts that we would substitute l with A, and by doing so we find that the rate $\frac{1}{A}\frac{d(cA)}{dt}$ is indeed proportional to the myosin concentration c (Figure 2.5.2) during the oscillation of an AS cell.



(a) Representative plot of normalized area and average myosin concentration c at the cell apical surface



(b) Plot of the rate $(1/Ac_0)$[d(cA)/dt] vs normalised concentration c for the data shown in Figure 2.5.2a

Figure 2.5.2: Plot of myosin intensity vs cell surface area in AS cells. Figure from [Dierkes et al., 2014]

Next, the dynamic equation for the the length l of one contractile unit can be written as follows:

$$\mu \frac{dl}{dt} = T_e - T(c) - K(l) \tag{2}$$

The tension generated by the contractile unit is a function of the concentration of force-producing molecules $T(c)$, while $K(l)$ is the elastic restoring force which depends on the force-deformation relationship of the spring element. $T(c)$ and $K(l)$ are assumed to be monotonic functions of their respective arguments; c and l. μ is a damping coefficient, and T_e is an external tension opposing deformation of the unit (Figure (B) 2.5.1).

At steady state, the external tension balances the internal tension such

that

$$T_e = T(c_0) + K(l_0)$$

Matter conservation and the assumption that $T(c)$ increases with c leads to an instability of the homogeneous state such that the contraction of the material leads to an increase in its density leading to an even stronger contractile force. This effect is balanced out by turnover restoring the reference concentration c_0 (please see schematic in Figure 2.5.3).

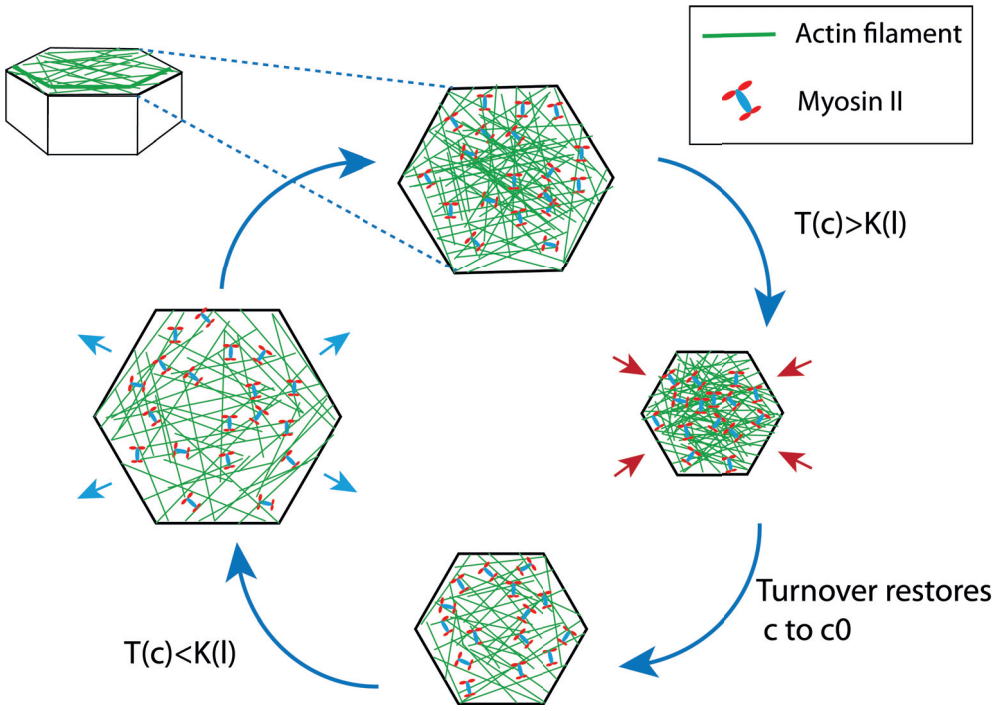


Figure 2.5.3: Schematic of AS cell oscillation driven by cortex turnover and elasticity

Next, in order to experimentally determine the turnover time of actin and myosin we carried out FRAP on both proteins (Movie_03a,b, Section

5.3 in Materials & Methods). We found the turnover half-life of myosin to be 6.3 s (n = 14) while that of actin was 25 s (n = 30). This suggests that the turnover of actin is more likely to be setting the turnover timescale of the cortex.

Further, the slope of the curve in Figure 2.5.2b allowed us to estimate the turnover of the cortex to be approximately 108 ± 66 s (n = 73), while the time scale $\frac{\mu}{k_1}$ is around 90 ± 50 s. In our paper [Dierkes et al., 2014], we have shown that that the period in the model is given by $\tau_0 = \sqrt{\frac{\tau\mu}{k_1}}$. With these estimates, the period at threshold would be $\tau_0 = 425 \pm 150$ s, which is on the same order of magnitude as the period of cell oscillation, $\tau_0 = 230 \pm 76$ s [Solon et al., 2009], and is consistent with our hypothesis.

2.5.2 How to generate waves?

As seen in Figure 2.4.2a, actomyosin networks in AS cells propagate across the surface area in a wave-like manner. In order to generate these waves, we considered a case where the single oscillating units described in 2.5.1 are now coupled to each other and the force producing elements are free to diffuse between the units. This allowed us to generate traveling waves (Figure 2.5.4).

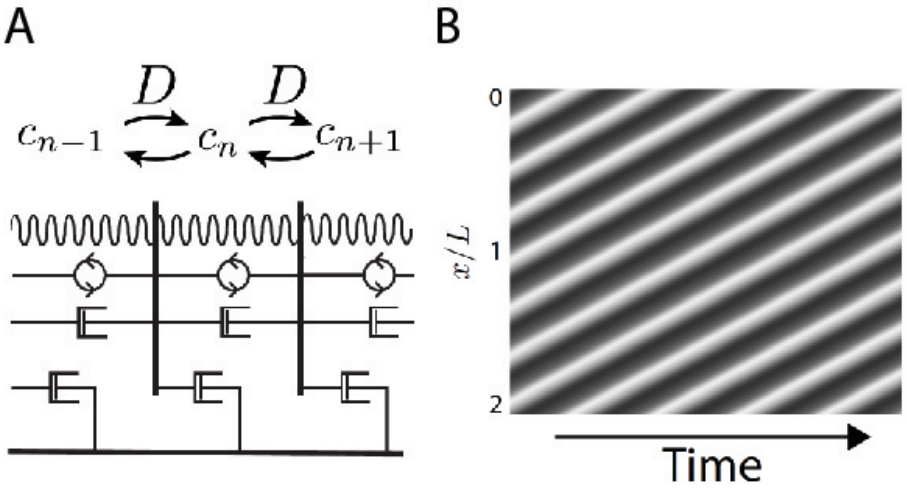


Figure 2.5.4: A. Schematic for a chain of oscillators with diffusion of force producing molecules between the units. B. Kymograph showing time evolution of concentration c. Figure from [Dierkes et al., 2014]

Summary:

1. We developed a biophysical model for AS cell shape pulsations that is based on intrinsic properties of the cell: Cortex turnover, active contractility by force producing molecules and cell elasticity. These three key ingredients were sufficient for generating stable oscillations.

2. We were able to generate waves that we observe in AS cells by coupling single oscillating units and adding a diffusion term whereby force producing elements are free to diffuse between the oscillating units.

2.6 Testing the model

2.6.1 Laser ablation of cortical waves to investigate tension in the system

In the model we consider that the tension T is generated by the contractile unit and depends on the concentration of force producing molecules, which we could consider to be myosin proteins. In order to obtain an idea about this relationship between tension and myosin concentration, we performed laser ablation on a propagating contraction (Movie_04). Ablating along a line on a contractile wave led to retraction of the region surrounding the cut; from the resulting images we obtained a kymograph of the ablation (Figure 2.6.1) which we used to extract data such as initial retraction velocity and myosin concentration. (Details in Section 5.4.1 in Materials & Methods)

Results of the ablation experiment suggested that tension generated by myosin has a positive correlation with the concentration of myosin, and this relationship is likely to be non-linear. This is a promising result since our model requires non-linearity of the system in order for stable oscillations to occur, and this non-linearity can originate either from tension or elasticity.

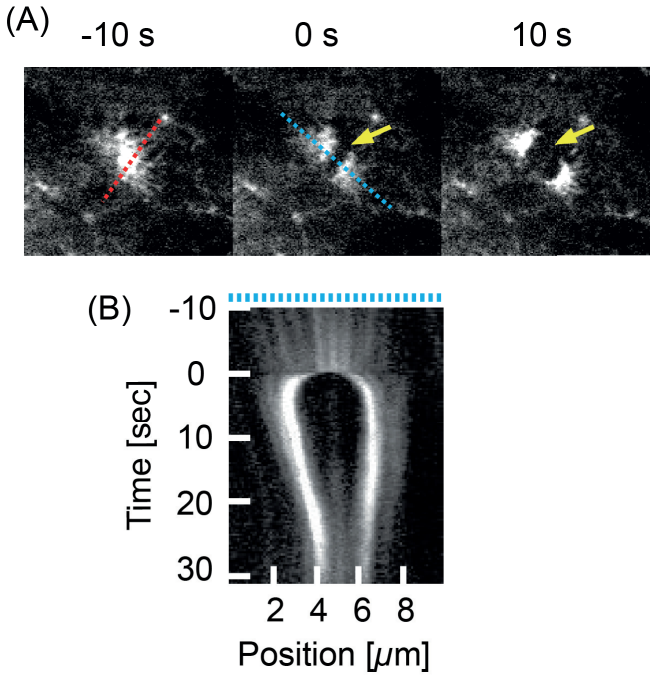


Figure 2.6.1: (A) Time lapse images of laser ablation being carried out on a contractile traveling wave in an embryo expressing Sqh-GFP. Red dotted line shows where the cut was performed and yellow arrows show retraction of surrounding region in response to the ablation. (B) Kymograph obtained by drawing a line (dotted cyan) across ablated region.

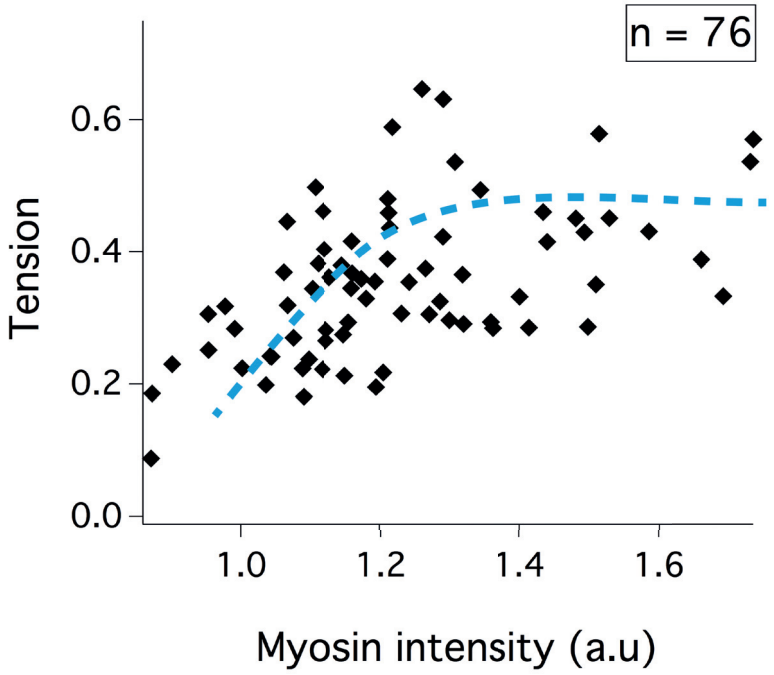


Figure 2.6.2: Plot of tension, T (or $T \propto \frac{A}{\tau}$) as a function of myosin concentration (M_c) shows a positive correlation between the two, that is likely to be saturating (Blue dashed line is just for a guide of the eye).

2.6.2 Pharmacological perturbation

In order to perturb the system, we injected embryos with pharmacological inhibitors of actin and myosin (Figure 2.6.3) followed by live imaging in order to study the dynamics of the perturbed actomyosin cortex and its effect on cell pulsation.

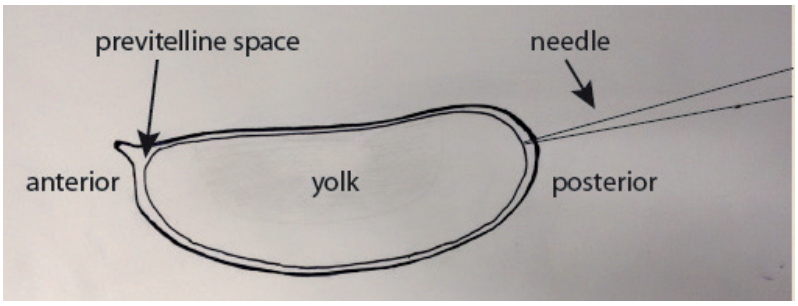
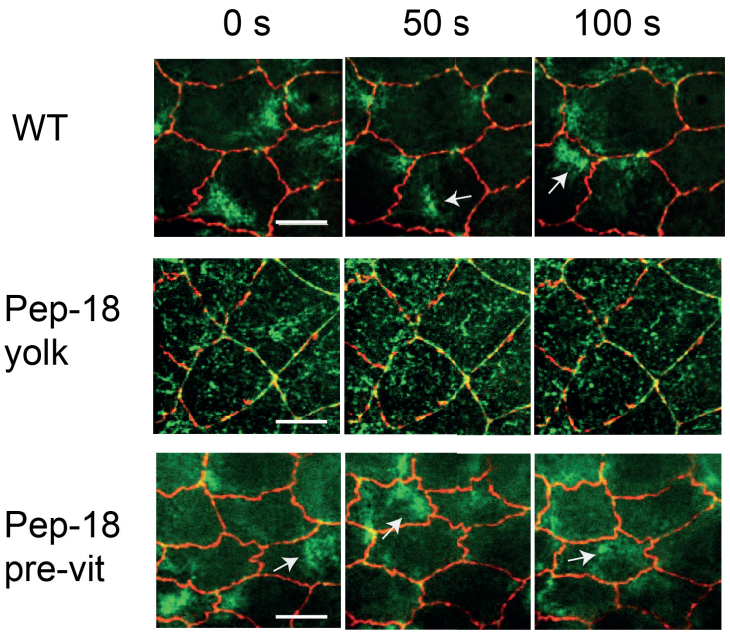


Figure 2.6.3: Schematic of microinjection

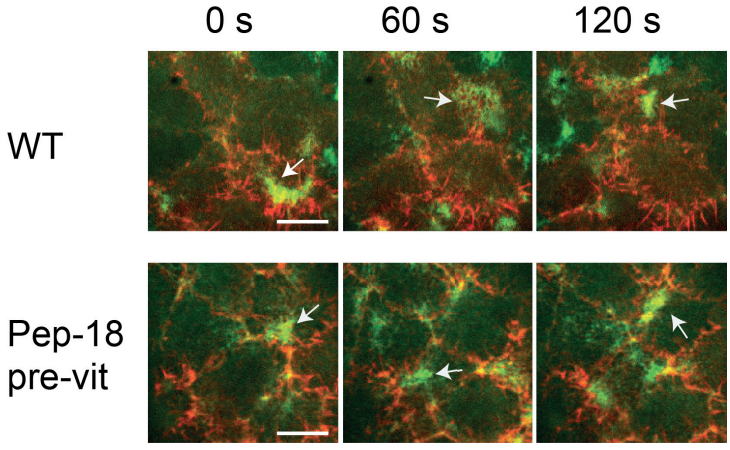
Peptide 18 inhibits myosin light chain kinase (MLCK) without interfering with calmodulin [Thomas J. Lukas et al., 1999] and has been shown to induce changes in cortical organisation consequently preventing sperm induced chromatin reorganisation in mouse oocyte [Deng et al., 2005]. Injection of $100\mu M$ peptide-18 in the yolk had no effects on actomyosin waves or cell pulsation, however, increasing concentration to $500\mu M$ led to complete inhibition of actomyosin waves 3 hours post-injection and death of injected embryos (n=9) (Figure 2.6.4a middle row).

Next, we injected the same concentration in the previtelline space and observed the embryos (n =5) for up to 3 hours post-injection but

there was no effect on the actomyosin waves (white arrows in Figure 2.6.4a). In order to visualise both myosin and actin activity we injected increasing concentrations of peptide-18 (5mM and 10 mM) in embryos expressing Tomato E-cadherin and Sqh-GFP (n =5) as well as those expresing sqh-GFP and Mcherry-Moe (n=5). However, we did not observe any phenotype that was striking or different from WT, and the waves appeared normal (white arrows in Figure 2.6.4b).



(a) Representative maximum projection images of cells from a WT embryo (top), embryo injected with 500 μM peptide-18 in yolk (middle), and 500μM peptide-18 in pre-vitelline space (bottom). Arrows show actomyosin waves. Cell junctions visualised with Tomato E-cadh and myosin with Sqh-GFP. Scale bar: 10μM



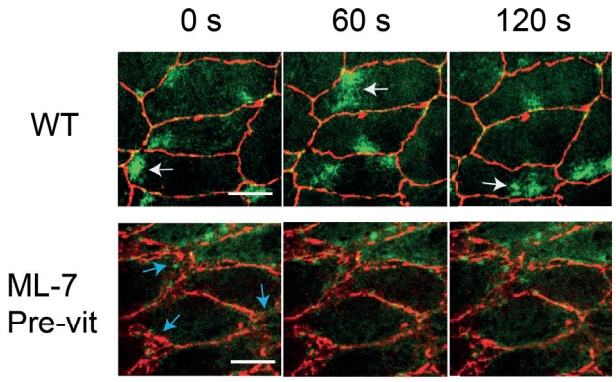
(b) Representative maximum projection images of cells from WT embryo (top) and embryo injected with 10mM peptide in pre-vitelline space (bottom). Arrows show actomyosin waves. Actin visualised with Mcherry-moe and myosin with Sqh-GFP. Scale bar: 10μM

Figure 2.6.4: Microinjection of MLCK inhibitor peptide-18

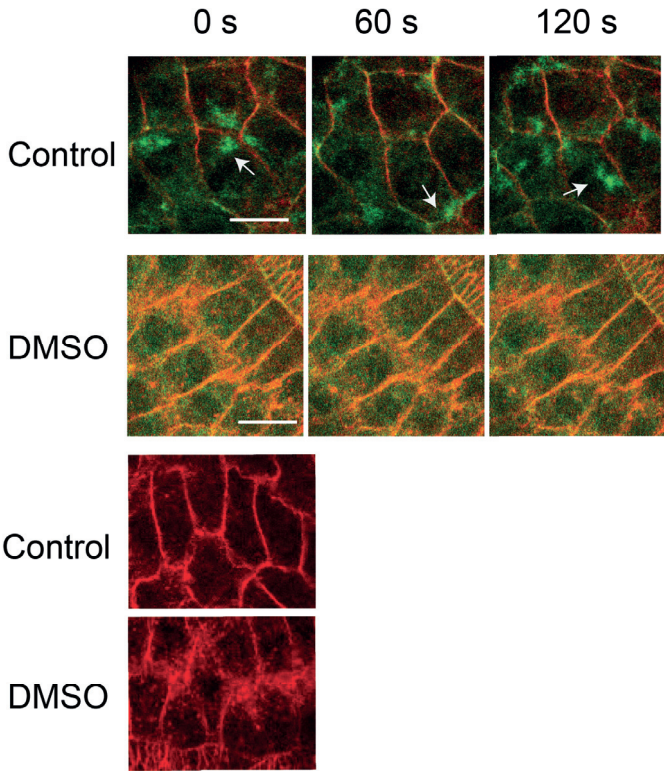
Next we used ML-7, a competitive inhibitor for ATP that has been shown to affect myosin in epithelial cell cultures (MCF-10A and MDCK) [Connell and Helfman, 2006]. However injection of $20mM$ ML-7 affected not only actomyosin waves and arrested cell pulsations but also disrupted cell-cell adhesion(Figure 2.6.5a).

To test if this cell junction defect was due to DMSO in which ML-7 had to be dissolved, we injected a control embryo expressing sqh-GFP with a dye FM4-64 (a membrane marker) and FM4-64 mixed with DMSO. We found that DMSO injected embryos (n=7) indeed showed an arrest in actomyosin waves and cell pulsation as well as disrupted cell junctions (Figure 2.6.5b).

Due to this observation, we discontinued injection of ML-7 as well Latrunculin-A which we were using at the time to perturb actin dynamics.



(a) Representative maximum projection images from WT embryo and embryo injected with $20mM$ ML-7 in pre-vitelline space. White arrows show waves in WT while blue arrows show disrupted cell junctions in ML-7 injected embryos. Cell junctions visualised with Tomato E-cadh and myosin with Sqh-GFP. Scale bar: $10\mu M$



(b) TOP: Representative maximum projection images from embryo injected with dye FM4-64 (control) and dye+DMSO in pre-vitelline space. Cell junctions visualised with dye FM4-64 and myosin with Sqh-GFP. White arrows show waves in control cells. BOTTOM: Cells from control embryo compared with those injected with DMSO. Scale bar: $10\mu M$

Figure 2.6.5: Microinjection of MLCK inhibitor ML-7 and test of DMSO

2.6.3 Molecular perturbation

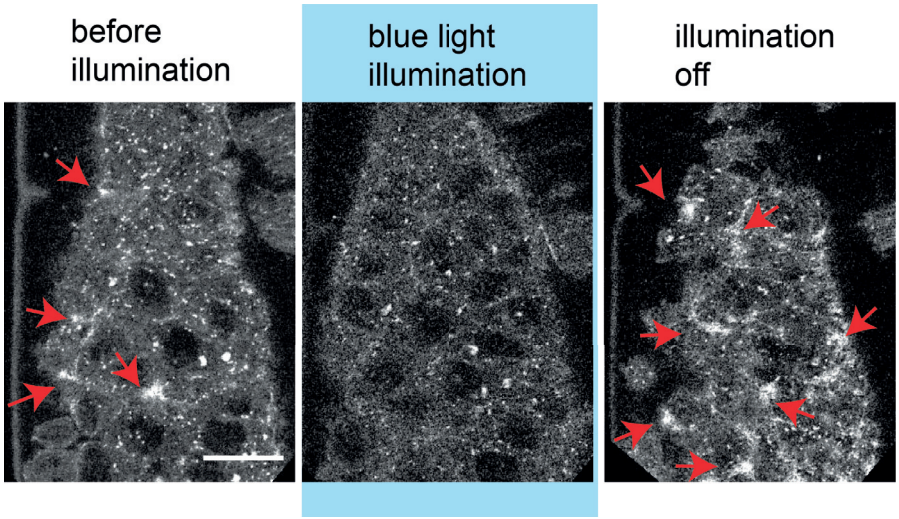
cAMP manipulation reveal mechanical coupling of AS cells

Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger involved in various cellular events and complex biological processes including intracellular signal transduction, activation of protein kinases and regulation of Ca2+ through the ion channels [Bugrim, 1999]. cAMP levels can be manipulated through PAC which was first identified and isolated from the photosensory organelle of the protozoan *Euglena gracilis*, and was found to be an important photoreceptor [Iseki et al., 2002]. PAC is composed of one sub-unit of photoactivated adenylate cyclase alpha (Pacα) and two sub-units of Pacβ, and these sub-units show adenylate-cyclase activity which is strongly and reversibly activated by blue light [Yoshikawa et al., 2005]. PAC activation was shown to increase cyclic adenosine monophosphate (cAMP) in HEK293, in vivo in Xenopus oocytes as well as modify *Drosophila* behaviour. Further, the activity of Pacα was found to be 100 times higher than Pacβ [Schröder-Lang et al., 2007].

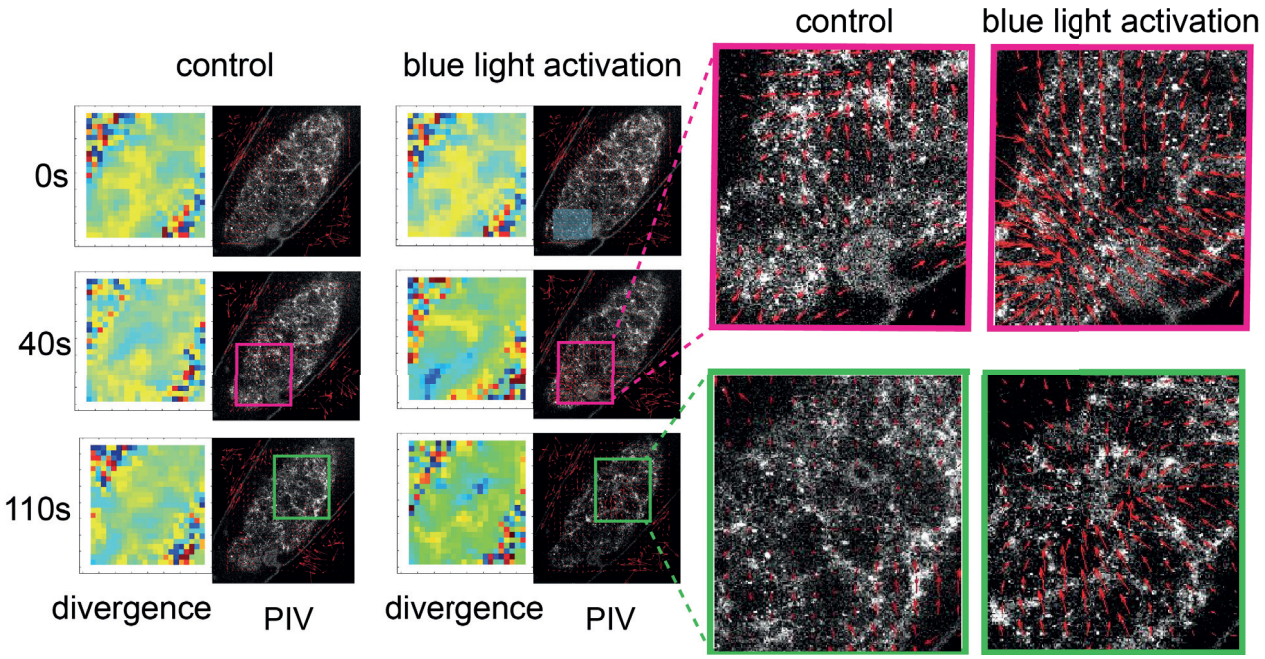
cAMP has been shown to affect dendritic formation in melanocytes by upregulating Rac activity and down-regulating Rho activity [Scott and Leopardi, 2003]. Rho and Rac are members of the family of guanosin triphosphate (GTP)-binding proteins that are known to regulate cy-

toskeletal reorganisation by binding to a variety of protein kinases as well as actin binding proteins [Sit and Manser, 2011, Nobes and Hall, 1995].

Therefore in order to manipulate cAMP we used the UAS-Gal4 system to express Pacα specifically in the amnioserosa. We visualised the tissue with Sqh-Cherry and used blue light (488nm) to activate the Pacα. Illuminating blue light for 2-5 minutes on an entire embryo appeared to induce a relaxation of the tissue (Figure 2.6.6a, middle image, Movie_05a) and decreased activity of actomyosin, which was followed by strong contraction of the actomyosin upon removal of blue light (Figure 2.6.6a, right image).



(a) Time-lapse images from a representative embryo expressing Sqh-Cherry in which blue light was illuminated on the whole embryo. Scale bar is 20μm



(b) PIV performed on control embryo (left) and divergence obtained from the PIV, on the right, the embryo was illuminated with blue light in a ROI (shaded blue box). Inset: Comparison of PIV obtained from control and light activated tissue. Note the arrows pointing inwards in the light activated tissue. Time 0s is the time at which the blue light is turned off.

Figure 2.6.6: Manipulation of PACα in the amnioserosa

The response of the tissue upon illumination with blue light led us to investigate a scenario where only a subset of the tissue was perturbed. This was to study whether cells in this tissue are mechanically coupled. For this, we illuminated a region of interest (ROI) that caused contraction in a subset of tissue around the ROI, we observed that this contraction triggered another contraction in the neighbouring tissue. This flow of contraction could be captured by particle image velocimetry (PIV), which allows the measurement of velocity fields by taking two images that have been captured a certain time apart and calculating the distance travelled by features on the images within this time period.

As shown in Figure 2.6.6b (as well as in Movie_05b), the arrows show contraction in the ROI after illumination with blue light, which is followed by another contraction in the nearby region. This can also be observed in the divergence plot which is obtained from the vector field of the PIV, in this case the divergence is negative (colour coded blue) since the vectors are pointing inwards. This result suggests that amnioserosa cells are mechanically coupled such that contraction in a region is able to influence the behaviour of cells in the neighbouring tissue.

The role of calcium in cell contractions has been previously addressed in a study on the amnioserosa [Hunter et al., 2014]. Further, cAMP and Ca2+ are highly interconnected at the level of second messenger

generation as well as at the level of the intracellular processes at which they target. cAMP can influence Ca2+ levels by acting on Ca2+ channels and pumps while Ca2+ can activate or inhibit certain isoforms of the adenylyl cyclase.

Therefore, in order to investigate the role of Ca2+ in actomyosin waves and cell pulsation, we injected the calcium chelator EGTA-AM (cell permeant) in the previtelline space of the embryos. A concentration of 100mM EDTA-AM did not show any effects on the waves (n=20) while 50% of embryos (n=13) injected with 300mM showed complete arrest of waves and consequently, cell pulsations (Figure).

Next we injected calcicludine, a peptide isolated from the venom of the green mamba (*Dendroaspis angusticeps*) that specifically blocks calcium channels. However, none of the embryos (n=5) showed any defects in actomyosin behaviour. However, further experiments and data are required in order to get a clearer understanding on the role of Ca2+ in actomyosin waves.

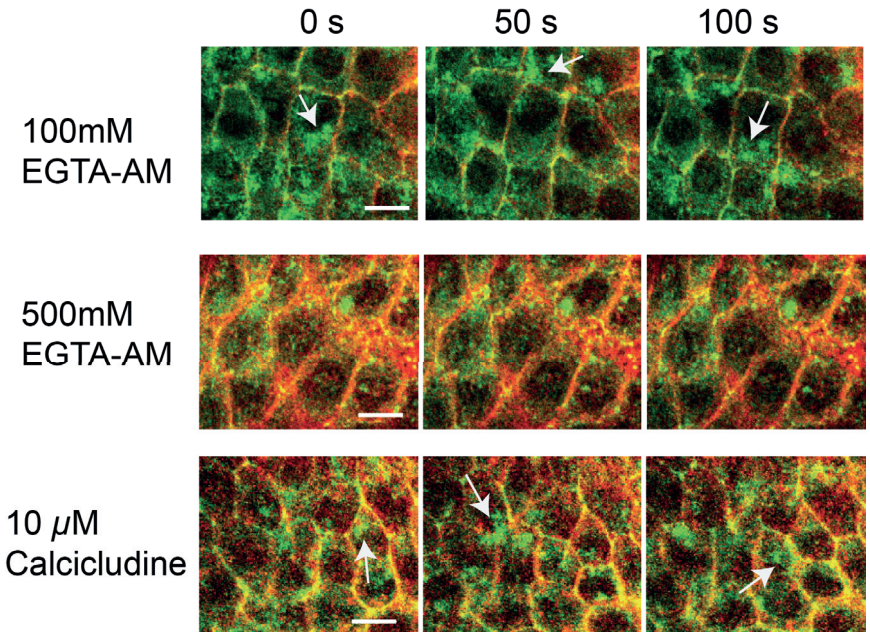


Figure 2.6.7: Top panel: Maximum projection images of cells from embryo micro-injected with 100mM EGTA-AM (top) show actomyosin waves (white arrows) which are not observed in those injected with 500mM (center). Bottom panel: Injection of Calcicludine (calcium channel inhibitor) had no effects on waves (white arrows). Scale bar 10 μM

Perturbing actomyosin dynamics reveal role of myosin activity in wave propagation

As mentioned earlier, Rho proteins regulate actomyosin contractility and reorganisation. The next approach we took to perturb Rho signalling pathway was by over-expressing DRhoGEF2 (*Drosophila* Rho guanine nucleotide exchange factor 2) which is an upstream regulator of this pathway. It has been previously shown that DRhoGEF2 regulates amnioserosa cell pulsations and apical constriction [Azevedo et al., 2011].

Overexpression of DRhoGEF2 in the AS with a Gal4 c332.3 led to

a change in actomyosin behaviour. In a WT cell, actomyosin contraction usually occurs in anti-phase with the neighbouring cell. However, in DRhoGEF2 expressing cells, contractions occur simultaneously in groups of cells (See frame at 80s in Figure 2.6.8, Movie_06). Further, actomyosin pulses form in discrete regions of a WT cell leading to anisotropic deformation of the area (WT, white arrows in Figure 2.6.8), while in DRhoGEF2, contraction formed across the entire cell at the same time and ended at the medial region of the cell, leading to an isotropic contraction of the area.

Next, to carry out perturbation more specific for myosin, we expressed a constitutively active form of the myosin light chain kinase (MLCKct). In this case, the actomyosin formed multiple puncti in the cells that did not propagate as waves (Figure 2.6.8, Movie_07). These two experiments suggest that the propagation of actomyosin pulses as waves is tightly regulated by the activity of myosin.

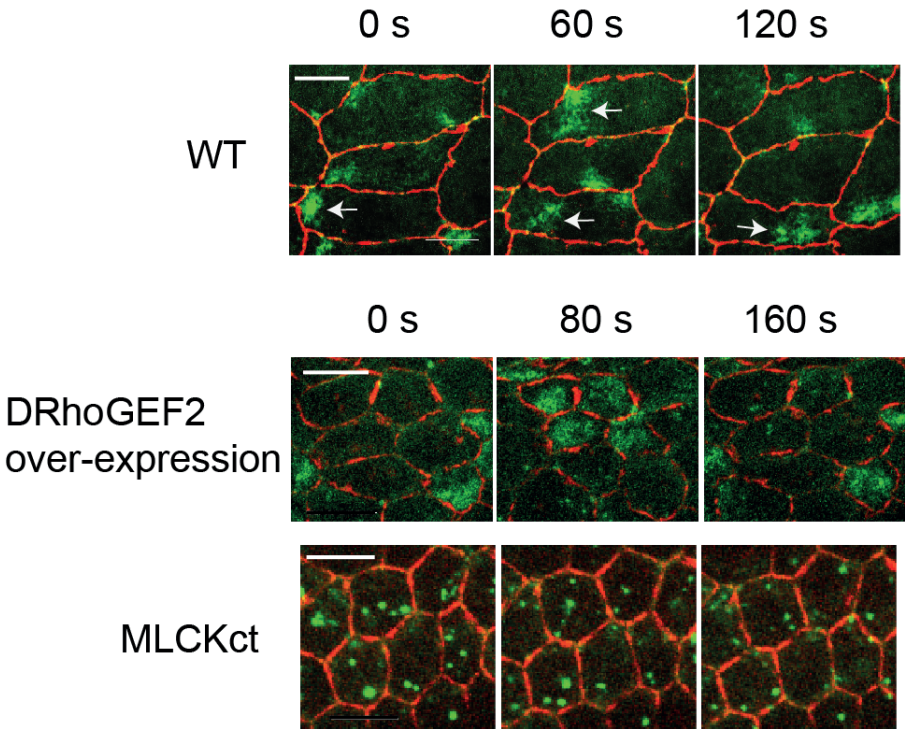
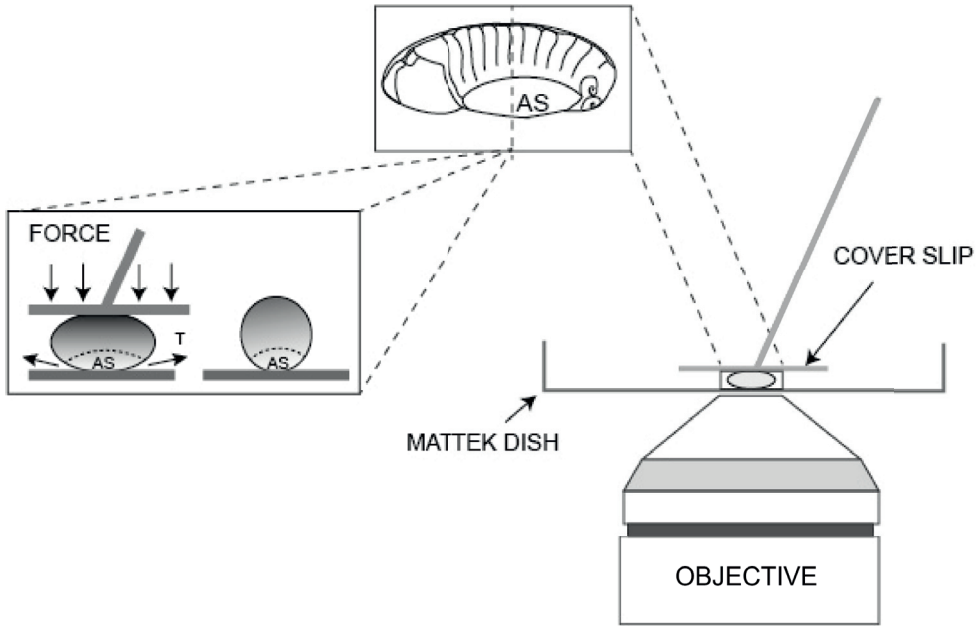


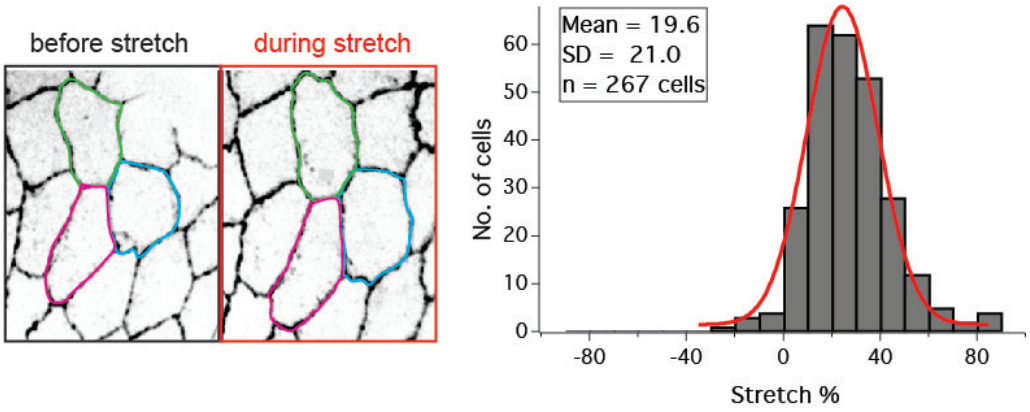
Figure 2.6.8: Actomyosin wave propagation is regulated by myosin activity: Time lapse images showing myosin pulse and waves (white arrows) in WT cells (top). In cells over expressing DRhoGEF2, no local pulses or waves are observed, instead myosin coalesce at the same time in multiple cells and contract in the centre of the cell (middle). Myosin waves are also absent in MLCKct, instead forming strong multiple puncti. Scale bar is $10\mu m$.

2.6.4 Mechanical perturbation

Mechanical perturbation is always a means to probe the physical properties of any system. However, there are still no well-established methods for tissue-scale mechanical stimulation of living embryos. For this reason we developed a novel method with which we could subject the tissue in an embryo to mechanical stress, and capture with live-imaging the response of cellular components to such stress. The set-up consisted of a micromanipulator to which a glass capillary was attached, the tip of the capillary was glued with a cover slip of 5mm diameter. With the cover slip we applied down-ward force on the embryo which was glued to a mattek dish and covered with 1% PBS solution. Since the volume of the embryo remains constant, this could lead to an increase in outward lateral tension that would pull on and stretch the tissue (Figure 2.6.9a).



(a) Experimental set-up for applying mechanical pressure on embryo



(b) Representative cells before and during stretch. Histogram shows instantaneous stretch (S_i) percentage.

Figure 2.6.9: Experimental set-up for stretching tissue and resulting increase in cell areas

The AS tissue was imaged on a confocal microscope for approximately 15 minutes before stretch application in order to capture cell shape changes and actomyosin behaviour. Imaging was continued while force was being applied on the embryo as well as after release allowing us to capture cellular behaviour with minimal loss of information. From the images obtained, cell outlines were segmented and tracked with a semi-automated software Packing Analyzer [Aigouy et al., 2010]. The resulting output was then used to extract data such as cell area and myosin intensity within the cell over time.

We found that we were indeed able to stretch the cell surface area by $\sim$20% (n = 267 cells). Stretch was calculated as either instantaneous (S_i) or average ($S_{av.}$) stretch, where $S_i = \frac{A(t_0)-A(t_0-1)}{A(t_0-1)}$ and $S_{av}=\frac{A(t_0)-<A(t)>}{<A(t)>}$.

Here, t_0 is the first time-point at which cells were stretched, and the mean $< A(t) >$ was calculated over a period of $\sim$5 minutes before stretch application. Both methods of calculating stretch yielded similar results. In the Figure 2.6.9b, the histogram is that of the instantaneous stretch.

Strikingly, application of stretch arrested cell shape pulsation and actomyosin activity (Figure 2.6.10, Movie_08a).

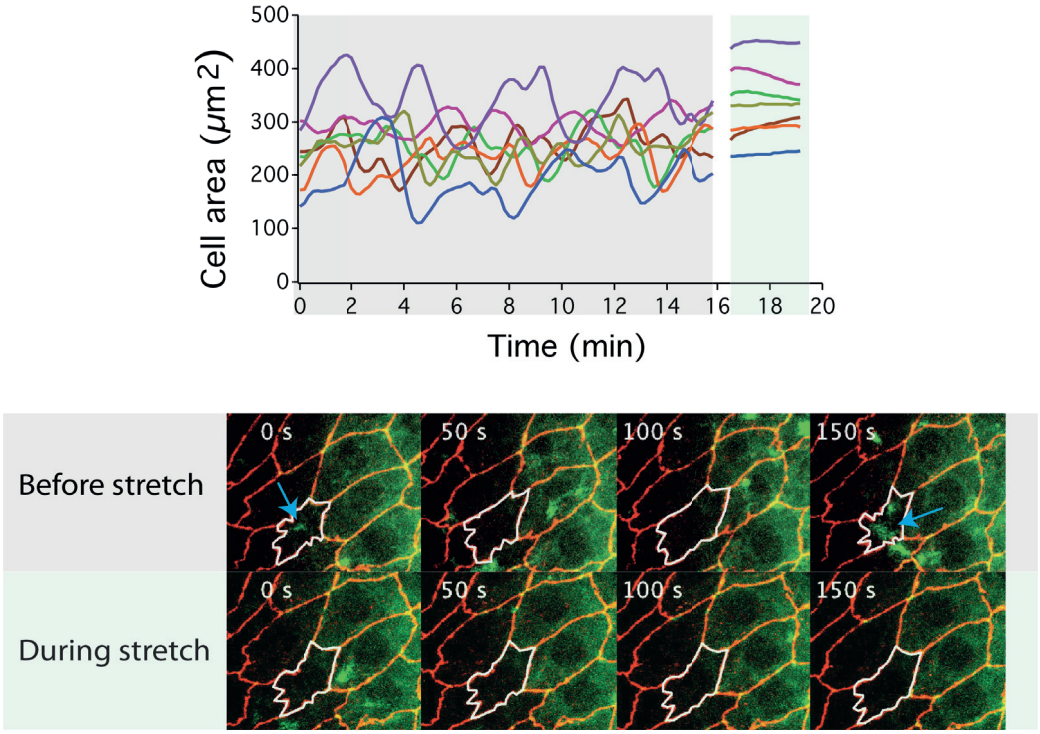


Figure 2.6.10: Top: Plot shows the area of a group of cells (from same embryo) over time. Each colour represents a cell. During the phase before stretch (grey shade), cell areas are fluctuating, while during application of stretch arrests cell pulsation (green shade). Bottom: Time lapse images of a representative cell (outlined in white) showing pulsation and actomyosin contraction (blue arrow) before stretch. During stretch, the same cell no longer show actomyosin waves or area fluctuation over time (Observe shape of white outline)

This suggested that we were able to force the cell areas into a new state and maintained in that regime. According to our cortex turnover model, the cortices would at some time-point reach an equilibrium with respect to cell area due to turnover. Thus, we assume all cells to be prepared in a similar initial state, with (i) their areas stretched to similar levels and (ii) their cortices at equilibrium concentration w.r.t cell areas. However, the cortices would reach this equilibrium state only if the timescale (T) of

stretch application was longer than or similar to the timescale of cortex turnover (τ) (Figure 2.6.13).

Indeed we observed that when stretch was released, there was a strong and simultaneous contraction of myosin at the apico-medial region of cells. This suggested that the cells had reached the same initial state where the cortices were in equilibrium with respect to the cell area.

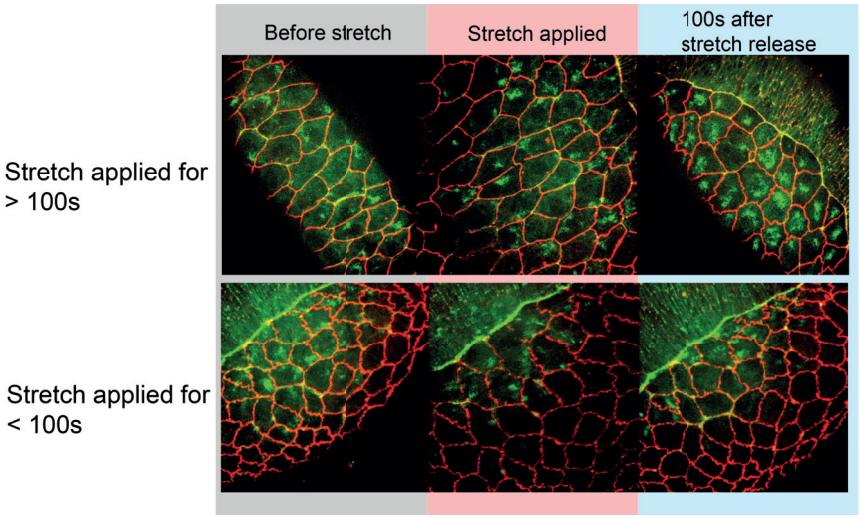
To verify, we applied stretch for different duration and observed that such response occurred only in situations where the time-scale of stretch application (T) was more than 100 seconds (Figure 2.6.11a, Movie_09a,b). This is consistent with our findings for the cortex turnover time-scale (τ) of 108 $\pm$ 66s as mentioned earlier in Section 2.5.1 of this chapter.

Next, we quantified this "synchronicity" based on the method of averaging trajectories to determine synchronicity of oscillating elements, whereby elements that are totally out of synch would output a flat trajectory while the output of elements that are highly synchronised would be similar to the original data.

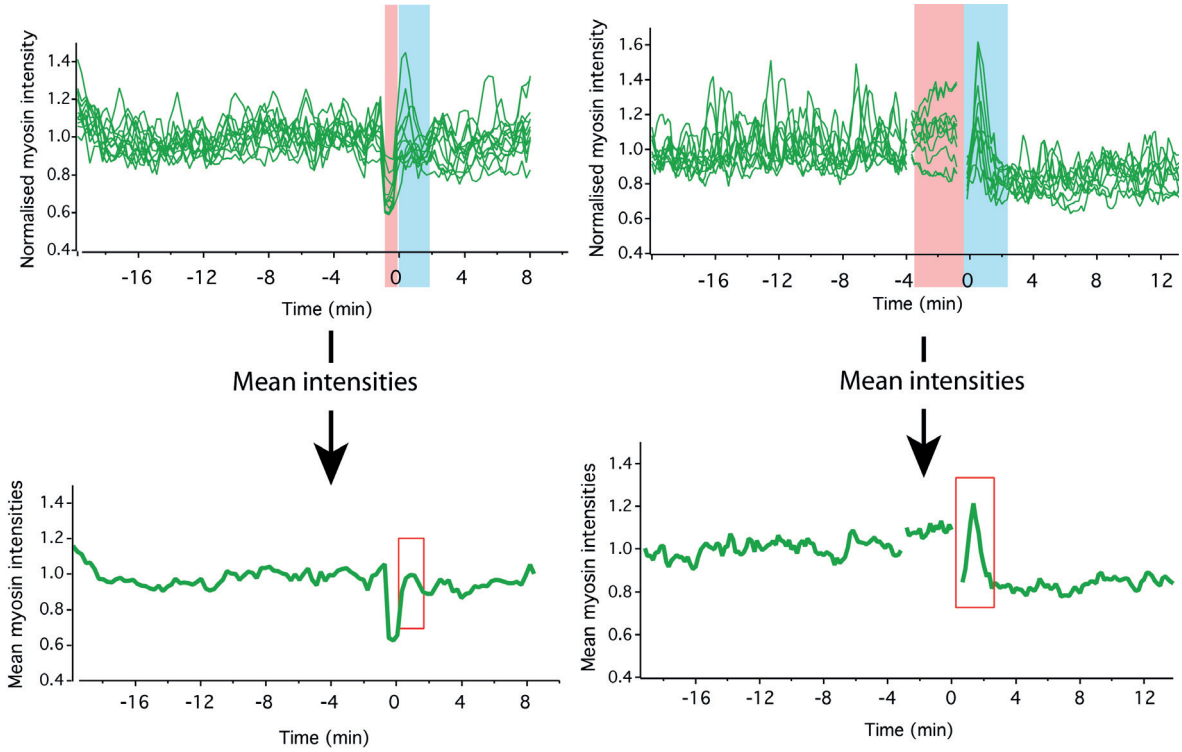
We averaged the areas and myosin intensities within the same cells to obtain their respective means from which we extract the ratio of the amplitude (A) of the output after release of stretch (red box in Figure 2.6.11b) and the standard deviation (ρ) of the mean area and myosin before application of stretch. From this we obtained a synchronisation

index, $S = (\frac{A}{\rho})$, also described in Figure 2.6.11b.

The synchronisation index was then plotted against the duration of stretch, which shows that synchronisation is stronger around 100s which then plateaus for longer durations of stretch.



(a) **Application of stretch for $T \geq \tau$ synchronises cells via turnover contraction upon release**: Time-lapse images showing cells before and during stretch, and 100s after release of stretch. In the top panel, embryo was stretched for $> 100s$, following release myosin strongly and simultaneously contract at the apico-medial region of cells. This is not observed in the case of the embryo that was stretched for $< 100s$ (lower panel). Cell junctions visualised with Tomato E-cadh and myosin with Sqh-GFP.



(b) Myosin intensities in a group of cells taken from the embryos shown in Figure (a) are averaged to obtain their respective mean intensities (pink shade is when stretch is applied, and blue shade is when stretch is released). From the mean intensity, we obtain the synchronisation index, $S = \frac{A}{\rho}$, where A is the amplitude after stretch release (red box), and ρ is the standard deviation of the mean intensity before stretch application.

Figure 2.6.11: Cortices are synchronised to a steady state via turnover in cells where shape pulsations have been arrested by stretching.

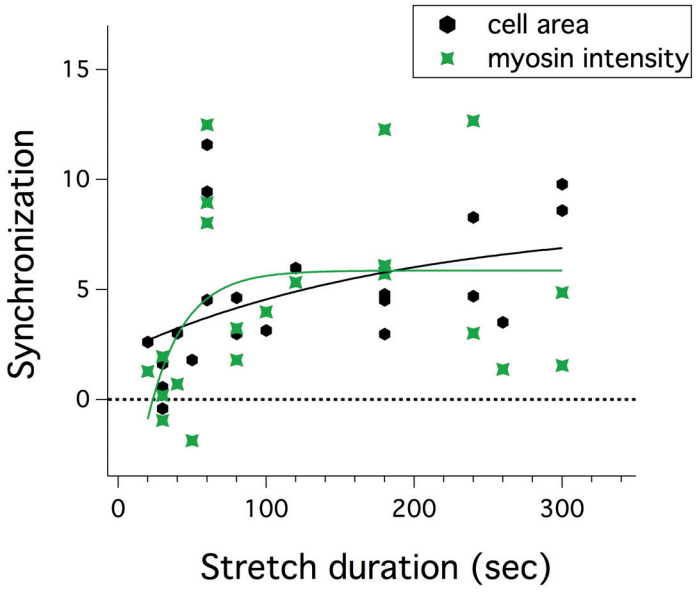


Figure 2.6.12: Synchronisation (S) plotted as a function of duration of stretch application. Each data point is an embryo. Plot shows synchronisation for both area and myosin.

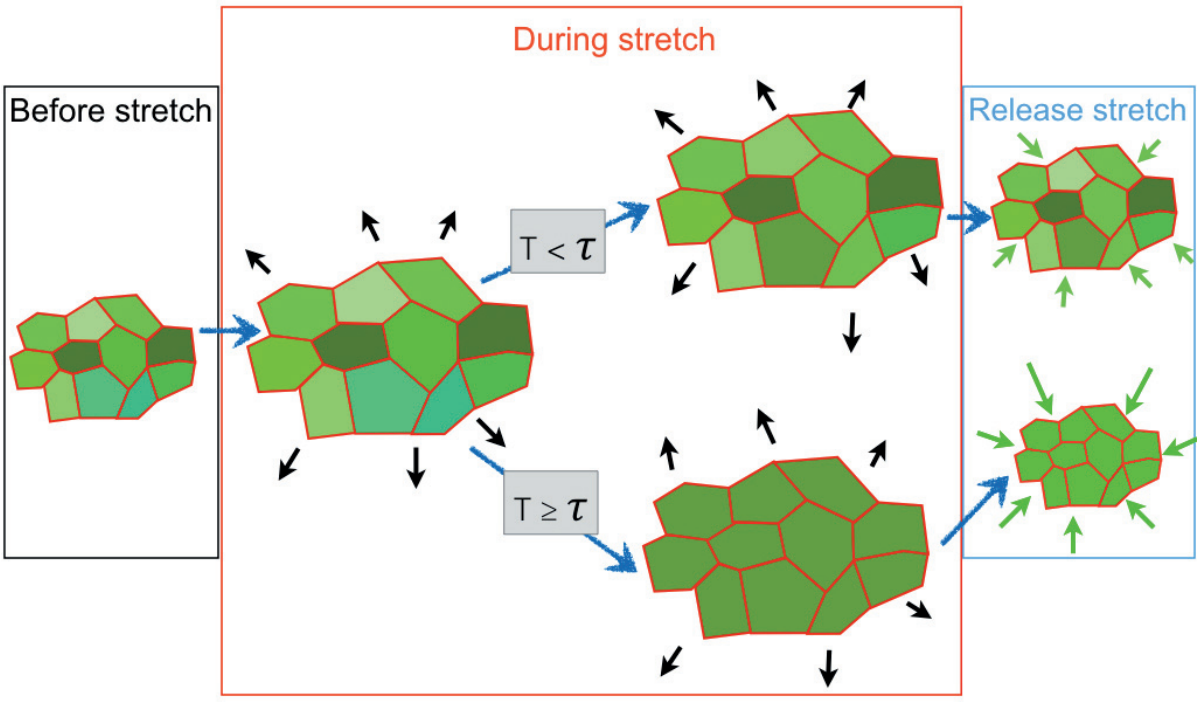


Figure 2.6.13: **Hypothesis for synchronisation of a group of cells based on cortex turnover**: Before stretch, cells will be at different stages (colour codes) of their oscillatory cycle while the cortices are constantly turning over w.r.t to the change in cell area. When stretch is applied, cells are forced into a new regime. Maintaining the cells in this regime for a timescale which is bigger than τ will bring the cortices in all cells to the same steady state due to turnover. T is duration of stretch application, while τ is cortex turnover time.

Summary

1. Laser ablation of contractile myosin waves revealed a non-linear relationship between tension and myosin concentration. This is consistent with our model's requirement for non-linearity of the system.

2. Inducing contraction in a subset of AS cells with an optogenetic tool led to contraction in the neighbouring cells suggesting a level of mechanical coupling in the AS tissue.

3. Based on results from molecular perturbation of the contractile machinery, we suggest that propagation of contractile actomyosin pulses is tightly regulated by activity of myosin.

4. We developed a novel technique with which we are able to stretch and maintain the areas of AS cells at a new state. Consistent with out model's prediction, such perturbation leads to the synchronisation of cells via cortex turnover.

Dierkes K, Sumi A, Solon J, Salbreux G. Spontaneous oscillations of elastic contractile materials with turnover. Phys Rev Lett. 2014 Oct 3;113(14):148102. DOI: 10.1103/PhysRevLett.113.148102

Chapter 3: A role for contractile actomyosin waves in junctional remodeling in constricting epithelial cells

3 A role for contractile actomyosin waves in junctional remodelling in constricting epithelial cells

3.1 Cellular response to mechanical force

Mechanical forces are ubiquitous around living organisms and cells are able to sense and respond to these forces. Many processes at the cellular, sub-cellular or molecular level are governed by mechanical signals that complement biochemical signals in order to co-ordinate and maintain multicellular organisation and homeostasis. For example, it has been shown that in fibroblasts, cortical stiffness varies with the stiffness of ECM whereby stiffer matrices promote generation of stiffer cortices [Solon et al., 2007]. Next, a study revealed that mechanical force can regulate gene expression - *Twist* is normally expressed only on the ventral most cells of the *Drosophila* blastoderm, however, subjecting the embryo to a 10% uniaxial stretch induced the expression of this gene around the entire dorsal-ventral region leading to the development of a deformed embryo [Farge, 2003].

These responses and changes in cellular organisation is possible due to the cells' ability to convert physical forces, at the molecular level, into biochemical signals. Researchers from a wide range of fields - molecular cell biology, developmental biology, physiology, engineering and bio-

physics have now identified a plethora of mechanosensitive molecules that are responsible for this process of energy conversion, known as mechanotransduction. Cellular and extracellular components that have been shown to respond to mechanical stimuli include, among others, stretch activated ion channels, extracellular matrix (ECM), integrins, caveolae and cytoskeletal structures such as cadherin, myosin and actin. These molecules have been identified by subjecting cells and their components to mechanical stimuli using techniques that are described in the next section.

3.2 Techniques for mechanical stimulation of cells

Due to the complexity of in vivo environment, most techniques that have been developed to study cellular response to external mechanical force and deformation rely on *in vitro* systems. These techniques usually involve cell cultures on which controlled mechanical forces such as hydrostatic pressure, substrate strain and fluid shear stress are applied. One of the earliest works involving the mechanical stimulation of cell cultures was carried out on endosteal cells from chick tibiae which were grown on substrates made of intercostal muscle that were then attached to neighbouring ribs. The endosteal cell cultures were compressed over several days as the muscle tissue degenerated and pulled the ribs inwards

[Glücksmann, 1939]. In the 1970s, new experimental techniques that allowed quantitative experimentation were developed. These include hydrostatic pressure application on suspended isolated cartilage or bone cells [Rodan et al., 1975a, Bourret and Rodan, 1976], pneumatic piston compression of chick embryo tibiae mounted on tuberculin syringe barrel [Rodan et al., 1975b] and tensile straining of rat calvarial cells cultured on collagen ribbons [Yeh and Rodan, 1984]. More recently a wide range of tools have been developed and can be broadly classified into three types depending on their ability to deform 1. molecular and sub-cellular components or a portion of the cell, 2. an entire cell and 3. group of cells.

1. **Deformation of molecular and sub-cellular components**: The commonly used methods for this type of deformation include optical trapping (OT), atomic force microscopy (AFM) and magnetic twisting cytometry (MTC) shown in (Figure 3.2.1 a, b, c). In OT, a laser beam is focused on a spot or "optical trap" in the plane of the specimen, that can hold beads to which a biological specimen can be attached. This method has been used to study sub-cellular components such as actin and myosin [Finer et al., 1994, 1995] as well as membranes of red blood cells [Sleep et al., 1999, Hénon et al., 1999]. In AFM, deformation on the cell surface is carried out with

the tip of a flexible cantilever and the applied force is estimated by calibrating the deflection of the tip. Examples of experiments where AFM has been used to probe responses of single cells to deformation has been well reviewed by Haase and Pelling [2015]. MTC involves the attachment of ferro-magnetic microbeads to cell surfaces that are then subjected to a magnetic field which twists the bead and deforms the cell surface. These methods can provide information about the elastic or viscoleastic properties of the cellular component Kasza and Zallen [2011].

2. **Deformation of entire cell**: For this kind of experiment, the methods that are commonly used are micropipette aspiration (MA) and micro-post array deformation (mPAD) (Figure 3.2.1 d, e). In MA, the surface of the cell (either a region of the cell or the entire cell) is deformed by suction through a micropipette and the elastic response of the cell is studied, usually without taking into account the friction between the cell membrane and the walls of the micropipette [Evans, 1973, Evans and Yeung, 1989, Hochmuth, 2000]. mPAD devices are usually designed with 3-D microposts made of flexible material. The microposts are coated with extracellular matrix proteins in order to enable attachment and growth of cells. As cells move, the posts bend which can be translated into traction

force using beam bending theory that then allows for quantitative measurement of force between the cell and its substrate [Tan et al., 2003, Cheng et al., 2013].

3. **Deformation of a group of cells**: In order to perturb entire population of cells, shear flow methods and stretching devices are used (Figure 3.2.1 f, g,h). In shear flow experiments, cells can either be plated on to parallel-plate flow chambers and subjected to laminar flow [Usami et al., 1993] or plated onto a stationary flat plate and laminar flow applied with a rotating inverted cone [Simmers et al., 2007]. Next, stretching devices that consist of elastic membranes can be coated with a cell layer and stretched uniaxially or biaxially [Shao et al., 2013, Tremblay et al., 2014]. Recently, Harris et al developed a novel method in which cells were cultured on sacrificial collagen scaffolds between two test rods, allowing them to form a monolayer. The scaffold was removed with collagenase, leaving a suspended cell monolayer as shown in Figure 3.2.1 (h). These suspended monolayers were then stretched by pulling the test rods apart with a micromanipulator and then imaged to study cell structure and subcellular organization response to stretch [Harris et al., 2013].

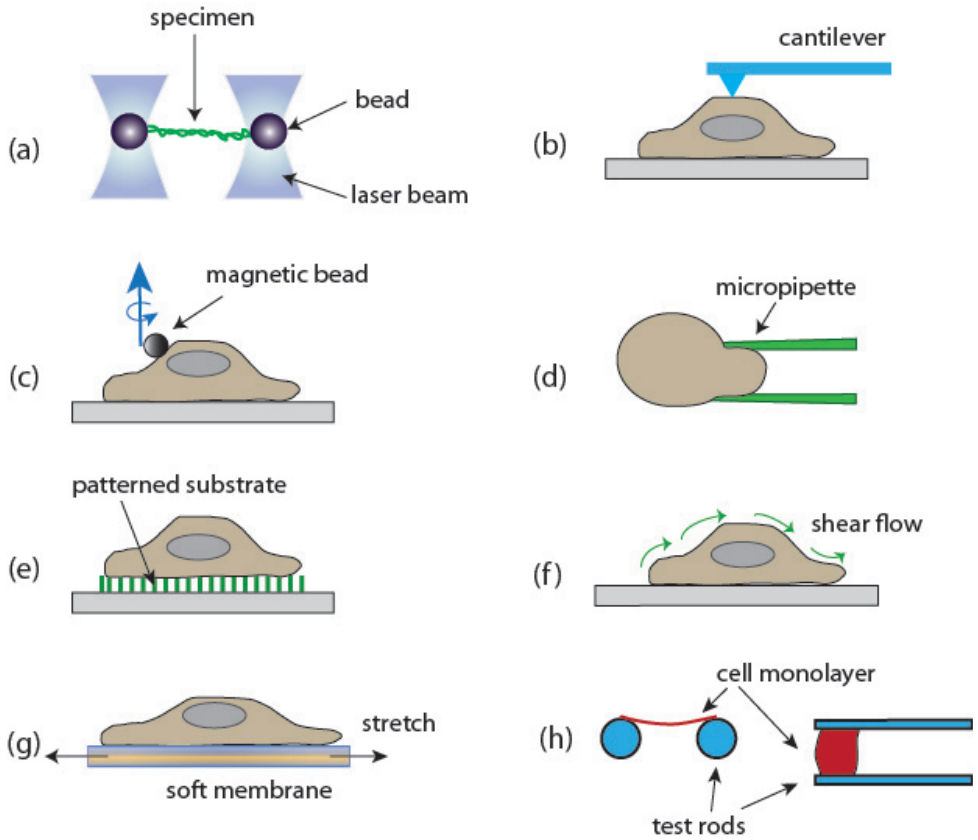


Figure 3.2.1: Techniques for mechanical force application: (a) Specimen is attached to two beads that are held by laser beam and deformed, (b) deformation carried out with a cantilever in AFM, (c) magnetic bead attached to a cell is stimulated by magnetic field, (d) suction with micropipette, (e) cell/group of cells are grown on micro-posts that are coated with ECM proteins, (f) cell/group of cells are subjected to deformation by shear flow (g) cell/group of cells are subjected to deformation by stretching the membrane on which they are plated, (h) A monolayer of cells is suspended between two test rods and stretched by moving the rods apart. Figure modified from [Ji and Bao, 2011] and [Harris et al., 2013].

The list mentioned above is not exhaustive, new techniques continue to be developed and added to the array of bioengineering tools and nano/micro-scale devices with which we can study the biophysical properties of the cell. These techniques have been widely used to study and assess the biophysical properties of cancer cells and stem cells and the

response of these cells to mechanical stimulation; one can find comprehensive reviews about work done on cancer cells Suresh [2007] as well as on stem cells [Sun et al., 2012, Kshitiz et al., 2012]. Similar studies have been done on cell cultures such as fibroblasts [Thoumine and Ott, 1997, Thoumine et al., 1999], MDCK cells [Harris et al., 2013], osteoblasts and HeLa cells [Charras and Horton, 2002, Charras et al., 2009], cells of mesenchymal lineage such as chondrocytes, osteoblasts and adipocytes [Darling et al., 2008] to name a few.

3.3 Cell cytoskeletal response to mechanical stress

So far a great deal of studies have looked at force generating components of the cell that is responsible for shape changes and rearrangement that sculpt a developing embryo, however, it is still not clear in which ways cells respond to mechanical stress. During tissue remodelling in embryogenesis, forces arise from various sources such as cell death, growth and division, as well as movements from neighbouring tissues. This means that tissues need to respond to extrinsic and intrinsic forces and ensure that the cells are returned to the preferred state of mechanical homeostasis. In this section we highlight the mechanisms by which tissues maintain integrity and dissipate stresses during mechanical perturbation of varying duration.

The response of cells and tissues to sudden and short time-scale (milliseconds to seconds) mechanical stimuli depends on the rate of redistribution of cytosol through a mesh of cytoskeleton, organelles and macromolecules. Such a response to short timescale mechanical stimuli is thought to give rise to a cell's poro-elastic property [Moeendarbary et al., 2013]. The other response of the cell to sudden stimuli is deformation of the plasma membrane, in order to prevent rupture and lysis, plasma membrane reserves are stored in folds called caveolae, that straighten upon stretch. Caveolae have been found to be associated with stress fibers and it has been suggested that this coupling provides cells with an important strategy for dealing with mechanical stress [Echarri and Del Pozo, 2015]. Conversely, the plasma membrane invaginates to form tubules and spheres upon sudden reduction of the cell surface areas through a process that is thought to be a purely mechanical process. These invaginations are instantaneous and their shapes are determined by the minimum elastic and adhesive energy required for storing membrane and liquid volume at the cell-substrate interface [Kosmalska et al., 2015].

At time-scales of few minutes, stress dissipation depends on the turnover of cortical components that allows the cytoskeletal network to adapt to mechanical perturbations [Sedzinski et al., 2011]. In this case, the pro-

tein structures that bear the load of external force undergo remodelling to allow the cells to adapt to the new environment [Mizuno et al., 2007]. It has been shown that an interplay of myosin power strokes along with a rapidly remodelling actin network leads to the regulation of force and cell shape in order for adaptation to environment stiffness [Étienne et al., 2015].

The response of the cortex to mechanical stress has been studied extensively in fibroblasts, epithelial and endothelial cells. Cultured endothelial cells react to uni-directional, laminar fluid shear stress by Rac1 and RhoA dependent reorganisation of their actin network and focal adhesion, such that the cells' long axis aligns in the direction of flow [Malek and Izumo, 1996, Tzima et al., 2005]. Epithelial cells have also been shown to preferentially orient their focal adhesions and stress fibers parallel to the direction of stretch [Saez et al., 2007]. Further, in fibroblasts, it was shown that the gradient of rigidity could dictate the direction of cell migration as cells were found to be migrating towards the stiffest area of the substrate [Lo et al., 2000]. In contrast, cyclic uni-axial stretching of various cell types led to an orientation that was perpendicular to the direction of strain. Endothelial cells [Dartsch and Betz, 1989, Naruse et al., 1998] and smooth muscle cells [Kanda et al., 1992] that were subjected to periodic uni-directional stretching and relaxing became elongated with

their long axis oriented perpendicular to the direction of stretch.

At longer time scales of minutes to hours, active cellular behaviours come into play to deal with cell shape changes resulting from deformation, and to dissipate stresses in these time-frames. Cellular processes such as neighbour exchange, division, fusion and extrusion allow a developing embryo to respond to and regulate tissue stress [Wyatt et al., 2016]. Thus, the response of cells and tissues to mechanical perturbation is achieved by different homeostatic mechanisms at different time-scales. We cannot be certain however, whether cytoskeletal responses that have been observed in cultured cells can accurately represent the response in living embryos.

A handful of studies have been carried out in embryos to study *in vivo* the mechanical properties of cellular components, for this, the commonly used methods are laser ablation [Colombelli and Solon, 2012], magnetic bead injection [Kumar and Shivashankar, 2012] as well as optical tweezers [Kapil Bambardekar, Raphaël Clément, Olivier Blanc, Claire Chardès, 2015]. However, there are still no well-established methods for tissue-scale mechanical stimulation of living embryos, for this reason we developed a method with which we could subject the tissue in an embryo to mechanical stress, and capture with live-imaging the response of cellular components to such stress.

We have looked at the cytoskeletal structures and adherens junctions in pulsatile AS cells that were stretched using the method described in Section 2.6.4 of Chapter 2. The results are presented below.

3.4 Results

All experiments leading to the following results were carried out by me, while the quantifications were carried out by Peran Hayes, a PhD student in our lab with whom I collaborated for this project. Figures obtained from his quantification will be clearly cited.

3.5 AS cell shape deformation induced by stretching

Using the method introduced in Section 2.6.4 of Chapter 2, we applied stretch on the AS tissue while continuing to image throughout the three phases of the experiment - before, during and after stretch release. This allowed us to capture cellular behaviour with minimal loss of information. From the images obtained, cell outlines were segmented and tracked with a semi-automated software Packing Analyzer [Aigouy et al., 2010]. The resulting output was then used to extract data such as area and myosin intensity over time.

In order to assess the deformation induced in cells by stretch, an "average cell" was obtained. For this, the centre of the cell was located and

distance from this point to the cell junction was measured for different angles to the anterior of the embryo. The distances for these angles were then averaged over all cells ($N = 87$) for 5 minutes during the three phases of the experiment: before and during stretch and after release of stretch. By plotting the distance from the junction as a function of the angle for the three phases, we see that the cells are stretched isotropically. Following release, the reduction in area of cells appear to be slightly anisotropic whereby the decrease is more significant in the dorso-ventral direction (Figure 3.5.1).

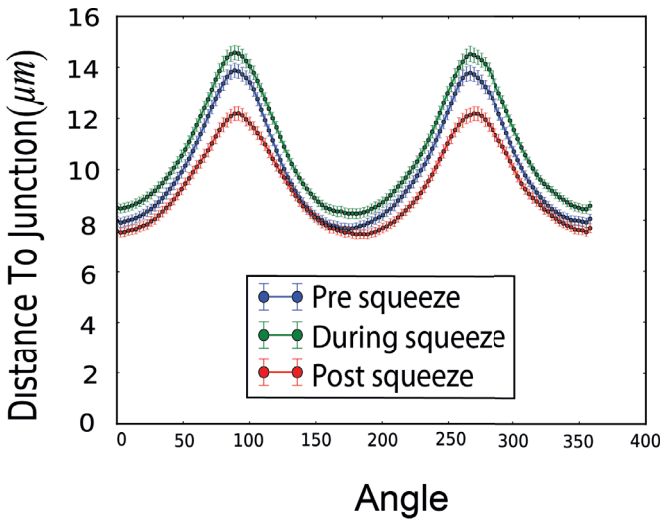


Figure 3.5.1: Plot of average cell showing deformation during stretching and release. The angles have been measured relative to anterior of embryo. Quantification by Peran Hayes.

3.6 AS cells display two states of actomyosin organisation that is stretch dependent

Application of stretch induced a change in the myosin behaviour from being random pulsatile waves to directed localisation at the cell junctions (Figure 3.6.1, middle row, Movie_10a).

To find out whether this is the case with actin, we applied mechanical force on embryos expressing Mcherry tagged to moesin (actin binding protein). The response of actin was similar in that the contractile pulses were no longer propagating as waves but rather localised to the junctions. Further, release of stretch led to a strong contraction of actin at the apico-medial region similar to myosin (Figure 3.6.2, Movie_10b).

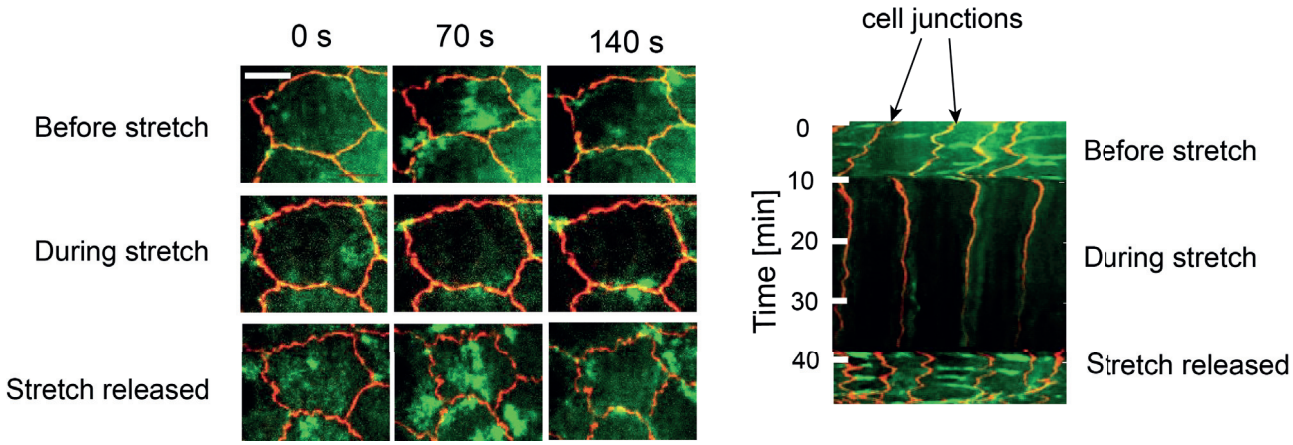


Figure 3.6.1: Left: A representative AS cell expressing Tomato e-cadherin (red) and Sqh GFP (green) before, during and after release of stretch application. Right: Kymograph of a subset of AS cells during stretching experiment. Scale bar: 10 μm

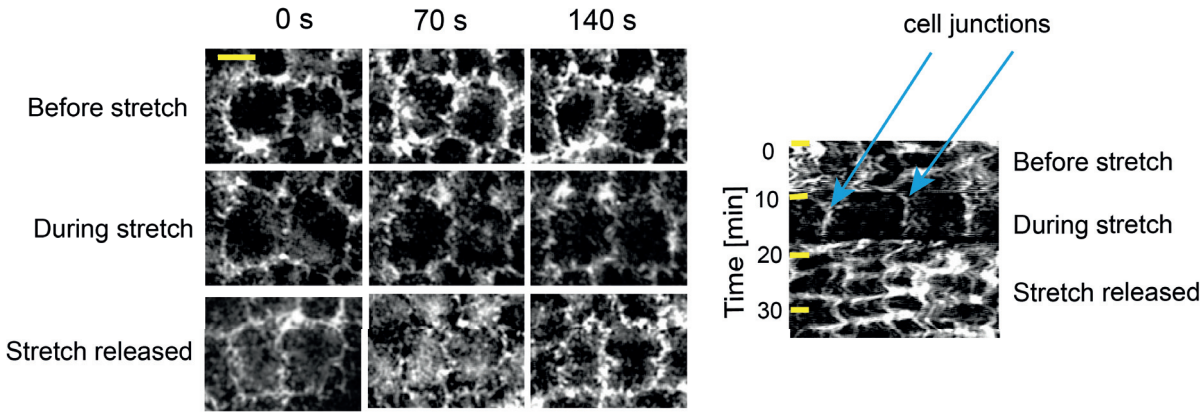
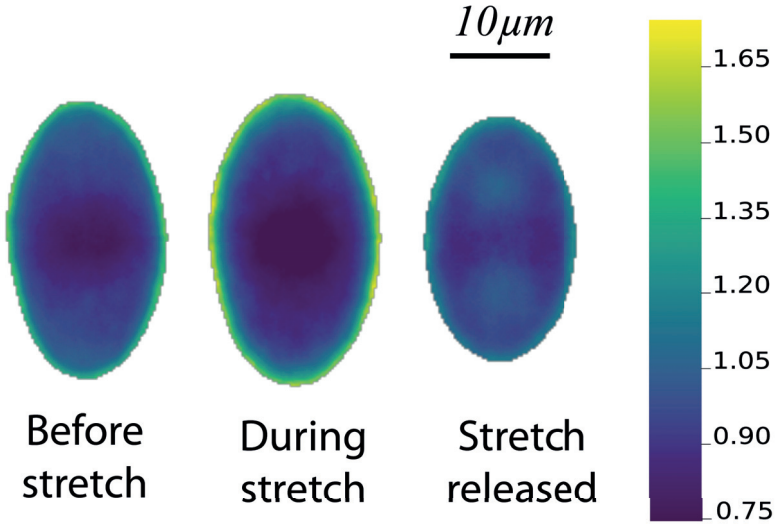


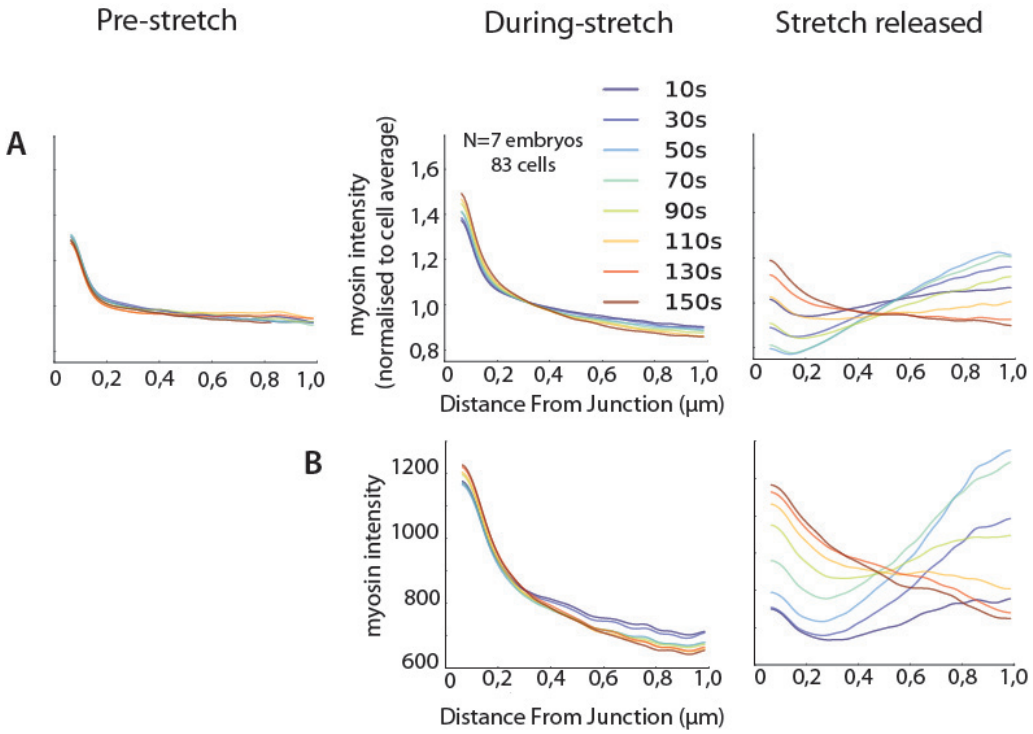
Figure 3.6.2: Actin behaviour during stretch application. Left: A subset of AS cells expressing Mcherry moesin (actin) before, during and after release of stretch application. Right: Kymograph of a subset of AS cells expressing Mcherry moesin during stretching experiment. Scale bar: 10 μm.

In order to quantify myosin localisation at the junctions, the average myosin distributions were overlaid onto the average cell (details in Section 5.7.2 in Materials and Methods) as shown in Figure 3.6.3a.

Next, the intensity of myosin was plotted as a function of the distance from the cell junction towards the centre of the cell to obtain myosin distribution before, during and after release of stretch (Figure 3.6.3b, Section 5.7.3 in Materials and Methods). The colour codes are for various time-points during which the intensities were extracted - blue being the earlier time-points and red the last time-point. These results indicate that in general, myosin localisation is higher at the junctions, however, during stretch, myosin intensity increases to an even higher level at the junctions as seen at time-point 150s during stretch (Middle plot in Figure 3.6.3b).



(a) Cells from a WT embryo superimposed to obtain an average cell before and during stretch and after release of stretch. Heat map shows the intensity of myosin.



(b) Plot of myosin intensity as a function of the distance from the junction. (A) Myosin intensity normalised to cell average, and (B) non-normalised version of the same plot. Different colours show the time-points at which the intensities of myosin were extracted.

Figure 3.6.3: Quantification of myosin relocalisation to cell junctions upon stretch application. Quantifications by Peran Hayes.

Upon release, there is a strong contraction of actomyosin at the medial region (Figure 3.6.1 and 3.6.2 at 70s). The quantification for this is shown in Figure 3.6.3b where we see an initial, rapid increase in myosin signal towards the medial region. This increase takes place over a period of around 70s following which the myosin distribution return to pre-stretch levels. As mentioned earlier, we used intensities normalised to average cell values, however that might prevent us from being able to determine whether only the medial myosin signal decreases, only junctional myosin signal increases, or both. Therefore, we plotted non-normalised versions which indicated that both processes are occurring (Figure 3.6.3, B). When stretch is removed, cells return to pre-stretch conditions with shape pulsations and actomyosin pulses and waves.

This suggests that we are able to manipulate the system such that it can exist in two states depending on stretch that is induced by mechanical force application- a state where cells exhibit pulsatile shape changes with flows of contractile actomyosin pulses and the other where the shape is stabilised and actomyosin is localised at the cell junctions.

3.6.1 System adapts to mechanical stress

Upon application of stretch for longer durations ($>$ 10 minutes), actomyosin waves resumed along with cell area pulsation (Figure 3.6.4),

suggesting a level of adaptability in the system. The cycle length (distance between two peaks or troughs) of these new area pulsations were longer: 7.8 minutes compared to 3.6 minutes in WT ($N = 20$), however, the amplitude of the new oscillations were similar to the WT w.r.t their areas ($N = 36$) as shown in Figure 3.6.5.

We speculate that the pulsations (cycle length) could be slower in the new oscillation due to the frictional force between the cells and the vitelline membrane as a result of applying downward force on the embryo. There are two likely mechanisms leading to adaptation of the system to stretch - either an up-regulation of exocytosis of junctional proteins such as cadherin such that the junction is brought back to a homeostatic state with respect to cell area, or a decrease in normal rate of junctional shrinkage via endocytosis. The maintenance of junctional length via endocytosis will be discussed in more detail in later sections.

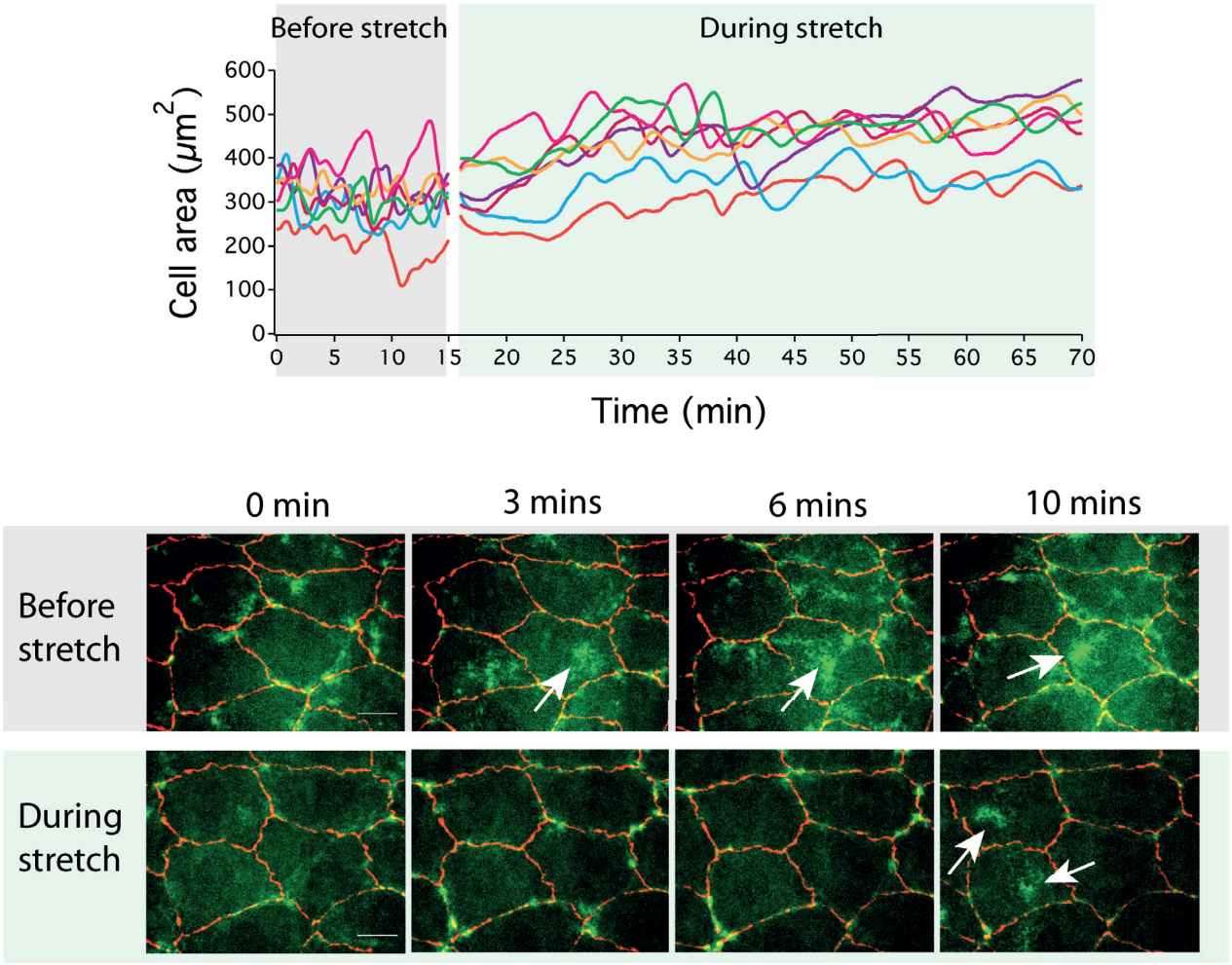


Figure 3.6.4: System shows adaptability to stretch application. Top: Plot shows the cell areas of a representative embryo during long duration stretch application. Bottom: Time-lapse images of cell shows actomyosin waves and cell area pulsation before stretch. During stretch, actomyosin waves reappear approximately 10 minutes in to application.

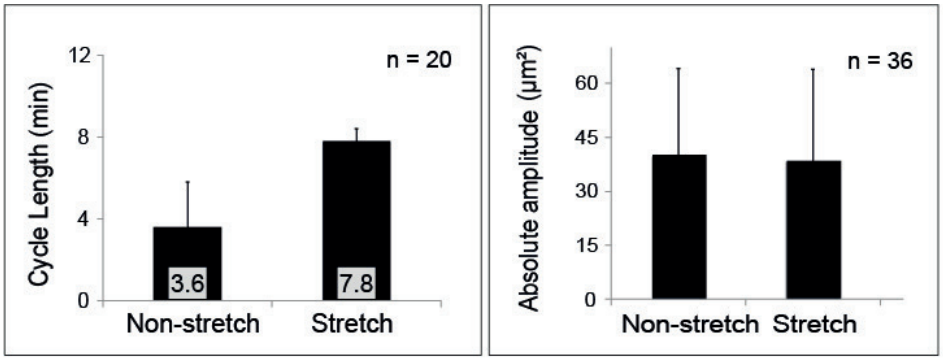


Figure 3.6.5: Cycle length and amplitude of cell pulsation in non-stretched and stretched cells

3.6.2 Inducing stretch in amnioserosa cell via genetic perturbation

Next, we asked whether we could induce a situation similar to mechanical stretching in AS cells using genetic approach. A previous study involving laser ablation has shown that the epidermis surrounding the dorsal gap is under tension and pulls in a direction opposite to closure. On the other hand amnioserosa contractility pulls inwards and counteracts this epidermal tension to facilitate closure ([Kiehart et al., 2000]). Therefore we speculated that decreasing contractility in the AS tissue would lead to the cells being stretched by the tension in the epidermis.

For this we inhibited myosin activity specifically in the AS by expressing MbsN300- a constitutively active form of the myosin-binding subunit of Myosin Light Chain (MLC) phosphatase. It has been shown that cells expressing MbsN300 have reduced apical surface tension ([Fischer et al.,

2014].

Cells expressing MbsN300 have a larger apical surface (Figure 3.6.7 and 3.6.10), which is consistent with previous observations ([Fischer et al., 2014, Saias et al., 2015]). These cells have reduced or no pulsing activity (Figure 3.6.6 and cells before stretch (blue line) in 3.6.10) similar to WT cells that have been stretched. In these cells, myosin activity is present albeit significantly more reduced than WT, and this activity is localised to the cell junctions rather than flowing across the surface area as contractile waves (Figure 3.6.6).

We speculate that the presence of myosin activity despite its down-regulation is due to the fact that we used an AS Gal4 driver (c332.3) that has a patchy expression pattern, which means that the phenotype generated is weaker.

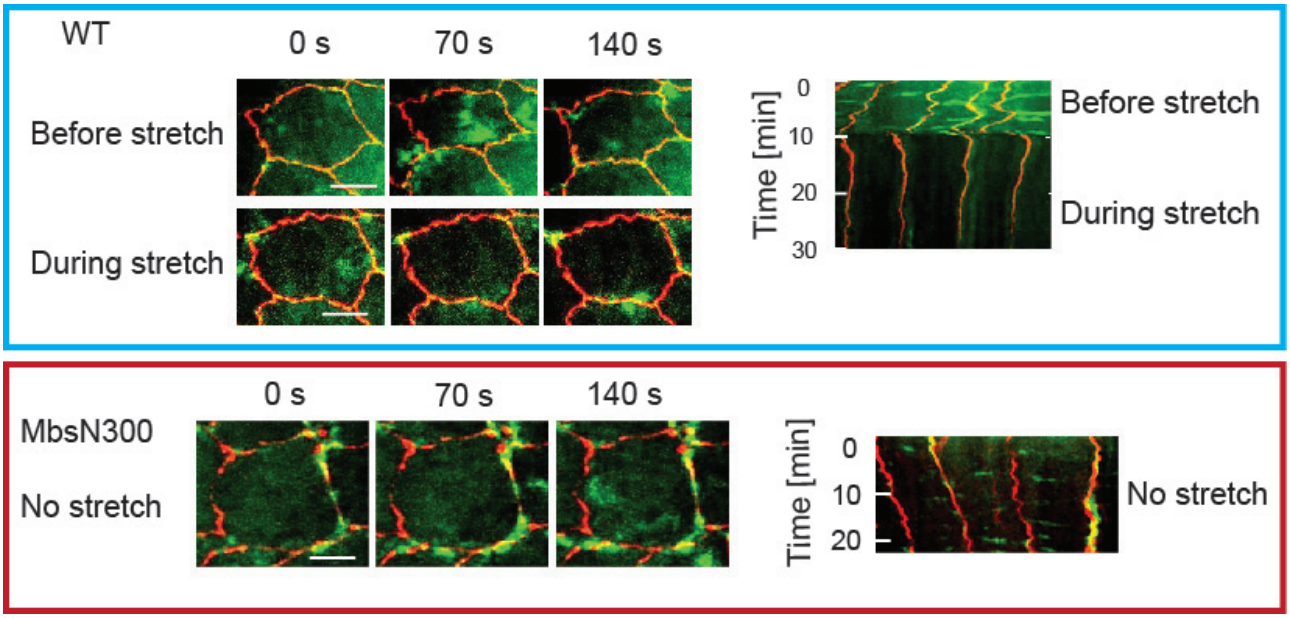


Figure 3.6.6: Blue panel: A representative wild type AS cell before stretching shows area pulsation with contractile myosin in apico medial region, when subjected to stretch, myosin relocates to junctions and cell area stays constant. expressing Tomato e-cadherin (red) and Sqh GFP (green) in a WT embryo subjected to stretching. myosin-downregulated (MbsN300) embryo. Right: Kymograph of a subset of AS cells expressing MbsN300. Scale bar: 10 μm

Next, we carried out quantifications similar to the WT stretched cells, and from this we find that the apical area of MbsN300 cells are approximately 40% larger than WT (Figure 3.6.7). The average cell shape is rounder compared to WT non-stretched and stretched cells that are more elongated in the dorso-ventral direction (Figure 3.6.8). Further, average myosin distribution is similar to the case where WT cells are stretched, as shown in Figure 3.6.9, in which myosin concentration is higher from around $0.4\mu m$ from the junctions as compared with $0.2\mu m$ in non-stretched cells.

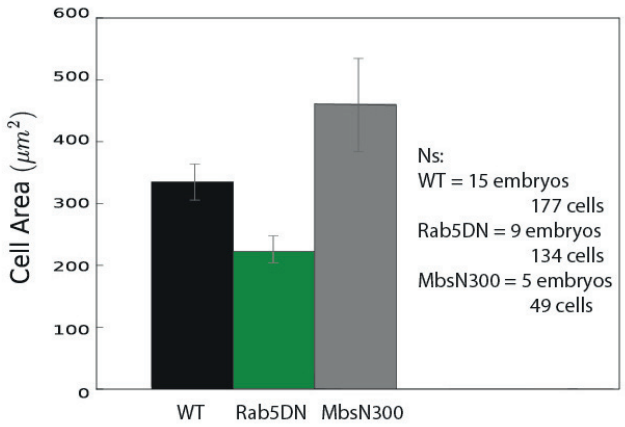


Figure 3.6.7: Cell areas of WT, Rab5DN and MbsN300

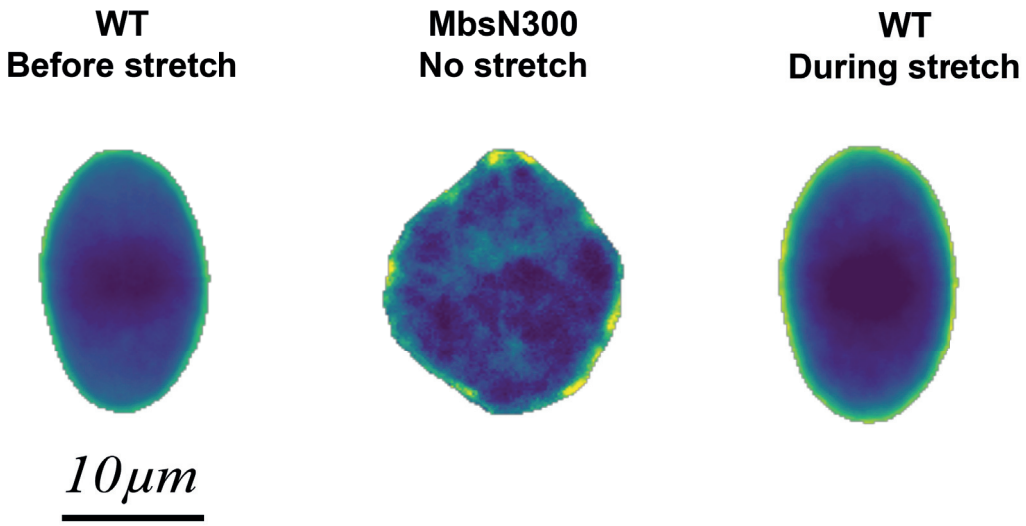


Figure 3.6.8: Average cell and myosin intensity of Mbsn300 (middle) compared with WT before (right) and during (left) stretch application. Quantification by Peran Hayes.

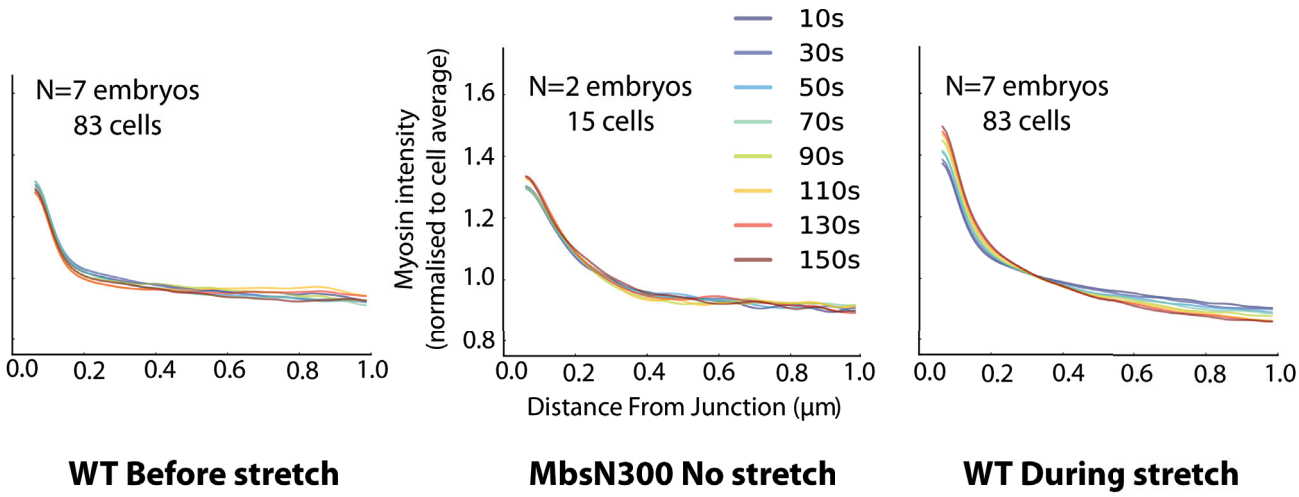


Figure 3.6.9: Plot of myosin intensity as a function of the distance from the junction for WT before stretch, MbsN300 no stretch and WT during stretch application. Different colours show the time-points at which the intensities of myosin were extracted. Quantification by Peran Hayes.

Application of stretch to MbsN300 cells led to similar response in terms of area and myosin concentration. However, upon release, there was no strong myosin contraction at the medial region that was observed in WT cells (Figure 3.6.1, compare with myosin peak in intensity of WT in Figure 3.6.10). In any case, these observations are taken from only one experiment and therefore we do not draw any conclusions form it.

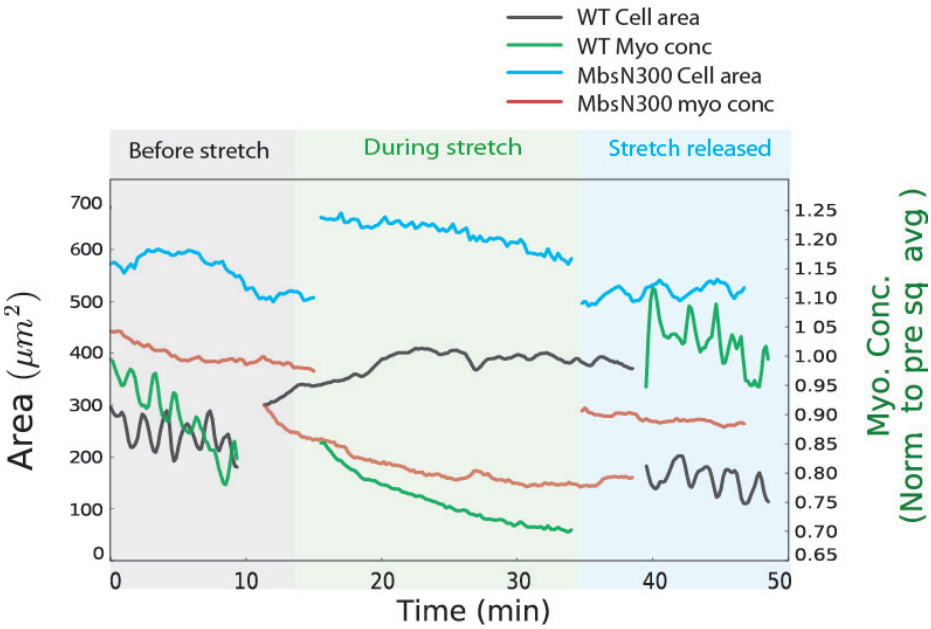


Figure 3.6.10: Cell area and myosin concentration of WT and Mbsn300 before and during stretch application and after stretch release. Quantification by Peran Hayes.

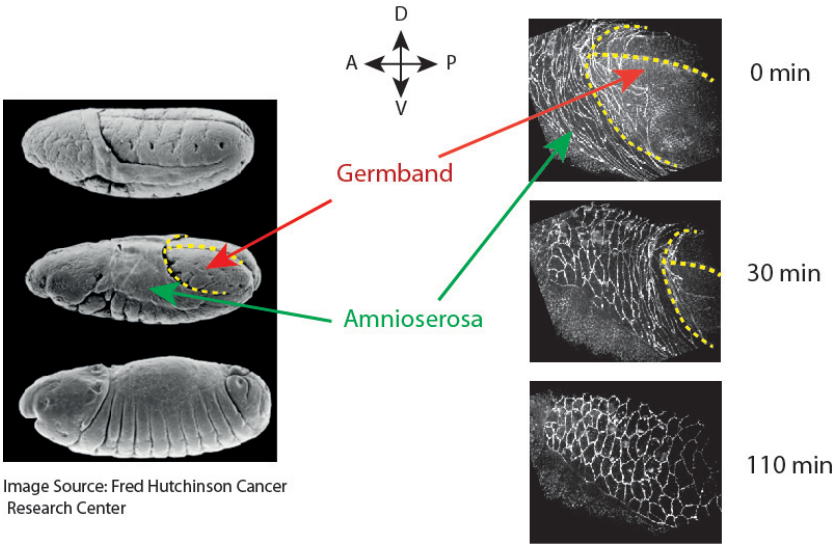
3.6.3 Early AS cells change states during development

During germband retraction, the stage that precedes dorsal closure (DC), AS cells are exposed as the germband begins to move towards the posterior region of the embryo. During this stage, we observed that AS cells have large surface areas and are highly elongated in the dorsoventral (D-V) direction.

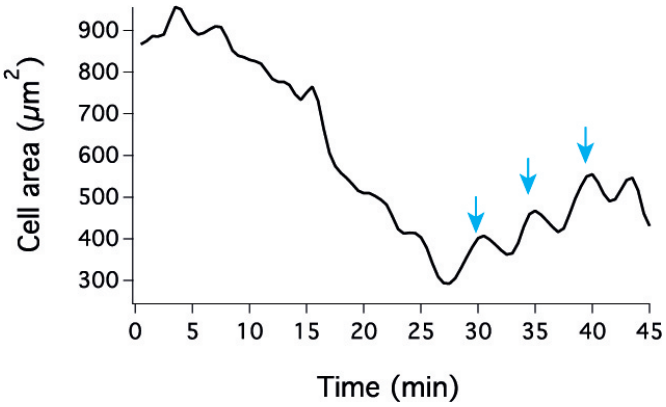
Quantifying the change in cell areas indicated that in the beginning, AS cells show weak fluctuations of their area and only begin to show regular oscillations when the areas have been reduced to approximately $400\mu m^2$ which is normally the size of AS cells during DC. Further, this onset of oscillation coincides with a change in the shape of the AS cells

from highly elongated to an isodiametric and hexagonal shape.

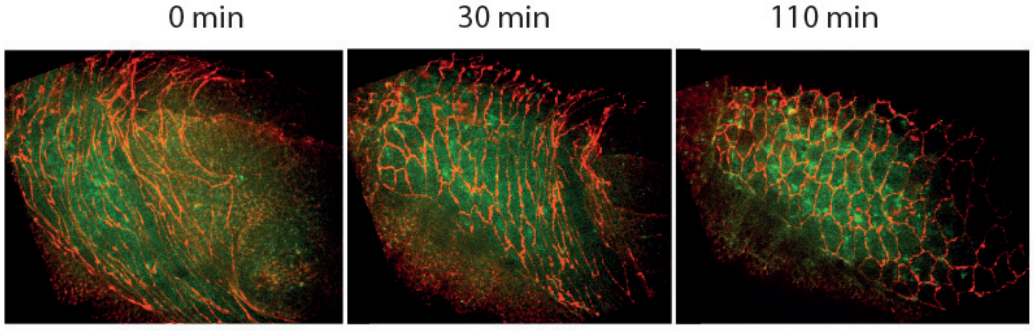
It is likely that pre-DC AS cells are stretched along the D-V axis, this, along with the fact that their areas are almost twice the size of cells at DC suggested a similarity to the case of cells stretched by mechanical force. Moreover, the onset of these oscillations is associated with myosin pulses and waves. Thus, it seems that AS cells change from one state to another as a part of the developmental process.



(a) Changes in AS cell shape during germband retraction. Embryo on the right is expressing Tomato-Ecad.



(b) Onset of oscillation in AS cells: Plot of area of a representative AS cell during GBR and early DC (Cell tracked from the embryo shown in (b). Onset of oscillation coincides with the decrease in cell area to a range which is normally the size of AS cells during DC.



(c) Embryo from Figure (a), now merged with myosin signal (Sqh-GFP). Myosin waves start to appear around 30 minutes in cells towards the anterior. A-P direction shown in Figure (a). This is around the time when cells begin to oscillate (Figure b).

Figure 3.6.11: Shape changes in pre-dorsal closure amnioserosa cells

The resolution at which we imaged the tissue (40X) was inadequate for capturing myosin intensity in pre-DC AS cells before onset of oscillation. Therefore, better resolution imaging needs to be done in order to to carry out quantifications on the localisation of myosin at this early stage.

In any case, it is likely that there is a change in actomyosin organisation and/or level of activity that is somehow correlated with cell shape and size. This could explain the onset of pulsations only when the cells have reached a certain size and shape.

Summary:

1. AS cells exhibit two states - shape oscillations associated with contractile actomyosin pulses and waves (state I), shape stabilised with myosin preferentially localised at the cell junctions (state II)

2. The change from state I to II can be induced by stretching cells via:

   (a) mechanical perturbation in which external force is added. This is reversible either by removal of the external force or maintaining the force for longer periods during which cells begin to reoscillate with a frequency lower than normal.

   (b) genetic perturbation in which contractile forces in the cells are decreased. This is irreversible.

3. Pre-dorsal closure AS cells change from state II to I - from cells that have large surface areas, are highly elongated (likely to be stretched), no shape oscillations and little actomyosin activity, to isodiametric, hexagonal shaped cells that oscillate with pulses and waves of actomyosin contractions.

3.7 Stretch is associated with myosin relocalisation to cell junctions

A closer look at cell junctions during stretch revealed strong enrichment of myosin at the junctions and vertices (Figure 3.7.1), especially in regions that appeared to have "gaps" in cadherin signal. The localisation of myosin seem to pull the junctions inwards as well as seal the gap (Kymograph in Figure 3.7.1).

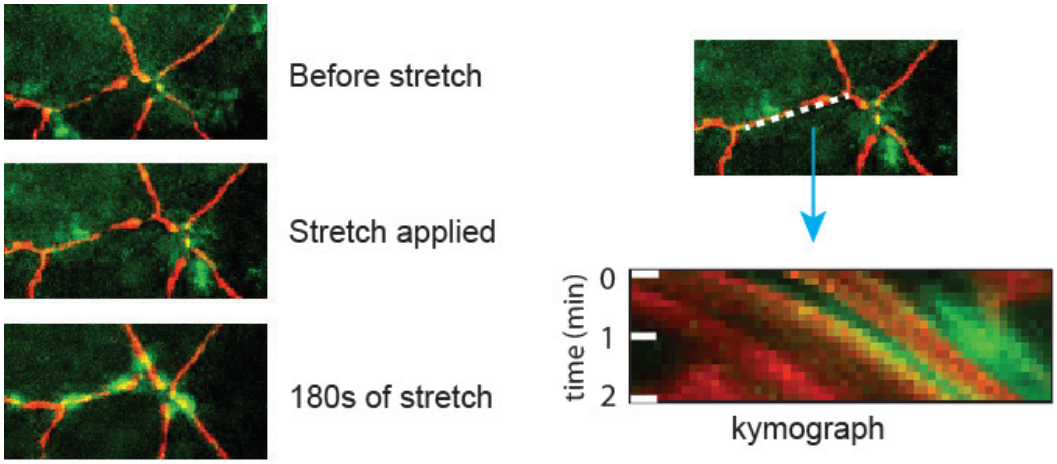


Figure 3.7.1: Right: Myosin relocalisation to stretched junctions. Timelapse images of a junction before stretch, at the point of stretch and 180s later during stretch. Left: Kymograph obtained by drawing a line on a cell junction, time is on the y-axis.

A number of studies have shown the recruitment of myosin II to regions of higher tension such as during cell intercalation in *Drosophila* embryo, as well as by micropipette aspiration on the pre-vitelline space [Fernandez-gonzalez et al., 2009], micropipette aspiration on dividing cells [Effler et al., 2006] and needled indentation on *Drosophila* embryo

[Pouille et al., 2009].

We hypothesised that a similar mechanism might be at play during the stretching of AS cells, and that stretch-induced tension might be responsible for the preferential localisation of myosin at the cell junctions. We quantified the levels of junctional stretch in order to find out whether it indeed correlates with myosin myosin enrichment. For this, the path length of a cell junction from one vertex to another was measured and the level of junctional stretch was defined as the ratio of the path length before and immediately after stretching. Myosin enrichment was defined as the ratio of the peak in junctional myosin concentration over 5 minutes during stretch and the concentration immediately after stretching. Using the Spearman's method which is a non-parametric version of Pearson method, we see a good correlation (Spearman's coefficient: 0.235 and p-value: 0.018) between junctional stretch and myosin enrichment. The Spearman method was chosen since this offers a measure of rank correlation and does not require the variables to be linear, only monotonic, which we believe is more likely to be the relationship between stretch and myosin enrichment.

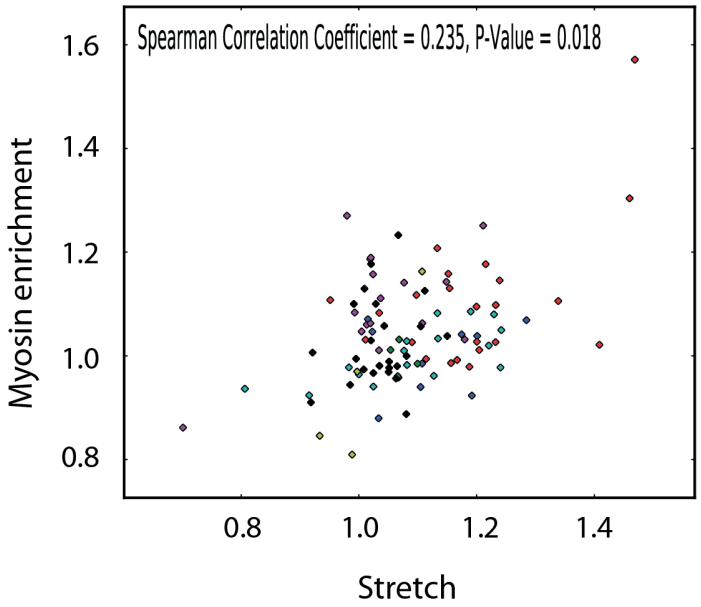


Figure 3.7.2: Plot of myosin enrichment at the junctions as a function of stretch. Myosin enrichment is defined as the ratio of myosin concentration at time-point 0 during stretch and Quantification by Peran Hayes.

This result allowed us to speculate that stretch induces the recruitment of myosin to cell junctions. One method for testing this hypothesis would be to increase junctional material in the cells such that despite application of stretch, the tension in the junctions would not increase to similar levels as a WT cell. We speculated that blocking endocytosis of cadherin would lead to its accumulation at the junctions thereby increasing junctional material. Cadherin endocytosis has been shown to be Rab5-dependent ([Kimura et al., 2006]) - Rab proteins play an essential role in the endocytic pathway and are classified into three types: Rab5 enrich early endosomes and has been shown to be a key regulator of

plasma membrane endocytosis, Rab7 are associated with late endosomes that will be degraded while Rab11 are associated with recycling endocytosed proteins to the membrane [Zerial and McBride, 2001]. Further, Rab5DN has previously been successfully used to block endocytosis in the *Drosophila* embryo to study the endocytic machinery during dorsal closure ([Mateus et al., 2011]).

Expression of Rab5DN specifically in the AS tissue led to a phenotype in which AS cells were highly wrinkled (Figure 3.7.3). This phenotype is consistent with previous observation ([Mateus et al., 2011]).

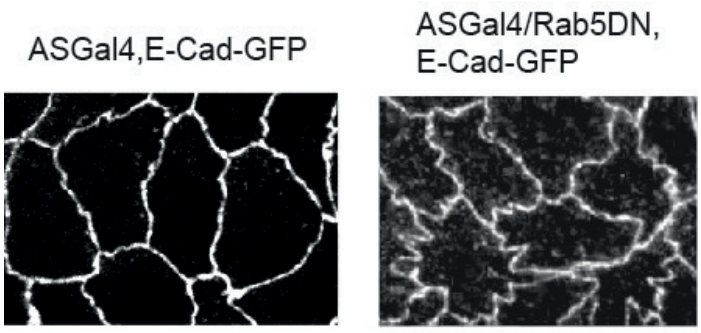


Figure 3.7.3: Comparison of WT and Rab5DN amnioserosa cells

Upon application of stretch on Rab5DN embryos, there was no myosin relocalisation to junctions and cells continue to show pulsations of their areas along with myosin waves (Figure 3.7.4). In order to verify that the Rab5DN cells have been stretched to similar levels as WT, we quantified the stretch percentage which is defined as the ratio of the cell area during stretch application and area before stretch (more details on this can be

found in Section 2.6.4 of Chapter 2). Plot (A) in Figure 3.7.5 shows the comparison of stretch percentage between WT and Rab5DN which indicates that the level of stretch in Rab5DN cells is consistent with that applied to WT cells.

Next, we obtained the average cell of Rab5DN using the same method mentioned in Section 3.5 which showed that these cells are elongated in the dorso-ventral (D-V) direction similar to WT cells. However, unlike WT cells that show an isotropic deformation in response to stretch, these cells displayed an anisotropic deformation whereby the increase in area was greater along the D-V direction. Next, when stretch was released Rab5DN cells returned to the same area and shape as before stretch, unlike the WT cells that contract dramatically to an area much smaller than pre-stretch (Figure 3.7.5). This significant decrease in the area of WT cells is associated with a strong contraction of the actomyosin at the apico-medial region of the cell (Image at 70s when stretch is released, Figure 3.6.1). Such contraction is not observed in Rab5DN cells (Image at 70s when stretch is released, Figure 3.7.4) and we could imagine that without the contractile force to pull junctions inwards, the cells would simply go back to pre-stretch area. It has to be mentioned here again that the WT cell areas do return to pre-stretch levels when they relax

after the strong contraction in response to stretch release.

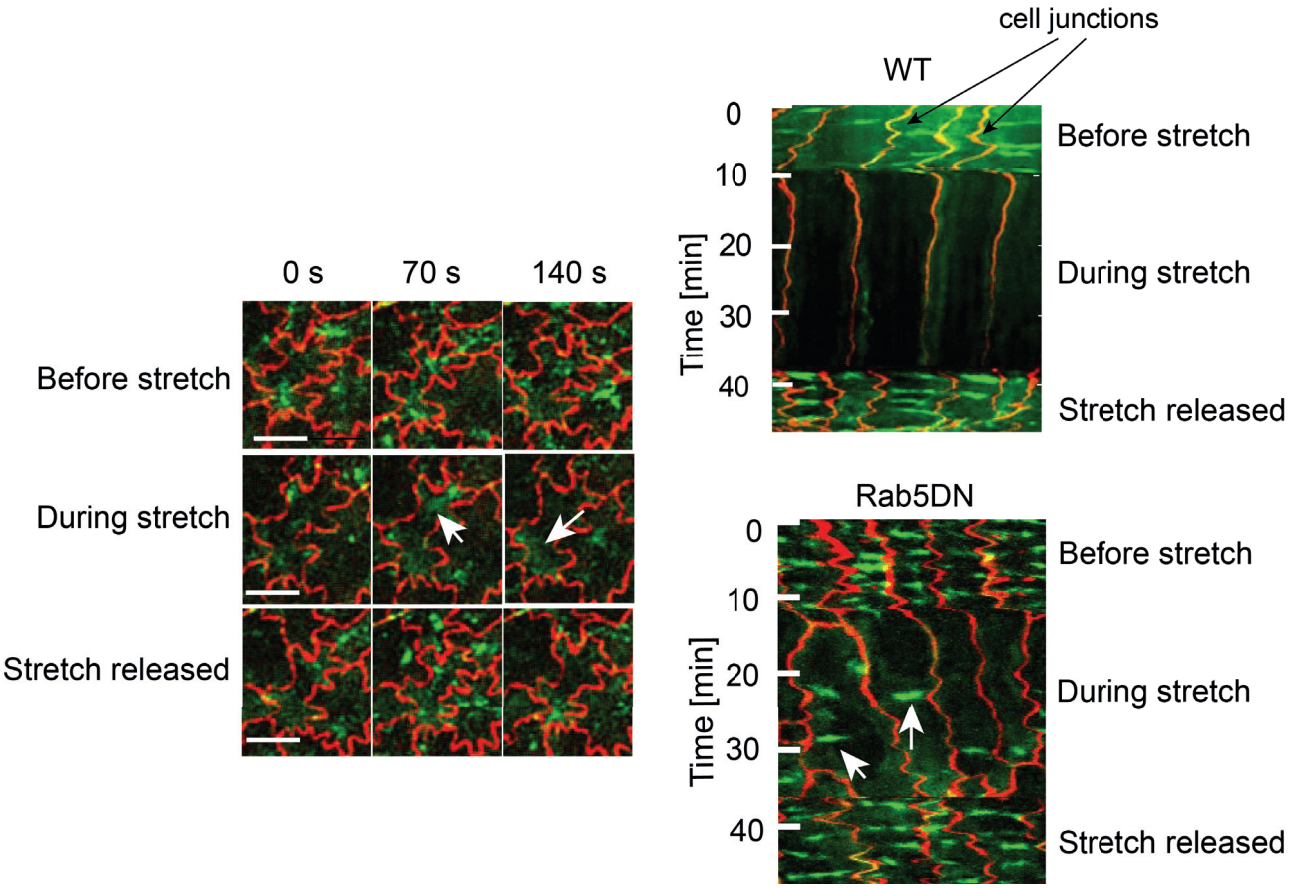


Figure 3.7.4: Timelapse of cells expressing Rab5DN before, during and after release of stretch application. Note the wrinkled appearance of cell junctions in the left panels and the presence of myosin waves (white arrows) during stretch in the kymograph which is absent in WT as seen in the kymograph (Right Top). Scale bar 10 μm

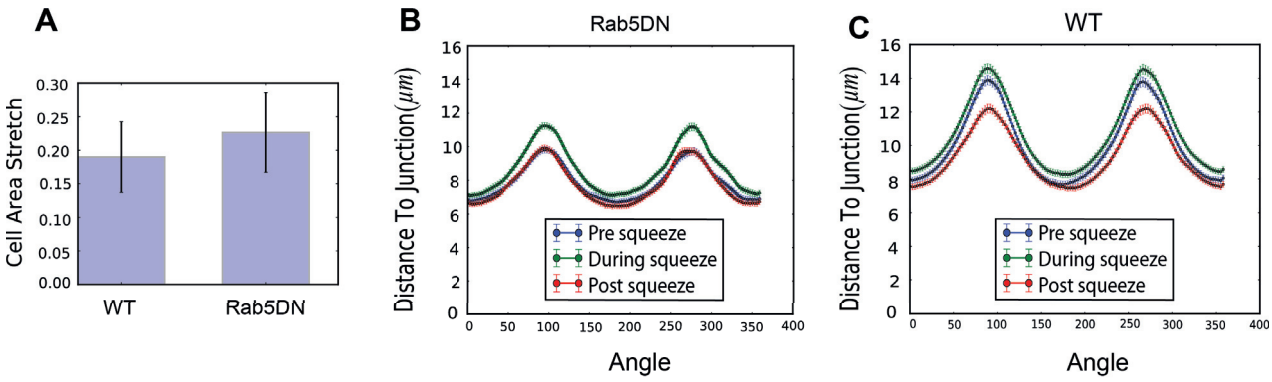


Figure 3.7.5: Deformation induced by stretch in cell area and shape in WT and Rab5DN cells: (A) Stretch percentage of WT and Rab5DN cells. (B) Average Rab5DN cell: these cells have areas smaller than WT and deformation is inhomogenous compared with WT in (C). Stretch leads to greater increase in Rab5DN cell area along D-V direction. Upon release, cells return to the pre-stretch state which is not the case in WT as shown in plot (C). Quantification by Peran Hayes.

Next, quantification of myosin distribution in Rab5DN cells showed that myosin localisation in these cells is fairly homogenous as we can see in Figure 3.7.6 and 3.7.7. We can also see from these plots that there is little change in myosin distribution during stretch and release. Further, focusing on single junctions show that the strong myosin localisation that occurs during stretch in WT is not seen in Rab5DN cells (Figure 3.7.8).

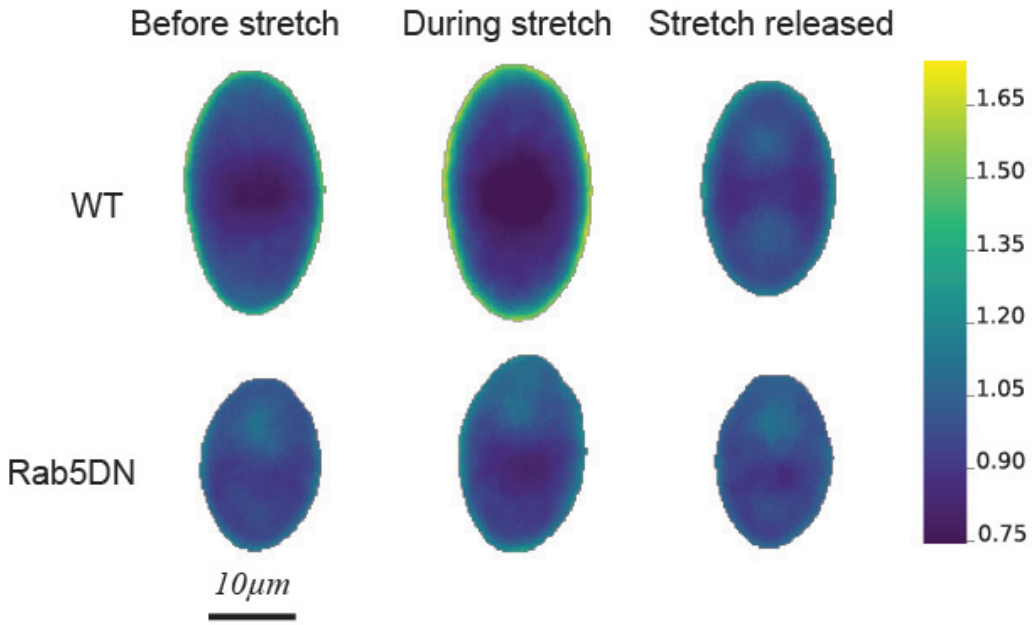


Figure 3.7.6: Average cell before and during stretch and after release of stretch for WT (top row) and Rab5DN expressing cells (bottom row). Heat map shows the intensity of myosin. Quantification by Peran Hayes.

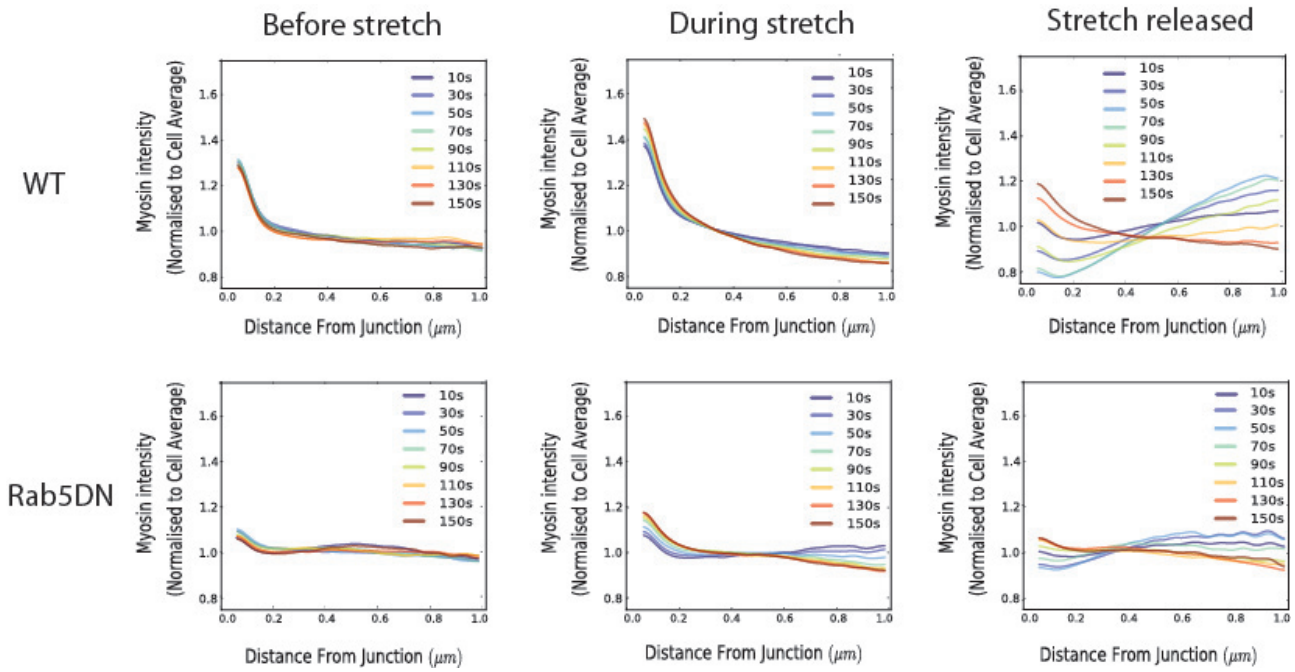


Figure 3.7.7: Plot of myosin intensity as a function of the distance from the junction for WT before and during stretch and after release of stretch for WT cells (top row) and Rab5DN expressing cells (bottom row). Different colours show the time-points at which the intensities of myosin were extracted. Quantification by Peran Hayes.

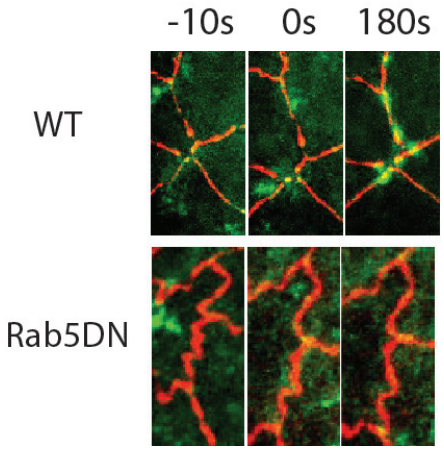


Figure 3.7.8: Comparison of junctions during stretch in WT and Rab5DN expressing cells: Timelapse images of representative junctions in WT and Rab5DN expressing cells before stretch (-10s), when stretch is applied (0s) and 180s later during stretch. One can notice how myosin relocalisation to junction does not occur in cells expressing Rab5DN.

To summarise, we hypothesise that tension due to stretch induces myosin recruitment to the junctions. First, our quantification indicates a positive correlation between level of junctional stretch and myosin enrichment. Next, we tested our hypothesis by blocking endocytosis (Rab5DN) thereby increasing junctional material, such that despite application of stretch similar to the case of WT, the junctions would not reach the same level of tension as WT. As expected, these cells had highly wrinkled junctions suggesting that junctional materials were not being removed as efficiently as WT. Indeed, upon stretch application, these cells continued to show area pulsation and myosin activity including waves. Further, a closer look at single junctions revealed that there was no myosin enrichment in the junctions in these cells unlike the WT cases.

This is also consistent with our earlier speculation in Section 3.6 that AS cells can exist in two states depending on stretch - a state where cells exhibit pulsatile shape changes with flows of contractile actomyosin pulses and the other where the shape is stabilised and actomyosin is localised at the cell junctions.

3.7.1 Myosin plays a role in maintaining tissue integrity

In Section 3.7, we have looked at myosin localisation at regions of "gaps" in the cadherin signal. These gaps are seen in wild-type scenarios as well

(Image "Before stretch" in Figure 3.7.1) and stretching leads to a further increase in the gaps.

However, in a number of cases, application of stretch led to intact cell junctions being pulled apart leading to a discontinuity, especially in the vertices (Figure 3.7.9). There was a clear and strong localisation of myosin at these regions that were eventually sealed, suggesting a role of myosin in maintaining tissue integrity. In order to investigate the response of actin we stretched cells that were expressing Mcherry tagged to moesin (an actin binding protein) and GFP tagged to α-catenin (which connects the cadherin complex to actin filaments). α-catenin GFP signal was similar to cadherin signal and actin localisation to site of junctional gap was similar to that of myosin (Figure

This behaviour of actomyosin was also seen in MbsN300 expressing cells in which junctions exhibited discontinuities in many cases during the process of dorsal closure. This is likely due to the fact that these cells have a decreased contractility and therefore are stretched and pulled apart by the opposing force of the epidermis. We observed that in these cases, myosin consistently and strongly localise at these "discontinuities" and pull junctions together. (Figure 3.7.11).

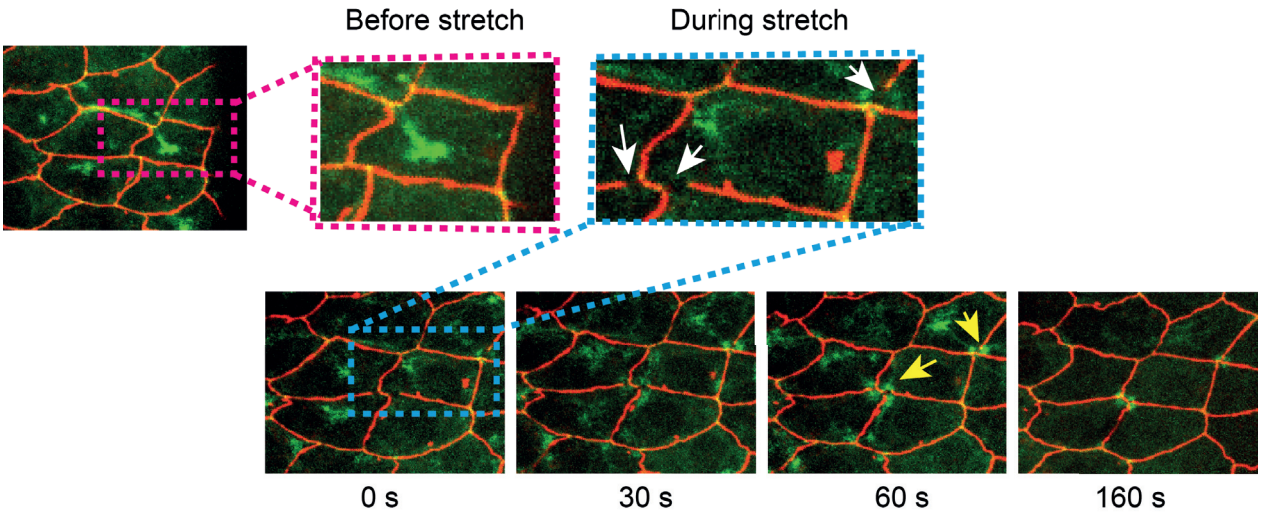


Figure 3.7.9: Myosin flow towards junctional breakage induced by high stretch: Close up of cell junctions before stretch (Pink inset) White arrows show where breakage occurs during stretch (Blue inset). Yellow arrows show enrichment of myosin at junctions where breakage occurred. Compare with other cell junctions and vertices. All time-points shown here are while stretch is still being applied.

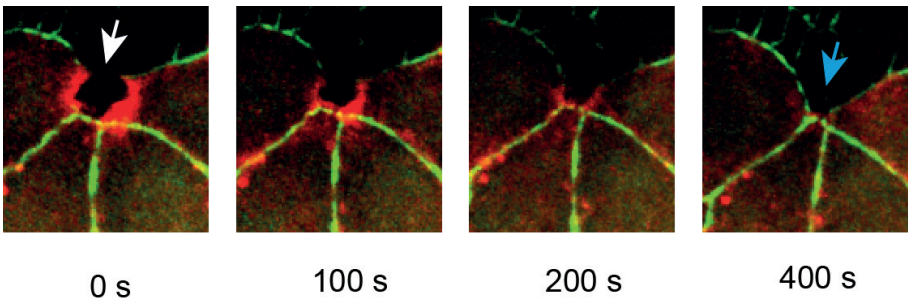


Figure 3.7.10: Actin flow towards junctional breakage induced by high stretch : A gap (white arrow) induced by stretching in a representative junction in cells expressing Mcherry -moe and α-catenin-GFP. Actin flows to the site of damage and seals the gap (blue arrow). Time-lapse images shown here are all during application of stretch.

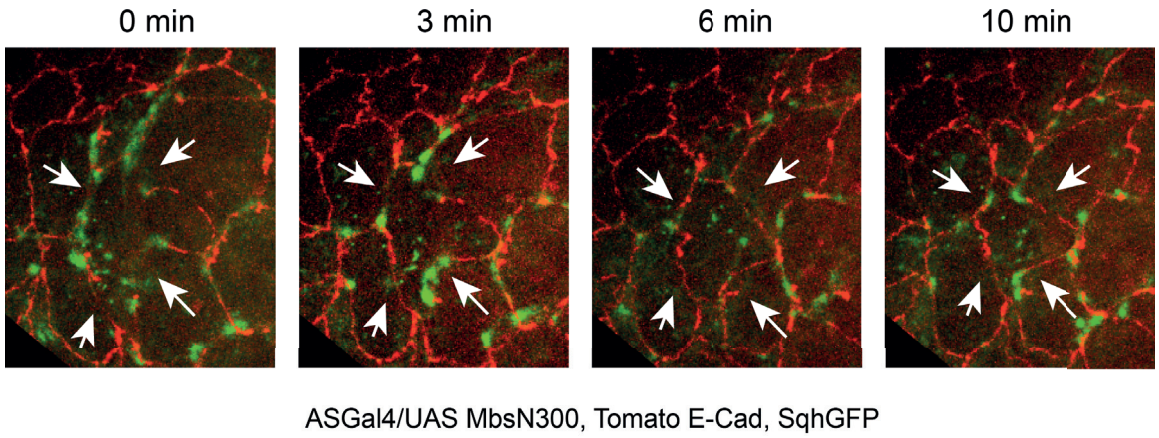


Figure 3.7.11: Cell junctional integrity in MbsN300 cells maintained by myosin contractility. White arrows point to regions of discontinuities in the cell junctions. Myosin consistently enrich in these regions over time and junctions are restored.

Based on these observations we speculate a role for actomyosin in maintaining tissue integrity by repairing regions where a discontinuity arises in the cell junctions. In the case of WT cells, such gaps form as a result of external force application, while in MbsN300 it is due to the decreased contractility in the cells that render them unable to counteract the pulling force of the epidermis and hence get highly stretched. We can imagine that in both cases, these gaps are likely to be regions of high tension induced by stretch and therefore the strong enrichment of myosin in these regions would be consistent with our hypothesis that recruitment of myosin is induced by stretch.

Two methods to test whether tension is indeed increased at the junctions were:

1. Laser ablation at the junctions or regions adjacent to gaps.

2. Observing and quantifying tension sensing proteins.

We were unable to carry out the first method owing to lack of an experimental set-up. For this, one would require a confocal microscope coupled to a laser cutter, and equipped with a stage that is wide and stable enough to hold the micromanipulator.

Therefore, we tried the second method by applying stretch on cells that were expressing Zyxin-YFP. Zyxin is a mechanosensitive protein that is localised at focal adhesions [Yoshigi et al., 2005]. It has been shown in *Drosophila* egg chambers that zyxin is a tension sensor. First, release of tension in stress fibers and focal adhesions by laser cutting led to translocation of zyxin away from the region of cut. Second, increasing tension with the cantilever of an atomic force microscope led to the acute enrichment of zyxin at the pulled stress fibers in a reversible manner [Colombelli et al., 2009].

We expected that upon stretch, zyxin would be enriched at regions of high tension. However, we did not observe any dramatic change in the intensity of zyxin signal upon stretch. This could be due to the fact that the level of our imaging resolution (60X) is not sensitive enough to capture increases/changes in zyxin intensity. In the study mentioned above Colombelli et al. [2009], imaging resolution (150X) was at the level of capturing individual stress fibers and zyxin localisation on single fibers.

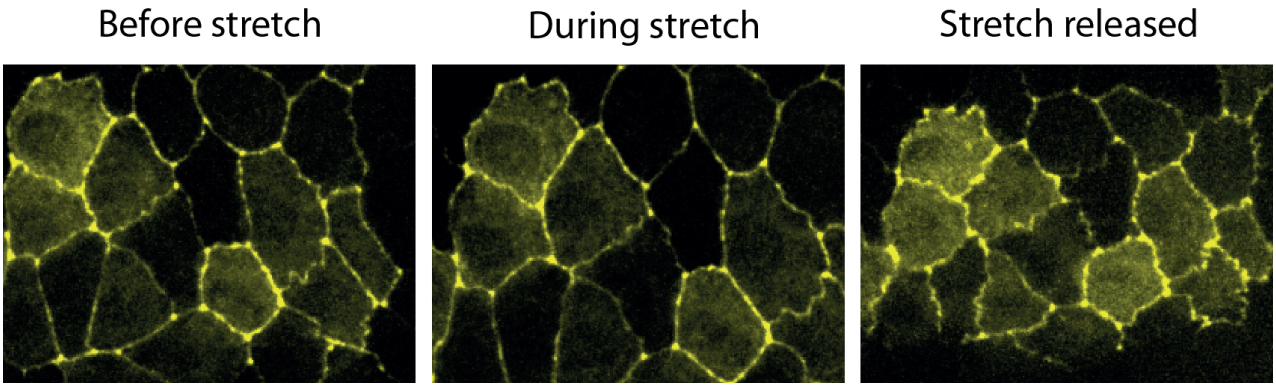


Figure 3.7.12: Application of stretch on cells expressing Zyxin-YFP

Summary:

1. Application of stretch induces a change in actomyosin behaviour from random pulsatile waves at cell apico-medial region to directed localisation at junctions.

2. We speculate the recruitment of myosin to regions of high tension induced by stretch. Quantification of junctional myosin localisation and stretch percentage indicated a positive correlation between the two.

3. In genetically perturbed cells with excessive junctional material, application of stretch and subsequent increase in area similar to WT levels does not induce junctional localisation of myosin, suggesting that these junctions are not sufficiently stretched/tensed. This confirms the speculation that myosin is recruited by stretch-induced tension.

3.8 Myosin plays a role in removal of excess cell junctional material

Release of stretch after long duration application led to contraction of the cell areas accompanied by wrinkling of junctions (Figure 3.8.2, at 1 minute). Wrinkles were quantified as the ratio of the distance between two vertices and the path length of the junction (Figure 3.8.1). These wrinkles were not straightened immediately but rather took up to 20 minutes. A closer look revealed consistent localisation of myosin at the wrinkled regions followed by straightening and shortening of the junctions (See frame at 20 minutes and inset in Figure 3.8.2).

This suggests that junction wrinkling upon release of stretch is not only due to the junctions being pulled inwards by the strong actomyosin contraction in the apico-medial region observed upon release, but that either, the normal rate of junctional shrinkage via endocytosis is decreased, or there is an up-regulation of exocytosis during stretch in order that cell junctions achieve a homeostatic level with respect to area. Either way, this could lead to an accumulation or increase in junctional material such that when cells are released from stretch, the ratio of area to junctional length is decreased hence leading to wrinkling of junctions.

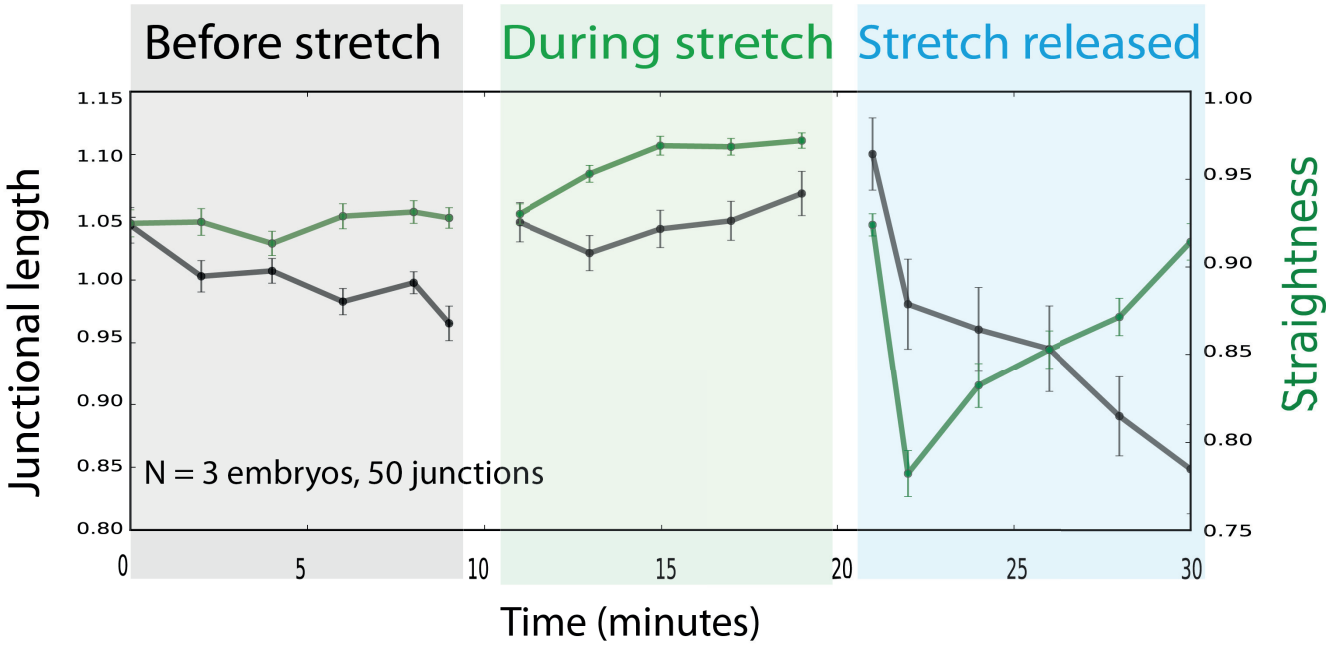


Figure 3.8.1: Plot of junctional length and straightness over time during stretch experiment. Junctional length is normalised to the average length before stretch application. Straightness is the ratio of the distance between two vertices and the path length of the junction. Quantification by Peran Hayes.

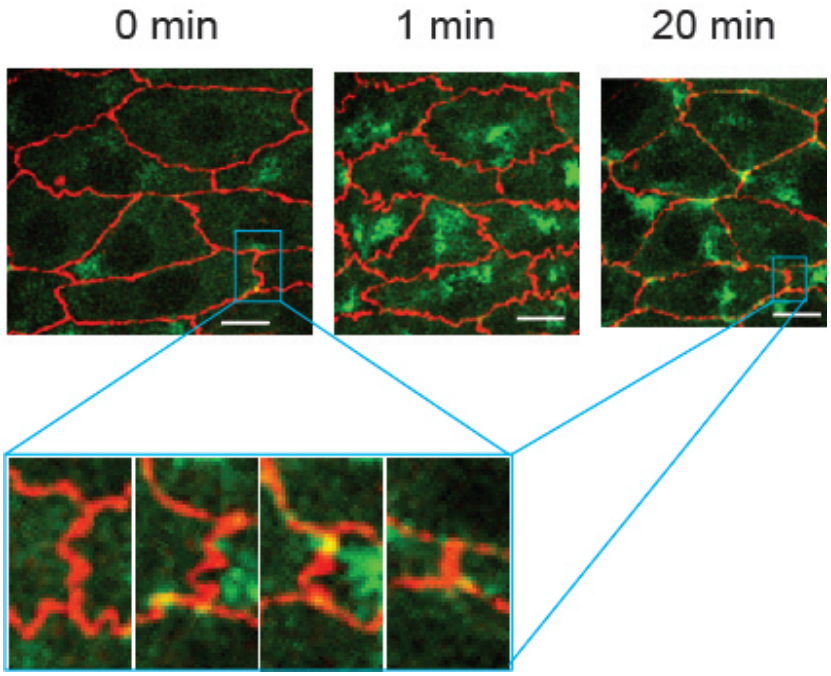


Figure 3.8.2: Timelapse of maximum projection images of a group of WT cells 0 minute after stretch release, followed by image at 1 minute during which cell junctions are highly wrinkled. Over the course of ˜20 minutes, junctions are straightened. Inset shows myosin activity around a representative junction that straightens and shortens over time.

This association of myosin localisation and junctional straightening and shortening allowed us to speculate a role for myosin in maintaining a homeostatic junctional length with respect to area. In order to investigate this further, we looked at AS junction behaviour during dorsal closure which is discussed in the next section.

3.9 Contractile myosin waves plays a role in junctional remodelling

We observed that throughout the process of dorsal closure, AS cells show a reduction in area, however, cell junctions maintained consistent thickness and straightness. This suggests a mechanism by which the homeostasis of cell junctions are maintained.

We then looked at AS cell behaviour during DC in embryos expressing Rab5DN (endocytosis blocked) and MbsN300 (myosin activity down-regulated) specifically in the AS. In embryos expressing Rab5DN, cell junctions had a wrinkled appearance and the areas were generally smaller than WT cells. As closure progressed, there was an apparent accumulation of junctional material as well as an increase in wrinkling. Embryos expressing Rab5DN (N=5) failed to close the dorsal gap as the actin cable at the leading edge of the epidermis as well as the cell junctions lost their integrity and the AS tissue disintegrated (Figure 3.9.1, middle panel).

Next, MbsN300 expressing cells had a larger surface area than WT, and exhibited discontinuities in cell junctions during the process of dorsal closure. As mentioned in Section 3.7.1, myosin strongly localised in these regions and repaired the gaps (Figure 3.7.11). However, these cells had defect in decreasing their surface areas and breaks in the junctions con-

tinued to appear throughout closure. In about 50% of the cases (N=5) , closure failed as the junctional integrity was compromised and the tissue appeared to collapse (Figure 3.9.1, bottom row). The rest of the embryos that appeared to have closed did not survive to larval stage.

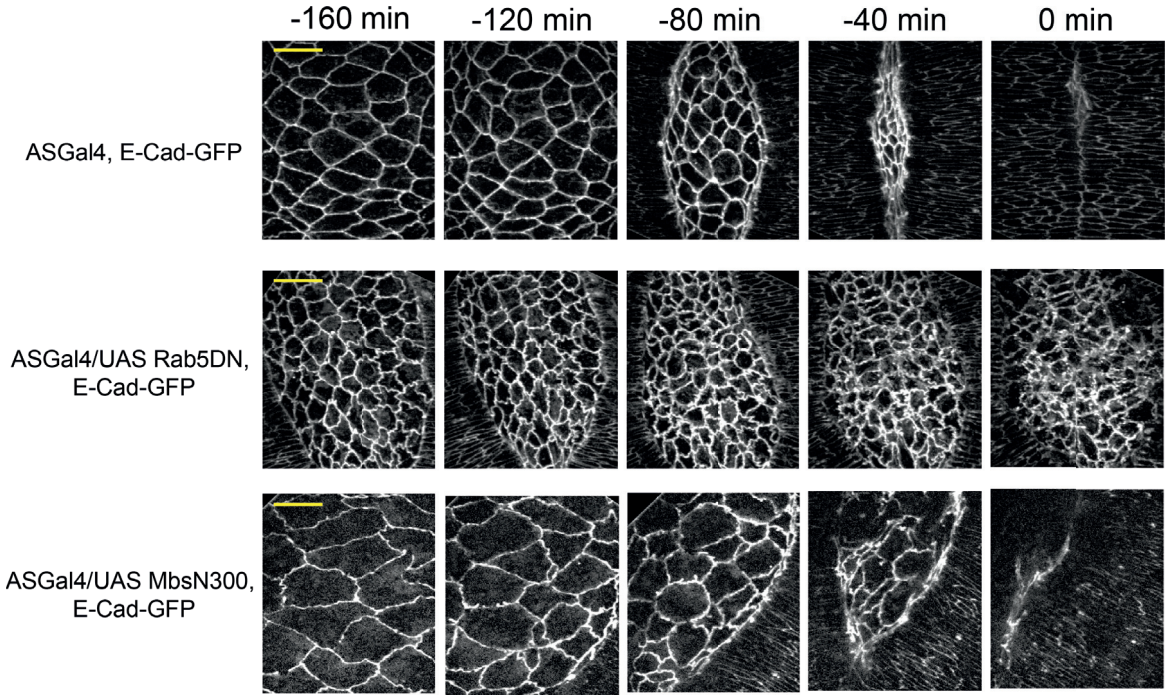


Figure 3.9.1: Time-lapse of amnioserosa cells during dorsal closure (DC) in WT compared with Rab5DN and MbsN300 embryos. Time-point 0 in WT is when closure is complete, while for Rab5DN and MbsN300 embryos this time-point is when the AS tissue started to disintegrate as DC failed. Scale bar is $20\mu m$.

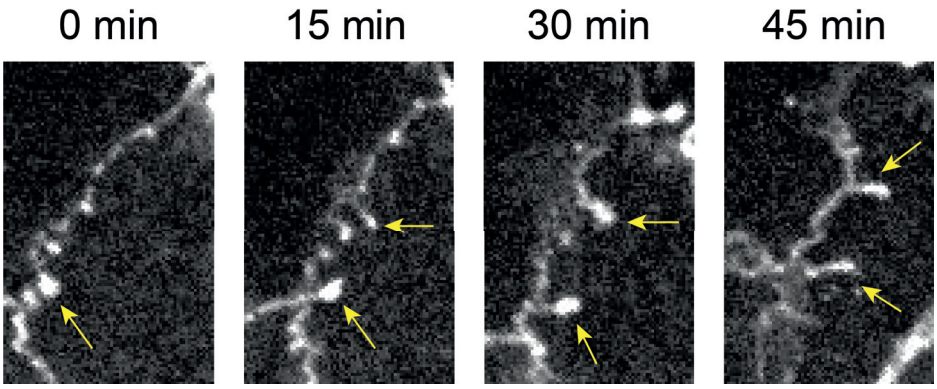


Figure 3.9.2: Tubule formation (yellow arrows) in junctions of cells expressing MbsN300

The failure to complete dorsal closure in these embryos might arise from other factors and we cannot correlate it with defects in cell contractility and junctional homeostasis alone. However, it is clear that in amnioserosa cells during dorsal closure, junctional homeostasis is being regulated such that there is a constant distribution of junctional material and the length is maintained at a steady state so that it is neither too floppy nor too taut.

It has been shown that cell junctions are remodelled through endocytosis of cadherin that can occur through either caveolin-mediated [Akhtar and Hotchin, 2001], macropinocytosis-like pathways [Bryant et al., 2007], or clathrin-mediated pathways [Kowalczyk and Nanes, 2012]. In addition, the role of myosin and its upstream regulators in cadherin localisation and endocytosis has been studied in *Drosophila* germband extension (GBE) [Levayer et al., 2011]. This process is driven by cell intercalation

during which cells exchange neighbours through planar polarised junction remodelling that is facilitated by shrinkage of vertical junctions (cell junctions that are oriented along the dorso-ventral axis of the *Drosophila* embryo). Cadherin localisation is planar polarised, whereby it is less abundant in these shrinking 'vertical' junctions. In this study, they showed that the planar polarised localisation of cadherin is due to up-regulation of clathrin and dynamin-mediated endocytosis, and that the recruitment of the endocytic machinery is driven upstream regulators of actomyosin activity. [Levayer et al., 2011, Levayer and Lecuit, 2013].

In embryos expressing MbsN300, cell junctions formed tubule-like structures that persisted throughout the process (Figure 3.9.2). This is likely due to defects in endocytosis of the junctional material as a consequence of reduced myosin activity.

In order to visualise changes in level of clathrin during and after release, we applied stretch on cells expressing clc-GFP (Clathrin light chain-GFP). However, we did not observe any striking changes in the intensity level or dynamics of clc-GFP during the experiment during application of stretch. Perhaps imaging at a higher resolution and closely following individual junctions and vesicles would yield a better result.

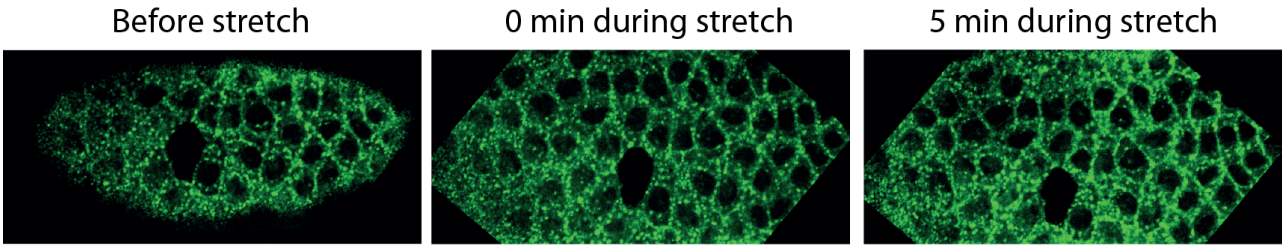


Figure 3.9.3: Application of stretch on AS tissue expressing clathrin-GFP (clc-GFP). The images shown here are - frame before stretch application, immediately after stretch, and 5 minutes during stretch.

In any case, it is likely that the mechanism responsible for the homeostasis of AS cell junctions is based on endocytosis of cadherin and we speculate a role for actomyosin waves in junctional remodelling of AS cells based on the following observations:

1. Myosin enrichment is associated with straightening and shortening of junctional length following release of stretch.

2. Distribution of junctional material and length relative to cell area remains constant in WT embryos throughout DC (dorsal closure).

3. Down-regulating myosin activity leads to junctional wrinkling and accumulation during the process of DC.

This allows us to present an answer to the long standing question on what could be the role of actomyosin waves in these cells. The seemingly random flows of contractile pulses could thus be a way of ensuring that

myosin is able to localise at junctions that require removal in order for its homeostasis to be maintained with respect to cell area.

Summary:

1. Following long duration stretch application, cell junctions were highly wrinkled upon release. The removal of these wrinkles was associated with strong and consistent flow of myosin to the junctions.

2. During DC in WT, the surface areas of AS cells showed a constant reduction till the entire tissue delaminated, however, junctional thickness and straightness were maintained at a steady level.

3. In cases where endocytosis was blocked, cell junctions became highly wrinkled with the reduction in surface areas during DC. While cells with down-regulated myosin activity exhibit tubule-like structures at junctions

Chapter 4: Discussion and future works

4 Discussion and future works

4.1 Biophysical mechanisms driving cell shape oscillations

The amnioserosa (AS) tissue of the *Drosophila* embryo has served as a model in several studies that have been aimed at understanding contractile epithelial cells and their physical and molecular mechanism during development [Kiehart et al., 2000, Solon et al., 2009, Martin et al., 2009, Gorfinkiel et al., 2009, Martin, 2010, Sokolow et al., 2012, Colombelli and Solon, 2012, Saravanan et al., 2013, Fischer et al., 2014]. An interesting feature of the cells in this tissue is the fluctuation of the actomyosin cortex coupled with oscillation of their surface areas for which detailed quantitative analysis have been carried out [Blanchard et al., 2010]. Moreover, a number of molecular mechanisms including PAR proteins, RhoGEF2 as well as ion channels have been suggested to regulate these fluctuations [David et al., 2010, Azevedo et al., 2011, Hunter et al., 2014]. However we still lack an understanding of the biophysical mechanisms that drive these oscillations. To address this, we developed a biophysical model for cell shape oscillation in amnioserosa cells based on cortex turnover which is an intrinsic property of the cell cortex. A similar model has been successfully used to account for cell shape oscillations in cells undergoing cytokinesis [Sedzinski et al., 2011].

We considered an oscillating unit of length l that consists of a spring and dashpot in parallel with an active contractile element. Our model is generic in that it consists only of three simple ingredients: cortex turnover, active tension and elasticity - all of which are intrinsic properties of contractile cells, thus, we hope that our model can be employed to capture the feature of other systems that show periodic shape changes during development.

To test our model we developed a method with which we were able to apply mechanical force on the embryo and stretch the AS cells. Thus, with this method, we were able to change the length l of the contractile unit and the external tension T_e. Interestingly, subjecting cells to external stretch led to an arrest in area oscillation. Our model predicted that fixing the length l at a certain value and maintaining it would synchronize cortices in all cells as the cortex would "catch up" with the change in area. However, this synchronisation would only occur if the duration of maintaining this new state for the cell is long enough for all the cortices to turn over. Indeed, this synchronisation was observed when stretch was released and the cortices of all stretched cells contracted simultaneously. Such response occurred only when the application of stretch was maintained for longer than 100 seconds, which is the timescale that we quantified as the cortex turnover time by extracting rate of change in

area and myosin concentration from experimental data.

The questions that remain to be answered are:

- Changing the ingredients of the model through genetic perturbation: Can we perturb the timescale of cortex turnover and the active tension and what would our model predict for this scenario? Cortex turnover involves the renewal of its constituent proteins such as actin, cross-linking proteins and myosin motors with varying timescales (Table in Introduction). A previous study Blanchard et al. [2010] has shown that the pattern of cell shape oscillations in the AS can be changed by perturbing the cortex through constitutive expression of myosin light chain kinase (MLCK) as well as diaphanous - *Drosophila* homolog of formin that nucleates actin filaments [Afshar et al., 2000]. More recently, a study involving FRAP has shown that expression of a constitutively active form of diaphanous - leads to an increased turnover rate of actin [Deng et al., 2015]. This suggests a possibility of affecting the cortex turnover rate by changing the expression levels of the constituent proteins. Next, the active tension in our oscillating unit comes from contractile forces of a "force-producing molecule", which we have considered to be myosin for our study. However, force generation is also possible through polymerisation of proteins such as actin [Theriot, 2000, Mogilner,

2006] and microtubule [Dogterom et al., 2005]. Perhaps, our model could be adapted to take into account these properties in contractile cells that exhibit area fluctuations in order to obtain a more encompassing picture for these cellular behaviours.

Specific future experiments

1. **Affecting turnover rate**:

   (a) Diaphanous is the *Drosophila* homolog of formin that nucleate F-actin both *de novo* as well as from the barbed ends of pre-existing actin filaments. A constitutively active form of diaphanous (DiaCA) can be expressed in the AS tissue with drivers Gal4c381 or c332.3 that have been recombined with Sqh-GFP or Moe-GFP and Tomato-Ecad. Sqh-GFP and Moe-GFP will allow visualisation of myosin and actin behaviour, and Tomato-Ecad will mark cell junctions allowing the segmentation and extraction of cell area information. Carry out FRAP of actin and myosin in DiaCA expressing cells to quantify turnover rate relative to WT. Plotting DiaCA cell area over time will show whether cell shape oscillations in these cells differ from WT in terms of amplitude and frequency.

   (b) The same experiment as above can be carried out for AS tissue expressing constitutively active form of myosin light chain kinase (MLCKct) that increases myosin activity. The next experiment can be done with DRhoGEG2 over-expression - RhoGEF2 is upstream of Rho signalling pathway and will therefore affect both actin and myosin dynamics. Our preliminary result already show that shape pulsation and actomyosin pulses in MLCKct cells

are greatly reduced or absent, while in DRhoGEF2 cells, contraction of actomyosin and cell shape is isotropic as opposed to discrete local contractions in the WT cells (Figure 2.6.8).

2. **Investigating tension generated by cytoskeletal components:**

   (a) Laser ablate actin and microtubules in WT as well as in perturbed cases such as DiaCA, RhoGEF2 and MLCKct expressing tissues. The initial retraction in response to ablation will provide information about tension in these filaments. Our imaging show that actin behave similar to myosin in terms of forming contractile pulses and waves. Thus, actin contractile pulse or a region close to it could be chosen for ablation. The reason for choosing pulses is because the fluorescence intensity is higher in these regions and allows visualisation of response to ablation. The motivation for carrying out laser ablation in perturbed scenarios is to study whether there is a correlation between cortex turnover and tension.

- What causes the arrest in cell shape pulsations during stretch? We speculate that it could be due to the increase in junctional tension by an external force or an increase in the friction between the AS tissue and the previtelline membrane, or both.

Preliminary results show that introducing an external force F that sufficiently increase the length l of the oscillating unit to a maximum would arrest oscillation. Further, in genetically perturbed cells with excessive

junctional material, application of stretch and subsequent increase in area similar to WT levels do not arrest cell shape pulsations, suggesting that these junctions are not sufficiently stretched/tensed. This indicates that increase in junctional tension, and the fact that this increase in tension is maintained by the force of stretch, prevents the cells from contracting.

Application of stretch for longer duration ($> 10mins$) led to re-appearance of oscillations. These new oscillations had a lower frequency than WT. This shows that, through a mechanism which is still not clear, cells are able to adapt to the change in the system, and overcome the potential friction between the tissue and pre-vitelline membrane. However, friction could still account for the fact that these new oscillations have a lower frequency than WT.

4.2 The two states of AS cells

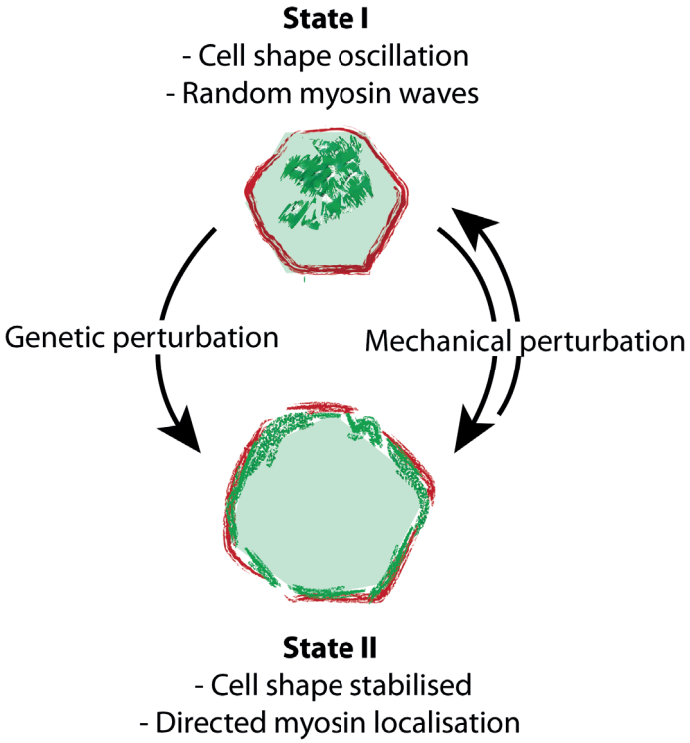


Figure 4.2.1: AS cells exhibit two states depending on actomyosin organisation. The change from state I to II can be induced through genetic and mechanical perturbation. This change in state is reversible in the case of mechanical perturbation by two ways - release of stretch and system adaptation.

AS cells exhibit two states - shape oscillations associated with contractile actomyosin pulses and waves (state I), shape stabilised with myosin preferentially localised at the cell junctions (state II). We found that cells can be forced from state I to II by stretching either through mechanical force or genetic perturbation (Figure 4.2.1).

Application of an external force to stretch the cells led to a change in state from I to II. However, this change is reversible in two ways:

1. Stretch release and 2. System adaptation. This adaptability of the system to stretch application occurred when stretch was maintained for longer periods (>10 minutes). The new oscillations have a lower frequency than WT oscillations which could be due to the friction between the tissue and the pre-vitelline membrane.

A likely mechanism leading to such adaptation is the remodelling of cell junctions via exocytosis of junctional proteins such as cadherin such that the junction is brought back to a homeostatic state with respect to cell area. Quantification of exocytic and endocytic proteins during long duration stretch is required in order to confirm this idea. This will also confirm whether it is endocytosis that is down-regulated rather than exocytosis being up-regulated.

Next, our results show that cells can be induced to state II in an irreversible manner through genetic perturbation - specific down-regulation of myosin activity by expressing a constitutively form of myosin phosphatase (MbsN300). We used an AS Gal4 driver (c332.2) with a patchy expression pattern such that myosin activity is not completely down-regulated. In these cells, myosin networks were markedly decreased compared to WT, and activity was restricted to the junctions where they were strongly enriched. Cell area pulsations were weak or absent and surface area was larger than WT with cell junctions being discontinuous

in many cases.

We speculate that these phenotypes were due to the fact that contractility in these cells are reduced which led them to being stretched by the pulling force of the epidermis.

Thus, by using either mechanical or genetic approach, subjecting AS cells to stretch leads to a change in state in which cell pulsations are arrested and this is associated with junctional localisation of myosin.

Next, we observed that there is likely to be a similar mechanism whereby cells change from one state to another - without external perturbation - during development. Early AS cells during germband retraction (GBR- the process that precedes DC) have large surface areas, are highly elongated and likely to be stretched in the D-V axis. During this time these cells do not show shape oscillations or strong myosin activity. Towards the end GBR, these cells have reduced their surface areas by half which coincides with shape oscillations and myosin pulses and waves. This is similar to the case where cells change their state following mechanical or genetic perturbation.

SPECIFIC FUTURE EXPERIMENTS

1. In order to verify whether endocytosis is down-regulated or exocytosis is up-regulated during long duration stretch application: Live imaging of AS tissue expressing UAS Rab5 GFP and Rab11-GFP, driven by AS Gal4, Tomato-Ecad (Rab11 is a recycling endosome that allows recently endocytosed proteins to the membrane). Imaging should be done before, during and after release of stretch to compare changes in numbers of vesicles.

2. To test the speculation that AS cells are able to adapt to stretch by addition of junctional/membrane material: Block exocytosis (Rab11DN). It would also be interesting to see whether in this case, cells are highly wrinkled upon release, and will shed light on what is happening at the cell junctions/membrane during stretch as mentioned above in point 1.

3. To investigate if pre-dorsal closure AS cells are indeed behaving similar to situations where cells are stretched via mechanical and genetic perturbation - Good temporal and spatial resolution of embryos expressing Sqh-GFP and Tomato-Ecad.

4.3 Stretch induced myosin localisation at cell junctions

In this part of the project, we show that stretch induces enrichment of myosin at cell junctions, and speculate that a mechanism driving this change in actomyosin behaviour is likely to be tension which we expect is increased at junctions and vertices by stretching. Our hypothesis is based on other studies that have shown recruitment of myosin to regions

of higher tension induced by mechanical perturbation [Effler et al., 2006, Pouille et al., 2009, Fernandez-gonzalez et al., 2009].

We test our hypothesis by blocking endocytosis of cadherin (Rab5DN) to "reduce tension" by generating excess junctional material. Indeed, in cells with excess junctional material, application of stretch levels similar to wild type (WT) does not change the behaviour of myosin which continue to flow in the apico-medial regions of the cell without localising to junctions.

SPECIFIC FUTURE EXPERIMENTS

1. Experimental set-up for laser ablation: Laser cutter coupled to a confocal microscope with a stage that is large and stable enough to support the micromanipulator. Then, laser ablation can be carried out while stretching cells to investigate the following questions:

   (a) Does stretch application increase tension in the cell junctions? This could be carried out in a WT embryo expressing adherens junction marker such as Tomato-Ecad or Ecad-GFP. Compare resulting initial retraction with non-stretch situation.

   (b) Is myosin recruited to regions of high tension? Ideally, this should be carried out in an embryo expressing Sqh-GFP and Tomato-Ecad (Reason for this combination is because Sqh-cherry forms aggregates and is not suitable for image analysis). During stretch, wait till myosin begins to enrich at junctions and vertices. Ablate the junction that is close to region of myosin enrichment.

This should release the tension in that junction/vertex. Does myosin move away from this region?

(c) Ablate cell junctions before and during stretch of these cells. Compare with WT.

Our next hypothesis is that myosin is involved in maintaining tissue integrity which we infer from experiments where previously intact junctions and vertices are pulled apart by high stretch to form clear discontinuities in cadherin signal. Strong myosin and actin enrichment in these regions was associated with sealing of the gaps and shortening of junctional length. A similar mechanism was observed in cells with down-regulated myosin activity (MbsN300) in which junctional integrity appears compromised and present a phenotype where the junctions are discontinuous in many cases. This is likely due to the fact that these cells have a decreased contractility and therefore are stretched and pulled apart by the opposing force of the epidermis.

We observed that during dorsal closure in Mbsn300 expressing embryos, myosin consistently localise at these "discontinuities" and pull junctions together. A recent study has shown the role of myosin in maintaining integrity at the zonula adherens (ZA) of epithelial cells. RhoA signalling is necessary for stable ZA, and myosin was found to be preventing its downregulation by scaffolding ROCK1 (Rho Kinase) that prevent recruitment of Rho suppressor [Priya et al., 2015].

Further, during epithelial wound healing, myosin is known to form a purse-string like contraction along with actin to pull the wound edges together. We can imagine that a similar mechanism is at play in the

amnioserosa in which myosin maintains the integrity of these cells as they pull on each other due to their contractile waves, as well as are being pulled on by the epidermis.

4.4 A role for myosin in removal of excess junctional material

Upon removal of long duration (up to 1 hour) stretch, cells show dramatic contraction of the actomyosin at the medial region accompanied by wrinkling of cell junctions. This wrinkling is not an immediate effect of cell contraction in response to stretch release since the wrinkles remain for up to 15 minutes before the junctions are straightened. Closer observation reveals consistent localisation of myosin at the junctions followed by removal of wrinkles and shortening of junctions. We hypothesise a role for myosin in removal of excess junctional material based on studies that have shown the regulation of cadherin endocytosis by actomyosin during cell intercalation in *Drosophila* embryo [Levayer et al., 2011, Levayer and Lecuit, 2013].

4.5 A role for myosin in AS junction remodelling

The response of actomyosin and junctions in amnioserosa cells to external stretch led us to investigate further these components during dorsal closure. We find that throughout closure, amnioserosa cells maintain a

homeostatic level of junctional material and length despite a constant reduction in area. In embryos where endocytosis is blocked (Rab5DN) or myosin activity is down-regulated (MbsN300), there is an accumulation of cell junction material as cells decrease their areas. This leads to wrinkling of junctions as well as appearance of tubule-like structures from the junctions. In these embryos, dorsal closure is impaired and even though other factors might be involved, we speculate that the regulation of cell junctions is required for normal progression of development.

Coupled with observations from our stretching experiment, we present a new hypothesis for regulation of junctions in amnioserosa cells by contractile actomyosin waves via endocytosis. This allows us to speculate a developmental role for contractile actomyosin pulses and waves which has been a long standing question in this area of study. The seemingly random flows of contractile pulses could be a way of ensuring that myosin is able to locate at junctions which needs to be removed in order for its homeostasis to be maintained.

An open question is whether myosin is able to sense specific junctions requiring endocytosis, or it is the contractile flow that precedes such that it buckles the junctions that are then removed. So far, the answer is not clear since the wrinkles that arise from contraction of cells in response to release of stretch are already present and these regions are where myosin

appears to consistently flow towards. Such myosin localisation is associated with the removal of what appears to be excess junctional material. On the other hand, in wild-type scenario, the case is often that myosin pulses at boundaries of cells is associated with wrinkling of the junctions due to contractile forces. In these cases, junctional materials are likely to be removed as it leads to shortening of the particular junction.

An experiment that could shed light on this question is the quantification and correlation of endocytic markers with myosin and cadherin localisation in WT amnioserosa cells during DC, during and after stretching for long periods as well as in cases where myosin activity is down-regulated.

Specific future experiments

1. To study the dynamics of endocytic machinery and its association with actomyosin:

   (a) During mechanical perturbation- Live imaging of AS tissue expressing proteins of the endocytic machinery such as UAS GFP-Rab5, UAS Clathrin-GFP (both light and heavy chain), driven by AS Gal4c381 or c332.3, myosin or actin Mcherry. Quantify puncti of endocytic markers and correlate with myosin localisation before, during and after release from long duration stretching.

   (b) During dorsal closure: For this, complete dorsal closure could be imaged in WT embryos expressing GFP tagged endocytic proteins driven by AS Gal4c381 or c332.3, myosin or actin Mcherry. While for embryos with down-regulated myosin activity (UAS MbsN300) driven by an AS Gal4c381 or c332.3, sqh-GFP or cadherin-GFP, one could inject Dextran Rhodamine. The levels of dextran coated vesicles in these embryos can then be compared with WT embryos expressing sqh-GFP or cadherin-GFP also injected with Dextran Rhodamine.

Conclusions

The development of an organism from a clump of cells involves dramatic remodelling and rearrangement of cells and tissues. Such changes are driven by forces that are generated by motor proteins and transmitted within and across cells via cytoskeletal elements and adhesion molecules. In order to understand how dynamic changes occurring at the subcellular level can account for the large scale changes leading to tissue morphogenesis, we need to study and analyze properties that are inherent to the cell and its constituents. One such property that has been found to be involved in a number of key morphogenetic processes and has been of much interest is the flow of actin and myosin.

However, this property varies from one system to another in terms of the dynamics and spatiotemporal organisation. This suggests that the mechanism and role of this cortical behaviour is also likely to be different. For instance, during *Drosophila* gastrulation, mesoderm invagination is driven by apical constriction of mesodermal cells. These cells exhibit periodic shape pulsations driven by centripetal actomyosin flows that are translated into apical constriction [Martin et al., 2009]. On the other hand, actomyosin flows observed in the yolk cell of gastrulating zebrafish embryo are non-pulsatile. These flows have been implicated in pulling the epithelial cell layer over the yolk cell [Behrndt et al., 2012].

However, in general, the mechanism that drive actomyosin pulses and waves and their roles in different developmental processes are still not clear. For this reason, we chose the amnioserosa cells of the *Drosophila* embryo as a model to study cell shape pulsations driven by contractile actomyosin pulses and waves. We addressed two key questions 1. What is the biophysical mechanism driving these cell shape oscillations? 2. What is the role of contractile actomyosin waves that are associated with these cell pulsations?

We were successful in developing a biophysical model for cell shape oscillation that was based on an intrinsic property of the cell cortex - constant turnover of its constituent components. We found that coupling three ingredients: 1. cortex turnover, 2. active contractility driven by force producing molecules and 3. cell elasticity was sufficient to generate stable oscillations. Further, we were able to generate waves by coupling the oscillating units and introducing a diffusion term to account for exchange of force producing molecules between the units.

The generic nature of our model means that it could be applied to other systems where actomyosin waves and shape oscillations have been observed.

Next, we developed a novel method with which we could apply large scale tissue deformation on a live embryo and capture *in vivo*, the re-

sponse of the cellular components to mechanical stress. With this method we observed a cross-talk between the cytoskeleton and cell junctions in amnioserosa cells. Based on our results, we propose a role for contractile actomyosin waves in AS cell junction homeostasis in two ways:

1. by relocalisation to regions of high tension at stretch-induced gaps in the junctions in order to maintain junctional integrity via contractile forces and

2. by pulling on and bringing together junctions with low tension that are too floppy, and facilitating removal of junctional materials by endocytic machinery (Figure 4.5.1).

Through this mechanism, AS cells undergoing constriction exist in a "Goldilocks zone" where junctional length is maintained relative to the cell area. In particular, we show that cells can be shifted to another state through mechanical and genetic perturbation where the cells are no longer pulsatile. In these cells, junctions are highly stretch and exhibit gaps to which myosin localise. The contractility of myosin maintains the integrity of these junctions and brings them back to the homeostatic state. On the other hand, when endocytosis is blocked, cell junctions accumulate material and become highly wrinkled thus shifting away from the Goldilocks zone. Myosin then facilitates the removal of junctional

material via endocytosis and returns the cell to the homeostatic state

In both situations, the likely mechanism by which myosin is able to sense regions of low or high tension is its mechanosensitive property (Discussed in Section 3.7, Chapter 3). Thus, the seemingly random flows of contractile pulses could in fact be a way for myosin to probe and sense regions that require either assistance or removal.

Till now, there has been no study that has presented a clear developmental role of cell shape oscillation. There have been a few speculations: it might be a way to ensure the equilibrium of the apical tension between neighboring cells, or it could simply be an epiphenomenon of the dynamics of actin and myosin [Blanchard et al., 2010]. Another idea was that these oscillations could be facilitating the process of dorsal closure, however, down-regulating myosin II activity and effectively abolishing these fluctuations led to a closure rate that was in fact faster than that of WT embryos [Saias et al., 2015] (Also mentioned in Section 2.2, Chapter 2).

Other open questions that remain are: Why do only some tissues have this property of cell shape pulsations during development? Or, why are these pulsations absent in tissue cultures? Our results show that cells can exist in two states depending on actomyosin organisation. Cell pulsations are associated with actomyosin pulses and waves, while in the scenario where actomyosin localises at the cell junctions, these pulsa-

tions are arrested. This association of shape pulsations with actomyosin organisation could explain why this pulsing phenomenon is not observed in all systems. Next, these pulsations have only been observed during morphogenesis in live embryos, where large scale tissue remodeling and rearrangements are taking place. An embryo is a complex system with many different processes occurring simultaneously, a change in shape or movement in one region is likely to affect the neighbouring tissue. This could be one reason cell shape pulsations have not (yet) been observed in tissue cultures.

Through this project, we have been able to gain a better understanding about the mechanism and role of cell shape pulsations driven by contractile actomyosin waves. However, as mentioned earlier, there are other systems that vary in several aspects and still need a deeper investigation. It is certainly possible that in some scenarios, depending on the structure of the system, there will be no connection between these cortical waves and junctional remodelling. It will be an exciting avenue of further study to investigate whether the mechanism proposed in this work is also at play in such analogous yet distinct systems.

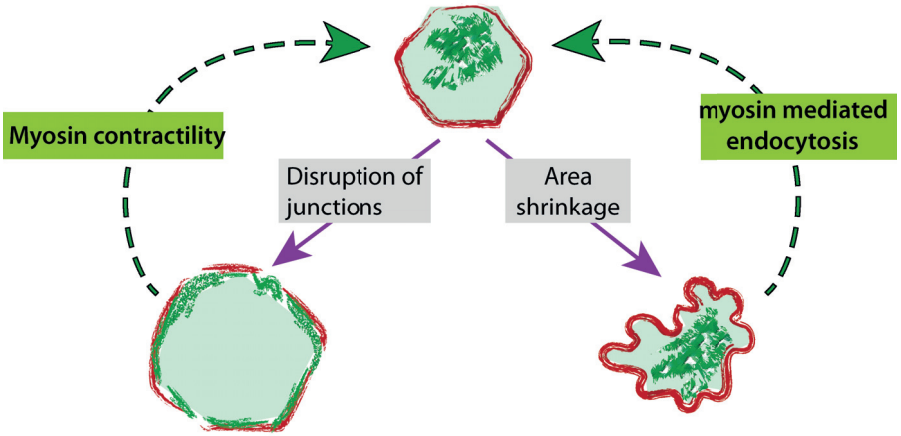


Figure 4.5.1: **Model for the dual role of myosin in maintaining junctional homeostasis:** *"Happy is the cell whose junctional length is maintained relative to its area"*. External forces can increase the cell area by stretching, thereby increasing tension in junctions and causing disruption. The contractility of myosin repairs and maintains junctional integrity bringing it back to the normal state. On the other hand, decrease in cell area can lead to a situation where the junctions become floppy due to an excess of junctional material. Here, myosin facilitates the removal of junctional material by coupling with the endocytic machinery and returns the cell to a homeostatic state.

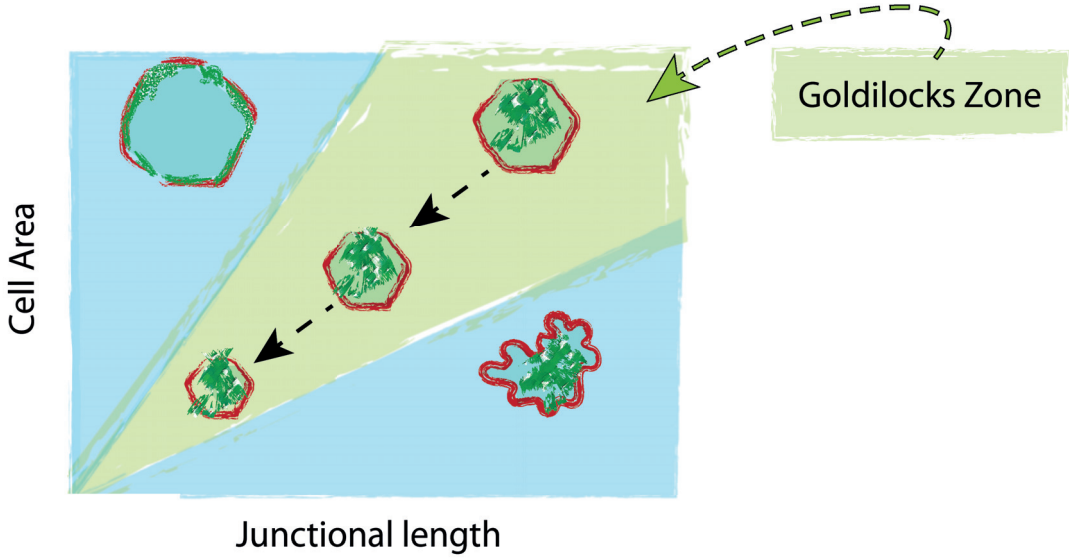


Figure 4.5.2: **Junctional homeostasis during constriction of AS cells**: The activity of myosin allows cells to remain in a "Goldilocks Zone" such that cells can undergo constriction by losing volume while junctional length is maintained relative to the change in cell area.

Chapter 5:Materials and Methods

5 Materials and Methods

5.1 *Drosophila* strains

The following *Drosophila* strains were used:

$Sqh^{AX3}; Sqh-GFP$ that I recombined with $w^*; DE-Cad :: Tomato$

$Sqh^{AX3}; Sqh-GFP$

$w;^* UAS-clcII-GFP$

$w^*; UAS-mCherry:Moe$

$w^*; UAS-PAC\alpha$

$w^*; UAS-MbsN300$

$w;^* UAS-MLCKct$

$w;^* UAS-Rab5DN$

$w;^* UAS-DRhoGEF2$

$w;^* UAS-\alpha-catenin-GFP$

$w;^* UAS-Zyxin-Ypet$

$w^*; Sqh-GFPc381-gal4$

$w^*; Sqh-Cherryc381-gal4$

$w;^* UAS-mCherry:Moe, c381-gal4$

5.2 Confocal microscopy

5.2.1 Sample preparation and imaging

Embryos were collected at 25 degrees overnight. They were then dechori-

onated with 50% bleach and washed with water. Embryos at the right stage were chosen under the fluorescent lamp and mounted depending on the experiment to be performed-

- **Normal confocal imaging:** mounted on a glass-bottomed culture dish (Mattek), covered with 1% agarose and immersed in 1% PBS.
- **Stretching experiment:** mounted on a glass-bottomed culture dish (Mattek) coated with heptane glue and immersed in PBS.
- **Micro-injection:** mounted on a rectangular cover glass that was coated with heptane glue, embryos were covered with halocarbon oil and the cover slip glued to a metal holder for imaging.

Imaging for stretching experiment and for normal wild-type embryos was carried out on Yokogawa CSU-X1 spinning disk on an inverted Olympus 1X81 microscope, lens U plan S Apo 60x 1.45 Oil.

For $PAC\alpha$ experiment, imaging was carried out on Leica SP5 AOBS microscope (Leica DMI 6000) with lens HCX PL APO CS 40x 1.25-0.75 Oil.

Next, embryos injected with dye FM4-64 were imaged on Leica TCS SP5 II CW-STED (inverted), 63x Oil.

5.3 Fluorescence recovery after photobleaching (FRAP)

FRAP is a technique that is used to study mobility of proteins in cells by bleaching a region within a cell and measuring the rate of recovery of fluorescence in the bleached region ([Axelrod et al., 1976]).

- FRAP on myosin was carried out on amnioserosa cells expressing Sqh-GFP, on the Yokogawa CSU-X1 spinning disk on an inverted Olympus 1X81 Microscope, with lens 100x, Oil. A square ROI of about $5\mu m^2$ was bleached within the AS cell with a 488nm laser beam. Images were taken every 1 second for ˜3 minutes before bleaching and ˜5 minutes after bleaching.

- FRAP on actin was done on amnioserosa cells expressing actin-GFP on a Spinning Disk coupled to a Zeiss C-Apo 63x/1.2 W lens (Advanced Digital Microscopy Facility, IRB. Barcelona). Imaging and bleaching parameters same as myosin FRAP experiment.

5.3.1 FRAP Analysis:

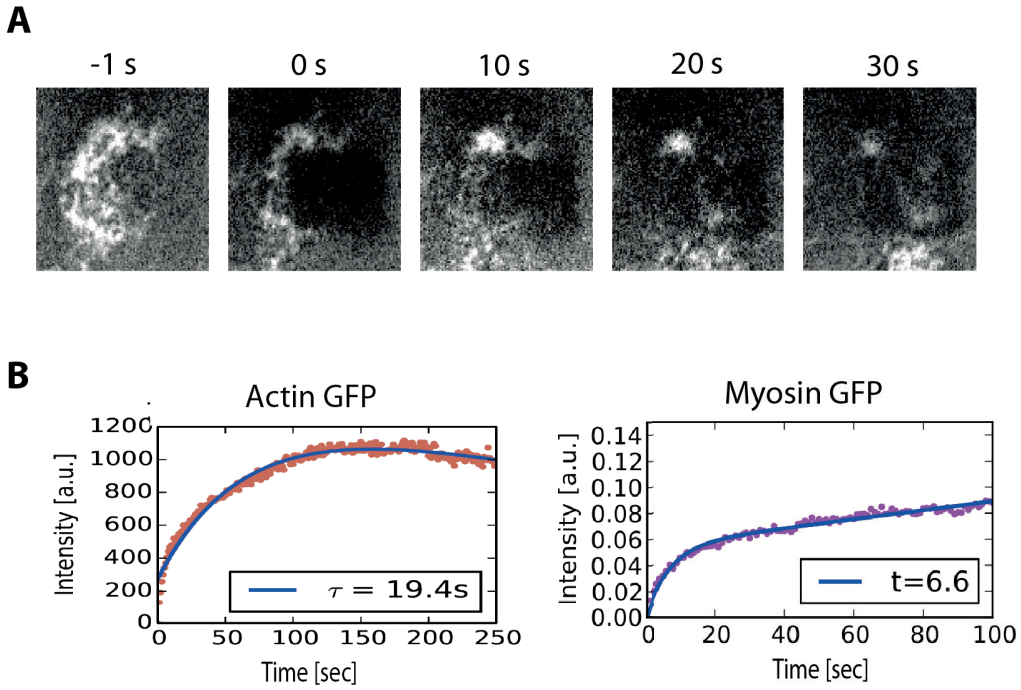


Figure 5.3.1: (A) Timelapse of photobleaching carried out on myosin-GFP. (B) Representative plots of recovery curve for actin and myosin-GFP

In order to quantify the recovery rate of fluorescence, the mean intensity within the ROI was extracted using a Python script that I wrote. Mean myosin intensity was plotted over time and the recovery curve was fitted with an exponential term:

$$I(t) = A(1 - e^{-\frac{t}{\tau}}) + B_1 t + B_2$$

Where,

A = amplitude or level of fluorescence recovery after photobleaching

e = Euler's number

τ= Time constant, which is a measure for the duration of fluorescence

recovery

t = time

The linear term $B_1t + B_2$ was added in order to account for the fact that the actomyosin network is not static but rather flow as waves.

5.4 Laser ablation

This experiment was carried out at the Advanced Digital Microscopy Facility, IRB. Barcelona.

Laser cutting was performed with a scanned 355-nm pulsed (470 ps)-laser, coupled through the port of an AxioVert 200 M and focused through a Zeiss C-Apo 63x/1.2 W lens. Fluorescence confocal images were acquired through a custom spinning disk unit coupled to the inverted microscope, with the excitation line at 488 nm of an Argon laser. Simultaneous fluorescence and transmission imaging was performed with two identical charge-coupled device cameras and a 50/50 splitter internal to the microscope stand. Fluorescence imaging was done at the rate of around 1-3 images/sec.

5.4.1 Analysis

First a kymograph was done on the result of ablation, and a ROI was cropped on the kymograph for 5 frames (5 seconds) before ablation. The mean intensity within the ROI was quantified and then normalised to

the mean intensity of a group of 4-5 cells surrounding region of ablation in order to obtain the myosin concentration (M_c)

Next, a profile of the relaxation was manually extracted from the kymograph, this profile was fitted with a single exponential using a Python script written by Peran Hayes. The initial retraction velocity V_0 was calculated as follows:

$f(t) = A(1 - e^{\frac{-t}{\tau}})$

$V_0 = \frac{A}{\tau}$

Here, A is retraction amplitude and τ, is the exponential decay time constant.

This initial retraction velocity V_0 was then plotted against M_c as a way of studying tension generated by myosin concentration.

5.5 Micro-injection

The needle for microinjection was made by pulling a glass capillary ($1 \times 90mm$) on a P-30 vertical micropipette puller (Sutter), and the tip of the pulled glass opened with EG-44-Narashige-Microgrinder (Digitimer).

Microinjection of embryos were carried out on a Femtojet Microinjector and Micromanipulator coupled to an inverted microscope (Leica DM IL LED).

Membrane marker dye FM4-64 and pharmacological inhibitors were

injected in previtelline space of embryos that were then immediately transferred to the microscope for live imaging. Distilled water was injected as control.

5.6 Set-up for stretch application

For application of force on embryos in order to stretch cells, a round cover glass ($5mm$ in diameter) was fixed to the end of a glass capillary ($1 \times 90mm$) with Super Glue. This was then attached to the pipette holder on a micromanipulator (Narashige) and stretch applied by manually adjusting the coarse and fine knobs.

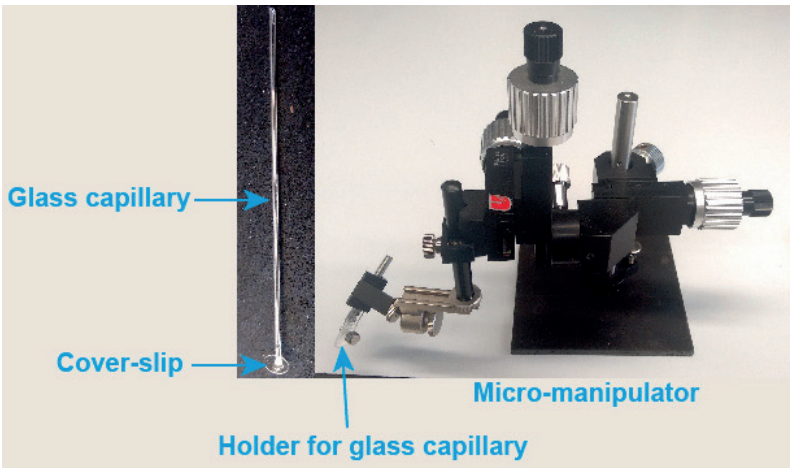


Figure 5.6.1: Set up for stretch application

The viability of embryos that had been subjected to mechanical pressure was also studied by allowing them to grow in the mattek dish. Despite a delay in dorsal closure (Figure 5.6.2), embryos developed into larvae, after which they were transferred into a vial containing standard

cornmeal medium, which then went on to develop into fertile adults.

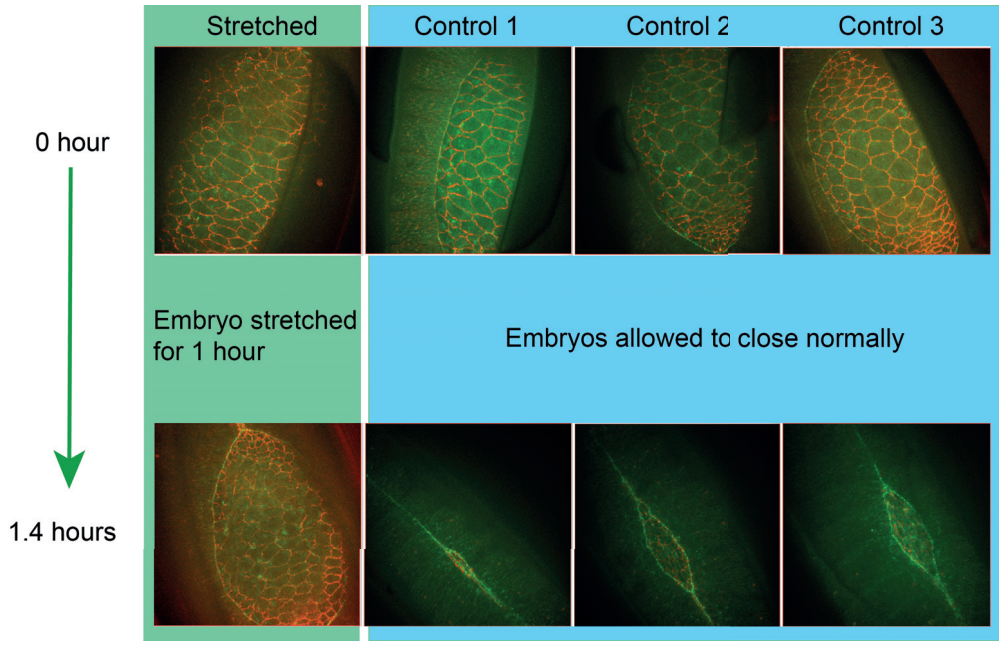


Figure 5.6.2: Comparison of DC in embryo subjected to stretch and controls. Controls were allowed to grow in the same conditions as the stretched by placing them inside the microscope chamber. Embryo was stretched for 1 hour and then allowed to close normally for 40 minutes before comparing with controls.

5.7 Image analysis

5.7.1 Cell segmentation

Amnioserosa cells were segmented and tracked using the software Packing Analyzer V2.0 [Aigouy et al., 2010], which designates a unique ID to each cell that has been segmented. A Python script was written by Kai Dierkes and Peran Hayes to extract data from a specific cell by referring to this ID. Data such as myosin intensity within a particular cell, perimeter, junctional length and straightness were extracted.

5.7.2 Average cell

For each cell the geometrical centroid of the apical area was located, and the distance from this point to the cell junction was measured for different angles to the anterior direction. Junctional distances for each angle were then averaged over all cells and all time points in the time frame of the first 5 minutes of each of the three phases of the squeezing experiment (pre-, during and post-stretch). The resultant average cell shape descriptions allow for assessment of the extent of stretch and relaxation occurring in cells upon pressure application and release for different directions relative to the embryo.

5.7.3 Junctional myosin

First, the average myosin localisations were overlaid onto the average

cell shape to generate an average cell image. Next, for all pixels within a cell's image, the relative distance of the pixel from the cell centroid to its border was measured. The relative distance was then mapped to a position on the average cell shape. Average sqh- GFP intensities for each position (normalised to the average intensity of the cell in order to avoid any effects from experimental variation, bleaching and signal drop upon squeezing) were then calculated; again taken for all cells and all time points in a 5-minute window, and this was carried out for cells in the 3 phases of the stretching experiment.

List of references

References

K Afshar, B Stuart, and S a Wasserman. Functional analysis of the Drosophila diaphanous FH protein in early embryonic development. *Development (Cambridge, England)*, 127(9):1887–97, may 2000. ISSN 0950-1991. URL http://www.ncbi.nlm.nih.gov/pubmed/10751177.

Benoît Aigouy, Reza Farhadifar, Douglas B Staple, Andreas Sagner, Jens-Christian Röper, Frank Jülicher, and Suzanne Eaton. Cell flow reorients the axis of planar polarity in the wing epithelium of Drosophila. *Cell*, 142(5):773–86, sep 2010. ISSN 1097-4172. doi: 10.1016/j.cell.2010.07.042. URL http://www.ncbi.nlm.nih.gov/pubmed/20813263.

N Akhtar and N A Hotchin. RAC1 regulates adherens junctions through endocytosis of E-cadherin. *Molecular biology of the cell*, 12(4):847–62, apr 2001. ISSN 1059-1524. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=32271{&}tool=pmcentrez{&}rendertype=abstract.

Bruce Alberts, Denis Bray, Julian Lewis, Martin Raff, Keith Roberts, and James D. Watson. *Molecular Biology of the Cell*. Garland Publishing, 1994. URL http://www.bioon.com/book/biology/mboc/mboc.cgi@action=figure{&}fig=16-71.htm.

Revathi Ananthakrishnan and Allen Ehrlicher. The forces behind cell movement. *International journal of biological sciences*, 3(5):303–17, jan 2007. ISSN 1449-2288. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1893118{&}tool=pmcentrez{&}rendertype=abstract.

Revathi Ananthakrishnan, Jochen Guck, Falk Wottawah, Stefan Schinkinger, Bryan

Lincoln, Maren Romeyke, Tess Moon, and Josef Käs. Quantifying the contribution of actin networks to the elastic strength of fibroblasts. *Journal of theoretical biology*, 242(2):502–16, sep 2006. ISSN 0022-5193. doi: 10.1016/j.jtbi.2006.03.021. URL http://www.ncbi.nlm.nih.gov/pubmed/16720032.

D Axelrod, D E Koppel, J Schlessinger, E Elson, and W W Webb. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophysical journal*, 16(9):1055–69, sep 1976. ISSN 0006-3495. doi: 10.1016/S0006-3495(76)85755-4. URL http://www.ncbi.nlm.nih.gov/pubmed/786399http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1334945.

Dulce Azevedo, Marco Antunes, Soren Prag, Xiaoyan Ma, Udo Hacker, G. Wayne Brodland, M. Shane Hutson, Jerome Solon, Antonio Jacinto, Shane Hutson, Jerome Solon, and Antonio Jacinto. DRhoGEF2 Regulates Cellular Tension and Cell Pulsations in the Amnioserosa during Drosophila Dorsal Closure. *PLoS ONE*, 6(9):e23964, sep 2011. ISSN 1932-6203. doi: 10.1371/journal.pone.0023964. URL http://dx.plos.org/10.1371/journal.pone.0023964http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3174941{&}tool=pmcentrez{&}rendertype=abstract.

Buzz Baum and Marios Georgiou. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *The Journal of cell biology*, 192(6):907–17, mar 2011. ISSN 1540-8140. doi: 10.1083/jcb.201009141. URL http://jcb.rupress.org/content/192/6/907.full.

Martin Behrndt, Julia Roensch, Stephan W Grill, and Carl-philipp Heisenberg. Forces Driving Epithelial Spreading in Zebrafish Gastrulation. 257:10–14, 2012. doi: 10.1126/science.1224143.

Claire Bertet, Lawrence Sulak, and Thomas Lecuit. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature*, 429(6992): 667–671, 2004. URL http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve{&}db=PubMed{&}dopt=Citation{&}list{_}uids=15190355.

D Bilder and N Perrimon. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature*, 403(6770):676–80, feb 2000. ISSN 0028-0836. doi: 10.1038/35001108. URL http://www.ncbi.nlm.nih.gov/pubmed/10688207.

Guy B Blanchard, Sughashini Murugesu, Richard J Adams, Alfonso Martinez-Arias, and Nicole Gorfinkiel. Cytoskeletal dynamics and supracellular organisation of cell shape fluctuations during dorsal closure. *Development (Cambridge, England)*, 137 (16):2743–52, aug 2010. ISSN 1477-9129. doi: 10.1242/dev.045872. URL http://www.ncbi.nlm.nih.gov/pubmed/20663818.

Nicolas Borghi, Olga G Shcherbakova, William I Weis, Beth L Pruitt, W James, Alexander R Dunn, Maria Sorokina, W James Nelson, Olga G Shcherbakova, William I Weis, Beth L Pruitt, W James Nelson, and Alexander R Dunn. Correction for Borghi et al., E-cadherin is under constitutive actomyosin-generated tension that is increased at cell-cell contacts upon externally applied stretch. *Proceedings of the National Academy of Sciences*, 109(46):19034–19034, oct 2012. ISSN 0027-8424. doi: 10.1073/pnas.1217417109. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3411997{&}tool=pmcentrez{&}rendertype=abstracthttp://www.pnas.org/cgi/doi/10.1073/pnas.1217417109.

M Bornens, M Paintrand, and C Celati. The cortical microfilament system of lymphoblasts displays a periodic oscillatory activity in the absence of microtubules: implications for cell polarity. *The Journal of cell biology*, 109(3):1071–83, sep 1989.

ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2115765{&}tool=pmcentrez{&}rendertype=abstract.

Floris Bosveld, Olga Markova, Boris Guirao, Charlotte Martin, Zhimin Wang, Anaëlle Pierre, Maria Balakireva, Isabelle Gaugue, Anna Ainslie, Nicolas Christophorou, David K. Lubensky, Nicolas Minc, and Yohanns Bellaïche. Epithelial tricellular junctions act as interphase cell shape sensors to orient mitosis. *Nature*, 530 (7591):495–498, feb 2016. ISSN 0028-0836. doi: 10.1038/nature16970. URL http://www.nature.com/doifinder/10.1038/nature16970.

L A Bourret and G A Rodan. The role of calcium in the inhibition of cAMP accumulation in epiphyseal cartilage cells exposed to physiological pressure. *Journal of cellular physiology*, 88(3):353–61, jul 1976. ISSN 0021-9541. doi: 10.1002/jcp.1040880311. URL http://www.ncbi.nlm.nih.gov/pubmed/178677.

D Bray and J G Whe. D. bray. 282(February):883–888, 1988.

J Brock, K Midwinter, J Lewis, and P Martin. Healing of incisional wounds in the embryonic chick wing bud: characterization of the actin purse-string and demonstration of a requirement for Rho activation. *The Journal of cell biology*, 135(4):1097–107, nov 1996. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2133375{&}tool=pmcentrez{&}rendertype=abstract.

David M Bryant, Markus C Kerr, Luke A Hammond, Shannon R Joseph, Keith E Mostov, Rohan D Teasdale, and Jennifer L Stow. EGF induces macropinocytosis and SNX1-modulated recycling of E-cadherin. *Journal of cell science*, 120(Pt 10): 1818–28, may 2007. ISSN 0021-9533. doi: 10.1242/jcs.000653. URL http://www.ncbi.nlm.nih.gov/pubmed/17502486.

A.E. Bugrim. Regulation of Ca2+release by cAMP-dependent protein kinase A mechanism for agonist-specific calcium signaling? *Cell Calcium*, 25(3):219–226, mar 1999. ISSN 01434160. doi: 10.1054/ceca.1999.0027. URL http://linkinghub.elsevier.com/retrieve/pii/S0143416099900271.

Fabien Cailliez and Richard Lavery. Cadherin mechanics and complexation: the importance of calcium binding. *Biophysical journal*, 89 (6):3895–903, dec 2005. ISSN 0006-3495. doi: 10.1529/biophysj.105.067322. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1366956{&}tool=pmcentrez{&}rendertype=abstract.

Lara Carvalho, António Jacinto, and Nina Matova. The Toll/NF-κB signaling pathway is required for epidermal wound repair in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, 111(50):E5373–82, dec 2014. ISSN 1091-6490. doi: 10.1073/pnas.1408224111. URL http://www.pnas.org/content/111/50/E5373.

Maureen Cetera, Guillermina R Ramirez-San Juan, Patrick W Oakes, Lindsay Lewellyn, Michael J Fairchild, Guy Tanentzapf, Margaret L Gardel, and Sally Horne-Badovinac. Epithelial rotation promotes the global alignment of contractile actin bundles during Drosophila egg chamber elongation. *Nature communications*, 5:5511, jan 2014. ISSN 2041-1723. doi: 10.1038/ncomms6511. URL http://www.nature.com/ncomms/2014/141121/ncomms6511/abs/ncomms6511.html.

Guillaume T Charras and Mike A Horton. Single cell mechanotransduction and its modulation analyzed by atomic force microscope indentation. *Biophysical journal*, 82(6):2970–81, jun 2002. ISSN 0006-3495. doi: 10.1016/S0006-3495(02)75638-5. URL http://www.ncbi.nlm.nih.gov/pubmed/12023220http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1302085.

Guillaume T. Charras, C.-K. Chi K. Hu, Margaret Coughlin, and Timothy J. Mitchison. Reassembly of contractile actin cortex in cell blebs. *The Journal of Cell Biology*, 175(3):477–490, 2006. ISSN 00219525. doi: 10.1083/jcb.200602085. URL http://www.jcb.org/cgi/doi/10.1083/jcb.200602085.

Guillaume T Charras, Timothy J Mitchison, and L Mahadevan. Animal cell hydraulics. *Journal of cell science*, 122(Pt 18):3233–41, sep 2009. ISSN 1477-9137. doi: 10.1242/jcs.049262. URL http://www.ncbi.nlm.nih.gov/pubmed/19690051http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2736862.

Qi Cheng, Zhe Sun, Gerald Meininger, and Mahmoud Almasri. PDMS Elastic Micropost Arrays for Studying Vascular Smooth Muscle Cells. *Sensors and actuators. B, Chemical*, 188:1055–1063, nov 2013. ISSN 0925-4005. doi: 10.1016/j.snb.2013.08.018. URL http://www.ncbi.nlm.nih.gov/pubmed/26451074http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4594632.

Seyeon Chung and Deborah J Andrew. The formation of epithelial tubes. *Journal of cell science*, 121(Pt 21):3501–4, nov 2008. ISSN 0021-9533. doi: 10.1242/jcs.037887. URL http://jcs.biologists.org/content/121/21/3501.

3 Ciara C. Reyes, 1 Meiyan Jin, 2 Elaina B. Breznau, 1 Rhogelyn Espino, 2 Ricard Delgado-Gonzalo, 4 Andrew B. Goryachev, and Ann L. Miller1. Anillin Regulates Cell-Cell Junction Integrity by Organizing Junctional Accumulation of Rho-GTP and Actomyosin - reyes1401.pdf. *Current biology : CB*, 24(11):1263–70, jun 2014. ISSN 1879-0445. doi: 10.1016/j.cub.2014.04.021. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4043929{&}tool=pmcentrez{&}rendertype=abstracthttp://bigwww.epfl.ch/publications/reyes1401.pdf.

Andrew G Clark, Kai Dierkes, and Ewa K Paluch. Monitoring actin cortex thickness

in live cells. *Biophysical journal*, 105(3):570–80, aug 2013. ISSN 1542-0086. doi: 10.1016/j.bpj.2013.05.057. URL http://www.ncbi.nlm.nih.gov/pubmed/23931305http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3736691.

M Clarke and J A Spudich. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. *Annual review of biochemistry*, 46:797–822, jan 1977. ISSN 0066-4154. doi: 10.1146/annurev.bi.46.070177.004053. URL http://www.annualreviews.org/doi/abs/10.1146/annurev.bi.46.070177.004053.

Julien Colombelli and Jérôme Solon. Force communication in multicellular tissues addressed by laser nanosurgery. *Cell and tissue research*, may 2012. ISSN 1432-0878. doi: 10.1007/s00441-012-1445-1. URL http://www.ncbi.nlm.nih.gov/pubmed/22622805.

Julien Colombelli, Achim Besser, Holger Kress, Emmanuel G Reynaud, Philippe Girard, Emmanuel Caussinus, Uta Haselmann, John V Small, Ulrich S Schwarz, and Ernst H K Stelzer. Mechanosensing in actin stress fibers revealed by a close correlation between force and protein localization. *Journal of cell science*, 122(Pt 10):1665–1679, 2009. ISSN 0021-9533. doi: 10.1242/jcs.054577.

Laureen E Connell and David M Helfman. Myosin light chain kinase plays a role in the regulation of epithelial cell survival. *Journal of cell science*, 119(Pt 11):2269–81, jun 2006. ISSN 0021-9533. doi: 10.1242/jcs.02926. URL http://www.ncbi.nlm.nih.gov/pubmed/16723733.

Geoffrey M Cooper. Structure and Organization of Actin Filaments. 2000.

R Craig, R Smith, and J Kendrick-Jones. Light-chain phosphorylation controls the conformation of vertebrate non-muscle and smooth muscle myosin molecules. *Nature*,

302(5907):436–9, jan 1983. ISSN 0028-0836. URL http://www.ncbi.nlm.nih.gov/pubmed/6687627.

J Dai, H P Ting-Beall, R M Hochmuth, M P Sheetz, and M A Titus. Myosin I contributes to the generation of resting cortical tension. *Biophysical journal*, 77(2):1168–76, aug 1999. ISSN 0006-3495. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1300408{&}tool=pmcentrez{&}rendertype=abstract.

Eric M Darling, Matthew Topel, Stefan Zauscher, Thomas P Vail, and Farshid Guilak. Viscoelastic properties of human mesenchymally-derived stem cells and primary osteoblasts, chondrocytes, and adipocytes. *Journal of biomechanics*, 41(2):454–64, 2008. ISSN 0021-9290. doi: 10.1016/j.jbiomech.2007.06.019. URL http://www.ncbi.nlm.nih.gov/pubmed/17825308http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2897251.

P C Dartsch and E Betz. Response of cultured endothelial cells to mechanical stimulation. *Basic research in cardiology*, 84(3):268–81, 1989. ISSN 0300-8428. URL http://www.ncbi.nlm.nih.gov/pubmed/2764859.

Daryl J V David, Alisa Tishkina, and Tony J C Harris. The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in Drosophila. *Development (Cambridge, England)*, 137(10):1645–55, may 2010. ISSN 1477-9129. doi: 10.1242/dev.044107. URL http://www.ncbi.nlm.nih.gov/pubmed/20392741.

Daryl J V David, Qiming Wang, James J Feng, and Tony J C Harris. Bazooka inhibits aPKC to limit antagonism of actomyosin networks during amnioserosa apical constriction. *Development (Cambridge, England)*, 140(23):4719–29, dec 2013. ISSN

1477-9129. doi: 10.1242/dev.098491. URL http://www.ncbi.nlm.nih.gov/pubmed/24173807.

Michael A Davis, Renee C Ireton, and Albert B Reynolds. A core function for p120-catenin in cadherin turnover. *The Journal of cell biology*, 163(3):525–34, nov 2003. ISSN 0021-9525. doi: 10.1083/jcb.200307111. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2173649{&}tool=pmcentrez{&}rendertype=abstract.

Manqi Deng, Carmen J. Williams, and Richard M. Schultz. Role of MAP kinase and myosin light chain kinase in chromosome-induced development of mouse egg polarity. *Developmental Biology*, 278(2):358–366, 2005. ISSN 00121606. doi: 10.1016/j.ydbio.2004.11.013.

Su Deng, Ingo Bothe, and Mary K Baylies. The Formin Diaphanous Regulates Myoblast Fusion through Actin Polymerization and Arp2/3 Regulation. *PLoS genetics*, 11(8):e1005381, aug 2015. ISSN 1553-7404. doi: 10.1371/journal.pgen.1005381. URL http://www.ncbi.nlm.nih.gov/pubmed/26295716http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4546610.

Kai Dierkes, Angughali Sumi, Jérôme Solon, and Guillaume Salbreux. Spontaneous oscillations from turnover of an elastic contractile material. 148102(OCTOBER):10, 2014. ISSN 10797114. doi: 10.1103/PhysRevLett.113.148102. URL http://arxiv.org/abs/1402.5390.

Marileen Dogterom, Jacob WJ Kerssemakers, Guillaume Romet-Lemonne, and Marcel E Janson. Force generation by dynamic microtubules. *Current Opinion in Cell Biology*, 17(1):67–74, 2005. ISSN 09550674. doi: 10.1016/j.ceb.2004.12.011.

Anthony D'Onofrio. Tissue Types Study Guide - Alyvea.com, 2009. URL http://www.alyvea.com/biologystudyguides/body-tissue-types.php.

Meghan K Driscoll, Wolfgang Losert, Ken Jacobson, and Maryna Kapustina. Spatiotemporal relationships between the cell shape and the actomyosin cortex of periodically protruding cells. *Cytoskeleton (Hoboken, N.J.)*, 72(6):268–81, jun 2015. ISSN 1949-3592. doi: 10.1002/cm.21229. URL http://www.ncbi.nlm.nih.gov/pubmed/26147497.

J C Dunlap. Molecular bases for circadian clocks. *Cell*, 96(2):271–90, jan 1999. ISSN 0092-8674. URL http://www.ncbi.nlm.nih.gov/pubmed/9988221.

Asier Echarri and Miguel A Del Pozo. Caveolae - mechanosensitive membrane invaginations linked to actin filaments. *Journal of cell science*, 128(15):2747–58, aug 2015. ISSN 1477-9137. doi: 10.1242/jcs.153940. URL http://www.ncbi.nlm.nih.gov/pubmed/26159735.

Janet C Effler, Yee-Seir Kee, Jason M Berk, Minhchau N Tran, Pablo A Iglesias, and Douglas N Robinson. Mitosis-specific mechanosensing and contractile-protein redistribution control cell shape. *Current biology : CB*, 16(19):1962–7, oct 2006. ISSN 0960-9822. doi: 10.1016/j.cub.2006.08.027. URL http://www.ncbi.nlm.nih.gov/pubmed/17027494http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2474462http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2474462{&}tool=pmcentrez{&}rendertype=abstract.

T T Egelhoff, R J Lee, and J A Spudich. Dictyostelium myosin heavy chain phosphorylation sites regulate myosin filament assembly and localization in vivo. *Cell*, 75(2):363–71, oct 1993. ISSN 0092-8674. URL http://www.ncbi.nlm.nih.gov/pubmed/7691416.

W Engl, B Arasi, L L Yap, J P Thiery, and V Viasnoff. Actin dynamics modulate mechanosensitive immobilization of E-cadherin at adherens junctions. *Nature cell biology*, 16(6):587–94, jun 2014. ISSN 1476-4679. doi: 10.1038/ncb2973. URL http://www.ncbi.nlm.nih.gov/pubmed/24859003.

Jocelyn Étienne, Jonathan Fouchard, Démosthène Mitrossilis, Nathalie Bufi, Pauline Durand-Smet, and Atef Asnacios. Cells as liquid motors: mechanosensitivity emerges from collective dynamics of actomyosin cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 112(9):2740–5, mar 2015. ISSN 1091-6490. doi: 10.1073/pnas.1417113112. URL http://www.ncbi.nlm.nih.gov/pubmed/25730854http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4352826.

E Evans and A Yeung. Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. *Biophysical journal*, 56 (1):151–60, jul 1989. ISSN 0006-3495. doi: 10.1016/S0006-3495(89)82660-8. URL http://www.ncbi.nlm.nih.gov/pubmed/2752085http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1280460.

E A Evans. New membrane concept applied to the analysis of fluid shear- and micropipette-deformed red blood cells. *Biophysical journal*, 13 (9):941–54, sep 1973. ISSN 0006-3495. doi: 10.1016/S0006-3495(73)86036-9. URL http://www.ncbi.nlm.nih.gov/pubmed/4733701http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1484376.

Emmanuel Farge. Mechanical induction of Twist in the Drosophila foregut/stomodeal primordium. *Current biology : CB*, 13(16):1365–77, aug 2003. ISSN 0960-9822. doi: 10.1016/s0960-9822(03)00576-1. URL http://www.ncbi.nlm.nih.gov/pubmed/12932320.

Rodrigo Fernandez-Gonzalez and Jennifer A Zallen. Wounded cells drive rapid epidermal repair in the early Drosophila embryo. *Molecular biology of the cell*, 24(20):3227–37, oct 2013. ISSN 1939-4586. doi: 10.1091/mbc.E13-05-0228. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3806660{&}tool=pmcentrez{&}rendertype=abstract.

Rodrigo Fernandez-gonzalez, Sérgio De Matos Simoes, Jens-christian Röper, Suzanne Eaton, and Jennifer A. Zallen. Myosin II Dynamics Are Regulated by Tension in Intercalating Cells. *Developmental Cell*, 17(5): 736–743, nov 2009. ISSN 15345807. doi: 10.1016/j.devcel.2009.09.003. URL http://www.cell.com/article/S1534580709003852/fulltexthttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2854079{&}tool=pmcentrez{&}rendertype=abstract.

Ryan W Fiehler and Tanya Wolff. Drosophila Myosin II, Zipper, is essential for ommatidial rotation. *Developmental biology*, 310(2):348–62, oct 2007. ISSN 0012-1606. doi: 10.1016/j.ydbio.2007.08.001. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2110880{&}tool=pmcentrez{&}rendertype=abstract.

J T Finer, R M Simmons, and J A Spudich. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature*, 368(6467):113–9, mar 1994. ISSN 0028-0836. doi: 10.1038/368113a0. URL http://www.ncbi.nlm.nih.gov/pubmed/8139653.

J T Finer, A D Mehta, and J A Spudich. Characterization of single actin-myosin interactions. *Biophysical journal*, 68(4 Suppl):291S–296S; discussion 296S–297S, apr 1995. ISSN 0006-3495. URL http://www.ncbi.nlm.nih.gov/pubmed/7787094http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1281952.

Sabine C Fischer, Guy B Blanchard, Julia Duque, Richard J Adams, Alfonso Martinez Arias, Simon D Guest, and Nicole Gorfinkiel. Contractile and mechanical properties of epithelia with perturbed actomyosin dynamics. *PloS one*, 9(4):e95695, jan 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0095695. URL http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095695.

Josef D Franke, Ruth A Montague, and Daniel P Kiehart. Nonmuscle myosin II generates forces that transmit tension and drive contraction in multiple tissues during dorsal closure. *Current Biology*, 15(24):2208–2221, 2005. URL http://www.ncbi.nlm.nih.gov/pubmed/16360683.

Scott E Fraser and Richard M Harland. The Molecular Metamorphosis of Experimental Embryology. *Cell*, 100(1):41–55, jan 2000. ISSN 00928674. doi: 10.1016/S0092-8674(00)81682-7. URL http://www.cell.com/article/S0092867400816827/fulltext.

W J Gallin. Evolution of the "classical" cadherin family of cell adhesion molecules in vertebrates. *Molecular biology and evolution*, 15(9):1099–107, sep 1998. ISSN 0737-4038. URL http://www.ncbi.nlm.nih.gov/pubmed/9729874.

F Gittes, B Mickey, J Nettleton, and J Howard. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *The Journal of cell biology*, 120(4):923–34, feb 1993. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2200075{&}tool=pmcentrez{&}rendertype=abstract.

A. Glücksmann. Studies on bone mechanics in vitro. II. The role of tension and pressure in chondrogenesis. *The Anatomical Record*, 73(1):39–55, jan 1939. ISSN

0003276X. doi: 10.1002/ar.1090730105. URL http://doi.wiley.com/10.1002/ar.1090730105.

D Godt and U Tepass. Drosophila oocyte localization is mediated by differential cadherin-based adhesion. *Nature*, 395(6700):387–91, sep 1998. ISSN 0028-0836. doi: 10.1038/26493. URL http://dx.doi.org/10.1038/26493.

Albert Goldbeter. Biological rhythms: clocks for all times. *Current biology : CB*, 18 (17):R751–R753, sep 2008. ISSN 0960-9822. doi: 10.1016/j.cub.2008.06.044. URL http://www.ncbi.nlm.nih.gov/pubmed/18786378.

M C Göpfert, A D L Humphris, J T Albert, D Robert, and O Hendrich. Power gain exhibited by motile mechanosensory neurons in Drosophila ears. *Proceedings of the National Academy of Sciences of the United States of America*, 102(2):325–30, jan 2005. ISSN 0027-8424. doi: 10.1073/pnas.0405741102. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=544284{&}tool=pmcentrez{&}rendertype=abstracthttp://www.pnas.org/content/102/2/325.full.

Nicole Gorfinkiel and Guy B Blanchard. Dynamics of actomyosin contractile activity during epithelial morphogenesis. *Current opinion in cell biology*, jul 2011. ISSN 1879-0410. doi: 10.1016/j.ceb.2011.06.002. URL http://www.ncbi.nlm.nih.gov/pubmed/21764278.

Nicole Gorfinkiel, Guy B Blanchard, Richard J Adams, and Alfonso Martinez Arias. Mechanical control of global cell behaviour during dorsal closure in Drosophila. *Development (Cambridge, England)*, 136(11):1889–98, jun 2009. ISSN 0950-1991. doi: 10.1242/dev.030866. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2680111{&}tool=pmcentrez{&}rendertype=abstract.

Minakshi Guha, Mian Zhou, Yu-li Wang, and Plantation Street. Cortical Actin Turnover during Cytokinesis Requires Myosin II. 15:732–736, 2005. doi: 10.1016/j.cub.2005.03.042.

Kristina Haase and Andrew E Pelling. Investigating cell mechanics with atomic force microscopy. *Journal of the Royal Society, Interface / the Royal Society*, 12(104):20140970, mar 2015. ISSN 1742-5662. doi: 10.1098/rsif.2014.0970. URL http://www.ncbi.nlm.nih.gov/pubmed/25589563http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4345470.

Jennifer M Halbleib and W James Nelson. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes & development*, 20(23):3199–214, dec 2006. ISSN 0890-9369. doi: 10.1101/gad.1486806. URL http://www.ncbi.nlm.nih.gov/pubmed/17158740.

F Hanakam, R Albrecht, C Eckerskorn, M Matzner, and G Gerisch. Myristoylated and non-myristoylated forms of the pH sensor protein hisactophilin II: intracellular shuttling to plasma membrane and nucleus monitored in real time by a fusion with green fluorescent protein. *The EMBO journal*, 15(12):2935–43, jun 1996. ISSN 0261-4189. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=450234{&}tool=pmcentrez{&}rendertype=abstract.

Andrew R Harris, Julien Bellis, Nargess Khalilgharibi, Tom Wyatt, Buzz Baum, Alexandre J Kabla, and Guillaume T Charras. Generating suspended cell monolayers for mechanobiological studies. *Nature protocols*, 8(12):2516–30, dec 2013. ISSN 1750-2799. doi: 10.1038/nprot.2013.151. URL http://www.ncbi.nlm.nih.gov/pubmed/24263091.

M Amanda Hartman and James A Spudich. The myosin superfamily at a glance.

Journal of cell science, 125(Pt 7):1627–32, apr 2012. ISSN 1477-9137. doi: 10.1242/jcs.094300. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3346823{&}tool=pmcentrez{&}rendertype=abstract.

Lior Haviv, David Gillo, Frederic Backouche, and Anne Bernheim-Groswasser. A cytoskeletal demolition worker: myosin II acts as an actin depolymerization agent. *Journal of molecular biology*, 375(2):325–30, jan 2008. ISSN 1089-8638. doi: 10.1016/j.jmb.2007.09.066. URL http://www.ncbi.nlm.nih.gov/pubmed/18021803.

Li He, Xiaobo Wang, Ho Lam Tang, and Denise J Montell. Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nature cell biology*, 12(12): 1133–1142, 2010. ISSN 1465-7392. doi: 10.1038/ncb2124.

S Hénon, G Lenormand, A Richert, and F Gallet. A new determination of the shear modulus of the human erythrocyte membrane using optical tweezers. *Biophysical journal*, 76(2):1145–51, feb 1999. ISSN 0006-3495. doi: 10.1016/S0006-3495(99)77279-6. URL http://www.ncbi.nlm.nih.gov/pubmed/9916046http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1300064.

Leah Herrgen, Saúl Ares, Luis G Morelli, Christian Schröter, Frank Jülicher, and Andrew C Oates. Intercellular coupling regulates the period of the segmentation clock. *Current biology : CB*, 20(14):1244–53, jul 2010. ISSN 1879-0445. doi: 10.1016/j.cub.2010.06.034. URL http://www.ncbi.nlm.nih.gov/pubmed/20637620.

S N Hird and J G White. Cortical and cytoplasmic flow polarity in early embryonic cells of Caenorhabditis elegans. *The Journal of cell biology*, 121(6):1343–55, jun 1993. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2119718{&}tool=pmcentrez{&}rendertype=abstract.

Robert M Hochmuth. Micropipette aspiration of living cells. *Journal of Biomechanics*,

33(1):15–22, jan 2000. ISSN 00219290. doi: 10.1016/S0021-9290(99)00175-X. URL http://linkinghub.elsevier.com/retrieve/pii/S002192909900175X.

Ginger Hunter. The Role of Mechanically Gated Ion Channels in Dorsal Closure During Drosophila morphogenesis. 2012.

Ginger L Hunter, Janice M Crawford, Julian Z Genkins, and Daniel P Kiehart. Ion channels contribute to the regulation of cell sheet forces during Drosophila dorsal closure. *Development (Cambridge, England)*, 141(2):325–34, jan 2014. ISSN 1477-9129. doi: 10.1242/dev.097097. URL http://www.ncbi.nlm.nih.gov/pubmed/24306105.

Miranda V Hunter, Donghoon M Lee, Tony J C Harris, and Rodrigo Fernandez-Gonzalez. Polarized E-cadherin endocytosis directs actomyosin remodeling during embryonic wound repair. *The Journal of cell biology*, 210(5):801–16, aug 2015. ISSN 1540-8140. doi: 10.1083/jcb.201501076. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4555830{&}tool=pmcentrez{&}rendertype=abstract.

M Shane Hutson, Yoichiro Tokutake, Ming-Shien Chang, James W Bloor, Stephanos Venakides, Daniel P Kiehart, and Glenn S Edwards. Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. *Science (New York, N.Y.)*, 300(5616):145–9, apr 2003. ISSN 1095-9203. doi: 10.1126/science.1079552. URL http://www.ncbi.nlm.nih.gov/pubmed/12574496.

Mineo Iseki, Shigeru Matsunaga, Akio Murakami, Kaoru Ohno, Kiyoshi Shiga, Kazuichi Yoshida, Michizo Sugai, Tetsuo Takahashi, Terumitsu Hori, and Masakatsu Watanabe. A blue-light-activated adenylyl cyclase mediates photoavoidance in Euglena gracilis. *Nature*, 415(6875):1047–51, feb 2002. ISSN 0028-0836. doi: 10.1038/4151047a. URL http://www.ncbi.nlm.nih.gov/pubmed/11875575.

Shin'ichi Ishiwata, Yuta Shimamoto, Madoka Suzuki, and Daisuke Sasaki. Regulation of muscle contraction by Ca2+ and ADP: focusing on the auto-oscillation (SPOC). *Advances in experimental medicine and biology*, 592:341–58, jan 2007. ISSN 0065-2598. URL http://www.ncbi.nlm.nih.gov/pubmed/17278378.

Youichi Iwai, Tadao Usui, Shinji Hirano, Ruth Steward, Masatoshi Takeichi, and Tadashi Uemura. Axon Patterning Requires D N-cadherin, a Novel Neuronal Adhesion Receptor, in the Drosophila Embryonic CNS. *Neuron*, 19(1):77–89, jul 1997. ISSN 08966273. doi: 10.1016/S0896-6273(00)80349-9. URL http://www.sciencedirect.com/science/article/pii/S0896627300803499.

Baohua Ji and Gang Bao. CELL AND MOLECULAR BIOMECHANICS :. *Acta Mechanica*, 24(1), 2011.

Frank Jülicher and Jacques Prost. Spontaneous Oscillations of Collective Molecular Motors. *Physical Review Letters*, 78(23):4510–4513, jun 1997. ISSN 0031-9007. doi: 10.1103/PhysRevLett.78.4510. URL http://journals.aps.org/prl/abstract/10.1103/PhysRevLett.78.4510.

K Kanda, T Matsuda, and T Oka. Two-dimensional orientational response of smooth muscle cells to cyclic stretching. *ASAIO journal (American Society for Artificial Internal Organs : 1992)*, 38(3):M382–5, 1992. ISSN 1058-2916. URL http://www.ncbi.nlm.nih.gov/pubmed/1457887.

Pierre-François Lenne Kapil Bambardekar, Raphaël Clément, Olivier Blanc, Claire Chardès. Direct laser manipulation reveals the mechanics of cell contacts in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 112(5):1416, 2015.

Karen E Kasza and Jennifer a Zallen. Dynamics and regulation of contractile actin-myosin networks in morphogenesis. *Current opinion in cell biology*, 23(1):30–8, feb 2011. ISSN 1879-0410. doi: 10.1016/j.ceb.2010.10.014. URL http://www.ncbi.nlm.nih.gov/pubmed/21130639.

Daniel P Kiehart, Catherine G Galbraith, Kevin A Edwards, Wayne L Rickoll, and Ruth A Montague. Multiple forces contribute to cell sheet morphogenesis for dorsal closure in Drosophila. *The Journal of Cell Biology*, 149(2):471–490, 2000. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2175161{&}tool=pmcentrez{&}rendertype=abstract.

K Kimura, M Ito, M Amano, K Chihara, Y Fukata, M Nakafuku, B Yamamori, J Feng, T Nakano, K Okawa, A Iwamatsu, and K Kaibuchi. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science (New York, N.Y.)*, 273(5272):245–8, jul 1996. ISSN 0036-8075. URL http://www.ncbi.nlm.nih.gov/pubmed/8662509.

T. Kimura, T. Sakisaka, T. Baba, T. Yamada, and Y. Takai. Involvement of the Ras-Ras-activated Rab5 Guanine Nucleotide Exchange Factor RIN2-Rab5 Pathway in the Hepatocyte Growth Factor-induced Endocytosis of E-cadherin. *Journal of Biological Chemistry*, 281(15):10598–10609, apr 2006. ISSN 0021-9258. doi: 10.1074/jbc.M510531200. URL http://www.jbc.org/cgi/doi/10.1074/jbc.M510531200.

Anita Joanna Kosmalska, Laura Casares, Alberto Elosegui-Artola, Joseph Jose Thottacherry, Roberto Moreno-Vicente, Víctor González-Tarragó, Miguel Ángel del Pozo, Satyajit Mayor, Marino Arroyo, Daniel Navajas, Xavier Trepat, Nils C Gauthier, and Pere Roca-Cusachs. Physical principles of membrane remodelling during cell mechanoadaptation. *Nature communications*, 6:7292, 2015. ISSN 2041-1723. doi: 10.

1038/ncomms8292. URL http://www.ncbi.nlm.nih.gov/pubmed/26073653http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4490354.

Mihály Kovács, Kavitha Thirumurugan, Peter J Knight, and James R Sellers. Load-dependent mechanism of nonmuscle myosin 2. *Proceedings of the National Academy of Sciences of the United States of America*, 104(24):9994–9, jun 2007. ISSN 0027-8424. doi: 10.1073/pnas.0701181104. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1885822{&}tool=pmcentrez{&}rendertype=abstract.

Andrew P Kowalczyk and Benjamin A Nanes. Adherens junction turnover: regulating adhesion through cadherin endocytosis, degradation, and recycling. *Subcellular biochemistry*, 60:197–222, jan 2012. ISSN 0306-0225. doi: 10.1007/978-94-007-4186-7_9. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4074012{&}tool=pmcentrez{&}rendertype=abstract.

Michael M Kozlov and Alexander D Bershadsky. Processive capping by formin suggests a force-driven mechanism of actin polymerization. *The Journal of cell biology*, 167(6):1011–7, dec 2004. ISSN 0021-9525. doi: 10.1083/jcb.200410017. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2172604{&}tool=pmcentrez{&}rendertype=abstract.

M Krieg, Y Arboleda-Estudillo, P-H Puech, J Käfer, F Graner, D J Müller, and C-P Heisenberg. Tensile forces govern germ-layer organization in zebrafish. *Nature cell biology*, 10(4):429–36, apr 2008. ISSN 1476-4679. doi: 10.1038/ncb1705. URL http://www.ncbi.nlm.nih.gov/pubmed/18364700.

Kshitiz, Deok-Ho Kim Kshitiz Jinseok Park Peter Kim Wilda Helen Adam J Engler Andre Levchenko, JinSeok Park, Peter Kim, Wilda Helen, Adam J Engler, Andre

Levchenko, and Deok-Ho Kim. Control of stem cell fate and function by engineering physical microenvironments. *Integr Biol (Camb)*, 4(9):1008–18, sep 2012. ISSN 1757-9708. URL http://www.ncbi.nlm.nih.gov/pubmed/23077731http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3476065.

Abhishek Kumar and G. V. Shivashankar. Mechanical Force Alters Morphogenetic Movements and Segmental Gene Expression Patterns during Drosophila Embryogenesis. *PLoS ONE*, 7(3):e33089, mar 2012. ISSN 1932-6203. doi: 10.1371/journal.pone.0033089. URL http://dx.plos.org/10.1371/journal.pone.0033089.

T L Le, A S Yap, and J L Stow. Recycling of E-cadherin: a potential mechanism for regulating cadherin dynamics. *The Journal of cell biology*, 146(1):219–32, jul 1999. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2199726{&}tool=pmcentrez{&}rendertype=abstract.

Terry Lechler and Elaine Fuchs. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature*, 437(7056):275–80, sep 2005. ISSN 1476-4687. doi: 10.1038/nature03922. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1399371{&}tool=pmcentrez{&}rendertype=abstract.

Thomas Lecuit, Pierre-François Lenne, and Edwin Munro. Force Generation, Transmission, and Integration During Cell and Tissue Morphogenesis. *Annual review of cell and developmental biology*, 27(June):157–84, nov 2010. ISSN 1530-8995. doi: 10.1146/annurev-cellbio-100109-104027. URL http://www.ncbi.nlm.nih.gov/pubmed/21740231.

Jen-Yi Lee and Bob Goldstein. Mechanisms of cell positioning during C. elegans gastru-

lation. *Development (Cambridge, England)*, 130(2):307–20, jan 2003. ISSN 0950-1991. URL http://www.ncbi.nlm.nih.gov/pubmed/12466198.

Jen-Yi Lee, Daniel J Marston, Timothy Walston, Jeff Hardin, Ari Halberstadt, and Bob Goldstein. Wnt/Frizzled signaling controls C. elegans gastrulation by activating actomyosin contractility. *Current biology : CB*, 16(20):1986–97, oct 2006. ISSN 0960-9822. doi: 10.1016/j.cub.2006.08.090. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2989422{&}tool=pmcentrez{&}rendertype=abstract.

Joanne M Leerberg, Guillermo A Gomez, Suzie Verma, Elliott J Moussa, Selwin K Wu, Rashmi Priya, Brenton D Hoffman, Carsten Grashoff, Martin A Schwartz, and Alpha S Yap. Tension-sensitive actin assembly supports contractility at the epithelial zonula adherens. *Current biology : CB*, 24(15):1689–99, aug 2014. ISSN 1879-0445. doi: 10.1016/j.cub.2014.06.028. URL http://www.cell.com/article/S0960982214007441/fulltext.

Loïc Legoff, Hervé Rouault, and Thomas Lecuit. A global pattern of mechanical stress polarizes cell divisions and cell shape in the growing Drosophila wing disc. *Development (Cambridge, England)*, 140(19):4051–9, oct 2013. ISSN 1477-9129. doi: 10.1242/dev.090878. URL http://www.ncbi.nlm.nih.gov/pubmed/24046320.

Peter Lenz and Lotte Søgaard-Andersen. Temporal and spatial oscillations in bacteria. *Nature reviews. Microbiology*, 9(8):565–77, aug 2011. ISSN 1740-1534. doi: 10.1038/nrmicro2612. URL http://dx.doi.org/10.1038/nrmicro2612.

Annalisa Letizia, Sol Sotillos, Sonsoles Campuzano, and Marta Llimargas. Regulated Crb accumulation controls apical constriction and invagination in Drosophila tracheal

cells. *Journal of cell science*, 124(Pt 2):240–51, jan 2011. ISSN 1477-9137. doi: 10.1242/jcs.073601. URL http://www.ncbi.nlm.nih.gov/pubmed/21172808.

R Lev Bar-Or, R Maya, L A Segel, U Alon, A J Levine, and M Oren. Generation of oscillations by the p53-Mdm2 feedback loop: a theoretical and experimental study. *Proceedings of the National Academy of Sciences of the United States of America*, 97(21):11250–5, oct 2000. ISSN 0027-8424. doi: 10.1073/pnas.210171597. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=17186{&}tool=pmcentrez{&}rendertype=abstract.

Romain Levayer and Thomas Lecuit. Biomechanical regulation of contractility: spatial control and dynamics. *Trends in cell biology*, 22(2):61–81, feb 2012. ISSN 1879-3088. doi: 10.1016/j.tcb.2011.10.001. URL http://www.ncbi.nlm.nih.gov/pubmed/22119497.

Romain Levayer and Thomas Lecuit. Oscillation and polarity of E-cadherin asymmetries control actomyosin flow patterns during morphogenesis. *Developmental cell*, 26(2):162–75, jul 2013. ISSN 1878-1551. doi: 10.1016/j.devcel.2013.06.020. URL http://www.ncbi.nlm.nih.gov/pubmed/23871590.

Romain Levayer, Anne Pelissier-Monier, and Thomas Lecuit. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. *Nature cell biology*, 13(5):529–40, may 2011. ISSN 1476-4679. doi: 10.1038/ncb2224. URL http://www.ncbi.nlm.nih.gov/pubmed/21516109.

Jormay Lim and Jean Paul Thiery. Epithelial-mesenchymal transitions: insights from development. *Development (Cambridge, England)*, 139(19):3471–86, oct 2012. ISSN 1477-9129. doi: 10.1242/dev.071209. URL http://www.ncbi.nlm.nih.gov/pubmed/22949611.

C M Lo, H B Wang, M Dembo, and Y L Wang. Cell movement is guided by the rigidity of the substrate. *Biophysical journal*, 79 (1):144–52, jul 2000. ISSN 0006-3495. doi: 10.1016/S0006-3495(00) 76279-5. URL http://www.ncbi.nlm.nih.gov/pubmed/10866943http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1300921.

Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. The Dynamics of Actin Assembly. 2000.

M Lombaerts, T van Wezel, K Philippo, J W F Dierssen, R M E Zimmerman, J Oosting, R van Eijk, P H Eilers, B van de Water, C J Cornelisse, and A-M Cleton-Jansen. E-cadherin transcriptional downregulation by promoter methylation but not mutation is related to epithelial-to-mesenchymal transition in breast cancer cell lines. *British journal of cancer*, 94(5):661–71, mar 2006. ISSN 0007-0920. doi: 10.1038/sj.bjc.6602996. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2361216{&}tool=pmcentrez{&}rendertype=abstract.

H P Lorenz and N S Adzick. Scarless skin wound repair in the fetus. *The Western journal of medicine*, 159(3):350–5, sep 1993. ISSN 0093-0415. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1011349{&}tool=pmcentrez{&}rendertype=abstract.

Kate MacCord. Epithelium, oct 2012. URL http://hpsrepository.asu.edu/handle/10776/3946.

R K Mahajan and J D Pardee. Assembly mechanism of Dictyostelium myosin II: regulation by K+, Mg2+, and actin filaments. *Biochemistry*, 35(48):15504–14, dec 1996. ISSN 0006-2960. doi: 10.1021/bi9618981. URL http://www.ncbi.nlm.nih.gov/pubmed/8952504.

Jean-Leon Maitre, Ritsuya Niwayama, Hervé Turlier, François Nédélec, and Takashi Hiiragi. Pulsatile cell-autonomous contractility drives compaction in the mouse embryo. *Nature cell biology*, pages Maitre, J.–L., Niwayama, R., Turlier, H., Nédélec,, jun 2015. ISSN 1476-4679. doi: 10.1038/ncb3185. URL http://www.ncbi.nlm.nih.gov/pubmed/26075357.

a M Malek and S Izumo. Mechanism of endothelial cell shape change and cytoskeletal remodeling in response to fluid shear stress. *Journal of cell science*, 109 (Pt 4: 713–726, 1996. ISSN 0021-9533.

Adam C Martin. Pulsation and stabilization: contractile forces that underlie morphogenesis. *Developmental biology*, 341(1):114–25, may 2010. ISSN 1095-564X. doi: 10.1016/j.ydbio.2009.10.031. URL http://www.ncbi.nlm.nih.gov/pubmed/19874815.

Adam C Martin, Matthias Kaschube, and Eric F Wieschaus. Pulsed contractions of an actin-myosin network drive apical constriction. *Nature*, 457(7228):495–9, jan 2009. ISSN 1476-4687. doi: 10.1038/nature07522. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2822715{&}tool=pmcentrez{&}rendertype=abstract.

Adam C Martin, Michael Gelbart, Rodrigo Fernandez-Gonzalez, Matthias Kaschube, and Eric F Wieschaus. Integration of contractile forces during tissue invagination. *The Journal of cell biology*, 188(5):735–49, mar 2010. ISSN 1540-8140. doi: 10.1083/jcb.200910099. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2835944{&}tool=pmcentrez{&}rendertype=abstract.

P Martin and J Lewis. Actin cables and epidermal movement in embryonic wound healing. *Nature*, 360(6400):179–83, nov 1992. ISSN 0028-0836. doi: 10.1038/360179a0. URL http://www.ncbi.nlm.nih.gov/pubmed/1436096.

Pascal Martin, D. Bozovic, Y. Choe, and A. J. Hudspeth. Spontaneous Oscillation by Hair Bundles of the Bullfrog's Sacculus. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 23(11):4533–48, jun 2003. ISSN 1529-2401. URL http://www.jneurosci.org/content/23/11/4533.abstracthttp://www.jneurosci.org/content/23/11/4533.short.

Ana Margarida Mateus, Nicole Gorfinkiel, Sabine Schamberg, and Alfonso Martinez Arias. Endocytic and recycling endosomes modulate cell shape changes and tissue behaviour during morphogenesis in Drosophila. *PloS one*, 6(4):e18729, jan 2011. ISSN 1932-6203. doi: 10.1371/journal.pone.0018729. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3077405{&}tool=pmcentrez{&}rendertype=abstract.

Mirjam Mayer, Martin Depken, Justin S Bois, Frank Jülicher, and Stephan W Grill. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. *Nature*, 467(7315):617–621, 2010. ISSN 0028-0836. doi: 10.1038/nature09376.

Nelson A. Medeiros, Dylan T. Burnette, and Paul Forscher. Myosin II functions in actin-bundle turnover in neuronal growth cones. *Nature cell biology*, 8(3):215–26, mar 2006. ISSN 1465-7392. doi: 10.1038/ncb1367. URL http://www.nature.com/doifinder/10.1038/ncb1367http://www.ncbi.nlm.nih.gov/pubmed/16501565.

Daisuke Mizuno, Catherine Tardin, C F Schmidt, and F C Mackintosh. Nonequilibrium mechanics of active cytoskeletal networks. *Science (New York, N.Y.)*, 315(5810):370–3, jan 2007. ISSN 1095-9203. doi: 10.1126/science.1134404. URL http://www.ncbi.nlm.nih.gov/pubmed/17234946.

Emad Moeendarbary, Léo Valon, Marco Fritzsche, Andrew R. Harris, Dale A. Moulding, Adrian J. Thrasher, Eleanor Stride, L. Mahadevan, and Guillaume T. Charras. The

cytoplasm of living cells behaves as a poroelastic material. *Nature Materials*, 12(3): 253–261, jan 2013. ISSN 1476-1122. doi: 10.1038/nmat3517. URL http://www.nature.com/doifinder/10.1038/nmat3517.

Alex Mogilner. On the edge: modeling protrusion. *Current Opinion in Cell Biology*, 18(1):32–39, 2006. ISSN 09550674. doi: 10.1016/j.ceb.2005.11.001.

Nicholas A M Monk. Oscillatory expression of Hes1, p53, and NF-kappaB driven by transcriptional time delays. *Current biology : CB*, 13(16):1409–13, aug 2003. ISSN 0960-9822. URL http://www.ncbi.nlm.nih.gov/pubmed/12932324.

Denise J Montell. Border-cell migration: the race is on. *Nature reviews. Molecular cell biology*, 4(1):13–24, jan 2003. ISSN 1471-0072. doi: 10.1038/nrm1006. URL http://www.ncbi.nlm.nih.gov/pubmed/12511865.

Miguel a. Moreno-Risueno and Philip N. Benfey. Time-based patterning in development: The role of oscillating gene expression. *Transcription*, 2(3):124–129, 2011. ISSN 2154-1264. doi: 10.4161/trns.2.3.15637.

Svetlana Mukhina, Yu-Li Wang, and Maki Murata-Hori. Alpha-actinin is required for tightly regulated remodeling of the actin cortical network during cytokinesis. *Developmental cell*, 13(4):554–65, oct 2007. ISSN 1534-5807. doi: 10.1016/j.devcel.2007.08.003. URL http://www.ncbi.nlm.nih.gov/pubmed/17925230http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2093944.

H A Müller and E Wieschaus. armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in Drosophila. *The Journal of cell biology*, 134(1):149–63, jul 1996. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2120925{&}tool=pmcentrez{&}rendertype=abstract.

Akankshi Munjal and Thomas Lecuit. Actomyosin networks and tissue morphogenesis. *Development (Cambridge, England)*, 141(9):1789–93, may 2014. ISSN 1477-9129. doi: 10.1242/dev.091645. URL http://www.ncbi.nlm.nih.gov/pubmed/24757001.

Edwin Munro, Jeremy Nance, James R Priess, and Friday Harbor. Cortical Flows Powered by Asymmetrical Contraction Transport PAR Proteins to Establish and Maintain Anterior-Posterior Polarity in the Early C . elegans Embryo. 7:413–424, 2004.

Kausalya Murthy and Patricia Wadsworth. Myosin-II-Dependent Localization and Dynamics of F-Actin during Cytokinesis. 15:724–731, 2005. doi: 10.1016/j.cub.2005.02.055.

K Naruse, T Yamada, and M Sokabe. Involvement of SA channels in orienting response of cultured endothelial cells to cyclic stretch. *The American journal of physiology*, 274(5 Pt 2):H1532–8, may 1998. ISSN 0002-9513. URL http://www.ncbi.nlm.nih.gov/pubmed/9612360.

W James Nelson. Remodeling epithelial cell organization: transitions between front-rear and apical-basal polarity. *Cold Spring Harbor perspectives in biology*, 1(1):a000513, jul 2009. ISSN 1943-0264. doi: 10.1101/cshperspect.a000513. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2742086{&}tool=pmcentrez{&}rendertype=abstract.

R Niederman and T D Pollard. Human platelet myosin. II. In vitro assembly and structure of myosin filaments. *The Journal of cell biology*, 67(1):72–92, oct 1975. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2109578{&}tool=pmcentrez{&}rendertype=abstract.

Tamako Nishimura and Masatoshi Takeichi. Shroom3-mediated recruitment of Rho

kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development (Cambridge, England)*, 135(8):1493–502, apr 2008. ISSN 0950-1991. doi: 10.1242/dev.019646. URL http://www.ncbi.nlm.nih.gov/pubmed/18339671.

C D Nobes and a Hall. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*, 81(1):53–62, apr 1995. ISSN 0092-8674. URL http://www.ncbi.nlm.nih.gov/pubmed/7536630.

Michael B O'Connor, David Umulis, Hans G Othmer, and Seth S Blair. Shaping BMP morphogen gradients in the Drosophila embryo and pupal wing. *Development (Cambridge, England)*, 133(2):183–93, jan 2006. ISSN 0950-1991. doi: 10.1242/dev.02214. URL http://dev.biologists.org/content/133/2/183.abstract.

H Oda, T Uemura, K Shiomi, A Nagafuchi, S Tsukita, and M Takeichi. Identification of a Drosophila homologue of alpha-catenin and its association with the armadillo protein. *The Journal of cell biology*, 121(5):1133–40, jun 1993. ISSN 0021-9525. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2119693{&}tool=pmcentrez{&}rendertype=abstract.

Ewa Paluch, Matthieu Piel, Jacques Prost, Michel Bornens, and Cécile Sykes. Cortical actomyosin breakage triggers shape oscillations in cells and cell fragments. *Biophysical journal*, 89(1):724–33, jul 2005. ISSN 0006-3495. doi: 10.1529/biophysj.105.060590. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1366569{&}tool=pmcentrez{&}rendertype=abstract.

Jacques Pecreaux, Jens-Christian Röper, Karsten Kruse, Frank Jülicher, Anthony A Hyman, Stephan W Grill, and Jonathon Howard. Spindle oscillations during asym-

metric cell division require a threshold number of active cortical force generators. *Current biology : CB*, 16(21):2111–22, nov 2006. ISSN 0960-9822. doi: 10.1016/j.cub.2006.09.030. URL http://www.cell.com/article/S0960982206022238/fulltext.

Mirna Perez-Moreno and Elaine Fuchs. Catenins: keeping cells from getting their signals crossed. *Developmental cell*, 11(5):601–12, nov 2006. ISSN 1534-5807. doi: 10.1016/j.devcel.2006.10.010. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2405914{&}tool=pmcentrez{&}rendertype=abstract.

P.-Y. Plaçais, M. Balland, T. Guérin, J.-F. Joanny, and P. Martin. Spontaneous Oscillations of a Minimal Actomyosin System under Elastic Loading. *Physical Review Letters*, 103(15):1–4, oct 2009. ISSN 0031-9007. doi: 10.1103/PhysRevLett.103.158102. URL http://link.aps.org/doi/10.1103/PhysRevLett.103.158102.

O J Pletjushkina, Z Rajfur, P Pomorski, T N Oliver, J M Vasiliev, and K a Jacobson. Induction of cortical oscillations in spreading cells by depolymerization of microtubules. *Cell motility and the cytoskeleton*, 48(4):235–44, apr 2001. ISSN 0886-1544. doi: 10.1002/cm.1012. URL http://www.ncbi.nlm.nih.gov/pubmed/11276073.

T D Pollard. Cytoskeletal functions of cytoplasmic contractile proteins. *Journal of supramolecular structure*, 5(3):317–34, jan 1976. ISSN 0091-7419. doi: 10.1002/jss.400050306. URL http://www.ncbi.nlm.nih.gov/pubmed/799215.

Karen L Pope and Tony J C Harris. Control of cell flattening and junctional remodeling during squamous epithelial morphogenesis in Drosophila. *Development (Cambridge, England)*, 135(13):2227–38, jul 2008. ISSN 0950-1991. doi: 10.1242/dev.019802. URL http://www.ncbi.nlm.nih.gov/pubmed/18508861.

Philippe-Alexandre Pouille, Padra Ahmadi, Anne-Christine Brunet, and Emmanuel Farge. Mechanical signals trigger Myosin II redistribution and mesoderm invagination

in Drosophila embryos. *Science signaling*, 2(66):ra16, 2009. ISSN 1937-9145. doi: 10.1126/scisignal.2000098.

Olivier Pourquié. The segmentation clock: converting embryonic time into spatial pattern. *Science (New York, N.Y.)*, 301(5631):328–30, jul 2003. ISSN 1095-9203. doi: 10.1126/science.1085887. URL http://www.ncbi.nlm.nih.gov/pubmed/12869750.

Leo S Price, Amra Hajdo-Milasinovic, Jun Zhao, Fried J T Zwartkruis, John G Collard, and Johannes L Bos. Rap1 regulates E-cadherin-mediated cell-cell adhesion. *The Journal of biological chemistry*, 279(34):35127–32, aug 2004. ISSN 0021-9258. doi: 10.1074/jbc.M404917200. URL http://www.ncbi.nlm.nih.gov/pubmed/15166221.

J W Pringle. The Croonian Lecture, 1977. Stretch activation of muscle: function and mechanism. *Proceedings of the Royal Society of London. Series B, Biological sciences*, 201(1143):107–30, may 1978. ISSN 0950-1193. URL http://www.ncbi.nlm.nih.gov/pubmed/27795.

Rashmi Priya, Guillermo a Gomez, Srikanth Budnar, Suzie Verma, Hayley L Cox, Nicholas a Hamilton, and Alpha S Yap. Feedback regulation through myosin II confers robustness on RhoA signalling at E-cadherin junctions. *Nature cell biology*, 17(10):1282–93, 2015. ISSN 1476-4679. doi: 10.1038/ncb3239. URL http://www.ncbi.nlm.nih.gov/pubmed/26368311.

Sophie Quintin, Christelle Gally, and Michel Labouesse. Epithelial morphogenesis in embryos: asymmetries, motors and brakes. *Trends in genetics : TIG*, 24(5):221–30, may 2008. ISSN 0168-9525. doi: 10.1016/j.tig.2008.02.005. URL http://www.ncbi.nlm.nih.gov/pubmed/18375008.

Matteo Rauzi, Pascale Verant, Thomas Lecuit, and Pierre-François Lenne. Nature and anisotropy of cortical forces orienting Drosophila tissue morphogenesis. *Nature cell*

biology, 10(12):1401–10, dec 2008. ISSN 1476-4679. doi: 10.1038/ncb1798. URL http://www.ncbi.nlm.nih.gov/pubmed/18978783.

Matteo Rauzi, Pierre-François Lenne, and Thomas Lecuit. Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature*, 468(7327):1110–4, dec 2010. ISSN 1476-4687. doi: 10.1038/nature09566. URL http://www.ncbi.nlm.nih.gov/pubmed/21068726.

R P Ray, K Arora, C Nüsslein-Volhard, and W M Gelbart. The control of cell fate along the dorsal-ventral axis of the Drosophila embryo. *Development (Cambridge, England)*, 113(1):35–54, sep 1991. ISSN 0950-1991. URL http://dev.biologists.org/content/113/1/35.abstract.

Elizabeth M Reichl, Yixin Ren, Mary K Morphew, Michael Delannoy, Janet C Effler, Kristine D Girard, Srikanth Divi, Pablo A Iglesias, Scot C Kuo, and Douglas N Robinson. Interactions between myosin and actin crosslinkers control cytokinesis contractility dynamics and mechanics. *Current biology : CB*, 18(7):471–80, apr 2008. ISSN 0960-9822. doi: 10.1016/j.cub.2008.02.056. URL http://www.ncbi.nlm.nih.gov/pubmed/18372178http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2361134.

Anne-Cécile Reymann, Rajaa Boujemaa-Paterski, Jean-Louis Martiel, Christophe Guérin, Wenxiang Cao, Harvey F Chin, Enrique M De La Cruz, Manuel Théry, and Laurent Blanchoin. Actin network architecture can determine myosin motor activity. *Science (New York, N.Y.)*, 336(6086):1310–4, jun 2012. ISSN 1095-9203. doi: 10.1126/science.1221708. URL http://www.ncbi.nlm.nih.gov/pubmed/22679097.

G A Rodan, L A Bourret, A Harvey, and T Mensi. Cyclic AMP and cyclic GMP: mediators of the mechanical effects on bone remodeling. *Science (New York, N.Y.)*,

189(4201):467–9, aug 1975a. ISSN 0036-8075. URL http://www.ncbi.nlm.nih.gov/pubmed/168639.

G A Rodan, T Mensi, and A Harvey. A quantitative method for the application of compressive forces to bone in tissue culture. *Calcified tissue research*, 18(2):125–31, jul 1975b. ISSN 0008-0594. URL http://www.ncbi.nlm.nih.gov/pubmed/1148895.

U Rowlatt. Intrauterine wound healing in a 20 week human fetus. *Virchows Archiv. A, Pathological anatomy and histology*, 381(3):353–61, mar 1979. ISSN 0340-1227. URL http://www.ncbi.nlm.nih.gov/pubmed/155931.

Alexandre Saez, Marion Ghibaudo, Axel Buguin, Pascal Silberzan, and Benoît Ladoux. Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. *Proceedings of the National Academy of Sciences of the United States of America*, 104(20):8281–6, may 2007. ISSN 0027-8424. doi: 10.1073/pnas.0702259104. URL http://www.ncbi.nlm.nih.gov/pubmed/17488828http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1895941.

Laure Saias, Jim Swoger, Arturo D'Angelo, Peran Hayes, Julien Colombelli, James Sharpe, Guillaume Salbreux, and Jérôme Solon. Decrease in Cell Volume Generates Contractile Forces Driving Dorsal Closure. *Developmental cell*, 33(5):611–21, jun 2015. ISSN 1878-1551. doi: 10.1016/j.devcel.2015.03.016. URL http://www.ncbi.nlm.nih.gov/pubmed/25982674.

Guillaume Salbreux, Guillaume Charras, and Ewa Paluch. Actin cortex mechanics and cellular morphogenesis. *Trends in cell biology*, 22(10):536–45, oct 2012. ISSN 1879-3088. doi: 10.1016/j.tcb.2012.07.001. URL http://www.ncbi.nlm.nih.gov/pubmed/22871642.

Surat Saravanan, C Meghana, and Maithreyi Narasimha. Local, cell-nonautonomous

feedback regulation of myosin dynamics patterns transitions in cell behavior: a role for tension and geometry? *Molecular biology of the cell*, 24(15):2350–61, aug 2013. ISSN 1939-4586. doi: 10.1091/mbc.E12-12-0868. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3727928{&}tool=pmcentrez{&}rendertype=abstract.

Jacob M Sawyer, Jessica R Harrell, Gidi Shemer, Jessica Sullivan-Brown, Minna Roh-Johnson, and Bob Goldstein. Apical constriction: a cell shape change that can drive morphogenesis. *Developmental biology*, 341 (1):5–19, may 2010. ISSN 1095-564X. doi: 10.1016/j.ydbio.2009.09.009. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2875788{&}tool=pmcentrez{&}rendertype=abstract.

Jessica K Sawyer, Nathan J Harris, Kevin C Slep, Ulrike Gaul, and Mark Peifer. The Drosophila afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junctions during apical constriction. *The Journal of cell biology*, 186(1):57–73, jul 2009. ISSN 1540-8140. doi: 10.1083/jcb.200904001. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2712996{&}tool=pmcentrez{&}rendertype=abstract.

Saskia Schröder-Lang, Martin Schwärzel, Reinhard Seifert, Timo Strünker, Suneel Kateriya, Jens Looser, Masakatsu Watanabe, U Benjamin Kaupp, Peter Hegemann, and Georg Nagel. Fast manipulation of cellular cAMP level by light in vivo. *Nature Methods*, 4(1):39–42, jan 2007. ISSN 1548-7091. doi: 10.1038/nmeth975. URL http://www.nature.com/doifinder/10.1038/nmeth975.

E C Schwarz, E M Neuhaus, C Kistler, A W Henkel, and T Soldati. Dictyostelium myosin IK is involved in the maintenance of cortical tension and affects motility and

phagocytosis. *Journal of cell science*, 113 (Pt 4:621–33, feb 2000. ISSN 0021-9533. URL http://www.ncbi.nlm.nih.gov/pubmed/10652255.

Glynis Scott and Sonya Leopardi. The cAMP signaling pathway has opposing effects on Rac and Rho in B16F10 cells: implications for dendrite formation in melanocytic cells. *Pigment cell research / sponsored by the European Society for Pigment Cell Research and the International Pigment Cell Society*, 16(2):139–48, apr 2003. ISSN 0893-5785. URL http://www.ncbi.nlm.nih.gov/pubmed/12622791.

Matthew P Scott. The Natural History of Genes. *Cell*, 100(1):27–40, jan 2000. ISSN 00928674. doi: 10.1016/S0092-8674(00)81681-5. URL http://www.cell.com/article/S0092867400816815/fulltext.

Jakub Sedzinski, Maté Biro, Annelie Oswald, Jean-Yves Tinevez, Guillaume Salbreux, and Ewa Paluch. Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. *Nature*, 476(7361):462–6, aug 2011. ISSN 1476-4687. doi: 10.1038/nature10286. URL http://www.ncbi.nlm.nih.gov/pubmed/21822289.

Yue Shao, Xinyu Tan, Roman Novitski, Mishaal Muqaddam, Paul List, Laura Williamson, Jianping Fu, and Allen P. Liu. Uniaxial cell stretching device for live-cell imaging of mechanosensitive cellular functions. *Review of Scientific Instruments*, 84(11):114304, 2013. ISSN 00346748. doi: 10.1063/1.4832977. URL http://scitation.aip.org/content/aip/journal/rsi/84/11/10.1063/1.4832977.

Daniel D. Shaye, Jordi Casanova, and Marta Llimargas. Modulation of intracellular trafficking regulates cell intercalation in the Drosophila trachea. *Nature Cell Biology*, 10(8):964–970, aug 2008. ISSN 1465-7392. doi: 10.1038/ncb1756. URL http://www.nature.com/doifinder/10.1038/ncb1756.

Michael B Simmers, Andrew W Pryor, and Brett R Blackman. Arterial shear stress

regulates endothelial cell-directed migration, polarity, and morphology in confluent monolayers. *American journal of physiology. Heart and circulatory physiology*, 293 (3):H1937–46, sep 2007. ISSN 0363-6135. doi: 10.1152/ajpheart.00534.2007. URL http://www.ncbi.nlm.nih.gov/pubmed/17586613.

Soon-Tuck Sit and Ed Manser. Rho GTPases and their role in organizing the actin cytoskeleton. *Journal of cell science*, 124(Pt 5):679–83, mar 2011. ISSN 1477-9137. doi: 10.1242/jcs.064964. URL http://www.ncbi.nlm.nih.gov/pubmed/21321325.

Paul Skoglund, Ana Rolo, Xuejun Chen, Barry M Gumbiner, and Ray Keller. Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network., aug 2008. ISSN 0950-1991. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2735133{&}tool=pmcentrez{&}rendertype=abstract.

J Sleep, D Wilson, R Simmons, and W Gratzer. Elasticity of the red cell membrane and its relation to hemolytic disorders: an optical tweezers study. *Biophysical journal*, 77(6):3085–95, dec 1999. ISSN 0006-3495. doi: 10.1016/S0006-3495(99)77139-0. URL http://www.ncbi.nlm.nih.gov/pubmed/10585930http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1300579.

Adam Sokolow, Yusuke Toyama, Daniel P. Kiehart, and Glenn S. Edwards. Cell ingression and apical shape oscillations during dorsal closure in Drosophila. *Biophysical Journal*, 102(5):969–979, 2012. ISSN 00063495. doi: 10.1016/j.bpj.2012.01.027. URL http://dx.doi.org/10.1016/j.bpj.2012.01.027.

Jérôme Solon, Ilya Levental, Kheya Sengupta, Penelope C Georges, and Paul A Janmey. Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophysical journal*, 93(12):4453–61, dec 2007. ISSN 1542-0086. doi: 10.1529/biophysj.

106.101386. URL http://www.ncbi.nlm.nih.gov/pubmed/18045965http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2098710.

Jerome Solon, Aynur Kaya-Copur, Julien Colombelli, and Damian Brunner. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell*, 137(7):1331–1342, 2009. URL http://www.ncbi.nlm.nih.gov/pubmed/19563762.

Daniel St Johnston. The art and design of genetic screens: Drosophila melanogaster. *Nature reviews. Genetics*, 3(3):176–88, mar 2002. ISSN 1471-0056. doi: 10.1038/nrg751. URL http://dx.doi.org/10.1038/nrg751.

Samantha J Stehbens, Andrew D Paterson, Matthew S Crampton, Annette M Shewan, Charles Ferguson, Anna Akhmanova, Robert G Parton, and Alpha S Yap. Dynamic microtubules regulate the local concentration of E-cadherin at cell-cell contacts. *Journal of cell science*, 119(Pt 9):1801–11, may 2006. ISSN 0021-9533. doi: 10.1242/jcs.02903. URL http://www.ncbi.nlm.nih.gov/pubmed/16608875.

Hugo Stocker and Peter Gallant. Getting started : an overview on raising and handling Drosophila. *Methods in molecular biology (Clifton, N.J.)*, 420:27–44, jan 2008. ISSN 1064-3745. doi: 10.1007/978-1-59745-583-1_2. URL http://www.ncbi.nlm.nih.gov/pubmed/18641939.

T P Stossel. Contractile proteins in cell structure and function. *Annual review of medicine*, 29:427–57, jan 1978. ISSN 0066-4219. doi: 10.1146/annurev.me.29.020178.002235. URL http://www.ncbi.nlm.nih.gov/pubmed/206188.

Yubing Sun, Christopher S Chen, and Jianping Fu. Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. *Annual review of biophysics*, 41:519–42, 2012. ISSN 1936-1238. doi: 10.1146/annurev-biophys-042910-155306.

URL http://www.ncbi.nlm.nih.gov/pubmed/22404680http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4123632.

Subra Suresh. Biomechanics and biophysics of cancer cells. *Acta biomaterialia*, 3(4):413–38, jul 2007. ISSN 1742-7061. doi: 10.1016/j.actbio.2007.04.002. URL http://www.ncbi.nlm.nih.gov/pubmed/17540628http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2917191.

D Sweeton, S Parks, M Costa, and E Wieschaus. Gastrulation in Drosophila: the formation of the ventral furrow and posterior midgut invaginations. *Development (Cambridge, England)*, 112(3):775–89, jul 1991. ISSN 0950-1991. URL http://dev.biologists.org/content/112/3/775.abstract.

John L Tan, Joe Tien, Dana M Pirone, Darren S Gray, Kiran Bhadriraju, and Christopher S Chen. Cells lying on a bed of microneedles: an approach to isolate mechanical force. *Proceedings of the National Academy of Sciences of the United States of America*, 100(4):1484–9, 2003. ISSN 0027-8424. doi: 10.1073/pnas.0235407100. URL http://www.pnas.org/content/100/4/1484.full.

U Tepass and E Knust. Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in Drosophila melanogaster. *Developmental biology*, 159(1):311–26, sep 1993. ISSN 0012-1606. doi: 10.1006/dbio.1993.1243. URL http://www.ncbi.nlm.nih.gov/pubmed/8365569.

U Tepass, E Gruszynski-DeFeo, T A Haag, L Omatyar, T Török, and V Hartenstein. shotgun encodes Drosophila E-cadherin and is preferentially required during cell rearrangement in the neurectoderm and other morphogenetically active epithelia. *Genes & development*, 10(6):672–85, mar 1996. ISSN 0890-9369. URL http://www.ncbi.nlm.nih.gov/pubmed/8598295.

Julie A. Theriot. The Polymerization Motor. *Traffic*, 1(1):19–28, jan 2000. ISSN 13989219. doi: 10.1034/j.1600-0854.2000.010104.x. URL http://doi.wiley.com/10.1034/j.1600-0854.2000.010104.x.

Thomas J. Lukas, Salida Mirzoeva, Urszula Slomczynska, , and D. Martin Watterson*. Identification of Novel Classes of Protein Kinase Inhibitors Using Combinatorial Peptide Chemistry Based on Functional Genomics Knowledge. 1999.

O Thoumine and A Ott. Time scale dependent viscoelastic and contractile regimes in fibroblasts probed by microplate manipulation. *Journal of cell science*, 110 (Pt 1: 2109–2116, 1997. ISSN 0021-9533.

O Thoumine, O Cardoso, and J J Meister. Changes in the mechanical properties of fibroblasts during spreading: a micromanipulation study. *European biophysics journal : EBJ*, 28(3):222–34, jan 1999. ISSN 0175-7571. URL http://www.ncbi.nlm.nih.gov/pubmed/10192936.

Yusuke Toyama, Xomalin G Peralta, Adrienne R Wells, Daniel P Kiehart, and Glenn S Edwards. Apoptotic force and tissue dynamics during Drosophila embryogenesis. *Science (New York, N.Y.)*, 321(5896):1683–6, sep 2008. ISSN 1095-9203. doi: 10.1126/science.1157052. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2757114{&}tool=pmcentrez{&}rendertype=abstract.

Dominique Tremblay, Sophie Chagnon-Lessard, Maryam Mirzaei, Andrew E Pelling, and Michel Godin. A microscale anisotropic biaxial cell stretching device for applications in mechanobiology. *Biotechnology letters*, 36(3): 657–65, mar 2014. ISSN 1573-6776. doi: 10.1007/s10529-013-1381-5. URL http://www.ncbi.nlm.nih.gov/pubmed/24129957http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3964308.

J J Tyson, K Chen, and B Novak. Network dynamics and cell physiology. *Nature reviews. Molecular cell biology*, 2(12):908–16, dec 2001. ISSN 1471-0072. doi: 10.1038/35103078. URL http://dx.doi.org/10.1038/35103078.

Eleni Tzima, Mohamed Irani-Tehrani, William B. Kiosses, Elizabetta Dejana, David A. Schultz, Britta Engelhardt, Gaoyuan Cao, Horace DeLisser, and Martin Alexander Schwartz. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*, 437(7057):426–431, sep 2005. ISSN 0028-0836. doi: 10.1038/nature03952. URL http://www.nature.com/doifinder/10.1038/nature03952.

Ryota Uehara, Gohta Goshima, Issei Mabuchi, Ronald D Vale, and James A Spudich. Report Determinants of Myosin II Cortical Localization during Cytokinesis. *Current Biology*, 20(12):1080–1085, 2010. ISSN 0960-9822. doi: 10.1016/j.cub.2010.04.058. URL http://dx.doi.org/10.1016/j.cub.2010.04.058.

S Usami, H H Chen, Y Zhao, S Chien, and R Skalak. Design and construction of a linear shear stress flow chamber. *Annals of biomedical engineering*, 21(1):77–83, 1993. ISSN 0090-6964. URL http://www.ncbi.nlm.nih.gov/pubmed/8434823.

Miguel Vicente-Manzanares, Xuefei Ma, Robert S Adelstein, and Alan Rick Horwitz. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nature reviews. Molecular cell biology*, 10(11):778–90, nov 2009. ISSN 1471-0080. doi: 10.1038/nrm2786. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2834236{&}tool=pmcentrez{&}rendertype=abstract.

Fay Wang, Karin Dumstrei, Thomas Haag, and Volker Hartenstein. The role of DE-cadherin during cellularization, germ layer formation and early neurogenesis in the Drosophila embryo. *Developmental biology*, 270(2):350–63, jun 2004. ISSN 0012-1606.

doi: 10.1016/j.ydbio.2004.03.002. URL http://www.sciencedirect.com/science/article/pii/S0012160604001745.

Shu-Yi Wei, Luis M Escudero, Fengwei Yu, Li-Hsun Chang, Li-Ying Chen, Yu-Huei Ho, Chiao-Ming Lin, Chin-Shun Chou, William Chia, Juan Modolell, and Jui-Chou Hsu. Echinoid is a component of adherens junctions that cooperates with DE-Cadherin to mediate cell adhesion. *Developmental cell*, 8(4):493–504, apr 2005. ISSN 1534-5807. doi: 10.1016/j.devcel.2005.03.015. URL http://www.ncbi.nlm.nih.gov/pubmed/15809032.

D A Winkelmann, L Bourdieu, A Ott, F Kinose, and A Libchaber. Flexibility of myosin attachment to surfaces influences F-actin motion. *Biophysical journal*, 68(6):2444–53, jun 1995. ISSN 0006-3495. doi: 10.1016/S0006-3495(95)80426-1. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1282154{&}tool=pmcentrez{&}rendertype=abstract.

William Wood, Antonio Jacinto, Richard Grose, Sarah Woolner, Jonathan Gale, Clive Wilson, and Paul Martin. Wound healing recapitulates morphogenesis in Drosophila embryos. *Nature cell biology*, 4(11):907–12, nov 2002. ISSN 1465-7392. doi: 10.1038/ncb875. URL http://dx.doi.org/10.1038/ncb875http://www.ncbi.nlm.nih.gov/pubmed/12402048.

Tom Wyatt, Buzz Baum, and Guillaume Charras. A question of time: tissue adaptation to mechanical forces. *Current opinion in cell biology*, 38:68–73, feb 2016. ISSN 1879-0410. doi: 10.1016/j.ceb.2016.02.012. URL http://www.ncbi.nlm.nih.gov/pubmed/26945098.

Jer-Yen Yang, Cong S Zong, Weiya Xia, Yongkun Wei, Mohamed Ali-Seyed, Zheng Li, Kristine Broglio, Donald A Berry, and Mien-Chie Hung. MDM2 promotes

cell motility and invasiveness by regulating E-cadherin degradation. *Molecular and cellular biology*, 26(19):7269–82, oct 2006. ISSN 0270-7306. doi: 10.1128/MCB.00172-06. URL http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1592879{&}tool=pmcentrez{&}rendertype=abstract.

Chih-Ko Yeh and Gideon A. Rodan. Tensile forces enhance prostaglandin E synthesis in osteoblastic cells grown on collagen ribbons. *Calcified Tissue International*, 36 (S1):S67–S71, mar 1984. ISSN 0171-967X. doi: 10.1007/BF02406136. URL http://link.springer.com/10.1007/BF02406136.

M Yoneda and K Dan. Tension at the surface of the dividing sea-urchin egg. *The Journal of experimental biology*, 57(3):575–87, dec 1972. ISSN 0022-0949. URL http://www.ncbi.nlm.nih.gov/pubmed/4675641.

Masaaki Yoshigi, Laura M Hoffman, Christopher C Jensen, H Joseph Yost, and Mary C Beckerle. Mechanical force mobilizes zyxin from focal adhesions to actin filaments and regulates cytoskeletal reinforcement. *The Journal of cell biology*, 171(2):209–15, oct 2005. ISSN 0021-9525. doi: 10.1083/jcb.200505018. URL http://www.ncbi.nlm.nih.gov/pubmed/16247023http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2171187.

Shinya Yoshikawa, Takeshi Suzuki, Masakatsu Watanabe, and Mineo Iseki. Kinetic analysis of the activation of photoactivated adenylyl cyclase (PAC), a blue-light receptor for photomovements of Euglena. *Photochemical & Photobiological Sciences*, 4(9):727, 2005. ISSN 1474-905X. doi: 10.1039/b417212d. URL http://xlink.rsc.org/?DOI=b417212d.

Shigehiko Yumura. Myosin II dynamics and cortical flow during contractile ring formation in Dictyostelium cells. pages 137–145, 2001. doi: 10.1083/jcb.200011013.

Marino Zerial and Heidi McBride. Rab proteins as membrane organizers. *Nature Reviews Molecular Cell Biology*, 2(2):107–117, feb 2001. ISSN 14710072. doi: 10.1038/35052055. URL http://www.nature.com/doifinder/10.1038/35052055.

Teresa Zulueta-Coarasa, Masako Tamada, Eun J Lee, and Rodrigo Fernandez-Gonzalez. Automated multidimensional image analysis reveals a role for Abl in embryonic wound repair. *Development (Cambridge, England)*, 141(14):2901–11, jul 2014. ISSN 1477-9129. doi: 10.1242/dev.106898. URL http://www.ncbi.nlm.nih.gov/pubmed/24948602.