From pulsatile apicomedial contractility to effective epithelial mechanics

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Abstract
We review recent developments in the understanding of the biomechanics of apicomedial actomyosin and how its contractility can tense and deform tissue. Myosin pulses are driven by a biochemical oscillator but how they are modulated by the mechanical context remains unclear. On the other hand, the emergence of tissue behaviour is highly dependent on the material properties of actin, on how strongly components are connected and on the influence of neighbouring tissues. We further review the use of constitutive equations in exploring the mechanics of epithelial apices dominated by apicomedial Myosin contractility.

1. Introduction

During embryonic development, epithelial tissues undergo a diverse array of movements such as bending, lengthening, migrating and folding that eventually give rise to the different shapes of our organs and body. Underlying this multicellular choreography is the actomyosin cytoskeleton, a dynamic system capable of a rich array of behaviours and able to respond and adapt to external constraints [1-3]. In the last few years, actomyosin contractility driven by medioapical pulsatile networks (Fig. 1a) has emerged as a powerful system to help understand cytoskeletal dynamics and how it translates through mechanics into specific cell and tissue deformations [4-7].

Pulsatile apicomedial actomyosin contractility was suggested from the oscillatory dynamics of non-muscle Myosin-II and F-actin reporters in epithelial cells undergoing apical contraction [8]. These oscillations are driven by the periodic assembly of Myosin, concentrated into an apicomedial 'focus' (Fig. 1a) and contracting an actin network in the apical cortex of the cells, followed by disassembly [9]. When the cortex is effectively bound across cell-cell junctions this pulsatile contractile activity generates changes in cell shape [10-13] (Fig. 1b). The coordination of thousands of cell shape changes throughout a tissue gives rise to macroscopic deformations that represent a tissue's morphogenetic phenotype. In three distinct tissues in the *Drosophila* embryo, for example, medioapical contractions drive tissue contraction in interestingly different ways (Fig. 1c-e; see legends of Fig.1 for a description of these three distinct morphogenetic processes). This mode of contractility has

also been observed in vertebrate systems [14-16] suggesting that this is a fundamental mechanism driving apical contraction.

Here, we review recent findings on how the interplay between biochemical and mechanical mechanisms drives actomyosin oscillatory contractions and how this local activity coordinates to generate global tissue contraction.

2. The control of Myosin pulsatility: Sub-cellular biochemical feedback

For the cell to generate contractile work, the Myosin-II motor has to be phosphorylated on its regulatory light chain to assemble into bipolar mini-filaments that bind and contract cortical actin. The regulation of Myosin phosphorylation has been shown to play a major role in the appearance of Myosin pulses. In several systems, both Rho kinase (Rok) and the Myosin phosphatase binding subunit (Mbs) co-localize with Myosin in pulses [17-19]. Cross-correlation analysis between these components revealed that while Rok co-occurred with Myosin, Mbs pulses were delayed relative to Myosin pulses [18]. The perturbation of kinase or phosphatase activities led to changes in the duration or frequency of Myosin pulses suggesting that a correct balance between these activities is required for pulsatile actomyosin contractions [17-20]. Given this conservation, it is not unreasonable to consider Rok/Myo/Mbs as an autonomous unit (Fig. 2a).

A major advance in the understanding of how actomyosin pulsatile activity arises came from the finding that Rho1/A, a regulator of Rok and Mbs, shows pulsatile dynamics as shown by a GFP-tagged sensor containing the Rho1/A binding domain of its downstream target Anillin [18,21]. While in germband cells Rho1/A oscillatory activity depends on the activity of its downstream target Myosin [18], recent data from *C. elegans* zygote and early embryo as well as *Drosophila* ventral furrow cells has shown that such activity is independent of Myosin II [21-23]. These observations have led to the idea that Rho1/A constitutes a critical upstream feed-forward pacemaker driving actomyosin pulsatility (Fig. 2a). Such oscillatory activities of RhoA GTPases have previously been described in contractile cells [24].

The mechanisms underlying Rho pulsation have been thoroughly investigated in the last few years. RhoGEF2, a guanine nucleotide-exchanging factor, was previously identified as a regulator of apical contraction during ventral furrow invagination and amnioserosa contraction [25,26]. More recently, RhoGEF2 was found to exhibit oscillatory behaviour in ventral furrow cells, with the peak in RhoGEF2 preceding the peak in Myosin. Overall, these results suggest that RhoGEF2 is a positive regulator of Rho1 activity [22]. On the other hand, RhoGAP71E in *Drosophila* and the RhoA GAP RGA-3 in *C. elegans* have been shown to act as negative regulators of Rho activity, being required for pulse disassembly [21,22]. Interestingly, it has been suggested that the ratio between RhoGEF and RhoGAP activities is crucial to enable the transition from pure oscillatory behaviour to sustained contraction, as this ratio increases during ventral furrow invagination due to the net increase in RhoGEF levels [22] (Fig. 2b).

What then determines the differential accumulation of RhoGEF and RhoGAP? A recent study on the role of GPCR signalling during *Drosophila* invagination and germband extension provides a clue of how this could be controlled [27]. GPCR signalling through the

heterotrimeric G-proteins Gα12/13, Gβ13F and Gγ1 activates Rho1/Rok leading to actomyosin activity both in the mesoderm and ectoderm. It is suggested that a different combination of ligands, receptors and downstream effectors can activate Myosin activity apicomedially or at the level of junctions in a tissue-specific manner. Moreover, these results also suggest that the transition from a pure oscillatory behaviour to a predominantly contractile behaviour could be driven by the quantitative activation of GPCR signalling. By regulating the levels of ligands like Fog and the number of GPCRs through which they signal, it would be possible to quantitatively control GPCR signalling, with low levels enabling pulsatile activity and high levels promoting the stabilization of the contracted state (Fig. 2b).

However, it should be noted that other pathways are likely to contribute to the control of these cell shape changes since in mutants for *fog*, Myosin is reduced but not abolished [26,28,29]. For example, Shroom, an actin-binding protein best known for its role in apical contraction during neural tube closure in vertebrates [30,31], is required for the junctional Myosin enrichment relative to the medial cortex as germband elongation progresses [32], suggesting that Shroom also contributes to the stabilization of Myosin pulsatile activity. Also, during invagination of the *Drosophila* salivary placode, non-centrosomal microtubules stabilise apico-medial actomyosin foci via the spectraplakin Shot [33].

3. Modelling medial pulsatility and possible mechanical feedback

Several theoretical models have been proposed to explore how actomyosin oscillations arise. In these models, different possible positive feedbacks driving the appearance and build-up of Myosin activity in foci, and different negative feedbacks driving the termination of pulses, are suggested. Starting with purely biochemical models for Myosin pulses, a hard-coded Rho1 oscillator has been used [23], and a model involving auto-activation of RhoA and delayed inactivation of RhoA through RGA-3/4 is able to produce pulsatile RhoA dynamics [21]. In both these examples, Myosin activity is downstream of the oscillator, in agreement with experimental evidence for a feed-forward autonomous Myosin control unit (Fig. 2a).

Other more mechanical models have attempted to generate actomyosin oscillations directly from their underlying interactions and dynamics. The accumulation of Myosin in foci has been modelled with a variety of positive feedback mechanisms, including cooperative actin bundling [20], advection or concentration of actomyosin [18], force-dependent activation of Rok [34] and there are good reasons to think that mechano-sensitive Myosin binding to actin filaments might also be involved [35]. In turn, the negative feedback process driving Myosin disassembly has been modelled as the disassembly of the actin network at high actin [18] or Myosin [20] densities, or as elastic resistance to further contraction originating either from the cell itself or the rest of the epithelium [36,37].

As reviewed above, there is no current requirement for mechanical feedback to initiate or sustain pulsatile Myosin foci, since a biochemical Rho1/A oscillator seems sufficient. This is not to say, however, that Myosin foci behave altogether independently of feedback from the cell and tissue scale. Some positive feedback by advection and negative feedback

through some limiting factor seems quite plausible, in addition to the biochemical oscillator [38]. Indeed, the rate of Myosin focus nucleation and its life-time are both reduced when targeting α-catenin [39]. Also, in the *Drosophila* posterior mid-gut, where contractions also begin with pulsatile actomyosin activity [40], myosin activation can be rescued in mutants by applying tissue tension to the mesoderm [41]. Moreover, mechanically-gated ion channels have been shown to have a role in dorsal closure, suggesting the existence of mechano-transduction mechanisms underlying cell-force production and coordination [42]. Hence, though the precise mechanisms remain unclear, there is some evidence and much scope for mechanical feedback on aspects of Myosin focus control. By contrast, the role of mechanics is rather more obvious in the effect of the Myosin focus on its actin substrate, which is the topic of the next section.

4. Myosin force transmission: Actin, adhesions and the surrounding tissue

A Myosin-II focus locally deforms its actin substrate, which in turn has the potential to contribute directly to tissue deformation, but only under certain circumstances. Because of this indirect link between active local stress and tissue stress it is useful to think of Myosin foci conferring a local 'pre-stress' [43], tensional forces that are opposed by resistance to deformation. Depending on this resistance, pre-stress effects are shared between an increase in global tissue stress and tissue deformation. Factors that mediate the transmission of Myosin pre-stress to tissue morphogenesis can be separated into (i) the material properties of the actin meshwork, including its turnover and the polarised nucleation of fibres, (ii) the connection of actin from cell to cell through adhesion complexes, (iii) the behaviour and response (e.g. stiffness, contraction) of the surrounding tissue (Fig. 2a,c,d, also Fig. 1e).

(i) Actin material properties
The organization of the medioapical actin network has recently started to be elucidated in ventral furrow cells in *Drosophila*. The localization of actin-binding proteins such as capping protein α and tropomodulin, that bind to barbed or pointed ends of F-actin filaments respectively, shows that the actin network is polarized with pointed ends enriched in the medioapical region and barbed ends enriched towards intercellular junctions [44]. This polarized actin network is dynamic and undergoes continuous turnover, with disassembly to allow network dispersal after its Myosin-driven condensation and de novo incorporation of actin monomers at barbed ends to maintain an actin network that is attached to intercellular junctions [45].

In *Drosophila* germband during axis extension there is an interesting co-dependence between the pulsing apicomedial actin [46] and junctional actin [47], with apico-medial myosin pulses feeding junctional myosin to drive cell intercalation [13]. The medioapical actin meshwork has been found to have a turnover half-life of 50-60 seconds [48]. This provides a mechanism for an intrinsic contractile ratchet, since Myosin foci occur at a sufficiently high frequency (80-100 sec intervals) that the actin network does not have time to relax back to its previous conformation and is maintained in a contracted configuration. It appears that this junctional actin ratchet combines with a Rab35 endocytotic ratchet to shorten cell interfaces, driving germband convergence [49].

To generate cell deformation, the medial actin network has to be connected to cell

membranes (Fig. 2c) [47]. A slipping of the medial cortex relative to the cell-cell junction in a so-called 'clutch' was observed in *C. elegans* embryos and possibly *Drosophila* mesoderm during gastrulation [50] . This slippage is a possible candidate for a mechanical regulator of focus behaviour. If the clutch is not engaged, the medial cortex will not feel the resistance of surrounding cells and will contract without deforming the cell apex (Fig 3c), whereas if it is engaged, the focus contraction will feel the mechanical resistance of neighbouring cells and deform the cell apex (Fig 3d). The strain rate of the medial actomyosin network can thus be larger than the strain rate of the cell when the clutch is disengaged. In [45], modulating actin turnover also creates a mismatch between the actin strain rate and the cell apex strain rate, causing holes between accumulated actin and adhesions. Thus Myosin work is being used up either in an internal flow of actin, without deformation [51], when an adhesion clutch is disengaged or when actin turnover is targeted, or to cell and tissue deformation if the clutch is engaged [50] and actin can turn over (Fig. 2a).

(ii) Connecting actin through cell-cell adhesion complexes
Intracellular stress must be transmitted across cell-cell adhesions if it is to contribute to tissue morphogenesis [52,53]. The ability of a Myosin focus to deform the cell apex has been found to decrease when cell-cell adhesion is targeted [39,53,54]. Perturbing the transmission of forces between a Myosin focus and cell-cell junctions by laser ablation results in a retraction of the focus away from the cut, with the motion of the focus guided by α-catenin and E-Cadherin [13] (Fig. 3e), showing that the Myosin focus is linked to the cell membrane. E-cadherin and α-catenin are also known to modulate Myosin focus duration [39,54,55] suggesting that force transmission through adhesion complexes feeds back on intracellular actin activity (Fig. 2a). It has been shown that "ratcheted" pulses, that is, contractions of cell area that are not cancelled entirely by subsequent relaxation, induce ratcheted pulses in neighbouring cells and are correlated with myosin stabilisation [56] (Fig. 2d). This implies some mechanical (or strain) communication between cells. However, mechanisms and causality relations remain elusive.

(iii) Mechanics of surrounding tissues and boundary conditions
Even if cell-intrinsic and cell-cell adhesion properties are appropriate to transmit Myosin pre-stress, generating cell deformation is still highly dependent on the surrounding tissue. Generally, embryonic tissues are under tension apically. For example, circular ablations lead to spectacular collapse of cells thus isolated from the tissue-scale stress (Fig. 3a) [57]. Thus a change in the Myosin pre-stress must bring the cell apex to a new mechanical balance with the current tissue tension. If neighbouring tissues are stiff and resist deformation, Myosin pre-stress will predominantly build tissue stress because it cannot generate contraction. Alternatively, if the surrounding tissue is compliant, Myosin pre-stress will predominantly result in tissue deformation [51,58]. Such mechanical constraints from surrounding tissues can be modelled as boundary conditions (Box 1, Fig. 2a).

Indeed, it has been shown that forces extrinsic to *Drosophila* epithelia affect their dynamics [59,60]. A useful way of seeing the dependence of the actomyosin response to boundary conditions is when these are anisotropic, or when they are perturbed, for example by using cauterization experiments [61]. Interestingly, neighbouring pairs of cells both with Myosin foci contract predominantly perpendicular to the orientation of the cell pair [62] (Fig. 3b). The strain rate at the cell-scale can thus be directed by mechanical cues,

although it is not known in this case whether the actin strain rate is also made anisotropic. In the ventral mesoderm of *Drosophila*, where stable structures of actomyosin follow on immediately from pulsations [40], these structures differ depending on the anisotropy of tissue stress. Ring-shaped Myosin foci emerge where anisotropic tissue stress is equalised by laser cuts across the orientation of greater stress (Fig. 3f).

Hence, cell deformation is the result of the interplay between Myosin foci and boundary conditions, through mechanical balance. Because mechanical forces equilibrate across the tissue almost instantaneously and because they couple the deformation and the tension in a non-trivial way, it is useful to formalise the interactions with equations. In the next section we therefore review how mechanical interactions have been formalised in tissues dominated by apicomedial contractility.

Box 1: Hydrodynamic length and boundary conditions

In embryos, at physiological time scales, any local change in mechanical stress instantaneously affects the tissue over a tissue-dependent distance, called the *hydrodynamic length*. This length is determined by the contact friction with neighbouring tissues and structures, reducing in the case of high friction. This length has been found to be of the order of tens of microns in some embryonic tissues [63,64] and thus can be much larger than the modelled region.

Imposing *boundary conditions* means summarising the mechanical behaviour of the surrounding tissue that will be felt by the modelled region, and locating it to the boundary of this region.

5. Constitutive equations of apicomedial material at different scales

A Constitutive Equation (CE) is a formal description of a material in terms of the relationship between stress and strain, mediated by the properties of the material. A CE details how a material would deform in response to a change in stress, or change its stress in response to an imposed deformation. For the apicomedial cortex in epithelia, various CEs have been used that are in essence quite similar. They all describe linear viscous or viscoelastic materials, with an additional active Myosin-based source term, the simplest example of which is as follows [65]:

$$\dot{\varepsilon} = \sigma/\eta - \sigma_{myo}/\eta \qquad \text{Eqn. 1}$$

where σ is the tissue stress, σ_{myo} is the Myosin pre-stress, the material property $\eta = \tau\kappa$, where κ is the bulk stiffness and τ a viscous relaxation time-scale, and $\dot{\varepsilon}$ is the strain (deformation) rate of the material. The pre-stress will tend to produce a contraction, which is a negative strain rate. $\dot{\varepsilon}$ can be measured directly from images [66,67] and σ_{myo} has been quantified as proportional to [62] or as a saturating Hill-function [23,68] of imaged Myosin fluorescence intensity. Access to the other parameters requires mechanical perturbation or force inference.

For viscoelastic materials, a $\dot{\sigma}/\kappa$ term is also required on the right-hand side of Eqn. 1 to describe material that is neither purely viscous nor purely elastic [4,48,62,64]. For material that is thought to have a goal size, a strain term, ε, is added to the left-hand side [4,68]. Myosin pre-stress, σ_{myo}, can be modulated by other factors such as actin–myosin

interactions [68,69] and Myosin activity may also affect actomyosin material properties κ and τ [51].

CEs can be employed at various scales, from sub-cellular actomyosin material up to the tissue as a material. At the sub-cellular scale, CEs can represent explicit cytoskeletal mechanisms [4,51], while at the tissue scale the CEs are a more phenomenological combination of many apicomedial cortices with varying amounts of Myosin activity, mediated by adhesion complexes [70]. At the whole embryo scale, the influence of *Drosophila* embryo geometry on patterns of stress and strain can be predicted in response to a given germband and posterior mid-gut pre-stress pattern [64].

CEs can evolve over developmental time. There is a transition over two hours of *Drosophila* dorsal closure of the amnioserosa from a viscous fluid-like apicomedial sheet with no net contraction to a contractile visco-elastic solid-like sheet [62]. This transition is accompanied by a stiffness doubling and a quadrupling of stress and is likely to be driven by a gradual increase in Myosin activity. The apicomedial material can usefully be compared to a standard linear solid (as in [4]), in which the fluid-like spring and dashpot in series dominate early dorsal closure, after which the solid-like spring in parallel progressively dominates. During the formation of the *Drosophila* ventral furrow, a much more rapid change in Myosin activity (over ~5 minutes) is likely to underlie a similar mechanical transition [56].

Boundary conditions and also a rule for how stress can vary across the tissue, a force balance equation, complete the mechanical description of a material, which allow equations of motion to be derived and explored. Boundary conditions need to be assumed or measured (see Box 1), unless the whole embryo is modelled [64]. For force balance, gradients in stress result from friction between the material and under- or overlying structures, scaled by the relative speeds of the layers (Fig. 4a, b). For example, the friction of the actin cortex with the underlying cytosol and/or yolk has been used in this way during *Drosophila* germband extension [4] and mesoderm invagination [71], in the *C. elegans* embryo cortex [23] and in addition to friction with the supra-apical vitelline membrane in a whole embryo model [64].

In the selection of CEs for apicomedial material, the emphasis so far has been on phenomenological simplicity rather than mechanistic realism. In principle a CE can include any number of linear, non-linear and cross terms, and distinct time-scales reflecting a rich zoo of possible materials [72]. These more complex models will no doubt start to be explored as our ability to perturb and infer the mechanics of *in vivo* tissues improves, as we now conclude.

6. Perspectives

The last few years have brought a major advance in the understanding of the biochemical mechanisms controlling pulsatile actomyosin activity. On the other hand, the mechanics of morphogenesis is still less well understood. A major limitation to progress in understanding mechanical feedback is our inability to visualise stress or material properties in vivo without perturbation, though the minimally invasive optical tweezing seems a promising approach [4,48]. Measuring retraction at different lengths from laser ablation cuts also seems a promising approach to understanding mechanics at multiple

scales [54,73]. An alternative is non-invasive force inference, using quantifiable visible information such as deformation rates at different scales along with Myosin dynamics [62,74,75].

Because of these limitations, feedback from tissue-level mechanics to cell deformation and Myosin focus dynamics, and possibly Myosin control, are currently under-explored. The literature offers a large array of perturbations (genetic and optogenetic, laser ablation, cauterization, tweezing) and observables related to either cell deformation or actomyosin pulsation (cell strain and strain rate, stabilisation of cell shape, focus life duration, actin flow) which are affected by mechanical perturbations (Fig. 3); however we are still lacking a unifying view of the mechanisms underlying these feedbacks. A future important step is to decide, in the light of the models, which combination of perturbations and observables could give definite answers to the role of mechano-chemical feedback during morphogenesis.

Conflicts of interest

None.

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Figure legends

Figure 1. Apicomedial Myosin pulses leading to tissue morphogenesis

(a) Cartoon of the apical actomyosin cortex with a Myosin focus in an epithelial cell. Myosin mini-filaments contract an actin meshwork that is connected via catenins to membrane bound E-Cadherin. **(b)** Fluctuation in Myosin-II fluorescence intensity (green) and apical cell area (black) over time for an example cell in the *Drosophila* amnioserosa. **(c)** *Drosophila* mesoderm invagination [76], ventral view. During gastrulation, ventral cells of the *Drosophila* embryo undergo pulsatile apical contraction that leads to ventral furrow formation and invagination of the mesoderm. **(d)** *Drosophila* germband extension [77-79] lateral view. Towards the end of gastrulation, lateral epidermal cells actively intercalate between one another while the tissue is pulled from the posterior by the posterior mid-gut invagination, resulting in a narrowing of the epidermis along the dorso-ventral axis and a lengthening in the antero-posterior axis. **(e)** *Drosophila* dorsal closure [80,81], lateral view. Midway through embryogenesis, the extra-embryonic dorsal amnioserosa undergoes pulsatile apicomedial pulsations to contract the lateral epidermis towards the dorsal midline and generate epidermal continuity. The amnioserosa contracts predominantly in the medio-lateral orientation due to anisotropic boundary conditions [82]. Anterior to the left in (c) – (e).

Figure 2. Myosin control and deformation across scales

(a) The Myosin focus pulsates in the apicomedial cortex under the control of an autonomous cellular unit of activators (described in Section 2). The Myosin focus locally pre-stresses actomyosin, changing the local mechanical balance and thus affecting actomyosin stress and strain, which in turn may feedback on Myosin focus build-up (e.g. by condensation of the focus). If catenins engage a mechanical clutch between apicomedial actomyosin and trans-membrane adhesion proteins, stress is transmitted to the whole cell and in turn to its neighbourhood. In this way, the mechanical balance is probing the mechanical resistance of the whole tissue, putting it under tension or deforming it, depending on the boundary conditions at its periphery. **(b)** The autonomous activator unit controls Myosin II pulsatile or stable activation, depending on RhoGEF or GPCR levels [18]. **(c)** Under the control of the activator unit, the Myosin focus nucleates, condenses and disassembles in the course of time across the apicomedial cortex of a cell. Mechanical forces may feedback on its condensation and movements across apicomedial actomyosin. Due to the Myosin-induced change in mechanical balance, and depending on the catenin clutch, the cell deforms in a pulsatile manner. **(d)** Ratcheted cell pulsations are affected by neighbouring cell deformations, possibly through mechanical balance (adapted from [56]).

Figure 3. Myosin pulses and resultant contractions and stress

(a) 'Cookie-cutter' ring ablation shows cells under apicomedial tension in the *Drosophila* amnioserosa [57]. **(b)** Neighbouring cells with Myosin foci contract predominantly perpendicular to the focus-focus orientation in the same tissue as (a) [62]. **(c)** Clutch between apicomedial actin and junctions not engaged in *C. elegans* embryo [50] allows slippage of actomyosin relative to cell-cell junction and leads to apicomedial actomyosin strain without cell deformation. **(d)** Clutch in (c) engaged cancels slippage and imposes the same deformation for apicomedial actomyosin and cell-cell junctions. **(e)** Laser cut of apicomedial actin in the *Drosophila* germband during Myosin focus activity leads to retraction of the focus away from cut, guided by junctional connections [13]. **(f)** Effect of tissue tension on the shape of Myosin foci [40]. Top, anisotropic tension, greatest along the

anterior-posterior axis (horizontal), leads to contraction in the perpendicular orientation and anisotropic focus shape. Bottom, when stress is rendered isotropic by laser cuts perpendicular to the orientation of greater tension, isotropic contraction and ring-shaped Myosin foci are observed.

Figure 4. Mechanical context and rheology of the *Drosophila* amnioserosa
(a) Dorsal view of *Drosophila* embryo during dorsal closure of the amnioserosa (top) and sagittal section through the dorsal half of the embryo (bottom). In the plane of the amnioserosa, the attached epidermis contributes to the boundary conditions, notably in the medio-lateral axis. The overlying vitelline membrane and underlying cell cytosol and yolk combine to impose a friction on the deformation of the apicomedial cortex. **(b)** Scheme for understanding the cell and tissue rheology of the amnioserosa, here concentrating on the medio-lateral connections and stress. The apicomedial cortex of each cell is modelled as a visco-elastic material, governed by the constitutive equation: $2\tau\,\kappa\,\dot{\epsilon} = \sigma + \tau\dot{\sigma} - \sigma_{myo}$, in which the strain rate is balanced by the stress and the contractile Myosin pre-stress (grey labels, see Section 5 for further definition of terms) [62]. The apicomedial cell cortex is connected through cell junctions in the plane and is subjected to a friction with over- and underlying tissues, portrayed here as out of plane springs (magenta labels). ECM: extracellular matrix.

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** Lecuit and colleagues show here the pulsatility of a reporter for Rho1, which is concurrent with peaks in Rok, Myosin II and Mbs (the latter with a slight delay). Interestingly, the pulsatility of Rho, Rok, Mbs as well as actin are dependent on Myo II activity leading the authors to suggest that pulsatility is not an autonomous property of a

biochemical pacemaker but rather the emergent property of a biomechanical network, in which advection-mediated positive feedback amplifies the recruitment of Rho1 and downstream factors, and the relaxation of the actomyosin network drives their dispersion.

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** Munro and colleagues propose here that RhoA pulsatility in the *C. elegans* embryo results from a combination of local autocatalytic activation of RhoA and a delayed negative feedback due to F-actin-dependent recruitment of RGA3/4, a negative regulator of RhoA. They observe that RhoA pulsatility is independent of Myosin and show that RGA3/4 pulses are delayed relative to RhoA pulses. Thus, it remains to be elucidated if the differences in the mechanism underlying Myosin pulsatility observed in *Drosophila* and *C. elegans* embryos are species-specific or whether these will become aligned in due course.

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** The authors follow the spatiotemporal pulsatile dynamics of RhoA, Myosin II and actin in the *C. elegans* zygote. They conclude that pulsations are not consistent with a model of mechanical feedback on RhoA, whereas assuming an autonomous oscillator leads to correct spatiotemporal patterns.

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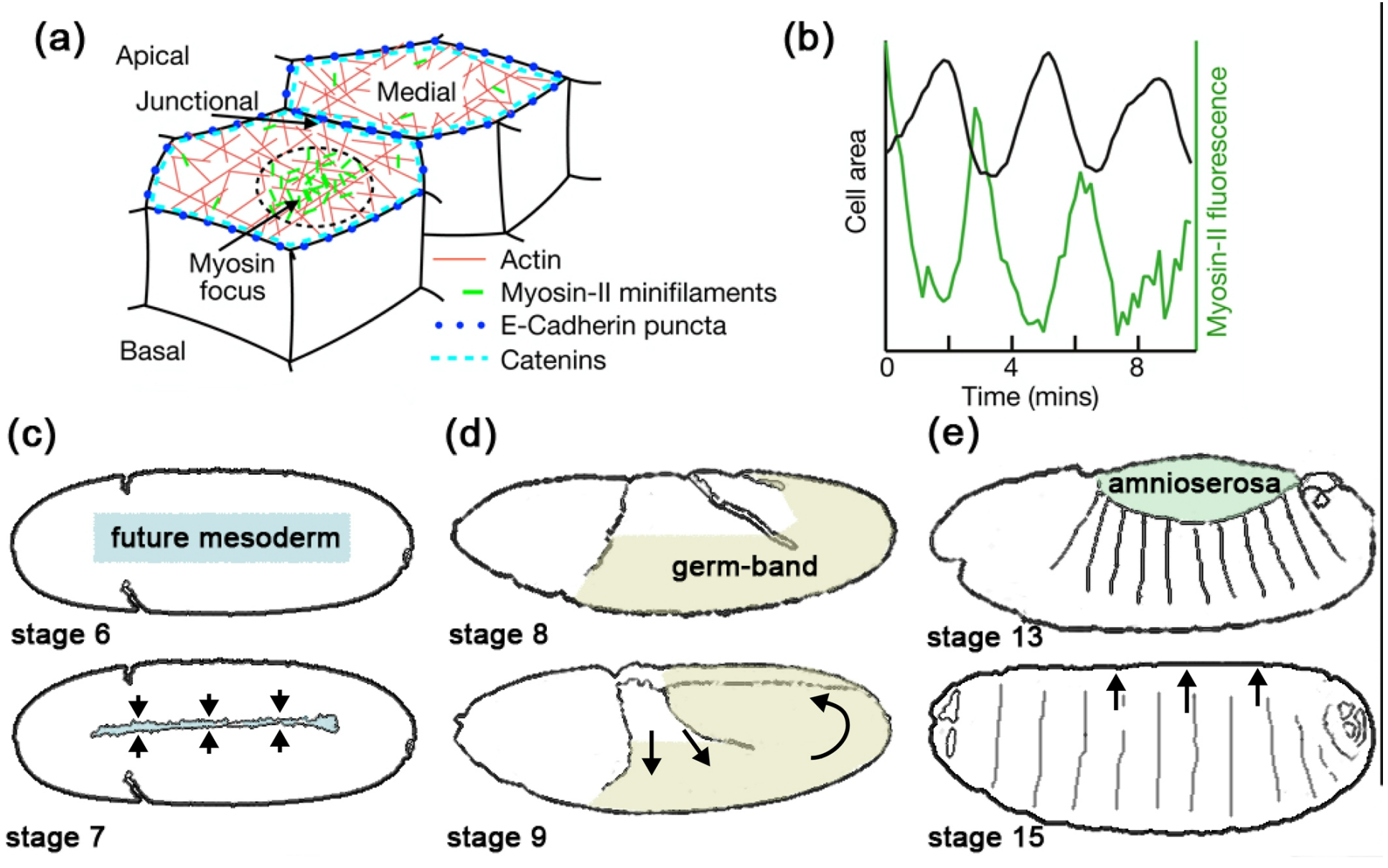
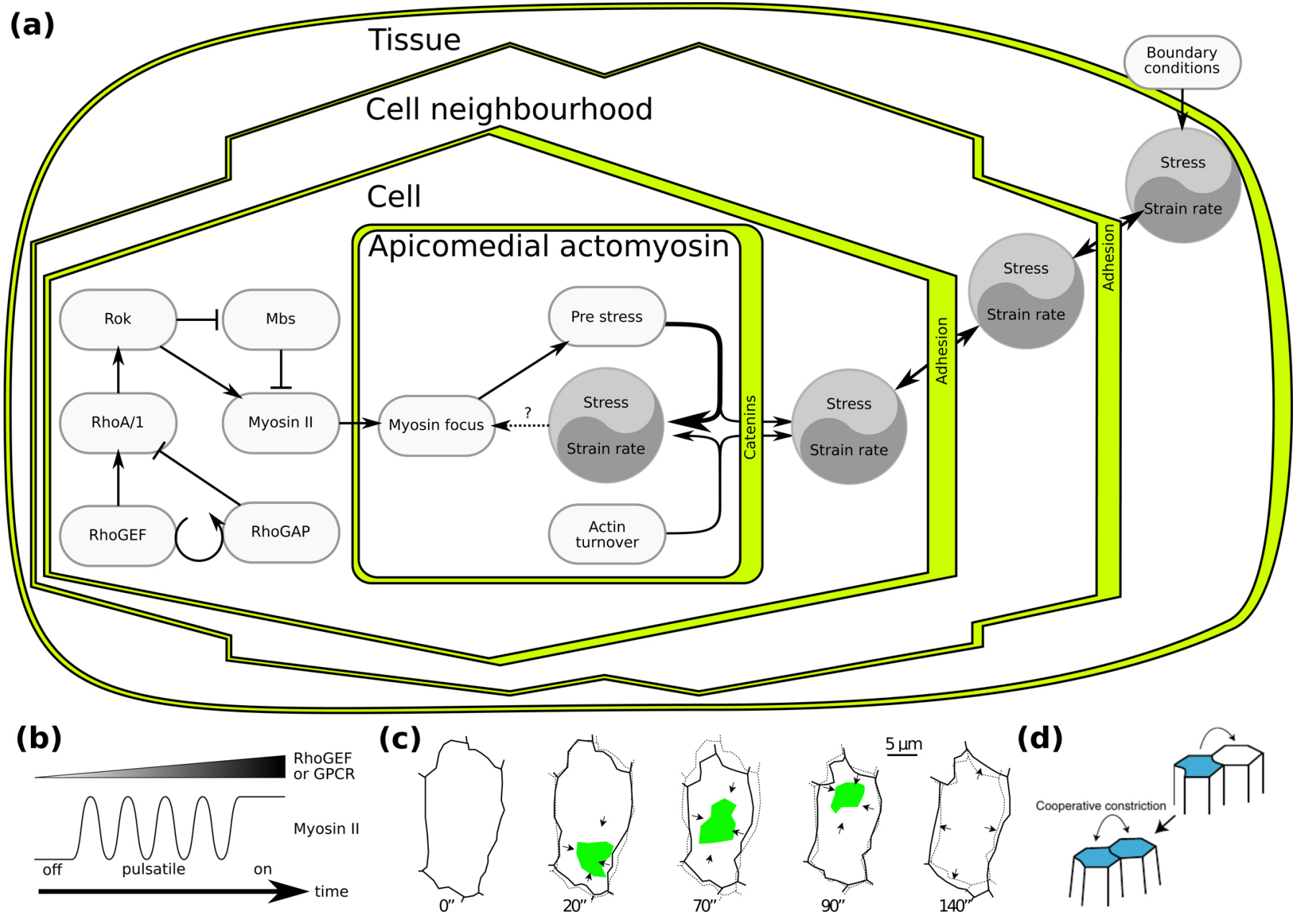
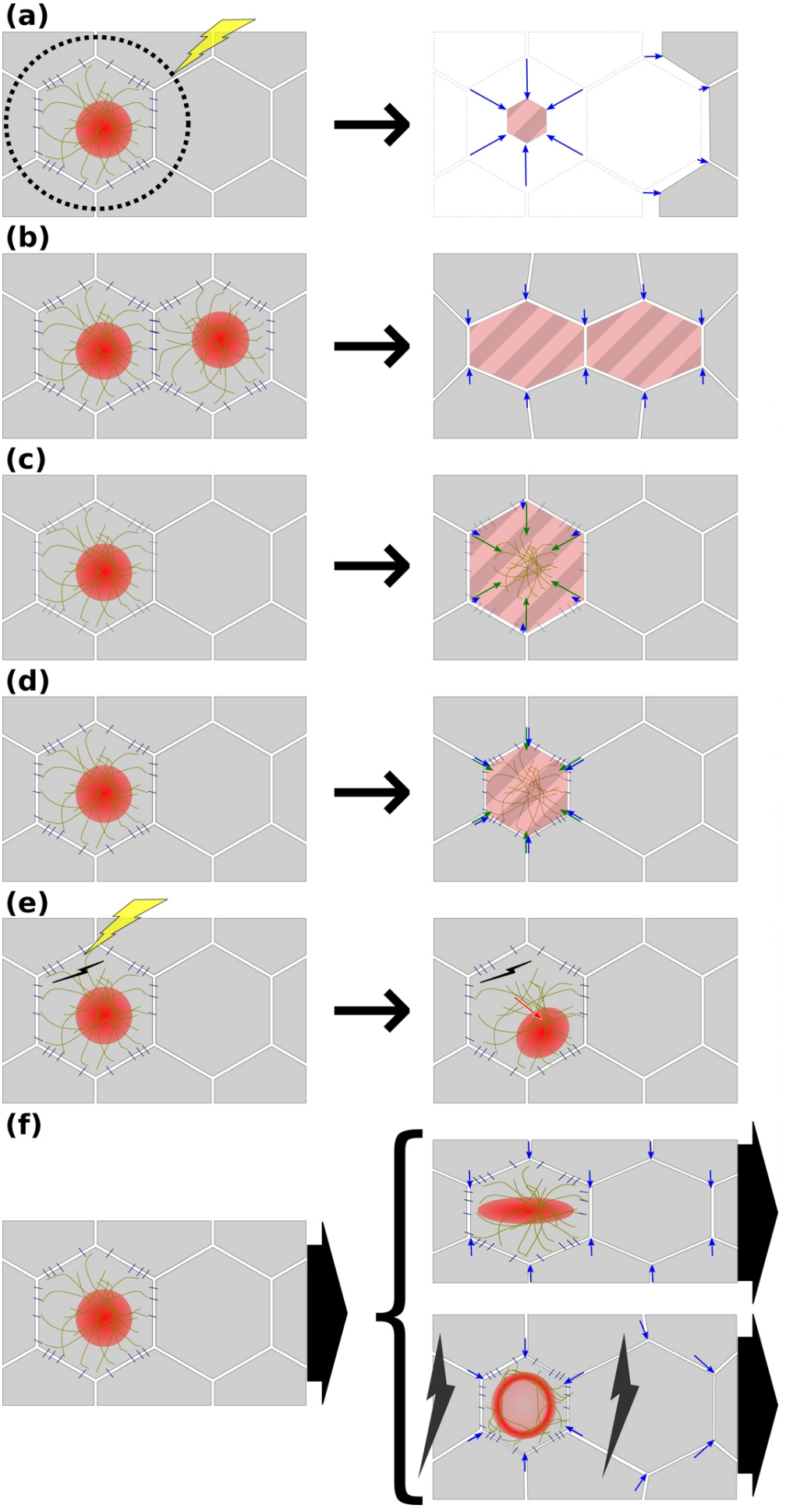

(a)
Apical
Junctional
Medial
Myosin
focus
Basal
Actin
Myosin-II minifilaments
E-Cadherin puncta
Catenins
(b)
Cell area
Myosin-II fluorescence
0
4
8
Time (mins)
(c)
future mesoderm
stage 6
stage 7
(d)
germ-band
stage 8
stage 9
(e)
amnioserosa
stage 13
stage 15


(a)
Tissue
Cell neighbourhood
Cell
Apicomedial actomyosin
Boundary conditions
Stress
Strain rate
Adhesion
Rok
Mbs
RhoA/1
Myosin II
RhoGEF
RhoGAP
Myosin focus
?
Pre stress
Actin turnover
Catenins
(b)
RhoGEF or GPCR
Myosin II
off
pulsatile
on
time
(c)
5 µm
0″
20″
70″
90″
140″
(d)
Cooperative constriction


(a)
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(f)

(a) Amnioserosa mechanical context

(b) Amnioserosa apicomedial cell and tissue rheology