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Membrane-Actin Interactions in Morphogenesis:Lessons Learned from *Drosophila* Cellularization

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Abstract

During morphogenesis, changes in the shapes of individual cells are harnessed to mold an entire tissue. These changes in cell shapes require the coupled remodeling of the plasma membrane and underlying actin cytoskeleton. In this review, we highlight cellularization of the *Drosophila* embryo as a model system to uncover principles of how membrane and actin dynamics are co-regulated in space and time to drive morphogenesis.

Keywords

Actin; Myosin-2; Cytokinesis; Endocytosis; Exocytosis; Membrane Trafficking; Cortical Compartments; Membrane Reservoir; Phosphoinositides

1. Introduction

Tissue morphogenesis is driven by collective change in the shapes of individual cells. As each cell changes shape, it must simultaneously remodel the plasma membrane and actin scaffold at its surface. What's more, the membrane and actin scaffold need to stay coupled to one another during this remodeling to ensure cell surface integrity, to generate forces and to catalyze further shape change [1, 2]. Yet, how coupled membrane and actin remodeling is achieved is still poorly understood. Over the course of the past two decades, cellularization of the *Drosophila* embryo has repeatedly informed us on the diversity of mechanisms employed by cells to integrate coincident membrane and actin remodeling during cell shape change. Within one hour, cellularization converts the single-cell syncytial fly embryo into a multicellular blastoderm composed of a primary epithelial sheet of thousands of columnar mononucleate cells (Fig. 1). Due to the massive cell surface reconstruction that accompanies

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this cell division process, actin and plasma membrane interactions are of paramount importance; and given that these interactions are also dramatic, they are noticeable and detectable by us as experimental observers. As such, cellularization has provided lessons that are both relevant to, and in some cases illuminating of, membrane and actin interactions occurring in more frequently studied examples of cell shape change and morphogenesis. These lessons have shown us: 1) how gene expression specifies coordination between actin and membrane remodeling during development; 2) how cell surface compartmentalization contributes to coupled actin and membrane remodeling; 3) how actin and endocytosis can feedback on each other, particularly in terms of actin controlling endocytic scission, and, conversely, endocytosis pruning actin/Myosin-2 (actomyosin) arrays; 4) how membrane trafficking, exocytosis and cell surface actin remodeling are linked; 5) how actin in a cell surface membrane reservoir is regulated to support cell growth; and, finally, 6) how the timing of actomyosin ring contractility is controlled by changes in plasma membrane phosphoinositide composition and associated actin filament (F-actin) crosslinkers. In this Review, we will detail these lessons by focusing on membrane-actin interactions during cellularization, as we offer our telling of how the *Drosophila* embryo is transformed from *"one cell to many"* [3].

We apologize in advance to colleagues whose findings were not included here due to space constraints. We also want to note that, while there is a broad literature to survey on cellularization, there are remarkably few discrepancies in results, despite the work having been done by numerous labs across the globe using many independent approaches. In fact, the reproducibility of results from different studies is quite striking – for example, many kinetic measurements match within a minute or two of each other – and attests to the stereotypical nature of cellularization between embryos and the unique power of this experimental model.

2. What is cellularization?

Cellularization is alternatively referred to as the first complete cytokinesis or the first tissue building event in the *Drosophila melanogaster* embryo. It serves to both package nuclei into individual cells, so fulfilling the cytokinesis descriptor; and at the same time generates the primary epithelial sheet that will fold into tissue layers and ultimately give rise to a hatching larva. Cellularization is observed during the embryogenesis of most insects but can be quite diverse in its timing and manner of execution [4-7]. Historically, cellularization has been most studied in *Drosophila*. Since it marks the conversion from syncytial to cellular development, when the mode of transmission of morphogens would necessarily change (*i.e.* from intra to inter-cellular conveyance), there has been significant interest in how differences in cellularization influence the genes and mechanisms of embryonic patterning between insects. We refer readers to several excellent reviews on this subject [8-10].

2.1 An overview of the process

It is the early syncytial development of the fly embryo that sets the stage for subsequent cellularization (Fig. 1). Immediately following fertilization, the embryonic nuclei undergo a total of nine rounds of division deep in the embryo interior with no intervening cytokinesis.

These nuclear divisions correspond to rapid cell cycles and mitosis. But, because cell cleavage is absent, these cycles are often called nuclear cycles (NC) rather than cell cycles. By the tenth NC (NC 10), which occurs 80-90 minutes post-fertilization at 25°C, the nuclei have been transported to the embryo surface and become anchored in a monolayer just beneath the plasma membrane [11]. These nuclei of the “syncytial blastoderm” stage embryo will continue to divide with no cytokinesis through NC 13, such that at the end of that cycle the monolayer contains ~6000 cortically positioned nuclei sharing one common cytoplasm (theoretically the number of nuclei should be greater, but a quality control mechanism acts to exclude nuclei with DNA damage by releasing them from the monolayer [12, 13]). Then, at the onset of NC 14, ~130 minutes post-fertilization and during interphase, cellularization begins with the plasma membrane simultaneously invaginating as furrows around every nucleus to ultimately generate a sheet of mononucleate, columnar epithelial cells (see Box 1 for organization of contents of newly forming cells). The whole cellularization process takes approximately one hour and the resulting cells are adherent to each other, polarized along their apical-basal axis, and will immediately begin to reorganize to build the multi-layered tissues of the gastrula.

The signals that initiate cellularization remain somewhat enigmatic. Unlike other developmental events in the early embryo, including insertion of G-phase into the cell cycles, the timing of cellularization is not tightly coupled to the nuclear-to-cytoplasmic ratio [14-17]. Rather, cellularization seems linked to a developmental timer of poorly defined molecular composition that somehow tracks the interval from fertilization to NC 14 [15, 17]; and cellularization is likely somehow entrained by the cell cycle since it is triggered just at the end of mitosis at NC 13 and beginning of interphase at NC 14 [18].

2.2 Genetic regulation of actin remodeling during cellularization

The successful initiation and progression of cellularization does critically depend on zygotic gene expression [17]. Multiple independent -omics analyses show that cellularization occurs concurrently with the embryo’s switch from full reliance on maternally loaded RNAs and proteins to activation of its own genome [19]. This maternal-to-zygotic transition at cellularization (MZT) corresponds with a major wave of transcription from the embryonic genome and a mass degradation of maternal products [20, 21]. However, prior to this full-throttled MZT, a relatively small cohort of genes are expressed from the zygotic genome, including patterning genes required for cell fate specification and five genes required for cellularization: *nullo, serendipity-α, slam, bottleneck* and *dunk* (*i.e.* the zygotic cellularization genes; Box 2).

Strikingly, each of the five zygotic cellularization genes encodes a protein that acts on the actin cytoskeleton at the level of actin filaments (F-actin) [22-27]. These proteins localize to the tips of invaginating plasma membrane furrows during cellularization and regulate or reinforce F-actin there; doing so either directly or by targeting maternally provided actin machinery, including Myosin-2 and Rho Guanine Nucleotide Exchange Factor-2 (RhoGEF2), which activates a RhoA GTPase homolog, Rho1 (Box 3). Loss of function of any one of the cellularization genes does not stop the onset of cellularization but does disrupt the coordination of F-actin and plasma membrane remodeling that is essential for

robust nuclear packaging [22-26, 28-35]. Loss of multiple cellularization genes leads to catastrophic failure of cellularization [28]. Finally, precocious expression of early zygotic genes, including the cellularization gene *slam,* supports precocious invagination of plasma membrane furrows in interphase of NC 13 [17], underscoring the critical F-actin remodeling activity that these zygotic actin regulators provide for cellularization.

The cellularization genes are, notably, only expressed in a pulse at the onset of cellularization, are downregulated immediately afterwards, and are not known to act at any other time in the *D. melanogaster* lifecycle [22, 30, 32-34, 36-38]. They represent a pathway in the early embryo that runs straight from genes to actin-membrane interactions and morphogenesis. The mechanisms to turn these genes on are highly regulated [39-41], as are, presumably, the mechanisms to turn them off. So, while it is not always clear in other systems if or how regulated gene expression informs coordinated actin and plasma membrane remodeling, cellularization offers an exceptionally unambiguous and direct path with which to interrogate this genotype to phenotype relationship.

3. How are the sides of the new cells built?

Cellularization yields a sheet of ~6000 columnar epithelial cells (Fig. 1). To achieve this new morphology, with the tall cells reaching a final length of 35 microns, the cell surface area of the embryo must expand by ~25 fold over the course of one hour – one of the most dramatic examples of rapid cell surface expansion that is known [42, 43]. Membrane growth is manifest by invagination of plasma membrane furrows that will be the lateral sides of the new cells (Fig. 1). Furrows take-on epithelial polarity coincident with invagination through an evolving refinement of cell surface compartments. Thus, we will now describe the intricate membrane handling that adds, sorts, and regulates material addition during furrow invagination. This membrane handling is always tightly coupled with actin remodeling, as the actin cytoskeleton contributes to both the active mechanical forces and furrow stability that is required for invagination.

3.1 Furrow positioning

A first necessity in cellularization is the specification of furrow positions around each of the thousands of nuclei that sit in a regularly arrayed monolayer at the embryo periphery (Fig. 1). To specify furrows, a highly efficient strategy is employed: Rather than select the position of all furrows from a blank slate, the embryo reuses landmarks at the cell surface from the prior mitosis at NC 13 (Fig. 2A) [22, 28]. These landmarks are the remains of transient “metaphase furrows”, which were previously assembled around the spindle of each dividing mother nucleus to prevent interaction between adjacent spindles [44]. These metaphase furrows mostly regress by the onset of cellularization, but their position is retained and serves as the site of growth for cellularization furrows [22, 28]. Because these cellularization furrows originate from an “old” position, they are called “old furrows”. This leaves only the interface between two daughter nuclei devoid of a furrow. Thus, a “new furrow” must be placed between the daughters. However, the daughter nuclei, themselves, do not define furrow position. Instead, each nucleus interacts with a centrosome pair (Box 1), and it is the centrosome pairs and their associated microtubule (MT) asters that determine

where new furrows will form [28, 45, 46]. Specifically, new furrows form where two as yet unconfined MT asters meet (Fig. 2A).

Asters inform furrow position and invagination during cellularization using mechanisms similar to those seen in cytokinesis [47]. At first, Myosin-2 filaments, and very likely other components, are recruited to old and new cellularization furrows via a flow of cytoplasm, called cortical flow, that streams just beneath the plasma membrane and moves from areas of lower cell surface tension to areas of higher tension (Fig. 2A) [22, 48-51]. As is the case at the onset of cellularization, cortical flow is often biased towards positions where MT asters meet [52-54]. Interestingly, this flow of Myosin-2 only takes place during the first several minutes of furrow initiation in cellularizing embryos and is somehow aided by the zygotic cellularization gene, Dunk, which localizes to furrows (Box 2) [22]. After that, F-actin and Myosin-2 accumulate at furrows using the same conserved regulators that drive furrow invagination in cytokinesis, including Rho1, RhoGEF2, Formin Family F-actin nucleator Diaphanous, and Pavarotti Kinesin Like Protein (PavKLP; Box 3) [55-59]. Within four to five minutes of entry into NC 14, all furrows look identical in their length (~3 microns), and they surround every nucleus [22, 28]. At this point, MT asters continue to contribute to the invagination of both old and new furrows by delivery of membrane and cortical actin regulators, as will be discussed in detail below.

3.2 Furrow compartmentalization and polarity

As cellularization gets underway, all furrows, old and new, continue to mature through both the recruitment of furrow components and the coincident polarization of these components into cortical compartments (Fig. 2A) [30, 44, 60]. Cortical compartments are distinct domains that form along the cell surface. These compartments include specific inventories of proteins embedded within the “actin cortex”, which is a 100-200 nm thick F-actin meshwork that underlies and associates with the plasma membrane [1, 2]. As is a feature of cortical compartments in general, the inventories of proteins per compartment impart F-actin organizing activities and/or define areas of discrete function along the cell surface. Compartment constituents can include specific F-actin nucleators and crosslinkers, as well as polarity cues, adhesion and signaling molecules, and transmembrane and plasma membrane-tethered proteins. Cortical compartment formation during cellularization is essential for sustained furrow ingression, depends on F-actin, and serves to partition specific F-actin and membrane remodeling events along the cell surface [25, 30, 44, 60, 61]. This compartmentalization also marks the first full expression of epithelial polarity in the embryo, with four domains – apical, subapical, lateral and basal – emerging along the cell surface (for precursors to this polarity, see Mavrakis *et al.*, 2014 [62]) [44, 63].

Compartmentalization of the furrow is rapid. Three proteins, Elmo, Sponge and Canoe, are early markers of the subapical compartment, defining it *de novo* in both new and old furrows, just as new furrows catch-up with their old counterparts [61]. Canoe, is a homolog of the F-actin binding protein Afadin, and Elmo and Sponge make a complex with guanine nucleotide exchange activity for the small GTPases Rac and Rap1 (Box 3) [64]. Despite knowing the identity of these recruited proteins, the exact mechanism of defining

the sub-apical compartment is not yet understood. What is clear is that formation of the subapical compartment at this early time promotes normal furrow ingression [61].

The basal compartment, at the leading tip of the furrows, is also quickly established within the first ten minutes of NC14 [61]. The zygotic cellularization gene Slam is one of the first proteins to arrive at this compartment and appears to concentrate there by two mechanisms. First, Slam is concentrated at the basal compartment by an unknown receptor that traffics there via a Rab11 GTPase and Recycling Endosomal (RE) route (Box 1, and detailed below in Section 3.3) [28]. Second, Slam drives a positive feedback loop to enhance its own recruitment at the basal compartment, so aiding fast polarization. In this feedback loop, a small initial amount of Slam protein at the basal compartment recruits *slam* mRNA. The mRNA is then locally translated to promote incorporation of more Slam protein [65, 66]. Within the compartment, Slam binds and recruits RhoGEF2, which activates Rho1 GTPase. Local Rho1 activation promotes F-actin polymerization via Diaphanous and Myosin-2 accumulation via Rho Kinase (Box 3) [56, 58, 67]. The resulting increases in F-actin levels within the compartment act as structural reinforcement for the compartment itself and the furrow overall. Two additional proteins, encoded by the zygotic cellularization genes, Nullo and Serendipity-α, also somehow localize to the nascent furrow tips, independent of Slam, and further promote rapid initial F-actin accumulation and compartmentalization [24, 25, 27, 28].

Once the sub-apical and basal compartments are established, all cortical domains are bounded and, thereafter, are distinct in actin and membrane remodeling activity [61]. Unlike other examples of cortical polarization systems – even those developing over the next several hours of fly embryo development – the continued refinement and maintenance of this early compartmentalization in cellularization does not at first involve mechanisms of direct mutual antagonism between proteins of different adjacent domains [68]. Instead, the fate of early polarization, and of cellularization itself, largely depends on actin-regulated endocytosis that is necessary to refine and maintain basal compartmentalization, specifically (Fig. 2B) [24, 25, 69-72]. Basal compartment refinement continues through the first 30 minutes of cellularization, at which point furrow lengths reach five microns, and in-plane diffusion of plasma membrane-associated proteins is completely blocked between the lateral and basal compartments [25].

Why is basal compartmentalization so important? As cellularization proceeds, the basal compartment can be considered the “business-end” of the furrow. Dunk, Slam, Nullo, and Serendipity-α expended their early activity to exclusively build-up actomyosin there and it is this compartment where the mechanical forces are generated to, first, drive furrow invagination; and second, contract and close-off the bottoms of the new cells (see Section 4, and Fig. 3A and 4A). To support invagination and closure, the compartment must have the right components present at the right levels. To regulate this composition, surplus membrane and protein are pruned away by endocytosis that depends on the N- and F-BAR Domain containing proteins Amphiphysin and Cip4/Toca-1, respectively; the ADP Ribosylation Factor (ARF) GTPase regulators, Steppke/Cytohesin and Stepping Stone; Dynamin; the F-actin nucleating Arp2/3 Complex; and F-actin itself (Box 3) [24, 69-72]. The topology of the compartment may be conducive to endocytosis because at its basal tip, there is high

membrane curvature where the two plasma membranes fold to directly face each other (Fig. 2B) [73]. Endocytosis from the furrow tip serves to remove excess membrane, so restraining invagination rate; and also to fine-tune amounts of contractile proteins by down-regulating Rho1 GTPase signaling to prevent premature contraction and closure of the bottom of the cells (see also Section 4) [69-72]. Thus, endocytosis regulates the composition and normal activity of this compartment, particularly in terms of controlling the kinetics of furrow invagination and actomyosin contraction.

At the same time, though, endocytosis at the basal compartment must be restrained. The source of this endocytic restraint is F-actin accumulation in the compartment [25]. Actin filaments are thought to prevent the endocytosis of too much membrane in the basal compartment by promoting efficient endocytic scission to limit vesicle size, and/or increasing resistance to membrane bending and engulfment [24, 72]. Otherwise, excess membrane is incorporated into endocytic tubules that elongate unchecked until they are somehow cleaved or simply break from the plasma membrane. Either loss-of-function of positive regulators of F-actin accumulation (e.g. Slam, Nullo and Diaphanous) or drug-mediated depletion of F-actin leads to loss of basal compartment identity, invasion of lateral domain components and reduced furrow stability such that a fraction of furrows regress [24, 25, 58, 71, 72]. If even a small number of furrows regress, then the embryo is less likely to successfully complete embryogenesis and hatch as a larva [27, 74]. So, while endocytosis is needed to regulate the actin cytoskeleton at the basal compartment, the right level of F-actin assembly is also needed to keep endocytosis in check. How this fine balance is struck remains unknown.

Finally, late in cellularization, the new cells are mostly built and are increasingly taking on epithelial characteristics [68]. The sub-apical compartment is targeted by exocytosis (see Section 3.3) [60] and contributes to assembly of apical adherens junctions [63, 75, 76]. Meanwhile, endocytosis again refines the composition of a compartment: This time the apical compartment. Large tubular membrane carriers endocytose from the apical compartment via a pathway that is likely clathrin-independent, based on geometry, but requires Dynamin and the Rab5 GTPase effector, Rabankaryin-5 (Fig. 2B) [77]. While the function of this tubular endocytosis is uncertain, one suggestion is that it ensures the proper morphology and composition of the apical compartment for upcoming apical events of gastrulation, such as actomyosin-dependent apical constriction. Consistent with this, tubular endocytosis is most prominent in the ventral region of the embryo where apical constriction drives internalization of the future mesoderm immediately following cellularization; and endocytosis is known to accompany apical constriction during morphogenesis in older fly embryos and during vertebrate neural tube formation [78-84]. Alternatively, tubular endocytosis might aid the transfer of apically localized junction components, including E-cadherin, to sub-apical positions where adherens junctions begin to form; or similar to Clathrin-independent carrier pathways (CLIC), tubular endocytosis could respond to the mechanical signal of membrane tension and trim away excess membrane to maintain cell surface homeostasis [85-87]. Unfortunately, until we understand if the apical endocytic carriers that form are selective for specific cargoes, it will be difficult to name a function. One thing that is clear is that these carriers are destined for early endosomes [77].

To summarize this section, cortical compartmentalization plays an outsized role in building the sides of new cells during cellularization. The compartments that form have their own distinct roles to play and the fidelity of the cellularization process overall depends on this functional polarity. Perhaps the role of compartmentalization is so prominent in cellularization due to the demanding extent of plasma membrane growth required to generate thousands of tall cells in a relatively short timeframe, or because the new cells must coincidentally become polarized as they grow in order to support epithelial integrity and gastrulation immediately after being built. In either case, cellularization serves as an excellent system to understand the mechanisms that facilitate compartmentalization. In particular, cellularization illuminates with clarity the interplay that can exist between actin remodeling and endocytosis in refining and maintaining cell polarity. This is a relationship now recognized as fundamental to cell shape change [88-91]. The two-way feedback seen between actin and endocytosis in the basal domain is striking in its ability to fine-tune both the invagination and contractile activities of furrows, showcasing how versatile endocytic mechanisms can be in tweaking diverse cell shape changes. Regarding endocytosis in both the basal and apical compartments, several outstanding questions remain: How is endocytosis itself regulated and polarized? What are the initiating cues? To what extent does endocytosis during cellularization represent active versus homeostatic processes? What materials are internalized and what are the post-endocytic routes for internalized membrane and proteins?

3.3 Invagination and material supplies for the furrows

The invagination of cellularization furrows is biphasic, proceeding in a slow phase followed by a fast phase, with each phase lasting ~30 minutes. Rates of slow and fast phase invagination are 0.2 ± 0.02 micron/minute and 1.2 ± 0.10 micron/minute, respectively, to achieve a final furrow length of ~35 microns (Fig. 3A) [42, 60, 92, 93]. The force driving furrow invagination in cellularizing embryos is not Myosin-2 based contraction, as is often the case in cytokinesis [46, 67, 94-96]. Instead, a leading model posits that much of the directed force required for furrow ingression is provided by plus-end directed microtubule motors, including PavKLP, that localize to the basal compartment and pull the furrow down along the inverted microtubule basket that hangs over each peripherally anchored nucleus (Box 1, Fig. 3A) [57, 59]. Relative to cytokinesis, furrow invagination during cellularization requires a huge influx of new materials including membrane and all the components necessary to assemble the actin cortex underneath [97]. Not surprisingly, a large amount of these materials – membrane and actin regulators – are delivered to the embryo surface via exocytosis of vesicles derived from biosynthetic pathways (Fig. 3B). Given the extreme cell surface expansion that accompanies cellularization, as well as its biphasic dynamics and execution over large cellular distances, study of these biosynthetic pathways has been highly conducive to showing us how exocytosis can be deployed to support the coupled and simultaneous remodeling of membrane and actin required for cell surface growth.

In a simple yet elegant set of pulse-chase labeling experiments in 2000, Lecuit and Wieschaus first mapped out the major insertion sites for exocytosis along the cell surface over the course of cellularization [60]. At different times during cellularization, they applied

a pulse of fluorescently labeled wheat germ agglutinin (WGA) to tag glycoproteins on the external surface of the embryo, and then characterized how the fluorescent WGA was displaced by insertion of unlabeled membrane from internal stores. They found that exocytosis is polarized and occurs at sites correlating with the cortical compartmentalization described above (Fig. 2 and 3B). In this mapping, earliest exocytosis at the embryo surface aids elaboration of furrow positions as basal compartments become increasingly distinct from the rest of the plasma membrane. As slow invagination commences, large-scale exocytosis occurs in the apical compartment. For the subsequent fast invagination phase, exocytosis targets the subapical compartment of the elongated furrows. While some details remain unclear, this initial map of exocytosis still serves as a framework to which three routes of membrane trafficking have been subsequently related (Fig. 3B). These routes, each culminating in delivery of material to the plasma membrane, can be thought of as partially distinct in timing, path and function.

One route of trafficking is necessary for forming and stabilizing the furrows at the onset of cellularization. If we designate routes by their most significant function, then this route might be considered the “furrow F-actin assembly route” because it predominantly contributes to building the F-actin cortex at furrows (Fig. 3B). This cortical F-actin is required for successful furrow formation and invagination [98]. Exocytosis via this route almost certainly depends on the exocyst complex for vesicle docking at the plasma membrane since exocyst component Secretory 5 (Sec5) and exocyst assembly factor Ras-Like Protein A (RalA) are needed for the earliest formation of furrows [99, 100]. Consistent with the WGA pulse-labeling experiments, both Sec5 and RalA localize apically at the onset of cellularization. This route is responsible for the eventual delivery of actin regulators to nascent furrows via a trajectory that involves Golgi and REs [28, 98, 99, 101]. RE’s are juxtaposed with centrosome pairs at each nucleus, placing them in proximity with the MT asters that emanate towards the cell surface (Box 1, Fig. 3B). At the RE, Rab11, Rab11 effector Nuclear Fall-Out/FIP3, and Dynamin contribute to the generation of Rab11-enriched vesicles carrying cargoes needed for the earliest steps in basal compartment establishment (Box 3) [28, 98, 99, 101]. One critically important cargo of these vesicles is a yet unidentified receptor of Slam [28]. As described in Section 3.2, delivery of this receptor to the cell surface is required for the first accumulation of Slam protein, followed by Slam-mediated recruitment of RhoGEF2 to support actomyosin assembly at forming basal compartments [26, 28, 98]. Another cargo is a Dystrophin homolog, Discontinuous Actin Hexagon (Dah) [102]. Dah is an actin binding protein that links the F-actin cortex and plasma membrane and is named according to the mutant phenotype arising from its loss of function [103, 104]. Specifically, in *dah* loss-of-function mutants, some furrows are not formed or are unstable and regress, resulting in breaks in the hexagonal furrow array (Fig. 4B).

A second route of trafficking is critical for delivery of a major portion of the membrane required for furrow invagination [42, 105]. Thus, this route might be considered a “membrane efflux route” (Fig. 3B). The extent to which this route involves REs is less clear [101], but certainly Golgi integrity and trafficking is essential. When Golgi-derived membrane secretion is inhibited by injecting embryos with the Golgi disrupting drug Brefeldin A (BFA) at the onset of cellularization, furrows fail to reach their normal 35

micron length [105-107]. Similarly, embryos show reduced furrow numbers and length following loss-of-function for the Golgin, Lava Lamp, Golgi-associated GTPase, Arf1, and signaling molecule Phospholipase D; all of which ensure normal Golgi organization (Box 3) [107-110]. Conversely, blocking furrow invagination in embryos results in a build-up of Golgi bodies in the cytoplasm [30, 105]. During normal cellularization, Golgi bodies undergo two rounds of apical trafficking that depend on Dynein and its interaction with Lava Lamp (Box 1) [107, 110]. These puncta move apically along the inverted basket MTs, consistent with the eventual insertion of membrane into the apical and sub-apical cell surface compartments reported by the WGA pulse-chase mapping experiments [60].

At first glance, it seems counterintuitive that Golgi bodies move and support exocytosis apically if their membrane is, indeed, destined for furrows. Why not dump membrane directly into invaginating furrows? The embryo has a surprising capacity to store a large stockpile of membrane in the form of dynamic protrusions or “microvilli” at the apical cell surface (Fig. 3A) [42, 43, 105]. Within this plasma membrane reservoir, which comprises the apical compartment, F-actin rapidly assembles and disassembles to push up excess membrane into a convoluted topology that can contain, at the peak of its form, as much as half of the membrane required for the entire cellularization process [42, 43, 105]. This microvillar reservoir “unfolds” over time and membrane slides along the plane of the cell surface into the invaginating furrows [42, 105]. During the slow and fast phases of furrow invagination, membrane is slowly or rapidly depleted from the reservoir, respectively [42]. If reservoir supply is limited by perturbation of exocytosis, or if reservoir unfolding is blocked, then furrow invagination is blocked [42, 105, 107, 110]. Similarly, if furrow invagination is blocked, then reservoir consumption is blocked [42, 105]. So, the path of new membrane to the furrows is indirect, flowing through an intermediate cell surface reservoir whose dynamics are linearly coupled to furrow invagination.

Plasma membrane reservoirs are broadly employed by all kinds of cells to modulate cell surface growth or shrinkage, and to buffer rapid tension changes caused by cell stretching or relaxation [97, 111, 112]. Cell surface reservoirs support development and tissue morphogenesis by managing membrane availability during cell constriction, expansion, division and motility. But, among known reservoirs, the cellularization reservoir is exceptional because it localizes to a specific location along the cell surface and its lifetime is completely stereotypical between embryos. This makes the reservoir highly tractable and has allowed an unprecedented mapping between exocytosis, reservoir dynamics and furrow invagination. Specifically, Golgi-derived exocytosis feeds the apical reservoir at cellularization onset and throughout the slow phase of invagination (Fig. 3B) [30, 60, 105, 107, 110]. Then, exocytosis rapidly redirects away from the apical compartment and to the subapical compartment during the fast phase of invagination (Fig. 3B) [60, 100, 113]. In carefully timed BFA injection experiments, it has been shown that blocking exocytosis during slow phase does not stop cellularization, but rather leads to premature depletion of the apical reservoir and shorter final furrow lengths [105, 107, 110]. Surprisingly, blocking Golgi-derived exocytosis at the beginning of the fast phase has little effect on reservoir dynamics or invagination [30, 60, 105, 107, 110]. Thus, the membrane deposited in the reservoir by apical exocytosis during the slow phase is sufficient, when unfolded, to provide

for full furrow invagination; whereas sub-apical exocytosis during the fast phase surely contributes to cellularization, but not by solely fueling invagination.

The tractability of the cellularization reservoir has also revealed the relationship between exocytosis, reservoir dynamics and F-actin dynamics (Fig. 3A) [105]. The protrusive microvilli of the reservoir are very heterogeneous in structure and are very short lived, with individual microvilli assembling and disassembling in less than 20 seconds. Each microvillus has an F-actin core that polymerizes and depolymerizes to support its individual dynamics. Looking over the course of cellularization, at the population level of the microvilli, F-actin levels in the reservoir are directly coupled to membrane levels in the reservoir, and F-actin depletion from the reservoir follows a slow phase and a fast phase linked to furrow invagination. Similar to what was found for membrane in the reservoir, blocking furrow invagination blocks F-actin depletion in the reservoir. Furthermore, blocking Golgi-derived exocytosis in slow phase leads to premature depletion of F-actin from the reservoir, suggesting that either the excess membrane or some component delivered with the membrane promotes actin polymerization within the cell surface reservoir. The ability to dissect these interactions between membrane and F-actin remodeling in a reservoir is, so far, completely unique to cellularization [105].

Finally, the third route of trafficking coincides with membrane insertion at the subapical compartment during the fast phase of furrow invagination (Fig. 3B) [60, 100, 113]. This route has been thoroughly characterized, although its function remains to be worked out. So, we will refer to this route as the “sub-apical addition route”. This route may contribute specialized membranes or components necessary for the final events of invagination or for the refinement of that compartment as epithelial polarity becomes increasingly distinct and adherens junctions assemble nearby [100, 113, 114]. This route involves the Golgi and REs, and terminates with insertion at exocyst tethering complexes, which clearly accumulate at the subapical compartment during the fast phase of cellularization [100, 113]. Exocytosis at these exocyst complexes requires the GTPase Rab8, which transforms from a cytoplasmic vesicular staining pattern to a cell surface associated pattern just at the onset of fast phase (Box 3) [99, 113].

To conclude this section, cellularization shares a reliance on exocytosis similar to cytokinesis and many examples of morphogenesis where cell growth occurs, including neuronal branching, tube formation, ciliogenesis, convergent extension and collective cell migration [90, 115-118]. Where possible, we emphasized the major contribution made by different trafficking routes during cellularization, focusing on the addition of actin regulators and membrane at the cell surface. Indeed, cellularization provides a clear demonstration that different trafficking routes can play distinct roles in adding one of these materials or the other to the expanding cell surface. However, it is critically important to note that these routes do not have a totally clear division of labor, as each vesicle that is exocytosed will add membrane as well as any cargoes that it carries. In fact, maybe this is the simplistic beauty of employing exocytosis as a means to change cell shape – actin and membrane remodeling are necessarily coupled. Despite what is known about each trafficking route during cellularization, we have yet to directly visualize vesicles as they move between compartments or insert at the plasma membrane. Nor do we know the full inventory of

cargoes that are carried. Other questions to be answered include: How distinct are each of the described pathways? How are trafficking and exocytosis regulated in space and time? By what mechanism is furrow invagination coupled to microvillar reservoir consumption? Finally, what is the role of subapical exocytosis during the fast phase of cellularization?

3.4 Furrow mechanics

Efforts to understand the mechanics of cellularization are confounded because the vitelline membrane, a protective proteinaceous shell that is required for viability, surrounds the embryo and limits direct access to the cell surface. Nonetheless, a few approaches have been employed to probe the mechanical properties of early embryos, including laser ablation of the cell surface to demonstrate in-plane tension, and microparticle injections for rheological analysis [22, 23, 95, 119-122]. (Yes. The protective shell can be penetrated to inject beads or other reagents, just not removed.)

Two studies are of note here because they begin to address the mechanical properties of the plasma membrane and associated actin cortex in cellularization furrows [119, 120]. Both efforts relied on the injection of magnetic particles into embryos, and then application of a directed magnetic force to assess the displacement of the particle and deformability of the furrow. Because the magnet is outside the embryo, these particle-based measurements are biased towards subapical furrow surfaces. In both studies, when a particle was pulled by the magnet against the side of a furrow, the furrow was pulled with it, and then release of the particle from the magnetic force showed snapping back of the particle and furrow surface to some partial extent [119, 120]. These results demonstrate the viscoelastic properties of the furrow. Interestingly, using a careful time-course of experiments, D'Angelo *et al.,* were able to demonstrate that the mechanical properties of the furrow change in a step-wise manner at the transition from the slow to fast phase of furrow invagination. Specifically, these authors report a rapid "softening" of the tissue upon fast phase (*i.e.* larger deformations are possible with reduced recoil). How plasma membrane and cortical F-actin interactions may influence this mechanical transition is an area of active pursuit.

4. How are the bottoms of the new cells built?

At this point it is important to recall that while it is easiest to picture invaginating cellularization furrows as lines in two-dimensions, they are in fact columns of growing membrane in three-dimensions. This means that the actomyosin structures at the basal compartments at the tips of the furrows are not points, but rather rings (Fig. 4A). Thus, the basal, bottom surface of the newly forming cells are, then, built via constriction of the actomyosin rings in the direction perpendicular to the invaginating furrows, and in a manner, again, similar to cytokinesis. However, during cellularization this actomyosin constriction requires additional regulatory steps as thousands of interconnected rings assemble simultaneously and need to coordinate the timing of their contractile behavior so that the final cells that are generated are of the same uniform size and morphology. Specifically, the major constriction of actomyosin that builds cell bottoms must be coordinated with furrow invagination to make cells of the right length and without impinging on the large ellipsoid nuclei (Fig. 4A and B) [32, 94, 96]. As such, cellularization represents

a case where coordination between actomyosin contractility and membrane remodeling is imperative to achieve stereotypical outcomes, and the prominence of regulatory mechanisms has made this coordination experimentally accessible.

The actomyosin constriction process during cellularization can be considered in three phases, which are distinguishable by the morphology and perimeter of rings, as well as the length of invaginating furrows with respect to the base of the nuclei (Fig. 4A). In early cellularization, there is an initial ring assembly phase that is coincident with furrow positioning and slow invagination. As discussed in Section 3.1, Dunk and Slam act sequentially to recruit Myosin-2 to the basal compartment via cortical flow and RhoGEF2-mediated recruitment, respectively [22, 26]. RhoGEF2 activates Rho1 and effector Rho Kinase, which acts synergistically with Death Associated Kinase (Drak), to phosphorylate and activate Spaghetti Squash/Myosin-2 Regulatory Light Chain (Box 3) [23, 56, 58, 67, 94, 123-125]. This activates Myosin-2 motor activity, which is required for timely ring assembly. Over 30 minutes, actomyosin bundles organize at the tips of the invaginating furrows into an interconnected array of hexagons (Fig. 4A). The zygotic cellularization gene product, Bottleneck accumulates at furrow tips and promotes F-actin bundling and formation of the hexagonal array, along with conserved F-actin crosslinker Filamin [23, 32]. Throughout the assembly phase, the hexagons must not constrict, lest they will pinch the nuclei, since furrow length has not yet surpassed nuclear length (Fig. 4B). To accomplish this modulation of contraction, the F-actin crosslinking activities of Bottleneck and Filamin antagonize Myosin-2 contractility, presumably by impeding actin filament sliding [23]. In addition, two Rho GTPase Activating Proteins, Cumberland GAP and GTPase Regulator Associated with FAK (GRAF) also act to downregulate Rho1 activity and limit Myosin-2 contractility to keep the hexagonal array open, interconnected, and mechanically rigid (Box 3) [23, 95, 126]. Next there is a middle rounding phase of ~10 minutes, coincident with the beginning of fast furrow invagination, when the hexagons convert to circular rings, and the rings individualize by resolving their connections with their neighbors (Fig. 4A). Rounding requires Myosin-2 motor activity [23, 67]; but, again, rings must remain fully open until the invaginating furrows reach the base of the nuclei. Thus, stringent modulation of Myosin-2 contractility continues. At the same time, F-actin regulators promote rounding and individualization by progressively remodeling actin filament number, length and curvature within rings. These F-actin regulators include the crosslinkers Anillin and Fimbrin, a severing protein Cofilin, and a filament-bending protein Peanut/Septin 3 (Box 3) [23, 67, 92, 93, 127]. Src Kinase (Src) may also influence filament number and length by regulating the gene expression of Actin 5C, an isoform of non-muscle actin that uniquely contributes to actomyosin contractility during cellularization [96, 128]. At the end of this phase, the open, individual actomyosin rings are positioned at the same apical/basal depth as the base of the nuclei (Fig. 4A).

Finally, there is a fast constriction phase in the last 20 minutes of cellularization when the round disconnected rings generate high tension and pull the attached plasma membrane to build the basal cell bottoms (Fig. 4A) [23, 67, 95]. Furrow invagination is also fast at this time and the nuclei are out of the way. Rather surprisingly, fast ring constriction is largely independent of Myosin-2 motor activity but relies heavily on both the proper depolymerization dynamics and architecture of F-actin in the ring [23, 67, 93]. In fact, the

switch from modulated to fast constriction is delayed 20 minutes in embryos with reduced Cofilin or Peanut/Septin 3 function [67]. At the end of this constriction phase, the rings are almost but not fully closed, remaining as “yolk plugs” until final closure in gastrulation (Fig. 4A).

Clearly, F-actin dynamics and architecture play a major role in setting the timing of actomyosin constriction throughout cellularization. These F-actin characteristics as well as Myosin-2 recruitment are coordinated with membrane trafficking and remodeling by numerous mechanisms. First, as detailed in Section 3.2, endocytosis plays an important role in trimming away excess contractile machinery from the furrow tips in early cellularization (Fig. 2A) [69, 70, 109]. Without this trimming, constriction occurs prematurely, pinching the nuclei and perturbing furrow invagination (Fig. 4B). Second, as described in Section 3.3, localization of Slam to basal compartments at furrow tips, and consequently localization of RhoGEF2 and all downstream targets including Myosin-2, is controlled by a Rab11 and RE-dependent membrane trafficking pathway (Fig. 3B) [26, 28, 98]. Third, changes in phosphoinositide levels at the plasma membrane of furrows influences F-actin crosslinker recruitment [129]. Chemical genetics and live imaging have shown that increasing the levels of $PI(4,5)P_2$ or decreasing the levels of $PI(3,4,5)P_3$ leads to the formation of short cells with nuclei trapped in prematurely constricted actomyosin rings, recapitulating the morphological alterations manifest in *bottleneck* loss-of-function embryos (Fig. 4B). What’s more, Bottleneck binds to $PI(3,4,5)P_3$, and $PI(3,4,5)P_3$ is required to recruit Bottleneck to furrow tips, suggesting a critical crosstalk between phosphoinositide composition at the furrow and the degree of ring constriction [129]. Fourth, and intriguingly, mitochondrial transport and fission/fusion events in the cytoplasm impact Myosin-2 activity in rings. Embryos mutant for the mitochondrial fission protein, Dynamin Related Protein-1 show enlarged mitochondria that fail to distribute evenly throughout the cytoplasm of newly forming cells (Box 1 and 3). These mutants also show a depletion of reactive oxygen species (ROS), and reduced Myosin-2 recruitment and constriction of rings [130]. In other contexts, ROS levels regulate Myosin-2 via Src and Rho Kinase, both of which localize to actomyosin rings during cellularization [131, 132].

In summary of this section, stringent coordination between actomyosin contractility and membrane remodeling is necessary during cellularization to build cells of the right length and morphology. This regulation occurs predominantly by controlling the timing of actomyosin remodeling and constriction. The actomyosin regulatory mechanisms identified so far, are broadly used in other examples of cell shape change and morphogenesis [47, 133-135]. Thus, while cellularization is a unique process, the lessons that it can teach regarding actomyosin contractility are broadly relevant. Interestingly, Dunk, Slam and Bottleneck do not have obvious homologs in higher organisms. However, their interactions with conserved machinery, such as RhoGEF2 and Myosin-2, and their conserved activities, such as directing cortical flow or F-actin bundling, still make them valuable in informing us on the mechanisms of actomyosin contractility. Surely, not all the actomyosin regulatory mechanisms or regulators of cellularization have been identified or fully elucidated yet, and so there is much room for further dissection of this system. For example, more work is needed to understand how $PI(4,5)P_2$ and $PI(3,4,5)P_3$ levels or availability might be changing during cellularization. How are these phosphoinositide levels modulated?

Given the importance of both $PI(4,5)P_2$ and $PI(3,4,5)P_3$ in regulating recruitment of many actin regulators to the plasma membrane and associated cortex [136], we anticipate that answering these questions will have impact well beyond cellularization. Finally, while all the membrane-actin coordination that we described here puts membrane activities upstream of actomyosin contractility, the relationship also acts in the reverse direction, too; at a minimum to pull the plasma membrane along with the constricting ring. Yet, we still know very little about this aspect of building cell bottoms during cellularization. Where does membrane come from for this specific cell surface expansion? What are the critical linkers between the actomyosin ring and growing plasma membrane? What is the nature of the final abscission event and how does this abscission impact gastrulation, which immediately follows cellularization?

5. Concluding Remarks

So far, cellularization has proven an exceptional experimental model for understanding how membrane and actin remodeling are coordinated during cell shape change and morphogenesis. Our goal here was to compile and highlight the numerous membrane-actin interactions that have been reported to date and present them within the timeline of cell-sculpting events required for cellularization. Both in our handling of the material, and in the literature overall, the temporal phases and events of cellularization have been treated as somewhat discrete for simplicity's sake. This discretization has also served to aid mathematical modeling of the process. However, as we move forward, a major challenge – and reward! – will be in learning what the molecular and mechanical connectors are between events, actin populations, cortical compartments, temporal phases, *et cetera.* For example: How does furrow invagination control disassembly and depletion of membrane reservoir F-actin? How does endocytosis "know" the amount of actomyosin machinery to trim away from the cell surface to achieve the right magnitude of contractility? How is actomyosin constriction held off until just the moment when furrows reach the base of the nuclei? What is the controlling clock? The next steps that we take together, as a community, to answer these more holistic questions will surely push our understanding of membrane-actin interactions into new and exciting territory.

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Box 1

Organization of newly forming cells during cellularization

Components, per nascent cell
Actin Cytoskeleton: F-actin organizes into at least four distinct populations, including (i) microvilli in apical compartments, (ii) cortical actin along furrow lengths, (iii) actomyosin rings in basal compartments, and (iv) actin rods in nuclei of heat stressed embryos [44, 74, 105, 136, 137]
Cell-Cell Junctions: In early cellularization, Cadherin/Catenin mediated bicellular “basal junctions” form at boundary of lateral and basal compartments; by late cellularization Cadherin/Catenin molecules concentrate at sub-apical compartments and are precursors to adherens junctions; tricellular junctions at cell corners aid transitions in cell packing morphologies during cellularization [36, 76, 138, 139]
Centrosomes: Apical to nucleus; duplicates early in cellularization and daughters separate to sit on either apical side of nuclei [140, 141]
Early Endosomes (EE): Rab5 enriched puncta sit in a pad basal to the nucleus; in late cellularization tubular EEs are internalized at apical compartments [24, 77]
Endoplasmic Reticulum (ER): Continuous membrane unit distributes around nucleus, but its components do not exchange between adjacent nuclei; concentrates apically by late cellularization [60, 106, 142]
Golgi: In the fruit fly embryo, Golgi is distributed as thousands of small puncta, sometimes referred to as Golgi bodies; Golgi bodies distribute around individual nuclei, but do not exchange between adjacent nuclei; trans Golgi bodies concentrate basal to nuclei and are transported apically by Dynein [60, 105-107, 110, 142]
Lipid Droplets: Puncta transported first basally then apically by MT motors during cellularization [143]
Microtubule (MT) Cytoskeleton: MTs originate from an apical centrosomal MT Organizing Center (MTOC) and are organized into at least two distinct populations, including (i) apical asters with plus-ends pointing to the PM, and (ii) basal baskets that hang over nuclei with plus-ends pointing to the embryo interior; in late cellularization MTs transition such that they become nucleated from apical acentrosomal MTOCs [138, 144, 145]
Mitochondria: Puncta that undergo fission and fusion; evenly distributed at early cellularization but become apically enriched by late cellularization [130]
Nuclei: Apically anchored, presumably by actin and microtubule-mediated mechanisms; nuclear shape changes from spherical to ellipsoid by late cellularization [146-148]
Plasma Membrane (PM): Before cellularization, PM apical to the nucleus is discrete and components do not exchange with PM of adjacent nuclei; from early cellularization PM is associated with underlying apical, subapical, lateral and basal cortical compartments [44, 62]
Recycling Endosomes (RE): Rab11 enriched puncta distribute around the apical centrosome pairs [101, 102]
Yolk Granules: Large granules packed in the embryo interior with associated yolk nuclei [11, 140]

Box 2

Zygotically Expressed Gene Products Required for Cellularization

Gene product	Cellularization function
Bottleneck (*D.m.* Bnk)	Negatively regulates actomyosin ring constriction at basal compartments [23, 32, 96, 129]
Dunk (*D.m.* Dunk)	Promotes Myosin-2 recruitment to furrows via cortical flow; remains localized in the basal compartment [22]
Nullo (*D.m.* Nullo)	Positively regulates F-actin levels at basal compartments, promotes assembly of basal junctions [24, 25, 36, 149]
Slow as Molasses (*D.m.* Slam)	Promotes Myosin-2 recruitment to basal compartment of furrows via recruitment of RhoGEF2 [26, 28, 30]
Serendipity-α (*D.m.* Sry-α)	Positively regulates F-actin levels at basal compartments, antagonizes Cofilin activity [27, 74]

Box 3

Maternally Supplied Gene Products Required for Cellularization

Gene product	Cellularization function
Abelson Kinase (*D.m.* Abl)	Negatively regulates Ena to modulate F-actin in microvilli [150]
ADP Ribosylation Factor 1 (*D.m.* Arf79F)	Monomeric GTPase; predominantly associates with Golgi; contributes to Golgi organization, vesicle budding [109]
Amphiphysin (*D.m.* Amph)	N-BAR protein; induces PM curvature; promotes endocytosis at basal compartment [24, 71]
Anillin (*D.m.* Anillin, Scraps)	F-actin crosslinker modulates actomyosin contraction [67, 92, 127]
Arp 2/3 Complex (*D.m.* Arp2, 3; ARPC1-5)	Complex of seven proteins; promotes F-actin nucleation; activated by nucleation promoting factors; enriched in microvilli [72, 150, 151]
Canoe/Afadin (*D.m.* Cno)	F-actin binding protein; promotes establishment of subapical compartment; links junction complexes to F-actin [61, 64]
Cdc42-Interacting Protein 4 (*D.m.* Cip4/Toca-1)	F-BAR protein; induces PM curvature; activates Arp2/3 and antagonizes Diaphanous at basal compartments [72]
Cofilin (*D.m.* Twinstar, Tsr)	F-actin binding and severing protein [67, 150]
Cumberland Gap (*D.m.* C-GAP/ RhoGAP71E)	GTPase activating protein; downregulates Rho1 activity to modulate actomyosin contraction [126]
Death Associated Kinase (*D.m.* Drak)	Phosphorylates Spaghetti Squash/Myosin-2 Regulatory Light Chain to activate Myosin-2 [123]
Diaphanous (*D.m.* Dia)	Formin-Family member; promotes F-actin polymerization; activated by Rho1 [55, 56]
Discontinuous Actin Hexagon (*D.m.* Dah)	Dystrophin homolog; tethers PM to F-actin [102-104]
Dynamin (*D.m.* Shibire, Shi)	Promotes vesicle scission during endocytosis and membrane trafficking from REs; regulates F-actin dynamics [24, 101, 152]
Dynamin Related Protein-1 (*D.m.* Drp-1)	Mitochondrial fission protein [130]
Dynein (*D.m.* Dhc64C)	Minus-end directed MT motor; transports membrane cargoes and mRNAs [107, 110, 143]
Elmo (*D.m.* Ced12)	Acts with Sponge to establish subapical compartment; provides guanine nucleotide exchange activity for Rac and Rap1 GTPases [61]
Enabled (D.m. Ena)	Actin nucleation promoting factor; promotes F-actin polymerization in microvilli [150]
Filamin (*D.m.* Cheerio, Cher)	F-actin crosslinker modulates actomyosin contraction [23]
Fimbrin (*D.m.* Fim)	F-actin crosslinker modulates actomyosin contraction [23]
GTPase Regulator Associated with FAK (*D.m.* GRAF)	GTPase activating protein; downregulates Rho1 activity to modulate actomyosin contraction [95]
Lava Lamp/Golgin (*D.m.* Lva)	Tethers Golgi-derived vesicles to Dynein for transport to PM [107, 110]
Myosin-2 (*D.m.* Zipper, Zip)	Plus-end directed actin motor; promotes actin-based contractility [94, 153, 154]
Nuclear Fall-Out/Arfophilin 2/ Rab11 Family-Interacting Protein 3 (*D.m.* Nuf)	Effector of Rab11 and Arf GTPases; promotes trafficking from REs [28, 98, 99]
Pavarotti Kinesin Like Protein (*D.m.* PavKLP)	Plus-end directed MT motor; transports membrane cargoes; promotes F-actin polymerization [57, 59, 155]
Peanut/Septin 3 (*D.m.* Pnt)	Heteromeric scaffolding protein; binds PM and F-actin; induces curvature of F-actin in actomyosin rings [92, 93, 156, 157]

Gene product	Cellularization function
Phospholipase D (*D.m.* Pld)	Promotes normal Golgi organization and trafficking [108]
Rab5 (*D.m.* Rab5)	Monomeric GTPase; regulates vesicle trafficking; predominantly at EEs and apical tubular carriers [24, 77, 101]
Rab8 (*D.m.* Rab8)	Monomeric GTPase; regulates vesicle trafficking; associates with exocyst at plasma membrane and at exocytic vesicles derived from REs and Golgi [113]
Rab11 (*D.m.* Rab11)	Monomeric GTPase; regulates vesicle trafficking; predominantly at REs [101, 102, 158]
Rabankaryin-5 (CG41099)	Effector of Rab5; promotes tubular carrier formation at the apical compartment in late cellularization, which facilitates pruning of excess membrane from disassembling microvilli and/or signaling for future actomyosin contractile events [77, 80]
Ras-Like Protein A (*D.m.* RalA)	Monomeric GTPase; facilitates exocyst assembly at apical and subapical compartments to support vesicle exocytosis throughout cellularization [99]
Rho1/RhoA GTPase (*D.m.* Rho1)	Monomeric GTPase; promotes F-actin polymerization and Myosin-2 motor activity [56, 58, 124]
Rho Guanine Nucleotide Exchange Factor-2 (*D.m.* RhoGEF2)	Guanine nucleotide exchange factor; activates Rho1 [56, 58, 98]
Rho Kinase (*D.m.* Rok)	Rho1 effector; phosphorylates Spaghetti Squash/Myosin-2 Regulatory Light Chain to activate Myosin-2 [67, 94]
Secretory 5 (*D.m.* Sec5)	Exocyst component; facilitates plasma membrane tethering and exocytosis of vesicles at apical and subapical compartments throughout cellularization [100, 113]
Spaghetti Squash (*D.m.* Sqh)	Myosin-2 Regulatory Light Chain; promotes Myosin-2 filament formation, localization and activation [94]
Sponge (*D.m.* Spg)	Acts with Elmo to establish subapical compartment; provides guanine nucleotide exchange activity for Rac and Rap1 GTPases [61]
Src Kinase (*D.m.* Src)	Promotes Actin 5C expression and actomyosin contraction [96, 159]
Stepping Stone (*D.m.* Sstn)	ARF regulator; acts with Step to promote endocytosis at basal compartment [69, 70, 109]
Steppke/Cytohesin (*D.m.* Step)	ARF guanine nucleotide exchange factor; acts with Sstn to promote endocytosis at basal compartment [69, 70, 109]
Syntaxin (*D.m.* Syx1A)	Promotes vesicle exocytosis; localizes to plasma membrane [160]

*For a breakdown of additional gene products per cortical compartment, see Schmidt and Grosshans, 2018 [44].

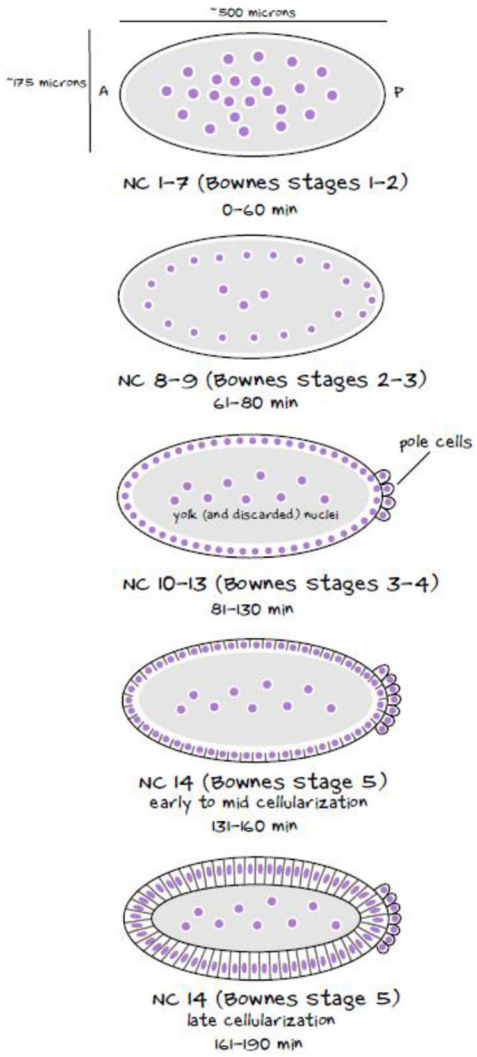

Fig. 1. Cross sectional view of the early developing *Drosophila* embryo.

Anterior (A) and posterior (P) poles indicated. Post-fertilization times (PF) at 25°C and Bownes Stage provided. Nuclei shown in purple. Nuclear divisions occur in the embryo interior during nuclear cycles (NC) 1-7. Nuclei are transported towards the embryo periphery during NC 8-9. Nuclei divide at the embryo periphery from NC 10-13, generating a regularly packed monolayer of ~6000 nuclei. Pole cells, which are the future germline, are emitted. At the onset of NC 14, cellularization starts and plasma membrane furrows form between every nucleus. Over one hour, furrows invaginate to make an epithelial sheet of mononucleate cells.

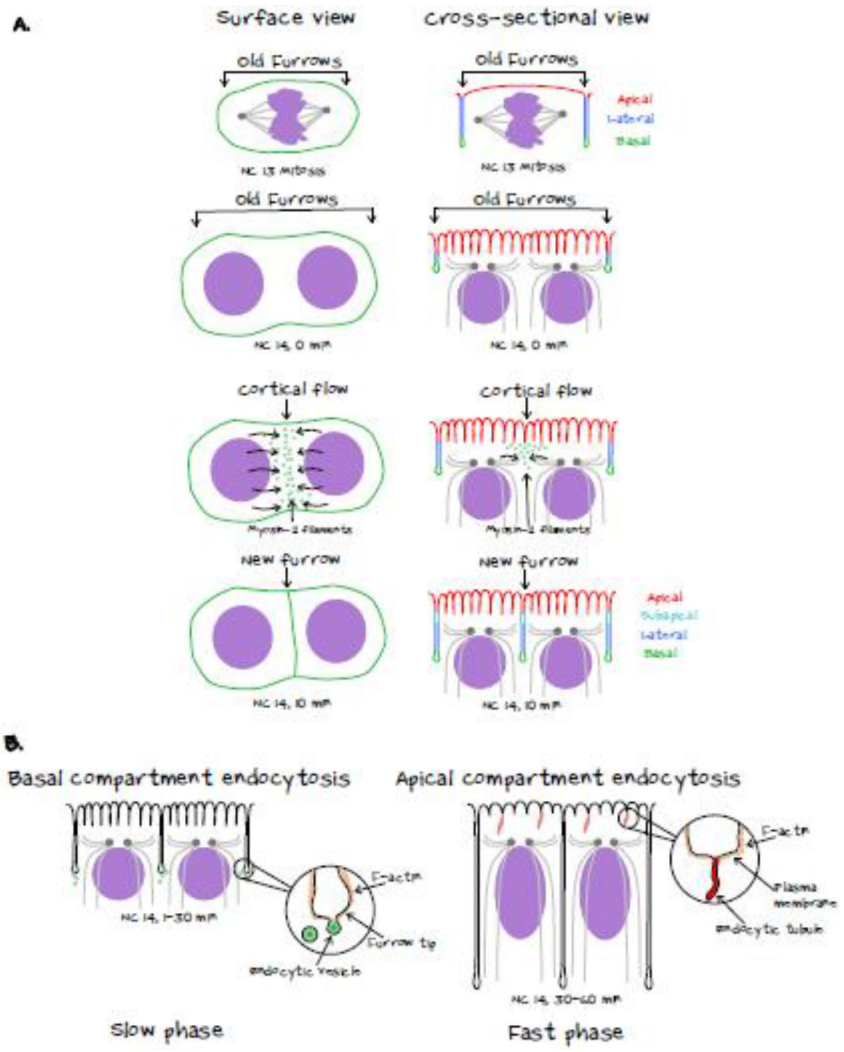

Fig. 2. Furrow assembly and compartmentalization during cellularization.

Chromosomes/nuclei and microtubules/MTOCs shown in purple and gray, respectively. (A) In mitosis of NC 13, metaphase furrows invaginate around every spindle, but no furrow forms at central spindles. At the end of NC 13, metaphase furrows partially regress. Once the embryo transitions to NC 14 and cellularization, remnants of metaphase furrows become “old furrows” and cortical flow drives recruitment of Myosin-2 and other components to assemble “new furrows” between daughter nuclei. As furrows form, they also polarize to generate distinct cortical compartments or domains as indicated. Establishment of a subapical domain is first seen at cellularization. (B) Endocytosis at the basal compartment in early cellularization and at the apical compartment in later cellularization ensures the proper membrane and protein complement per compartment.

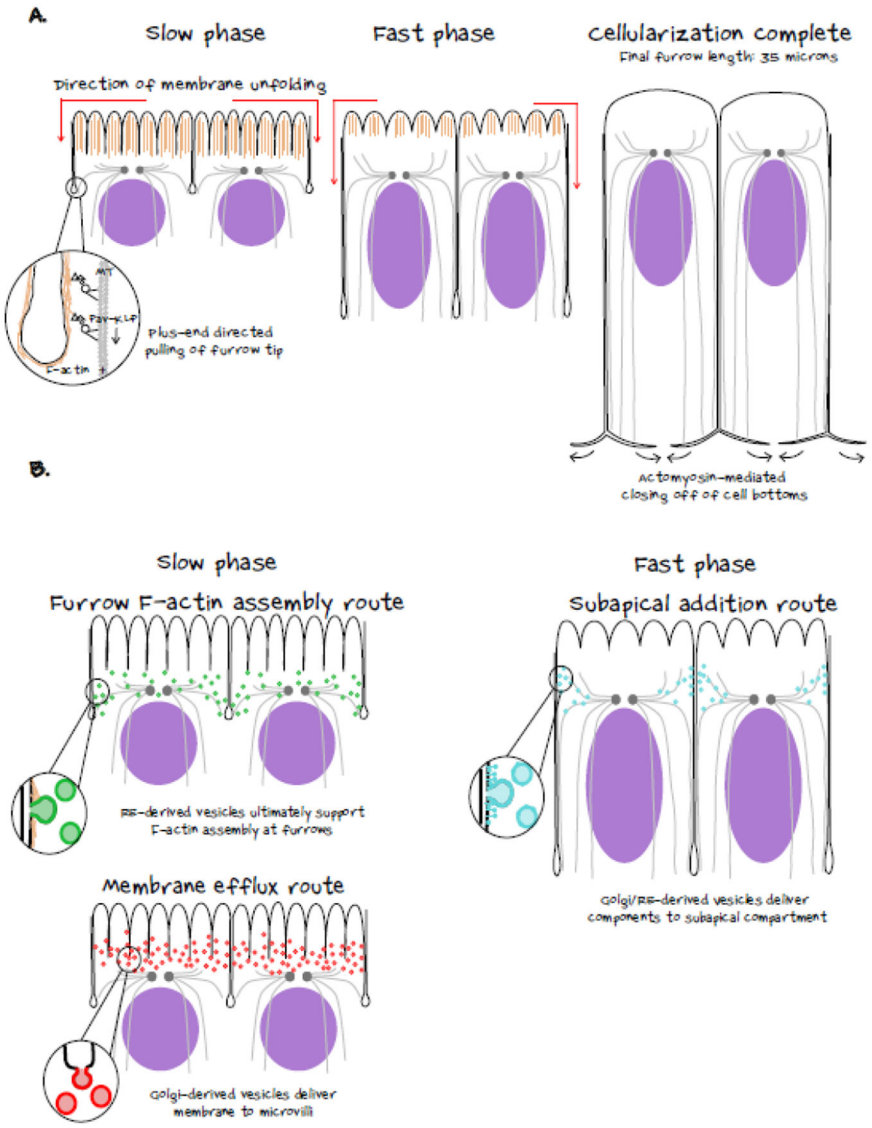

Fig. 3. Membrane handling and exocytosis during cellularization.

Nuclei and microtubules/MTOCs shown in purple and gray, respectively. (A) F-actin shown in orange. In addition to exocytosis, an apical microvillar membrane reservoir also unfolds to fuel furrow growth (*i.e.* picture a wavy string straightening out to cover more distance). Pulling forces exerted at the furrow tips by plus-end directed microtubule motors may aid unfolding. Membrane and microvillar F-actin depletion are both linearly coupled to furrow ingression. By late cellularization the reservoir is gone, and the tops of cells are flat. (B) Three membrane trafficking routes, culminating in exocytosis, deliver components to the cell surface as indicated.

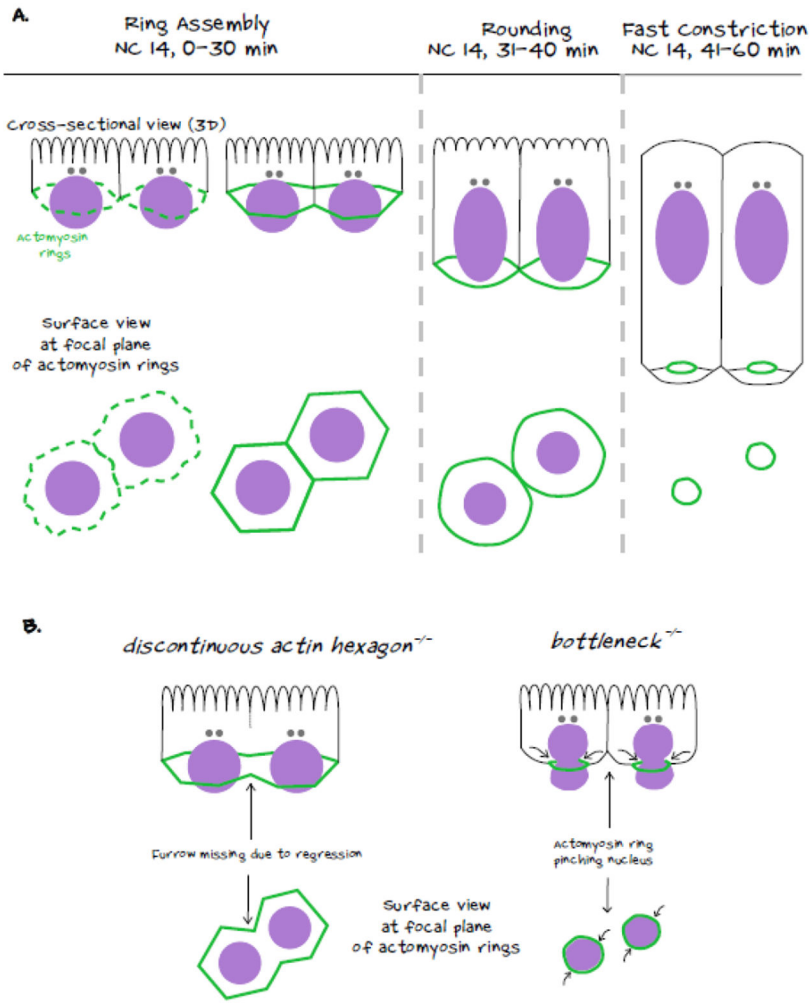

Fig. 4. Actomyosin constriction during cellularization.

Nuclei and actomyosin shown in purple and green, respectively. Centrosomes shown in gray. (A) Actomyosin ring constriction occurs in three phases, as indicated, to close off the bottom of cells during cellularization. (B) Two conspicuous phenotypes are seen when either the actomyosin ring does not assemble properly (left) or ring constriction is mis-regulated (right). In loss of function mutants for *discontinuous actin hexagon,* and other mutants/perturbations that lead to an F-actin deficit in the ring, furrows are weakened and sometimes regress, generating multinucleate cells. In loss of function mutants for *bottleneck,* and other mutants/perturbation that lead to premature constriction of actomyosin rings, the nuclei are pinched by the ring and cellularization fails.