Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Cytoskeleton (Hoboken). Author manuscript; available in PMC 2016 June 30.

Published in final edited form as:

Cytoskeleton (Hoboken). 2015 May ; 72(5): 207–224. doi:10.1002/cm.21228.

Drosophila comes of age as a model system for understanding the function of cytoskeletal proteins in cells, tissues, and organisms

Avital A. Rodal[1], **Steven J. Del Signore**[1], and **Adam C. Martin**[2]

[1]Department of Biology, Brandeis University, Waltham, MA 02453

[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, 02139

Keywords

Drosophila; actin; microtubule; oocyte; myoblast; myosin

For the last one hundred years, *Drosophila melanogaster* has been a powerhouse genetic system for understanding mechanisms of inheritance, development, and behavior in animals. In recent years, advances in imaging and genetic tools have led to *Drosophila* becoming one of the most effective systems for unlocking the sub-cellular functions of proteins (and particularly cytoskeletal proteins) in complex developmental settings. In this review, written for non-*Drosophila* experts, we will discuss critical technical advances that have enabled these cell biological insights, highlighting three examples of cytoskeletal discoveries that have arisen as a result: (1) regulation of Arp2/3 complex in myoblast fusion, (2) cooperation of the actin filament nucleators Spire and Cappuccino in establishment of oocyte polarity, and (3) coordination of supracellular myosin cables. These specific examples illustrate the unique power of *Drosophila* both to uncover new cytoskeletal structures and functions, and to place these discoveries in a broader *in vivo* context, providing insights that would have been impossible in a cell culture model or *in vitro*. Many of the cellular structures identified in *Drosophila* have clear counterparts in mammalian cells and tissues, and therefore elucidating cytoskeletal functions in *Drosophila* will be broadly applicable to other organisms.

Drosophila as a model for the discovery of cytoskeletal genes, pathways, and mechanisms

In vitro and cell culture models have provided and continue to provide tremendous insight into the biophysical and biochemical properties of the actin cytoskeleton and its regulators. However, many questions remain regarding how these factors are employed *in vivo* to generate the incredible diversity of cytoskeletal structures and architectures that promote normal development and physiology. To fully understand the regulation and function of these cytoskeletal structures, we must (1) have a complete inventory of the molecules that regulate their construction, (2) understand the mechanisms that coordinate the activity of each regulator in space and time, and (3) understand how these regulators and resultant cytoskeletal assemblies affect (and are themselves affected by) the dynamic, multicellular

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

environment of a developing organism. *Drosophila* is a uniquely ideal system with which to answer these questions. Here, we will provide an overview of the technical capabilities that empower *Drosophila* as a model to investigate these questions, briefly noting several discoveries that have emerged from each, followed by more detailed case studies in myoblast fusion, oocyte polarity, and supracellular actomyosin cables (**Table 1**).

Drosophila has obvious advantages as a model system: low cost to culture, fast generation time, high degree of gene conservation with mammals (but with the benefit of limited gene redundancy), optically accessible embryos, and complex yet tractable development. These advantages have enabled decades of genetic screens to identify many of the key cytoskeletal proteins and their regulators [Nusslein-Volhard and Wieschaus 1980; Schupbach and Wieschaus 1989], resulting in a large collection of mutant alleles that are readily available to the *Drosophila* community from labs and stock centers. Further, an explosion of high-throughput RNAi screens in *Drosophila* cell lines has identified many new potential cytoskeletal actors and regulators encoded by the fly genome [Rogers et al. 2003; Kiger et al. 2003]. These genetic approaches serve not only to identify genes, but also establish functional interactions amongst them, on the basis of similar phenotypes for specific mutants. For example, defects in the easily observed external mechanosensory bristles (**Figure 1A**), led to the identification and characterization of the actin crosslinkers *forked* and *singed*/fascin [Tilney and DeRosier 2005]. Other key actin regulators were identified through their requirement for oocyte maturation [Hudson and Cooley 2002]. This process involves the dumping of cytoplasm from a set of interconnected support or 'nurse' cells into the developing oocyte, through actin based 'ring canal' pores (**Figure 1A**). In addition to these ring canals, cables of bundled actin are required to restrain nurse cell nuclei and prevent clogging of the ring canals. Screens for defective oocyte maturation led to the identification of distinct sets of cytoskeletal regulators for each structure: the villin and fascin homologs Quail and Singed are required for F-actin bundle organization, while the F-actin crosslinking proteins Filamin and Kelch are necessary components of the ring canals [Hudson and Cooley 2002]. These findings provided early clues for how specific groups of regulators might coordinate to produce specific actin-based structures.

In addition to identifying regulators of particular cytoskeletal structures, the range of well-characterized developmental processes in *Drosophila* provides a means to identify novel F-actin regulators that may be required only in a subset of cell or tissue contexts. In a genetic screen for mutants affecting border cell migration, an established model of collective cell migration (**Figure 1A** and [Montell et al. 2012]), the Montell lab identified the gene *psidin* as a novel F-actin binding protein required for normal protrusive dynamics in the migrating border cells. Importantly, they showed that the mammalian homolog of *psidin*, C12orf30, exhibited a similar requirement for collective migration of wounded MCF10A monolayers [Kim et al. 2011].

While the ease and power of *Drosophila* for discovering new pathways and mechanisms is clear, its value as a tool for biomedical research ultimately depends on the degree to which these discoveries apply to mammalian systems. Indeed, an incredible volume of work has consistently demonstrated that findings in flies greatly advance our understanding of human development, physiology, and disease (for an excellent discussion, see [Wangler et al.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2015]). Among cytoskeletal proteins and their regulators, this conservation is seen at the level of gene and protein structure and function [Rohn et al. 2011], as well as at the level of particular cytoskeletal structures. For example, the bundles of F-actin observed in the mechanosensory bristles and nurse cell cables (see above) share many structural similarities with brush border microvilli and hair cell stereocilia [DeRosier and Tilney 2000]. Furthermore, each structure depends upon the concerted action of at least two actin bundling proteins, each of which is conserved from flies to humans. It is worth noting that there is also value in systems for which there are no clear analogs of a cellular structure. For example, the dynamic actin cones that drive *Drosophila* spermatocyte individualization exhibit a complex, three-dimensional morphology, and require the concerted action of highly conserved regulators of both branched and linear F-actin [Fabian and Brill 2012]. For cases such as these, the differences between systems provide as much value for the cell biologist as the similarities, as they reveal the adaptability of conserved cytoskeletal regulators and structures. Thus, diversity is itself a tool that can provide valuable insights into the full range of molecular interactions that govern cytoskeletal structure and function.

Technical advances that have brought *Drosophila* developmental studies to the subcellular level

Toolkit for dissecting molecular mechanism in vivo

While genetic screens have identified critical cytoskeletal regulators by mutant phenotype, in order to fully explore their roles in complex developmental processes it is ultimately necessary to reverse engineer mutants and fusion proteins to perform mechanistic studies in animals. Historically, the relative inefficiency of homologous recombination has hampered the generation of allelic series via reverse genetics. However, recent advances in genome modification have made *Drosophila* an excellent developmental system for making targeted mutations to disrupt specific molecular interactions. First, the development of a site-specific integration system using the bacteriophage ΦC31 integrase allows *Drosophila* researchers to target transgenes to well-defined genomic “landing-sites”. Thus, a congenic allelic series can be generated to perform structure-function analyses, as was recently performed for the *Drosophila* α-catenin gene [Desai et al. 2013]. Second, bacterial recombineering can be performed using a library of Bacterial Artificial Chromosomes (BACs) in order to modify genomic loci in bacteria. Edited genomic regions as large as 120 kb can be integrated using ΦC31-mediated insertion. A limitation of this technique is the need to recombine transgenes with mutants in the endogenous gene locus. Third, RNAi can be an effective mechanism for silencing *Drosophila* genes in the context of the animal, especially in genetic backgrounds with increased Dicer activity [Mohr and Perrimon 2012]. Finally, the synthesis of these existing technologies with new genome editing techniques such as TALENs and CRISPR will facilitate engineering of endogenous loci, rendering *Drosophila* an even more powerful system for dissecting molecular functions [Liu et al. 2012; Ren et al. 2013; Port et al. 2014].

Techniques for temporal and tissue-specific manipulation of gene function

Many mutants have tissue- and developmental stage-specific phenotypes that are obscured by mutant phenotypes at other stages or in other tissues. This is particularly an issue for the large number of cytoskeletal mutants that result in early embryonic lethality, precluding

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

studies of gene function in tissues that do not appear until later stages of development. Further, for many gene products, *Drosophila* embryos are heavily loaded with a maternal contribution of RNA or protein. In these cases, manipulation of the embryonic genome does not deplete the gene product. To overcome this obstacle, Drosophilists have developed multiple tools to manipulate both endogenous genes and transgenes with a high degree of spatiotemporal specificity.

The first widely used tool is the modular GAL4-UAS system, which is used to drive tissue and/or stage specific expression of transgenes (**Figure 1B** and [Brand and Perrimon 1993]). The system utilizes the yeast transcriptional activator GAL4 under the control of endogenous or synthetic *Drosophila* genomic enhancers, paired with transgenic constructs in which a gene of interest is fused to the Upstream Activating Sequence for GAL4 (UAS) (**Figure 1B**). Thousands of publicly available GAL4 drivers and UAS lines afford the Drosophila researcher tremendous flexibility in manipulating gene function simply by mating the desired GAL4-driver and UAS-transgene lines together. Additionally, use of a temperature sensitive inhibitor of GAL4 can enhance temporal control of gene expression [McGuire et al. 2003]. Moreover, complementary modular systems have been developed to allow for simultaneous manipulation of transgene expression in multiple tissues in the same animal [Venken et al. 2011]. The GAL4-UAS system also enables RNAi-mediated depletion of genes in a tissue-specific manner using RNA hairpins cloned downstream of the UAS sequence. To facilitate experiments using this approach, multiple publicly available genome-wide collections of UAS-driven RNAi lines have already been generated [Dietzl et al. 2007; Ni et al. 2011], and can work against maternally provided as well as zygotic gene products [Sopko et al. 2014]. These technologies have enabled the execution of targeted screens to identify regulators of particular developmental processes or cytoskeletal structures. For instance, using a mechanosensory bristle specific GAL4 line in combination with UAS-driven dominant negative constructs, Rab35 was identified as a novel regulator of the actin crosslinker singed/fascin. This unexpected functional relationship was found to be conserved in NIH 3T3 fibroblasts, where Rab35 mediated fascin recruitment and filopodia formation [Zhang et al. 2009].

A second widely used tool is a highly efficient method for generating homozygous mutant clones in heterozygous tissue, using FLP-mediated mitotic recombination (**Figure 1B**). The FLP recombinase induces efficient and specific recombination between FRT DNA sequences in flies. In this method, animals heterozygous for a mutant are induced to undergo mitotic recombination at FRT sites located near the centromere, producing homozygous mutant cells that can expand into clones within the otherwise heterozygous (and thus phenotypically normal) tissue [Xu and Rubin 1993]. This technique has been further extended with the MARCM (Mosaic Analysis with a Repressible Cell Marker) technique, which combines the FLP/FRT system with GAL4/UAS [Lee and Luo 2001] to improve the system in two ways. First, MARCM allows for greatly improved visualization of mutant cells, which has been particularly advantageous in the study of cytoskeletal process such as neurite outgrowth and branching [Ng et al. 2002]. Second, the MARCM system allows researchers to combine clonal loss of function analysis with a UAS mediated transgene or RNAi overexpression for more sophisticated experimental designs. In addition, FLP-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mediated recombination allows generation of mutant germline clones within the ovary, to produce mutant oocytes (and subsequently embryos) without a maternal load of RNA or protein [Chou et al. 1993].

Finally, several techniques have recently become available for acute manipulation of protein function in living *Drosophila*. These include photoactivatable proteins, in which a native protein is fused to a photosensitive inhibitory domain of a plant phototropin (e.g. paRac1 [Wang et al. 2010]), and photo-degradation of a genetically tetracysteine-tagged protein of interest (chromophore-assisted light inactivation or CALI) [Marek and Davis 2002]. Finally, protein levels can be precisely controlled via inducible expression of a ubiquitin-ligating single-chain anti-GFP antibody, which targets a GFP-tagged protein of interest for degradation by the proteasome [Caussinus et al. 2012]. Thus, *Drosophila* researchers have at their disposal a number of tools for temporal and spatial control of gene expression and function in the animal.

Microscopy: Connecting molecules, cells, and tissues

The improvement in the quality of microscopes and the development of fluorescent probes to detect cellular structures have dramatically pushed forward our ability to visualize developmental events, and to connect multicellular behaviors with dynamic cellular and molecular processes. *Drosophila* embryos are ~200 μm in diameter and ~500 μm long, and fixed embryos (and other tissues) can be effectively imaged by confocal microscopy for most applications, without the need for deeper light penetration. Confocal imaging has therefore been the workhorse for *Drosophila* biology. In the past, Drosophilists relied most heavily on imaging fixed samples, but newer microscopy modalities, greater access to both single-photon and multi-photon confocals, and improved diversity of fluorescent proteins have placed a greater emphasis on live-cell imaging [Winter and Shroff 2014]. Recently, live imaging of developmental events that were previously studied extensively with fixed samples revealed novel mechanisms for cell and tissue morphogenesis. These include, but are not limited to: pulsatile cell contractions driving tissue contraction and epithelial sheet movement throughout embryogenesis [Martin et al. 2009; Solon et al. 2009; Rauzi et al. 2010; He et al. 2010], polarized migration and revolution of a continuous epithelial sheet [Haigo and Bilder 2011], and dynamics of synapse assembly [Schmid et al. 2008]. The availability of many different wavelength fluorophores for multi-channel imaging has allowed dynamic changes in cell and tissue shape to be correlated with specific cytoskeletal structures [Kremers et al. 2011]. For instance, cell rearrangements that result in extension of the embryonic tissue during germband extension have been connected in real-time with the enrichment of junctional proteins and myosin motors to complementary domains [Bertet et al. 2004; Blankenship et al. 2006; Simoes et al. 2010; Rauzi et al. 2010]. Other innovations, such as photo-activatable and photo-convertible fluorophores (photoactivatable GFP (PA-GFP), mEOS, Dendra, and Dronpa) have allowed for the analysis of protein dynamics during developmental processes, including the dynamics of adherens junctions during epithelial elongation, and of the plasma membrane during early cleavage divisions [Cavey et al. 2008; Mavrakis et al. 2009].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The combination of genetic techniques with live imaging in Drosophila has particularly advanced our understanding of how complex multicellular environments influence actin based behaviors previously studied in cultured cells, such as cytokinesis and cell migration. By utilizing a diverse range of clonally expressed fluorescent proteins to analyze cell division within an epithelium, it was shown that cytokinesis of epithelial cells is not a unicellular process driven exclusively by the cytokinetic ring, but rather is a multicellular process involving at least three distinct actin based structures [Founounou et al. 2013; Guillot and Lecuit 2013; Herszterg et al. 2013]. Another striking example of coordinated cell behavior is seen in the collective cell migration of border cells (**Figure 1A**). In this system, protrusive dynamics are largely restricted to the leading cell. A FRET based biosensor revealed that leader cells exhibit higher levels of Rac activity than trailing cells. Spatiotemporal manipulation of Rac activity using photoactivatable constructs confirmed that Rac activity in a single cell is sufficient to polarize the entire border cell cluster [Wang et al. 2010]. To understand how this polarization is coordinated amongst cells, subsequent studies utilized cell specific genetic manipulations with the GAL4/UAS system to show that this coordination requires a feedback loop between Rac and E-Cadherin-based adhesion of migrating border cells and the surrounding nurse cells [Ramel et al. 2013; Cai et al. 2014].

In addition to confocal microscopy, *Drosophila* is amenable to other microscopy techniques, which have expanded the range of spatial scales that can be studied by light microscopy. A recent study used total internal reflection microscopy to analyze the resorption of microvilli at the onset of gastrulation [Fabrowski et al. 2013]. Super-resolution techniques such as structured illumination have been used to examine cytoskeletal structures at junctions, and the organization of large ribonucleoprotein-containing structures that are trafficked by the cytoskeleton to set up embryonic polarity [Roper 2012; Weil et al. 2012]. The application of newer “super-resolution” technologies, such as STORM (stochastic optical reconstruction microscopy) or STED (stimulated emission depletion) is allowing Drosophilists to bridge the gap between molecular events and multicellular behaviors [Kittel et al. 2006; Ehmann et al. 2014]. Finally, the emergence of selective-plane illumination microscopy (SPIM) and lattice light-sheet microscopy promises to increase the scale of imaging, in some cases allowing entire organisms or organs to be viewed undergoing morphogenesis with sufficient resolution to view cellular and subcellular changes [Keller et al. 2010; Rebollo et al. 2014; Chen et al. 2014]. Similar advances in the analysis and modeling of live imaging data have allowed researchers to functionally link these spatial scales, identifying reciprocal interactions between the molecular cues that establish polarity at the subcellular level and tissue level mechanical forces and morphological dynamics [Aigouy et al. 2010; Bosveld et al. 2012].

In summary, the low cost and 'open source' nature of *Drosophila* allow for the execution of both broad and targeted screens, permitting saturating identification of genes and characterization of genetic pathways that control particular cytoskeletal based processes. Molecular genetic techniques allow for mechanistically directed reverse engineering of genes, as well as increasingly precise tissue and stage-specific functional dissection of these pathways. Finally, advances in imaging and quantitative analysis synergize with these capabilities to render *Drosophila* a tool uniquely suited to understand not only the functions

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

of particular cytoskeletal regulators, but also the effect of cell and tissue context on the output of these regulatory networks. We will now discuss in detail three case studies in which these strengths facilitated discoveries that would have been impossible using other systems.

Case studies in *Drosophila* cell biology

1. Cooperation of actin assembly factors in myoblast fusion

Cell-cell fusion is a fundamental process that is critical for the development and regeneration of many syncytial tissues, and requires multiple cell types and positional cues that have been difficult to fully recapitulate in cultured cells [Abmayr and Pavlath 2012; Kim et al. 2015a]. Recent studies have taken advantage of the powerful tools in *Drosophila* to illuminate how the actin cytoskeleton drives these fusion events *in vivo*. Syncytial muscle formation in the *Drosophila* embryo depends on cell-cell fusion, and occurs when fusion-competent myoblasts (FCM) fuse with a single muscle founder cell (FC) or a growing myotube (**Figure 2** and reviewed in [Abmayr and Pavlath 2012; Kim et al. 2015a]). This process of myoblast fusion begins with cell migration and heterotypic cell-cell adhesion, and culminates in the formation of a “fusogenic synapse” [Doberstein et al. 1997; Kesper et al. 2007; Richardson et al. 2007; Sens et al. 2010]. During this process, F-actin structures called “actin foci” become enriched at the center of a ring of cell adhesion molecules in the FCM, forming a ~2 μm dimple with dynamic finger-like projections that invade the FC or growing myotube [Kesper et al. 2007; Richardson et al. ; Sens et al. 2010; Haralalka et al. 2011l; Haralalka et al. 2014], (**Figure 2A)**. These structures at the fusion site have been termed “podosome-like structures” (PLS; [Sens et al. 2010]) due to their structural and functional similarity to extracellular matrix-remodeling podosomes and invadopodia, which are observed in cultured cells and are also defined by a core of branched F-actin surrounded by a ring of cell adhesion molecules [Schachtner et al. 2013]. On the FC side of the fusion site, a thin actin sheath forms around the PLS to increase mechanical tension at the junction between the cells [Kim et al. 2015b]. After 5-30 min, fusion pores form as the actin foci disappear, followed by pore expansion and complete cell fusion [Richardson et al. 2007; Sens et al. 2010]. The discovery of this series of events was made possible by a powerful combination of genetic screens to identify the molecular players (reviewed in [Abmayr and Pavlath 2012; Kim et al. 2015a].), tissue-specific live and fixed imaging that differentiated the organization and dynamics of actin in the FC compared to the FCM [Richardson et al. 2007; Sens et al. 2010; Haralalka et al. 2011; Jin et al. 2011], and ultrastructural analysis of the fusing cells [Doberstein et al. 1997; Kim et al. 2007; Sens et al. 2010]. Here, we will focus only on the cytoskeletal mechanisms underlying actin foci in the FCM, as they illustrate the power of using *Drosophila* for cytoskeletal discovery.

Extensive genetic screens and directed tests have identified mutants defective in myoblast fusion, including mutations in the actin-nucleation promoting Actin-related protein 2/3 (Arp2/3) complex [Massarwa et al. 2007; Richardson et al. 2007; Berger et al. 2008]. The Arp2/3 complex itself is inactive, and requires stimulation by nucleation promoting factors (NPFs) to assemble branched actin networks (**Figure 2B** and [Rotty et al. 2013]). Two distinct NPFs, from the Wiskott-Aldrich syndrome protein (WASP) and WAVE (WASP-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

family verprolin homology protein) families, are required for PLS function. Mutations in *WASp* and its regulator *dWIP*/*Solitary*/*Sltr* (the homolog of WASp-interacting protein (WIP)) lead to fusion defects [Schafer et al. 2007; Kim et al. 2007; Massarwa et al. 2007; Berger et al. 2008; Gildor et al. 2009], as do mutations in *SCAR* (the homolog of WAVE), and its regulator *kette* (the homolog of NAP1) [Schroter et al. 2004; Richardson et al. 2007; Berger et al. 2008; Gildor et al. 2009]. Several lines of evidence suggest that *WASp* and *SCAR* function together in the FCM. First, dWIP is detectable only in the FCM [Kim et al. 2007], suggesting that it functions there and not in the FC. Further, tissue-specific rescue experiments utilizing FC and FCM specific GAL4 lines demonstrated that dWIP and WASp are required primarily in the FCM [Schafer et al. 2007; Massarwa et al. 2007; Sens et al. 2010], while SCAR is required in both the FC and the FCM for normal fusion.

WASp and SCAR represent functionally distinct activators of actin polymerization through the Arp2/3 complex, with different potencies of Arp2/3 activation and different cellular regulatory mechanisms (**Figure 2B**, reviewed in [Rotty et al. 2013]). Therefore, the discovery that both are involved in the F-actin focus during myoblast fusion raised the important question of how their activities were coordinated. The formin Diaphanous (Dia), which stimulates the formation of unbranched actin filaments, has also been implicated in myoblast fusion, and localizes to finger-like protrusions from the PLS [Haralalka et al. 2014]. This system provides a unique model to study the interplay between these converging actin assembly regulators in a novel cytoskeletal ultrastructure, distinct from the widely studied leading edge of migrating cells. Here we will focus on the distinct functions of SCAR and WASp.

Both SCAR/Kette and WASp/WIP modules localize to actin foci. Though both are thought to stimulate actin assembly via Arp2/3 complex, WASp and SCAR mutants surprisingly fail to disassemble actin foci. Further, mutations in each gene yield different phenotypes: loss of SCAR/Kette causes enlargement rather than depletion of actin foci, while loss of WASp has no effect on focus size [Richardson et al. 2007; Gildor et al. 2009, Sens, 2010 #1507; Mukherjee et al. 2011]. These results suggest that WASp and SCAR play specific roles in the function, and not just assembly, of the focus. Examination of double mutants between these two NPFs revealed both shared and separable roles. *dWIP SCAR* and *SCAR WASp* double mutants exhibit enhanced fusion defects and actin focus defects, suggesting additive roles for the WASp and SCAR modules in the function of actin foci [Berger et al. 2008; Gildor et al. 2009; Sens et al. 2010; Bothe et al. 2014]. However, a closer look demonstrated that single mutants in the two NPFs have distinct ultrastructural phenotypes. The PLS of WASp or dWIP mutants fails to efficiently invade the FC, and finger-like protrusions observed by electron microscopy are short or collapsed. Further, dWIP mutants exhibit an accumulation of secretory vesicles [Kim et al. 2007]. In contrast, the ultrastructure of the PLS in *kette* mutants appears normal, though subsequent fusion is blocked, likely due to the requirement of the Scar complex in founder cells [Sens et al. 2010].

Mechanistic insights into the role and function of WASp in actin foci have come from recent studies of *blown fuse* (*blow*) mutants [Jin et al. 2011]. These were originally identified based on a myoblast fusion defect [Doberstein et al. 1997], but the molecular function of Blow, a PH (pleckstrin homology)-domain containing protein, was unknown. Consistent with a role

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in the PLS, Jin et al. found that Blow interacts indirectly with the FCM specific cell adhesion molecule Sticks and Stones (Sns), and interacts directly with dWIP [Jin et al. 2011]. WIP binds to tightly to WASp and regulates its stability and targeting to cellular structures [Anton et al. 2007]. Interestingly, Blow competes with WASp for dWIP binding, locally destabilizing the WASp-WIP complex. However, *blow* mutants do not simply cause an increase in the steady state accumulation of WASp-WIP and F-actin. Instead, an elegant set of cell specific genetic rescue experiments combined with FRAP of WASp, dWIP, and F-actin within the PLS, revealed that *blow* mutants dramatically decrease the dynamic turnover of all three components [Jin et al. 2011]. This important and surprising result suggests that rapid turnover of the WASp/dWIP complex and actin structures, rather than actin assembly alone, is required to maintain function of the PLS. This may be due to a need to recycle WASp for additional rounds of Arp2/3 activation, or alternatively to alleviate inhibition of filament elongation by the previously characterized actin filament barbed end-capping activity of WASp near the plasma membrane [Co et al. 2007; Khanduja and Kuhn 2014].

Additional insights into the role of SCAR in fusion came recently with the development of a heterologous assay for cell fusion in *Drosophila* S2R+ cells, which do not normally undergo fusion [Shilagardi et al. 2013]. S2R+ cells can be induced to fuse very efficiently by co-expression of the *C. elegans* fusogenic protein Eff-1 with the FCM-specific cell adhesion molecule Sns, or with α-PS2 integrin, which is not thought to be involved in myoblast fusion *in vivo*. Sns drives efficient S2 cell fusion, and is required in only one of the fusion partner S2 cells to promote fusion, much like its role in myoblasts. The authors used STORM to investigate the structure of the fusing cells. They found that the F-actin foci generated by Sns consist of Sns-containing invasive fingers extending from one cell into its fusion partner, while α-PS2 integrin-dependent fusion generates numerous separated hair-like projections along a broad contact zone, anchored at their basal side by α-PS2. Interestingly RNAi of WASp, SCAR or WIP abolishes Sns-dependent cell fusion, while only SCAR is required for α-PS2 integrin-dependent fusion, further indicating distinct functions for NPFs. While SCAR alone can promote protrusion formation and fusion at adhesion sites, the additional activities of the WASp/WIP complex permit formation of a broad contact zone and a large, robust focus. These different properties may arise from previously described inherent differences in the NPF and branching activities of WASp and SCAR [Zalevsky et al. 2001; Yarar et al. 2002; Kang et al. 2010; Sweeney et al. 2014], from additional effects on the actin network (e.g. barbed end capping by WASp [Co et al. 2007; Khanduja and Kuhn 2014]), or perhaps on differential efficiency or spatial control of activation for each NPF.

In conclusion, studies in *Drosophila* have demonstrated that myoblast fusion is asymmetric, driven primarily by a protrusive, podosome-like structure emanating from the fusion competent myoblasts. Assembly of a robust, dynamic F-actin network requires the combined activities of SCAR and WASp/WIP. SCAR functions together with WASp/WIP (and perhaps other NPFs) to generate a functional actin focus, while a dynamic WASp/WIP complex, regulated by Blow, is required to maintain rapid turnover within the PLS, forming a dynamic structure that can effectively invade the FC. Filopodia-like structures emanating

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

from the focus could also involve the activities of formins such as Dia. Finally, SCAR may be involved in the final steps of fusion pore formation, though more work needs to be done to resolve its exact function at this step. In addition to defining the molecular roles of actin regulators, these studies have suggested several new functions for dynamic branched actin networks in cell fusion, including driving robust invasion of the FC, increasing apposed surface area for pore generation, exocytic vesicle traffic, extracellular matrix remodeling, and potentially in formation of the fusion pore itself (**Figure 2C**).

This case study exemplifies how *Drosophila* was used to determine how a unique cytoskeletal structure is remodeled by the coordinated actions of functionally distinct regulators, to produce an ordered series of events in the context of a developing tissue. Tools available in *Drosophila* enabled these studies, which used analysis of tissue specificity of gene function in FCs and FCMs, ultrastructural observations, and perhaps most importantly, live imaging of the PLS in wild type and mutant backgrounds. This foundational work in *Drosophila* has inspired similar studies in mammals [Abmayr and Pavlath 2012; Kim et al. 2015a]. These insights could also enhance our understanding of analogous invasive structures, such as podosomes and invadopodia, which are structurally similar to the fusogenic synapse and also require WASp function [Linder et al. 2011]. Thus, myoblast fusion may provide one of the few *in vivo* systems where these structures, which are important in human physiology and disease, are experimentally accessible.

2. Big Cell polarity

Cell polarity is the fundamental process by which cells establish positional information within the cortex and cytoplasm. In the *Drosophila* oocyte, mRNAs encoding patterning genes must become polarized within the oocyte cortex to establish the dorsal-ventral (D-V) and anterior-posterior (A-P) axes of the future embryo (**Figures 1A and 3A**). For example, posterior localization of *oskar* and *nanos* mRNAs is critical for establishing posterior identity in the embryo and the eventual germline of the adult [Ephrussi et al. 1991; Gavis and Lehmann 1992]. Because the *Drosophila* oocyte reaches lengths of greater than 100 μm, diffusion-based mechanisms for establishing and maintaining cell polarity appear to be insufficient. Instead, efficient localization of *oskar* and *nanos* mRNA depends on the dynamic rearrangement and activity of the oocyte microtubule (MT) cytoskeleton (reviewed in [Riechmann and Ephrussi 2001]). *Oskar* mRNA is localized to the posterior pole during mid-oogenesis via directed transport along a gradient of MTs along the A-P axis, with their plus ends biased towards the posterior [Parton et al. 2011]. Then, at late stages of oogenesis, the MT cytoskeleton is reorganized into subcortical bundles that promote cytoplasmic streaming and mixing of the oocyte yolk, as cytoplasm is deposited from supporting nurse cells [Theurkauf et al. 1992]. Cytoplasmic streaming in the oocyte facilitates *nanos* mRNA delivery from the nurse cell cytoplasm to the posterior pole, where it becomes anchored [Forrest and Gavis 2003]. The timing of MT cytoskeleton reorganization is critical for polarity because premature induction of cytoplasmic streaming disrupts the anterior-posterior MT gradient and *oskar* localization [Theurkauf 1994]. Here, we will focus on the interesting observation that the timing of MT reorganization is regulated by the actin cytoskeleton.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Drosophila genetics served as a powerful tool to identify the important regulators of oocyte polarity, because maternal effect mutants that disrupt cell polarity result in clear morphological defects in the embryo [Schupbach and Wieschaus 1989]. Two genes identified in genetic screens, *cappuccino* (*capu*) and *spire* (*spir*), encode proteins that mediate actin filament assembly in the oocyte [Manseau and Schupbach 1989]. Capu is the sole *Drosophila* member of the FMN-family of formin proteins, which nucleate unbranched actin filaments while remaining associated with the barbed end, and enhance the rate of filament elongation in the presence of the abundant actin-monomer binding protein profilin [Goode and Eck 2007]. Capu can also bind to MTs and cross-link MTs and actin filaments [Rosales-Nieves et al. 2006; Quinlan et al. 2007]. Spir nucleates actin filament assembly, via tandem actin-binding WH2 domains that stabilize actin oligomers [Quinlan et al. 2007; Bosch et al. 2007; Rasson et al. 2015]. Removal of either Capu or Spir results in premature cytoplasmic streaming, which disrupts the localization of mRNA to the posterior pole [Theurkauf 1994]. An important advance came with the identification of unexpected a cytoplasmic actin mesh network and cortical actin bundles [Dahlgaard et al. 2007; Wang and Riechmann 2008], whose disappearance correlates with MT bundling and the onset of cytoplasmic streaming (**Figure 3B**) [Dahlgaard et al. 2007; Wang and Riechmann 2008]. Both Capu and Spir were shown to be required for formation of this mesh [Dahlgaard et al. 2007] (**Figure 3B**). The cytoplasmic actin mesh and/or cortical bundles appear to anchor MTs with their minus ends embedded in the cortex [Wang and Riechmann 2008], and may passively restrict MT bundling by functioning like a sieve. Because MT bundle formation in *capu* mutants is suppressed by kinesin heavy chain mutants, Spir-Capu actin assembly and/or actin-MT cross-linking appear to restrict MTs from being swept into alignment by motor-driven cytoplasmic flows [Dahlgaard et al. 2007].

Critical insights into the mechanisms of actin mesh formation came from combining *in vivo* and *in vitro* studies to answer the following questions: How and why are two nucleators needed to assemble a cytoplasmic actin mesh? Are there separate requirements for the different activities (e.g. nucleation and elongation) of these actin regulators? Because Spir and Capu loss of function mutants share the same phenotype, it came as a surprise that Spir potently inhibits both the actin nucleation and actin-MT cross-linking activities of Capu *in vitro* [Rosales-Nieves et al. 2006; Quinlan et al. 2007] (**Figure 3C**). Spir interacts directly with the Capu tail through its KIND domain (with nanomolar affinity) to inhibit Capu nucleation, MT binding, and F-actin bundling [Quinlan et al. 2007; Vizcarra et al. 2011]. In contrast, Spir nucleation activity is enhanced by Capu binding, even when Capu itself is blocked from nucleation [Quinlan et al. 2007]. Further, Capu is autoinhibited through an interaction between its N-terminus and a site at its C-terminal tail that overlaps with the Spir binding site [Bor et al. 2012]. Thus, Spir binding could also help release Capu from autoinhibition, or regulate the processivity of Capu at the barbed end ([Vizcarra et al. 2014] and **Figure 3C**). It is tempting to speculate that the Spir-Capu complex functions as a unit to nucleate an actin filament whose barbed end is then handed-off to Capu to elongate (**Figure 3C**); however, the signal for triggering such a handoff after nucleation has not yet been identified.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Systematic phenotypic analysis of a congenic allelic series of Capu and Spir mutants provided insight into how interactions between these proteins might regulate actin network assembly and cytoplasmic streaming in the oocyte. Expression of Spir or Capu point mutants that disrupt their interactions (**Figure 3C**) fail to rescue fertility in *spir* and *capu* null mutants, respectively, demonstrating that their interaction is required *in vivo* [Quinlan 2013]. In addition, *capu* point mutants isolated in forward genetic screens were sequenced, and the mutant proteins biochemically characterized, revealing that *in vivo* phenotypes did not correlate directly with nucleation activity. However, Capu alleles with relatively wild-type nucleation activity, but with reduced barbed end elongation rate and processivity, exhibited defects in oocyte development [Yoo et al. 2015]. These results suggest a model where Spir and Capu physically interact, but then separate, such that Capu-mediated enhancement of actin elongation can assemble a cytoplasmic actin mesh [Quinlan 2013; Bor et al. 2014]. This model has recently been supported by *in vitro* observations for Spir and Capu's mammalian homologs, Spire and Formin 2. These two proteins work together to produce large numbers of short filaments through a suggested sequential “handoff” mechanism in which the actin filament barbed end oscillates between phases of elongation (formin-bound) and phases of paused growth (Spire-bound) ([Montaville et al. 2014] and **Figure 3C**). In fact, Spire and Formin 2 are required for the generation of an actin mesh in mouse oocytes that is responsible for asymmetric spindle positioning and polar body formation [Pfender et al. 2011]. Thus, the importance of Spire and formin proteins to oocyte development, elucidated in the powerful *Drosophila* system, is likely conserved throughout metazoan evolution.

A critical outstanding question is what regulates the timing of Capu and Spir activity and/or protein levels in the oocyte. Cortical Spir disappears at the onset of streaming and Spir overexpression prevents streaming, suggesting that destruction or delocalization of Spir determines the timing of the observed changes in the actin cytoskeleton [Dahlgaard et al. 2007; Quinlan et al. 2007]. The *Drosophila* RhoA GTPase can bind Capu and Spir [Rosales-Nieves et al. 2006]. Thus, RhoA activation could control distinct activities of the Capu/Spir complex. It will be important to establish where and when RhoA is activated in the oocytes and how RhoA activity modulates the Capu/Spir complex. One possible mechanism involves the RhoA effector Wiskott-Aldrich Syndrome Protein and SCAR Homolog (WASH), an Arp2/3 complex activator that interacts with Capu, Spir, and RhoA and is important to prevent premature streaming [Liu et al. 2009]. WASH-family proteins mediate actin assembly in endosomal compartments, suggesting a possible platform for cytoplasmic mesh assembly or a role for vesicle trafficking in regulating a cortical signal. An intriguing possibility is that changes in actin network architecture involve a switch from unbranched actin to branched actin polymerization.

This case study illustrates how a novel and conserved cytoskeletal structure was discovered in *Drosophila*, and how cell biological and genetic tools were used to lay the foundation for discovering its mechanism of assembly and function. These findings inspired complementary studies of the mammalian *proteins in vitro* and *in vivo*, and set the stage for discovery of the full complement of actin structures that are synthesized in *Drosophila* and mammalian oocytes.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Supracellular actomyosin cables

During development, epithelial cells of different cell types can sort into lineage-restricted compartments that fail to intermix, despite the continuous cellular rearrangement and cell division that occurs in growing tissues. Compartment boundaries are often not obviously demarcated in a tissue, but do deviate from the normal hexagonal packing of epithelia by forming a straight, smooth interface that minimizes contact area between different cell types (**Figure 4A and B**). Because of the precise apposition of different cell types, compartment boundaries often serve as sources for morphogens that provide in-plane positional information to pattern the surrounding epithelial tissue [Dahmann et al. 2011]. In addition, smooth interfaces similar to compartment boundaries are found to encircle organ primordia. The encircled primordia can undergo out-of-plane bending to form tubular structures, suggesting a possible mechanical role for interfaces between different cell types during morphogenesis [Laplante and Nilson 2006; Nishimura et al. 2007; Osterfield et al. 2013; Roper 2012]. Cell sorting and boundary formation has most commonly been attributed to differential adhesion between different cell types (for review of models for boundaries see [Fagotto 2014]). However, investigation of multiple types of boundaries in *Drosophila* has identified actomyosin cables that are assembled at the interface between tissue types (**Figure 4A**) [Kiehart et al. 2000; Jacinto et al. 2002; Major and Irvine 2006; Laplante and Nilson 2006; Nishimura et al. 2007]. Thus, in addition to differential adhesion, the juxtaposition of different tissue types can induce the assembly of a specialized cytoskeletal structure that may play important roles at the boundary between the two cell types.

The range of approaches available to *Drosophila* biologists has been invaluable to identify actomyosin cables at boundaries, dissect their function, and identify possible mechanisms that trigger their assembly. In this case, biophysical approaches have been applied in combination with *Drosophila* genetics, tissue specific gene expression, and live cell imaging coupled with quantitative image analysis. This combination has led to physical models for actomyosin cable function at boundaries, illustrating the versatility of *Drosophila* as a cell biological and biophysical system. Similar to myoblast fusion, *Drosophila* compartment boundaries provide a physiological system to understand how cross talk between cell-cell adhesion and the cytoskeleton is used to polarize cytoskeletal activity within a cell. In addition, boundary-specific actomyosin cables provide a unique system to investigate how cytoskeletal assembly and dynamics are coordinated between cells to form a supracellular cytoskeletal structure.

For many years cell sorting and boundary formation were explained by the differential adhesion hypothesis (DAH), which posits that cells express different levels of adhesion molecules that result in different cell affinities and lead to cell sorting, similar to separation of immiscible liquids [Steinberg 1963]. However, in most cases, *Drosophila* genetics failed to identify clear differences in levels of adhesion molecules that function in boundary formation *in vivo*. For this developmental problem, cell biological and biophysical approaches provided a key insight. Actin and non-muscle myosin II (MyoII) were found enriched at compartment boundaries at different developmental stages (**Figure 4A**) and inhibition of MyoII activity resulted in the mixing of cells across the boundary (**Figure 4C**) [Major and Irvine 2005; Major and Irvine 2006; Landsberg et al. 2009; Monier et al. 2010].

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Furthermore, laser-cutting experiments revealed that the actomyosin cable had a clear effect on the mechanical properties of the boundary. This method employs a UV laser to ablate cytoskeletal structures, and the resulting recoil velocity of the surrounding tissue is proportional to tension in the cable immediately prior to cutting [Hutson et al. 2003]. These experiments revealed that the tension of interfaces at compartment boundaries is higher than interfaces within either compartment, suggesting that MyoII contractility increases interfacial tension specifically along the boundary interface [Landsberg et al. 2009; Aliee et al. 2012]. These studies provided some of the first experimental evidence that cortical tension is a key mechanism of cell sorting and boundary formation, beyond differential adhesion. Importantly, myosin contractility has also been implicated in cell sorting and boundary formation in *Xenopus* and zebrafish, suggesting that cortical contractility is a conserved mechanism for maintaining a compartment boundary [Krieg et al. 2008; Rohani et al. 2011; Fagotto et al. 2013].

How could increased tension on interfaces shared by two different cell types prevent mixing between compartments? One model proposes that the cable serves as a barrier that prevents cells from pushing into and invading the opposing compartment during cell division (**Figure 4C - Model 1**) [Monier et al. 2010]. To dissect the specific role for MyoII at the compartment boundary, chromophore-assisted laser inactivation (CALI) was used to inhibit MyoII activity specifically and acutely at the boundary interface [Monier et al. 2010]. Inhibition of MyoII at parasegment boundaries in the *Drosophila* embryo allowed cells from one compartment to mix with the opposite cell type, in a manner that depended on cell division. This result suggested that a polarized increase in tension at the compartment boundary functions to restrict cell mixing that normally results from cell division (**Figure 4C – Model 1**). An alternative model is that cell mixing occurs in the absence of cell division by cell rearrangements (e.g. cells intercalate and change neighbors). This model emerged from studies of cell dynamics at compartment boundaries of the abdominal epidermis. In this different tissue, the authors combined live imaging with automated image analysis and modeling to determine that cell mixing was associated with neighbor exchanges between cells (**Figure 4C – Model 2**). Increased tension along the compartment boundary biased the directionality of cellular rearrangements such that a straight interface was preserved without cell mixing [Umetsu et al. 2014a]. One or both of these mechanisms could operate in different tissues to maintain boundaries.

What is the instructive signal that triggers polarized actomyosin cable assembly along boundary interfaces? While differential cell affinity has not been shown to directly contribute to cell sorting at *Drosophila* compartment boundaries, as formulated in the DAH, differential expression of cell adhesion molecules (CAMs) has been shown to regulate actomyosin cable formation during tissue morphogenesis. Expression of a protein that mediates homophilic adhesion has been shown to result in the formation of an actomyosin cable at the boundary between cells expressing the homophilic adhesion molecule and those not expressing the molecule (**Figure 4D**). This situation has been demonstrated for two different CAMs shown to exhibit homophilic adhesion, the immunoglobulin (Ig) domain-containing CAM, Echinoid (Ed, nectin homologue) [Wei et al. 2005; Laplante and Nilson 2006], and the apical domain protein Crumbs (Crbs) [Roper 2012]. Because of the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

homophilic binding properties of Ed and Crbs, there is an intrinsic polarity to Ed+ or Crbs+ cells at a boundary with cells not expressing the CAM (**Figure 4D**). Ed is depleted at interfaces between Ed+ and Ed− cells, and ectopic Ed expression in tissue that is normally Ed− cells results in uniform Ed localization and the loss of actomyosin cable formation [Laplante and Nilson 2011]. The intracellular domains of homophilic CAMs such as Ed and Crumbs could recruit negative regulators of MyoII to non-boundary interfaces, resulting in polarized MyoII assembly at the boundary between cell types. In contrast to differential expression of a homophilic adhesion molecule, a recent study of the early *Drosophila* embryo demonstrated that interfaces between cell types expressing different Toll family receptors assemble actomyosin cables, presumably through heterophilic interactions that activate MyoII contractility (**Figure 4D**) [Pare et al. 2014]. Thus, differential expression of either homophilic or heterophilic cell surface receptors could instruct the polarized assembly of an actomyosin cable at interfaces between different cell types. Further supporting such a model, heterophilic interactions between membrane-linked ephrin receptors and Eph receptor tyrosine kinase promote boundary formation between anterior and posterior domains of the *Drosophila* imaginal disc and between segments of the vertebrate hindbrain [Umetsu et al. 2014b; Cooke and Moens 2002]. Notably, boundary formation in the hindbrain was recently shown to depend on supracellular actomyosin cable formation as well, in a study likely inspired by findings in *Drosophila* [Calzolari et al. 2014].

A striking feature of actomyosin cables at boundaries is that the cables connect between cells to form a continuous supracellular structure. Thus, while CAMs could provide an initial cue for myosin cable assembly, it is possible that other mechanisms operate to reinforce this cue and coordinate myosin assembly between cells. A recent *Drosophila* study of supracellular myosin cable formation during cell-cell intercalation suggests that tension can modulate myosin dynamics and coordinate myosin assembly between cells. During germband extension, a process that drives the elongation of the embryonic tissue, multiple cell interfaces align to form supracellular myosin cables. The supracellular cables connecting the interfaces between 5 or more cells contract to form a pizza-pie-like arrangement of cells (called a rosette) that contact each other at a central point, where cells then establish new contacts to extend the tissue [Blankenship et al. 2006]. A combination of live imaging and biophysical perturbations has provided compelling evidence that cable tension coordinates myosin recruitment: Laser ablation experiments revealed that mechanical tension is higher in supracellular cables than in isolated myosin-containing interfaces [Fernandez-Gonzalez et al. 2009]. Further, FRAP studies using myosin::GFP showed that myosin in supracellular cables is less mobile than in isolated interfaces, suggesting that tension modulates myosin dynamics. Consistent with this, laser ablation causes a loss of myosin intensity, and microaspiration of the tissue increases myosin intensity. The mechanical sensitivity of myosin dynamics could result directly from the mechanical properties of the myosin motor itself. For instance, MyoII exhibits a stress dependent cortical stabilization in regions of cell stress [Ren et al. 2009]. Future experiments in which the myosin motor is molecularly perturbed will be needed to dissect the mechanism of this feedback. Alternatively, other processes could contribute to polarized myosin assembly. A recent structure-function analysis of the myosin activator Rho-associated and coiled-coil kinase (ROCK) identified the Shroom-binding domain of ROCK as being critical

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

for ROCK polarity in cells [Simoes et al. 2014]. Shroom is an actin-binding protein that is essential for neural tube closure and appears to function as a key regulator of ROCK in combination with RhoA [Nishimura and Takeichi 2008]. Shroom localization requires binding to F-actin, suggesting that actomyosin assembly could reinforce a polarity cue by recruiting Shroom and thus ROCK.

In contrast to the previous examples, the finding that mechanical tension serves as a physical barrier to cell mixing emerged not from genetic screens, but rather from the novel observation of actomyosin cables in a range of developing tissues. A combination of incisive genetic manipulations, mechanical perturbations, and quantitative live imaging demonstrate that in a broad range of contexts, boundary formation between tissues depends upon increased interfacial tension mediated by a supracellular actomyosin cable. The range of experimentally accessible developmental processes in *Drosophila* provides attractive model systems to continue to investigate the mechanisms that coordinate adhesion, cytoskeletal assembly, and biomechanical feedback during tissue development. As in other systems, emerging findings that the physical mechanisms of boundary formation are similar in vertebrate development [Fagotto et al. 2013; Calzolari et al. 2014] suggest that discoveries in the fly will continue to shed light on mechanisms of development and physiology in more complex organisms.

Conclusion

The three case studies described in this review illustrate the power of the *Drosophila* system for cytoskeletal discovery. While *in vitro* and cellular studies have elucidated many important mechanisms for cytoskeletal control of cell shape, motility, and subcellular compartmentalization, these phenomena only describe a small fraction of the roles of the cytoskeleton in unique and diverse tissues in an animal. Recent advances in microscopy and genetic manipulation have launched *Drosophila* into position to uncover the many roles of the cytoskeleton in assembling the diverse cellular and multicellular structures that have enabled the explosion of eukaryotic life.

Acknowledgements

We thank E. Chen, J. Coravos, J. Eskin, B. Goode, and M. Quinlan for advice and input on this manuscript. AAR is supported by NIH/NINDS (DP2 NS082127), and a Pew Scholar Award (A.A.R.). ACM is supported by NIH/NIGMS (R01-GM084947) and a Research Scholar Award from the American Cancer Society.

REFERENCES

Abmayr SM, Pavlath GK. Myoblast fusion: lessons from flies and mice. Development. 2012; 139(4): 641–56. [PubMed: 22274696]

Aigouy B, Farhadifar R, Staple DB, Sagner A, Roper JC, Julicher F, Eaton S. Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. Cell. 2010; 142(5):773–86. [PubMed: 20813263]

Aliee M, Roper JC, Landsberg KP, Pentzold C, Widmann TJ, Julicher F, Dahmann C. Physical mechanisms shaping the *Drosophila* dorsoventral compartment boundary. Curr Biol. 2012; 22(11): 967–76. [PubMed: 22560616]

Anton IM, Jones GE, Wandosell F, Geha R, Ramesh N. WASP-interacting protein (WIP): working in polymerisation and much more. Trends Cell Biol. 2007; 17(11):555–62. [PubMed: 17949983]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Berger S, Schafer G, Kesper DA, Holz A, Eriksson T, Palmer RH, Beck L, Klambt C, Renkawitz-Pohl R, Onel SF. WASP and SCAR have distinct roles in activating the Arp2/3 complex during myoblast fusion. J Cell Sci. 2008; 121(Pt 8):1303–13. [PubMed: 18388318]

Bertet C, Sulak L, Lecuit T. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. Nature. 2004; 429(6992):667–71. [PubMed: 15190355]

Blankenship JT, Backovic ST, Sanny JS, Weitz O, Zallen JA. Multicellular rosette formation links planar cell polarity to tissue morphogenesis. Dev Cell. 2006; 11(4):459–70. [PubMed: 17011486]

Bor B, Bois JS, Quinlan ME. Regulation of the formin Cappuccino is critical for polarity of *Drosophila* oocytes. Cytoskeleton. 2014; 72(1):1–15. [PubMed: 25557988]

Bor B, Vizcarra CL, Phillips ML, Quinlan ME. Autoinhibition of the formin Cappuccino in the absence of canonical autoinhibitory domains. Mol Biol Cell. 2012; 23(19):3801–13. [PubMed: 22875983]

Bosch M, Le KH, Bugyi B, Correia JJ, Renault L, Carlier MF. Analysis of the function of Spire in actin assembly and its synergy with formin and profilin. Mol Cell. 2007; 28(4):555–68. [PubMed: 18042452]

Bosveld F, Bonnet I, Guirao B, Tlili S, Wang Z, Petitalot A, Marchand R, Bardet PL, Marcq P, Graner F. Mechanical control of morphogenesis by Fat/Dachsous/Four-jointed planar cell polarity pathway. Science. 2012; 336(6082):724–7. others. [PubMed: 22499807]

Bothe I, Deng S, Baylies M. PI(4,5)P2 regulates myoblast fusion through Arp2/3 regulator localization at the fusion site. Development. 2014; 141(11):2289–301. [PubMed: 24821989]

Brand AH, Perrimon N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 1993; 118(2):401–15. [PubMed: 8223268]

Cai D, Chen SC, Prasad M, He L, Wang X, Choesmel-Cadamuro V, Sawyer JK, Danuser G, Montell DJ. Mechanical feedback through E-cadherin promotes direction sensing during collective cell migration. Cell. 2014; 157(5):1146–59. [PubMed: 24855950]

Calzolari S, Terriente J, Pujades C. Cell segregation in the vertebrate hindbrain relies on actomyosin cables located at the interhombomeric boundaries. EMBO J. 2014; 33(7):686–701. [PubMed: 24569501]

Caussinus E, Kanca O, Affolter M. Fluorescent fusion protein knockout mediated by anti-GFP nanobody. Nat Struct Mol Biol. 2012; 19(1):117–21. [PubMed: 22157958]

Ctavey M, Rauzi M, Lenne PF, Lecuit T. A two-tiered mechanism for stabilization and immobilization of E-cadherin. Nature. 2008; 453(7196):751–6. [PubMed: 18480755]

Chen BC, Legant WR, Wang K, Shao L, Milkie DE, Davidson MW, Janetopoulos C, Wu XS, Hammer JA 3rd, Liu Z. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. Science. 2014; 346(6208):1257998. others. [PubMed: 25342811]

Chou TB, Noll E, Perrimon N. Autosomal P[ovoD1] dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. Development. 1993; 119(4):1359–69. [PubMed: 8306893]

Co C, Wong DT, Gierke S, Chang V, Taunton J. Mechanism of actin network attachment to moving membranes: barbed end capture by N-WASP WH2 domains. Cell. 2007; 128(5):901–13. [PubMed: 17350575]

Cooke JE, Moens CB. Boundary formation in the hindbrain: Eph only it were simple. Trends Neurosci. 2002; 25(5):260–7. [PubMed: 11972963]

Dahlgaard K, Raposo AA, Niccoli T, St Johnston D. Capu and Spire assemble a cytoplasmic actin mesh that maintains microtubule organization in the *Drosophila* oocyte. Dev Cell. 2007; 13(4): 539–53. [PubMed: 17925229]

Dahmann C, Oates AC, Brand M. Boundary formation and maintenance in tissue development. Nat Rev Genet. 2011; 12(1):43–55. [PubMed: 21164524]

DeRosier DJ, Tilney LG. F-actin bundles are derivatives of microvilli: What does this tell us about how bundles might form? J Cell Biol. 2000; 148(1):1–6. [PubMed: 10629213]

Desai R, Sarpal R, Ishiyama N, Pellikka M, Ikura M, Tepass U. Monomeric alpha-catenin links cadherin to the actin cytoskeleton. Nat Cell Biol. 2013; 15(3):261–73. [PubMed: 23417122]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. Nature. 2007; 448(7150):151–6. others. [PubMed: 17625558]

Doberstein SK, Fetter RD, Mehta AY, Goodman CS. Genetic analysis of myoblast fusion: blown fuse is required for progression beyond the prefusion complex. J Cell Biol. 1997; 136(6):1249–61. [PubMed: 9087441]

Ehmann N, van de Linde S, Alon A, Ljaschenko D, Keung XZ, Holm T, Rings A, DiAntonio A, Hallermann S, Ashery U. Quantitative super-resolution imaging of Bruchpilot distinguishes active zone states. Nat Commun. 2014; 5:4650. others. [PubMed: 25130366]

Ephrussi A, Dickinson LK, Lehmann R. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. Cell. 1991; 66(1):37–50. [PubMed: 2070417]

Fabian L, Brill JA. Drosophila spermiogenesis: Big things come from little packages. Spermatogenesis. 2012; 2(3):197–212. [PubMed: 23087837]

Fabrowski P, Necakov AS, Mumbauer S, Loeser E, Reversi A, Streichan S, Briggs JA, De Renzis S. Tubular endocytosis drives remodelling of the apical surface during epithelial morphogenesis in *Drosophila*. Nat Commun. 2013; 4:2244. [PubMed: 23921440]

Fagotto F. The cellular basis of tissue separation. Development. 2014; 141(17):3303–18. [PubMed: 25139853]

Fagotto F, Rohani N, Touret AS, Li R. A molecular base for cell sorting at embryonic boundaries: contact inhibition of cadherin adhesion by ephrin/ Eph-dependent contractility. Dev Cell. 2013; 27(1):72–87. [PubMed: 24094740]

Fernandez-Gonzalez R, Simoes S, Roper JC, Eaton S, Zallen JA. Myosin II dynamics are regulated by tension in intercalating cells. Dev Cell. 2009; 17(5):736–43. [PubMed: 19879198]

Forrest KM, Gavis ER. Live imaging of endogenous RNA reveals a diffusion and entrapment mechanism for nanos mRNA localization in *Drosophila*. Curr Biol. 2003; 13(14):1159–68. [PubMed: 12867026]

Founounou N, Loyer N, Le Borgne R. Septins regulate the contractility of the actomyosin ring to enable adherens junction remodeling during cytokinesis of epithelial cells. Dev Cell. 2013; 24(3): 242–55. [PubMed: 23410939]

Gavis ER, Lehmann R. Localization of nanos RNA controls embryonic polarity. Cell. 1992; 71(2): 301–13. [PubMed: 1423595]

Gildor B, Massarwa R, Shilo BZ, Schejter ED. The SCAR and WASp nucleation-promoting factors act sequentially to mediate *Drosophila* myoblast fusion. EMBO Rep. 2009; 10(9):1043–50. [PubMed: 19644501]

Goode BL, Eck MJ. Mechanism and function of formins in the control of actin assembly. Annu Rev Biochem. 2007; 76:593–627. [PubMed: 17373907]

Guillot C, Lecuit T. Adhesion disengagement uncouples intrinsic and extrinsic forces to drive cytokinesis in epithelial tissues. Dev Cell. 2013; 24(3):227–41. [PubMed: 23410938]

Haglund K, Nezis IP, Stenmark H. Structure and functions of stable intercellular bridges formed by incomplete cytokinesis during development. Commun Integr Biol. 2011; 4(1):1–9. [PubMed: 21509167]

Haigo SL, Bilder D. Global tissue revolutions in a morphogenetic movement controlling elongation. Science. 2011; 331(6020):1071–4. [PubMed: 21212324]

Haralalka S, Shelton C, Cartwright HN, Guo F, Trimble R, Kumar RP, Abmayr SM. Live Imaging Provides New Insights on Dynamic F-Actin Filopodia and Differential Endocytosis during Myoblast Fusion in *Drosophila*. PLoS One. 2014; 9(12):e114126. [PubMed: 25474591]

Haralalka S, Shelton C, Cartwright HN, Katzfey E, Janzen E, Abmayr SM. Asymmetric Mbc, active Rac1 and F-actin foci in the fusion-competent myoblasts during myoblast fusion in *Drosophila*. Development. 2011; 138(8):1551–62. [PubMed: 21389053]

He L, Wang X, Tang HL, Montell DJ. Tissue elongation requires oscillating contractions of a basal actomyosin network. Nat Cell Biol. 2010; 12(12):1133–42. [PubMed: 21102441]

Herszterg S, Leibfried A, Bosveld F, Martin C, Bellaiche Y. Interplay between the dividing cell and its neighbors regulates adherens junction formation during cytokinesis in epithelial tissue. Dev Cell. 2013; 24(3):256–70. [PubMed: 23410940]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Hudson AM, Cooley L. Understanding the function of actin-binding proteins through genetic analysis of *Drosophila* oogenesis. Annu Rev Genet. 2002; 36:455–88. [PubMed: 12429700]

Hutson MS, Tokutake Y, Chang MS, Bloor JW, Venakides S, Kiehart DP, Edwards GS. Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. Science. 2003; 300(5616):145–9. [PubMed: 12574496]

Jacinto A, Wood W, Woolner S, Hiley C, Turner L, Wilson C, Martinez-Arias A, Martin P. Dynamic analysis of actin cable function during *Drosophila* dorsal closure. Curr Biol. 2002; 12(14):1245–50. [PubMed: 12176336]

Jin P, Duan R, Luo F, Zhang G, Hong SN, Chen EH. Competition between Blown fuse and WASP for WIP binding regulates the dynamics of WASP-dependent actin polymerization in vivo. Dev Cell. 2011; 20(5):623–38. [PubMed: 21571220]

Kang H, Wang J, Longley SJ, Tang JX, Shaw SK. Relative actin nucleation promotion efficiency by WASP and WAVE proteins in endothelial cells. Biochem Biophys Res Commun. 2010; 400(4): 661–6. [PubMed: 20816932]

Keller PJ, Schmidt AD, Santella A, Khairy K, Bao Z, Wittbrodt J, Stelzer EH. Fast, high-contrast imaging of animal development with scanned light sheet-based structured-illumination microscopy. Nat Methods. 2010; 7(8):637–42. [PubMed: 20601950]

Kesper DA, Stute C, Buttgereit D, Kreiskother N, Vishnu S, Fischbach KF, Renkawitz-Pohl R. Myoblast fusion in *Drosophila* melanogaster is mediated through a fusion-restricted myogenic-adhesive structure (FuRMAS). Dev Dyn. 2007; 236(2):404–15. [PubMed: 17146786]

Khanduja N, Kuhn JR. Processive acceleration of actin barbed-end assembly by N-WASP. Mol Biol Cell. 2014; 25(1):55–65. [PubMed: 24227886]

Kiehart DP, Galbraith CG, Edwards KA, Rickoll WL, Montague RA. Multiple forces contribute to cell sheet morphogenesis for dorsal closure in *Drosophila*. J Cell Biol. 2000; 149(2):471–90. [PubMed: 10769037]

Kiger AA, Baum B, Jones S, Jones MR, Coulson A, Echeverri C, Perrimon N. A functional genomic analysis of cell morphology using RNA interference. J Biol. 2003; 2(4):27. [PubMed: 14527345]

Kim JH, Cho A, Yin H, Schafer DA, Mouneimne G, Simpson KJ, Nguyen KV, Brugge JS, Montell DJ. Psidin, a conserved protein that regulates protrusion dynamics and cell migration. Genes Dev. 2011; 25(7):730–41. [PubMed: 21406550]

Kim JH, Jin P, Duan R, Chen EH. Mechanisms of myoblast fusion during muscle development. Curr Opin Genet Dev. 2015a; 32:162–170. [PubMed: 25989064]

Kim JH, Ren Y, Ng WP, Li S, Son S, Kee YS, Zhang S, Zhang G, Fletcher DA, Robinson DN. Mechanical tension drives cell membrane fusion. Dev Cell. 2015b; 32(5):561–73. others. [PubMed: 25684354]

Kim S, Shilagardi K, Zhang S, Hong SN, Sens KL, Bo J, Gonzalez GA, Chen EH. A critical function for the actin cytoskeleton in targeted exocytosis of prefusion vesicles during myoblast fusion. Dev Cell. 2007; 12(4):571–86. [PubMed: 17419995]

Kittel RJ, Wichmann C, Rasse TM, Fouquet W, Schmidt M, Schmid A, Wagh DA, Pawlu C, Kellner RR, Willig KI. Bruchpilot promotes active zone assembly, Ca2+ channel clustering, and vesicle release. Science. 2006; 312(5776):1051–4. others. [PubMed: 16614170]

Kremers GJ, Gilbert SG, Cranfill PJ, Davidson MW, Piston DW. Fluorescent proteins at a glance. J Cell Sci. 2011; 124(Pt 2):157–60. [PubMed: 21187342]

Krieg M, Arboleda-Estudillo Y, Puech PH, Kafer J, Graner F, Muller DJ, Heisenberg CP. Tensile forces govern germ-layer organization in zebrafish. Nat Cell Biol. 2008; 10(4):429–36. [PubMed: 18364700]

Landsberg KP, Farhadifar R, Ranft J, Umetsu D, Widmann TJ, Bittig T, Said A, Julicher F, Dahmann C. Increased cell bond tension governs cell sorting at the *Drosophila* anteroposterior compartment boundary. Curr Biol. 2009; 19(22):1950–5. [PubMed: 19879142]

Laplante C, Nilson LA. Differential expression of the adhesion molecule Echinoid drives epithelial morphogenesis in *Drosophila*. Development. 2006; 133(16):3255–64. [PubMed: 16854971]

Laplante C, Nilson LA. Asymmetric distribution of Echinoid defines the epidermal leading edge during *Drosophila* dorsal closure. J Cell Biol. 2011; 192(2):335–48. [PubMed: 21263031]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Lee T, Luo L. Mosaic analysis with a repressible cell marker (MARCM) for *Drosophila* neural development. Trends Neurosci. 2001; 24(5):251–4. [PubMed: 11311363]

Linder S, Wiesner C, Himmel M. Degrading devices: invadosomes in proteolytic cell invasion. Annu Rev Cell Dev Biol. 2011; 27:185–211. [PubMed: 21801014]

Liu J, Li C, Yu Z, Huang P, Wu H, Wei C, Zhu N, Shen Y, Chen Y, Zhang B. Efficient and specific modifications of the *Drosophila* genome by means of an easy TALEN strategy. J Genet Genomics. 2012; 39(5):209–15. others. [PubMed: 22624882]

Liu R, Abreu-Blanco MT, Barry KC, Linardopoulou EV, Osborn GE, Parkhurst SM. Wash functions downstream of Rho and links linear and branched actin nucleation factors. Development. 2009; 136(16):2849–60. [PubMed: 19633175]

Luxton GW, Gomes ER, Folker ES, Vintinner E, Gundersen GG. Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. Science. 2010; 329(5994):956–9. [PubMed: 20724637]

Lye CM, Sanson B. Tension and epithelial morphogenesis in *Drosophila* early embryos. Curr Top Dev Biol. 2011; 95:145–87. [PubMed: 21501751]

Major RJ, Irvine KD. Influence of Notch on dorsoventral compartmentalization and actin organization in the *Drosophila* wing. Development. 2005; 132(17):3823–33. [PubMed: 16049109]

Major RJ, Irvine KD. Localization and requirement for Myosin II at the dorsal ventral compartment boundary of the *Drosophila* wing. Dev Dyn. 2006; 235(11):3051–8. [PubMed: 17013876]

Manseau LJ, Schupbach T. *cappuccino* and *spire*: two unique maternal-effect loci required for both the anteroposterior and dorsoventral patterns of the *Drosophila* embryo. Genes Dev. 1989; 3(9):1437–52. [PubMed: 2514120]

Marek KW, Davis GW. Transgenically encoded protein photoinactivation (FlAsH-FALI): acute inactivation of synaptotagmin I. Neuron. 2002; 36(5):805–13. [PubMed: 12467585]

Martin AC, Kaschube M, Wieschaus EF. Pulsed contractions of an actin-myosin network drive apical constriction. Nature. 2009; 457(7228):495–9. [PubMed: 19029882]

Massarwa R, Carmon S, Shilo BZ, Schejter ED. WIP/WASp-based actin polymerization machinery is essential for myoblast fusion in *Drosophila*. Dev Cell. 2007; 12(4):557–69. [PubMed: 17419994]

Mavrakis M, Rikhy R, Lippincott-Schwartz J. Plasma membrane polarity and compartmentalization are established before cellularization in the fly embryo. Dev Cell. 2009; 16(1):93–104. [PubMed: 19154721]

McGuire SE, Le PT, Osborn AJ, Matsumoto K, Davis RL. Spatiotemporal rescue of memory dysfunction in *Drosophila*. Science. 2003; 302(5651):1765–8. [PubMed: 14657498]

Mohr SE, Perrimon N. RNAi screening: new approaches, understandings, and organisms. Wiley Interdiscip Rev RNA. 2012; 3(2):145–58. [PubMed: 21953743]

Monier B, Pelissier-Monier A, Brand AH, Sanson B. An actomyosin-based barrier inhibits cell mixing at compartmental boundaries in *Drosophila* embryos. Nat Cell Biol. 2010; 12(1):60–5. [PubMed: 19966783]

Montaville P, Jegou A, Pernier J, Compper C, Guichard B, Mogessie B, Schuh M, Romet-Lemonne G, Carlier MF. Spire and Formin 2 synergize and antagonize in regulating actin assembly in meiosis by a ping-pong mechanism. PLoS Biol. 2014; 12(2):e1001795. [PubMed: 24586110]

Montell DJ, Yoon WH, Starz-Gaiano M. Group choreography: mechanisms orchestrating the collective movement of border cells. Nat Rev Mol Cell Biol. 2012; 13(10):631–45. [PubMed: 23000794]

Mukherjee P, Gildor B, Shilo BZ, VijayRaghavan K, Schejter ED. The actin nucleator WASp is required for myoblast fusion during adult *Drosophila* myogenesis. Development. 2011; 138(11): 2347–57. [PubMed: 21558381]

Ng J, Nardine T, Harms M, Tzu J, Goldstein A, Sun Y, Dietzl G, Dickson BJ, Luo L. Rac GTPases control axon growth, guidance and branching. Nature. 2002; 416(6879):442–7. [PubMed: 11919635]

Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou D, Shim HS, Tao R, Handler D, Karpowicz P. A genome-scale shRNA resource for transgenic RNAi in *Drosophila*. Nat Methods. 2011; 8(5):405–7. others. [PubMed: 21460824]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Nishimura M, Inoue Y, Hayashi S. A wave of EGFR signaling determines cell alignment and intercalation in the *Drosophila* tracheal placode. Development. 2007; 134(23):4273–82. [PubMed: 17978004]

Nishimura T, Takeichi M. Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. Development. 2008; 135(8):1493–502. [PubMed: 18339671]

Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. Nature. 1980; 287(5785):795–801. [PubMed: 6776413]

Osterfield M, Du X, Schupbach T, Wieschaus E, Shvartsman SY. Three-dimensional epithelial morphogenesis in the developing *Drosophila* egg. Dev Cell. 2013; 24(4):400–10. [PubMed: 23449472]

Pare AC, Vichas A, Fincher CT, Mirman Z, Farrell DL, Mainieri A, Zallen JA. A positional Toll receptor code directs convergent extension in *Drosophila*. Nature. 2014; 515(7528):523–7. [PubMed: 25363762]

Parton RM, Hamilton RS, Ball G, Yang L, Cullen CF, Lu W, Ohkura H, Davis I. A PAR-1-dependent orientation gradient of dynamic microtubules directs posterior cargo transport in the *Drosophila* oocyte. J Cell Biol. 2011; 194(1):121–35. [PubMed: 21746854]

Pfender S, Kuznetsov V, Pleiser S, Kerkhoff E, Schuh M. Spire-type actin nucleators cooperate with Formin-2 to drive asymmetric oocyte division. Curr Biol. 2011; 21(11):955–60. [PubMed: 21620703]

Port F, Chen HM, Lee T, Bullock SL. Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*. Proc Natl Acad Sci U S A. 2014; 111(29):E2967–76. [PubMed: 25002478]

Quinlan ME. Direct interaction between two actin nucleators is required in *Drosophila* oogenesis. Development. 2013; 140(21):4417–25. [PubMed: 24089467]

Quinlan ME, Hilgert S, Bedrossian A, Mullins RD, Kerkhoff E. Regulatory interactions between two actin nucleators, Spire and Cappuccino. J Cell Biol. 2007; 179(1):117–28. [PubMed: 17923532]

Ramel D, Wang X, Laflamme C, Montell DJ, Emery G. Rab11 regulates cell-cell communication during collective cell movements. Nat Cell Biol. 2013; 15(3):317–24. [PubMed: 23376974]

Rasson AS, Bois JS, Pham DS, Yoo H, Quinlan ME. Filament assembly by Spire: key residues and concerted actin binding. J Mol Biol. 2015; 427(4):824–39. [PubMed: 25234086]

Rauzi M, Lenne PF, Lecuit T. Planar polarized actomyosin contractile flows control epithelial junction remodelling. Nature. 2010; 468(7327):1110–4. [PubMed: 21068726]

Rebollo E, Karkali K, Mangione F, Martin-Blanco E. Live imaging in *Drosophila*: The optical and genetic toolkits. Methods. 2014; 68(1):48–59. [PubMed: 24814031]

Ren X, Sun J, Housden BE, Hu Y, Roesel C, Lin S, Liu LP, Yang Z, Mao D, Sun L. Optimized gene editing technology for *Drosophila* melanogaster using germ line-specific Cas9. Proc Natl Acad Sci U S A. 2013; 110(47):19012–7. others. [PubMed: 24191015]

Ren Y, Effler JC, Norstrom M, Luo T, Firtel RA, Iglesias PA, Rock RS, Robinson DN. Mechanosensing through cooperative interactions between myosin II and the actin crosslinker cortexillin I. Curr Biol. 2009; 19(17):1421–8. [PubMed: 19646871]

Richardson BE, Beckett K, Nowak SJ, Baylies MK. SCAR/WAVE and Arp2/3 are crucial for cytoskeletal remodeling at the site of myoblast fusion. Development. 2007; 134(24):4357–67. [PubMed: 18003739]

Riechmann V, Ephrussi A. Axis formation during *Drosophila* oogenesis. Curr Opin Genet Dev. 2001; 11(4):374–83. [PubMed: 11448623]

Rogers SL, Wiedemann U, Stuurman N, Vale RD. Molecular requirements for actin-based lamella formation in *Drosophila* S2 cells. J Cell Biol. 2003; 162(6):1079–88. [PubMed: 12975351]

Rohani N, Canty L, Luu O, Fagotto F, Winklbauer R. EphrinB/EphB signaling controls embryonic germ layer separation by contact-induced cell detachment. PLoS Biol. 2011; 9(3):e1000597. [PubMed: 21390298]

Rohn JL, Sims D, Liu T, Fedorova M, Schock F, Dopie J, Vartiainen MK, Kiger AA, Perrimon N, Baum B. Comparative RNAi screening identifies a conserved core metazoan actinome by phenotype. J Cell Biol. 2011; 194(5):789–805. [PubMed: 21893601]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Roper K. Anisotropy of Crumbs and aPKC drives myosin cable assembly during tube formation. Dev Cell. 2012; 23(5):939–53. [PubMed: 23153493]

Roper K. Supracellular actomyosin assemblies during development. Bioarchitecture. 2013; 3(2):45–9. [PubMed: 23760352]

Rosales-Nieves AE, Johndrow JE, Keller LC, Magie CR, Pinto-Santini DM, Parkhurst SM. Coordination of microtubule and microfilament dynamics by *Drosophila* Rho1, Spire and Cappuccino. Nat Cell Biol. 2006; 8(4):367–76. [PubMed: 16518391]

Rotty JD, Wu C, Bear JE. New insights into the regulation and cellular functions of the ARP2/3 complex. Nat Rev Mol Cell Biol. 2013; 14(1):7–12. [PubMed: 23212475]

Schachtner H, Calaminus SD, Thomas SG, Machesky LM. Podosomes in adhesion, migration, mechanosensing and matrix remodeling. Cytoskeleton (Hoboken). 2013; 70(10):572–89. [PubMed: 23804547]

Schafer G, Weber S, Holz A, Bogdan S, Schumacher S, Muller A, Renkawitz-Pohl R, Onel SF. The Wiskott-Aldrich syndrome protein (WASP) is essential for myoblast fusion in *Drosophila*. Dev Biol. 2007; 304(2):664–74. [PubMed: 17306790]

Schmid A, Hallermann S, Kittel RJ, Khorramshahi O, Frolich AM, Quentin C, Rasse TM, Mertel S, Heckmann M, Sigrist SJ. Activity-dependent site-specific changes of glutamate receptor composition in vivo. Nat Neurosci. 2008; 11(6):659–66. [PubMed: 18469810]

Schroter RH, Lier S, Holz A, Bogdan S, Klambt C, Beck L, Renkawitz-Pohl R. kette and blown fuse interact genetically during the second fusion step of myogenesis in *Drosophila*. Development. 2004; 131(18):4501–9. [PubMed: 15342475]

Schupbach T, Wieschaus E. Female sterile mutations on the second chromosome of *Drosophila* melanogaster. I. Maternal effect mutations. Genetics. 1989; 121(1):101–17. [PubMed: 2492966]

Sens KL, Zhang S, Jin P, Duan R, Zhang G, Luo F, Parachini L, Chen EH. An invasive podosome-like structure promotes fusion pore formation during myoblast fusion. J Cell Biol. 2010; 191(5): 1013–27. [PubMed: 21098115]

Shilagardi K, Li S, Luo F, Marikar F, Duan R, Jin P, Kim JH, Murnen K, Chen EH. Actin-Propelled Invasive Membrane Protrusions Promote Fusogenic Protein Engagement During Cell-Cell Fusion. Science. 2013; 340(6130):359–63. [PubMed: 23470732]

Simoes S, Blankenship JT, Weitz O, Farrell DL, Tamada M, Fernandez-Gonzalez R, Zallen JA. Rho-kinase directs Bazooka/Par-3 planar polarity during *Drosophila* axis elongation. Dev Cell. 2010; 19(3):377–88. [PubMed: 20833361]

Simoes S, Mainieri A, Zallen JA. Rho GTPase and Shroom direct planar polarized actomyosin contractility during convergent extension. J Cell Biol. 2014; 204(4):575–89. [PubMed: 24535826]

Solon J, Kaya-Copur A, Colombelli J, Brunner D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. Cell. 2009; 137(7):1331–42. [PubMed: 19563762]

Sopko R, Foos M, Vinayagam A, Zhai B, Binari R, Hu Y, Randklev S, Perkins LA, Gygi SP, Perrimon N. Combining genetic perturbations and proteomics to examine kinase-phosphatase networks in *Drosophila* embryos. Dev Cell. 2014; 31(1):114–27. [PubMed: 25284370]

Steinberg MS. Reconstruction of tissues by dissociated cells. Some morphogenetic tissue movements and the sorting out of embryonic cells may have a common explanation. Science. 1963; 141(3579):401–8. [PubMed: 13983728]

Sweeney MO, Collins A, Padrick SB, Goode BL. A novel role for WAVE1 in controlling actin network growth rate and architecture. Mol Biol Cell. 2014; 26(3):495–505. [PubMed: 25473116]

Theurkauf WE. Premature microtubule-dependent cytoplasmic streaming in *cappuccino* and *spire* mutant oocytes. Science. 1994; 265(5181):2093–6. [PubMed: 8091233]

Theurkauf WE, Smiley S, Wong ML, Alberts BM. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. Development. 1992; 115(4):923–36. [PubMed: 1451668]

Tilney LG, DeRosier DJ. How to make a curved *Drosophila* bristle using straight actin bundles. Proc Natl Acad Sci U S A. 2005; 102(52):18785–92. [PubMed: 16357198]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Umetsu D, Aigouy B, Aliee M, Sui L, Eaton S, Julicher F, Dahmann C. Local increases in mechanical tension shape compartment boundaries by biasing cell intercalations. Curr Biol. 2014a; 24(15): 1798–805. [PubMed: 25065753]

Umetsu D, Dunst S, Dahmann C. An RNA interference screen for genes required to shape the anteroposterior compartment boundary in *Drosophila* identifies the Eph receptor. PLoS One. 2014b; 9(12):e114340. [PubMed: 25473846]

Venken KJ, Simpson JH, Bellen HJ. Genetic manipulation of genes and cells in the nervous system of the fruit fly. Neuron. 2011; 72(2):202–30. [PubMed: 22017985]

Vizcarra CL, Bor B, Quinlan ME. The role of formin tails in actin nucleation, processive elongation, and filament bundling. J Biol Chem. 2014; 289(44):30602–13. [PubMed: 25246531]

Vizcarra CL, Kreutz B, Rodal AA, Toms AV, Lu J, Zheng W, Quinlan ME, Eck MJ. Structure and function of the interacting domains of Spire and Fmn-family formins. Proc Natl Acad Sci U S A. 2011; 108(29):11884–9. [PubMed: 21730168]

Wang X, He L, Wu YI, Hahn KM, Montell DJ. Light-mediated activation reveals a key role for Rac in collective guidance of cell movement in vivo. Nat Cell Biol. 2010; 12(6):591–7. [PubMed: 20473296]

Wang Y, Riechmann V. Microtubule anchoring by cortical actin bundles prevents streaming of the oocyte cytoplasm. Mech Dev. 2008; 125(1-2):142–52. [PubMed: 18053693]

Wangler MF, Yamamoto S, Bellen HJ. Fruit flies in biomedical research. Genetics. 2015; 199(3):639–53. [PubMed: 25624315]

Wei SY, Escudero LM, Yu F, Chang LH, Chen LY, Ho YH, Lin CM, Chou CS, Chia W, Modolell J. Echinoid is a component of adherens junctions that cooperates with DE-Cadherin to mediate cell adhesion. Dev Cell. 2005; 8(4):493–504. others. [PubMed: 15809032]

Weil TT, Parton RM, Herpers B, Soetaert J, Veenendaal T, Xanthakis D, Dobbie IM, Halstead JM, Hayashi R, Rabouille C. *Drosophila* patterning is established by differential association of mRNAs with P bodies. Nat Cell Biol. 2012; 14(12):1305–13. others. [PubMed: 23178881]

Winter PW, Shroff H. Faster fluorescence microscopy: advances in high speed biological imaging. Curr Opin Chem Biol. 2014; 20:46–53. [PubMed: 24815857]

Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. Development. 1993; 117(4):1223–37. [PubMed: 8404527]

Yarar D, D'Alessio JA, Jeng RL, Welch MD. Motility determinants in WASP family proteins. Mol Biol Cell. 2002; 13(11):4045–59. [PubMed: 12429845]

Yoo H, Roth-Johnson EA, Bor B, Quinlan ME. *Drosophila* Cappuccino alleles provide insight into formin mechanism and role in oogenesis. Mol Biol Cell. 2015; 26(10):1875–86. [PubMed: 25788286]

Zalevsky J, Lempert L, Kranitz H, Mullins RD. Different WASP family proteins stimulate different Arp2/3 complex-dependent actin-nucleating activities. Curr Biol. 2001; 11(24):1903–13. [PubMed: 11747816]

Zhang J, Fonovic M, Suyama K, Bogyo M, Scott MP. Rab35 controls actin bundling by recruiting fascin as an effector protein. Science. 2009; 325(5945):1250–4. [PubMed: 19729655]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

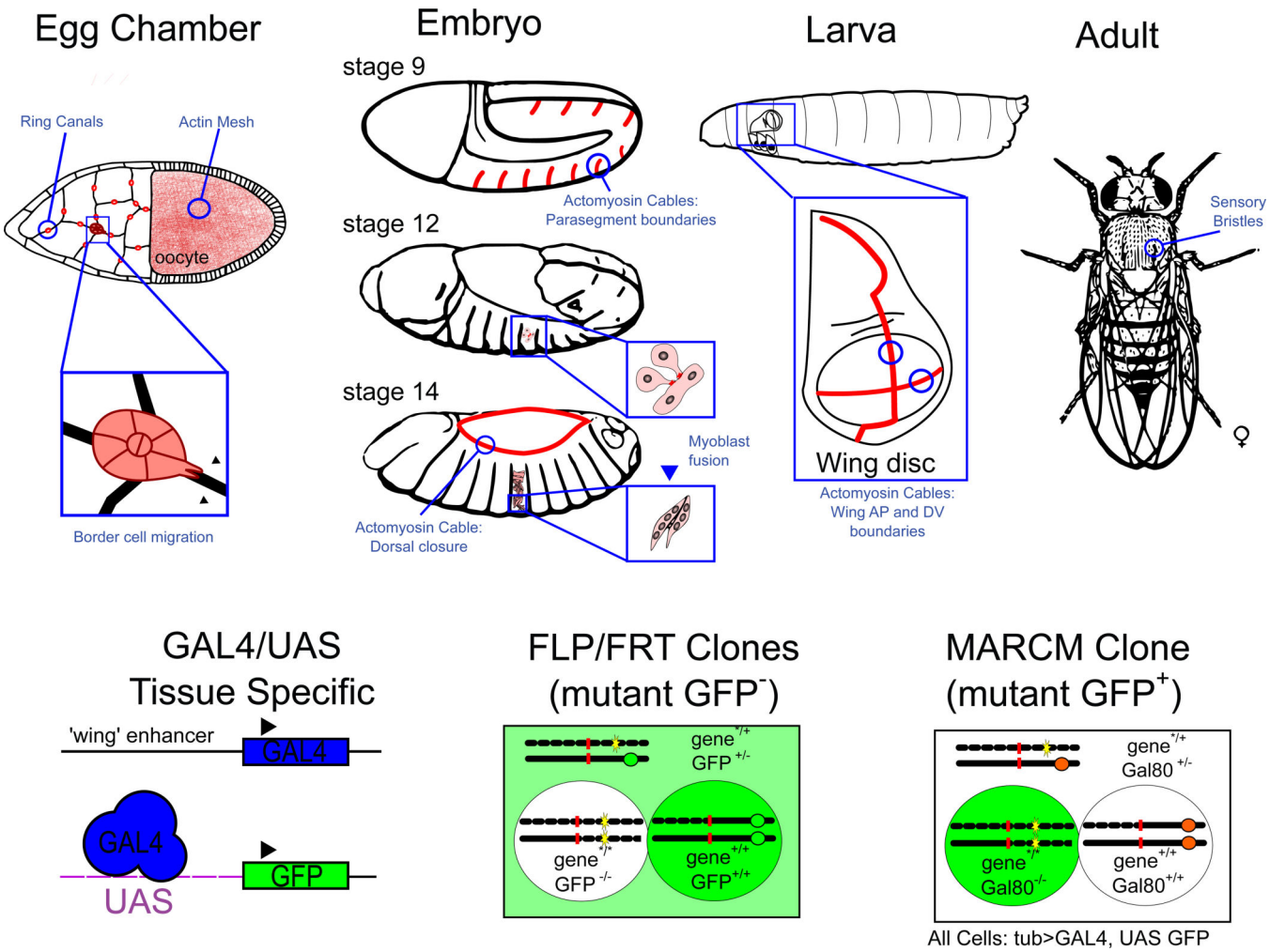

Figure 1.

(A) Schematic of developmental stages and cellular processes in *Drosophila* that are highlighted in this review; F-actin structures are highlighted in red. In the developing egg chamber, ring canals and border cell migration serve as important models to investigate the assembly of complex F-actin structures and collective cell migration, respectively. In the oocyte, an F-actin mesh is required to control the timing of cytoplasmic streaming and oocyte polarity (see Case Study 2). During myoblast fusion in the embryo, F-actin foci are required for the formation of multinucleate muscle fibers, and provide insight into the coordination of actin nucleator activities (see Case Study 1). Actomyosin cables (see Case Study 3) mediate cell sorting at the embryonic parasegment boundary, larval wing imaginal disc, and pupal abdominal epidermis (not shown), and contribute to epithelial sheet movements during embryonic dorsal closure. Lastly, the adult mechanosensory bristle is a highly accessible system that has been used to identify genes required for the formation of F-actin bundles similar to those found in brush border microvilli and hair cell stereocilia. Together, these models provide a range of systems to investigate the complex regulation and activity of cytoskeletal regulators *in vivo*, and contribute to our understanding of highly related processes and structures in vertebrates (summarized in Table 1). **(B)** Schematic of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

tools available for temporal and spatial regulation of gene function in *Drosophila*. (Left) The GAL4/UAS system consists of the heterologous transcriptional activator GAL4 and a target gene of interest under the control of the upstream activating sequence (UAS). (Center and Right) FLP/FRT and MARCM systems to induce mitotic clones. Both systems utilize the FLP recombinase and target FRT sites (red boxes) to induce homozygous mutant and wild type cells in an otherwise heterozygous background, but differ in how clones are marked. In the FLP/FRT example, heterozygous tissue is marked by one copy of GFP (green shading). Following recombination, one daughter cell receives two copies of the mutant allele (yellow star) and zero copies of GFP (green oval), resulting in a negatively marked clone (white). The other daughter cell forms a wild type 'twin spot' containing two copies of GFP (dark green) and two wild type alleles of the gene of interest. In the MARCM system, all cells contain a ubiquitously expressed GAL4, a UAS-GFP, and a single copy of the GAL4 inhibitor GAL80 (orange oval). Following recombination, one daughter cell receives two copies of the mutant allele and zero copies of GAL80, thus allowing expression of GFP and resulting in a positively marked homozygous mutant clone (dark green). All other cells remain GFP negative due to the presence of one (heterozygous) or two (twin spot) copies of GAL80.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

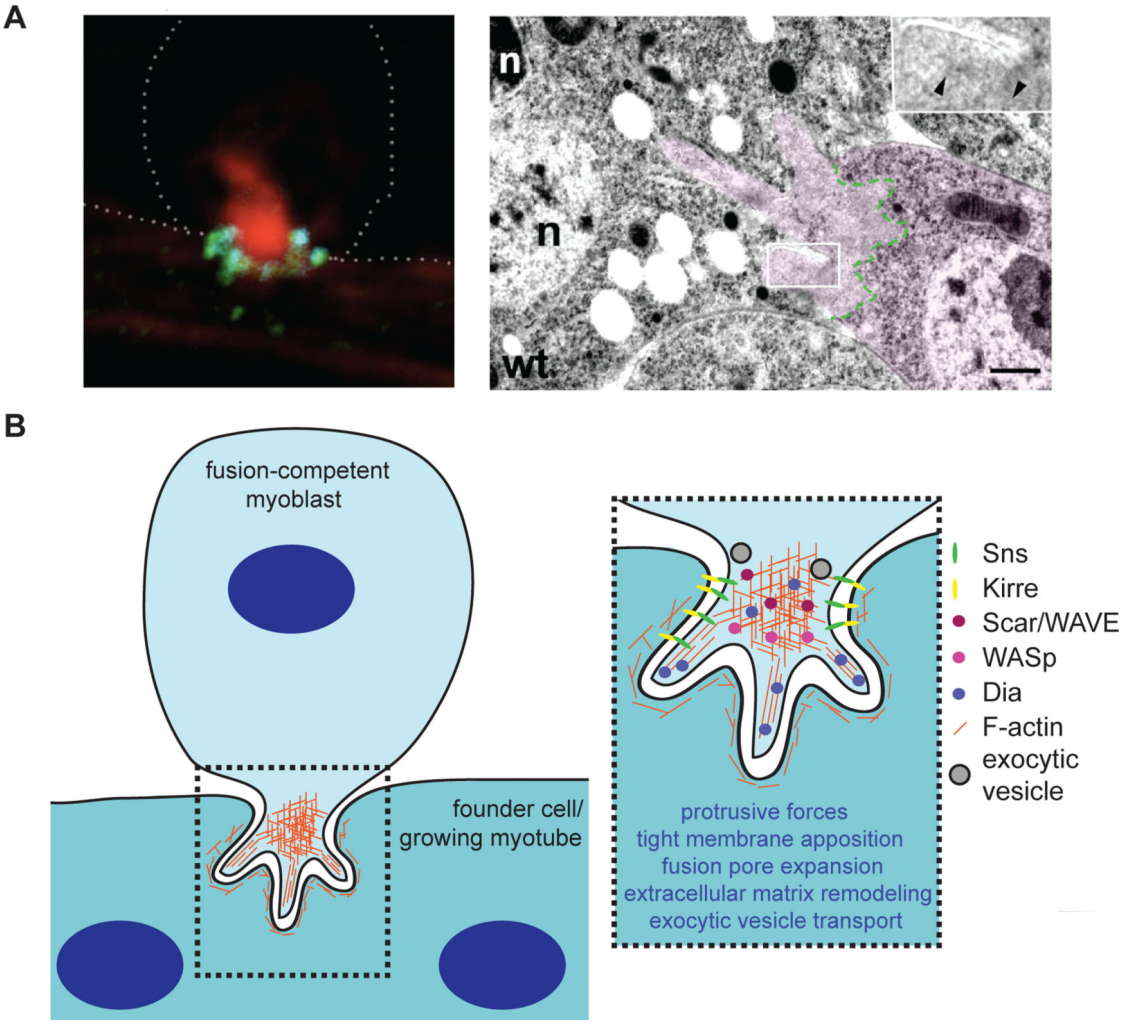

Figure 2.

(A) Electron micrograph of high-pressure-frozen/freeze substituted wild type (wt) stage 13 myoblast (pseudocolored pink) fusing with a myotube. The boundaries of the F-actin focus are indicated with a dashed green line. Inset shows actin filaments and n indicates myotube nuclei. Scale bar is 500 nm. ©2010 Sens et al. Originally published in *Journal of Cell Biology*. 191:1013-1027. doi:10.1083/jcb.201006006. **(B)** Schematic of regulatory mechanisms for the Arp2/3 NPFs WASp and SCAR/WAVE. GBD: GTPase-binding domain; VCA: Arp2/3-activating Verprolin-Central-Acidic Domain. **(C)** Schematic of a fusion-competent myoblast forming a podosome-like structure (PLS) on a growing myotube (nuclei in blue). Magnified area shows a model for the localization of various actin nucleators and cell adhesion proteins (Sns in the fusion-competent myoblast and Kirre in the founder cell/myotube) proximal to the actin focus, and potential roles for the PLS in fusion.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

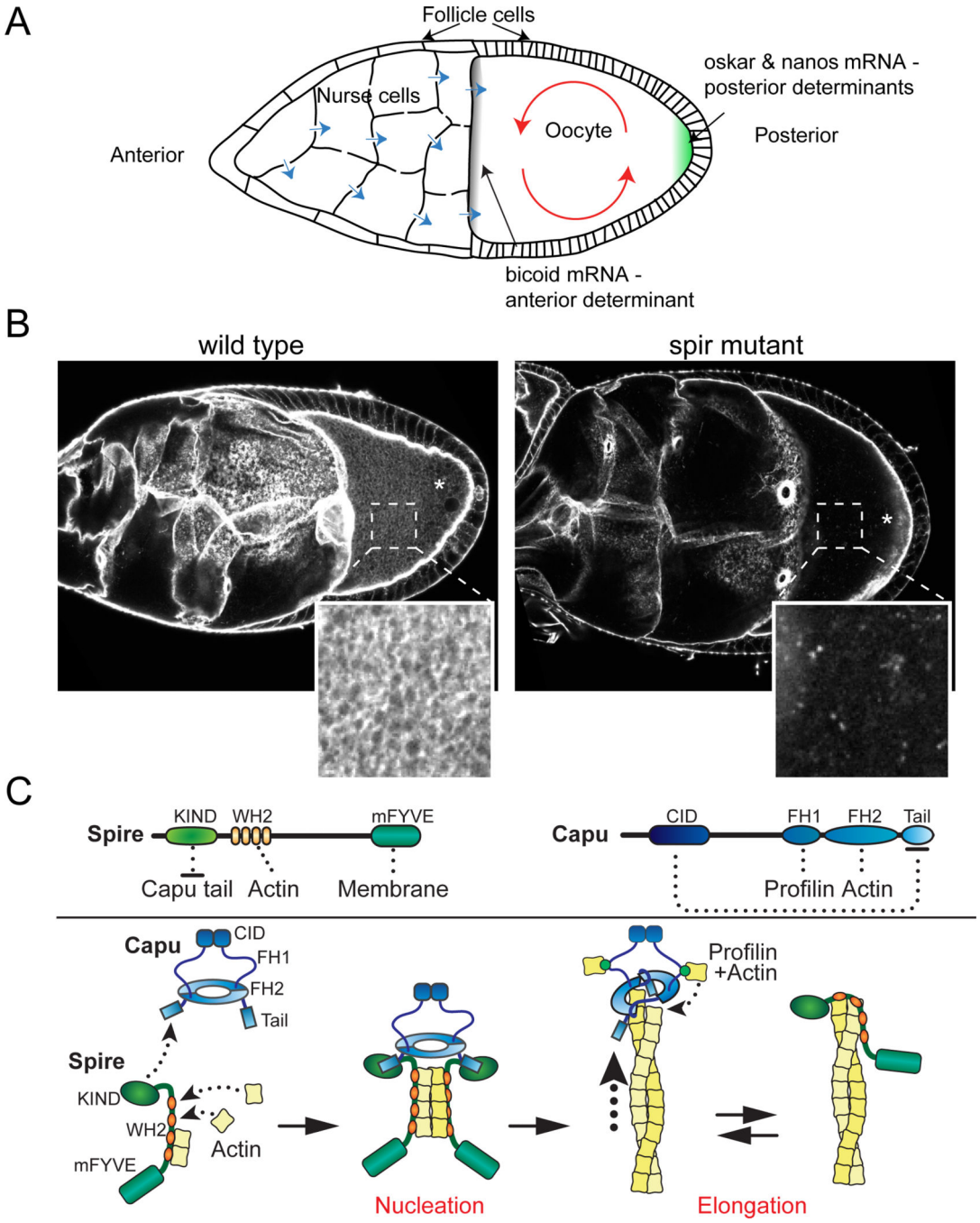

Figure 3.

Cross-talk between actin and microtubule cytoskeletons regulates oocyte cell polarity. (**A**) mRNA localization in the developing oocyte. mRNA is transported into the oocyte (blue arrows) and anchored to defined regions, establishing the polarity of the embryo. *oskar* mRNA localization to the posterior pole requires the microtubule cytoskeleton (not shown). Other mRNAs (i.e. *nanos*) deposited into the oocyte at a later stage require cytoplasmic streaming (mixing of nurse cell cytoplasm with oocyte yolk, red arrows) for efficient localization. (**B**) A cytoplasmic actin mesh is present in the oocyte prior to cytoplasmic

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

streaming. Image on left is a wild-type egg chamber stained with phalloidin (* indicates the oocyte). Image on right is a *spir* mutant egg chamber stained with phalloidin, which lacks the actin mesh and exhibits premature cytoplasmic streaming. Images courtesy of M. Quinlan. (**C**) Domain structure and interactions between Capu and Spir (top). The Spir kinase inactive domain (KIND) binds to the Capu tail domain and inhibits nucleation and processive elongation. This interaction also stimulates Spir nucleation activity via the WASp homology 2 (WH2) domains. Capu also has an N-terminal Capu inhibitory domain (CID), which binds to its tail domain (overlapping the Spir binding site) and inhibits Capu activity. Model for the function of Capu and Spir during actin assembly (bottom). Initially, Capu is bound to Spir, stimulating Spir nucleation activity and promoting the formation of an actin filament. Next, Spir releases Capu, relieving Capu inhibition and promoting Capu association with the actin barbed end, which enhances actin filament elongation. Subsequently, the barbed end cycles between Spir-bound (stalled) and Capu-bound (elongating) states. Images courtesy of Julian Eskin.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

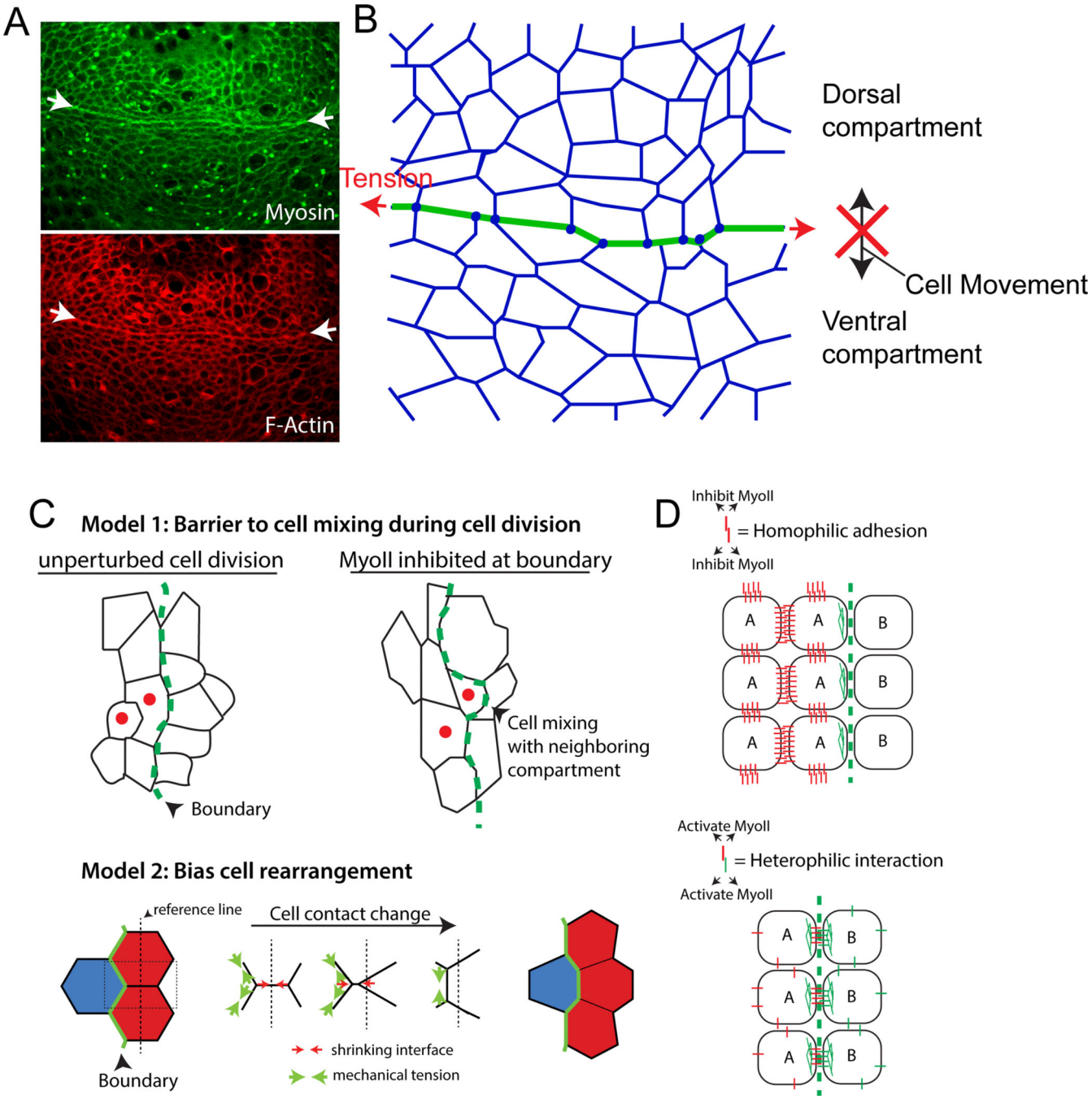

Figure 4.

A supracellular actomyosin cable is the basis for forming tissue compartments. (**A**) Images showing Myosin (green) and F-actin (red) in imaginal wing discs. The dorsal-ventral compartment boundary is indicated (white arrows). Image courtesy of K. Irvine. Originally presented in Major and Irvine, Dev. Dyn. 1996. (**B**) Cartoon illustrating shapes of cells along a compartment boundary. An actomyosin cable assembles along the compartment boundary (green) and presents cell mixing between compartments. (**C**) Models for how a supracellular actomyosin cable prevents mixing between compartments. Model 1 shows a

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

cell that has divided along the boundary (red dots indicate daughter cells) in an embryo with a normal functioning boundary (left) or an embryo in which myosin along the boundary has been inhibited by CALI (right). Model 1 is adapted from [Monier et al. 2010]. Model 2 shows how tension along the boundary can bias cell-cell rearrangements. Model 2 is adapted from [Umetsu et al. 2014a]. (**D**) Homophilic or heterophilic interactions can trigger actomyosin assembly at interfaces between cell types. Homophilic interactions can polarize actomyosin contractility to an interface by inhibiting actomyosin in the rest of the cell. In contrast, heterophilic interactions that activate actomyosin can trigger cable formation at a boundary.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Examples of actin-based structures in Drosophila development, and their mammalian counterparts.

Drosophila Model	Actin Structure/Process	Related Mammalian Systems	References
Myoblast fusion	Podosome-like structure	Myoblast fusion	[Abmayr and Pavlath 2012; Kim et al. 2015a].
		Invadopodia	[Linder et al. 2011]
		Podosomes	[Schachtner et al. 2013]
Oocyte cytoplasmic streaming	Actin meshwork	Oocyte spindle positioning	[Hudson and Cooley 2002] [Pfender et al. 2011]
Nurse cell dumping	Ring canals	Intercellular bridges	[Hudson and Cooley 2002] [Haglund et al. 2011]
	F-actin bundles	Nuclear positioning	[Luxton et al. 2010]
Compartment boundaries	Actomyosin Cables	Hindbrain segmentation	[Lye and Sanson 2011]
Dorsal closure	Actomyosin Cables	Wound healing	[Roper 2013]
Mechanosensory bristles	F-actin bundles	Brush border Hair cell stereocilia	[Tilney and DeRosier 2005]
Border cell migration	Group cell migration	Neural crest migration Cancer metastasis	[Montell et al. 2012]